KELLEY & FIRESTEIN'S

# Textbook of Rheumatology



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# Textbook of Rheumatology

Tenth Edition


**GARY S. FIRESTEIN, MD**
Distinguished Professor of Medicine
Dean and Associate Vice Chancellor, Translational Medicine
University of California, San Diego School of Medicine
La Jolla, California

**RALPH C. BUDD, MD**
Professor of Medicine and Microbiology and Molecular Genetics
Director, Vermont Center for Immunology and Infectious Diseases
University of Vermont College of Medicine Distinguished University Professor
The University of Vermont College of Medicine
Burlington, Vermont

**SHERINE E. GABRIEL, MD, MSc**
Professor and Dean
Rutgers Robert Wood Johnson Medical School
CEO, Robert Wood Johnson Medical Group
Emeritus Professor, Mayo Clinic
New Brunswick, New Jersey

**IAIN B. McINNES, PhD, FRCP, FRSE, FMedSci**
Muirhead Professor of Medicine
Arthritis Research UK Professor of Rheumatology
Director of Institute of Infection, Immunity, and Inflammation
College of Medical, Veterinary, and Life Sciences
University of Glasgow
Glasgow, United Kingdom

**JAMES R. O'DELL, MD**
Bruce Professor and Vice Chair of Internal Medicine
University of Nebraska Medical Center
Chief of Rheumatology
Department of Medicine
Omaha Veterans Affairs
Omaha, Nebraska


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1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

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*Publishing Manager:* Michael Houston
*Content Strategist:* Maureen Iannuzzi
*Senior Content Development Specialist:* Janice Gaillard
*Publishing Services Manager:* Trish Tannian
*Senior Project Manager:* Amanda Mincher
*Design Direction:* Brian Salisbury

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TOR has borne the name of Dr. Kelley with pride for decades, recognizing his foresight and scholarship in creating the premier clinical and academic text in the discipline. With the passage of time, just as rheumatology has advanced, so too TOR has evolved. This has occurred driven particularly by the vision and energy of Dr. Firestein, our Editor-in-Chief. The co-editors consider it timely to acknowledge Dr. Firestein's remarkable contribution by renaming the textbook for this, its tenth edition.

Sincerest thanks to my wonderful wife, Linda, and our children, David and Cathy, for their patience and support. Also, the editorial help of our three Cavalier King Charles puppies, Winston, Humphrey, and Punkin, was invaluable.

**Gary S. Firestein**

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**Sherine E. Gabriel**

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**Iain B. McInnes**

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**James R. O'Dell**

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# Contributors


**Steven Abramson, MD**
New York University Langone Medical Center
New York, New York
*Pathogenesis of Osteoarthritis*

**KaiNan An, PhD**
Emeritus Professor
Department of Orthopedic Surgery
Mayo Clinic
Rochester, Minnesota
*Biomechanics*

**Felipe Andrade, MD, PhD**
Associate Professor of Medicine
Department of Medicine
Division of Rheumatology
The Johns Hopkins University School of Medicine
Baltimore, Maryland
*Autoantibodies in Rheumatoid Arthritis*

**Stacy P. Ardoin, MD, MS**
Associate Professor of Adult and Pediatric Rheumatology
Ohio State University
Nationwide Children's Hospital
Columbus, Ohio
*Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis*

**Anne Barton, MBChB, MSc, PhD, FRCP**
Professor of Rheumatology
Centre for Musculoskeletal Research
The University of Manchester
Manchester, United Kingdom
*Genetics of Rheumatic Diseases*

**Robert P. Baughman, MD**
Professor of Medicine
Department of Internal Medicine
University of Cincinnati
Cincinnati, Ohio
*Sarcoidosis*

**Dorcas E. Beaton, BScOT, MSc, PhD**
Scientist
Musculoskeletal Health and Outcomes Research
Li Ka Shing Knowledge Institute
St Michael's Hospital
Senior Scientist
Institute for Work and Health
Associate Professor
Institute of Health Policy Management and Evaluation
University of Toronto
Toronto, Ontario, Canada
*Assessment of Health Outcomes*

**Helen M. Beere, PhD**
Department of Immunology
St. Jude Children's Research Hospital
Memphis, Tennessee
*Immunologic Repercussions of Cell Death*

**Javier Beltran, MD, FACR**
Chairman
Department of Radiology
Maimonides Medical Center
Brooklyn, New York
*Osteonecrosis*

**David Bending, PhD**
University College London Institute of Child Health
London, United Kingdom
*Etiology and Pathogenesis of Juvenile Idiopathic Arthritis*

**Robert M. Bennett, MD, FRCP, MACR**
Professor of Medicine
Oregon Health and Science University
Portland, Oregon
*Overlap Syndromes*

**Bonnie L. Bermas, MD**
Director, Clinical Lupus Center
Department of Rheumatology
Brigham and Women's Hospital
Associate Professor of Medicine
Harvard Medical School
Boston, Masachusetts
*Pregnancy and Rheumatic Diseases*

**George Bertsias, MD, PhD**
Lecturer
Rheumatology, Clinical Immunology, and Allergy
University of Crete
Iraklio, Greece
*Treatment of Systemic Lupus Erythematosus*

**Nina Bhardwaj, MD, PhD**
Director, Immunotherapy
Hess Center for Science and Medicine
Professor of Medicine
Division of Hematology and Oncology
Tisch Cancer Institute
Icahn School of Medicine at Mount Sinai
New York, New York
*Dendritic Cells: General Overview and Role in Autoimmunity*

**Johannes W.J. Bijlsma, MD, PhD**
Professor
Rheumatology and Clinical Immunology
University Medical Center Utrecht
Utrecht, Netherlands
*Glucocorticoid Therapy*

**Linda K. Bockenstedt, MD**
Harold W. Jockers Professor of Medicine
Internal Medicine/Rheumatology
Yale University School of Medicine
New Haven, Connecticut
*Lyme Disease*

**Maarten Boers, MSc, MD, PhD**
Department of Epidemiology and Biostatistics
Amsterdam Rheumatology and Immunology Center
VU University Medical Center
Amsterdam, Netherlands
*Assessment of Health Outcomes*

**Eric Boilard, PhD**
Universite Laval
CHU de Quebec
Quebec, Canada
*Platelets*

**Francesco Boin, MD**
Associate Professor of Medicine
Director, UCSF Scleroderma Center
Division of Rheumatology
University of California, San Francisco
San Francisco, California
*Clinical Features and Treatment of Scleroderma*

**Dimitrios T. Boumpas, MD, FACP**
Professor of Internal Medicine
National and Kapodistrian University of Athens, Medical School
Professor of Internal Medicine
4th Department of Medicine
Attikon University Hospital
Affiliated Investigator
Immunobiology
Biomedical Research Foundation of the Academy of Athens
Athens, Greece
Affiliated Investigator
Developmental and Functional Biology
Institute of Molecular Biology and Biotechnology – FORTH
Iraklion, Greece
*Treatment of Systemic Lupus Erythematosus*

**David L. Boyle, BA**
Professor of Medicine
University of California, San Diego
La Jolla, California
*Biologic Markers in Clinical Trials and Clinical Care*

**Sean Bradley, PhD**
Research Fellow
Department of Medicine
Division of Rheumatology
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts
*Principles of Signaling*

**Matthew Brown, MD, FRACP**
Professor of Immunogenetics
Princess Alexandra Hospital
University of Queensland Diamantina Institute
Translational Research Institute
Brisbane, Queensland, Australia
*Ankylosing Spondylitis*

**Maya Buch, MB CHB, PhD, MRCP**
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
*Arthrocentesis and Injection of Joints and Soft Tissue*

**Christopher D. Buckley, MBBS, DPhil**
Arthritis Research UK Professor of Rheumatology
Rheumatology Research Group
Institute of Inflammation and Ageing
University of Birmingham
Queen Elizabeth Hospital
Birmingham, United Kingdom
*Fibroblasts and Fibroblast-like Synoviocytes*

**Ralph C. Budd, MD**
Professor of Medicine and Microbiology and Molecular Genetics
Director, Vermont Center for Immunology and Infectious Diseases
University of Vermont College of Medicine Distinguished University Professor
The University of Vermont College of Medicine
Burlington, Vermont
*T Lymphocytes*
*Amyloidosis*

**Nathalie Burg, MD**
Instructor in Clinical Investigation
The Rockefeller University
New York, New York
*Neutrophils*

**Christopher M. Burns, MD**
Associate Professor of Medicine
Department of Medicine
Geisel School of Medicine at Dartmouth
Staff Rheumatologist
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire
*Clinical Features and Treatment of Gout*

**Amy C. Cannella, MD, MS**
Associate Professor
University of Nebraska Medical Center
Veterans Affairs Medical Center
Omaha, Nebraska
*Traditional DMARDS: Methotrexate, Leflunomide, Sulfasalazine, and Hydroxychloroquine and Combination Therapies*
*Clinical Features of Rheumatoid Arthritis*

**John D. Carter, MD**
Professor of Medicine
Department of Internal Medicine
Division of Rheumatology
University of South Florida Morsani School of Medicine
Tampa, Florida
*Undifferentiated Spondyloarthritis*

**Eliza F. Chakravarty, MD, MS**
Associate Member
Arthritis and Clinical Immunology
Oklahoma Medical Research Foundation
Oklahoma City, Oklahoma
*Musculoskeletal Syndromes in Malignancy*

**Soumya D. Chakravarty, MD, PhD, FACP, FACR**
Assistant Professor in Rheumatology
Department of Medicine
Drexel University College of Medicine
Philadelphia, Pennsylvania
*Arthritis Accompanying Endocrine and Metabolic Disorders*

**Christopher Chang, MD, PhD, MBA**
Clinical Professor of Medicine
Associate Director, Allergy and Immunology Fellowship Program
Division of Rheumatology, Allergy, and Clinical Immunology
University of California, Davis
Davis, California
*Osteonecrosis*

**Joseph S. Cheng, MD, MS**
Director, Neurosurgery Spine Program
Department of Neurosurgery
Vanderbilt University
Nashville, Tennessee
*Neck Pain*

**Christopher P. Chiodo, MD**
Foot and Ankle Division Chief
Department of Orthopedic Surgery
Brigham and Women's Hospital
Boston, Massachusetts
*Foot and Ankle Pain*

**Sharon Chung, MD, MAS**
Assistant Professor in Residence
Division of Rheumatology
Department of Medicine
University of California, San Francisco
San Francisco, California
*Anti-neutrophil Cytoplasmic Antibody–Associated Vasculitis*

**Leslie G. Cleland, MBBS, MD**
Department of Medicine
University of Adelaide School of Medicine
Faculty of Health Sciences
Adelaide, South Australia, Australia
*Nutrition and Rheumatic Diseases*

**Stanley Cohen, MD**
Program Director
Rheumatology
Presbyterian Hospital
Clinical Professor
Internal Medicine
University of Texas Southwestern Medical School
Medical Director
Metroplex Clinical Research Center
Dallas, Texas
*Novel Intra-cellular Targeting Agents in Rheumatic Disease*

**Robert A. Colbert, MD, PhD**
Senior Investigator
National Institute of Arthritis, Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
*Etiology and Pathogenesis of Spondyloarthritis*

**Paul P. Cook, MD**
Professor of Medicine
Department of Medicine
Brody School of Medicine at East Carolina University
Greenville, North Carolina
*Bacterial Arthritis*

**Joseph E. Craft, MD**
Paul B. Beeson Professor of Medicine
Chief of Rheumatology
Professor of Immunobiology
Director, Investigative Medicine Program
Yale University School of Medicine
New Haven, Connecticut
*Anti-nuclear Antibodies*

**Leslie J. Crofford, MD**
Professor of Medicine
Director, Division of Rheumatology and Immunology
Vanderbilt University
Nashville, Tennessee
*Fibromyalgia*
*Biology and Therapeutic Targeting of Prostanoids*

**Bruce N. Cronstein, MD**
Paul R. Esserman Professor of Medicine
New York University School of Medicine
New York, New York
*Acute Phase Reactants and the Concept of Inflammation*

**Mary K. Crow, MD**
Joseph P. Routh Professor of Rheumatic Diseases in Medicine
Weill Cornell Medical College
Physician-in-Chief
Benjamin M. Rosen Chair in Immunology and Inflammation Research
Medicine and Research
Hospital for Special Surgery
New York, New York
*Etiology and Pathogenesis of Systemic Lupus Erythematosus*

**Cynthia S. Crowson, MS**
Associate Professor of Medicine
Assistant Professor of Biostatistics
Department of Health Sciences Research
Division of Rheumatology
Mayo Clinic
Rochester, Minnesota
*Cardiovascular Risk in Inflammatory Rheumatic Disease*

**Kirsty L. Culley, PhD**
Research Division
Hospital for Special Surgery
New York, New York
*Cartilage and Chondrocytes*

**Gaye Cunnane, PhD, MB, FRCPI**
Department of Medicine
Trinity College Dublin
Department of Rheumatology
St. James's Hospital
Dublin, Ireland
*Relapsing Polychondritis*
*Hemachromatosis*

**Maria Dall'Era, MD**
Associate Professor of Medicine
Division of Rheumatology
University of California, San Francisco
San Francisco, California
*Clinical Features of Systemic Lupus Erythematosus*

**Erika Darrah, PhD**
Assistant Professor of Medicine
Department of Medicine, Division of Rheumatology
The Johns Hopkins University School of Medicine
Baltimore, Maryland
*Autoantibodies in Rheumatoid Arthritis*

**John M. Davis III, MD, MS**
Associate Professor of Medicine
Division of Rheumatology
Department of Medicine
Mayo Clinic College of Medicine
Rochester, Minnesota
*History and Physical Examination of the Musculoskeletal System*

**Cosimo De Bari, MD, PhD, FRCP**
Musculoskeletal Research Program
Institute of Medical Sciences
University of Aberdeen
Aberdeen, United Kingdom
*Regenerative Medicine and Tissue Engineering*

**Francesco Dell'Accio, MD, PhD, FRCP**
Professor of Musculoskeletal Regenerative Medicine and Rheumatology
Queen Mary University of London
William Harvey Research Institute
London, United Kingdom
*Regenerative Medicine and Tissue Engineering*

**Betty Diamond, MD**
Autoimmune and Musculoskeletal Disease
The Feinstein Institute for Medical Research
Manhasset, New York
*B Cells*

**Paul E. Di Cesare, MD**
Carlsbad, California
*Pathogenesis of Osteoarthritis*

**Rajiv Dixit, MD**
Clinical Professor of Medicine
University of California, San Francisco
San Francisco, California
Director
Northern California Arthritis Center
Walnut Creek, California
*Low Back Pain*

**Joost P.H. Drenth, MD, PhD**
Professor of Gastroenterology
Department of Gastroenterology
Radboud University Medical Centre
Nijmegen, the Netherlands
*Familial Autoinflammatory Syndromes*

**Michael L. Dustin, PhD**
Professor
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Kennedy Institute of Rheumatology
Oxford, United Kingdom
Research Professor
Pathology
New York University School of Medicine
New York, New York
*Adaptive Immunity and Organization of Lymphoid Tissues*

**Hani S. El-Gabalawy, MD**
Professor of Internal Medicine and Immunology
University of Manitoba
Winnipeg, Manitoba, Canada
*Synovial Fluid Analyses, Synovial Biopsy, and Synovial Pathology*

**Musaab Elmamoun, MBBS, MRCPI**
Clinical Research Fellow
Department of Rheumatology
St. Vincent's University Hospital
University College Dublin
Dublin, Ireland
*Psoriatic Arthritis*

**Alan R. Erickson, MD**
Associate Professor of Rheumatology
University of Nebraska
Omaha, Nebraska
*Clinical Features of Rheumatoid Arthritis*

**Doruk Erkan, MD**
Associate Physician-Scientist
Barbara Volcker Center
Hospital for Special Surgery
Weill Cornell Medicine
New York, New York
*Anti-phospholipid Syndrome*

**Stephen Eyre, PhD**
Senior Lecturer
Centre for Musculoskeletal Research
The University of Manchester
Manchester, United Kingdom
*Genetics of Rheumatic Diseases*

**Antonis Fanouriakis, MD**
Rheumatology and Clinical Immunology
Attikon University Hospital
Athens, Greece
*Treatment of Systemic Lupus Erythematosus*

**David T. Felson, MD, MPH**
Professor of Medicine and Epidemiology
Boston University School of Medicine
Boston, Masachusetts
Professor of Medicine and Public Health
University of Manchester
Manchester, Lancashire, United Kingdom
*Treatment of Osteoarthritis*

**Max Field, MD, FRCP**
Associate Academic (Retired)
Division of Immunology, Institute of Infection, Immunology, and Immunity
College of Medical, Veterinary, and Life Sciences
University of Glasgow
Glasgow, United Kingdom
*Acute Monoarthritis*

**Andrew Filer, BSc, MBChB, PhD**
Senior Lecturer
Institute of Inflammation and Ageing
The University of Birmingham
Honorary Consultant Rheumatologist
Department of Rheumatology
University Hospitals Birmingham NHS Foundation Trust
Birmingham, United Kingdom
*Fibroblasts and Fibroblast-like Synoviocytes*

**Gary S. Firestein, MD**
Distinguished Professor of Medicine
Dean and Associate Vice Chancellor, Translational Medicine
University of California, San Diego School of Medicine
La Jolla, California
*Synovium*
*Etiology and Pathogenesis of Rheumatoid Arthritis*

**Felicity G. Fishman, MD**
Assistant Professor
Department of Orthopaedics and Rehabilitation
Yale University School of Medicine
New Haven, Connecticut
*Hand and Wrist Pain*

**Oliver FitzGerald, MD, FRCPI, FRCP(UK)**
Newman Clinical Research Professor
Rheumatology
St. Vincent's University Hospital and Conway Institute
University College Dublin
Dublin, Ireland
*Psoriatic Arthritis*

**John P. Flaherty, MD**
Professor of Medicine
Northwestern University Feinberg School of Medicine
Chicago, Illinois
*Mycobacterial Infections of Bones and Joints*
*Fungal Infections of Bones and Joints*

**César E. Fors Nieves, MD**
Department of Medicine
Division of Rheumatology
New York University School of Medicine
New York, New York
*Acute Phase Reactants and the Concept of Inflammation*

**Karen A. Fortner, PhD**
Assistant Professor of Medicine
The University of Vermont College of Medicine
Burlington, Vermont
*T Lymphocytes*

**Sherine E. Gabriel, MD, MSc**
Professor and Dean
Rutgers Robert Wood Johnson Medical School
CEO, Robert Wood Johnson Medical Group
Emeritus Professor, Mayo Clinic
New Brunswick, New Jersey
*Cardiovascular Risk in Inflammatory Rheumatic Disease*

**Philippe Gasque, PhD**
Professor of Immunology
UM134 (PIMIT) and LICE-OI (CHU)
University of La Réunion
St. Denis, Réunion
*Viral Arthritis*

**M. Eric Gershwin, MD, MACR, MACP**
The Jack and Donald Chia Distinguished Professor of Medicine
Chief, Division of Rheumatology, Allergy, and Clinical Immunology
University of California, Davis
Davis, California
*Osteonecrosis*

**Heather S. Gladue, DO**
Assistant Professor of Internal Medicine
Division of Rheumatology
Emory University
Atlanta, Georgia
*Inflammatory Diseases of Muscle and Other Myopathies*

**Mary B. Goldring, PhD**
Senior Scientist, Research Division
Hospital for Special Surgery
Professor of Cell and Developmental Biology
Weill Cornell Graduate School of Medical Sciences
Weill Cornell Medical College
New York, New York
*Biology of the Normal Joint*
*Cartilage and Chondrocytes*

**Steven R. Goldring, MD**
Chief Scientific Officer Emeritus
Hospital for Special Surgery
Weill Cornell Medical College
New York, New York
*Biology of the Normal Joint*

**Yvonne M. Golightly, PT, MS, PhD**
Research Assistant Professor
Department of Epidemiology
Faculty Member
Thurston Arthritis Research Center
Injury Prevention Research Center
Human Movement Science Program
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina
*Clinical Research Methods in Rheumatic Disease*

**Stuart Goodman, MD, PhD, FRCSC, FACS, FBSE**
Robert L. and Mary Ellenburg Professor of Surgery
Orthopaedic Surgery and (by courtesy) Bioengineering
Stanford University
Stanford, California
*Hip and Knee Pain*

**Siamon Gordon, MB, ChB, PhD**
Sir William Dunn School of Pathology
University of Oxford
Oxford, United Kingdom
*Mononuclear Phagocytes*

**Walter Grassi, MD**
Professor of Rheumatology
Clinica Reumatologica
Università Politecnica delle Marche
Ancona, Italy
*Imaging in Rheumatic Disease*

**Douglas R. Green, PhD**
Peter C. Doherty Endowed Chair of Immunology
Department of Immunology
St. Jude Children's Research Hospital
Memphis, Tennessee
*Immunologic Repercussions of Cell Death*

**Adam Greenspan, MD, FACR**
Professor of Radiology and Orthopedic Surgery
Section of Musculoskeletal Imaging
Department of Radiology
University of California, Davis School of Medicine
Sacramento, California
*Osteonecrosis*

**Peter Gregersen, MD**
Professor of Rheumatology
Robert S. Boas Center for Genomics and Human Genetics
The Feinstein Institute for Medical Research
Manhasset, New York
*Genetics of Rheumatic Diseases*

**Christine Grimaldi, PhD**
Boehringer Ingelheim Pharmaceuticals
Ridgefield, Connecticut
*B Cells*

**Luiza Guilherme, PhD**
Professor
Heart Institute (InCor)
School of Medicine, University of São Paulo
Institute for Immunology Investigation
National Institute for Science and Technology
São Paulo, Brazil
*Rheumatic Fever and Post-streptococcal Arthritis*

**Rula A. Hajj-Ali, MD**
Associate Professor
Cleveland Clinic Lerner College of Medicine
Cleveland, Ohio
*Primary Angiitis of the Central Nervous System*

**Dominik R. Haudenschild, PhD**
Associate Professor
Department of Orthopaedic Surgery
University of California, Davis
Sacramento, California
*Pathogenesis of Osteoarthritis*

**David B. Hellmann, MD**
Vice Dean and Chairman
Department of Medicine
The Johns Hopkins Bayview Medical Center
Baltimore, Maryland
*Giant Cell Arteritis, Polymyalgia Rheumatica, and Takayasu's Arteritis*

**Rikard Holmdahl, MD, PhD**
Professor of Medical Biochemistry and Biophysics
Karolinska Institutet
Stockholm, Sweden
*Experimental Models for Rheumatoid Arthritis*

**Joyce J. Hsu, MD, MS**
Clinical Associate Professor
Pediatric Rheumatology
Stanford University School of Medicine
Stanford, California
*Clinical Features and Treatment of Juvenile Idiopathic Arthritis*

**James I. Huddleston III, MD**
Associate Professor of Orthopaedic Surgery
Adult Reconstruction Service Chief
Department of Orthopaedic Surgery
Stanford University Medical Center
Stanford, California
*Hip and Knee Pain*

**Alan P. Hudson, PhD**
Professor Emeritus
Department of Immunology and Microbiology
Wayne State University School of Medicine
Detroit, Michigan
*Undifferentiated Spondyloarthritis*

**Thomas W.J. Huizinga, MD, PhD**
Professor of Rheumatology
Leiden University Medical Center
Leiden, Netherlands
*Early Synovitis and Early Undifferentiated Arthritis*

**Gene G. Hunder, MS, MD**
Professor Emeritus
Division of Rheumatology
Department of Medicine
Mayo Clinic College of Medicine
Rochester, Minnesota
*History and Physical Examination of the Musculoskeletal System*

**Maura D. Iversen, BSc, MPH, DPT, SD, FNAP, FAPTA**
Professor and Chair
Department of Physical Therapy, Movement, and Rehabilitation Sciences
Northeastern University
Senior Instructor
Department of Medicine
Harvard Medical School
Behavioral Scientist
Section of Clinical Sciences
Division of Rheumatology, Immunology, and Allergy
Brigham and Women's Hospital
Boston, Massachusetts
*Introduction to Physical Medicine, Physical Therapy, and Rehabilitation*

**Johannes W.G. Jacobs, MD, PhD**
Associate Professor
Rheumatology and Clinical Immunology
University Medical Center Utrecht
Utrecht, Netherlands
*Glucocorticoid Therapy*

**Ho Jen, MDCM, FRCPC**
Diagnostic Radiology and Nuclear Medicine
Associate Clinical Professor
Radiology and Diagnostic Imaging
University of Alberta
Edmonton, Alberta, Canada
*Imaging in Rheumatic Disease*

**Joanne M. Jordan, MD, MPH**
Joseph P. Archie, Jr., Eminent Professor of Medicine
Executive Associate Dean for Faculty Affairs and Leadership Development
Department of Medicine
Chief
Division of Rheumatology, Allergy, and Immunology
Director
Thurston Arthritis Research Center
University of North Carolina
Chapel Hill, North Carolina
*Clinical Research Methods in Rheumatic Disease*
*Clinical Features of Osteoarthritis*

**Joseph L. Jorizzo, MD**
Professor of Clinical Dermatology
Weill Cornell Medical College
New York, New York
Professor, Former, and Founding Chair
Department of Dermatology
Wake Forest University School of Medicine
Winston Salem, North Carolina
*Behçet's Disease*

**Jorge Kalil, MD**
Full Professor
Heart Institute (InCor)
Institute for Immunology Investigation
National Institute for Science and Technology
Clinical Immunology and Allergy
Department of Clinical Medicine
School of Medicine, University of São Paulo
São Paulo, Brazil
*Rheumatic Fever and Post-streptococcal Arthritis*

**Kenton Kaufman, PhD, PE**
Director
Biomechanics/Motion Analysis Laboratory
Mayo Clinic
Rochester, Minnesota
*Biomechanics*

**William S. Kaufman, MD**
Dermatology Associates, PA
Wilmington, North Carolina
*Behçet's Disease*

**Arthur Kavanaugh, MD**
Professor of Medicine
Center for Innovative Therapy
Division of Rheumatology, Allergy, and Immunology
University of California, San Diego School of Medicine
La Jolla, California
*Anti-cytokine Therapies*

**Robert T. Keenan, MD, MPH**
Assistant Professor of Medicine
Duke University School of Medicine
Durham, North Carolina
*Etiology and Pathogenesis of Hyperuricemia and Gout*

**Tony Kenna, BSc (Hons), PhD**
Senior Research Fellow
The University of Queensland Diamantina Institute
Translational Research Institute
Brisbane, Queesland, Australia
*Ankylosing Spondylitis*

**Darcy A. Kerr, MD**
Assistant Professor of Pathology
University of Miami Miller School of Medicine
University of Miami Hospital
Miami, Florida
*Tumors and Tumor-like Lesions of Joints and Related Structures*

**Alisa E. Koch, MD**
Senior Medical Fellow
Eli Lilly
Adjunct Professor of Rheumatology
University of Michigan
Ann Arbor, Michigan
*Cell Recruitment and Angiogenesis*

**Dwight H. Kono, MD**
Professor of Immunology
Department of Immunology and Microbial Science
The Scripps Research Institute
La Jolla, California
*Autoimmunity*

**Peter Korsten, MD**
Resident Physician
Department of Nephrology and Rheumatology
University Medical Center Göttingen
Göttingen, Germany
*Sarcoidosis*

**Deborah Krakow, MD**
Professor
Departments of Orthopaedic Surgery, Human Genetics, and OB/GYN
David Geffen School of Medicine
University of California, Los Angeles
Los Angeles, California
*Heritable Diseases of Connective Tissue*

**Svetlana Krasnokutsky, MD, MSci**
Assistant Professor of Medicine
Rheumatology
New York University School of Medicine
New York, New York
*Etiology and Pathogenesis of Hyperuricemia and Gout*

**Floris P.J.G. Lafeber, PhD**
Professor
Rheumatology and Clinical Immunology
University Medical Center Utrecht
Utrecht, Netherlands
*Hemophilic Arthropathy*

**Robert G.W. Lambert, MB BCh, FRCR, FRCPC**
Professor and Chair
Radiology and Diagnostic Imaging
University of Alberta
Edmonton, Alberta, Canada
*Imaging in Rheumatic Disease*

**Nancy E. Lane, MD**
Director of Center for Musculoskeletal Health
Department of Internal Medicine
University of California, Davis School of Medicine
Sacramento, California
*Metabolic Bone Disease*

**Carol A. Langford, MD, MHS**
Director, Center for Vasculitis Care and Research
Department of Rheumatic and Immunologic Diseases
Cleveland Clinic Lerner College of Medicine
Cleveland, Ohio
*Primary Angiitis of the Central Nervous System*

**Daniel M. Laskin, BS, DDS, MS, DSc (hon)**
Professor and Chairman Emeritus
Department of Oral and Maxillofacial Surgery
Virginia Commonwealth University Schools of Dentistry and Medicine
Richmond, Virginia
*Temporomandibular Joint Pain*

**Gerlinde Layh-Schmitt, PhD**
Senior Investigator
National Institute of Arthritis, Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
*Etiology and Pathogenesis of Spondyloarthritis*

**Lela A. Lee, MD**
Clinical Professor of Dermatology and Medicine
University of Colorado School of Medicine
Denver, Colorado
*Skin and Rheumatic Diseases*

**Tzielan C. Lee, MD**
Clinical Associate Professor
Pediatric Rheumatology
Stanford University School of Medicine
Stanford, California
*Clinical Features and Treatment of Juvenile Idiopathic Arthritis*

**Michael D. Lockshin, MD**
Director, Barbara Volcker Center
Hospital for Special Surgery
Weill Cornell Medicine
New York, New York
*Anti-phospholipid Syndrome*

**Carlos J. Lozada, MD**
Professor of Clinical Medicine
Division of Rheumatology
University of Miami Miller School of Medicine
Miami, Florida
*Rheumatic Manifestations of Hemoglobinopathies*

**Ingrid E. Lundberg, MD, PhD**
Rheumatology Unit
Department of Medicine, Solna
Karolinska University Hospital
Stockholm, Sweden
*Inflammatory Diseases of Muscle and Other Myopathies*

**Raashid Luqmani, DM, FRCP, FRCPE**
Professor of Rheumatology
Consultant Rheumatologist
Nuffield Orthopaedic Centre
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Science
University of Oxford
Oxford, United Kingdom
*Polyarteritis Nodosa and Related Disorders*

**Frank P. Luyten, MD**
Professor of Rheumatology
University Hospitals KU
Leuven, Belgium
*Regenerative Medicine and Tissue Engineering*

**Reuven Mader, MD**
Head, Rheumatic Diseases Unit
Ha'Emek Medical Center
Afula, Israel
Associate Clinical Professor
The B. Rappaport Faculty of Medicine
The Technion Institute of Technology
Haifa, Israel
*Proliferative Bone Diseases*

**Walter Maksymowych, MB ChB, FACP, FRCP(C)**
Professor of Medicine
University of Alberta
Edmonton, Alberta, Canada
*Ankylosing Spondylitis*

**Joseph A. Markenson, MD, MS, MACR**
Professor of Clinical Medicine
Medicine/Rheumatology
Joan and Sanford Weill Medical College of Cornell University
Attending Physician
Medicine/Rheumatology
Hospital for Special Surgery
New York, New York
*Arthritis Accompanying Endocrine and Metabolic Disorders*

**Scott David Martin, MD**
Associate Professor of Orthopedics
Harvard Medical School
Research Associate
Professor and Chairman of Orthopedics
Orthopedics
Brigham and Women's Hospital
Boston, Massachusetts
*Shoulder Pain*

**Eric L. Matteson, MD, MPH**
Professor of Medicine
Division of Rheumatology and Division of Epidemiology
Mayo Clinic College of Medicine
Rochester, Minnesota
*Cancer Risk in Rheumatic Diseases*

**Laura McGregor, MBChB, MRCP**
Consultant Rheumatologist
Centre for Rheumatic Diseases
Glasgow Royal Infirmary
Glasgow, United Kingdom
*Acute Monoarthritis*

**Iain B. McInnes, PhD, FRCP, FRSE, FMedSci**
Muirhead Professor of Medicine
Arthritis Research UK Professor of Rheumatology
Director of Institute of Infection, Immunity, and Inflammation
College of Medical, Veterinary, and Life Sciences
University of Glasgow
Glasgow, United Kingdom
*Cytokines*

**Elizabeth K. McNamara, MD**
Wilmington VA Health Care Center
Wilmington, North Carolina
*Behçet's Disease*

**Ted R. Mikuls, MD, MSPH**
Umbach Professor of Rheumatology
Department of Internal Medicine
Division of Rheumatology and Immunology
University of Nebraska Medical Center
Veterans Affairs Nebraska
Western-Iowa Health Care System
Omaha, Nebraska
*Urate-Lowering Therapy*
*Clinical Features of Rheumatoid Arthritis*

**Mark S. Miller, PhD**
Assistant Professor
Department of Kinesiology
University of Massachusetts
Amherst, Massachusetts
*Muscle: Anatomy, Physiology, and Biochemistry*

**Pedro Azevedo Ming, MD, PhD**
Assistant Professor of Rheumatology
Faculdade Evangelicade Medicina
Department of Rheumatology
Curitiba, Brazil
*Rheumatic Fever and Post-streptococcal Arthritis*

**Kevin G. Moder, MD**
Associate Professor of Medicine
Division of Rheumatology
Department of Medicine
Mayo Clinic College of Medicine
Rochester, Minnesota
*History and Physical Examination of the Musculoskeletal System*

**Paul A. Monach, MD, PhD**
Chief, Rheumatology Section
Veterans Affairs Boston Healthcare System
Associate Professor
Section of Rheumatology
Boston University School of Medicine
Boston, Massachusetts
*Anti-neutrophil Cytoplasmic Antibody–Associated Vasculitis*

**Vaishali R. Moulton, MD, PhD**
Assistant Professor
Department of Medicine
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts
*Principles of Signaling*

**Kanneboyina Nagaraju, DVM, PhD**
Professor of Integrative Systems Biology and Pediatrics
Department of Integrative Systems Biology
George Washington University School of Medicine
Director, Research Center for Genetic Medicine
Children's National Medical Center
Washington, D.C.
*Inflammatory Diseases of Muscle and Other Myopathies*

**Amanda E. Nelson, MD, MSCR, RhMSUS**
Assistant Professor of Medicine
Division of Rheumatology, Allergy, and Immunology
Member
Thurston Arthritis Research Center
University of North Carolina
Chapel Hill, North Carolina
*Clinical Features of Osteoarthritis*

**Peter A. Nigrovic, MD**
Associate Professor of Medicine
Harvard Medical School
Staff Pediatric Rheumatologist
Division of Immunology
Boston Children's Hospital
Director, Center for Adults with Pediatric Rheumatic Illness
Division of Rheumatology, Immunology, and Allergy
Brigham and Women's Hospital
Boston, Massachusetts
*Mast Cells*
*Platelets*

**Kiran Nistala, MD, MRCPCH, PhD**
University College London Institute of Child Health
Great Ormond Street Hospital for Children NHS Trust
London, United Kingdom
*Etiology and Pathogenesis of Juvenile Idiopathic Arthritis*

**James R. O'Dell, MD**
Bruce Professor and Vice Chair of Internal Medicine
University of Nebraska Medical Center
Chief of Rheumatology
Department of Medicine
Omaha Veterans Affairs
Omaha, Nebraska
*Traditional DMARDS: Methotrexate, Leflunomide, Sulfasalazine, and Hydroxychloroquine and Combination Therapies*
*Treatment of Rheumatoid Arthritis*

**Yasunori Okada, MD, PhD**
Professor of Pathology
Keio University School of Medicine
Tokyo, Japan
*Proteinases and Matrix Degradation*

**Mikkel Østergaard, MD, PhD, DMSc**
Professor
Copenhagen Center for Arthritis Research
Center for Rheumatology and Spine Diseases
Rigshospitalet, University of Copenhagen
Copenhagen, Denmark
*Imaging in Rheumatic Disease*

**Miguel Otero, BS**
Research Division
Hospital for Special Surgery
New York, New York
*Cartilage and Chondrocytes*

**Bradley M. Palmer, PhD**
Research Assistant Professor
Department of Molecular Physiology and Biophysics
The University of Vermont College of Medicine
Burlington, Vermont
*Muscle: Anatomy, Physiology, and Biochemistry*

**Richard S. Panush, MD, MACP, MACR**
Professor of Medicine
Division of Rheumatology
Department of Medicine
Keck School of Medicine at University of Southern California
Los Angeles, California
*Occupational and Recreational Musculoskeletal Disorders*

**Stanford L. Peng, MD, PhD**
Clinical Assistant Professor
Division of Rheumatology
University of Washington
Seattle, Washington
*Anti-nuclear Antibodies*

**Shiv Pillai, MD, PhD**
Professor of Medicine and Health Sciences and Technology
Harvard Medical School
Ragon Institute of MGH, MIT, and Harvard
Boston, Massachusetts
*$IgG_4$-Related Disease*

**Michael H. Pillinger, MD**
Professor of Medicine and Biochemistry and Molecular Pharmacology
New York University School of Medicine
Section Chief, Rheumatology
Veterans Affairs New York Harbor Health Care System–New York Campus
New York, New York
*Neutrophils*
*Etiology and Pathogenesis of Hyperuricemia and Gout*

**Annette Plüddemann, MSc, PhD**
Nuffield Department of Primary Care Health Sciences
University of Oxford
Oxford, United Kingdom
*Mononuclear Phagocytes*

**Gregory R. Polston, MD**
Clinical Professor
Chief, VA Pain Service
Department of Anesthesiology
University of California, San Diego
San Diego, California
*Analgesic Agents in Rheumatic Disease*

**Steven A. Porcelli, MD**
Murray and Evelyne Weinstock Professor and Chair
Department of Microbiology and Immunology
Albert Einstein College of Medicine
Bronx, New York
*Innate Immunity*

**Mark D. Price, MD**
Department of Orthopedic Surgery
Massachusetts General Hospital
Boston, Massachusetts
*Foot and Ankle Pain*

**Ann M. Reed, MD**
William Cleland Professor and Chair
Department of Pediatrics
Duke University
Durham, North Carolina
*Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis*

**John D. Reveille, MD**
Professor and Director
Rheumatology
University of Texas Health Science Center at Houston
Houston, Texas
*Rheumatic Manifestations of Human Immunodeficiency Virus Infection*

**Angela B. Robinson, MD, MPH**
Assistant Professor of Pediatrics
Rainbow Babies and Children's Hospital/Case Medical Center
Cleveland, Ohio
*Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis*

**Philip Robinson, MBChB, PhD, FRACP**
Research Fellow
Centre for Neurogenetics and Statistical Genomics
University of Queensland
Specialist Rheumatologist
Department of Rheumatology
Royal Brisbane and Women's Hospital
Brisbane, Queensland, Australia
*Ankylosing Spondylitis*

**William H. Robinson, MD, PhD**
Associate Professor
Stanford University
Staff Physician
VA Palo Alto Health Care System
Palo Alto, California
*Biologic Markers in Clinical Trials and Clinical Care*

**Goris Roosendaal, MD, PhD**
Van Creveldkliniek
Center for Benign Hematology, Thrombosis, and Hemostasis
University Medical Center Utrecht
Utrecht, the Netherlands
*Hemophilic Arthropathy*

**Antony Rosen, MB, ChB, BSc (Hons)**
Mary Betty Stevens Professor of Medicine
Professor of Pathology
Director, Division of Rheumatology
The Johns Hopkins University School of Medicine
Baltimore, Maryland
*Autoantibodies in Rheumatoid Arthritis*

**James T. Rosenbaum, AB, MD**
Professor of Ophthalmology, Medicine, and Cell Biology
Oregon Health and Science University
Chenoweth Chair of Ophthalmology
Legacy Devers Eye Institute
Portland, Oregon
*The Eye and Rheumatic Diseases*

**Andrew E. Rosenberg, MD**
Vice Chair, Director of Bone and Soft Tissue Pathology
Department of Pathology
University of Miami Miller School of Medicine
Miami, Florida
*Tumors and Tumor-like Lesions of Joints and Related Structures*

**Eric M. Ruderman, MD**
Professor of Medicine/Rheumatology
Northwestern University Feinberg School of Medicine
Chicago, Illinois
*Mycobacterial Infections of Bones and Joints*
*Fungal Infections of Bones and Joints*

**Kenneth G. Saag, MD, MSc**
Jane Knight Lowe Professor of Medicine
Division of Clinical Immunology and Rheumatology
Department of Medicine
University of Alabama at Birmingham
Birmingham, Alabama
*Clinical Research Methods in Rheumatic Disease*

**Jane E. Salmon, MD**
Collette Kean Research Chair
Professor of Medicine
Weill Cornell Medicine
Hospital for Special Surgery
New York, New York
*Anti-phospholipid Syndrome*

**Lisa R. Sammaritano, MD**
Associate Professor of Clinical Medicine
Department of Rheumatology
Hospital for Special Surgery
Weill Cornell Medicine
New York, New York
*Pregnancy and Rheumatic Diseases*

**Jonathan Samuels, MD**
Assistant Professor of Medicine
Rheumatology
New York University Langone Medical Center
New York, New York
*Pathogenesis of Osteoarthritis*

**Christy I. Sandborg, MD**
Professor of Pediatric Rheumatology
Stanford University School of Medicine
Stanford, California
*Clinical Features and Treatment of Juvenile Idiopathic Arthritis*

**Amr H. Sawalha, MD**
Division of Rheumatology
Department of Internal Medicine
Center for Computational Medicine and Bioinformatics
University of Michigan
Ann Arbor, Michigan
*Epigenetics of Rheumatic Diseases*

**Amit Saxena, MD**
Department of Medicine
Division of Rheumatology
New York University School of Medicine
New York, New York
*Acute Phase Reactants and the Concept of Inflammation*

**Georg Schett, MD**
Professor of Medicine
Department of Internal Medicine 3
Rheumatology and Immunology
Friedrich Alexander University Erlangen–Nuremberg
Erlangen, Germany
*Biology, Physiology, and Morphology of Bone*

**Roger E.G. Schutgens, MD, PhD, MSc**
Van Creveldkliniek
Center for Benign Hematology, Thrombosis, and Hemostasis
University Medical Center Utrecht
Utrecht, Netherlands
*Hemophilic Arthropathy*

**†David C. Seldin, MD, PhD**
Wesley and Charlotte Skinner Professor for Research in Amyloidosis
Professor of Medicine and Microbiology
Boston University School of Medicine
Chief, Section of Hematology-Oncology
Boston Medical Center
Boston, Massachusetts
*Amyloidosis*

**Binita Shah, MD, MS**
Instructor of Medicine
New York University School of Medicine
Attending Physician
VA New York Harbor Health Care System–New York Campus
New York, New York
*Neutrophils*

**Keith A. Sikora, MD**
Senior Investigator
National Institute of Arthritis, Musculoskeletal and Skin Diseases
National Institutes of Health
Bethesda, Maryland
*Etiology and Pathogenesis of Spondyloarthritis*

**Anna Simon, MD, PhD**
Associate Professor
Internal Medicine
Section of Infectious Diseases
Radboud University Medical Centre
Nijmegen, the Netherlands
*Familial Autoinflammatory Syndromes*

**Dawd S. Siraj, MD, MPH&TM**
Clinical Professor of Medicine
Division of Infectious Diseases
Brody School of Medicine
East Carolina University
Greenville, North Carolina
*Bacterial Arthritis*

**Linda S. Sorkin, PhD**
Professor
Anesthesiology
University of California, San Diego
La Jolla, California
*Neuronal Regulation of Pain and Inflammation*

**E. William St. Clair, MD**
Professor of Medicine and Immunology
Chief, Division of Rheumatology and Immunology
Duke University Medical Center
Durham, North Carolina
*Sjögren's Syndrome*

**Lisa K. Stamp, MBChB, FRACP, PhD**
Department of Medicine
University of Otago, Christchurch
Christchurch, New Zealand
*Nutrition and Rheumatic Diseases*

**John H. Stone, MD, MPH**
Professor of Medicine
Harvard Medical School
The Edward Fox Chair in Medicine
Director, Clinical Rheumatology
Massachusetts General Hospital
Boston, Massachusetts
*Classification and Epidemiology of Systemic Vasculitis*
*Immune Complex–Mediated Small-Vessel Vasculitis*
*$IgG_4$-Related Disease*

**Abel Suarez-Fueyo, PhD**
Research Fellow
Department of Medicine
Division of Rheumatology
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts
*Principles of Signaling*

**Camilla I. Svensson, MS, PhD**
Assistant Professor
Physiology and Pharmacology
Karolinska Institutet
Stockholm, Sweeden
*Neuronal Regulation of Pain and Inflammation*

**Nadera J. Sweiss, MD**
Associate Professor of Medicine
Division of Pulmonary, Critical Care, Sleep, and Allergy
Division of Rheumatology
University of Illinois at Chicago
Chicago, Illinois
*Sarcoidosis*

**Carrie R. Swigart, MD**
Associate Professor
Department of Orthopaedics and Rehabilitation
Yale University School of Medicine
New Haven, Connecticut
*Hand and Wrist Pain*

†Deceased.

**Zoltán Szekanecz, MD, PhD, DSc**
Professor of Rheumatology, Immunology, and Medicine
Institute of Medicine
University of Debrecen
Faculty of Medicine
Debrecen, Hungary
*Cell Recruitment and Angiogenesis*

**Stephen Tait, D.Phil**
Principal Investigator
Cancer Research United Kingdom Beatson Institute
Institute of Cancer Sciences
University of Glasgow
Glasgow, United Kingdom
*Metabolic Regulation of Immunity*

**Antoine Tanne, PhD**
Research Fellow
Division of Hematology and Oncology
Tisch Cancer Institute
Icahn School of Medicine at Mount Sinai
New York, New York
*Dendritic Cells: General Overview and Role in Autoimmunity*

**Peter C. Taylor, MA, PhD, FRCP**
Professor
Kennedy Institute of Rheumatology
Botnar Research Centre
Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Sciences
University of Oxford
Oxford, United Kingdom
*Cell-Targeted Biologics and Emerging Targets: Rituximab, Abatacept, and Other Biologics*

**Robert Terkeltaub, MD**
Chief of Rheumatology Section
Veterans Affairs Healthcare System
San Diego, California
Professor of Medicine
Rheumatology, Allergy, and Immunology
University of California, San Diego
San Diego, California
*Calcium Crystal Disease: Calcium Pyrophosphate Dihydrate and Basic Calcium Phosphate*

**Argyrios N. Theofilopoulos, MD**
Professor and Vice-Chairman
Department of Immunology and Microbial Science
The Scripps Research Institute
La Jolla, California
*Autoimmunity*

**Thomas S. Thornhill, MD**
John B. and Buckminster Brown Professor of Orthopaedic Surgery
Brigham and Women's Hospital
Boston, Massachusetts
*Shoulder Pain*

**Kathryn S. Torok, MD**
Assistant Professor of Pediatric Rheumatology
University of Pittsburgh
Pittsburgh, Pennsylvania
*Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis*

**Michael J. Toth, PhD**
Associate Professor of Medicine
The University of Vermont College of Medicine
Burlington, Vermont
*Muscle: Anatomy, Physiology, and Biochemistry*

**Elaine C. Tozman, MD**
Associate Professor of Clinical Medicine
Rheumatology and Immunology
University of Miami Miller School of Medicine
Miami, Florida
*Rheumatic Manifestations of Hemoglobinopathies*

**Leendert A. Trouw, PhD**
Department of Rheumatology
Leiden University Medical Center
Leiden, Netherlands
*Complement System*

**George C. Tsokos, MD**
Professor and Chief
Department of Medicine
Division of Rheumatology
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts
*Principles of Signaling*

**Peter Tugwell, MD, MSc**
Professor of Medicine and Epidemiology and Community Medicine
University of Ottawa
Senior Scientist
Clinical Epidemiology Program
Ottawa Hospital Research Institute
Director, Centre for Global Health
University of Ottawa
Co-Director
WHO Collaborating Centre for Knowledge Translation and Health Technology Assessment in Health Equity
Bruyere Institute
Ottawa, Canada
*Assessment of Health Outcomes*

**Zuhre Tutuncu, MD**
Assistant Clinical Professor
Department of Rheumatology
University of California, San Diego
Rheumatology
Scripps Coastal Medical Center
San Diego, California
*Anti-cytokine Therapies*

**Shivam Upadhyaya, BA**
Orthopedic Surgery
Boston University School of Medicine
Boston Medical Center
Brigham and Women's Hospital
Boston, Massachusetts
*Shoulder Pain*

**Annette H.M. Van Der Helm-Van Mil, MD, PhD**
Internist, Rheumatologist
Department of Rheumatology
Leiden University Medical Center
Leiden, the Netherlands
*Early Synovitis and Early Undifferentiated Arthritis*

**Sjef van der Linden, MD, PhD**
Professor of Rheumatology
Department of Internal Medicine
Division of Rheumatology
Maastricht University Medical Center
Maastricht, Limburg, Netherlands
Professor of Rheumatology
VieCuri Hospital
Venlo Venray, Limburg, Netherlands
*Ankylosing Spondylitis*

**Jos W.M. Van Der Meer, MD, PhD**
Professor of Medicine
Radboud University Medical Centre
Nijmegen, the Netherlands
*Familial Autoinflammatory Syndromes*

**Jacob M. Van Laar, MD, PhD**
Professor and Chair
Rheumatology and Clinical Immunology
University Medical Center Utrecht
Utrecht, the Netherlands
*Immunosuppressive Drugs*

**Heather Van Meter, MD, MS**
Assistant Professor of Pediatric Rheumatology
Division of Pediatrics
Duke Univeristy
Durham, North Carolina
*Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis*

**Ronald F. van Vollenhoven, MD, PhD**
Director
Amsterdam Rheumatology and Immunology Center
Professor and Chief
Department of Rheumatology and Clinical Immunology
Academic Medical Center
University of Amsterdam
Professor and Chief
Department of Rheumatology
VU University Medical Center
Amsterdam, Netherlands
*Evaluation and Differential Diagnosis of Polyarthritis*

**Lize F.D. van Vulpen, MD**
PhD Student
Rheumatology and Clinicial Immunology
Van Creveldkliniek
Center for Benign Hematology, Thrombosis, and Hemostasis
University Medical Center Utrecht
Utrecht, Netherlands
*Hemophilic Arthropathy*

**John Varga, MD**
John and Nancy Hughes Professor
Department of Medicine
Northwestern University Feinberg School of Medicine
Chicago, Illinois
*Etiology and Pathogenesis of Scleroderma*

**Samera Vaseer, MBBS**
Assistant Professor
University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma
*Musculoskeletal Syndromes in Malignancy*

**Raul Vasquez-Castellanos, MD**
Clinical Instructor of Neurological Surgery
Vanderbilt University Medical Center
Nashville, Tennessee
*Neck Pain*

**Douglas J. Veale, MD, FRCPI, FRCP (Lon)**
Director of Translational Research Medicine
Dublin Academic Medical Centre
Professor of Medicine
University College Dublin
Fellow
Conway Institute of Biomolecular and Biomedical Medicine
Dublin, Ireland
*Synovium*

**Richard J. Wakefield, BD, MD, FRCP**
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
*Arthrocentesis and Injection of Joints and Soft Tissue*

**Mark S. Wallace, MD**
Professor of Clinical Anesthesiology
Chair, Division of Pain Medicine
Department of Anesthesiology
University of California, San Diego
San Diego, California
*Analgesic Agents in Rheumatic Disease*

**Ruoning Wang, PhD**
Principal Investigator
Center for Childhood Cancer and Blood Disease
The Research Institute at Nationwide Children's Hospital
Assistant Professor
Department of Pediatrics
The Ohio State University School of Medicine
Columbus, Ohio
*Metabolic Regulation of Immunity*

**Tingting Wang, PhD**
Postdoctoral Researcher
Center for Childhood Cancer and Blood Disease
The Research Institute at Nationwide Children's Hospital
Columbus, Ohio
*Metabolic Regulation of Immunity*

**David M. Warshaw, PhD**
Professor and Chair
Department of Molecular Physiology and Biophysics
The University of Vermont College of Medicine
Burlington, Vermont
*Muscle: Anatomy, Physiology, and Biochemistry*

**Lucy R. Wedderburn, MD, MA, PhD, FRCP**
Professor in Paediatric Rheumatology
UCL Institute of Child Health
University College London
Great Ormond Street Hospital NHS Trust
London, United Kingdom
*Etiology and Pathogenesis of Juvenile Idiopathic Arthritis*

**Victoria P. Werth, MD**
Professor of Dermatology
University of Pennsylvania
Chief
Department of Dermatology
Philadelphia Veterans Affairs Medical Center
Philadelphia, Pennsylvania
*Skin and Rheumatic Diseases*

**Fredrick M. Wigley, MD**
Martha McCrory Professor of Medicine
Division of Rheumatology
The Johns Hopkins University School of Medicine
Baltimore, Maryland
*Clinical Features and Treatment of Scleroderma*

**David Wofsy, MD**
Professor of Medicine and Microbiology/Immunology
University of California, San Francisco
San Francisco, California
*Clinical Features of Systemic Lupus Erythematosus*

**Frank A. Wollheim, MD, PhD, FRCP**
Emeritus Professor
Department of Rheumatology
Lund University
Lund, Sweden
*Enteropathic Arthritis*

**Elisabeth Wondimu, BS**
Research Division
Hospital for Special Surgery
New York, New York
*Cartilage and Chondrocytes*

**Cyrus Wong, MD**
Texas Health Physicians Group
North Texas Neurosurgical and Spine Center
Fort Worth, Texas
*Neck Pain*

**Robert L. Wortmann, MD, FACP, MACR**
Professor Emeritus
Department of Medicine
Geisel School of Medicine at Dartmouth
Lebanon, New Hampshire
*Clinical Features and Treatment of Gout*

**Edward Yelin, PhD**
Professor of Medicine
University of California, San Francisco
San Francisco, California
*Economic Burden of Rheumatic Diseases*

**Ahmed Zayat, MBBCH, MSc, MD, MRCP**
Leeds Institute of Rheumatic and Musculoskeletal Medicine
University of Leeds
Leeds, United Kingdom
*Arthrocentesis and Injection of Joints and Soft Tissue*

**Yong-Rui Zou, PhD**
Autoimmune and Musculoskeletal Disease
The Feinstein Institute for Medical Research
Manhasset, New York
*B Cells*

**Robert B. Zurier, MD**
Professor of Medicine
Chief of Rheumatology, Emeritus
University of Massachusetts Medical School
Worcester, Massachusetts
Investigator
Autoimmunity and Musculoskeletal Disease Center
The Feinstein Institute for Medical Research
Manhasset, New York
*Prostaglandins, Leukotrienes, and Related Compounds*

# Preface

*Whither textbooks: Learning in the internet era*

The tenth edition of the *Textbook of Rheumatology* (TOR10) returns with the entire editorial team from the previous edition and a contributing group of truly outstanding experts in the field. Once again, we believe that this represents an authoritative—actually, *the* authoritative—resource for rheumatology trainees, clinicians, and researchers.

One key question that we are asked and actually ask ourselves is, "Why produce a textbook when you can log onto the internet and download a review article or original research in an instant?" Traditionalists may relish the shiny pages of a beautiful book, but this seems antiquated in the e-book era. However, we contend that a tome like TOR10 provides an entirely different experience for the reader. It is "moderated" by the editors; authors are carefully vetted, and their chapters are reviewed by multiple experts. The organization of TOR10 is also quite distinct. We do not divide a disease or topic into multiple short chapters written by several authors and covering narrow areas; instead, we purposely keep chapters broad enough to provide an integrated view of the literature. That does not mean that TOR10 chapters are broad or superficial. On the contrary, the level of scholarship is extraordinary, and the authors demonstrate their total mastery of the topic. We have continued the principle of the founding editors to provide comprehensive references so that trainees appreciate the original, classic papers in our field. The publication cycle of textbooks is longer than the rapid fire of specific scientific discoveries or the theory du jour. This offers the distinct advantage of pause for thought. Paracelsus declared the physician's greatest friend to be time. TOR10 delivers a considered appraisal over time of the evolving general concepts of rheumatology.

Even so, textbooks might seem anachronistic at this point. Not so for TOR10! We have migrated more and more to online or e-versions. While textbook paper copy distribution drifts downward, online access as measured by "clicks" has soared. These observations confirm our basic premise that our specialty still demands the content and care that goes into creating a unique resource like TOR10.

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**The Editors**

CHAPTER 1

# Biology of the Normal Joint



Steven R. Goldring • Mary B. Goldring

**KEY POINTS**

- Condensation of mesenchymal cells, which differentiate into chondrocytes, results in formation of the cartilage anlagen, which provides the template for the developing skeleton.
- During development of the synovial joint, growth differentiation factor–5 regulates interzone formation, and interference with movement of the embryo during development impairs joint cavitation.
- Members of the bone morphogenetic protein/transforming growth factor-β, fibroblast growth factor, and Wnt families and the parathyroid hormone–related peptide/Indian hedgehog axis are essential for joint development and growth plate formation.
- The synovial lining of diarthrodial joints is a thin layer of cells lacking a basement membrane and consisting of two principal cell types—macrophages and fibroblasts.
- The articular cartilage receives its nutritional requirements via diffusion from the synovial fluid, and interaction of the cartilage with components of the synovial fluid contributes to the unique low-friction surface properties of the articular cartilage.

## CLASSIFICATION OF JOINTS

Human joints, which provide the structures by which bones join with one another, may be classified according to the histologic features of the union and the range of joint motion. Three classes of joint design exist: (1) synovial or diarthrodial joints (Figure 1-1), which articulate with free movement, have a synovial membrane lining the joint cavity, and contain synovial fluid; (2) amphiarthroses, in which adjacent bones are separated by articular cartilage or a fibrocartilage disk and are bound by firm ligaments, permitting limited motion (e.g., the pubic symphysis, intervertebral disks of vertebral bodies, distal tibiofibular articulation, and sacroiliac joint articulation with pelvic bones); and (3) synarthroses, which are found only in the skull (suture lines) where thin, fibrous tissue separates adjoining cranial plates that interlock to prevent detectable motion before the end of normal growth, yet permit growth in childhood and adolescence.

Joints also can be classified according to the connective tissues that are present. Symphyses have a fibrocartilaginous disk separating bone ends that are joined by firm ligaments (e.g., the symphysis pubis and intervertebral joints). In synchondroses, the bone ends are covered with articular cartilage, but no synovium or significant joint cavity are present (e.g., the sternomanubrial joint). In syndesmoses, the bones are joined directly by fibrous ligaments without a cartilaginous interface (the distal tibiofibular articulation is the only joint of this type outside the cranial vault). In synostoses, bone bridges are formed between bones, producing ankylosis.

The synovial joints are classified further according to their shapes, which include ball-and-socket (hip), hinge (interphalangeal), saddle (first carpometacarpal), and plane (patellofemoral) joints. These configurations reflect the varying functions, with the shapes and sizes of the opposing surfaces determining the direction and extent of motion. The various designs permit flexion, extension, abduction, adduction, or rotation. Certain joints can act in one (humeroulnar), two (wrist), or three (shoulder) axes of motion.

This chapter concentrates on the developmental biology and relationship between structure and function of a "prototypic," "normal" human diarthrodial joint—the joint in which arthritis is most likely to develop. Most research that has been performed concerns the knee because of its accessibility, but other joints are described when appropriate.

## DEVELOPMENTAL BIOLOGY OF THE DIARTHRODIAL JOINT

Skeletal development is initiated by the differentiation of mesenchymal cells that arise from three sources: (1) neural crest cells of the neural ectoderm that give rise to craniofacial bones; (2) the sclerotome of the paraxial mesoderm, or somite compartment, which forms the axial skeleton; and (3) the somatopleure of the lateral plate mesoderm, which yields the skeleton of the limbs.[1] The appendicular skeleton develops in the human embryo from limb buds, which are first visible at approximately 4 weeks of gestation. Structures resembling adult joints are generated at approximately 4 to 7 weeks of gestation.[2] Many other crucial phases of musculoskeletal development follow, including vascularization of epiphyseal cartilage (8 to 12 weeks), appearance of villous folds in synovium (10 to 12 weeks), evolution of bursae (3 to 4 months), and the appearance of periarticular fat pads (4 to 5 months).

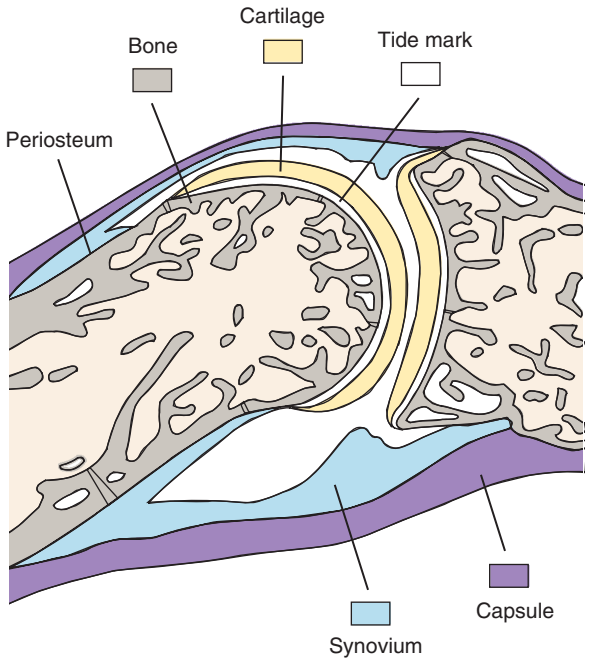



**Figure 1-1** A normal human interphalangeal joint, in sagittal section, as an example of a synovial, or diarthrodial, joint. The tidemark represents the calcified cartilage that bonds articular cartilage to the subchondral bone plate. *(From Sokoloff L, Bland JH:* The musculoskeletal system. *Baltimore, Williams & Wilkins, 1975. Copyright 1975, the Williams & Wilkins Co, Baltimore.)*

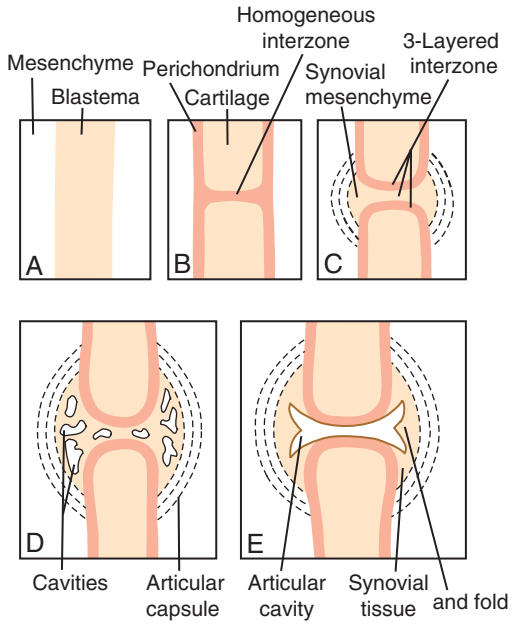



**Figure 1-2** The development of a synovial joint. **A,** Condensation. Joints develop from the blastema, not the surrounding mesenchyme. **B,** Chondrification and formation of the interzone. The interzone remains avascular and highly cellular. **C,** Formation of synovial mesenchyme. Synovial mesenchyme forms from the periphery of the interzone and is invaded by blood vessels. **D,** Cavitation. Cavities are formed in the central and peripheral interzone and merge to form the joint cavity. **E,** The mature joint. *(From O'Rahilly R, Gardner E: The embryology of movable joints. In Sokoloff L, editor:* The joints and synovial fluid, *vol 1, New York, Academic Press, 1978.)*

The upper limbs develop approximately 24 hours earlier than the analogous portions of the lower limbs. Proximal structures, such as the glenohumeral joint, develop before more distal ones, such as the wrist and hand. As a consequence, insults to embryonic development during limb formation affect a more distal portion of the upper limb than of the lower limb. Long bones form as a result of replacement of the cartilage template by endochondral ossification. The stages of limb development are well described by O'Rahilly and Gardner[2,3] and are shown in Figure 1-2. The developmental sequence of the events occurring during synovial joint formation and some of the regulatory factors and extra-cellular matrix components involved are summarized in Figure 1-3. The three main stages in joint development are interzone formation, cavitation, and morphogenesis, as described in detail in several reviews.[4-9]

## INTERZONE FORMATION AND JOINT CAVITATION

The structure of the developing synovial joint and the process of joint cavitation have been described in many classic studies performed on the limbs of mammalian and avian embryos.[10] In the human embryo, cartilage condensations, or chondrifications, can be detected at stage 17, when the embryo is small—approximately 11.7 mm long.[2,3] In the region of the future joint, after formation of the homogeneous chondrogenic interzone at 6 weeks (stages 18 and 19), a three-layered interzone is formed at approximately 7 weeks (stage 21), which consists of two chondrogenic, perichondrium-like layers that cover the opposing surfaces of the cartilage anlagen and are separated by a narrow band of densely packed cellular blastema that remains and forms the interzone. Cavitation begins in the central interzone at about 8 weeks (stage 23).

Although the cellular events associated with joint formation have been recognized for many years, only more recently have the genes regulating these processes been elucidated.[6,7,9] These genes include growth differentiation factor (GDF)-5 (also known as cartilage-derived morphogenetic protein-1) and Wnt14 (also known as Wnt9a), which are involved in early joint development. Two major roles have been proposed for Wnt14. First, it acts at the onset of joint formation as a negative regulator of chondrogenesis. Second, it facilitates interzone formation and cavitation by inducing the expression of GDF-5, autotaxin, lysophosphatidic acid, the bone morphogenetic protein (BMP) antagonist, chordin, and the hyaluronan receptor, CD44.[4,11] Paradoxically, application of GDF-5 to developing joints in mouse embryo limbs in organ culture causes joint fusion,[12] suggesting that temporospatial interactions among distinct cell populations are important for the correct response. The current view is that GDF-5 is required at the early stages of condensations, where it stimulates recruitment and differentiation of chondrogenic cells, and later, when its expression is restricted to the interzone.

The distribution of collagen types and proteoglycans in developing avian and rodent joints has been characterized histologically and by immunohistochemistry and in situ hybridization.[9,13,14] Types I and III collagen characterize the

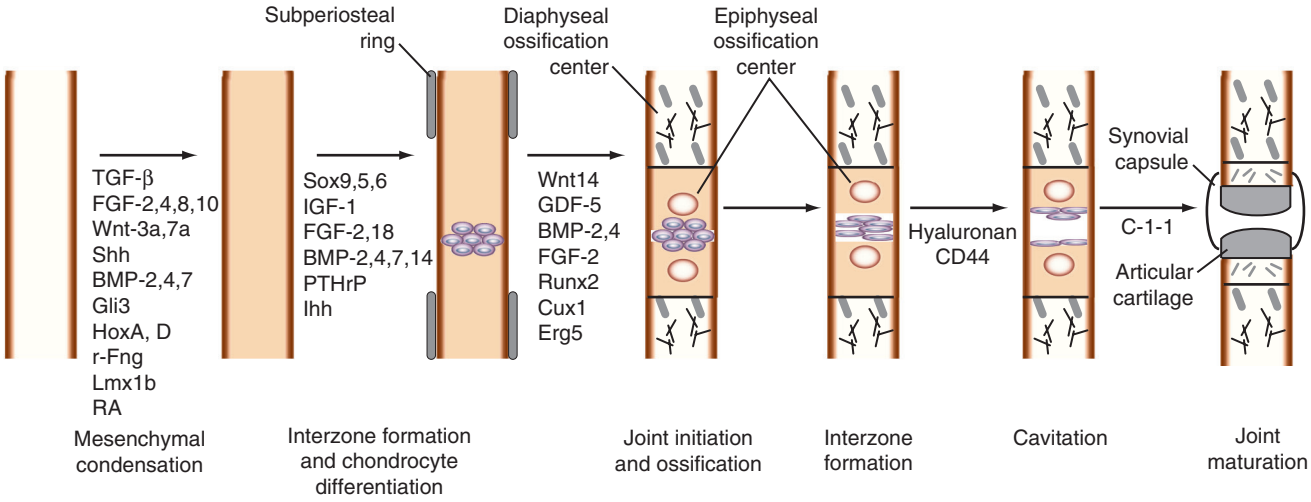



**Figure 1-3** Development of long bones and diathrodial joint formation from cartilage anlagen. BMP, Bone morphogenetic protein; C-C-1, Erg3 variant; CD44, cell determinant 44; Cux, cut-repeat homeobox protein; Erg, ETS-related gene 5; FGF, fibroblast growth factor; GDF, growth and differentiation factor; Gli, glioma-associated oncogene homolog; Hox, homeobox; IGF, insulin-like growth factor; Ihh, Indian hedgehog; Lmx1b, LIM homeodomain transcription factor 1b; PTHrP, parathyroid hormone–related protein; RA, retinoic acid; r-Frg, radical fringe; Runx, runt domain binding protein; Shh, Sonic hedgehog; Sox, SRY-related high mobility group-box protein; TGF-β, transforming growth factor-β; Wnt, wingless type.

matrix produced by mesenchymal cells, which switch to the production of types II, IX, and XI collagens that typify the cartilaginous matrix at the time of condensation. The messenger RNAs (mRNAs) encoding the small proteoglycans, biglycan and decorin, may be expressed at this time, but the proteins do not appear until after cavitation in the regions destined to become articular cartilage. The interzone regions are marked by the expression of genes encoding type IIA collagen by chondrocyte progenitors in the perichondrial layers, type IIB and XI collagens by differentiated chondrocytes in the cartilage anlagen, and type I collagen in the interzone and in the developing capsule and perichondrium (Figure 1-4).[15]

The interzone region contains cells in two outer layers, where they are destined to differentiate into chondrocytes and become incorporated into the epiphyses, and in a thin intermediate zone where they are programmed to undergo joint cavitation and may remain as articular chondrocytes.[8] These early chondrocytes arise from the same population, but unlike the other chondrocytes of the anlagen, they do not activate matrilin-1 expression and are destined to form the articular surface.[16] Fluid and macromolecules accumulate in this space and create a nascent synovial cavity. Blood vessels appear in the surrounding capsulosynovial blastemal mesenchyme before separation of the adjacent articulating surfaces. Although it was first assumed that these interzone cells should undergo necrosis or programmed cell death (apoptosis), many investigators have found no evidence of DNA fragmentation preceding cavitation. In addition, no evidence exists that metalloproteinases are involved in loss of tissue strength in the region undergoing cavitation. Instead, the actual joint cavity seems to be formed by mechanospatial changes induced by the synthesis of hyaluronan via uridine diphosphoglucose dehydrogenase (UDPGD) and hyaluronan synthase. Interaction of hyaluronan CD44 modulates cell migration, but the accumulation of hyaluronan and the associated mechanical influences play the major role in forcing the cells apart and inducing rupture of the intervening extra-cellular matrix by tensile forces. This mechanism accounts partially for observations that joint cavitation is incomplete in the absence of movement.[17-19] Equivalent data from human embryonic joints are difficult to obtain,[20] but in all large joints in humans, complete joint cavities are apparent at the beginning of the fetal period.

## CARTILAGE FORMATION AND ENDOCHONDRAL OSSIFICATION

The skeleton develops from the primitive, avascular, densely packed cellular mesenchyme, termed the *skeletal blastema*. Common precursor mesenchymal cells divide into chondrogenic, myogenic, and osteogenic lineages that determine the differentiation of cartilage centrally, muscle peripherally, and bone. The surrounding tissues, particularly epithelium, influence the differentiation of mesenchymal progenitor cells to chondrocytes in the cartilage anlagen. The cartilaginous nodules appear in the middle of the blastema, and simultaneously cells at the periphery become flattened and elongated to form the perichondrium. In the vertebral column, cartilage disks arise from portions of the somites surrounding the notochord, and nasal and auricular cartilage and the embryonic epiphysis form from the perichondrium. In the limb, the cartilage remains as a resting zone that later becomes the articular cartilage, or it undergoes terminal hypertrophic differentiation to become calcified (growth plate formation) and is replaced by bone (endochondral ossification). The latter process requires extra-cellular matrix remodeling and vascularization (angiogenesis). These events are controlled exquisitely by cellular interactions with the surrounding matrix, growth and differentiation factors, and other environmental factors that initiate or suppress cellular signaling pathways and transcription of specific genes in a temporospatial manner.

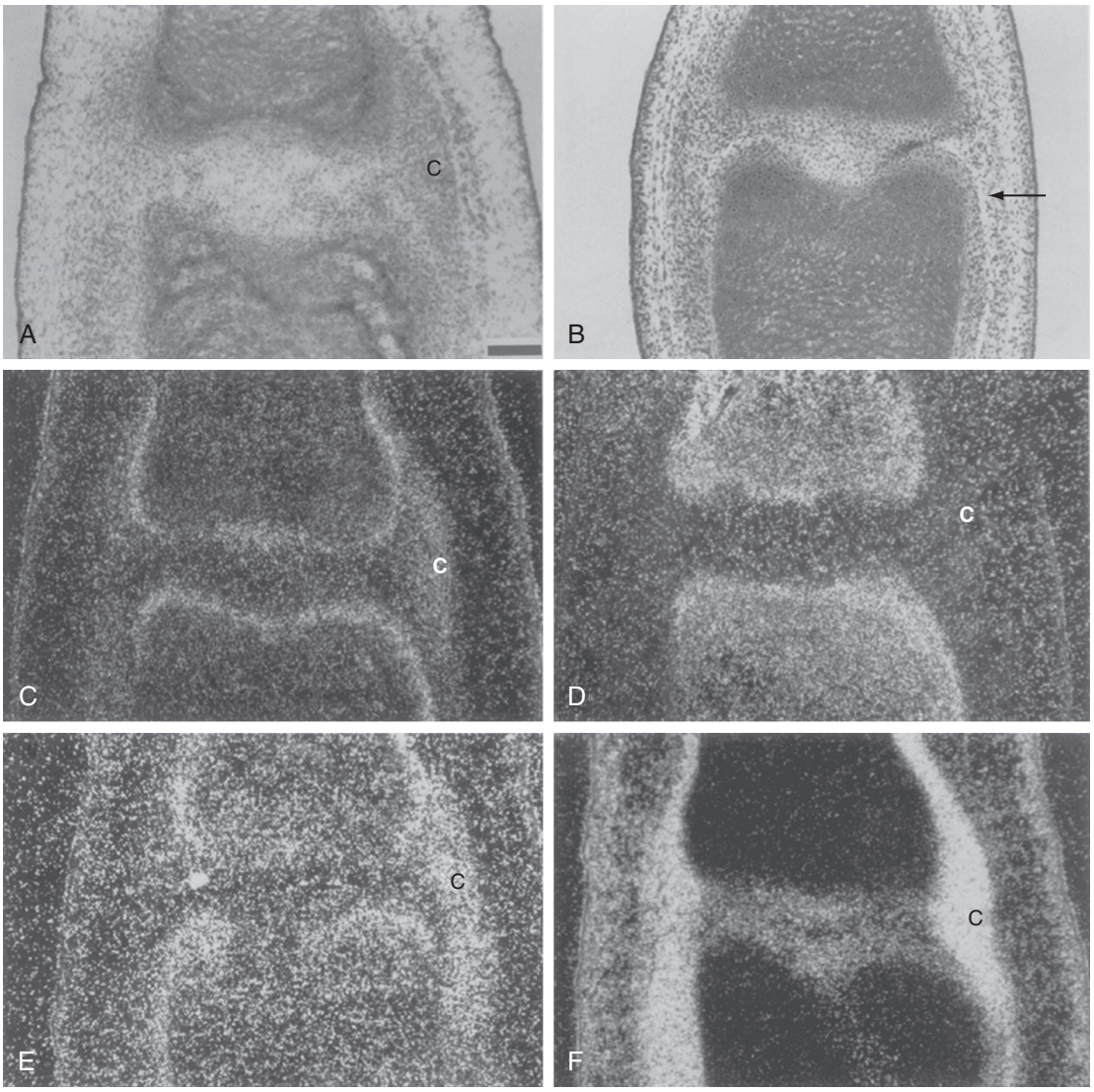



**Figure 1-4** In situ hybridization of a 13-day-old (stage 39) chicken embryo middle digit, proximal interphalangeal joint, midfrontal sections. **A,** Bright-field image showing developing joint and capsule (C). **B,** Equivalent paraffin section of opposite limb of same animal, showing onset of cavitation laterally *(arrow)*. **C,** Expression of type IIA collagen messenger RNA (mRNA) in articular surface cells, perichondrium, and capsule. **D,** Type IIB collagen mRNA is expressed only in chondrocytes of the anlagen. **E,** Type XI collagen mRNA is expressed in the surface cells, perichondrium, and capsule, with lower levels in chondrocytes. **F,** Type I collagen mRNA is present in cells of the interzone and capsule. **C** through **F** images are dark field. Calibration bar = 1 µm. *(From Nalin AM, Greenlee TK Jr, Sandell LJ: Collagen gene expression during development of avian synovial joints: transient expression of types II and XI collagen genes in the joint capsule.* Develop Dyn *203:352–362, 1995.)*

## Condensation and Limb-Bud Formation

Formation of the cartilage anlage occurs in four stages: (1) cell migration, (2) aggregation regulated by mesenchymal-epithelial cell interactions, (3) condensation, and (4) chondrocyte differentiation. Interactions with the epithelium determine mesenchymal cell recruitment and migration, proliferation, and condensation.[2,3,21] The aggregation of chondroprogenitor mesenchymal cells into precartilage condensations was first described by Fell[22] and depends on signals initiated by cell-cell and cell-matrix interactions, the formation of gap junctions, and changes in the cytoskeletal architecture. Before condensation, the prechondrocytic mesenchymal cells produce extra-cellular matrix that is rich in hyaluronan and type I collagen and type IIA collagen, which contains the exon-2–encoded aminopropeptide found in noncartilage collagens. The initiation of condensation is associated with increased hyaluronidase activity and the transient upregulation of versican, tenascin, syndecan, the cell adhesion molecules, neural cadherin (N-cadherin) and neural cell adhesion molecule (NCAM), which facilitate cell-cell interactions.[21,23]

Before chondrocyte differentiation, the cell-matrix interactions are facilitated by the binding of fibronectin to syndecan, thus downregulating NCAM and setting the condensation boundaries. Increased cell proliferation and extra-cellular matrix remodeling, with the disappearance of type I collagen, fibronectin, and N-cadherin and the appearance of tenascins, matrilins, and thrombospondins, including cartilage oligomeric matrix protein, initiate the transition from chondroprogenitor cells to a fully committed chondrocyte.[1,23-25] N-cadherin and NCAM disappear in

differentiating chondrocytes and are detectable later only in perichondrial cells. The differentiated chondrocytes then proliferate and become prehypertrophic when they stop proliferating and undergo the complex process of hypertrophic maturation. Whether the resting differentiated chondrocytes remain within cartilage elements in articular joints or other progenitors are the source of the articular chondrocyte has not been clarified completely.

Much of the current understanding of limb development is based on early studies in chickens and more recently in mice. The regulatory events are controlled by interacting patterning systems involving homeobox (Hox) transcription factors and fibroblast growth factor (FGF), hedgehog, transforming growth factor-β (TGF-β)/BMP, and Wnt pathways, each of which functions sequentially over time (see Figure 1-3).[11,26-29] The HoxA and HoxD gene clusters, which are crucial for the early events of limb patterning in the undifferentiated mesenchyme, are required for the expression of FGF-8 and Sonic hedgehog (Shh),[30] which modulate the proliferation of cells within the condensations.[21] BMP-2, BMP-4, and BMP-7 coordinately regulate the patterning of limb elements within the condensations depending on the temporal and spatial expression of BMP receptors and BMP antagonists, such as noggin and chordin, as well as the availability of BMP- and TGF–β-induced SMADs (signaling mammalian homologues of *Drosophila* mothers against decapentaplegic).[31] BMP signaling is required for the formation of precartilaginous condensations and for the differentiation of precursors into chondrocytes,[32,33] acting in part by opposing effects on FGF actions.[34] Growth of the condensation ceases when noggin inhibits BMP signaling and permits differentiation to chondrocytes. The cartilage formed serves as a template for formation of cartilage elements in the vertebra, sternum, and rib, and for limb elongation or endochondral bone formation.

## Molecular Signals in Cartilage Morphogenesis and Growth Plate Development

The cartilage anlagen grow by cell division and deposition of the extra-cellular matrix and by apposition of proliferating cells from the inner chondrogenic layer of the perichondrium. The nuclear transcription factor, Sox9, is one of the earliest markers expressed in cells undergoing condensation and is required for the subsequent stage of chondrogenesis characterized by the deposition of matrix containing collagens II, IX, and XI and aggrecan in the cartilage anlagen.[35] The expression of SOX proteins depends on BMP signaling via BMPR1A and BMPR1B, which are functionally redundant and active in chondrocyte condensations, but not in the perichondrium.[32] Sox5 and Sox6 are required for the expression of Col9a1, aggrecan, link protein, and Col2a1 during chondrocyte differentiation.[36] The runt-domain transcription factor, Runx2 (also known as core binding factor, Cbfa1), is expressed in all condensations, including those that are destined to form bone.

Throughout chondrogenesis, the balance of signaling by BMPs and FGFs determines the rate of proliferation and the pace of the differentiation.[28,34,37] In the long bones, long after condensation, BMP-2, BMP-3, BMP-4, BMP-5, and BMP-7 are expressed primarily in the perichondrium, and only BMP-7 is expressed in the proliferating chondrocytes.[37] BMP-6 is found later exclusively in hypertrophic chondrocytes along with BMP-2. More than 23 FGFs have been identified thus far.[38] The specific ligands that activate each FGF receptor (FGFR) during chondrogenesis in vivo have been difficult to identify because the signaling depends on the temporal and spatial location of not only the ligands but also the receptors.[39] FGFR2 is upregulated early in condensing mesenchyme and is present later in the periphery of the condensation along with FGFR1, which is expressed in surrounding loose mesenchyme. FGFR3 is associated with proliferation of chondrocytes in the central core of the mesenchymal condensation and overlaps with FGFR2. Proliferation of chondrocytes in the embryonic and postnatal growth plate is regulated by multiple mitogenic stimuli, including FGFs, which converge on cyclin D1.[40]

Early studies indicated that FGFR3 could serve as a master inhibitor of chondrocyte proliferation via Stat1 and the cell cycle inhibitor p21. More recent work has shown that FGFR3 activation downregulates AKT activity to decrease proliferation[41] and MEK activation leads to decreased chondrocyte differentiation.[42] The physiologic FGFR3 ligands are not known, but FGF-9 and FGF-18 are good candidates because they bind FGFR3 in vitro and are expressed in the adjacent perichondrium and periosteum, forming a functional gradient.[28,43] FGF-18–deficient mice have an expanded zone of proliferating chondrocytes similar to that in FGFR3-deficient mice, and FGF-18 can inhibit Indian hedgehog (Ihh) expression. As the growth plate develops, FGFR3 disappears and FGFR1 is upregulated in the prehypertrophic and hypertrophic zones, where FGF-18 and FGF-9 regulate vascular invasion by inducing vascular endothelial growth factor (VEGF) and VEGFR1 and terminal differentiation.[38,44,45]

The proliferation of chondrocytes in the lower proliferative and prehypertrophic zones is under the control of a local negative feedback loop involving signaling by parathyroid hormone–related protein (PTHrP) and Ihh.[46] Ihh expression is restricted to the prehypertrophic zone, and the PTHrP receptor is expressed in the distal zone of periarticular chondrocytes. The adjacent, surrounding perichondrial cells express the Hedgehog receptor patched (Ptch), which, upon Ihh binding, similar to Shh in the mesenchymal condensations, activates Smo and induces Gli transcription factors, which can feedback regulate Ihh target genes in a positive (*Gli1* and *Gli2*) or negative (*Gli3*) manner.[47,48] Ihh induces expression of PTHrP in the perichondrium, and PTHrP signaling stimulates cell proliferation via its receptor expressed in the periarticular chondrocytes.[28,49] More recent evidence indicates that Ihh also acts independently of PTHrP on periarticular chondrocytes to stimulate differentiation of columnar chondrocytes in the proliferative zone, whereas PTHrP acts by preventing premature differentiation into prehypertrophic and hypertrophic chondrocytes, suppressing premature expression of Ihh.[50,51] Ihh and PTHrP, by transiently inducing proliferation markers and repressing differentiation markers, function in a temporospatial manner to determine the number of cells that remain in the chondrogenic lineage versus the number that enter the endochondral ossification pathway.[46] Components of the extra-cellular matrix also contribute to regulation of the different stages of growth plate development, including chondrogenesis and terminal differentiation, by interacting

with signaling molecules and chondrocyte cell surface receptors.[52]

## Endochondral Ossification

The development of long bones from the cartilage anlagen occurs by a process termed *endochondral ossification*, which involves terminal differentiation of chondrocytes to the hypertrophic phenotype, cartilage matrix calcification, vascular invasion, and ossification (see Figure 1-3).[28,49,53] This process is initiated when the cells in the central region of the anlage begin to hypertrophy, increasing cellular fluid volume by almost 20 times. Ihh plays a pivotal role in regulating endochondral bone formation by synchronizing perichondrial maturation with chondrocyte hypertrophy, which is essential for initiating the process of vascular invasion. Ihh is expressed in prehypertrophic chondrocytes as they exit the proliferative phase and enter the hypertrophic phase, at which time they begin to express the hypertrophic chondrocyte marker, type X collagen and alkaline phosphatase. These cells are responsible for laying down the cartilage matrix that subsequently undergoes mineralization. Wnt/β-catenin signaling promotes chondrocyte maturation by a BMP-2–mediated mechanism and induces chondrocyte hypertrophy partly by enhancing matrix metalloproteinase (MMP) expression and potentially by enhancing Ihh signaling and vascularization.[54]

Runx2, which serves as a positive regulatory factor in chondrocyte maturation to hypertrophy, is expressed in the adjacent perichondrium and in prehypertrophic chondrocytes, but less in late hypertrophic chondrocytes, overlapping with Ihh, COL10A1, and BMP-6. IHH induces Gli transcription factors, which interact with Runx2 and BMP-induced Smads, to regulate transcription and expression of *COL10A1*.[55] An essential role for Runx2 in the process of chondrocyte hypertrophy is supported by the observation that the terminal differentiation is blocked in Runx2-deficient mice. A member of the myocyte enhancer factor (MEF) 2 family, MEF2C, stimulates hypertrophy partly by increasing Runx2 expression.[56] The class II histone deacetylase, HDAC4, prevents premature hypertrophy by directly suppressing the activities of Runx2 and MEF2C.[57] HDAC4 is in turn regulated by PTHrP and salt-inducible kinase 3 (SIK3).[58,59] Sox9,[60] FOXA2 and FoxA3,[61] Runx3,[62] Zfp521,[63] and peroxisome proliferator-activated receptor γ (PPARγ[64]) are also important transcriptional regulators of chondrocyte hypertrophy. MMP-13, a downstream target of Runx2, is expressed by terminal hypertrophic chondrocytes, and MMP-13 deficiency results in significant interstitial collagen accumulation, leading to the delay of endochondral ossification in the growth plate with increased length of the hypertrophic zone.[65,66]

Runx2 also is required for transcription activation of *COL10A1*, the gene encoding type X collagen, which is the major matrix component of the hypertrophic zone in the embryo and in the postnatal growth plate. Mutations in the *COL10A1* gene are associated with the dwarfism observed in human chondrodysplasias. These mutations affect regions of the growth plate that are under great mechanical stress, and it has been suggested that the defect in skeletal growth may be due partly to alteration of the mechanical integrity of the pericellular matrix in the hypertrophic zone, although a role for defective vascularization also has been proposed. The extra-cellular matrix remodeling that accompanies chondrocyte terminal differentiation is thought to induce an alteration in the environmental stress experienced by hypertrophic chondrocytes, which eventually undergo apoptosis.[67] Whether chondrocyte hypertrophy with cell death is the ultimate fate of hypertrophic chondrocytes or whether hypertrophy is a transient process that precedes osteogenesis has been a subject of debate. However, recent genetic lineage tracing studies suggest that hypertrophic chondrocytes can survive at the chondro-osseous junction and become osteoblasts and osteocytes.[68,69]

Cartilage is an avascular tissue, and because the developing growth plate is relatively hypoxic, hypoxia inducible factor (HIF)-1α is important for survival as chondrocytes transition to hypertrophy. Under normoxia, the cell content of HIF-1α, -2α , and -3α is low because of oxygen-dependent hydroxylation by prolyl-hydroxylases, resulting in ubiquitination and degradation by the proteasome. In contrast, under hypoxia, prolyl-hydroxylase activity is reduced and the α subunits heterodimerize with the constitutive β-subunit members known as aryl hydrocarbon receptor nuclear translocators (ARNTs). HIFs are transcription factors that bind to hypoxia-responsive elements (HREs) in responsive genes. HIF-2α regulates endochondral ossification processes by directly targeting HREs within the promoters of the *COL10A1*, *MMP13*, and *VEGFA* genes.[70]

Vascular invasion of the hypertrophic zone is required for the replacement of calcified cartilage by bone. VEGF acts as an angiogenic factor to promote vascular invasion by specifically activating local receptors, including Flk1, which is expressed in endothelial cells in the perichondrium or surrounding soft tissues; neuropilin 1 (Npn1), which is expressed in late hypertrophic chondrocytes; or Npn2, which is expressed exclusively in the perichondrium. VEGF is expressed as three different isoforms: VEGF188, a matrix-bound form, is essential for metaphyseal vascularization, whereas the soluble form, VEGF120 (VEGFA), regulates chondrocyte survival and epiphyseal cartilage angiogenesis, and VEGF164 can be either soluble or matrix bound and may act directly on chondrocytes via Npn2. VEGF is released from the extra-cellular matrix by MMPs, including MMP-9, membrane-type (MT)1-MMP (MMP-14), and MMP-13. MMP-9 is expressed by endothelial cells that migrate into the central region of the hypertrophic cartilage.[71] MMP-14, which has a broader range of expression than MMP-9, is essential for chondrocyte proliferation and secondary ossification, whereas MMP-13 is found exclusively in late hypertrophic chondrocytes. Perlecan (Hspg2), a heparan sulfate proteoglycan in cartilage matrix, is required for vascularization in the growth plate through its binding to the VEGFR of endothelial cells, permitting osteoblast migration into the growth plate.[72]

A number of ADAM (a disintegrin and metalloproteinase) proteinases are also emerging as important regulators in growth plate development. For example, ADAM10 is a principle regulator of Notch signaling, which modulates endochondral ossification via RBPjk in chondrocytes[73] and promotes osteoclastogenesis at the chondro-osseous junction by regulating endothelial cell organization in the developing bone vasculature.[74] ADAM17 is the critical proteinase mediating cellular shedding of TNF but also the epidermal

growth factor receptor (EGFR) ligands, including TGF-α. The EGFR signaling pathway induced by EGF and TGF-α plays a crucial role in the remodeling of the growth plate, where inactivation of EGFR results in the inability of hypertrophic chondrocytes to degrade the surrounding collagen matrix and to attract osteoclasts to invade and remodel the advancing growth plate under control of the osteoclast differentiation factor receptor activator of nuclear factor κB (NFκB) ligand (RANKL).[75,76] Mice lacking ADAM17 in chondrocytes *(Adam17ΔCh)* show an expanded hypertrophic zone in the growth plate,[77] essentially phenocopying mice with defects in EGFR signaling in chondrocytes.[75] Tight regulation of EGFR signaling is important for cartilage and joint homeostasis, as shown in mice with cartilage-specific deletion of the mitogen-inducible gene 6 (MIG-6), a scaffold protein that binds EGFR and targets it for internalization and degradation.[78] These events of cartilage matrix remodeling and vascular invasion are required for the migration and differentiation of osteoclasts and osteoblasts, which remove the mineralized cartilage matrix and replace it with bone.

## DEVELOPMENT OF THE JOINT CAPSULE AND SYNOVIUM

The interzone and the contiguous perichondrial envelope, of which the interzone is a part, contain the mesenchymal cell precursors that give rise to other joint components, including the joint capsule, synovial lining, menisci, intracapsular ligaments, and tendons.[7-9] The external mesenchymal tissue condenses as a fibrous capsule. The peripheral mesenchyme becomes vascularized and is incorporated as the synovial mesenchyme, which differentiates into a pseudomembrane at about the same time as cavitation begins in the central interzone (stage 23, approximately 8 weeks). The menisci arise from the eccentric portions of the articular interzone. In common usage, the term *synovium* refers to the true synovial lining and the subjacent vascular and areolar tissue, up to—but excluding—the capsule. Synovial lining cells can be distinguished as soon as the multiple cavities within the interzone begin to coalesce. At first, these cells are exclusively fibroblast-like (type B) cells.

As the joint cavity increases in size, synovial-lining cell layers expand by proliferation of fibroblast-like cells and recruitment of macrophage-like (type A) cells from the circulation. The synovial lining cells express the hyaluronan receptor CD44 and UDPGD, the levels of which remain elevated after cavitation. This increased activity likely contributes to the high concentration of hyaluronan in joint fluids. Further synovial expansion results in the appearance of synovial villi at the end of the second month, early in the fetal period, which greatly increases the surface area available for exchange between the joint cavity and the vascular space. Cadherin 11 is an additional molecule expressed by synovial lining cells.[79,80] It is essential for establishment of synovial lining architecture during development, where its expression correlates with cell migration and tissue outgrowth of the synovial lining.

The role of innervation in the developing joint is not well understood. A dense capillary network develops in the subsynovial tissue, with numerous capillary loops that penetrate into the true synovial lining layer. The human synovial microvasculature is already innervated by 8 weeks (stage 23) of gestation, around the time of joint cavitation. Evidence of neurotransmitter function is not found until much later, however, with the appearance of the sensory neuropeptide substance P at 11 weeks. The putative sympathetic neurotransmitter, neuropeptide Y, appears at 13 weeks of gestation, along with the catecholamine-synthesizing enzyme tyrosine hydroxylase. The finding that the *Slit2* gene, which functions for the guidance of neuronal axons and neurons, is expressed in the mesenchyme and in peripheral mesenchyme of the limb bud (stages 23 to 28) suggests that innervation is an integral part of synovial joint development.[81]

## DEVELOPMENT OF NONARTICULAR JOINTS

In contrast to articular joints, the temporomandibular joint develops slowly, with cavitation at a crown-rump length of 57 to 75 mm (i.e., well into the fetal stage). This slow development may occur because this joint develops in the absence of a continuous blastema and involves the insertion between bone ends of a fibrocartilaginous disk that arises from muscular and mesenchymal derivatives of the first pharyngeal arch. However, many of the same genes as those involved in articular joint development are involved in morphogenesis and growth of the temporomandibular joint.[82]

The development of other types of joints, such as synarthroses, is similar to that of diarthrodial joints except that cavitation does not occur, and synovial mesenchyme is not formed. In these respects, synarthroses and amphiarthroses resemble the "fused" peripheral joints induced by paralyzing chicken embryos, and they may develop as they do because relatively little motion is present during their formation.[83]

The intervertebral disk consists of a semiliquid nucleus pulposus (NP) in the center, surrounded by a multilayered fibrocartilaginous annulus fibrosus (AF), which is sandwiched between the cartilaginous end plates (EPs).[84] Between the EPs lies the vertebral body consisting of the growth plate, which later disappears, and the primary and secondary centers of ossification that fuse together. The cells in the NP arise from the embryonic notochord and the notochord orchestrates somatogenesis, from which arises the ventral mesenchymal sclerotome that forms the AF of the intervertebral disk, as well as the vertebral bodies and ribs.[84] The NP acts as the center for controlling cell differentiation in the AF and EP through Shh signaling, which is regulated by WNT signaling and, in turn, promotes growth and differentiation through downstream transcription factors, Brachyury and Sox9, and gene expression of extra-cellular matrix components.[85,86] The proteoglycans and collagens expressed during development of the intervertebral disk have been mapped and reflect the complex structure-function relationships that allow flexibility and resistance to compression in the spine.[87]

## DEVELOPMENT OF ARTICULAR CARTILAGE

In the vertebrate skeleton, cartilage is the product of cells from three distinct embryonic lineages. Craniofacial cartilage is formed from cranial neural crest cells; the cartilage of the axial skeleton (intervertebral disks, ribs, and sternum)

forms from paraxial mesoderm (somites); and the articular cartilage of the limbs is derived from the lateral plate mesoderm.[1] In the developing limb bud, mesenchymal condensations, followed by chondrocyte differentiation and maturation, occur in digital zones, whereas undifferentiated mesenchymal cells in the interdigital web zones undergo cell death. Embryonic cartilage is destined for one of several fates: It can remain as permanent cartilage, as on the articular surfaces of bones, or it can provide a template for the formation of bones by endochondral ossification. During development, chondrocyte maturation expands from the central site of the original condensation, which forms the cartilage anlage resembling the shape of the future bone, toward the ends of the forming bones. During joint cavitation, the peripheral interzone is absorbed into each adjacent cartilaginous zone, evolving into the articular surface. The articular surface is destined to become a specialized cartilaginous structure that does not normally undergo vascularization and ossification.[4,8,9]

More recent evidence indicates that postnatal maturation of the articular cartilage involves an appositional growth mechanism originating from progenitor cells at the articular surface, rather than an interstitial mechanism. During formation of the mature articular cartilage, the differentiated articular chondrocytes synthesize the cartilage-specific matrix molecules, such as type II collagen and aggrecan (see Chapter 3). Through the processes described previously, the articular joint spaces are developed and lined on all surfaces either by cartilage or by synovial lining cells. These two different tissues merge at the enthesis, the region at the periphery of the joint where the cartilage melds into bone, and where ligaments and the capsule are attached. In the postnatal growth plate, the differentiation of the perichondrium also is linked to the differentiation of the chondrocytes in the epiphysis to form the different zones of the growth plate, contributing to longitudinal bone growth. Once the growth plate closes in the human joint, the adult articular cartilage must be maintained by the resident chondrocytes with low-turnover production of matrix proteins.[88,89]

## ORGANIZATION AND PHYSIOLOGY OF THE MATURE JOINT

The unique structural properties and biochemical components of diarthrodial joints make them extraordinarily durable load-bearing devices. The mature diarthrodial joint is a complex structure, influenced by its environment and mechanical demands (see Chapter 6). Joints have structural differences that are determined by their different functions. The shoulder joint, which demands an enormous range of motion, is stabilized primarily by muscles, whereas the hip, which requires motion and antigravity stability, has an intrinsically stable ball-and-socket configuration. The components of the "typical" synovial joint are the synovium, muscles, tendons, ligaments, bursae, menisci, articular cartilage, and subchondral bone. The anatomy and physiology of muscles are described in detail in Chapter 5.

## SYNOVIUM

The synovium, which lines the joint cavity, is the site of production of synovial fluid that provides the nutrition for the articular cartilage and lubricates the cartilage surfaces. The synovium is a thin membrane between the fibrous joint capsule and the fluid-filled synovial cavity that attaches to skeletal tissues at the bone-cartilage interface and does not encroach on the surface of the articular cartilage. It is divided into functional compartments: the lining region (synovial intima), the subintimal stroma, and the neurovasculature (Figure 1-5). The synovial intima, also termed *synovial lining*, is the superficial layer of the normal synovium that is in contact with the intra-articular cavity. The synovial lining is loosely attached to the subintima, which contains blood vessels, lymphatics, and nerves. Capillaries and arterioles generally are located directly underneath the synovial intima, whereas venules are located closer to the joint capsule.

A transition from loose to dense connective tissue occurs from the joint cavity to the capsule. Most cells in the normal subintimal stroma are fibroblasts and macrophages, although

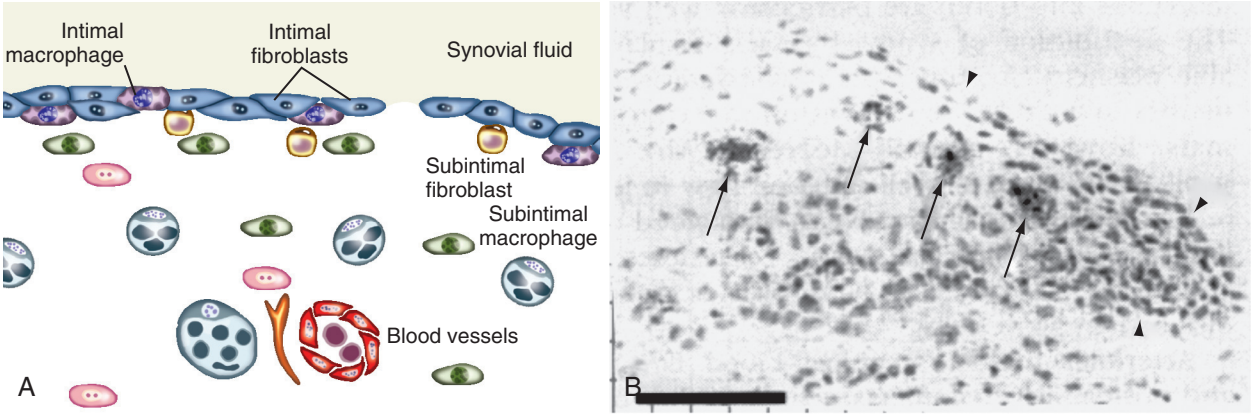



**Figure 1-5** **A,** Schematic representation of normal human synovium. The intima contains specialized fibroblasts expressing vascular cell adhesion molecule-1 (VCAM-1) and uridine diphosphoglucose (UDPG) and specialized macrophages expressing FcγRIIIa. The deeper subintima contains unspecialized counterparts. **B,** Microvascular endothelium in human synovium contains receptors for the vasodilator/growth factor substance P. Silver grains represent specific binding of [$^{125}$I]Bolton Hunter–labeled substance P to synovial microvessels *(arrows)*. *Arrowheads* indicate the synovial surface. Emulsion-dipped in vitro receptor autoradiography preparations with hematoxylin and eosin counterstain. Calibration bar = 1 μm. ***(A,** from Edwards JCW: Fibroblast biology: development and differentiation of synovial fibroblasts in arthritis.* Arthritis Res *2:344–347, 2000.)*

adipocytes and occasional mast cells are present.[90] These compartments are not circumscribed by basement membranes but nonetheless have distinct functions; they are separated from each other by chemical barriers, such as membrane peptidases, which limit the diffusion of regulatory factors between compartments. Synovial compartments are unevenly distributed within a single joint. Vascularity is high at the enthesis where synovium, ligament, and cartilage coalesce. Far from being a homogeneous tissue in continuity with the synovial cavity, synovium is highly heterogeneous, and synovial fluid may be poorly representative of the tissue-fluid composition of any synovial tissue compartment. In rheumatoid arthritis, the synovial lining of diarthrodial joints is the site of the initial inflammatory process. This lesion is characterized by proliferation of the synovial lining cells, increased vascularization, and infiltration of the tissue by inflammatory cells, including B and T lymphocytes, plasma cells, and activated macrophages (see Chapter 69).[91-94] The roles of synovitis and synovial angiogenesis are also of current interest in relation to the progression and severity of joint damage in osteoarthritis (OA).[95,96]

## Synovial Lining

The synovial lining, a specialized condensation of mesenchymal cells and extra-cellular matrix, is located between the synovial cavity and stroma. In normal synovium, the lining layer is two to three cells deep, although intra-articular fat pads usually are covered by only a single layer of synovial cells, and ligaments and tendons are covered by synovial cells that are widely separated. At some sites, lining cells are absent, and the extra-cellular connective tissue constitutes the lining layer. Such "bare areas" become increasingly frequent with advancing age. Although the synovial lining is often referred to as the synovial membrane, the term *membrane* is more correctly reserved for endothelial and epithelial tissues that have basement membranes, tight intercellular junctions, and desmosomes. Instead, synovial lining cells lie loosely in a bed of hyaluronate interspersed with collagen fibrils; this is the macromolecular sieve that imparts the semipermeable nature of the synovium. The absence of any true basement membrane is a major determinant of joint physiology.

Early electron microscopic studies characterized lining cells as macrophage-derived type A and fibroblast-derived type B cells. High UDPGD activity and CD55 are used to distinguish type B synovial cells, whereas nonspecific esterase and CD68 typify type A cells. Normal synovium is lined predominantly by fibroblast-like synoviocytes, whereas macrophage-like synovial cells compose only 10% to 20% of lining cells (see Figure 1-5).[92]

Type A, macrophage-like synovial cells contain vacuoles, a prominent Golgi apparatus, and filopodia, but they have little rough endoplasmic reticulum. These cells express numerous cell surface markers of the monocyte-macrophage lineage, including CD16, CD45, CD11b/CD18, CD68, CD14, CD163, and the immunoglobulin (Ig)G Fc receptor, FcγRIIIa.[90,92] Synovial intimal macrophages are phagocytic and may provide a mechanism by which particulate matter can be cleared from the normal joint cavity. Similar to other tissue macrophages, these cells have little capacity to proliferate and are likely localized to the joint during development. The op/op osteopetrotic mouse that is deficient in macrophages because of an absence of macrophage colony-stimulating factor also lacks synovial macrophages, suggesting that type A synovial cells are of a common lineage with other tissue macrophages. Although they represent only a small percentage of the cells in the normal synovium, the macrophages are recruited from the circulation during synovial inflammation, partly from subchondral bone marrow through vascular channels near the enthesis.

The type B, fibroblast-like synoviocytes contain fewer vacuoles and filopodia than type A cells and have abundant protein-synthetic organelles.[92] Similar to other fibroblasts, lining cells express genes encoding extra-cellular matrix components, including collagens, sulfated proteoglycans, fibronectin, fibrillin-1, and tenascin, and they express intracellular and cell surface molecules such as vimentin and CD90 (Thy-1). They have the potential to proliferate, although proliferation markers are rarely seen in normal synovium. In contrast to stromal fibroblasts, synovial intimal fibroblasts express UDPGD and synthesize hyaluronan, an important constituent of synovial fluid.[90] They also synthesize lubricin, which, together with hyaluronan, is necessary for the low-friction interaction of cartilage surfaces in the diarthrodial joint. Synovial lining cells bear abundant membrane peptidases on their surface that are capable of degrading a wide range of regulatory peptides, such as substance P and angiotensin II.

Normal synovial lining cells also express a rich array of adhesion molecules, including CD44, the principal receptor for hyaluronan; vascular cell adhesion molecule (VCAM)-1; intercellular adhesion molecule (ICAM)-1; and CD55 (decay-accelerating factor).[92] They are essential for cellular attachment to specific matrix components in the synovial lining region, preventing loss into the synovial cavity of cells subjected to deformation and shear stresses during joint movement. Adhesion molecules such as VCAM-1 and ICAM-1 potentially are involved in the recruitment of inflammatory cells during the evolution of arthritis. Cadherins mediate cell-cell adhesion between adjacent cells of the same type. The identification of cadherin-11 as a key adhesion molecule that regulates the formation of the synovial lining during development and the synoviocyte function postnatally has provided the opportunity to examine its role in inflammatory joint disease.[79] Cadherin-11 is highly expressed in fibroblast-like cells at the pannus-cartilage interface in rheumatoid synovium, where it plays a role in the invasive properties of the synovial fibroblasts,[97] and treatment with a cadherin-11 antibody or a cadherin-11 fusion protein reduces synovial inflammation and cartilage erosion in an animal model of arthritis.[80]

## Synovial Vasculature

The subintimal synovium contains blood vessels, providing the blood flow that is required for solute and gas exchange in the synovium itself and for the generation of synovial fluid.[98] The avascular articular cartilage also depends on nutrition in the synovial fluid, derived from the synovial vasculature. The vascularized synovium behaves similar to an endocrine organ, generating factors that regulate synoviocyte function and serving as a selective gateway that recruits cells from the circulation during stress and

inflammation. Finally, synovial blood flow plays an important role in regulating intra-articular temperature.

The synovial vasculature can be divided, on morphologic and functional grounds, into arterioles, capillaries, and venules. In addition, lymphatics accompany arterioles and larger venules.[90] Arterial and venous networks of the joint are complex and are characterized by arteriovenous anastomoses that communicate freely with blood vessels in periosteum and periarticular bone. As large synovial arteries enter the deep layers of the synovium near the capsule, they give off branches, which bifurcate again to form "microvascular units" in the subsynovial layers. The synovial lining region, the surfaces of intra-articular ligaments, and the entheses (in the angle of ligamentous insertions into bone) are particularly well vascularized.[99]

The distribution of synovial vessels, which were formed largely as a result of vasculogenesis during development of the joint, displays considerable plasticity. Vasculogenesis is a dynamic process that depends on the cellular interactions with regulatory factors and the extra-cellular matrix, which are also important in angiogenesis. In inflammatory arthritis, the density of blood vessels decreases relative to the growing synovial mass, creating a hypoxic and acidotic environment.[100,101] Angiogenic factors such as VEGF, acting via VEGF receptors 1 and 2 (Flt1 and Flk2), and basic FGF promote proliferation and migration of endothelial cells, a process that is facilitated by matrix-degrading enzymes and adhesion molecules such as integrin $\alpha v\beta 3$ and E-selectin, expressed by activated endothelial cells. Vessel maturation is facilitated by angiopoietin-1 acting via the Tie-2 receptor. The angiogenic molecules are restricted to the capillary epithelium in normal synovium, but their levels are elevated in inflamed synovium in perivascular sites and areas remote from vessels.[102,103]

### Regulation of Synovial Blood Flow

Synovial blood flow is regulated by intrinsic (autocrine and paracrine) and extrinsic (neural and humoral) systems. Locally generated factors, such as the peptide vasoconstrictors angiotensin II and endothelin-1, act on adjacent arteriolar smooth muscle to regulate regional vascular tone.[99] Normal synovial arterioles are richly innervated by sympathetic nerves containing vasoconstrictors, such as norepinephrine and neuropeptide Y, and by "sensory" nerves that also play an efferent vasodilatory role by releasing neuropeptides, such as substance P and calcitonin gene–related peptide (CGRP). Arterioles regulate regional blood flow. Capillaries and postcapillary venules are sites of fluid and cellular exchange. Correspondingly, regulatory systems are differentially distributed along the vascular axis. Angiotensin-converting enzyme, which generates angiotensin II, is localized predominantly in arteriolar and capillary endothelia and decreases during inflammation. Specific receptors for angiotensin II and for substance P are abundant on synovial capillaries, with lower densities on adjacent arterioles. Dipeptidyl peptidase IV, a peptide-degrading enzyme, is specifically localized to the cell membranes of venular endothelium. The synovial vasculature is not only functionally compartmentalized from the surrounding stroma but also highly specialized along its arteriovenous axis. Other unique characteristics of the normal synovial vasculature include the presence of inducible nitric oxidase synthase–independent 3-nitrotyrosine, a reaction product of peroxynitrite, and the localization of the synoviocyte-derived CXCL12 chemokine on heparan sulfate receptors on endothelial cells, suggesting physiologic roles for these molecules in normal vascular function.

## JOINT INNERVATION

Dissection studies have shown that each joint has a dual nerve supply, consisting of specific articular nerves that penetrate the capsule as independent branches of adjacent peripheral nerves and articular branches that arise from related muscle nerves. The definition of joint position and the detection of joint motion are monitored separately and by a combination of multiple inputs from different receptors in varied systems. Nerve endings in muscle and skin and in the joint capsule mediate sensation of joint position and movement. Normal joints have afferent (sensory) and efferent (motor) innervations consisting of both unmyelinated and sensory thick myelinated A fibers in ligaments, fibrous capsule, menisci and adjacent periosteum, where they are thought to function primarily as sensors for pressure and movements. Sensory A and C fibers terminate as free nerve endings in the fibrous capsule, adipose tissue, ligaments, menisci, and the adjacent periosteum, where they are thought to act as nociceptors and contribute to the regulation of synovial microvascular function.

In normal synovium a dense network of fine unmyelinated nerve fibers follow the courses of blood vessels and extend into the synovial lining layers. These nerve fibers do not have specialized endings and are slow-conducting fibers; they may transmit diffuse, burning, or aching pain sensation. Sympathetic nerve fibers surround blood vessels, particularly in the deeper regions of normal synovium, and contain and release classic neurotransmitters, such as norepinephrine, and neuropeptides that are markers of sensory nerves including substance P, CGRP, neuropeptide Y, and vasoactive intestinal peptide.[98,104]

Afferent nerves containing substance P also have an efferent role in the synovium. Substance P is released from peripheral nerve terminals into the joint, and specific, G protein–coupled receptors for substance P are localized to microvascular endothelium in normal synovium. Abnormalities of articular innervation that are associated with inflammatory arthritis may contribute to the failure of synovial inflammation to resolve. Excessive local neuropeptide release may result in the loss of nerve fibers as a result of neuropeptide depletion. Synovial tissue proliferation without concomitant growth of new nerve fibers may lead to an apparent partial denervation of synovium. Studies in patients suggest that free nerve endings containing substance P may modulate inflammation and the pain pathway in OA. Afferent nerve fibers from the joint play an important role in the reflex inhibition of muscle contraction. Trophic factors generated by motor neurons, such as the neuropeptide CGRP, are important in maintaining muscle bulk and a functional neuromuscular junction. Decreases in motor neuron trophic support during articular inflammation probably contribute to muscle wasting.

Mechanisms of joint pain have been reviewed in detail.[105-107] In a noninflamed joint, most sensory nerve

fibers do not respond to movement within the normal range; these fibers are referred to as silent nociceptors. In an acutely inflamed joint, however, these nerve fibers become sensitized by mediators such as bradykinin, neurokinin 1, and prostaglandins (peripheral sensitization), and as a result, normal movements induce pain. Pain sensation is upregulated or downregulated further in the central nervous system, at the level of the spinal cord and in the brain, by central sensitization and "gating" of nociceptive input. Although the normal joint may respond predictably to painful stimuli, a poor correlation often exists between apparent joint disease and perceived pain in persons with chronic arthritis. Pain associated with joint movements within the normal range is a characteristic symptom described by patients with chronically inflamed joints caused by rheumatoid arthritis. Chronically inflamed joints may not be painful at rest, however, unless they are acutely inflamed.

The expression of substance P and CGRP are upregulated by nerve growth factor (NGF), which belongs to a family of neurotrophins that regulated neuronal growth during embryonic development.[108] Postnatally, NGF and the neurotrophins regulate neuronal regeneration and pain perception. In addition to promoting nerve growth and mediating pain perception, NGF can act together with VEGF to promote blood vessel formation. Angiogenesis and nerve growth thus are linked by common pathways involving NGF, VEGF, and neuropeptides such as CGRP, neuropeptide Y, and semiphorins.[98,109,110]

## TENDONS

Tendons are functional and anatomic bridges between muscle and bone.[111,112] Tendons focus the force of a large mass of muscle into a localized area on bone and, by splitting to form numerous insertions, may distribute the force of a single muscle to different bones. Tendons are formed of longitudinally arranged collagen fibrils embedded in an organized, hydrated proteoglycan matrix with blood vessels, lymphatics, and fibroblasts.[113] Cross-links between adjacent collagen chains or molecules contribute to the tensile strength of the tendon. Tendon collagen fibrillogenesis is initiated during early development by a highly ordered process of alignment involving the actin cytoskeleton and cadherin-11.[114,115] Many tendons, particularly those with a large range of motion, run through vascularized, discontinuous sheaths of collagen lined with mesenchymal cells resembling synovium. Gliding of tendons through their sheaths is enhanced by hyaluronic acid produced by the lining cells. Tendon movement is essential for the embryogenesis and maintenance of tendons and their sheaths.[116,117] Degenerative changes appear in tendons, and fibrous adhesions form between tendons and sheaths when inflammation or surgical incision is followed by long periods of immobilization. At the myotendinous junction, recesses between muscle cell processes are filled with collagen fibrils, which blend into the tendon. At its other end, collagen fibers of the tendon typically blend into fibrocartilage, mineralize, and merge into bone through a fibrocartilaginous transition zone termed the *enthesis*, or insertion site.[118]

Tendon fibroblasts synthesize and secrete collagens, proteoglycans, and other matrix components, such as fibronectin and tenascin C, as well as MMPs and their inhibitors, which can contribute to the breakdown and repair of tendon components.[113] Collagen fibrils in tendon are composed primarily of type I collagen with some type III collagen, but there are regional differences in the distribution of other matrix components. The compressed region contains the small proteoglycans, biglycan, decorin, fibromodulin, and lumican, as well as the large proteoglycan versican. The major components in the tensile region of the tendon are decorin, microfibrillar type VI collagen, fibromodulin, and the proline and arginine-rich end leucine-rich repeat protein (PRELP). The presence of cartilage oligomeric matrix protein, aggrecan, and biglycan and collagen types II, IX, and XI is indicative of fibrocartilage. The collagen fiber orientation at the tendon-to-bone enthesis is important for maintaining microarchitecture by reducing the stress concentrations and shielding the outward splay of the insertion from the highest stresses.[119] Understanding the structure has implications for tendon repair because motion between a tendon graft and bone tunnel may impair early graft incorporation and lead to tunnel widening secondary to bone resorption.[120,121]

Failure of the muscle-tendon apparatus is rare; when it does occur, it is the result of enormous, quickly generated forces across a joint and usually occurs near the tendon insertion into bone.[122] Factors that may predispose to tendon failure are aging processes, including loss of extra-cellular water and the increase in intermolecular cross-links of collagen; tendon ischemia; iatrogenic factors, including injection of glucocorticoids; and deposition of calcium hydroxyapatite crystals within the collagen bundles. Alterations in collagen fibril composition and structure are associated with tendon degeneration during aging and may predispose to OA. Understanding the contributions in tendon development of Sox9, scleraxis, Mohawk, members of the TGF-β/BMP superfamily, including GDF-5, and Wnt/β-catenin, and Ihh signaling[123,124] may point to strategies for tendon repair.[122,125]

## LIGAMENTS

Ligaments, which provide a stabilizing bridge between bones, permit a limited range of movement.[126] Ligaments often are recognized only as hypertrophied components of the fibrous joint capsule and are structurally similar to tendons.[127] Although the fibers are oriented parallel to the longitudinal axis of both tissues, the collagen fibrils in ligaments are nonparallel and arranged in fibers that are oriented roughly along the long axis in a wavy, undulating pattern, or "crimp," which can straighten in response to load. Some ligaments have a higher ratio of elastin to collagen (1 : 4) than do tendons (1 : 50), which permits a greater degree of stretch. Ligaments also have larger amounts of reducible cross-links, more type III collagen, slightly less total collagen, and more glycosaminoglycans compared with tendons. The cells in ligaments seem to be more metabolically active than the cells in tendons and have more plump cellular nuclei and higher DNA content.

During postnatal growth, the development of ligament attachment zones involves changes in the ratios and distribution of types I, III, and V collagen and the synthesis of type II collagen and proteoglycans by fibrochondrocytes

that develop from ligament cells at the attachment zone. Attachment zones are believed to permit gradual transmission of the tensile force between ligament and bone.

Ligaments play a major role in the passive stabilization of joints, aided by the capsule and, when present, menisci. In the knee, the collateral and cruciate ligaments provide stability when there is little or no load on the joint. As compressive load increases, there is an increasing contribution to stability from the joint surfaces themselves and the surrounding musculature. Injured ligaments generally heal, and structural integrity is restored by contracture of the healing ligament so it can act again as a stabilizer of the joint.

## BURSAE

The many bursae in the human body facilitate gliding of one tissue over another, much as a tendon sheath facilitates movement of its tendon. Bursae are closed sacs, lined sparsely with mesenchymal cells that are similar to synovial cells, but they are generally less well vascularized than synovium. Most bursae differentiate concurrently with synovial joints during embryogenesis. Throughout life, trauma or inflammation may lead to the development of new bursae, hypertrophy of previously existing ones, or communication between deep bursae and joints. In patients with rheumatoid arthritis, communications may exist between the subacromial bursae and the glenohumeral joint, between the gastrocnemius or semimembranosus bursae and the knee joint, and between the iliopsoas bursa and the hip joint. It is unusual, however, for subcutaneous bursae, such as the prepatellar bursa or olecranon bursa, to develop communication with the underlying joint.[128]

## MENISCI

The meniscus, a fibrocartilaginous, wedge-shaped structure, is best developed in the knee but also is found in the acromioclavicular and sternoclavicular joints, the ulnocarpal joint, and the temporomandibular joint. Until more recently, menisci were thought to have little function and a quiescent metabolism with no capability of repair, although early observations indicated that removal of menisci from the knee could lead to premature arthritic changes in the joint. Evidence from arthroscopic studies of patients with anterior cruciate ligament insufficiency indicates that disease of the medial meniscus correlates with that of the medial femoral cartilage. The meniscus is now considered to be an integral component of the knee joint that has important functions in joint stability, load distribution, shock absorption, and lubrication.[129,130]

The microanatomy of the meniscus is complex and age dependent.[131] The characteristic shapes of the lateral and the medial menisci are achieved early in prenatal development. At that time, the menisci are cellular and highly vascularized; with maturation, vascularity decreases progressively from the central margin to the peripheral margin. After skeletal maturity, the peripheral 10% to 30% of the meniscus remains highly vascularized by a circumferential capillary plexus and is well innervated. Tears in this vascularized peripheral zone may undergo repair and remodeling. The central portion of the mature meniscus is an avascular fibrocartilage, however, without nerves or lymphatics, consisting of cells surrounded by an abundant extra-cellular matrix of collagens, chondroitin sulfate, dermatan sulfate, and hyaluronic acid. Tears in this central zone heal poorly, if at all.

Collagen constitutes 60% to 70% of the dry weight of the meniscus and is mostly type I collagen, with lesser amounts of types III, V, and VI. A small quantity of cartilage-specific type II collagen is localized to the inner, avascular portion of the meniscus. Collagen fibers in the periphery are mostly circumferentially oriented, with radial fibers extending toward the central portion. Elastin content is around 0.6%, and proteoglycan content is around 2% to 3% dry weight. Aggrecan and decorin are the major proteoglycans in the adult meniscus. Decorin is the predominant proteoglycan synthesized in the meniscus from young persons, whereas the relative proportion of aggrecan synthesis increases with age. Although the capacity of the meniscus to synthesize sulfated proteoglycans decreases after the teenage years, the age-related increases in expression of decorin and aggrecan mRNA suggest that the resident cells are able to respond quickly to alterations in the biomechanical environment.

The meniscus was defined originally as a fibrocartilage, based on the rounded or oval shape of most of the cells and the fibrous microscopic appearance of the extra-cellular matrix. Based on molecular and spatial criteria, three distinct populations of cells are recognized in the meniscus of the knee joint[132]:

1. The fibrochondrocyte is the most abundant cell in the middle and inner meniscus, synthesizing primarily type I collagen and relatively small amounts of type II and III collagens. It is round or oval in shape and has a pericellular filamentous matrix containing type VI collagen.
2. The fibroblast-like cells lack a pericellular matrix and are located in the outer portion of the meniscus. They are distinguished by long, thin, branching cytoplasmic projections that stain for vimentin. They make contact with other cells in different regions via connexin 43–containing gap junctions. The presence of two centrosomes, one associated with a primary cilium, suggests a sensory, rather than motile, function that could enable the cells to respond to circumferential tensile loads rather than compressive loads.
3. The superficial zone cells have a characteristic fusiform shape with no cytoplasmic projections. The occasional staining of these cells in the uninjured meniscus with α-actin and their migration into surrounding wound sites suggest that they are specialized progenitor cells that may participate in a remodeling response in the meniscus and surrounding tissues.

Cell lineage tracing and gene profiling studies in mouse embryos have provided insight into the complexity of the meniscus and how it was formed.[133,134] Researchers have considerable interest in using this information to develop new strategies for meniscal generation.

## MATURE ARTICULAR CARTILAGE

Articular cartilage is a specialized connective tissue that covers the weight-bearing surfaces of diarthrodial joints.

The principal functions of cartilage layers covering bone ends are to permit low-friction, high-velocity movement between bones, to absorb the transmitted forces associated with locomotion, and to contribute to joint stability. Lubrication by synovial fluid provides frictionless movement of the articulating cartilage surfaces. Chondrocytes (see Chapter 3) are the single cellular component of adult hyaline articular cartilage and are responsible for synthesizing and maintaining the highly specialized cartilage matrix macromolecules. The cartilage extra-cellular matrix is composed of an extensive network of collagen fibrils, which confers tensile strength, and an interlocking mesh of proteoglycans, which provides compressive stiffness through the ability to absorb and extrude water. Numerous other noncollagenous proteins also contribute to the unique properties of cartilage (Table 1-1). Histologically, the tissue appears to be fairly homogeneous and clearly distinguished from the calcified cartilage and underlying subchondral bone (Figure 1-6). However, this appearance is misleading because significant topographical and regional differences exist in the molecular organization and composition of the articular cartilage, as described in Chapter 3.

**TABLE 1-1** Extra-cellular Matrix Components of Articular Cartilage*

| |
|---|
| **Collagens** |
| Type II |
| Type IX |
| Type XI |
| Type VI |
| Types XII, XIV |
| Type X (hypertrophic chondrocyte) |
| **Proteoglycans** |
| Aggrecan |
| Versican |
| Link protein |
| Biglycan (DS-PGI) |
| Decorin (DS-PGII) |
| Epiphycan (DS-PGIII) |
| Fibromodulin |
| Lumican |
| Proline/arginine-rich and leucine-rich repeat protein (PRELP) |
| Chondroadherin |
| Perlecan |
| Lubricin (SZP) |
| **Other Noncollagenous Proteins (Structural)** |
| Cartilage oligomeric matrix protein (COMP) or thrombospondin-5 |
| Thrombospondin-1 and thrombospondin-3 |
| Cartilage matrix protein (matrilin-1) and matrilin-3 |
| Fibronectin |
| Tenascin-C |
| Cartilage intermediate layer protein (CILP) |
| Fibrillin |
| Elastin |
| **Other Noncollagenous Proteins (Regulatory)** |
| Glycoprotein (gp)-39, YKL-40 |
| Matrix Gla protein (MGP) |
| Chondromodulin-I (SCGP) and chondromodulin-II |
| Cartilage-derived retinoic acid–sensitive protein (CD-RAP) |
| Growth factors |
| **Cell Membrane–Associated Proteins** |
| Integrins (α1β1, α2β1, α3β1, α5β1, α6β1, α10β1, αvβ3, αvβ5) |
| Anchorin CII (annexin V) |
| Cell determinant 44 (CD44) |
| Syndecan-1, 3, and 4 |
| Discoidin domain receptor 2 |

*The collagens, proteoglycans, and other noncollagenous proteins in the cartilage matrix are synthesized by chondrocytes at different stages during development and growth of cartilage. In mature articular cartilage, proteoglycans and other noncollagen proteins are turned over slowly, whereas the collagen network is stable unless exposed to proteolytic cleavage. Proteins that are associated with chondrocyte cell membranes also are listed because they permit specific interactions with extra-cellular matrix proteins. The specific structure-function relationships are discussed in Chapter 3 and described in Table 3-1.

DS-PG, Dermatan sulfate proteoglycan; SCGP, small cartilage–derived glycoprotein; SZP, superficial zone protein; YKL-40, 40KD chitinase 3-like glycoprotein.

## SUBCHONDRAL BONE

Subchondral bone is not a homogeneous tissue and consists of a layer of compact cortical bone and an underlying system of cancellous bone organized into a trabecular network.[135,136] The subchondral bone is separated from the overlying articular cartilage by a thin zone of calcified cartilage. The so-called tidemark defines the transition zone between the articular and calcified cartilage. This complex biocomposite of bone and calcified cartilage provides an optimal system for distributing loads that are transmitted from the weight-bearing surfaces that are lined by the hyaline articular cartilage. Although the tidemark was originally believed to form a barrier to fluid flow, evidence shows that biologically active molecules can transit this zone, providing a mechanism by which products produced by chondrocytes or bone cells can influence the activity of the other cell type.[137,138] In addition, further communication is provided via products released from vascular elements in channels that penetrate the calcified cartilage from the adjacent marrow space.[139] Under physiologic conditions the composition and structural organization of the subchondral bone and calcified cartilage are optimally adapted to transfer loads, but several conditions can lead to changes in the structural and functional properties of these tissues.

The subchondral bone undergoes continuous structural reorganization throughout postnatal life. These alterations are mediated by the coordinated activity of bone-resorbing osteoclasts and bone-forming osteoblasts that remodel and adapt the bone in response to local biomechanical and biological signals.[140] Several lines of evidence have established that osteocytes are the bone cell type that plays a key role in regulating the bone remodeling process.[141,142] Osteocytes are distributed throughout the mineralized bone matrix, forming an interconnected network that is ideally positioned to sense and respond to local and systemic stimuli. These effects are mediated via both cell-cell interactions with osteoclasts and osteoblasts but also via signaling through the release of soluble mediators. These products include RANKL, the essential regulator of osteoclast differentiation and activity and its inhibitor osteoprotegerin (OPG),[143,144] as well as additional mediators, including prostanoids, nitric oxide, nucleotides, and a broad spectrum of growth factors and cytokines.[145] In addition to these factors, osteocytes also produce sclerostin and Dickkopf-related protein 1 (DKK-1), which are potent inhibitors of the Wnt/β-catenin pathway that contributes to the regulation of osteoblast-mediated bone formation.[146] The release of RANKL and OPG and the Wnt pathway regulators, DKK-1 and sclerostin, play a major role in controlling the

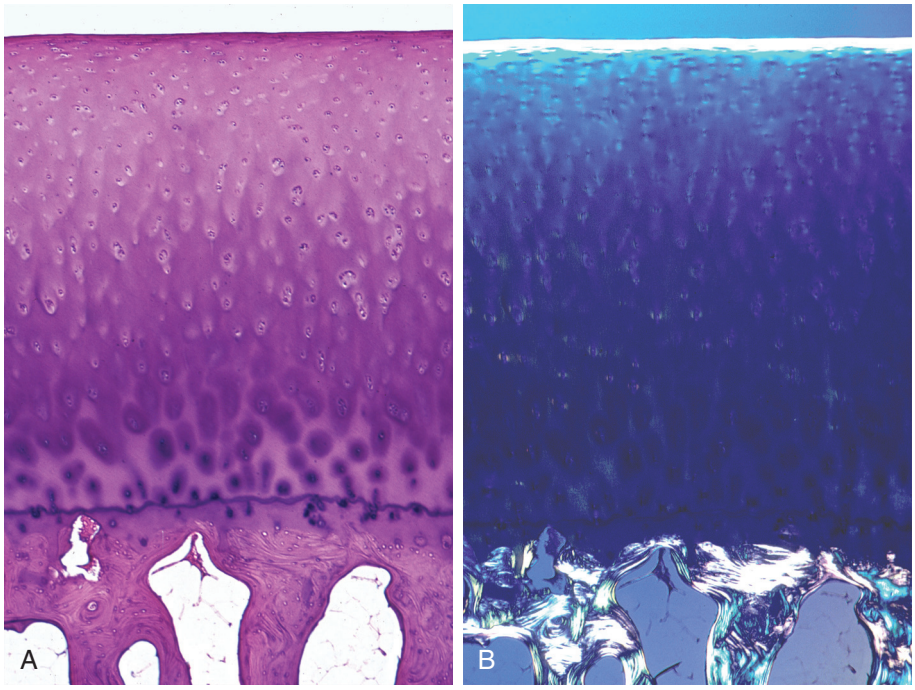



**Figure 1-6** Representative sections of normal human adult articular cartilage, showing nearly the same field in plain **(A)** and polarized **(B)** light. Note the clear demarcation of the articular cartilage from the calcified cartilage below the tidemark and the underlying subchondral bone. (Hematoxylin-eosin stain; original magnification ×60.) *(Courtesy Edward F. DiCarlo, MD, Pathology Department, Hospital for Special Surgery, New York, N.Y.)*

adaptation of the subchondral bone to alterations in mechanical loading in both physiologic and pathologic conditions.

The maintenance of the structural and functional integrity of articular cartilage and subchondral bone under physiologic loading is evidence of the unique and intimate interaction of these tissues, but controversy remains with regard to the relationship between them in the pathogenesis of OA.[147] Radin and Rose[148] proposed that the initiation of early alterations in articular cartilage is caused by an increase in subchondral bone stiffness that adversely affects the function of articular chondrocytes, leading to deterioration in the properties of the articular cartilage and susceptibility to mechanical disruption. Alternatively, it has been proposed that changes in subchondral bone stiffness may be a result of cartilage deterioration.[149-151] The alterations in subchondral bone and cartilage that accompany the osteoarthritic process are not restricted to these tissues but also affect the zone of calcified cartilage, where there is evidence of vascular invasion, advancement of the calcified cartilage, and duplication of the tidemark that contributes to a decrease in articular cartilage thickness.[152,153] The penetration of the vascular channels from the subchondral bone and calcified cartilage into the deep zones of the articular cartilage permit exchange of fluids and soluble mediators between these tissues, providing an additional mechanism by which the subchondral bone and articular cartilage can affect the activity of cells within each of these tissues. These structural alterations in the articular cartilage and periarticular bone may also lead to modification of the contours of the adjacent articulating surfaces, further contributing to the adverse biomechanical environment.[148,154-156]

## SYNOVIAL FLUID AND NUTRITION OF JOINT STRUCTURES

The volume and composition of synovial fluid are determined by the properties of the synovium and its vasculature. Fluid in normal joints is present in small quantities (2.5 mL in the normal knee) sufficient to coat the synovial surface but not sufficient to separate one surface from the other. Tendon sheath fluid and synovial fluid are biochemically similar. Both are essential for the nutrition and lubrication of adjacent avascular structures, including tendons and articular cartilage, and for limiting adhesion formation and maintaining movement. Characterization and measurement of synovial fluid constituents have proved useful for the identification of locally generated regulatory factors, markers of cartilage turnover, and the metabolic status of the joint, as well as for the assessment of the effects of therapy on cartilage homeostasis. However, interpretation of such data requires an understanding of the generation and clearance of synovial fluid and its various components.

## GENERATION AND CLEARANCE OF SYNOVIAL FLUID

Synovial fluid concentrations of a protein represent the net contributions of synovial blood flow, plasma concentration, microvascular permeability, and lymphatic removal and its production and consumption within the joint space. Synovial fluid is a mixture of a protein-rich ultrafiltrate of plasma and hyaluronan synthesized by synoviocytes.[91] Generation of this ultrafiltrate depends on the differences between intracapillary and intra-articular hydrostatic pressures and

between colloid osmotic pressures of capillary plasma and synovial tissue fluid. Fenestrations (i.e., small pores covered by a thin membrane) in the synovial capillaries and the macromolecular sieve of hyaluronic acid facilitate rapid exchange of small molecules, such as glucose and lactate, assisted—in the case of glucose—by an active transport system. Proteins are present in synovial fluid at concentrations inversely proportional to molecular size, with synovial fluid albumin concentrations being about 45% of those in plasma. Concentrations of electrolytes and small molecules are equivalent to those in plasma.[157]

Synovial fluid is cleared through lymphatics in the synovium, assisted by joint movement. In contrast to ultrafiltration, lymphatic clearance of solutes is independent of molecular size. In addition, constituents of synovial fluid, such as regulatory peptides, may be degraded locally by enzymes, and low-molecular-weight metabolites may diffuse along concentration gradients into plasma. The kinetics of delivery and removal of a protein must be determined (e.g., using albumin as a reference solute) to assess the significance of its concentration in the joint.[158]

Hyaluronic acid is synthesized by fibroblast-like synovial lining cells, and it appears in high concentrations in synovial fluid at around 3 g/L, compared with a plasma concentration of 30 μg/L.[91] Lubricin, a glycoprotein that assists articular lubrication, is another constituent of synovial fluid that is generated by the lining cells.[159] It is now believed that hyaluronan functions in fluid-film lubrication, whereas lubricin is the true boundary lubricant in synovial fluid (see later discussion). Because the volume of synovial fluid is determined by the amount of hyaluronan, water retention seems to be the major function of this large molecule.

Despite the absence of a basement membrane, synovial fluid does not mix freely with extra-cellular synovial tissue fluid.[96] Hyaluronan may trap molecules within the synovial cavity by acting as a filtration screen on the surface of the synovial lining, resisting the movement of synovial fluid out from the joint space. Synovial fluid and its constituent proteins have a rapid turnover time (around 1 hour in normal knees), and equilibrium is not usually reached among all parts of the joint. Tissue fluid around fenestrated endothelium reflects plasma ultrafiltrate most closely, with a low content of hyaluronate compared with synovial fluid. Alternatively, locally generated or released peptides, such as endothelin and substance P, may attain much higher perivascular concentrations than those measured in synovial fluid. However, the turnover time for hyaluronan in the normal joint (13 hours) is an order of magnitude slower than that of small solutes and proteins. Association with hyaluronan may result in trapping of solutes within synovial fluid.

In normal joints, intra-articular pressures are slightly subatmospheric at rest (0 to −5 mm Hg). During exercise, hydrostatic pressure in the normal joint may decrease further. Resting intra-articular pressures in rheumatoid joints are around 20 mm Hg, whereas during isometric exercise, they may increase to greater than 100 mm Hg, well above capillary perfusion pressure and, at times, above arterial pressure. Repeated mechanical stresses can interrupt synovial perfusion during joint movement, particularly in the presence of a synovial effusion.

## SYNOVIAL FLUID AS AN INDICATOR OF JOINT FUNCTION

In the absence of a basement membrane separating synovium or cartilage from synovial fluid, measurements of synovial fluid may reflect the activity of these tissues. A wide range of regulatory factors and products of synoviocyte metabolism and cartilage breakdown may be generated locally within the joint, resulting in marked differences between the composition of synovial fluid and plasma ultrafiltrate. Because little capacity exists for the selective concentration of solutes in synovial fluid, solutes that are present at higher concentrations than in plasma are probably synthesized locally. It is necessary, however, to know the local clearance rate to determine whether the solutes present in synovial fluid at lower concentrations than in plasma are generated locally.[157,158] Because clearance rates from synovial fluid may be slower than those from plasma, synovial fluid levels of drugs or urate may remain elevated after plasma levels have declined.

Plasma proteins are less effectively filtered in inflamed synovium, perhaps because of increased size of endothelial cell fenestrations or because interstitial hyaluronate-protein complexes are fragmented by enzymes associated with the inflammatory process. Concentrations of proteins, such as α2 macroglobulin (the principal proteinase inhibitor of plasma), fibrinogen, and IgM, are elevated in inflammatory synovial fluids (see Figure 1-7), as are associated protein-bound cations. Membrane peptidases may limit the

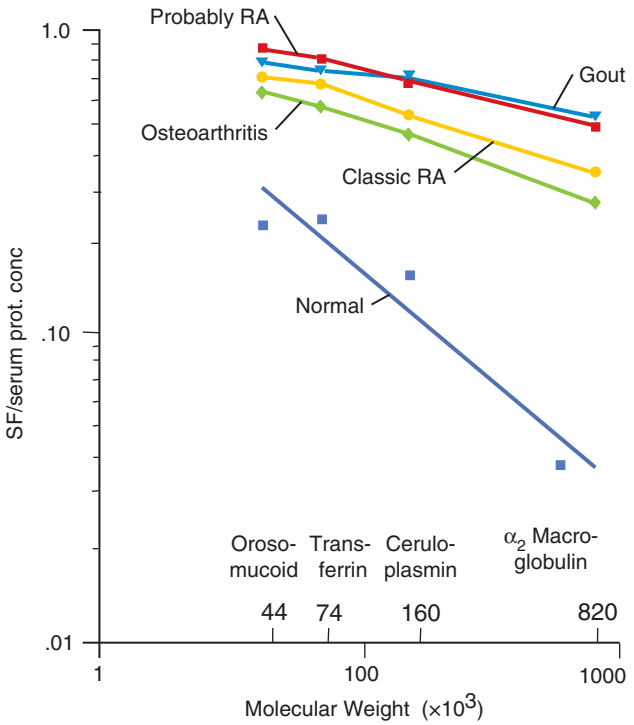



**Figure 1-7** Ratio of the concentration of proteins in synovial fluid to that found in serum, plotted as a function of molecular weight. Larger proteins are selectively excluded from normal synovial fluid, but this macromolecular sieve is less effective in diseased synovium. Prot. conc., Protein concentration; RA, rheumatoid arthritis; SF, synovial fluid. *(From Kushner I, Somerville JA: Permeability of human synovial membrane to plasma proteins.* Arthritis Rheum *14:560, 1971. Reprinted with permission of the American College of Rheumatology.)*

diffusion of regulatory peptides from their sites of release into synovial fluid. In inflammatory arthritis, fibrin deposits may retard flow between the tissue and the liquid phase.

Recently, Sohn and coworkers[160] analyzed synovial fluids and sera from a small series of patients with OA and rheumatoid arthritis using mass spectrometry and multiplex bead-based immunoassays. They identified more than a hundred proteins that were increased in the synovial fluid of patients with OA compared with healthy subjects. Of interest, they found that more than one-third of the proteins in the OA synovial fluid were plasma proteins. They speculated that the presence of these plasma proteins in the synovial fluid could be related to alterations in the endothelial barrier associated with local inflammation in the synovial tissue.

Gobezie and co-workers[161] utilized high-throughput mass spectroscopy-based proteomic analysis to define the protein expression profiles of high abundance synovial fluid proteins in healthy subjects and in patients with early and late OA. They identified 18 proteins that were significantly differentially expressed between the osteoarthritic and control groups. Although all of the differentially expressed proteins were present in the blood and could therefore enter the joint through alterations in vascular permeability associated with the disease state, these molecules were also products of synovial cells and chondrocytes, suggesting that they could be locally produced within the joint. More recently, Ritter et al.[162] utilized a more sensitive method based on gel electrophoresis and mass spectrometry to examine the synovial fluid proteome from patients with OA. They compared the proteomic results with mRNA expression profiles of joint tissues and demonstrated that many of the proteins were derived from synovium or cartilage, providing direct evidence that cells within the joint were a source of the synovial fluid products. Proteins associated with oxidative damage and activation of mitogen-activated protein kinases were among the high-abundance molecules in the OA synovial fluids. They also identified members of the proinflammatory complement cascade in the synovial fluid. Of interest, these molecules have been implicated in the pathophysiology of both OA and rheumatoid arthritis.[163]

## LUBRICATION AND NUTRITION OF THE ARTICULAR CARTILAGE

### Lubrication

Synovial fluid serves as a lubricant for articular cartilage and a source of nutrition for the chondrocytes. Lubrication is essential for protecting cartilage and other joint structures from friction and shear stresses associated with movement under loading. There are two basic categories of joint lubrication. In fluid-film lubrication, cartilage surfaces are separated by an incompressible fluid film, and hyaluronan functions as the lubricant. In boundary lubrication, specialized molecules attached to the cartilage surface permit surface-to-surface contact while decreasing the coefficient of friction. During loading, a noncompressible fluid film is trapped between opposing cartilage surfaces and prevents the surfaces from touching. Irregularities in the cartilage surface and its deformation during compression may augment this trapping of fluid. This stable film is approximately 0.1-μm thick in the normal human hip joint, but it can be much thinner in the presence of inflammatory synovial fluids or with increased cartilage porosity.[91]

Lubricin is the major boundary lubricant in the human joint.[159] Lubricin is a glycoprotein, also called superficial zone protein or proteoglycan 4, that is synthesized by synovial cells, chondrocytes, meniscus, and tendon cells.[164,165] It has a molecular weight of 225,000, a length of 200 nm, and a diameter of 1 to 2 nm. Dipalmitoyl phosphatidylcholine, which constitutes 45% of the lipid in normal synovial fluid, acts together with lubricin as a boundary lubricant. In *boundary lubrication*, lubricin functions as a phospholipid carrier via a mechanism that is common to all tissues and protects the cartilage by reducing pathologic deposition of proteins at the cartilage surface.[166] The importance of lubricin in preserving cartilage homeostasis is illustrated by the study of persons with loss-of-function mutations in the lubricin gene, resulting in the camptodactyl-arthopathy-coxa vara-pericarditis syndrome, which is associated with the development of severe premature OA.[167] Of interest, long-term overexpression of lubricin in animal models of OA protects against both age-related and post-traumatic OA through inhibition of transcriptional programs that promote cartilage catabolism and chondrocyte hypertrophy.[168]

### Nutrition

As observed by Hunter in 1743,[169] normal adult articular cartilage contains no blood vessels. Vascularization of cartilage would be expected to alter its mechanical properties. Blood flow would be repeatedly occluded during weight bearing and exercise, with reactive oxygen species generated during reperfusion, resulting in repeated damage to cartilage matrix and chondrocytes. Chondrocytes synthesize specific inhibitors of angiogenesis that maintain articular cartilage as an avascular tissue.[170-173] As a result of the lack of adjacent blood vessels, the chondrocyte normally lives in a hypoxic and acidotic environment, with extra-cellular fluid pH values around 7.1 to 7.2,[174] and it uses anaerobic glycolysis for energy production.[175,176] High lactate levels in normal synovial fluid, compared with paired plasma measurements, partially reflect this anaerobic metabolism.[176] The two sources of nutrients for articular cartilage are the synovial fluid and subchondral blood vessels.

The synovial fluid and, indirectly, the synovial lining, through which synovial fluid is generated, are the major sources of nutrients for articular cartilage. Nutrients may enter cartilage from synovial fluid either by diffusion or by mass transport of fluid during compression-relaxation cycles.[177] Molecules as large as hemoglobin (65 kDa) can diffuse through normal articular cartilage,[178] and the solutes needed for cellular metabolism are much smaller. Diffusion of uncharged small solutes, such as glucose, is not impaired in matrices containing large amounts of glycosaminoglycans, and diffusivity of small molecules through hyaluronate is enhanced.[179,180]

Intermittent compression may serve as a pump mechanism for solute exchange in cartilage. The concept has arisen from observations that joint immobilization or dislocation leads to degenerative changes. In contrast, exercise increases solute penetration into cartilage in experimental systems.[178] During weight bearing, fluid escapes from the

load-bearing region by flow to other cartilage sites. When the load is removed, cartilage re-expands and draws back fluid, exchanging nutrients with waste materials.[181]

In a growing child, the deeper layers of cartilage are vascularized, such that blood vessels penetrate between columns of chondrocytes in the growth plate. It is likely that nutrients diffuse from these tiny end capillaries through the matrix to chondrocytes. Diffusion from subchondral blood vessels is not considered a major route for the nutrition of normal adult articular cartilage because of the barrier provided by its densely calcified lower layer. Nonetheless, partial defects may normally exist in this barrier,[182] and in arthritis, neovascularization of the deeper layers of articular cartilage may contribute to cartilage nutrition and to entry of inflammatory cells and cytokines.[139,152,183]

## CONCLUSION

Normal human synovial joints are complex structures that comprise interacting connective tissue elements that permit constrained and low-friction movement of adjacent bones. The development of synovial joints in the embryo is a highly ordered process involving complex cell-cell and cell-matrix interactions that lead to the formation of the cartilage anlage and interzone and joint cavitation. Understanding of the cellular interactions and molecular factors involved in cartilage morphogenesis and limb development has provided clues to understanding the functions of the synovium, articular cartilage, and associated structures in the mature joint.

The synovial joint is uniquely adapted to respond to environmental and mechanical demands. The synovial lining is composed of two to three cell layers, with no basement membrane separating the lining cells from the underlying connective tissue. The synovium produces synovial fluid, which provides nutrition and lubrication to the avascular articular cartilage. Normal articular cartilage contains a single cell type, the articular chondrocyte, which is responsible for maintaining the integrity of the extracellular cartilage matrix. This matrix consists of a complex network of collagens, proteoglycans, and other noncollagenous proteins, which provide tensile strength and compressive resistance. Proper distribution and relative composition of these proteins is required for the function of cartilage in protecting the subchondral bone from adverse environmental influences.

Maintenance of the unique composition and organization of each joint tissue is crucial for normal joint function, which is compromised in response to inflammation, biomechanical injury, and aging. Knowledge of the normal structure-function relationships within joint tissues is essential for understanding the pathogenesis and consequences of joint diseases.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 2



# Synovium

Douglas J. Veale • Gary S. Firestein

**KEY POINTS**

- **The synovium provides nutrients to cartilage and produces lubricants for the joint.**
- **The intimal lining of the synovium includes macrophage-like and fibroblast-like synoviocytes.**
- **The sublining in normal synovium contains scattered immune cells, fibroblasts, blood vessels, and fat cells.**
- **Fibroblast-like synoviocytes in the intimal lining produce specialized enzymes that synthesize lubricants such as hyaluronic acid.**

## STRUCTURE

The synovium is a membranous structure that extends from the margins of articular cartilage and lines the capsule of diarthrodial joints, including the temporomandibular joint[1] and the facet joints of vertebral bodies (Figure 2-1).[2] The healthy synovium covers intra-articular tendons and ligaments, as well as fat pads, but not articular cartilage or meniscal tissue. Synovium also ensheaths tendons where they pass beneath ligamentous bands and bursas that cover areas of stress such as the patella and the olecranon. The synovial membrane is divided into two general regions: the intima, or synovial lining, and the subintima, otherwise referred to as the *sublining*. The intima represents the interface between the cavity containing synovial fluid and the subintimal layer. No well-formed basement membrane separates the intima from the subintima. In contrast to the pleura or pericardium, it is not a true lining because it lacks tight junctions, epithelial cells, and a well-formed basement membrane. The subintima is composed of fibrovascular connective tissue and merges with the densely collagenous fibrous joint capsule.

### Synovial Lining Cells

The synovial intimal layer is composed of synovial lining cells (SLCs), which are arrayed on the luminal aspect of the joint cavity. SLCs, termed *synoviocytes*, are one to three cells deep, depending on the anatomic location, and extend 20 to 40 μm beneath the lining layer surface. The major and minor axes of SLCs measure 8 to 12 μm and 6 to 8 μm, respectively. The SLCs are not homogeneous and are conventionally divided into two major populations, namely, type A (macrophage-like) synoviocytes and type B (fibroblast-like) synoviocytes.[3]

#### Ultrastructure of Synovial Lining Cells

Transmission electron microscopic analysis shows that the intimal cells form a discontinuous layer, and thus the subintimal matrix can directly contact the synovial fluid (Figure 2-2). The existence of two distinct cell types—type A and type B SLCs—was originally described by Barland and associates,[4] and several lines of evidence, including animal models, detailed ultrastructural studies, and immunohistochemical analyses, indicate that these cells represent macrophages (type A SLCs) and fibroblasts (type B SLCs). Studies of SLC populations in a variety of species, including humans, have found that macrophages make up anywhere from 20% and fibroblast-like cells approximately 80% of the lining cell.[5,6] The existence of the two cell types has been substantiated by similar findings in a wide variety of species, including hamsters, cats, dogs, guinea pigs, rabbits, mice, rats, and horses.[6-14]

Distinguishing different cell populations that form the synovial lining requires immunohistochemistry or transmission light microscopy. At an ultrastructural level, type A cells are characterized by a conspicuous Golgi apparatus, large vacuoles, and small vesicles, and they contain little rough endoplasmic reticulum, giving them a macrophage-like phenotype (Figure 2-3A and B). The plasma membrane of type A cells possesses numerous fine extensions, termed *filopodia*, that are characteristic of macrophages. Type A cells occasionally cluster at the tips of the synovial villi; this uneven distribution explains, at least in part, early reports that suggested that type A cells were the predominant intimal cell type.[4,8] However, the distribution is highly variable and can differ depending on the joint evaluated or even within an individual joint.

Type B SLCs have prominent cytoplasmic extensions that extend onto the surface of the synovial lining (Figure 2-3C and D).[15] Frequent invaginations are seen along the plasma membrane, and a large indented nucleus relative to the area of the surrounding cytoplasm is also a feature. Type B cells have abundant rough endoplasmic reticulum widely distributed in the cytoplasm, and the Golgi apparatus, vacuoles, and vesicles are generally inconspicuous, although some cells have small numbers of prominent vacuoles at their apical aspect. Type B SLCs are known to contain longitudinal bundles of different-sized filaments, supporting their classification as fibroblasts. Desmosomes and gap-like junctions have been described in rat, mouse, and rabbit synovium, but the existence of these structures in human SLCs has never been documented. Although occasional reports describe an intermediate synoviocyte phenotype, it

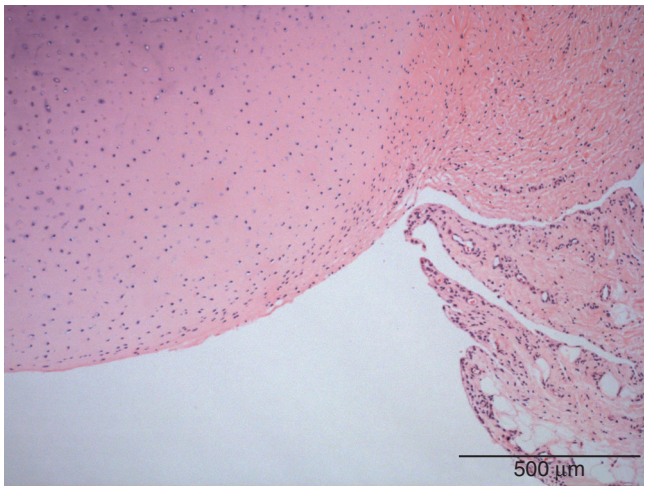



**Figure 2-1** The cartilage-synovium junction. Hyaline articular cartilage occupies the *left half* of this image, and fibrous capsule and synovial membrane occupy the *right half*. A sparse intimal lining layer with a fibrous subintima can be observed extending from the margin of the cartilage across the capsular surface to assume a more cellular intimal structure with areolar subintima.




**Figure 2-2** Transmission electron photomicrograph of synovial intimal lining cells. The cell *on the left* exhibits the dendritic appearance of a synovial intimal fibroblast (type B cell). Other overlying fibroblast dendrites can be observed. Intercellular gaps allow the synovial fluid to be in direct contact with the synovial matrix.

is likely that these cells are functionally conventional type A or B cells.[16,17]

## Immunohistochemical Profile of Synovial Cells

**Synovial Macrophages.** Synovial macrophages and fibroblasts express lineage-specific molecules that can be detected by immunohistochemistry. Synovial macrophages express common hematopoietic antigen CD45 (Figure 2-4A); monocyte/macrophage receptors CD163 and CD97; and lysosomal enzymes CD68 (Figure 2-4B), neuron-specific esterase, and cathepsins B, L, and D. Cells expressing CD14, a molecule that acts as a co-receptor for the detection of bacterial lipopolysaccharide and is expressed by circulating monocytes and monocytes newly recruited to tissue, are rarely seen in the healthy intimal layer, but small numbers are found close to venules in the subintima.[18-24]

The Fcγ receptor, FcγRIII (CD16), which is expressed by Kupffer cells of the liver and type II alveolar macrophages of the lung, is expressed on a subpopulation of synovial macrophages.[25-27] The synovial macrophage population also expresses the class II major histocompatibility complex (MHC) molecule, which plays an important role in the immune response. More recently, the macrophages, which are responsible for the removal of debris, blood, and particulate material from the joint cavity and possess antigen-processing properties, have been found to express Z39Ig, a complement-related protein that is a cell surface receptor and immunoglobulin superfamily member involved in the induction of human leukocyte antigen, DR subregion (HLA-DR) and implicated in phagocytosis and antigen-mediated immune responses.[28-30]

Expression of the β2 integrin chains CD18, CD11a, CD11b, and CD11c varies; CD11a and CD11c may be absent or weakly expressed on a few lining cells.[31,32] Osteoclasts, which are tartrate resistant and acid phosphatase positive and express the αVβ3 vitronectin and calcitonin receptors, do not appear in the normal synovium.

**Synovial Intimal Fibroblasts.** Synovial intimal and subintimal fibroblasts are indistinguishable by light microscopy. They generally are considered to be closely related in terms of cell lineage, but because of their different microenvironments, they do not always share the same phenotype. They possess prominent synthetic capacity and produce the essential joint lubricants hyaluronic acid (HA) and lubricin.[33] Intimal fibroblasts express uridine diphosphoglucose dehydrogenase (UDPGD), an enzyme involved in HA synthesis that is a relatively specific marker for this cell type. UDPGD converts UDP-glucose to UDP-glucuronate, one of the two substrates required by HA synthase for assembly of the HA polymer.[34] CD44, the nonintegrin receptor for HA, is expressed by all SLCs.[32,35,36]

Synovial fibroblasts also synthesize normal matrix components, including fibronectin, laminin, collagens, proteoglycans, lubricin, and other identified and unidentified proteins. They have the capacity to produce large quantities of metalloproteinases, metalloproteinase inhibitors, prostaglandins, and cytokines. This capacity must provide essential biologic advantages, but the complex physiologic mechanisms relevant to normal function are incompletely delineated. Expression of selected adhesion molecules on synovial fibroblasts probably facilitates the trafficking of some cell populations, such as neutrophils, into the synovial fluid and the retention of others, such as mononuclear leukocytes, in the synovial tissue. Expression of metalloproteinases, cytokines, adhesion molecules, and other cell surface molecules is strikingly increased in inflammatory states.

Specialized intimal fibroblasts express many other molecules that also might be expressed by the intimal macrophage population or by most subintimal fibroblasts, including decay-accelerating factor (CD55), vascular cell adhesion molecule–1,[33,37-40] and cadherin-11.[41,42] PGP.95, a neuronal marker, might be specific for type B synoviocytes in some species.[43] Decay-accelerating factor, which is also expressed on many other cells (most notably erythrocytes), as well as bone marrow cells, interacts with CD97, a glycoprotein that is present on the surface of activated leukocytes, including intimal macrophages, and is thought to be involved in signaling processes early after leukocyte activation.[44,45] In contrast, FcγRIII is expressed by macrophages only when they are in close contact with decay-accelerating factor–positive fibroblasts or decay-accelerating factor–coated fibrillin-1 microfibrils in the extra-cellular matrix.[26]

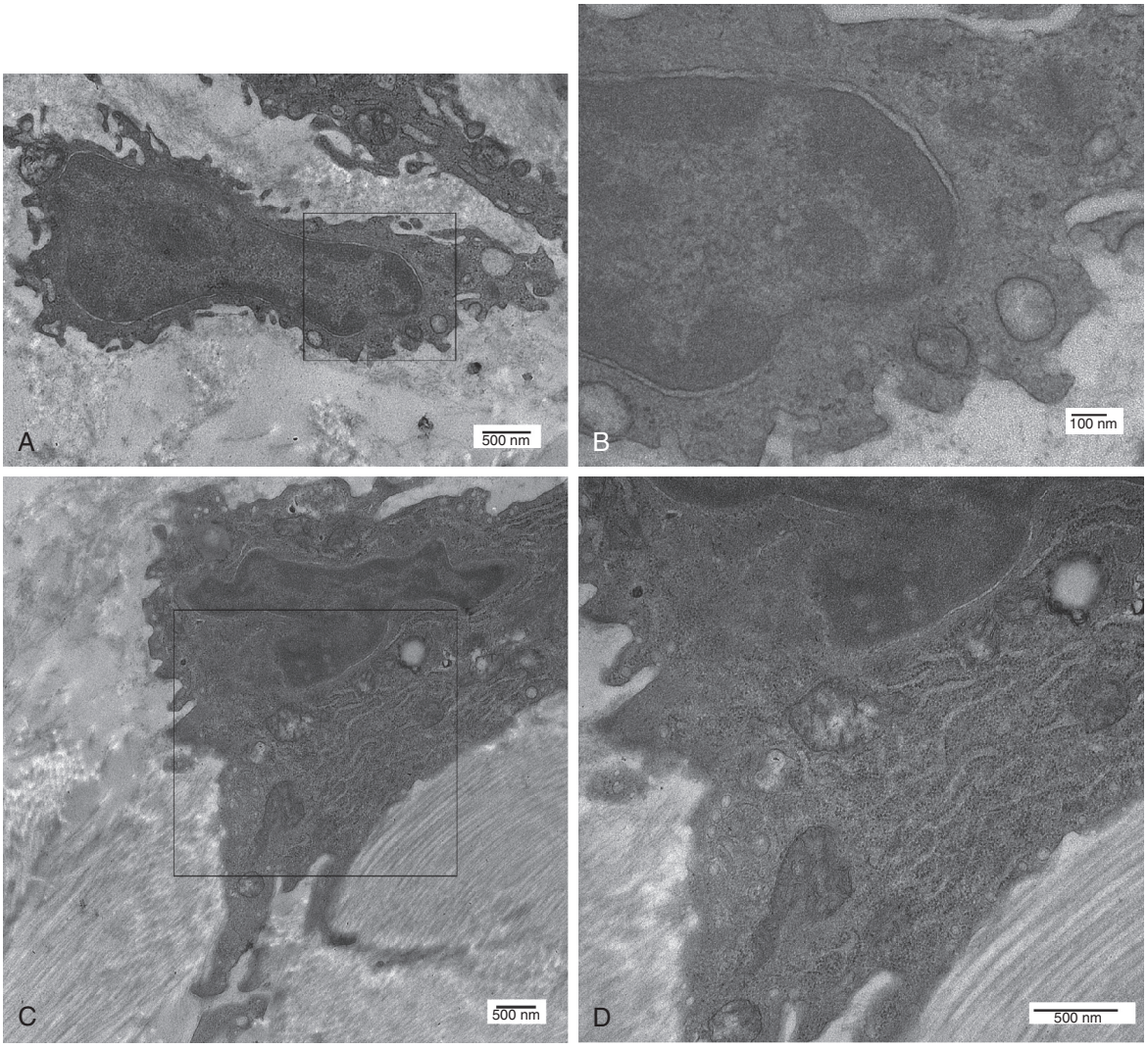



**Figure 2-3** Transmission electron photomicrographs of synovial intimal macrophages (type A cells) and fibroblasts (type B cells). **A,** Low-powered magnification shows the surface fine filopodia, characteristic of macrophages, and a smooth-surfaced nucleus. **B,** The *boxed area* in **A** is shown at a higher magnification, revealing numerous vesicles, characteristic of macrophages. Absence of rough endoplasmic reticulum also is noted. **C,** The convoluted nucleus along with the prominent rough endoplasmic reticulum *(boxed area)* is characteristic of a synovial intimal fibroblast (type B cell). **D,** The rough endoplasmic reticulum is shown at greater magnification.

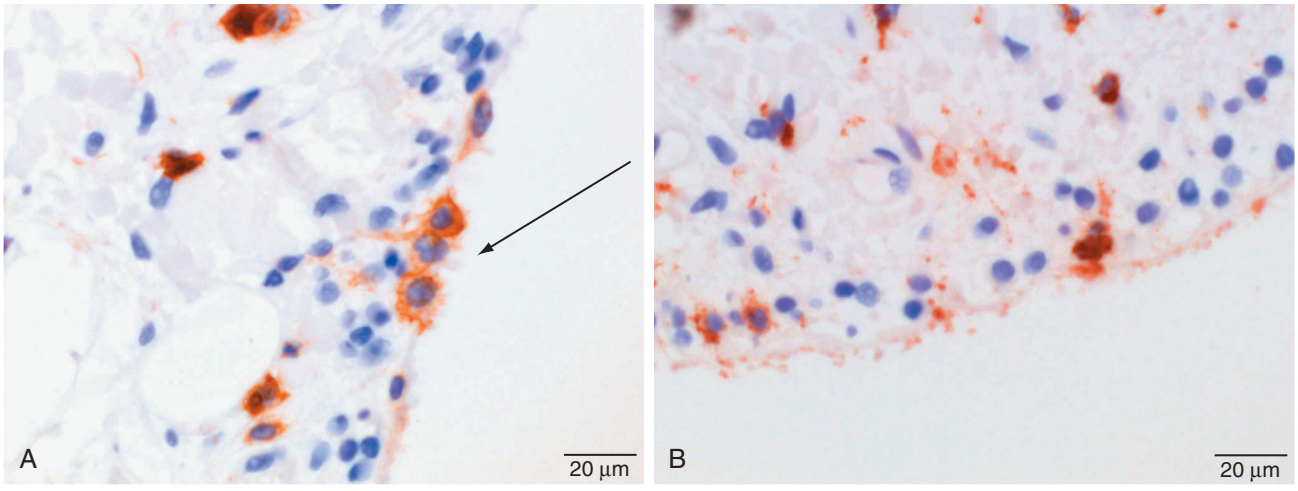



**Figure 2-4** Photomicrographs depicting synovial intimal macrophages by immunohistochemistry. Macrophages are decorated with CD45 (*arrow* in **A**) and CD68 **(B)**, which are markers that identify hematopoietic cells (CD45) and macrophages (CD68).

Toll-like receptors (TLRs) are also expressed on intimal fibroblasts, including TLR2, which is activated by serum amyloid A (among other ligands), leading to angiogenesis and cell invasion that is mediated, at least in part, via the Tie2 signaling pathway.[46,47] Cadherins are a class of tissue-restricted transmembrane proteins that play important roles in homophilic intercellular adhesion and are involved in maintaining the integrity of tissue architecture. Cadherin-11, which was cloned from rheumatoid arthritis (RA) synovial tissue, is expressed in normal synovial intimal fibroblasts but not in intimal macrophages. Fibroblasts transfected with cadherin-11 form a lining-like structure in vitro, which implicates this molecule in the architectural organization of the synovial lining.[41,42,48] This suggestion is supported by the observation that cadherin-deficient mice have a hypoplastic synovial intimal lining and are resistant to inflammatory arthritis.[49] When fibroblasts expressing cadherin-11 are embedded in laminin microparticles, they migrate to the surface and form an intimal lining–like structure.[50] If macrophage lineage cells are included in the culture, they can co-localize with fibroblasts on the surface. Therefore, the organization of the synovial lining, including the distribution of type A and B cells, is orchestrated by fibroblast-like synoviocytes.

β1 and β3 integrins are present on all SLCs, forming receptors for laminin (CD49f and CD49b), types I and IV collagen (CD49b), vitronectin (CD51), CD54 (a member of the immunoglobulin superfamily), and fibronectin (CD49d and CD49e). CD31 (platelet–endothelial cell adhesion molecule), a member of the immunoglobulin superfamily expressed on endothelial cells, platelets, and monocytes, is weakly expressed on SLCs.[32]

## Turnover of Synovial Lining Cells

Proliferation of SLCs in humans is low; when normal human synovial explants have a labeling index of approximately 0.05% to 0.3%[51] when exposed to 3H thimidine.

This labeling index bears a striking contrast to labeling indices of approximately 50% for bowel crypt epithelium. Similar evidence of low proliferation has been found in the synovium of rats and rabbits. The proportion of SLCs expressing the proliferation marker Ki67 is between 1 in 2800 and 1 in 30,000, confirming the relatively slow rate of in situ proliferation.[52] Proliferating cells are generally synovial fibroblasts,[22,53] a finding consistent with the concept that type A synovial cells are terminally differentiated macrophages. Mitotic activity of SLCs is low in inflammatory conditions, such as RA—a condition associated with SLC hyperplasia. Some investigators[54] have reported only rare mitotic figures in RA synovium samples.

Apart from the knowledge that synovial fibroblasts proliferate slowly, little is known about their natural life span, recruitment, or mode of death. Apoptosis is likely involved with maintaining synovial homeostasis, but cultured fibroblast-like synoviocytes tend to be resistant to apoptosis, and very few intimal lining cells display evidence of completed apoptosis by ultrastructural analysis or by labeling for fragmented DNA. The paucity of normal synovium samples for evaluation and the rapid clearance of apoptotic cells could confound the analysis.[55]

## Origin of Synovial Lining Cells

There is little doubt that the type A SLC population is bone marrow–derived and represents cells of the mononuclear phagocyte system.[4] Studies in the Beige (bg) mouse, which harbors a homozygous mutation that confers the presence of giant lysosomes in macrophages, have confirmed the bone marrow origin of these cells.[56,57] Normal mice with bone marrow depleted through irradiation were rescued with bone marrow cells obtained from the bg mouse. Electron microscopic analysis of the synovium from recipient animals revealed that type A SLCs contained the giant lysosomes of the donor bg mouse and that these structures were never identified in type B cells. These findings provide powerful evidence that (1) type A SLCs represent macrophages, (2) they are recruited from the bone marrow, and (3) they are a distinct lineage from type B SLCs.

In addition to immunohistochemistry, several lines of evidence support the concept that type A SLCs are recruited from the bone marrow:

- The osteopetrotic (op/op) mouse, a spontaneously occurring mutant that fails to produce macrophage colony-stimulating factor because of a missense mutation in the *CSF1* gene,[58-60] has low numbers of circulating and resident macrophage colony-stimulating factor–dependent macrophages, including those in the synovium.
- Type A cells in rat synovium do not populate the joint until after the development of synovial blood vessels.[22]
- Type A SLCs are conspicuous around vessels in the synovium in neonatal mice.[6]
- When synovial explants are placed in culture, the reduction in type A SLCs is explained in part by their migration into the culture medium—an observation that reflects the process of migration of macrophages into the synovial fluid in vivo.[1,61]
- Macrophages constitute up to 80% of the cells found around venules in inflammatory conditions such as RA and are cleared rapidly (<48 hours) after successful treatment but will re-accumulate from the circulation if relapse occurs.[62]

Type B intimal cells represent a resident fibroblast population in the synovial lining, but little is known about the cells from which they derive and about how their recruitment is regulated. The existence of mesenchymal stem cells in the synovium suggests that these cells might differentiate into the synovial lining fibroblast. To date, a specific transcription factor directing mesenchymal stem cell differentiation into the synovial fibroblast, similar to factors required for commitment by this multipotential population into bone (cbfa-1), cartilage (Sox-9), and fat (peroxisome proliferator-activated receptor γ [PPARγ]), has not been identified.

Several important signaling pathways are activated in the inflamed synovium, including nuclear factor-κB (NF-κB), Janus kinase/signal transducer and activator of transcription (JAK/STAT), Notch, and hypoxia-inducible factor 1, α subunit (HIF-1α). NF-κB is a key transcriptional regulator in the inflamed synovium.[63] NF-κB signaling is complex and may be activated by cytokines, cell surface

adhesion molecules, and hypoxia.[63,64] NF-κB activation could facilitate synovial hyperplasia by promoting proliferation and inhibiting apoptosis of RA fibroblast-like synoviocytes. One of the key roles of NF-κB is to protect RA fibroblast-like synoviocytes against apoptosis, possibly by countering the cytotoxicity of tumor necrosis factor (TNF) and Fas ligand.[65]

JAK/STAT, Notch, and HIF-1α signaling pathways are also evident in inflamed synovium. STAT3 expression in the synovium correlates with synovitis and is activated by interleukin (IL)-6[66] but also indirectly by TNF. Notch signaling pathway components are predominantly localized to perivascular/vascular regions[67] and are regulated by vascular endothelial growth factor (VEGF) and ang2, which is consistent with the role of mediation of angiogenesis by Notch in inflammation and cancer.[67,68] Interestingly, hypoxia induces activation of phospho (p)-STAT3/p-STAT1, NF-κB, and Notch in synovial cells.[69] Furthermore, Notch/HIF-1α interactions in RA synoviocytes are in part mediated through STAT3 activation,[70] possibly through competition of STAT3 with von Hippel–Lindau tumor suppressor for binding to HIF-1α. Although no direct link between NF-κB and HIF-1α has been demonstrated in the inflamed joint, preferential activation of the canonical NF-κB pathway occurs in RA synovial tissue obtained from patients with more hypoxic joints.[69]

## Subintimal Layer

SLCs are not separated from the underlying subintima by a well-formed basement membrane composed of the typical trilaminar structure seen beneath epithelial mucosa. Nevertheless, most components of basement membrane are present in the extra-cellular matrix surrounding SLCs. These components include tenascin X, perlecan (a heparan sulfate proteoglycan), type IV collagen laminin, and fibrillin-1.[71,72] Of note is the absence of laminin-5 and integrin α3β3γ2, which are components of epithelial hemidesmosomes.[73]

The subintima is composed of loose connective tissue of variable thickness and variable proportions of fibrous/collagenous and adipose tissue, depending on the anatomic site. Under normal healthy conditions, inflammatory cells are virtually absent from the subintima, apart from a sprinkling of macrophages and scattered mast cells.[74] Human synovial tissue is a rich source of mesenchymal stem cells, and although it is unknown which compartment contains this cell population, some cells have the ability to self-renew and differentiate into bone, cartilage, and fat in vitro—a phenomenon that reflects the ability of the cell to regenerate in vivo.[75-77]

Three categories of subintima are well defined: areolar, fibrous, and fatty/adipose types. Under the light microscope, areolar-type subintima, the most commonly studied, generally is found in larger joints in which there is free movement (Figure 2-5A). It is composed of fronds with a cellular intimal lining and loose connective tissue in the subintima, with little in the way of dense collagen fibers, and a rich vasculature. The fibrous subintima is composed of scant, dense, fibrous, poorly vascularized connective tissue, and it has an attenuated layer of SLCs (Figure 2-5B). The adipose type, which contains abundant mature fat cells and has a single layer of SLCs, is seen more commonly with aging and in intra-articular fat pads (Figure 2-5C).

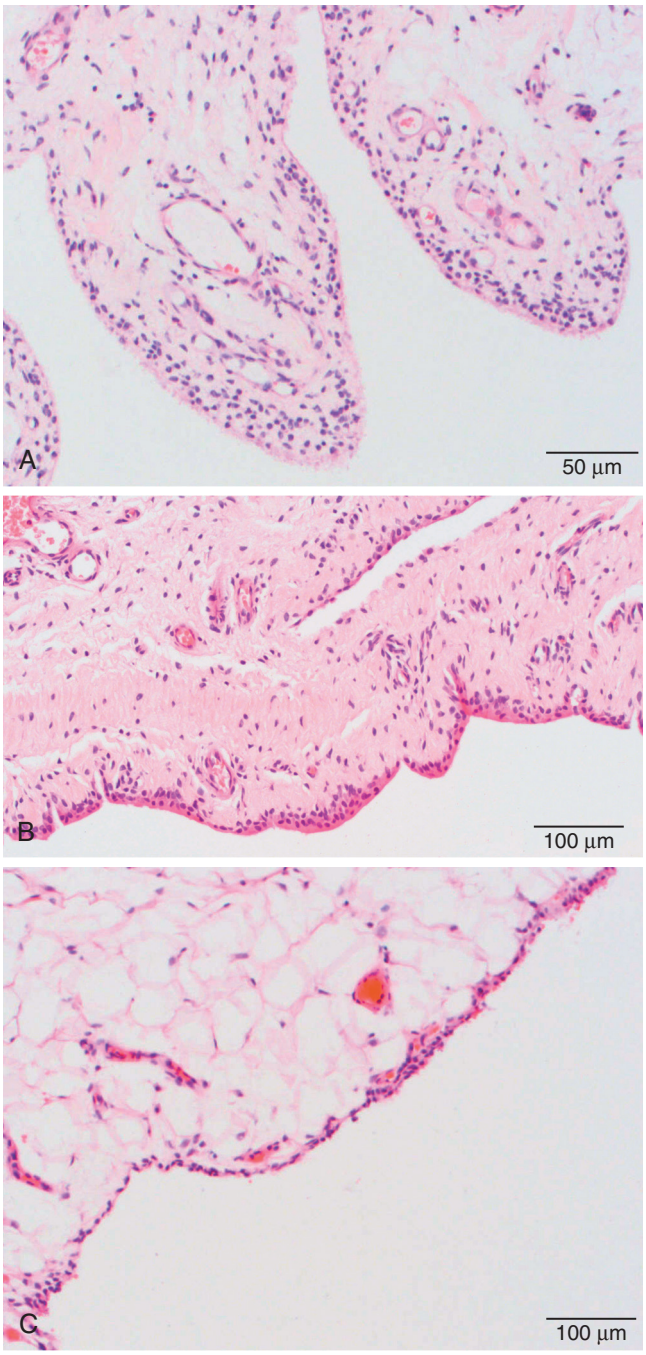



**Figure 2-5** Photomicrographs of different morphologic types of synovial tissue. All photomicrographs show an intimal layer of one to two cells in depth. **A,** The areolar synovium is composed of villous fronds. Beneath the intimal lining layer is cellular loose fibrovascular fatty subintima. **B,** The fibrous synovium comprises dense collagenous material in the subintimal layer. **C,** The subintimal layer of the fatty synovial tissue is composed of less cellular mature adipose tissue with little collagen deposition.

The subintima contains types I, III, V, and VI collagen, glycosaminoglycans, proteoglycans, and extra-cellular matrices, including tenascin and laminins. Integrin receptors for collagens, laminin, and vitronectin are absent or at best weakly expressed by subintimal cells. In contrast,

receptors for fibronectin (CD49d and CD49e) are detected, and CD44, the HA receptor, is strongly expressed in most subintimal cells. β2 integrins are largely limited to perivascular areas, particularly in the subintimal zone, as is CD54.[78]

### Subintimal Vasculature

The vascular supply to the synovium is provided by many small vessels and is shared in part by the joint capsule, epiphyseal bone, and other perisynovial structures. Arteriovenous anastomoses communicate freely with the vascular supply to the periosteum and to periarticular bone. As large synovial arteries enter the deep layers of the synovium near the capsule, they branch to form microvascular units in the more superficial subsynovial layers. Precapillary arterioles probably play a major role in controlling circulation to the lining layer. The surface area of the synovial capillary bed is large, and because it runs only a few cell layers deep to the surface, it has a role in trans-synovial exchange of molecules. The intimal lining, however, is devoid of blood vessels. While few in number, studies have shown that vessels in the normal synovium have an intact pericyte layer, suggesting vessel stability, in contrast to the inflamed joint, where a mix of mature and immature vessels were observed. Neural cell adhesion molecule (NCAM) deficiency and oxidative DNA damage suggest that vessels may remain in a plastic state even after pericyte recruitment.[79,80] After TNF blockade, synovial blood vessels become more stable and resemble normal synovium.

Numerous physical factors influence synovial blood flow. Heat promotes blood flow through synovial capillaries. Exercise enhances synovial blood flow to normal joints but may reduce the clearance rate of small molecules from the joint space. Experiments have shown a substantial vascular reserve capacity in normal articulations. Immobilization reduces synovial blood flow, and pressure on the synovial membrane can act to tamponade the synovial blood supply.

Vascular endothelial lining cells express CD34 and CD31 (Figure 2-6A). They also express receptors for the major components of basement membrane, including laminin and collagen IV, and the integrin receptors CD49a (laminin and collagen receptors), CD49d (fibronectin receptor), CD41, CD51 (vitronectin receptor), and CD61 (the β3 integrin subunit). Endothelial cells express CD44, the HA receptor, and CD62P (P-selectin), which acts as a receptor that supports binding of leukocytes to activated platelets and endothelium. They are only weakly positive in uninflamed synovium, however, for expression of CD54 (intercellular adhesion molecule–1), a receptor for β2 integrins expressed by many leukocytes. The endothelial cells of capillaries in the superficial zone of the subintima are strongly positive for HLA-DR expression by immunohistochemistry, whereas cells in the larger vessels in the deep aspect of the membrane are negative.[32,34]

Hypoxia might be a key driver of endothethlial cell activation and blood vessel formation in the inflamed joint. This theory was originally proposed in 1970,[81] when a synovial fluid electrode was used to demonstrate that a partial pressure of $O_2$ in a knee joint affected by RA was 26.5 mm Hg, which was significantly lower than that in joints affected by osteoarthritis (42.9 mm Hg) or traumatic effusions (63 mm Hg). This observation was supported by studies showing increased glycolytic metabolism in the joint suggestive of increased metabolic activity. Low $pO_2$ in the inflammed synovial membrane was confirmed with $pO_2$ probes, with mean levels approximately 3% compared with normal joints at 7%.[82] The degree of hypoxia in synovium affected by RA and normal synovium was inversely related to the number of blood vessels observed and their level of maturity. In patients responding to TNF blockade, the $pO_2$ increased, thus improving oxygenation to a level similar to that of normal joints.

### Subintimal Lymphatics

Detailed analysis of the number and distribution of lymphatic vessels has been made possible by the use of the antibody to the lymphatic vessel endothelial HA receptor (LYVE-1) (Figure 2-6B).[83] This antibody is highly specific for lymphatic endothelial cells in lymphatic vessels and lymph node sinuses and does not react with endothelial cells of capillaries and other blood vessels that express CD34 and factor VIII–related antigen. Expression of LYVE-1 in lymphatic endothelial cells has been used as a marker to show that lymphatic vessels are less common in the fibrous synovium compared with areolar and adipose variants of human subsynovial tissue. Detection of this molecule reveals that lymphatics are present in the superficial, intermediate, and deeper layers of synovial membrane in synovium from healthy persons or patients with osteoarthritis and joints affected by RA, although the number in the superficial subintimal layer is low in normal synovium. Little difference in the distribution and number is noted between normal and osteoarthritis synovium, which is characterized by lack of villous hypertrophy. Lymphatic channels are plentiful, however, in the subintimal layer in the presence of villous edema hypertrophy and chronic inflammation.

### Subintimal Nerve Supply

The synovium has a rich network of sympathetic and sensory nerves. The former, which are myelinated and detected with the antibody against S-100 protein, terminate close to blood vessels, where they regulate vascular tone (Figure 2-6C through E). Sensory nerves respond to proprioception and pain via large myelinated nerve fibers and via small (<5 μm) unmyelinated or myelinated fibers with unmyelinated free nerve ends (nociceptors). The latter are immunoreactive in the synovium for neuropeptides, including substance P, calcitonin gene–related peptide, and vasoactive intestinal peptides.[84,85]

## FUNCTION

Synthetic and protective functions of individual synovial cell populations are multiple and complex. The composite synovial structure, which includes cell populations and their products, vasculature, nerves, and the intercellular matrix, possesses several specialized functions that are essential for normal joint movement, synovial fluid formation, chondrocyte nutrition, and cartilage protection at multiple

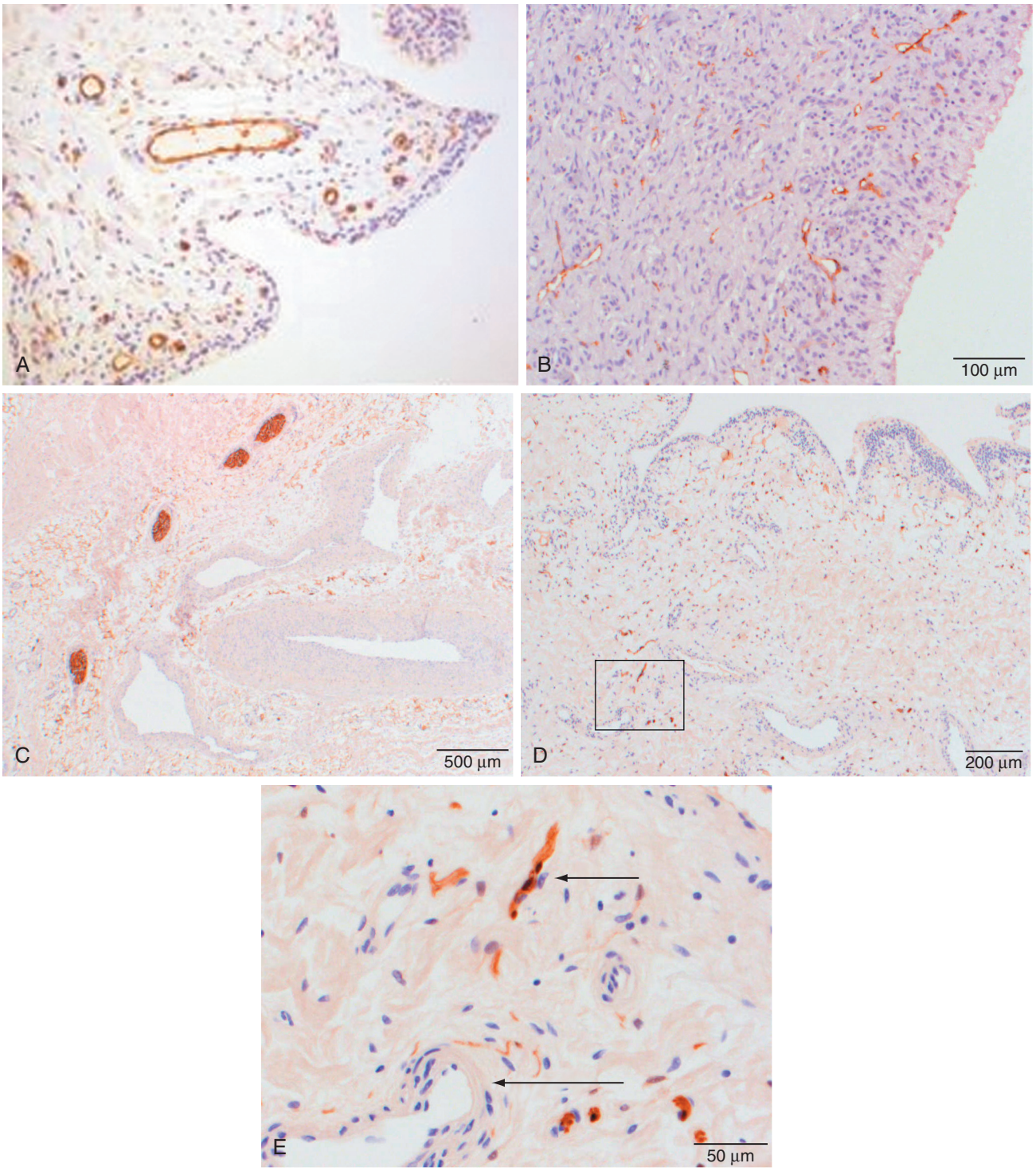



**Figure 2-6** Photomicrographs of synovium show lymphovascular and nervous structures by immunohistochemistry. **A** and **B,** Areolar synovium featuring thin-walled vessels are highlighted with antibody to CD31 **(A),** and lymphatic vessels in an inflamed synovium are highlighted with antibody to lymphatic vessel endothelial HA receptor (LYVE-1) **(B)**. **C,** Deep in the synovial subintima, close to the joint capsule, medium-sized neurovascular bundles are present with nerves highlighted by antibody to S-100. **D,** Within the more superficial synovium, small nerves decorated with S-100 are identified. **E,** The *boxed area* in **D** is shown at a higher magnification. The *upper arrow* is directed at a nerve; the *lower arrow* is directed at a small vessel.

anatomic locations. These functions must be preserved over a lifetime to maintain maximal mobility and independence. Absence of essential constituents of synovial fluid or inadequate cartilage protection results in early articular malfunction, which may progress to local or generalized joint failure.

## Joint Movement

Four characteristics of the synovium are essential for joint movement: deformability, porosity, nonadherence, and lubrication. In a healthy person, the synovium is a highly deformable structure that facilitates movement between

other adjacent, nondeformable structures within the joint. This unique facility of the synovium to enable movement between tissues rather than within tissues has been emphasized[86] and can be attributed to the presence of a free surface that allows synovial tissue to remain separated from adjacent tissues. The ensuing space is maintained by the presence of synovial fluid.

### Deformability

The deformability of normal synovium is considerable because it must accommodate the extreme positional range available to the joint and its adjacent tendons, ligaments, and capsule. When a finger is flexed, the palmar synovium of each interphalangeal joint contracts while the dorsal synovium expands, and as the finger extends, the reverse mechanism occurs. This normal contraction and expansion of synovium seems to involve a folding and unfolding component and an elastic stretching and relaxation of the tissue. During repeated rapid movement, the synovial lining cannot be pinched between cartilage surfaces and for it to successfully retain its integrity and the integrity of synovial blood vessels and lymphatics. Deformability also limits the extent of synovial ischemia-reperfusion injury during joint motion by maintaining a relatively low intra-articular pressure.

### Porosity

The synovial microvasculature and the intimal lining must be porous to permit robust diffusion of nutrients to cartilage. The structure of the intimal lining is ideal for this requirement because of the relatively disorganized basement membrane and lack of tight junctions. Plasma components freely diffuse into the intra-articular space, and most plasma components, including proteins, are present in synovial fluid at about one-third to one-half the plasma concentration.

### Nonadherence

The third important characteristic of the synovium that facilitates joint movement is its nonadherence to opposing surfaces. Intimal cells on the synovial surface adhere to underlying cells and matrix but do not adhere to opposing synovial and cartilage surfaces. The mechanism that preserves this phenomenon of nonadherence is unknown and might involve the arrangement of cell surface and tissue matrix molecules, such as collagen, fibronectin, and HA. Alternatively, nonadherence may result in part from regular movement of the normal synovial lining.

### Lubrication

The fourth characteristic of synovium that is essential for joint motion is an efficient lubrication mechanism to facilitate movement of cartilage on cartilage. The mechanisms of joint lubrication are complex and are an integral component of synovial physiology. In an articulating joint, cartilage is subjected to numerous compressive and frictional forces every day. Friction and wear can never be eliminated from a functioning joint. Adult chondrocytes do not normally divide in vivo, and damaged cartilage has limited capacity for self-repair. For a joint to maintain its function throughout a lifetime of use, protective biologic mechanisms, such as lubrication, help minimize wear and damage that result from normal daily activities. Synovial membrane may also contribute to concentration of lubricants in synovial fluid, because it is a semi-permeable membrane. These functions have recently been replicated by a polytetrafluoroethylene membrane that can be used in a bioreactor system to modulate lubricant retention in bioengineered synovial fluid. Synoviocytes adherent to such membranes may serve as a source of lubricant and a barrier for lubricant transport.[87] Furthermore, cytokines can stimulate normal lubricant production 40- to 80-fold in such bioreactor systems (Figure 2-7).[88]

*Boundary lubrication* refers to the protective effect of particular lubricating molecules adsorbing to a surface and repelling its opposing interface.[89] Bearing surfaces must generate a mutual repulsion to be lubricated in the boundary mode. Boundary lubricants exert their effects by changing the physicochemical characteristics of a surface, and they reduce articular friction and wear by providing a smooth and slippery coating. Friction is reduced by an interposed

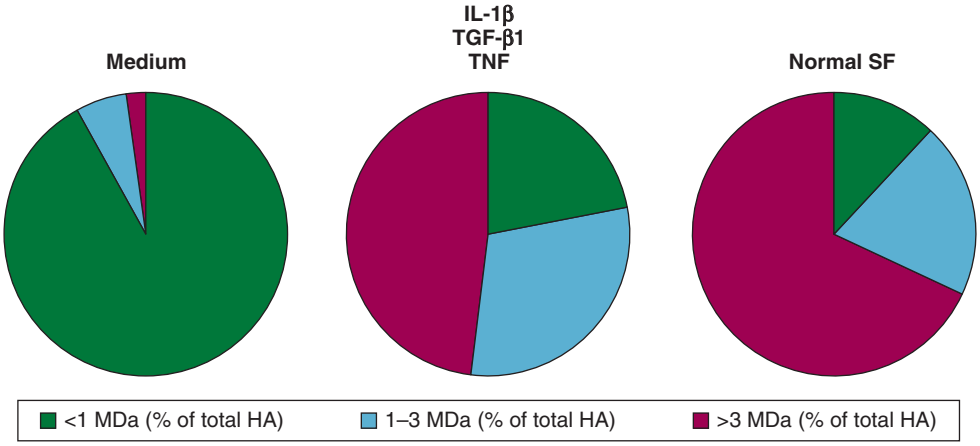



**Figure 2-7** The molecular size of synovial fluid hyaluronic acid (HA) (Normal SF), supernatants of cultured synoviocytes (Medium), and synoviocytes stimulated with IL-1β, TGF-β1 TNF. Note that cells stimulated with the cytokine cocktail closely approximate HA of normal SF compared with control cells, with high molecular weight species that promote a low friction environment. *(Data from Blewis ME, Lao BJ, Schumacher BL, et al: Interactive cytokine regulation of synoviocyte lubricant secretion.* Tissue Eng Part A *16:1329–1337, 2010.)*

film of protective fluid that allows one surface to ride freely over another. The cartilage matrix is integral to this phenomenon because it is fluid filled and compressible. Loaded cartilage extrudes lubricant fluid from its surface, and expressed fluid contributes to the separation of the two articulating surfaces. Scanning electron microscopy has shown a continuous film of fluid, only 100 nm thick, that separates one surface from the other, preventing direct abrasive contact.[90] This ultrathin coating of lubricant resists distraction of the two articulating surfaces, enhancing joint stability. In healthy joints, another essential advantage of an intra-articular lubrication system is the effective prevention of pinching of adjacent, well-vascularized synovial membrane, a feature that is lost in the inflamed joint in which synovial membrane adheres to the cartilage surface.

**Hyaluronic Acid.** HA, a high-molecular-weight polysaccharide, is a major component of synovial fluid and cartilage.[91] It is produced in large amounts by mechanosensitive, fibroblast-like synoviocytes.[92,93] HA, which has three mammalian forms designated HAS1, HAS2, and HAS3,[94] is synthesized by HA synthase at the plasma membrane and is extruded directly into the extra-cellular compartment. HA synthase activity and HA secretion are stimulated by pro-inflammatory cytokines, including IL-1β and TGF-β.[92,95,96] Interestingly, although the levels of cytokines are increased in arthritic joints, the synovial fluid concentration of HA decreases.[97] HA is also synthesized by many other skeletal cells and is an important component of extracellular matrices. It is simultaneously a solid phase matrix element of cartilage and other tissues and a fluid phase element in the synovial space under normal and abnormal conditions.

HA has many biologic functions, which include effects on cell growth, migration, and adhesion. The regulatory role of HA is mediated through HA-binding proteins and receptors, including CD44, which are present on the cell surfaces of chondrocytes, lymphocytes, and other mononuclear cell populations. HA plays a crucial role in morphogenesis and in wound healing. HA also is a vital structural component of the synovial lining, and it has an essential role in the induction of joint cavitation during embryogenesis. HA, which is produced by synovium, was originally thought to be primarily a joint lubricant, and it is generally accepted that it plays a major physiologic role in maintaining synovial fluid viscosity. HA is important in normal joint function, not least through its capacity to provide effective shock absorption. It has been suggested that HA is a particularly important viscohydrodynamic lubricant at low-load interfaces, such as synovium-on-synovium and synovium-on-cartilage.[98] Synovial fluid HA, acting in combination with albumin, has a role in the attenuation of fluid loss from the joint cavity, particularly during periods of increased pressure, which can occur during sustained joint flexion.[99-101]

**Lubricin.** Compelling evidence suggests that lubricin, which was first described in the 1970s,[102] is the factor primarily responsible for boundary lubrication of diarthrodial joints.[103] Lubricin, a large secreted, mucin-like proteoglycan with an apparent molecular weight of 280 kDa, is a product of the gene proteoglycan 4 *(PRG4)*. It is a major component of synovial fluid and is present at the cartilage surface. The gene is highly expressed by human synovial fibroblasts and by superficial zone chondrocytes.[104] Lubricin is closely related to superficial zone protein, megakaryocyte-stimulating factor, and hemangiopoietin, which are encoded by the same gene but can differ in terms of post-translational modification. Superficial zone protein is expressed by SLCs and by superficial zone chondrocytes at the cartilage surface but not by intermediate or deep zone chondrocytes.[105] It has been suggested that lubricin may bind to the much longer hyaluronate polymers, distributing shear stress and stabilizing essential lubricant molecules.[106]

In an experimental model, lubricin seemed to have multiple functions in articulating joints and tendons, including protection of cartilage surfaces from protein deposition and cell adhesion and inhibition of synovial cell overgrowth.[107] *Prg4*$^{-/-}$ mice, which were consistently normal at birth, showed progressive loss of superficial zone chondrocytes and increasing synovial cell hyperplasia (Figure 2-8). The essential role of lubricin in maintaining joint integrity was shown by the identification of disease-causing mutations in patients with the autosomal-recessive disorder camptodactyly–arthropathy–coxa vara–pericarditis (CACP) syndrome.[108] CACP is a large joint arthropathy associated with the absence of lubricin from synovial fluid and ineffective boundary lubrication provided by the synovial fluid (Figure 2-9).[106,109] In other studies of lubricin biology and joint integrity, experimental injury resulted in reduced synovial fluid lubricin concentrations, decreased boundary lubricating ability, and increased cartilage matrix degradation, each of which could be attributed to trauma-induced inflammatory processes.[104]

Other investigators have argued against the primacy of lubricin in joint lubrication by proposing that surface-active phospholipid, which is also secreted by intimal fibroblasts, is the essential boundary lubricant that reduces cartilage friction to remarkably low levels.[110] It was hypothesized that lubricin acts as the carrier of surface-active phospholipid to articular cartilage but is not the lubricant per se, a function that is similar to that of the well-characterized alveolar surfactant binding proteins in the lung.

## Synovial Fluid Formation

In healthy people a constant volume of synovial fluid is important during joint movement as a cushion for synovial tissue and as a reservoir of lubricant for cartilage. Many of the soluble components and proteins in synovial fluid exit the synovial microcirculation through pores or fenestrations in the vascular endothelium, then diffuse through the interstitium before entering the joint space. Synovial fluid is in part a filtrate of plasma to which additional components, including HA and lubricin, are added and removed by the SLCs (Figure 2-10). As noted earlier, concentrations of electrolytes and small molecules in synovial fluid are similar to those in plasma. Synovial permeability to most small molecules is determined by a process of free diffusion through the double barrier of endothelium and interstitium, limited mainly by the intercellular space between SLCs. For most small molecules, synovial permeability is inversely related to the dimensions of the molecule.

Experimental evidence suggests that the exchange of small solutes is determined predominantly by the synovial

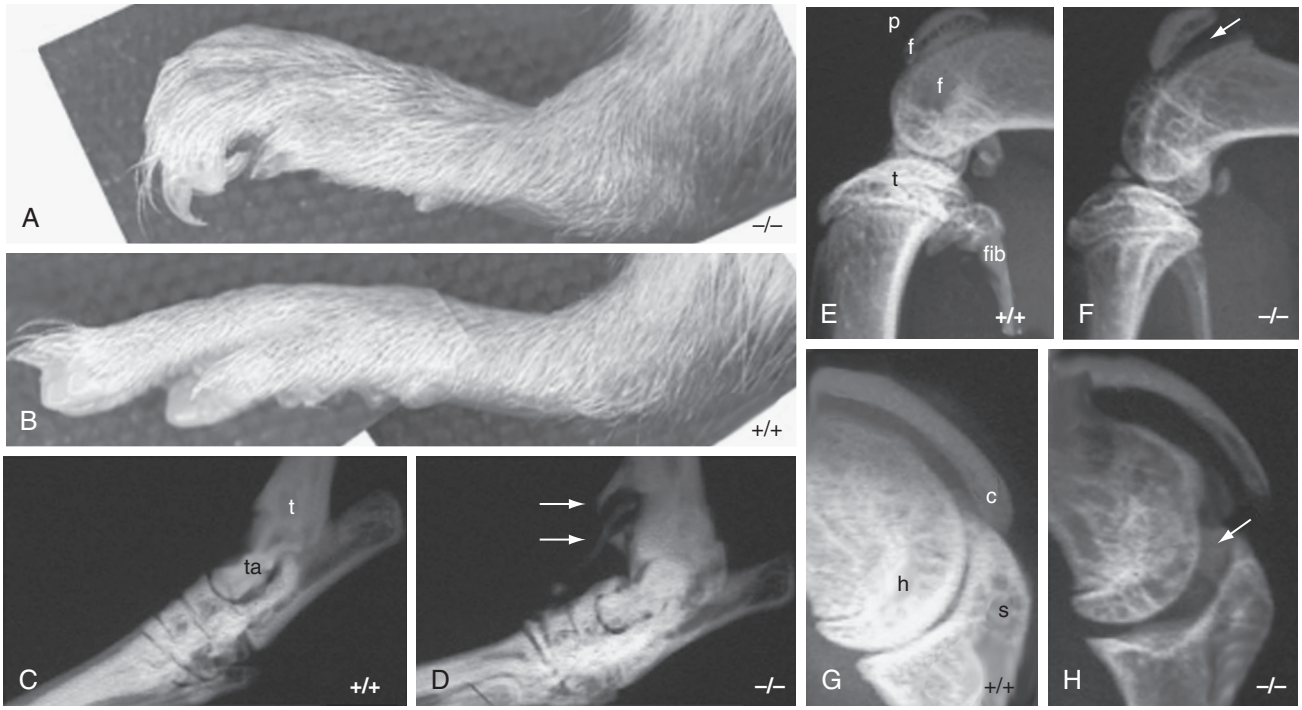



**Figure 2-8** Clinical appearance and radiographic changes in *Prg4*$^{-/-}$ mice. **A** and **B,** Photographs of the hind paws of 6-month-old *Prg4*$^{-/-}$ **(A)** and wild-type **(B)** mice. Note the curved digits in the mutant mouse and swelling at the ankle joint. **C** and **D,** Radiographs of the ankle joint of 9-month-old wild-type **(C)** and *Prg4*$^{-/-}$ mice **(D)**. Structures corresponding to the tibia (t) and talus (ta) are indicated. Note the calcification of structures adjacent to the ankle (*arrows* in **D**). **E,** Lateral knee radiograph of a 4-month-old wild-type mouse. Structures corresponding to the patella (p), femoral condyle (f), tibial plateau (t), and fibula (fib) are indicated. **F,** Lateral knee radiograph of a 4-month-old *Prg4*$^{-/-}$ mouse. Note the increased joint space between the patella and femur *(arrow)* and osteopenia of the patella, femoral condyles, and tibial plateau. **G,** Shoulder radiograph of a 4-month-old wild-type mouse. Structures corresponding to the humeral head (h), glenoid fossa of the scapula (s), and lateral portion of the clavicle (c) are indicated. **H,** Shoulder radiograph of a 4-month-old *Prg4*$^{-/-}$ mouse. Note the increased joint space between the humerus and scapula *(arrow)* and osteopenia of the humeral head. *(From Rhee DK, Marcelino J, Baker M, et al: The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth.* J Clin Invest *115:622–631, 2005.)*

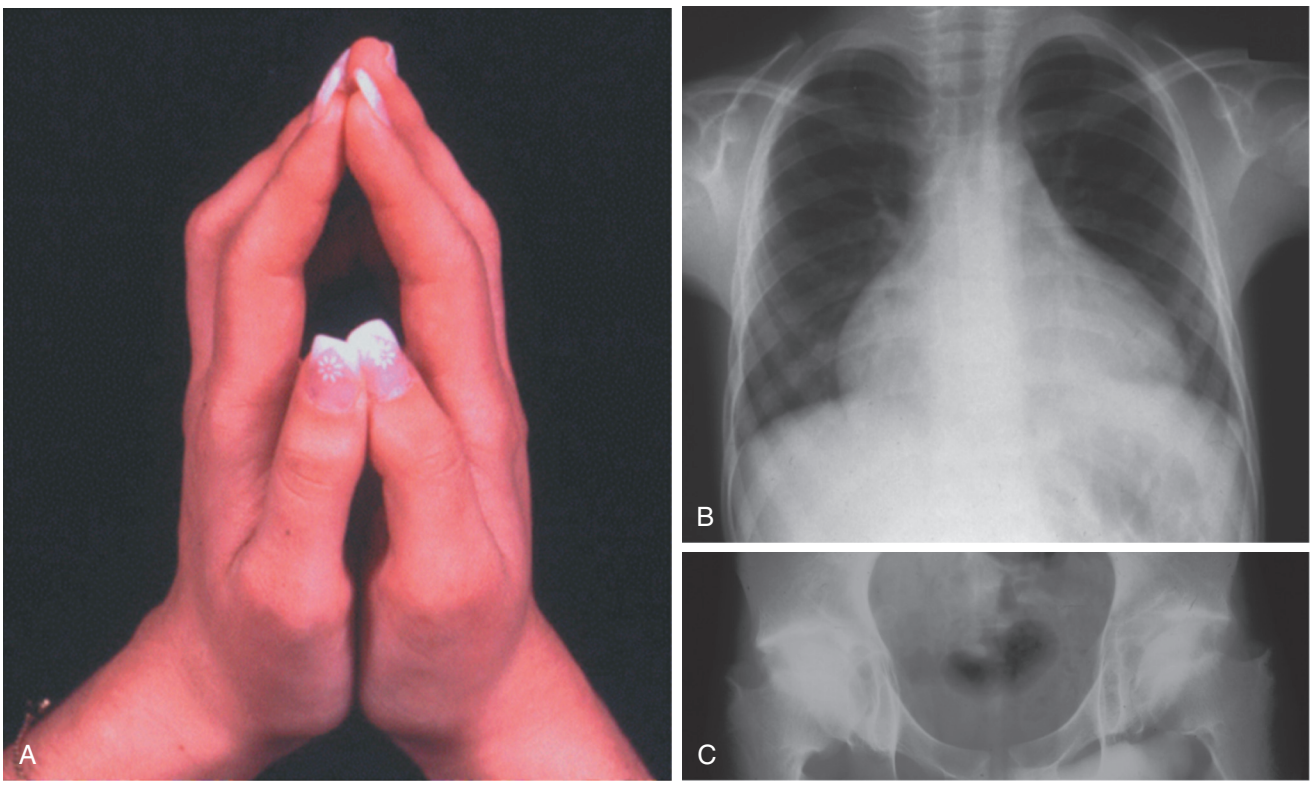



**Figure 2-9** Clinical features of camptodactyly–arthropathy–coxa vara–pericarditis (CACP) syndrome. **A,** The characteristic deformity of the hands is shown. **B,** A chest radiograph shows an enlarged cardiac outline caused by pericarditis. **C,** radiograph of the pelvis highlights coxa vara in a boy with CACP. *(**B** and **C,** Courtesy Ronald Laxer, MD, Hospital for Sick Children, Toronto, Ontario, Canada.)*

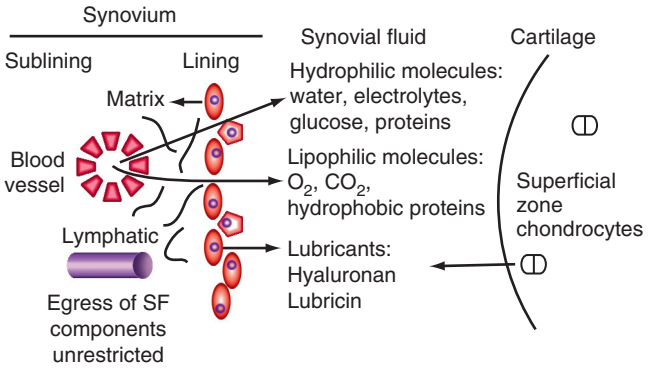



**Figure 2-10** Schematic representation of the formation of synovial fluid (SF). Many of the soluble components and proteins in SF exit the synovial subintimal microcirculation through pores or fenestrations in the vascular endothelium, then diffuse through the interstitium before entering the joint space. Synovial permeability to most small molecules is determined by a process of free diffusion through the double barrier of endothelium and interstitium, limited mainly by the intercellular space between synovial lining cells. Fat-soluble molecules can diffuse through, and between, cell membranes; their passage across the synovial surface is less restricted. Additional components, including hyaluronan and lubricin, are produced by synovial lining cells.

interstitium and that permeability to proteins is mainly determined by the microvascular endothelium. The synovium should not be regarded as simply an inert membrane but as a complex regulatory tissue system. The small physiologic molecules that traverse the endothelium of synovial blood vessels and diffuse through intercellular spaces of the synovial lining before entering the synovial fluid include water, glucose, and many other essential nutrients and waste tissue metabolites. Evidence suggests that passage of some solutes across the synovium is facilitated by specific transport systems that possibly provide a "pump" mechanism capable of moving water out of the joint space.

Plasma proteins are able to cross the endothelium, traversing the synovial interstitium and entering the synovial fluid. The efficiency of this process is determined by the molecular size of the protein and the diameter of the endothelial pores. Smaller proteins, such as albumin, enter easily, whereas larger molecules, such as fibrinogen, gain access with greater difficulty. In contrast, the clearance or removal of proteins and other synovial fluid constituents is unrestricted and considerably more efficient through lymphatic drainage. The synovial fluid concentration of any protein reflects the dynamic balance between ingress and egress at a given time. Because egress is more efficient than ingress, joint space pressure is normally subatmospheric. Negative intra-articular pressure is thought to be important in maintaining joint stability. The synovial fluid-to-serum ratio of plasma proteins is inversely related to the molecular size of the protein. When the joint becomes inflamed, greater endothelial permeability permits more profuse ingress of all proteins, and the most obvious changes are noted in the concentrations of larger molecules. Increased synovial fluid volume also reduces the stability of the joint.

In contrast to hydrophilic molecules, fat-soluble molecules can diffuse through and between cell membranes, and their passage across the synovial surface is less restricted. The entire surface area of the synovium is available to lipophilic molecules that diffuse into and out of the joint space. Physiologically, the most important fat-soluble molecules are the respiratory gases, oxygen and carbon dioxide. When the joint is inflamed, synovial fluid may exhibit low partial pressure of oxygen, high partial pressure of carbon dioxide, decreased pH, and increased lactate production.[82] The resultant hypoxia and acidosis can have serious implications for the synovial microcirculation and chondrocyte metabolism.

## Chondrocyte Nutrition

Another important function of synovium is to enhance the nutrition of chondrocytes, which reside in articular cartilage (see Chapter 3). Because articular cartilage is avascular, delivery of nutrients to chondrocytes and removal of metabolic breakdown products from the cartilage are believed to occur through synovial fluid and synovial tissue arterioles and venules, as well as through subchondral bone. Morphologic, physiologic, and pathologic studies have confirmed that solutes pass easily from the synovial fluid into cartilage and that cartilage does not survive without synovial fluid contact in vivo. Within the cartilage matrix, three potential mechanisms for nutrient transfer have been proposed: diffusion, active transport by chondrocytes, and pumping by intermittent compression of cartilage matrix. A large proportion of hyaline cartilage lies within 50 μm of a synovial surface and its rich supply of blood vessels. Chondrocytes are oxygen sensitive and are well adapted to living in hypoxic conditions. Low oxygen tension promotes expression of the chondrocyte phenotype and cartilage-specific matrix formation. Reactive oxygen species also may play a crucial role in the regulation of some normal chondrocytic activities, such as cell activation, proliferation, and matrix remodeling.

## CONCLUSION

The normal human synovial membrane is a highly specialized, multifunctional organ that is vital for mobility and survival. The intimal layer is composed of two distinct cell phenotypes with characteristics of macrophage and fibroblast lineages. Synovial macrophages express CD45, CD163 and CD97, CD68, neuron-specific esterase, and cathepsins B, L, and D. Cells expressing CD14 are rarely seen in the healthy intimal layer. FcγRIII (CD16), expressed by Kupffer cells of the liver and type II alveolar macrophages of the lung, is expressed on a subpopulation of synovial macrophages. Synovial macrophages also express the class II MHC molecule and play a central role in phagocytosis and in antigen-mediated immune responses.

Synovial intimal fibroblasts possess prominent synthetic capacity and produce the essential joint lubricants HA and lubricin. They also synthesize normal matrix components, including fibronectin, laminin, collagens, proteoglycans, lubricin, and other identified and unidentified proteins. They have the capacity to produce large quantities of metalloproteinases, metalloproteinase inhibitors, prostaglandins, and cytokines. Expression of selected adhesion molecules on synovial fibroblasts probably facilitates the trafficking of some cell populations, such as polymorphs, into synovial

fluid, and the retention of others, such as mononuclear leukocytes, within synovial tissue.

The subintimal layer is composed of a loose connective tissue matrix and contains branching blood and lymphatic vessels, a nerve supply, and a variety of resident cell populations, including infiltrating macrophages and fibroblasts. The nerve supply is important in regulating synovial blood flow. Lymphatic vessels allow egress of metabolic breakdown products from the synovium and synovial fluid. The structure of the subintimal layer varies according to the anatomic location and the local functional requirements.

Coordinated functions of the composite synovial membrane are essential for normal joint movement, formation of synovial fluid, nutrition of chondrocytes, and protection of cartilage. These functions must be preserved over a lifetime at multiple anatomic locations. Absence of essential constituents of synovial fluid, such as lubricin, or inadequate cartilage protection results in early articular malfunction, which may progress to variable degrees of joint failure. The characteristics of lubricin deficiency have been elegantly described in animal models and in humans. Additional studies may define novel clinical categories of degenerative polyarthritis that are associated with other specific disorders of synovial membrane function.



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CHAPTER 3



# Cartilage and Chondrocytes

Mary B. Goldring • Kirsty L. Culley • Elisabeth Wondimu • Miguel Otero

**KEY POINTS**

- **Articular cartilage matrix is heterogeneous and contains numerous extra-cellular matrix (ECM) proteins, of which the large aggregating proteoglycan aggrecan and types II, IX, and XI collagen are the major constituents.**
- **The collagen network of cartilage confers tensile strength, and aggrecan provides resistance to compression.**
- **Adult articular chondrocytes are nonmitotic cells that survive at low oxygen tension in the absence of a vascular supply.**
- **In response to trauma or inflammation, the metabolic activity of the chondrocyte is increased in response to catabolic and anabolic factors that regulate remodeling of the ECM.**
- **Under physiologic conditions, the chondrocyte maintains low-turnover repair of proteoglycans, but the repair capacity, responses to anabolic factors, cell survival, and quality of the matrix decline with age.**

Hyaline cartilage, including the articular cartilage of diarthrodial joints, consists of a single cellular component, the chondrocyte, which is embedded in a unique and complex matrix. Adult articular chondrocytes are considered to be fully differentiated cells that maintain matrix constituents in a low-turnover state of equilibrium. Chondrocytes serve diverse functions during development and postnatal life. In the embryo, the chondrocyte arises from mesenchymal progenitors from diverse sources, including the cranial neural crest of the neural ectoderm, cephalic mesoderm, sclerotome of the paraxial mesoderm, and somatopleure of the lateral plate mesoderm, depending on the ultimate location of the cartilage. The chondrocyte synthesizes the templates, or cartilage anlagen, through a process termed *chondrogenesis*.

After mesenchymal condensation and chondroprogenitor cell differentiation, the chondrocytes undergo proliferation, terminal differentiation to chondrocyte hypertrophy, and apoptosis through a process termed *endochondral ossification*, whereby the hypertrophic cartilage is replaced by bone. A similar sequence of events occurs in the postnatal growth plate and leads to rapid growth of the skeleton. Processes that control the different stages of skeletal development are described in Chapter 1.

In adults, the anatomic distribution of cartilage is restricted primarily to the joints, trachea, and nasal septum, where the major function is structural support. In joints, cartilage has the additional function of providing low-friction articulation. Adult articular cartilage comprises a specialized matrix of collagens, proteoglycans, and other cartilage-specific and nonspecific proteins. Adult articular chondrocytes, which are remnants of the resting, or reserve, chondrocytes that laid down the original cartilage matrix during chondrogenesis, are inactive metabolically, partially because of the absence of a vascular supply and innervation in the tissue. The clinical importance of the adult chondrocyte resides in its capacity to respond to mechanical stimuli, growth factors, and cytokines that may influence normal homeostasis in a positive or negative manner. In persons with rheumatoid arthritis (RA), cartilage destruction occurs primarily in areas contiguous with the proliferating synovial pannus, although evidence indicates that the chondrocyte can respond to the inflammatory milieu and participate in degrading its own matrix. In persons with osteoarthritis (OA), the chondrocyte plays a key role by reacting to structural changes in the surrounding cartilage matrix through the production of catabolic cytokines and anabolic factors, which act in an autocrine-paracrine manner.[1,2] Nevertheless, the chondrocyte has limited capacity, which declines with age, to regenerate the normal cartilage architecture with zonal variations in the matrix network that was formed originally. This chapter focuses on the structure and function of normal articular cartilage and the role of the chondrocyte in maintaining cartilage homeostasis and responding to adverse environmental insults that may modify cartilage integrity.

## CARTILAGE STRUCTURE

Normal articular cartilage is a specialized tissue characterized macroscopically by its milky, shelled-almond (hyaline) appearance. It is an avascular tissue nourished by diffusion from the vasculature of the subchondral bone and from the synovial fluid. Articular cartilage is more than 70% water, and it is hypocellular compared with other tissues; chondrocytes constitute only 1% to 2% of its total volume. Most of the dry weight of cartilage consists of two components: type II collagen and the large aggregating proteoglycan, aggrecan. Several "minor" collagens and small proteoglycans also contribute to the unique structural organization of the cartilage matrix.[3,4]

Organic constituents represent only about 20% of the wet weight of cartilage. Collagen, primarily type II, accounts for approximately 15% to 25% of the wet weight and about half of the dry weight except in the superficial zone, where it represents most of the dry weight. Proteoglycans, primarily aggrecan, account for 10% of the wet weight and about 25% of the dry weight. The highly cross-linked type II

collagen–containing fibrils form a systematically oriented network that traps the highly negatively charged proteoglycan aggregates. Histochemical analysis of cartilage shows that proteoglycans can be stained reliably with Safranin O, Toluidine blue, or Alcian blue, although at low substrate concentrations, these methods are not stoichiometric.[5] Collagen also can be stained efficiently, but differentiation of collagen types requires immunostaining with specific antibodies.

Despite its thinness (≤7 mm) and apparent homogeneity, mature articular cartilage is a heterogeneous tissue with four distinct regions: (1) the superficial tangential (or gliding) zone, (2) the middle (or transitional) zone, (3) the deep (or radial) zone, and (4) the calcified cartilage zone, which is located immediately below the tidemark and above the subchondral bone (Figure 3-1).[4] In the superficial zone, the chondrocytes are flattened, with thin collagen fibrils in tangential array, a high concentration of the small proteoglycan decorin, and a low concentration of aggrecan. The middle zone, composing 40% to 60% of cartilage weight, consists of rounded chondrocytes surrounded by radial bundles of collagen fibrils that are thicker than in other zones. In the deep zone, chondrocytes frequently are grouped in columns or clusters.

Cell density progressively decreases from the surface to the deep zone, where it is one-half to one-third the density in the superficial zone; chondrocytes in the deep and middle zones have a cell volume that is twice that of superficial chondrocytes. Water is 75% to 80% of the wet weight in the superficial zone and progressively decreases to 65% to 70% with increasing depth. Compared with the middle and deep zones, greater amounts of collagen relative to proteoglycans are present in the superficial zone, and type I collagen may be present, in addition to type II collagen. With increasing depth, the proportion of proteoglycan increases to 50% of the dry weight in the deep zone. The calcified zone is formed as a result of endochondral ossification and persists after growth plate closure, with the histologically defined tidemark defining the boundary with the articular cartilage.[6] The calcified zone serves as an important mechanical buffer between uncalcified articular cartilage and subchondral bone (Figure 3-2).

The physical properties of articular cartilage are determined by the unique fibrillar collagen network, which

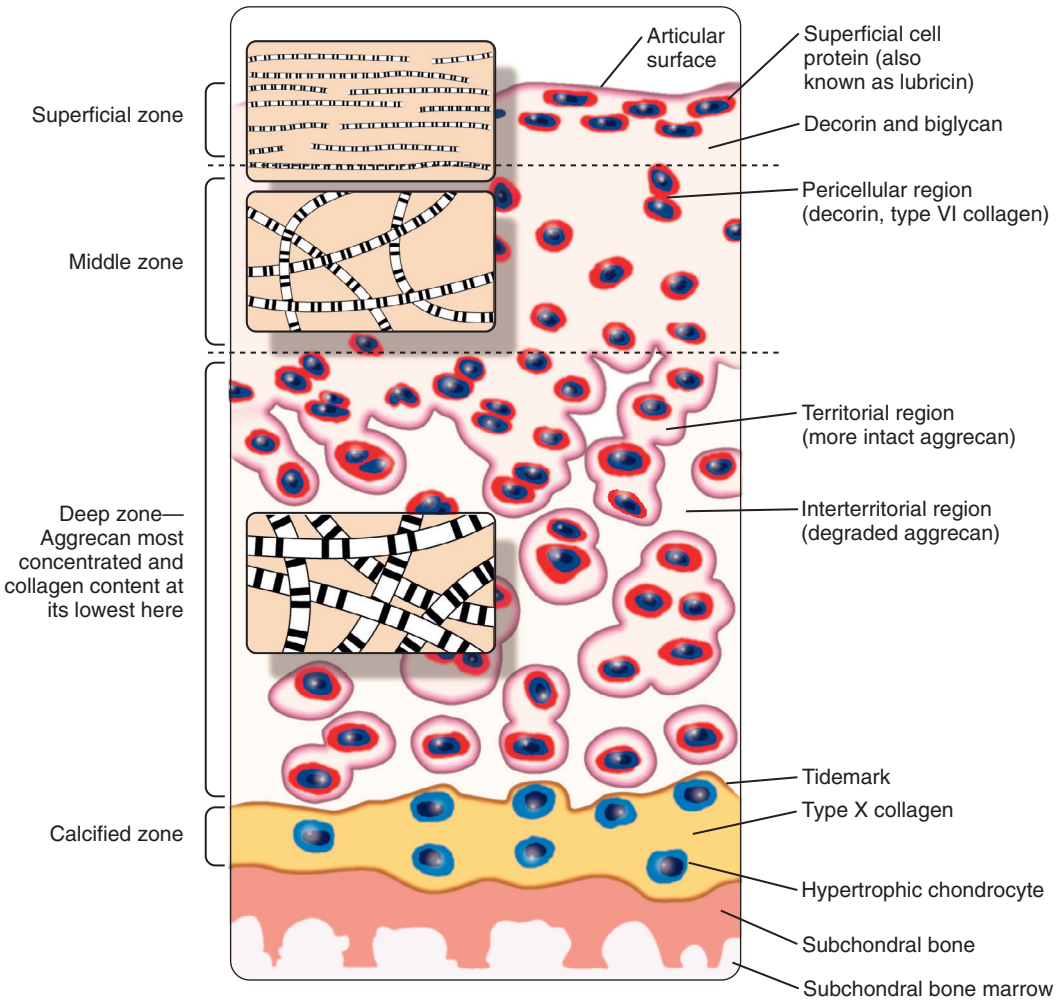



**Figure 3-1** The structure of human adult articular cartilage showing zones of cellular distribution and the pericellular, territorial, and interterritorial regions of matrix organization. Insets show the relative diameters and orientations of collagen fibrils in the different zones. The positions of the tidemark and subchondral bone and other special features of matrix composition also are noted. *(From Poole AR, Kojima T, Yasuda T, et al: Composition and structure of articular cartilage: a template for tissue repair.* Clin Orthop Relat Res *[391S]:S26–S33, 2001. Copyright Lippincott Williams & Wilkins.)*

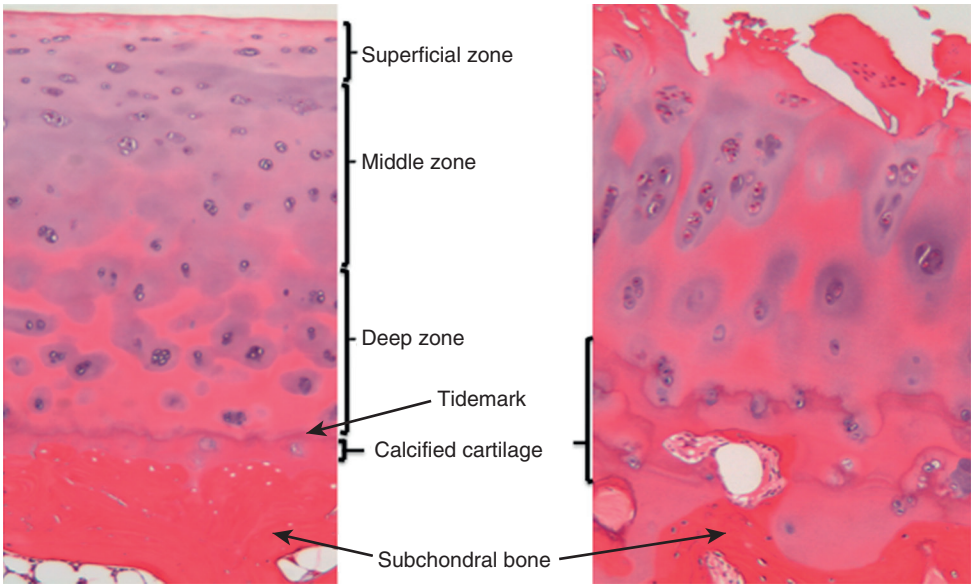



**Figure 3-2** The composition and cellular organization of healthy human adult articular cartilage *(left panel)* is more complex than it looks in this histologic section showing chondrocytes distributed in matrix with a homogeneous appearance and the clear demarcation (tidemark) between the articular cartilage and a thin zone of calcified cartilage next to the underlying subchondral bone. During the development of osteoarthritis, the normally quiescent chondrocytes become activated and undergo a phenotypic shift, resulting in surface fibrillation and degradation of cartilage matrix, the appearance of chondrocyte clusters, increased cartilage calcification associated with tidemark advancement, or duplication, and vascular penetration from the subchondral bone *(right panel). (Histology [Safranin O staining] courtesy Cecilia Dragomir, Hospital for Special Surgery, New York, NY; 10× magnification; modified from Goldring MB, Marcu KB: Epigenomic and microRNA-mediated regulation in cartilage development, homeostasis, and osteoarthritis.* Trends Mol Med *18:109–118, 2012.)*

provides tensile strength, interspersed with proteoglycan aggregates that bestow compressive resilience.[7,8] Proteoglycans are associated with large quantities of water bound to the hydrophilic glycosaminoglycan chains. This cartilaginous extra-cellular matrix (ECM), with its tightly bound water, provides a high degree of resistance to deformation by compressive forces. The capacity to resist compressive forces is associated with the ability to extrude water as the cartilage compresses. When compression is released, the proteoglycans (now depleted of balancing counter ions that were removed with the water) contain sufficient fixed charge to reabsorb osmotically the water and small solutes into the matrix, which then rebounds to its original dimensions.

## STRUCTURE-FUNCTION RELATIONSHIPS OF CARTILAGE MATRIX COMPONENTS

ECM components synthesized by chondrocytes include highly cross-linked fibrils of triple-helical type II collagen molecules that interact with other collagens, aggrecan, small proteoglycans, and other cartilage-specific and nonspecific matrix proteins (Table 3-1).[3,4,9] The importance of these structural proteins may be observed in heritable disorders, such as chondrodysplasias, or in transgenic animals in which mutations or deficiencies in cartilage genes result in cartilage abnormalities. Deficiencies or disruptions in genes that encode the cartilage-specific collagens result, in some cases, in premature OA.[10] Knowledge of the composition of the cartilage matrix has permitted the development of methods for identifying molecular markers in serum and synovial fluid that can be used to monitor changes in cartilage metabolism and to assess cartilage damage in OA or RA.[11] Changes in the structural composition of cartilage can markedly affect its biomechanical properties (see Chapter 6).

### Cartilage Collagens

The major component of the collagen network in adult articular cartilage is the triple-helical type II collagen molecule, which is composed of three identical α chains $(\alpha 1[II])_3$. These molecules are assembled in fibrils in a quarter-stagger array that can be observed by electron microscopy.[4,12] These fibrils are thinner than type I collagen–containing fibrils in skin because of the higher numbers of hydroxylysine residues that can form cross-links and the presence of other collagen and noncollagen components in the fibril. Type IIB collagen in articular cartilage is a product of alternative splicing and lacks a 69 amino acid, cysteine-rich domain of the amino-terminal propeptide, which is encoded by exon 2 in the human type II collagen gene *(COL2A1)*.[13] This domain is found in type IIA procollagen, which is expressed by chondroprogenitor cells during development, and in the amino propeptides of other interstitial collagen types, and it may play a feedback-inhibitory role in collagen biosynthesis.[14] The reappearance of type IIA collagen in the midzone pericellular matrix and type X collagen, the hypertrophic chondrocyte marker, in the deep zone of OA cartilage suggests reversion to a developmental phenotype in an attempt to repair the damaged matrix.

Although collagens VI, IX, XI, XII, and XIV are quantitatively minor components, they may have important structural and functional properties. Collagens IX and XI are relatively specific to cartilage, whereas collagens VI, XII, and XIV are widely distributed in other connective tissues.

**TABLE 3-1** Extra-cellular Matrix Components of Cartilage

| Molecule | Structure | Function and Location |
|---|---|---|
| **Collagens** | | |
| Type II | $[\alpha1(II)]_3$; fibril-forming | Tensile strength; major component of collagen fibrils |
| Type IX | [α1(IX)α2(IX)α3(IX)]; single CS or DS chain; α1(II) gene encodes α3(IX); FACIT | Tensile properties, interfibrillar connections; cross-links to surface of collagen fibril, NC4 domain projects into matrix |
| Type XI | [α1(XI)α2(XI)α3(XI)]; fibril-forming | Nucleation/control of fibril formation; within collagen fibril |
| Type VI | [α1(VI)α2(VI)α3(VI)]; microfibrils | Forms microfibrillar network; binds hyaluronan, biglycan, and decorin; pericellular |
| Type X | $[\alpha1(X)]_3$; hexagonal network | Support for endochondral ossification; hypertrophic zone and calcified cartilage |
| Type XII | $[\alpha1(XII)]_3$; FACIT large cruciform NC3 domain | Associated with type I collagen fibrils in perichondrium and articular surface |
| Type XIV | $[\alpha1(XIV)]_3$; FACIT | Associated with type I collagen; superficial zone |
| Type XVI | $[\alpha1(XVI)]_3$; FACIT | Integrates with collagen II/XI fibrils |
| Type XXVII | COL27A1: 156 kb, 61 exons | Fibril-forming; developing cartilage |
| **Proteoglycans** | | |
| Aggrecan | 255 kDa core protein; CS/KS side chains; C-terminal EGF and lectin-like domains | Compressive stiffness through hydration of fixed charge density; binding through G1 domain to HA stabilized by link protein |
| Versican | 265 to 370 kDa core protein; CS/DS side chains; C-terminal EGF, C-type lectin, and CRP-like domains | Low levels in articular cartilage throughout life; calcium-binding and selectin-like properties |
| Perlecan | 400 to 467 kDa core protein; HS/CS side chains; no HA binding | Cell-matrix adhesion; pericellular |
| Biglycan | 38 kDa; LRR core protein with two DS chains (76 kDa) | Binds collagen VI and TGF-β; pericellular |
| Decorin | 36.5 kDa; LRR core protein with one CS or DS side chain (100 kDa) | Controls size/shape of collagen fibrils, binds collagen II and TGF-β; interterritorial |
| Asporin | 40 kDa; LRR core protein; N-terminal extension of 15 aspartate residues | Binds collagen, modulates TGF-β function |
| Fibromodulin | 42 kDa; containing KS chains in central LRR region and N-terminal tyrosine sulfate domains | Same as decorin |
| Lumican | 38 kDa; structure similar to fibromodulin | Same as decorin |
| PRELP | 44 kDa; LRR core protein; proline-rich and arginine-rich N-terminal binding domain for heparin and HS | Mediates cell binding through HS in syndecan |
| Chondroadherin | 45 kDa; LRR core protein without N-terminal extension | Binding to cells via α2β1 integrin |
| **Other Molecules** | | |
| HA (hyaluronan) | 1000 to 3000 kDa | Retention of aggrecan within matrix |
| Link protein | 38.6 kDa | Stabilizes attachment of aggrecan G1 domain to HA |
| COMP | 550 kDa; five 110 kDa subunits; thrombospondin-like | Interterritorial in articular cartilage; stabilizes collagen network or promotes collagen fibril assembly; calcium binding |
| CMP (or matrilin-1); matrilin-3 | Three 50 kDa subunits with vWF and EGF domains | Tightly bound to aggrecan in immature cartilage |
| CILP | 92 kDa; homology with nucleotide pyrophosphohydrolase without active site | Restricted to middle/deep zones of cartilage; increase in early and late OA |
| Glycoprotein (gp)-39, YKL-40, or chitinase 3-like protein 1 (CH3L1) | 39 kDa; chitinase homology | Marker of cartilage turnover; chondrocyte proliferation; superficial zone of cartilage |
| Fibronectin | Dimer of 220 kDa subunits | Cell attachment and binding to collagen and proteoglycans; increased in OA cartilage |
| Tenascin-C | Six 200 kDa subunits forming hexabrachion structure | Binds syndecan-3 during chondrogenesis; angiogenesis |
| SZP, lubricin, or PRG 4 | 225 kDa, 200 nm length | Joint lubrication; superficial zone only |

**TABLE 3-1** Extra-cellular Matrix Components of Cartilage—cont'd

| Molecule | Structure | Function and Location |
|---|---|---|
| **Membrane Proteins** | | |
| CD44 | Integral membrane protein with extra-cellular HS/CS side chains | Cell-matrix interactions; binds HA |
| Syndecan-1, -3, -4 | N-terminal HS attachment site; cytoplasmic tyrosine residues | Syndecan-3 is receptor for tenascin-C during cartilage development; cell-matrix interactions |
| Annexin V (anchorin CII) | 34 kDa; homology to calcium-binding proteins calpactin and lipocortin | Cell surface attachment to type II collagen; calcium binding |
| Integrins (α1, α2, α3, α5, α6, α10; β1, β3, β5) | Two noncovalently linked transmembrane glycoproteins (α and β subunits) | Cell-matrix binding: α1β1/collagen I or VI, α2β1 or α3β1/collagen II, α5β1/fibronectin; intra-cellular signaling |
| Discoidin domain receptor 2 | Receptor tyrosine kinase | Binds native type II collagen fibrils; Ras/ERK signaling |
| TRPV4 | $Ca^{2+}$ channel | Mechanosensor |
| Connexin 43 | ATP release channel | Mechanosensor; primary cilia |

ATP, Adenosine triphosphate; CILP, cartilage intermediate layer protein; CMP, cartilage matrix protein; COMP, cartilage oligomeric matrix protein; CRP, complement regulatory protein; CS, chondroitin sulfate; DS, dermatan sulfate; EGF, epidermal growth factor; FACIT, fibril-associated collagens with interrupted triple helices; HA, hyaluronic acid; HS, heparan sulfate; KS, keratan sulfate; LRR, leucine-rich repeat; NC, noncollagen; OA, osteoarthritis; PRELP, proline-rich and arginine-rich end leucine-rich repeat protein; PRG, proteoglycan; SZP, superficial zone protein; TGF-β, transforming growth factor-β; TRPV4, transient receptor potential vanilloid 4; vWF, von Willebrand factor.

Collagen VI is present as microfibrils in the pericellular matrix; it may play a role in cell attachment and interacts with other matrix proteins, such as hyaluronan, perlecan, biglycan, monomers or small aggregates of aggrecan, and type IX collagen, which are located there exclusively or at higher amounts than in the interterritorial matrix.[15] Small amounts of collagen III are present in cartilage, and collagens VI and III may increase in OA cartilage.[3]

Type IX collagen is a proteoglycan and a collagen because it contains a chondroitin sulfate chain attachment site in one of the noncollagen domains. The helical domains of the type IX collagen molecule form covalent cross-links with type II collagen telopeptides and are attached to the fibrillar surface, as observed by the electron microscope. Type IX collagen may function as a structural intermediate between type II collagen fibrils and the proteoglycan aggregates, serving to enhance the mechanical stability of the fibril network and resist the swelling pressure of the trapped proteoglycans. Destruction of type IX collagen accelerates cartilage degradation and loss of function.

The α3 chain of type XI collagen has the same primary sequence as the α1(II) chain, and the heterotrimeric type XI collagen molecule is buried in the same fibril as type II collagen. Type XI collagen may have a role in regulating fibril diameter. The more recently discovered nonfibrillar fibril-associated collagens with interrupted triple helices (FACIT), XII and XIV, which are structurally related to type IX collagen, do not form fibrils by themselves but co-aggregate with fibril-forming collagens and modulate the packing of collagen fibers through domains projecting from their surfaces.

## Cartilage Proteoglycans

The major proteoglycan in articular cartilage is the large aggregating proteoglycan, or aggrecan, which consists of a core protein of 225 to 250 kDa with covalently attached side chains of glycosaminoglycans, including approximately 100 chondroitin sulfate chains, 30 keratan sulfate chains, and shorter *N*-linked and *O*-linked oligosaccharides.[3] Link protein, a small glycoprotein, stabilizes the noncovalent linkage between aggrecan and hyaluronic acid (also called *hyaluronan*) to form the proteoglycan aggregate that may contain 100 aggrecan monomers. The G1 and G2 N-terminal globular domains of aggrecan and its C-terminal G3 domain have distinct structural properties that function as integral parts of the aggrecan core protein and contribute cleavage products that accumulate with age or in OA. The G2 domain is separated from G1 by a linear interglobular domain and has two proteoglycan tandem repeats. The G3 domain contains sequence homologies to epidermal growth factor, lectin, and complement regulatory protein, and it participates in growth regulation, cell recognition, intra-cellular trafficking, and the recognition, assembly, and stabilization of the ECM. About half of the aggrecan molecules in adult cartilage lack the G3 domain, probably as a result of proteolytic cleavage during matrix turnover. Small quantities of other large proteoglycans are found in cartilage, including versican, which forms aggregates with hyaluronic acid, and perlecan, which is nonaggregating; however, these proteoglycans function primarily during skeletal development, when versican is expressed in prechondrogenic condensations and perlecan is expressed in the cartilage anlagen after expression of type II collagen and aggrecan.[16,17]

The nonaggregating small proteoglycans are not specific to cartilage, but in cartilage they serve specific roles in matrix structure and function, primarily by modulating collagen-fibril formation.[3,18] Of the more than 10 leucine-rich repeat (LRR) proteoglycans discovered thus far, only osteoadherin is not present in cartilage. The 24 amino acid central LRR domain is conserved, but the N-terminal and C-terminal domains have patterns of cysteine residues involved in intrachain disulfide bonds that distinguish the four subfamilies: (1) biglycan, decorin, fibromodulin, and lumican; (2) keratocan and proline and arginine-rich end

leucine-rich repeat protein (PRELP); (3) chondroadherin; and (4) epiphycan/PG-Lb and mimecan/osteoglycin. Biglycan may have one or two glycosaminoglycan chains—chondroitin sulfate and/or dermatan sulfate—attached near the N-terminus through two closely spaced serine-glycine dipeptides. Decorin contains only one chondroitin sulfate or dermatan sulfate chain. Fibromodulin and lumican contain keratan sulfate chains linked to the central domain of the core protein and several sulfated tyrosine residues in the N-terminus. Negatively charged glycosaminoglycan side chains contribute to the fixed charge density of the matrix and, together with the highly anionic tyrosine-sulfation sites, permit multiple-site linkage between adjacent collagen fibrils, stabilizing the network. Decorin, the most extensively studied LRR proteoglycan, binds to collagens II, VI, XII, and XIV and to fibronectin and thrombospondin. Biglycan, decorin, and fibromodulin bind transforming growth factor (TGF)-β and the epidermal growth factor receptor and may modulate growth, remodeling, and repair. PRELP and chondroadherin may regulate cell-matrix interactions through binding to syndecan and α2β1 integrin.

### Other Extra-cellular Matrix and Cell Surface Proteins

Several other noncollagenous matrix proteins may play important roles in determining cartilage matrix integrity.[19] Cartilage oligomeric matrix protein (COMP), a member of the thrombospondin family, is a disulfide-bonded, pentameric, 550 kDa, calcium-binding protein that constitutes approximately 10% of the noncollagenous, nonproteoglycan protein in normal adult cartilage. COMP is located in the interterritorial matrix of adult articular cartilage, where it interacts with the COL3 and NC4 domains of type IX collagen that protrude from the fibril, stabilizing the collagen network. COMP is pericellular in the proliferating region of the growth plate, where it may have a role in cell-matrix interactions.[20] The cartilage matrix protein (or matrilin-1) and matrilin-3 are expressed in cartilage at certain stages of development. Matrilin-1 is present in the pericellular matrix of adult articular cartilage and matrilin-1, -2, and -3 may be upregulated in articular cartilage in the presence of OA.[21]

Tenascin-C, a glycoprotein that is regulated in development, is characteristic of nonossifying cartilage. Similar to fibronectin, alternative splicing of tenascin-C messenger RNA (mRNA) gives rise to different protein products at different stages of chondrocyte differentiation. Both proteins are increased in OA cartilage and may serve specific functions in remodeling and repair.[22] The cartilage intermediate-layer protein (CILP) is expressed by chondrocytes in the middle to deep zones of articular cartilage as a precursor protein. When cleaved during secretion, CILP has structural similarities with nucleotide pyrophosphohydrolase, although it lacks the catalytic site, and it may play a role in pyrophosphate metabolism and calcification.[23] Asporin is related to decorin and biglycan and, similar to those other LRR proteins, may interact with and sequester growth factors such as TGF-β.[24] YKL-40/HC-gp39, also known as *chitinase 3–like protein 1*, is found only in the superficial zone of normal cartilage and stimulates proliferation of chondrocytes and synovial cells.[25] Chitinase 3–like protein 1 is induced by inflammatory cytokines and may function as a feedback regulator because it inhibits cytokine-induced cellular responses. Synthesis or release of these proteins or fragments is often increased in cartilage that is undergoing repair or remodeling, and they have been investigated as markers of cartilage damage in arthritis.

## STRUCTURE, CLASSIFICATION, AND NORMAL FUNCTION OF CHONDROCYTES

### Structure

The characteristic feature of the chondrocyte embedded in cartilage matrix is its rounded or polygonal structure. The exception occurs at tissue boundaries, such as the articular surfaces of joints, where chondrocytes may be flattened or discoid. Intra-cellular features, including a rough endoplasmic reticulum, a juxtanuclear Golgi apparatus, and deposition of glycogen, are characteristic of a synthetically active cell. The cell density of full-thickness, human, adult, femoral condyle cartilage is maintained at 14.5 (±3.0) × $10^3$ cells/$mm^2$ from age 20 to 30 years. Although senescence of chondrocytes occurs with aging, mitotic figures are not observed in normal adult articular cartilage.

The structure, density, and synthetic activity of an adult chondrocyte vary according to its position within the different zones of articular cartilage.[4] In the region of highest cell density, the superficial zone, cells are flattened and are oriented parallel to the surface, along with the collagen fibers. Chondrocytes within the middle zone appear larger and more rounded and display a random distribution within the matrix, where the collagen fibers also are more randomly arranged. Chondrocytes in the deeper zones form columns that, along with the collagen fibers, are oriented perpendicular to the cartilage surface. Chondrocytes may exhibit different behaviors depending on their position within the different layers, and these zonal differences in synthetic properties may persist in primary chondrocyte cultures. The primary cilia are important for spatial orientation of cells in developing growth plate and are sensory organelles in chondrocytes. Primary cilia are centers for wingless type (Wnt) and hedgehog signaling and contain mechanosensitive receptors, including the transient receptor potential vanilloid 4 (TRPV4), a $Ca^{2+}$ channel, and connexin 43, an adenosine triphosphate (ATP) release channel.[26,27]

### Classification: Cell Origin and Differentiation

Chondrocytes arise in the embryo from mesenchymal origin during *chondrogenesis*, which is the earliest phase of skeletal development involving mesenchymal cell recruitment, migration, and condensation and differentiation of mesenchymal chondroprogenitor cells.[28,29] As described in detail in Chapter 1, chondrogenesis results in the formation of cartilage anlagen, or templates, at sites where skeletal elements form. This process is controlled by cell-cell and cell-matrix interactions and by growth and differentiation factors that initiate or suppress cellular signaling pathways and transcription of specific genes in a temporospatial manner (Figure 3-3).[30]

Vertebrate limb development is controlled by interacting patterning systems involving fibroblast growth factor (FGF),

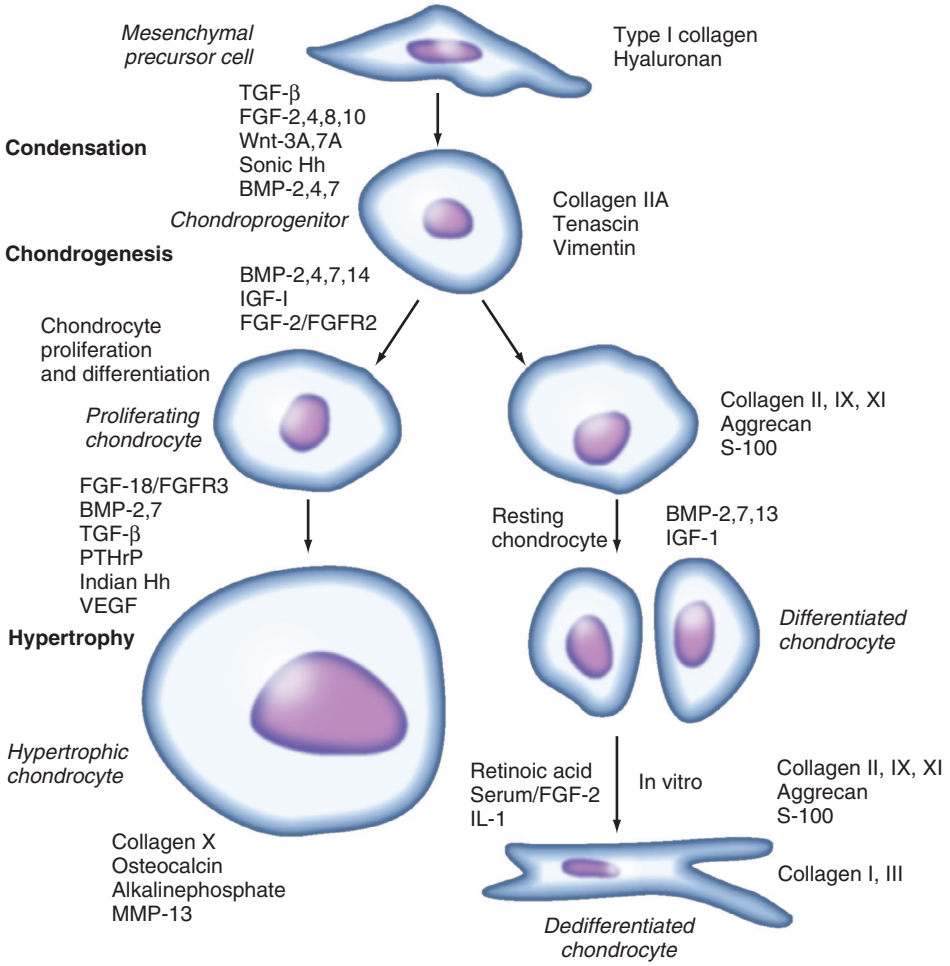



**Figure 3-3** Schematic representation of cellular phenotypes associated with developmental fates during condensation, chondrogenesis, chondrocyte proliferation, differentiation, and hypertrophy. Some of the regulatory factors active at different stages are listed to the left of the arrows. The major extra-cellular matrix genes are listed to the right of each cell type in which they are differentially expressed. BMP, Bone morphogenetic protein; FGF, fibroblast growth factor; Hh, hedgehog; IGF, insulin-like growth factor; IL-1, interleukin-1; MMP, matrix metalloproteinase; PTHrP, parathyroid hormone–related protein; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; Wnt, wingless type.

hedgehog, bone morphogenetic protein (BMP), TGF-β, and Wnt pathways.[31-34] Wnt signaling, via the canonical β-catenin pathway and activation of T cell factor (TCF)/Lef transcription factors, functions in a cell-autonomous manner to induce osteoblast differentiation and suppress chondrocyte differentiation in early chondroprogenitors. During chondrogenesis, Wnt/β-catenin acts at two stages: at low levels to promote chondroprogenitor differentiation, and later at high levels to promote chondrocyte hypertrophic differentiation and subsequent endochondral ossification. The transcription factor, Sry-type high-mobility group box 9 (Sox9), is an early marker of the differentiating chondrocyte that is required for the onset of expression of type II collagen, aggrecan, and other cartilage-specific matrix proteins, such as type IX collagen.[32] Two other members of the SOX family, L-Sox5 and Sox6, are not present in early mesenchymal condensations but are required during overt chondrocyte differentiation, forming heterodimers that induce transcription more efficiently than Sox9 by itself. Expression of SOX proteins depends on signaling through mammalian homologs of *Drosophila* mothers against decapentaplegic (SMADs), which are functionally redundant and active in differentiating chondrocytes. Other transcription factors such as Gata4/5/6 and Nkx3.2 may interact directly or indirectly with Sox9 to upregulate the expression of *COL2A1* aggrecan (*ACAN*) and other cartilage-specific genes at early stages of chondrogenesis.[35]

In the embryonic or postnatal epiphyseal growth plates, molecules that promote matrix remodeling and angiogenesis facilitate endochondral ossification, whereby bone replaces the calcified cartilaginous matrix in the hypertrophic zone (see Chapter 1). Differentiated chondrocytes that remain in the reserve, or resting, zone become the cartilage elements in articular joints, or they can proliferate and undergo the complex process of terminal differentiation to hypertrophy marked by type X collagen. Indian hedgehog and parathyroid hormone–related protein (PTHrP) transiently induce proliferation and repress differentiation, determining the number of cells that enter the hypertrophic maturation pathway. The Runt domain transcription factor, Runx2 (also known as *core binding factor* or Cbfa1), serves as a positive regulatory factor in chondrocyte maturation to the hypertrophic phenotype and subsequent osteogenesis.[36] Runx2 is expressed in the adjacent perichondrium and in

prehypertrophic chondrocytes, but less in late hypertrophic chondrocytes, and is required for expression of type X collagen and other markers of terminal differentiation.[28,35]

Numerous other transcription factors positively or negatively regulate chondrocyte terminal differentiation by controlling, in part, the expression or activity of Runx2.[35,36] BMP-induced Smad1 and interactions between Smad1 and Runx2 are required for the induction of chondrocyte hypertrophy. Histone deacetylase 4 (HDAC4), which is expressed later in prehypertrophic chondrocytes, prevents premature chondrocyte hypertrophy by interacting with Runx2 and inhibiting its activity.[37] The hypoxia-inducible factor (HIF)-1α contributes to chondrocyte survival during hypertrophic differentiation, partially because of its regulation of vascular endothelial cell growth factor (VEGF) expression, and it prevents premature hypertrophy by directly suppressing Runx2 activity. The transcription factors involved in regulating gene expression during late-stage hypertrophy, in addition to Runx2, include Runx3, myocyte enhancer factor (MEF) 2C and 2D, Foxa2 and FoxA3, and Zfp521.[28,35]

One major function of the chondrocyte is growth of the skeleton through increased cell proliferation, production of ECM, and cell volume through hypertrophy. Lineage tracing studies indicate that Sox9-expressing cells are precursors for both articular and growth plate chondrocytes. Furthermore, cells expressing *Tgfbr2*, the gene encoding the TGF-β receptor II in the interzone, can be traced to synovial lining, superficial meniscus, and ligaments and may persist as reserve progenitor cells that could later participate in regeneration. Finally, Gdf5, matrilin-1, and PTHrP appear to specify the eventual border between the articular cartilage surface and the joint space.[29] After cessation of growth, the resting chondrocyte remains as part of the supporting structures in articular, tracheal, and nasal cartilages, indicating that the fate of a chondrocyte depends on origin and location.[31]

## Normal Function of the Adult Articular Chondrocyte

The mature articular chondrocyte embedded in its ECM is a resting cell with no detectable mitotic activity and a low rate of synthetic activity. Because articular cartilage is not vascularized, the chondrocyte must rely on diffusion from the articular surface or subchondral bone for exchange of nutrients and metabolites. Chondrocytes maintain active membrane transport systems for exchange of cations, including $Na^+$, $K^+$, $Ca^{2+}$, and $H^+$, whose intra-cellular concentrations fluctuate with load and changes in the composition of the cartilage matrix. The chondrocyte cytoskeleton is composed of actin, tubulin, and vimentin filaments, and the composition of these filament systems varies in the different cartilage zones.

Chondrocyte metabolism operates at low oxygen tension within the cartilage matrix, ranging from 10% at the surface to less than 1% in the deep zone. The consumption of oxygen by cartilage on a per-cell basis is only 2% to 5% of that in the liver or kidney, although the amounts of lactate produced are comparable. Chondrocytes do not normally contain abundant mitochondria, energy metabolism depends strongly on the glucose supply, and the energy requirements may be modulated by mechanical stress. Glucose serves as the major energy source for chondrocytes and as an essential precursor for glycosaminoglycan synthesis. Facilitated glucose transport in chondrocytes is mediated by several distinct glucose transporter proteins (GLUTs) that may be constitutively expressed (GLUT3 and GLUT8) or induced by cytokines (GLUT1 and GLUT6). Proteomic studies of chondrocytes have identified intra-cellular proteins known to be involved in cell organization, energy protein fate, metabolism, and cell stress. The relative expression of these proteins may determine the capacity of chondrocytes to survive in cartilage matrix and to modulate metabolic activity in response to environmental changes.

When cultured in a range of oxygen tensions between severe hypoxia (0.1% oxygen) and normoxia (21% oxygen), chondrocytes adapt to low oxygen tension by upregulating HIF-1α. Hypoxia via HIF-1α can stimulate chondrocytes to express GLUTs, angiogenic factors such as VEGF, and numerous genes associated with cartilage anabolism and chondrocyte differentiation.[38] In the growth plate, hypoxia and HIF-1α are associated with type II collagen production. HIF-1α is expressed in normal and OA articular cartilage, where it maintains tonic activity during physiologic hypoxia in the deeper layers associated with increased proteoglycan synthesis. In contrast to other tissues, however, HIF-1α is not completely degraded in cartilage, when normoxic conditions are applied. Long-term systemic hypoxia (13%) may downregulate collagen and aggrecan gene expression in articular cartilage, whereas hyperoxia (55% oxygen) may increase the breakdown of cartilage collagens in articular cartilage in the presence of vascularized rheumatoid synovium. By modulating the intra-cellular expression of survival factors such as HIF-1α, chondrocytes have a high capacity to survive in the avascular cartilage matrix and to respond to environmental changes.[38] Recent studies indicate that another hypoxia-inducible factor, HIF-2α, can become activated by pro-inflammatory cytokines and stress in the avascular, hypoxic environment of articular cartilage.[39,40]

The chondrocyte maintains a steady-state metabolism secondary to equilibrium between anabolic processes and catabolic processes that results in the normal turnover of matrix molecules. In normal adult articular cartilage, the turnover of matrix components is low. The turnover of collagen has been estimated to occur with a half-life greater than 100 years. In contrast, glycosaminoglycan constituents on the aggrecan core protein are more readily replaced; the half-life of aggrecan subfractions has been estimated to range from 3 months to 24 years. Other cartilage ECM components including biglycan, decorin, COMP, tenascins, and matrilins that were incorporated previously into the matrix during development also may be synthesized by chondrocytes under low-turnover conditions. Regional differences in the remodeling activities of chondrocytes have been noted, however, and matrix turnover may be more rapid in the immediate pericellular zones. The metabolic potential of these cells is indicated by their capacity to proliferate in culture and to synthesize matrix proteins after enzymatic release from the cartilage even of elderly persons.

The complex composition of the articular cartilage matrix is more difficult for the chondrocyte to replicate if

severe damage to the collagen network occurs. During initial stages of OA, chondrocytes in vivo respond to structural changes in the surrounding cartilage matrix by increasing cell proliferation and synthesis of matrix proteins, proteinases, and anabolic and catabolic factors. The aberrant behavior of OA chondrocytes is reflected in the appearance of fibrillations, matrix depletion, cell clusters, and changes in quantity, distribution, or composition of matrix proteins. Evidence of phenotypic modulation is reflected in increases in collagens I and III and the appearance of the hypertrophic chondrocyte marker, type X collagen, and other markers of terminal differentiation, suggesting recapitulation of a developmental program.[2,41]

Genomic and proteomic analyses of global gene expression in cartilage have revealed increased anabolism reflected in increased levels of *COL2A1* and other cartilage-specific mRNAs in late OA, but also noncartilage collagens and other matrix proteins. Nevertheless, the capacity of the adult articular chondrocyte to regenerate the normal cartilage matrix architecture is limited, and the damage becomes irreversible if the destructive process is not interrupted.

## CULTURE MODELS FOR STUDYING CHONDROCYTE METABOLISM

Primary cultures of articular chondrocytes have served as useful models for studying the mechanisms controlling responses to growth factors and cytokines.[42,43] A challenge to researchers in cartilage biology is the maintenance of chondrocyte structure and cartilage-specific gene expression during in vitro studies. In confluent primary monolayer cultures, chondrocytes maintain a rounded, polygonal structure (Figure 3-4), but progressive loss of cartilage phenotype is seen with the passage of time and after subculture. High-density cultures maintain the gene expression and synthesis of cartilage-specific matrix proteins until they are subcultured, although gene expression of type II collagen is generally more labile than that of aggrecan. During this loss of phenotype or dedifferentiation, chondrocytes lose their rounded, polygonal structure and express some, but not all, characteristics of the fibroblast phenotype, such as type I collagen. It is possible to expand cultures through a limited number of subcultures and to "redifferentiate" the cells in three-dimensional culture systems, in which chondrocytes regain structure; cessation of proliferation is associated with increased expression of cartilage-specific matrix proteins. Alternatively, explant cultures of articular cartilage in which chondrocytes remain encased within their own ECM have been used as in vitro models to study cartilage biochemistry and metabolism, as described in the next section.

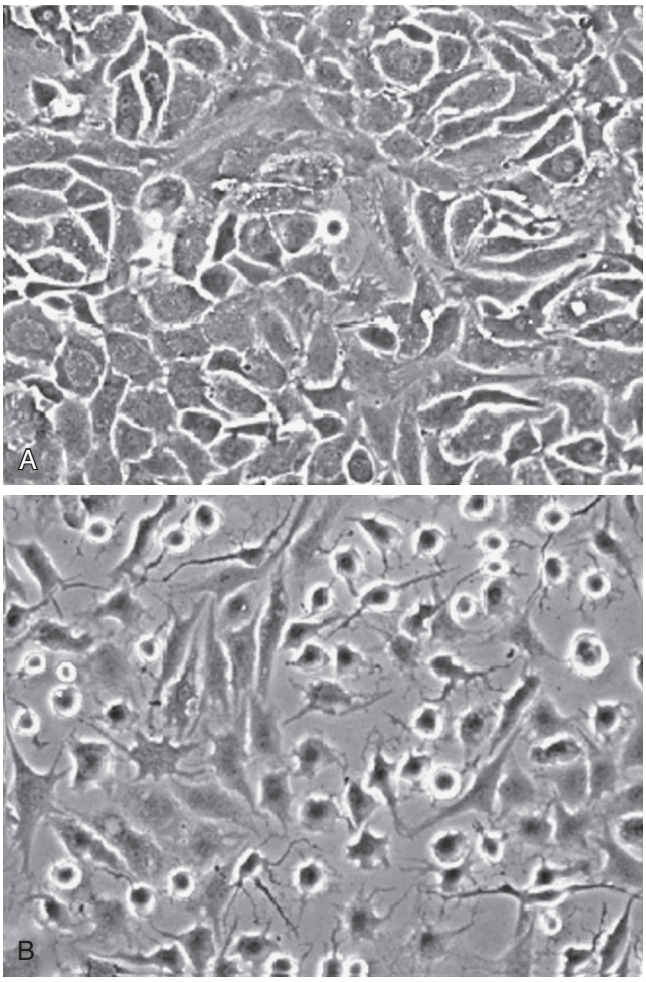



**Figure 3-4** Structure of human articular chondrocytes grown in monolayer culture on plastic. Chondrocytes were isolated from articular cartilage and cultured in growth medium containing 10% fetal calf serum until confluent. The cultures were changed to serum-free defined medium, IL-1β was added the next day, and incubation was continued for 24 hours. **A,** Untreated chondrocytes display the characteristic cobblestone structure. **B,** IL-1β–treated cultures respond with a dramatic morphologic change.

### Articular Chondrocytes

#### Cartilage Explant (Organ) Cultures

Based on the pioneering work of Dame Honor Fell, who showed that it was possible to maintain pieces of cartilage in culture, the explant culture system was developed to characterize chondrocyte function in cartilage from various species, including humans, at different ages. Early work in bovine cartilage established the mechanisms of biosynthesis of cartilage proteoglycans under the influence of different serum concentrations and determined the turnover rate whereby the chondrocyte could maintain the balance between anabolic and catabolic pathways. Methods developed for measuring the proteoglycan content in cartilage, such as incorporation of sulfate $^{35}S$ into newly synthesized proteoglycans or spectrophotometric quantitation of Alcian blue staining, have been used widely as standard assays for assessing cartilage metabolism. Cartilage organ cultures also maintain constant levels of type II collagen during several weeks of culture, along with the characteristic structure and banding pattern of collagen fibrils. These cultures have been useful for studying the regulation of cartilage matrix synthesis and degradation by proteinases, inflammatory cytokines, retinoic acid, and anabolic growth factors.[44]

#### Monolayer Cultures

Primary monolayer cultures of chondrocytes isolated from young animals that maintain the cartilage-specific phenotype at least throughout primary culture are easily obtained

and have been used widely to assess differentiated chondrocyte functions. When chondrocytes are isolated from their matrix and cultured in monolayer, they adhere to the culture dish and readily respond to serum growth factors that stimulate proliferation of normally quiescent cells. Freshly isolated human articular or costal chondrocytes express cartilage-specific type II collagen and continue to do so for several days to weeks in primary monolayer culture. In addition to cartilage-specific collagens and aggrecan, chondromodulin and protein S-100 are useful markers expressed in primary chondrocyte cultures. Identification of cell surface markers that determine chondrogenic capacity may enable the enrichment of subpopulations for further characterization.

Early attempts to culture chondrocytes from various animal and human sources were frustrated by the tendency of these cells to acquire a fibroblast-like structure associated with the appearance of type I collagen synthesis. When plated at high density, the cells maintain a polygonal, although flattened, structure. At low plating densities, with prolonged culture, and upon expansion in serial subculture, the cells gradually assume a more elongated, "fibroblast-like" structure. Early work suggested that this change in structure is associated with loss of phenotype, whereby the synthesis of cartilage-specific matrix molecules, such as type II collagen and aggrecan, decreases or disappears. This "dedifferentiated phenotype," which has been described thus far only in vitro, is marked by the appearance of synthesis of type I and type III collagens, and it can be accelerated by plating the cells at low densities or by treating with cytokines such as interleukin (IL)-1 or retinoic acid. The substrate on which chondrocytes are plated can influence the differentiation capacity of articular chondrocytes.[45] Culture expansion in medium with low concentration of glucose can also preserve the chondrocyte phenotype in monolayer and promote the capacity of chondrocytes to form a cartilage-like matrix in three-dimensional culture.[43]

The use of chondrocytes of adult human origin in studies related to the pathogenesis of joint disease has been problematic because the source of the cartilage cannot be controlled, sufficient numbers of cells are not readily obtained through random operative procedures, and the phenotypic stability of adult human chondrocytes is lost more quickly upon expansion in serial monolayer cultures than in cells of juvenile human or embryonic or postnatal animal origin. Serum-free defined media of varying compositions, but usually including insulin, have been used, frequently in combination with monolayer and other culture systems, as mentioned in the next section.

### Three-Dimensional Culture Systems

Early studies showed that the phenotype can be maintained if isolated chondrocytes are placed in suspension cultures in spinner flasks or in dishes coated with nonadherent substrates. Freshly isolated or subcultured chondrocytes also can be embedded in three-dimensional matrices, such as collagen gels or sponges, agarose, or alginate.[42] In these three-dimensional matrices, chondrocytes have a normal spherical shape, synthesize and secrete abundant cell-associated ECM components, and may maintain phenotypic stability for several months. Because articular chondrocytes are unable to proliferate in suspension or three-dimensional culture, expansion in monolayer culture followed by transfer to alginate culture, for example, has been used as a strategy to obtain sufficient numbers of differentiated chondrocytes for study. After prolonged culture in monolayer, however, the chondrogenic potential of dedifferentiated chondrocytes may irreversibly be lost.

The high-density pellet culture system, which was originally developed to study growth plate hypertrophy, has been used as a three-dimensional model because it permits articular chondrocytes to deposit a well-organized ECM that contains type II collagen and aggrecan.[45,46] These pellet cultures show *phenotypic plasticity*, comparable with that of mesenchymal stem cells undergoing chondrogenesis, by recapitulating aspects of chondrocyte hypertrophy.[43,46] Isolated chondrons containing one or more chondrocytes within a capsule of pericellular matrix also have been used for in vitro studies of chondrocyte metabolism within a three-dimensional environment.[47]

## INTERACTIONS OF CHONDROCYTES WITH THE EXTRA-CELLULAR MATRIX

Chondrocytes in vivo respond to structural changes in the cartilage ECM. The ECM not only provides a framework for chondrocytes suspended within it, but its constituents interact with cell surface receptors and provide signals that regulate many chondrocyte functions (Figure 3-5).[48]

### Integrins

The most prominent of the ECM receptors are the integrins, which bind specifically to different cartilage matrix components and induce the formation of intra-cellular signaling complexes that regulate cell proliferation, differentiation, survival, and matrix remodeling. Integrins also may serve as mechanoreceptors that mediate responses to normal and abnormal loading of cartilage.[49] Chondrocytes express many different integrins that interact with cartilage ECM ligands, although most are not specific to this cell type. They include integrins that are receptors for collagen ($\alpha1\beta1$, $\alpha2\alpha1$, $\alpha3\alpha1$, and $\alpha10\alpha1$), fibronectin ($\alpha5\alpha1$, $\alpha v\alpha3$, and $\alpha v\alpha5$), and laminin ($\alpha6\alpha1$). The integrin $\alpha1\alpha1$ has broader ligand specificity than the other collagen-binding integrins and mediates chondrocyte adhesion to pericellular type VI collagen and to the cartilage matrix protein, matrilin-1. The $\alpha2\alpha1$ integrin also binds to chondroadherin. The $\alpha v$-containing integrins bind to vitronectin and osteopontin, in addition to serving as alternative fibronectin receptors. The $\alpha5\alpha1$ and $\alpha v\alpha3$ integrins serve as receptors for different conformations of COMP.

Because $\alpha1\alpha1$, $\alpha2\alpha1$, and $\alpha10\alpha1$ are receptors for cartilage-specific type II collagen, there is great interest in determining whether they mediate differential responses of chondrocytes to changes in the ECM resulting from normal loading or pathologic changes. The $\alpha5\alpha1$ integrin is the prominent integrin in human adult articular cartilage. Depending on the method of analysis, adult chondrocytes also express $\alpha1\alpha1$ and $\alpha v\alpha5$ integrins accompanied by weaker expression of $\alpha3\alpha1$ and $\alpha v\alpha3$. Normal adult articular chondrocytes express little or no $\alpha2\alpha1$, whereas expression of $\alpha2\alpha1$ and $\alpha3\alpha1$ integrins is associated with a

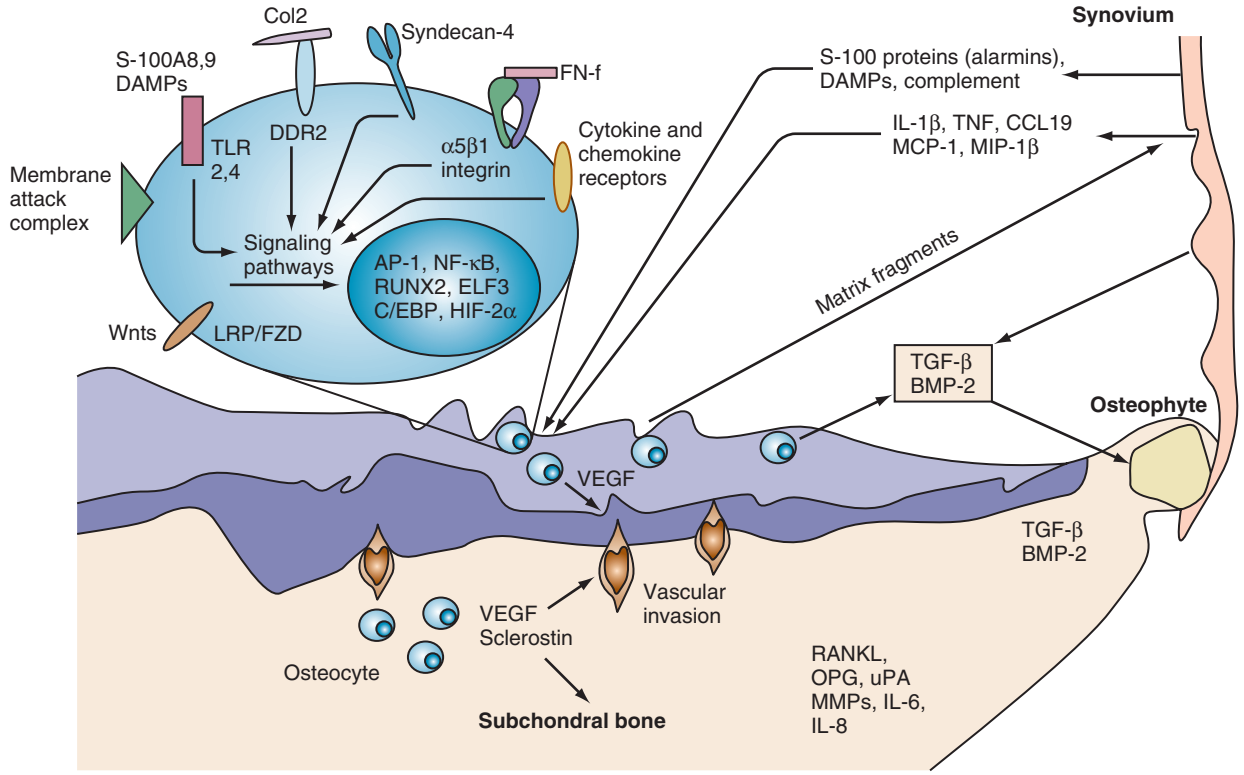



**Figure 3-5** Selected factors involved in the osteoarthritic process in the synovium, cartilage, and bone. Proteins including S-100 proteins (alarmins) and damage-associated molecular pattern molecules (DAMPs), cytokines (interleukin [IL]-1β, tumor necrosis factor [TNF], and IL-15), chemokines (C-C motif ligand19 [CCL19], monocyte chemotactic protein-1 [MCP-1], and macrophage inhibitory protein [MIP-1β]), and complement components released from the synovium can stimulate articular chondrocytes through activation of various cell surface receptors, including Toll-like receptors (TLRs) and cytokine and chemokine receptors, or by formation of the complement membrane attack complex. Other factors that activate cartilage matrix destruction include binding of native type II collagen to discoidin domain receptor 2 (DDR2); fibronectin fragments to the α5β1 integrin; wingless type (Wnt) proteins to LRP/frizzled (FZD) complexes; and extra-cellular ligands to the heparin sulfate proteoglycan syndecan-4. Syndecan-4 may also act by targeting A Disintegrin and Metalloproteinase with Thrombospondin Motifs-5 (ADAMTS-5) to the cell surface. Various signaling pathways lead to activation of a set of transcription factors that translocate to the nucleus and regulate expression of matrix-degrading enzymes and inflammatory mediators. Matrix fragments released from the cartilage can stimulate further synovitis. Production of vascular endothelial growth factor (VEGF) in cartilage and bone stimulates vascular invasion from subchondral bone into the zone of calcified cartilage. VEGF, sclerostin, receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL), osteoprotegerin (OPG), urokinase-type plasminogen activator (uPA), matrix metalloproteinase (MMP)s, IL-6, and IL-8 mediate bone remodeling and potentially diffuse to the cartilage to also promote cartilage matrix destruction. Transforming growth factor (TGF)-β and bone morphogenic protein (BMP)-2 produced in the synovium, cartilage, and bone stimulate osteophyte formation. AP-1, Activator protein 1; C/EBP, CCAAT enhancer binding protein; HIF, hypoxia-inducible factor. *(Modified from Loeser RF, Goldring SR, Scanzello CR, Goldring MB: Osteoarthritis: a disease of the joint as an organ.* Arthritis Rheum *64:1697–1707, 2012.)*

proliferative phenotype, as in fetal chondrocytes and in chondrosarcoma and chondrocyte cell lines. In growth plate chondrocytes, α5α1, αvα5, and α10α1 are important for joint formation, chondrocyte proliferation, hypertrophy, and survival. Knockout of α1 integrin results in severe growth plate abnormalities and chondrodysplasias, whereas α1 integrin knockout mice develop OA without growth plate abnormalities. Importantly, α10α1 is the critical collagen receptor in skeletal development.[50]

Cellular binding to immobilized ECM proteins or integrin receptor aggregation with activating antibodies can promote numerous intra-cellular signaling events.[49] As in other cell types, integrin signaling is mediated by interaction with intra-cellular protein tyrosine kinases, such as pp125 focal adhesion kinase (FAK) and pyk2Pyk2, which interact with the integrin cytoplasmic tail and induce a conformational change in the receptor subunits. Changes in organization of the cytoskeleton are associated with the formation of integrin signaling complexes, which contain scaffolding proteins such as talin, paxillin, and α-actinin, in addition to FAK and the integrin-linked kinase (ILK). Mice lacking ILK in cartilage display chondrodysplasia, a phenotype similar to that of cartilage-specific β1 integrin knockout mice.

Cooperative signaling among integrins and growth factors is a fundamental mechanism in the regulation of cellular functions.[49] Integrin aggregation and receptor occupancy enhance phosphorylation of growth factor receptors and activation of mitogen-activated protein kinases (MAPKs), notably extra-cellular signal-regulated kinase (ERK)-1 or ERK-2.[51] Integrins mediate the effects of mechanical forces through activation of cell signaling, a process termed *mechanotransduction*.[52]

Normal chondrocytes use α5β1 as a mechanoreceptor. As the primary fibronectin receptor, α5β1 plays a role in cartilage degradation by binding to fibronectin fragments that upregulate matrix metalloproteinases (MMPs), such as MMP-3 and MMP-13. Chondrocyte adhesion to

fibronectin or binding to fibronectin fragments also increases the production of cytokines and other catabolic or inflammatory mediators through a mechanism requiring reactive oxygen species (ROS). The specificity of these responses depends on the relative expression of α-integrin subunits on the chondrocyte cell surface.

### Other Cell Surface Receptors in Chondrocytes

Other integral membrane proteins found in chondrocytes include cell determinant 44 (CD44), annexins, syndecans, and discoidin domain receptor 2 (DDR2). CD44, a receptor for hyaluronan, binds collagen and fibronectin. Through specific interactions with hyaluronan, CD44 has a role in the assembly, organization, and maintenance of the chondrocyte pericellular matrix. CD44 expression is upregulated in chondrocytes in articular cartilage from patients with RA and in experimental OA. Chondroprotective agents and anabolic factors may ameliorate cartilage matrix destruction by reducing CD44 fragmentation that disrupts hyaluronan-CD44 interactions and has adverse effects on the pericellular matrix.[53,54]

Annexin V, also known as *annexin CII*, is a 34 kDa integral membrane protein that binds type II collagen and shares extensive homology with the calcium-binding proteins calpactin and lipocortin. Annexins II, V, and VI have been detected in chondrocytes, where they likely play roles in physiologic mineralization of skeletal tissues and in pathologic mineralization of articular cartilage. Annexin V was first detected in chick cartilage and was described as a type II collagen–binding protein that anchors chondrocytes to the ECM. In growth plate chondrocytes, annexins are required for calcium ion uptake and subsequent mineralization.[55] Annexin A6 is highly expressed in OA cartilage and plays a role in catabolic signaling.[56]

Syndecans have important roles during cartilage development and homeostasis. Syndecans link to the cell surface via glycosyl phosphatidylinositol and bind growth factors, proteinases and inhibitors, and matrix molecules through heparan sulfate side chains on the extra-cellular domain.[57] Syndecan-1, syndecan-3, and syndecan-4 are upregulated in OA cartilage. Syndecan-4 is a positive effector of aggrecan activity through controlling the synthesis of the stromelysin, MMP-3.

In contrast to integrins, which bind collagen fragments, DDR2 binds specifically to type II and X collagen fibrils, leading to activation of its integral receptor tyrosine kinase. DDR2 is upregulated in OA cartilage and induces specifically the expression of MMP-13 associated with cleavage of type II collagen. The serine proteinase, high temperature requirement A1 (HTRA1), which is induced by TGF-β and increased in OA articular cartilage, is responsible for disrupting the pericellular matrix, thereby exposing DDR2 to activation by fibrillar type II collagen.[58,59]

## ANGIOGENIC AND ANTIANGIOGENIC FACTORS

Adult articular cartilage is among the few avascular tissues in mammalian organisms; this property and the presence of angiogenesis inhibitors make it resistant to vascular angiogenesis and invasion by inflammatory and neoplastic cells. Troponin I, MMP inhibitors, chondromodulin-I, and endostatin, a 20 kDa proteolytic fragment of type XVIII collagen, all function as endogenous angiogenic inhibitors in cartilage. In conditions in which extensive remodeling of ECM occurs, as in arthritis, the cartilage becomes susceptible to invasion by vascular endothelial and mesenchymal cells from the synovium and subchondral bone.[60] VEGF, which is an essential mediator of angiogenesis during endochondral ossification, is induced by inflammatory cytokines, hypoxia, and mechanical overload.[38,51] In OA, in which abnormal biomechanics and joint effusions cause severe hypoxia, chondrocytes may produce VEGF, inducing angiogenesis at the chondro-osseous junction and contributing to expansion of the calcified cartilage, tidemark duplication, and cartilage thinning.[61] TGF-β–mediated angiogenesis in the subchondral bone may be among the earliest events driving OA,[62] where microcracks produced by mechanical stress and exacerbation of naturally occurring pores provide conduits for vascular invasion into the calcified cartilage and diffusion of small molecules.[63] In RA, ingrowth of blood vessels and synovial pannus into cartilage contributes to degradation of the cartilage matrix. Gene expression profiling analyses comparing inflamed and noninflamed areas of synovium from the same patients with OA identified *STC1*, encoding stanniocalcin-1, a molecule that plays roles in angiogenic sprouting via the VEGF/VEGF receptor 2 pathway, as the most highly upregulated gene in inflamed synovial membrane.[64] Articular cartilage is aneural, and the sensory nerve fibers observed in the vascular channels that are associated with osteochondral angiogenesis may constitute a potential source of symptomatic pain.[65] The clinical importance of these observations is supported by the report that VEGF blockade with bevacizumab inhibits post-traumatic OA in a rabbit model with pain relief associated with prevention of both synovitis and angiogenesis.[66]

## ROLES OF GROWTH AND DIFFERENTIATION (ANABOLIC) FACTORS IN NORMAL CARTILAGE METABOLISM

Growth and differentiation factors generally are considered positive regulators of homeostasis of mature articular cartilage because of their capacity to stimulate chondrocyte anabolic activity and, in some cases, inhibit catabolic activity.[41,67] The best-characterized anabolic factors in the context of their production and action in articular cartilage include insulin-like growth factor (IGF)-I and members of the FGF and TGF-β/BMP families. The PTHrP, Ihh, and Wnt/β-catenin pathways have been implicated in maintenance of cartilage homeostasis or OA disease processes. Many of these factors also regulate chondrogenesis and endochondral ossification during skeletal development.[28,33,35] In adult cartilage, their expression declines with age—a risk factor for OA—and their activities are downregulated.[67]

### Insulin-like Growth Factor

IGF-I was first described as *somatomedin C*, a serum factor controlling sulfate incorporation by articular cartilage in vitro, and it was later found to have the specific capacity to stimulate or maintain chondrocyte phenotype in vitro

by promoting the synthesis of type II collagen and aggrecan. IGF-I is a competence factor for cell proliferation that is categorized more appropriately as a differentiation factor because its limited mitogenic activity seems to depend on the presence of other growth factors, such as FGF-2, a progression factor. IGF-I is considered an essential mediator of cartilage homeostasis through its capacity to stimulate proteoglycan synthesis, promote chondrocyte survival, and oppose the activities of catabolic cytokines in cooperation with other anabolic factors such as BMP-7. IGF-I and insulin can activate cell signaling via the IGF-I tyrosine kinase receptor or the type I insulin receptor at concentrations proportional to their binding affinities.[67]

Specific IGF-binding proteins (IGF-BPs) that do not recognize insulin also regulate IGF-I activity. Chondrocytes at different stages of differentiation express IGF-I and IGF receptors and different arrays of IGF-BPs, providing a unique system by which IGF-I can exert different regulatory effects on these cells. IGF-BP-2 is a positive regulator that increases proteoglycan synthesis in chondrocytes, whereas binding of IGF-BP-3 to IGF-I negatively regulates the anabolic functions of IGF-I. IGF-BP-3 may also directly inhibit chondrocyte proliferation in an IGF-independent manner.

In OA cartilage, the normal anabolic function of IGF-I may be disrupted because chondrocytes from animals with experimental arthritis and from patients with OA are hyporesponsive to IGF-I, despite normal or increased IGF-I receptor levels. This hyporesponsiveness has been attributed to increased levels of IGF-BPs that may interfere with IGF-I actions and overproduction of nitric oxide through disruption of integrin signaling, reduction of phosphorylation of the IGF-I receptor, stimulation of cyclic guanosine monophosphate production, or suppression of mitochondrial oxidative metabolism. IGF-I can oppose the effects of inflammatory cytokines that promote cartilage degradation and inhibit proteoglycan synthesis.

## Fibroblast Growth Factor

Members of the FGF family, including FGF-2, FGF-4, FGF-8, FGF-9, FGF-10, and FGF-18, together with the FGF receptors, FGFR1, FGFR2, FGFR3, and FGFR4, coordinate patterning and cell proliferation during chondrogenesis and endochondral ossification in embryonic and postnatal growth plates.[33] The most extensively studied is FGF-2, or basic FGF, which is a potent mitogen for adult articular chondrocytes, but findings regarding its effects on the synthesis of cartilage matrix are contradictory, showing stimulation, inhibition, or no effect on proteoglycan synthesis.

The FGFs are generally considered homeostatic factors in joint tissues.[68,69] FGF-2 stored in the adult cartilage matrix is released with mechanical injury or with loading, suggesting a mechanism for modulating chondrocyte proliferation and anabolic activity. Different FGF receptors in cartilage may mediate opposing effects: FGF-2 promotes cartilage protection via FGFR3 and cartilage destruction via FGFR1.[70] Of the four receptors, FGFR1 and FGFR3 are the most abundant, and the ratio of FGFR3 to FGFR1 is reduced in OA cartilage. Furthermore, cartilage-specific deletion of the *Fgfr1* gene attenuates articular cartilage degeneration in mice.[71]

In cartilage development, FGF-18 negatively regulates chondrocyte proliferation and terminal differentiation in the growth plate via FGFR3. In articular cartilage, FGF-18 has a role in the maintenance of homeostasis via FGFR3 and protects against loading-induced damage in cartilage explants ex vivo.[72] In vivo, intra-articular administration of FGF-18 protects against cartilage damage in a rat model of injury-induced arthritis.[73] Thus there is considerable interest in the potential of anabolic factors such as FGF-18 for enhancing cartilage regeneration with tissue engineering approaches.[74] A recent proof-of-concept trial with human recombinant FGF-18, sprifermin, showed statistically significant dose-dependent improvement in prespecified secondary structural endpoints by magnetic resonance imaging (MRI), radiographic joint space narrowing, and Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain scores.[75]

## TGF-β/BMP Superfamily

Activities of the TGF-β/BMP superfamily in the skeleton were first discovered by Marshall Urist as constituents of demineralized bone that induced new bone formation when implanted into extraskeletal sites in rodents. These bioactive morphogens subsequently were extracted, purified, and cloned and were found to regulate the early commitment of mesenchymal cells to chondrogenic and osteogenic lineages during cartilage development and endochondral bone formation (Table 3-2). The TGF-β/BMP superfamily includes activins, inhibins, müllerian duct inhibitory substance, nodal, glial-derived neurotrophic factor, osteogenic protein-1 (OP-1) (or BMP-7), and growth differentiation

**TABLE 3-2** Bone Morphogenetic Protein Superfamily

| BMP | Other Names | Potential Function |
|---|---|---|
| BMP-2 | BMP-2A | Cartilage and bone morphogenesis |
| BMP-3 | Osteogenin, GDF-10 | Bone formation |
| BMP-4 | BMP-2B | Cartilage and bone morphogenesis |
| BMP-5 | | Bone morphogenesis |
| BMP-6 | Vgr-1 | Cartilage hypertrophy |
| BMP-7 | OP-1 | Cartilage and bone morphogenesis |
| BMP-8 | OP-2 | Bone morphogenesis |
| BMP-9 | GDF-2 | Cartilage morphogenesis |
| BMP-10 | | Unknown |
| BMP-11 | GDF-11 | Unknown |
| BMP-12 | GDF-7, CDMP-3 | Cartilage morphogenesis |
| BMP-13 | GDF-6, CDMP-2 | Cartilage morphogenesis |
| BMP-14 | GDF-5, CDMP-1 | Cartilage morphogenesis |

BMP, Bone morphogenetic protein; CDMP, cartilage-derived morphogenetic protein; GDF, growth and differentiation factor; OP, osteogenic protein; Vgr-1, vegetal-related-1.

factors (GDFs), also called *cartilage-derived morphogenetic proteins* (CDMPs). In addition to regulating cartilage condensation and chondrocyte differentiation, members of this superfamily play key roles in site specification and cavitation of synovial joints (see Chapter 1) and in the development of other organ systems. Many of these factors, including BMP-2, -6, -7, -9, TGF-β, and CDMP-1, are able to induce chondrogenic differentiation of mesenchymal progenitor cells in vitro. They also may have direct effects on mature articular chondrocytes in vivo and in vitro and are important for cartilage maintenance.[34]

### Transforming Growth Factor-β

TGF-β was so named because it was discovered that it could transform cells to grow in soft agar. TGF-β is not a potent inducer of chondrocyte proliferation, however; rather, it controls early mesenchymal cell condensation, as well as chondrocyte differentiation at early and late stages of chondrogenesis and endochondral ossification. Both inhibition and stimulation of the synthesis of aggrecan and type II collagen by TGF-β have been observed in vitro. However, TGF-β by itself cannot rescue the type II collagen phenotype when cells have undergone dedifferentiation during serial passaging. Levels of TGF-β measured in synovial fluids of persons with OA and RA may reflect anabolic processes in cartilage and other joint tissues. TGF-β may promote anabolism by inducing the expression of tissue inhibitors of MMP (TIMPs).

TGF-β1, TGF-β2, and TGF-β3 generally are considered to be potent stimulators of proteoglycan and type II collagen synthesis in primary chondrocytes and cartilage explants in vitro. However, TGF-β signaling can play both protective and deleterious roles in OA.[76] Although intra-articular injection of TGF-β stimulates proteoglycan synthesis and limits cartilage damage in inflammatory arthritis models, injection or adenovirus-mediated delivery of TGF-β1 may result in adverse effects in joint tissues, such as osteophyte formation, swelling, and synovial hyperplasia. Administration of agents that block TGF-β activity, such as the soluble form of TGF-βRII, inhibitory SMADs, or the physiologic antagonist, latency-associated peptide-1, increases proteoglycan loss and cartilage damage in experimental models of OA.

Recent studies have also identified aberrant TGF-β signaling in bone as a key pathway in OA.[77] In the anterior cruciate ligament (ACL) transection model of OA, high doses of a TGF-β inhibitor administered systemically promoted cartilage proteoglycan loss, whereas lower doses prevented the migration and/or localization of mesenchymal stem cells (MSCs), osteoprogenitors, and osteoblasts in the subchondral bone of operated limbs and attenuated neovascularization and cartilage loss.[62] Transgenic expression of active TGF-β in osteoblasts induced the formation of nestin-positive MSCs in clusters, whereas knockout of *Tgfbr2* in nestin-positive MSCs prevented MSC migration to the subchondral bone and normalized bone parameters, cartilage homeostasis, and limb function. Furthermore, implantation of a TGF-β–specific antibody in the subchondral bone attenuated the OA changes in both the cartilage and bone, suggesting that bone-targeted therapies may be useful in some forms of the disease.[62]

### Bone Morphogenetic Proteins

BMPs constitute a large subclass of the TGF-β superfamily essential for normal appendicular skeletal and joint development.[78] The isolation and cloning of the first BMP family members from bone prompted a search for cartilage-derived BMPs, or CDMPs—CDMP-1, -2, and -3—which are classified as GDF-5, -6, and -7. The BMPs may be divided into four distinct subfamilies based on the similarity of primary amino acid sequences:

1. BMP-2 and -2B (BMP-4), which are 92% identical in the 7-cysteine region
2. BMP-3 (osteogenin) and -3B (GDF-10)
3. BMP-5, -6, -7 (OP-1), BMP-8 (OP-2), BMP-9 (GDF-2), BMP-10, and BMP-11 (GDF-11)
4. BMP-12 (GDF-7 or CDMP-3), BMP-13 (GDF-6 or CDMP-2), BMP-14 (GDF-5 or CDMP-1), and BMP-15.

BMP-1 is not a member of this family but is an astacin-related MMP that cleaves the BMP inhibitor chordin and acts as a procollagen C-proteinase.

Several BMPs, including BMP-2, -7 (OP-1), and GDF-5/CDMP-1, can stimulate differentiation of mesenchymal precursors into chondrocytes and promote the differentiation of hypertrophic chondrocytes. BMP-2, -4, -6, -7, -9, and -13 can enhance the synthesis of type II collagen and aggrecan by articular chondrocytes in vitro. BMP-2 also is expressed in normal and OA articular cartilage, and it is a molecular marker, along with type II collagen and FGFR3, for the capacity of adult articular chondrocyte cultures to form stable cartilage in vivo. BMP-7 is expressed in mature articular cartilage and is possibly the strongest anabolic stimulus for adult chondrocytes in vitro because it increases aggrecan and type II collagen synthesis more strongly than IGF-I. In addition, BMP-7 reverses many of the catabolic responses induced by IL-1β, including induction of MMP-1 and -13, downregulation of TIMP, and downregulation of proteoglycan synthesis in primary human articular chondrocytes. CDMP-2 is found in articular cartilage, skeletal muscle, placenta, and hypertrophic chondrocytes of the epiphyseal growth plate. CDMP-1 and -2 maintain the synthesis of type II collagen and aggrecan in mature articular chondrocytes, although they are less effective initiators of chondrogenesis than other BMPs in early progenitor cell populations in vitro.

BMPs have pleiotropic effects in vivo, however, acting in a concentration-dependent manner. While initiating chondrogenesis in the limb bud, they generally set the stage for bone morphogenesis. Several BMPs also are true morphogens for other tissues, such as kidney, eye, heart, and skin.

## Receptors, Signaling Molecules, and Antagonists that Mediate Chondrocyte Responses to Growth and Differentiation Factors

Major pathways activated by the growth and differentiation factors previously discussed involve members of the ERK1/2, p38 MAPK, and phosphatidylinositol 3′-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog (AKT) pathways.[79] As in other cell types, FGF family members

activate kinases of the ERK1/2 and p38 MAPK cascades in chondrocytes. Specific inhibitors of these pathways block FGF-2–induced and FGF-18–induced mitogenesis in chondrocytes and prevent FGF-2 induction of Sox9 in primary chondrocytes. The PI3K pathway is required for the stimulation of proteoglycan synthesis by IGF-I in primary human articular chondrocytes, whereas the ERK1/2 pathway acts as a negative regulator.[67]

TGF-β and BMP family members transduce signals through the formation of heteromeric complexes of ligand-specific receptors, which have serine-threonine kinase activity. The specificity of subsequent signals is determined mainly by the type I receptors. Type I and type II receptors are required for signal transduction. Seven types of type I receptors, called *activin receptor–like kinases* (ALKs), have been identified in mammals and have similar structures. TGF-β interacts with TGFβRII, which recruits a TGF-β type I receptor (principally TGFβRI) to form a heterotrimeric receptor complex. The constitutively active TGFβRII kinase phosphorylates TGFβRI at serine and threonine residues. Three type I receptors, type IA BMP (BMPR-IA or ALK-3), BMPR-IB (ALK-6), and ALK-2, mediate BMP signaling. Although BMPR-1 receptors are able to bind ligand in the absence of type II BMP receptors, cooperativity has been shown in binding assays. On ligand binding, analogous to TGFβRI and TGFβRII, type I BMP receptors are phosphorylated by the type II BMP receptors, which include activin (Act)RII, ActRIIB, and ALK. Spatial and temporal differences in the distribution of these receptors in different tissues can govern the response patterns to different members of the TGF-β/BMP family.

The canonical SMAD pathway mediates TGF-β and BMP signaling through phosphorylation of receptor-activated SMADs (R-SMADs), which are related to the *Drosophila* mothers against decapentaplegic (MAD) and nematode SMA signaling molecules. TGFβRI receptors (ALK-4, -5, and -7) phosphorylate Smad2 and Smad3 transcription factors. BMPs act primarily through ALK-1, -2, -3, and -6 to activate Smad1, Smad5, and Smad8 and generally promote chondrocyte hypertrophic differentiation. The R-SMADs form complexes with the common Smad4 and translocate to the nucleus, where they bind to SMAD-specific DNA binding sites in the promoters of target genes.

The major route of TGF-β signaling in healthy articular cartilage is through canonical ALK-5, which activates Smad2/3 to inhibit chondrocyte hypertrophy, but when TGF-β interacts with ALK-1, it signals through Smad1/5/8 and induces MMP-13 and other degradative enzymes[76] and nerve growth factor (NGF).[80] Increase of the ALK-1/ALK-5 ratio during aging[76] may account for the aberrant chondrocyte responses associated with the development of OA and off-target effects of TGF-β injections in joints that result in osteophyte formation and synovial fibrosis.

Further regulation is provided by inhibitory SMADs, including Smad6 and Smurf1 and Smurf2, which primarily inhibit Smad1/5/8 signaling by accelerating the proteosomal breakdown of phosphorylated Smads. The aberrant hypertrophic differentiation in Smad3-deficient mice can be rescued by restoring TGF-β–activated kinase 1 (TAK1) and activating transcription factor (ATF)-2 signaling,[81] but inhibition of TAK1 or Janus kinase (JAK) can rescue impaired differentiation of MSCs in OA.[82]

BMP antagonists play important roles in spatial and temporal regulation of BMP activities during skeletal development. Originally discovered in *Xenopus*, they act as antagonists by determining the bioavailability of BMPs for binding to BMP receptors.[83] The roles of noggin and chordin seem to be crucial for determining boundaries during joint morphogenesis. They display different spatial and temporal patterns of expression, binding affinities, and susceptibility to proteinases that release BMP. BMPs bind to chordin and noggin via cysteine-rich domains that are similar to domains in the N-terminal propeptides of fibrillar procollagens I, II, III, and V, which also bind BMPs and are susceptible to cleavage by MMPs. BMP may be released from chordin by cleavage with MMPs or BMP-1/tolloid, whereas noggin binds BMP with high affinity and cannot be cleaved to release BMP.

Follistatin, gremlin, chordin, and chordin-like 2 are upregulated in OA cartilage. Follistatin, which has been linked to inflammatory processes; gremlin, which is associated with hypertrophic phenotype; and chordin appear at different stages of OA and with different topographic distribution. Because each antagonist binds preferentially to different BMPs, the differential expression may serve as a feedback mechanism to balance anabolic activities at different stages.

The Wnt/β-catenin pathway plays a role in both cartilage and bone development and disease.[84] The cellular responses are governed by the availability of Wnt ligands in different tissues and their use of distinct, canonical versus noncanonical, pathways over the course of differentiation and during disease.[84] The Wnt antagonists, including sclerostin (SOST), originally described as osteocyte-specific, dickkopf-related protein-1 (DKK1), and secreted frizzled-related protein 1 (sFRP1), have been explored as regulators and biomarkers of articular cartilage homeostasis, because they may decrease hypertrophic conversion and expression of proteinases in vitro and, in some cases, in vivo.[85-88]

## ROLE OF THE CHONDROCYTE IN CARTILAGE DISEASE

The chondrocyte, the unique cell type in mature cartilage, maintains a stable equilibrium between the synthesis and the degradation of matrix components. During aging and in the presence of joint diseases, such as RA and OA, this equilibrium is disrupted, and the rate of loss of collagens and proteoglycans from the matrix exceeds the rate of deposition of newly synthesized molecules. Cartilage destruction in OA is believed to be chondrocyte mediated in response to biomechanical insult and may occur directly or indirectly through the production of cytokines and cartilage matrix–degrading proteinases in cartilage and other joint tissues[89] (Figure 3-6).

Cartilage destruction in RA occurs primarily in areas contiguous with the proliferating synovial pannus as a result of the release and activation of proteinases from the synovial cells and, to some extent, at the cartilage surface, which is exposed to matrix-degrading enzymes from polymorphonuclear neutrophils in the synovial fluids. In addition to the direct action of proteinases, RA synovial tissues contribute indirectly to cartilage loss by releasing cytokines and other mediators that act on the chondrocytes to

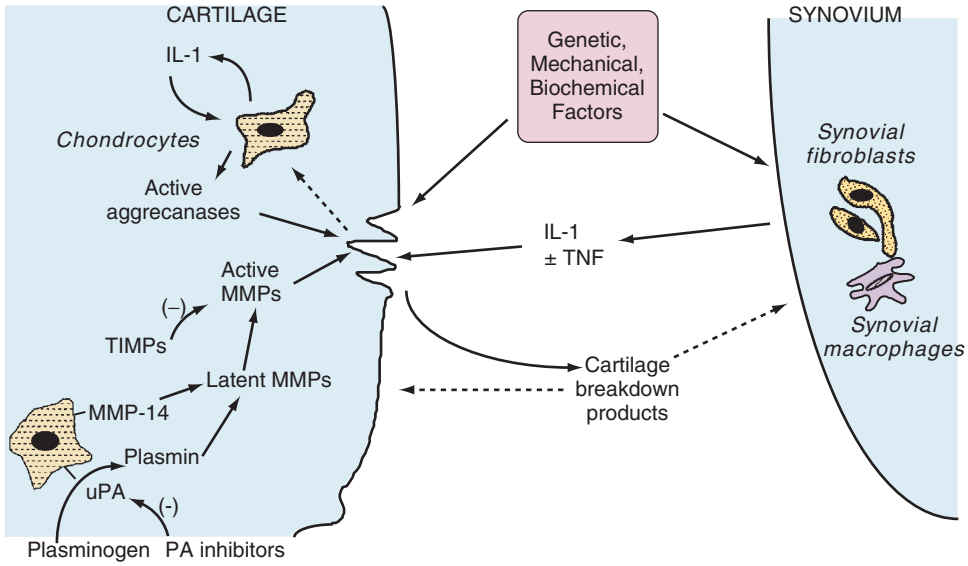



**Figure 3-6** The role of chondrocyte-derived proteinases in cartilage destruction in osteoarthritis. Although studies in vitro and in vivo have shown that the chondrocyte can respond directly to mechanical loading, to catabolic cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF), and to cartilage breakdown products, the initiating signals and their relative importance have not been defined clearly. MMP, Matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; uPA, urinary plasminogen activator. *(Modified from Goldring MB: Osteoarthritis and cartilage: the role of cytokines.* Curr Rheumatol Rep *2:459–465, 2000.)*

produce dysregulation of chondrocyte function. Understanding of basic cellular mechanisms regulating chondrocyte responses to inflammatory cytokines has been inferred from numerous studies in vitro using cultures of cartilage fragments or isolated chondrocytes and is supported by studies in experimental models of inflammatory arthritis, such as collagen-induced arthritis and antigen-induced arthritis in mice.[90-92]

Direct analysis of cartilage or chondrocytes from patients with OA undergoing joint replacement has yielded more information than is available from patients with RA, in whom cartilage damage is extensive. These studies indicate that chondrocytes produce not only pro-inflammatory cytokines but also inhibitory and anabolic cytokines that modulate responses. The impact of cytokines on chondrocyte function, particularly with respect to their various roles in cartilage destruction, has been reviewed extensively.[1,2,48] Studies of cartilage degradation in genetically modified mouse strains that are resistant to or accelerate the development of OA spontaneously with aging or when subjected to excessive biomechanical injury have yielded information about the molecular effectors of the disease and potential targets for therapy.[90,93-95]

## Cartilage Matrix–Degrading Proteinases

Chondrocytes synthesize and secrete MMPs in latent forms, which are activated outside the cells via activation cascades. An important cascade in cartilage is initiated by plasmin, the product of plasminogen activator activity, which may be produced by the chondrocyte; plasmin activates latent stromelysin (MMP-3), an activator of latent collagenases. In early studies, chondrocytes were among the first identified sources of TIMP-1, and they are now known to synthesize additional TIMPs, including TIMP-3, which is the major inhibitor of aggrecanase activities. TIMPs and MMPs detected in synovial fluids reflect the adaptive response to the local imbalance caused by increased production of active MMPs by chondrocytes and other joint tissues. Collagenases 1, 2, and 3 (MMP-1, -8, and -13); gelatinases (MMP-2 and -9); stromelysin-1 (MMP-3); membrane type 1 (MT1)-MMP (MMP-14); and the aggrecanases, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and ADAMTS-5, specifically degrade native collagens and proteoglycans in cartilage matrix (Table 3-3).[96,97]

MMPs, aggrecanases, and the cleavage fragments generated by them are localized in regions of cartilage degradation and are detected in synovial fluids and cartilage of persons with either OA or RA.[1,2] Expression of MMP-13 in OA and RA cartilage and its ability to degrade type II collagen more effectively suggest a major role for this enzyme in cartilage degradation. Postnatal overexpression of constitutively active MMP-13 in cartilage in mice produces OA-like changes in knee joints, and knockout of the gene encoding MMP-13 protects cartilage against surgically induced OA.[98] Deficiency of DDR2, a collagen receptor whose activation is associated with upregulation of MMP-13, attenuates development of OA induced by destabilization of the medial meniscus (DMM) surgery.[58] Deficiencies of the Runx2 and HIF-2α transcription factors that are key regulators of MMP-13 also protect against OA development or progression.[40] Nuclear factor-κB (NF-κB) transcription factors in response to their upstream activating kinases can directly modulate the amplitude of MMP-13 expression under inflammatory, mechanical, and oxidative stress.[99]

Although elevated levels of MMPs in RA synovial fluids likely originate from the synovium, intrinsic chondrocyte-derived chondrolytic activity is present at the cartilage-pannus junction and in deeper zones of cartilage matrix in

**TABLE 3-3** Chondrocyte Proteinases that Mediate Degradation of Cartilage Matrix

| Proteinase Class | Cartilage Matrix Substrates | Activity |
|---|---|---|
| **Matrix Metalloproteinases** | | |
| Collagenases (MMP-1, MMP-8, MMP-13) | Collagens I, II<br>Aggrecan core protein | Fibrillar domain, 3/4 from N-terminus N-telopeptide (MMP-13)<br>$Asn^{341}$-$Phe^{342}$ IGD |
| Stromelysins (MMP-3, MMP-10) | Aggrecan core protein<br>Collagens IX, XI<br>Link protein, fibronectin<br>pro-MMPs, pro-TNF | $Asn^{341}$-$Phe^{342}$ IGD<br>Telopeptide region |
| Gelatinases (MMP-2, MMP-9) | Collagens II, XI<br>Proteoglycans, link protein | Telopeptide or denatured collagen chains |
| MT-MMPs<br>MT-MMP-1 (MMP-14), MT-MMP-2, MT-MMP-3, MT-MMP-4 (MMP-15, MMP-16, MMP-17) | Collagen II<br>Fibronectin, aggrecan<br>Pro-MMP-2<br>Pro-MMP-13<br>Pro-TNF | Telopeptide |
| Matrilysin (MMP-7) | Link protein | |
| Enamelysin (MMP-20) | COMP, link protein | |
| **Aggrecanases** | | |
| ADAMTS-1, ADAMTS-4, ADAMTS-5 | Aggrecan core protein IGD | $Glu^{373}$-$Ala^{374}$, $Glu^{1545}$-$Gly^{1546}$, $Glu^{1714}$-$Gly^{1715}$, $Glu^{1819}$-$Ala^{1820}$, $Glu^{1919}$-$Leu^{1920}$ |
| **Serine** | | |
| Plasminogen activators (tPA, uPA) | Aggrecan, fibronectin, proMMPs | Activation of plasminogen gives rise to plasmin |
| Cathepsin G | Aggrecan, collagen II, proMMPs | |
| HTRA1 | Matrilin 3, fibronectin, biglycan, fibromodulin, COMP, collagen VI | Degrades pericellular matrix |
| **Cysteine** | | |
| Cathepsins B, K, L, S | Collagens IX, XI<br>Link protein, aggrecan | Telopeptides (optimal pH 4.0-6.5) |
| **Aspartate** | | |
| Cathepsin D | Phagocytosed ECM components | In lysosomes (optimal pH 3.0-6.0) |

ADAMTS, A disintegrin and metalloproteinase with thrombospondin-1 domains; COMP, cartilage oligomeric matrix protein; ECM, extra-cellular matrix; HTRA1, high temperature requirement A1; IGD, interglobular domain; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; proMMP, proenzyme form of MMP; TNF, tumor necrosis factor; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

some RA specimens. MMP-1 is expressed at lower levels than MMP-3 and MMP-13 in the RA synovial pannus but is also produced by chondrocytes. MMP-10, similar to MMP-3, activates procollagenases and is produced by the synovium and chondrocytes in response to inflammatory cytokines. MT1-MMP (MMP-14), produced principally by the synovial tissue, is important for synovial invasiveness and may also serve as an activator of other MMPs produced by chondrocytes.[96]

Several of the MMPs, including MMP-3, -8, -14, -19, and -20, are capable of degrading proteoglycans. Members of the reprolysin-related proteinases of the ADAM family, particularly ADAMTS-4 and ADAMTS-5, are now regarded as the principal mediators of aggrecan degradation.[100,101] The activities of MMPs and aggrecanases are complementary, however. Of the aggrecanases, ADAMTS-5 is associated with increased susceptibility to OA, as shown in *Adamts5*-deficient mice. TIMP-3, but not TIMP-1, -2, or -4, is a potent inhibitor of ADAMTS-4 and -5 in vitro, and TIMP-3 deficiency results in mild cartilage degradation similar to that seen in persons with OA.[102]

Cysteine proteinases, cathepsins B, K, S, and L, and the aspartic proteinase, cathepsin D, are lysosomal enzymes that may play a secondary role in cartilage degradation via intracellular digestion of products released by other proteinases.[103] Cathepsin B also may have a role in extra-cellular degradation of collagen telopeptides, collagens IX and XI, and aggrecan. Cathepsin K is expressed in synovial fibroblasts on the cartilage surface at the pannus-cartilage junction and is upregulated by inflammatory cytokines. Among the known cathepsins, cathepsin K is the only proteinase that is capable of hydrolyzing type I and type II collagens at multiple sites within the triple-helical regions, and its requirement for acidic pH may be provided by the microenvironment between the synovial pannus and the cartilage.[97]

Other MMPs, including MMP-16 and MMP-28, and ADAM family members, including ADAM-17/TNF converting enzyme (TACE), are expressed by chondrocytes, but their roles in adult cartilage have yet to be defined.[104,105] Identification of the precise roles of these proteinases and their endogenous inhibitors in chondrocyte-mediated cartilage degradation has provided the opportunity to develop targeted therapies that interfere with the activities of aggrecanases or MMPs without disrupting normal physiology.[106,107] MicroRNAs are of current interest as novel endogenous regulators targeting proteinases in cartilage, and epigenetic changes involving DNA methylation and

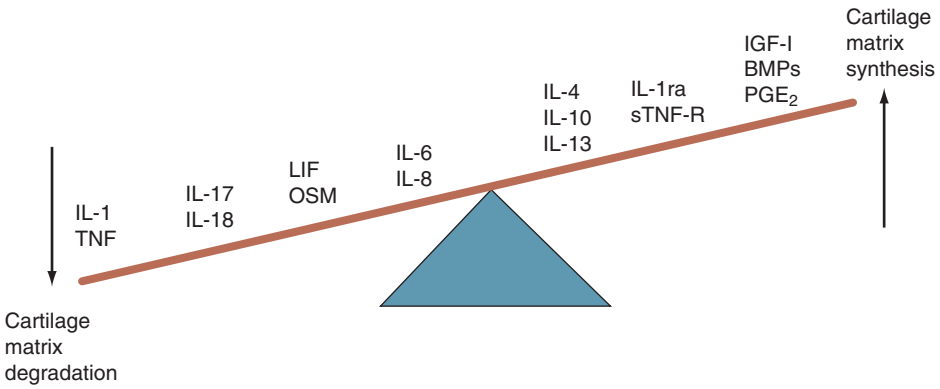



**Figure 3-7** The cytokine balance in cartilage metabolism. Soluble mediators toward the left of the balance promote the loss of cartilage matrix. Mediators on the right side prevent the synthesis or actions of catabolic cytokines and prevent loss of cartilage matrix. Anabolic factors, including insulin-like growth factor (IGF)-I and bone morphogenetic proteins (BMPs), along with prostaglandin $E_2$ ($PGE_2$), maintain or promote cartilage matrix synthesis. IL-1, Interleukin-1; IL-1ra, IL-1 receptor antagonist; LIF, lymphocyte-activating factor; OSM, oncostatin M; sTNF-R, soluble tumor necrosis factor receptor; TNF, tumor necrosis factor. *(Modified from Goldring MB: Osteoarthritis and cartilage: the role of cytokines.* Curr Rheumatol Rep *2:459–465, 2000.)*

**TABLE 3-4** Cytokines that Regulate Cartilage Destruction

| | |
|---|---|
| Catabolic | IL-1<br>TNF<br>IL-17<br>IL-18 |
| Modulatory | IL-6<br>Leukemia inhibitory factor<br>Oncostatin M<br>IL-11 |
| Inhibitory | IL-4<br>IL-10<br>IL-13<br>IL-1 receptor antagonist |

IL, Interleukin; TNF, tumor necrosis factor.

histone modifications that differentially affect gene expression in cartilage are of current interest.[108,109]

## Balance of Cytokines in Cartilage Destruction

The roles of cytokines in cartilage metabolism must be considered in the context of the entire joint.[48] Sources of cytokines in the joint include the synovial macrophages and infiltrating mononuclear cells in the synovium, as well as the chondrocytes themselves.[110] Of the cytokines that affect cartilage metabolism, most are pleiotropic factors that were identified originally as immunomodulators but were found to regulate cellular functions in cells of mesenchymal origin. IL-1 and TNF not only stimulate chondrocytes to synthesize cartilage matrix–degrading proteinases, they also regulate matrix protein synthesis and cell proliferation. Considerable redundancy and overlap in biologic activities exist among the individual cytokines, and they do not act alone, but rather in synergy or partnership with or in opposition to other cytokines via cytokine networks. In addition to IL-1 and TNF, other catabolic cytokines have been characterized, as have inhibitory or anabolic cytokines produced by the chondrocytes themselves or by other cells in joint tissues (Table 3-4). Investigations in vitro and in vivo have begun to sort out the complexities of the cytokine networks and to determine how the balance in normal homeostasis can be restored when it is disrupted (Figure 3-7). Studies of type II collagen–induced arthritis and other types of induced arthritis in transgenic animals with overexpressed or deleted genes encoding cytokines, their receptors, or activators have provided further insight into the roles of these factors in cartilage destruction.

### Interleukin-1 and Tumor Necrosis Factor

IL-1 and TNF are the predominant catabolic cytokines involved in the destruction of articular cartilage. Early work first identified IL-1 as a soluble factor, termed *catabolin*, in supernatants of normal, noninflamed porcine synovial fragment cultures that stimulated chondrocytes to degrade the surrounding cartilage matrix. Similar activities in culture supernatants from mononuclear cells and rheumatoid synovium were attributed to IL-1, and the catabolin isoforms were identified as IL-1α and IL-1β. Since those early findings, numerous studies in vitro and in vivo indicate that IL-1 and TNF, originating primarily from the inflamed synovium, are the predominant catabolic cytokines involved in the destruction of articular cartilage in RA.[111]

Major events in OA pathogenesis occur within the cartilage itself, and evidence suggests that chondrocytes participate in this destructive process not only by responding to cytokines released from other joint tissues but also by synthesizing them.[1,2] They may be exposed continuously to the autocrine and paracrine effects of IL-1 and other inflammatory mediators at high local concentrations. Chondrocytes in OA cartilage, especially those in clonal clusters, are positive for IL-1 immunostaining and express IL-1β converting enzyme (caspase-1) and type 1 IL-1 receptor (IL-1R1). IL-1 co-localizes with TNF, MMP-1, -3, -8, -13, and type II collagen cleavage epitopes in regions of matrix depletion in OA cartilage. The increased sensitivity of OA chondrocytes to IL-1 and TNF may be associated with increased levels of IL-1R1 and p55 TNF receptor at localized sites. These cytokines, MMPs, and type II collagen cleavage epitopes are observed in regions of matrix depletion in OA and RA cartilage in humans and in animal models.

The assumption has been that *IL1B* mRNA expressed in chondrocytes of OA cartilage could result in translation to pro-IL-1β and secretion of IL-1β. However, the inflammasome complex, consisting of NLRP3, ASC, and the IL-1β activator caspase-1, does not appear to be active in cartilage, thereby preventing secretion and autocrine activity of this cytokine. Furthermore, deficiency of genes encoding NLRP3 or IL-1β did not prevent catabolic responses induced by TNF, lipopolysaccharide, or mechanical load in cartilage explants from knockout mice.[112] These results suggest that locally produced IL-1β is not available to act in an autocrine or paracrine manner in cartilage, but rather this cytokine could be released from the synovium and other tissues to elicit chondrocyte responses.[112]

Originally known as *cachectin*, TNF produces many effects on chondrocytes in vitro that are similar to those of IL-1β, including stimulation of the production of matrix-degrading proteinases and suppression of cartilage matrix synthesis. Although IL-1β is 100-fold to 1000-fold more potent on a molar basis than TNF, strong synergistic effects occur at low concentrations of the two cytokines together, eliciting more severe cartilage damage than injection of either cytokine alone. The concept that TNF drives acute inflammation, whereas IL-1β has a pivotal role in sustaining inflammation and cartilage erosion, has been derived from work in animal models of RA using cytokine-specific neutralizing antibodies, soluble receptors, or receptor antagonists and in transgenic or knockout mouse models.

### Cytokine Networks

The cytokine networks that affect chondrocytes must also be considered within the context of the entire joint.[1,2,9,48] IL-1β and TNF can induce chondrocytes to produce other pro-inflammatory cytokines, including IL-6, leukemia inhibitory factor, IL-17, IL-18, and chemokines. IL-6 seems to play a dual role by increasing expression of IL-1ra, soluble TNF receptor, and TIMPs, while also enhancing immune cell function and inflammation. The activity of IL-6 requires soluble IL-6 receptor to synergize with IL-1 to stimulate expression of MMPs and ADAMTS and to downregulate *COL2A1* and aggrecan in cultured chondrocytes. IL-6 knockout mice are more susceptible to cartilage degeneration during aging, however, suggesting that this cytokine may play a protective role in normal physiology.

Other members of the IL-6 family that act via receptors that heterodimerize with gp130 also may serve modulatory roles. IL-11 shares several actions of IL-6, including stimulation of TIMP production, without affecting MMP production by chondrocytes, and it may inhibit cartilage destruction. Leukemia inhibitory factor (LIF) participates in a positive feedback loop by increasing the production of IL-6 by chondrocytes. Oncostatin M (OSM), a product of macrophages and activated T cells, is a potent stimulator of chondrocyte production of MMPs and aggrecanases in synergism with IL-1β or TNF.

IL-17 and IL-18 are potent catabolic factors that stimulate the production of IL-1β, MMPs, and other pro-inflammatory and catabolic factors in human chondrocytes. IL-17 is produced by activated T helper (Th) type 1, or $CD4^+$, lymphocytes and binds to a receptor that is not related to any known cytokine receptor family and can act in synergy with other cytokines. IL-17 can drive T cell–dependent erosive arthritis in TNF-deficient and IL-1ra knockout mice, and treatment of collagen-induced arthritis or antigen-induced arthritis mice with neutralizing IL-17 antibody effectively inhibits cartilage destruction in those models of RA. IL-18 is produced by macrophages, and its receptor shares homology with IL-1RI. IL-4, IL-10, IL-13, and the naturally occurring IL-1Ra are classified as inhibitory cytokines because they decrease the production and activities of catabolic and pro-inflammatory cytokines in chondrocytes in vitro and suppress cartilage destruction in vivo (see Table 3-4). IL-4 and IL-10 inhibit cartilage-degrading proteinases and reverse some effects of catabolic cytokines in vitro; together they produce synergistic suppression of cartilage destruction in vivo. The efficacy of IL-4, IL-10, and IL-13 in retarding cartilage damage may be related in part to their stimulatory effects on IL-1ra production, and their therapeutic application has been proposed as a means of restoring the cytokine balance in RA. IL-1ra is capable of blocking the actions of IL-1 if added at sufficiently high concentrations in vitro and was among the first agents to be developed for anti-cytokine therapy. However, TNF inhibitor therapy is more effective in RA than systemic IL-1ra, and intra-articular IL-1ra in OA joints requires delivery systems that maintain sufficiently high concentrations or local production.[113]

### Other Mediators

Chondrocytes in OA cartilage, especially those in clonal clusters, also express the pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and receptor for advanced glycation endproducts (RAGE). They are activated by ligands such as damage-associated molecular patterns (DAMPs), also known as alarmins, including high mobility group box-1 (HMGB1), S-100A8 (MRP8, calgranulin A) and S-100A9 (MRP14, calgranulin B), serum amyloid A (SAA), collagen and proteoglycan constituents, and calcium pyrophosphate or hydroxyapatite crystals. DAMPs or other products released from damaged cartilage are evidence of adaptive immunity due to interactions of T cells,[91] but during established, chronic OA their role in innate immunity, in which they promote the initiation and perpetuation of low-grade inflammation in the OA joint, is of current interest.[114,115]

S-100A8, S-100A9, and S-100A11 have critical roles in inflammatory arthritis and OA.[116,117] Activation of TLR or RAGE signaling drives inflammation-associated matrix catabolism through increased expression of inflammatory and catabolic genes, including MMP-3, MMP-13, and nitric oxide synthase (NOS)2 and increased ROS.[115,118] Pro-inflammatory cytokines, prostaglandins, ROS, and nitric oxide also promote oxidative stress and chondrocyte apoptosis by altering mitochondrial function.[119]

Other candidate innate effectors of the disease include the complement proteins, which are detected in synovial fluids of persons with OA by proteomic analysis and can be activated by DAMPs.[120] The classical, mannose-binding lectin, and alternative complement pathways all converge on C3 to activate the membrane attack complex (MAC)

formed from the complement effector C5b-C9. Knockout of the C5 and C6 components of MAC or pharmacologic treatment with CR2-fH attenuates cartilage damage in the medial meniscectomy mouse model.[121]

The peroxisome proliferation-activated receptors (PPARs) are a family of transcription factors that play roles in a broad spectrum of physiologic and pathologic processes, including skeletal homeostasis. PPARγ is activated by the endogenous ligand 15-deoxy-$\Delta^{12,14}$ prostaglandin $J_2$ ($PGJ_2$). PPARγ activation opposes the induction of cyclooxygenase (COX)-2, inducible (i)NOS, and MMPs and the suppression of aggrecan synthesis by IL-1. PPARα agonists may also protect chondrocytes against IL-1–induced responses by increasing the expression of IL-1ra. In OA, the focus has been on the roles of PPARγ in obesity and inflammation and its capacity to decrease cartilage degradation in animal models of OA.[122-125]

Among the proteins that may be induced in chondrocytes in response to abnormal stimuli is the cytokine-induced suppressor of cytokine signaling 3 (SOCS3), which acts as a feedback regulator to ameliorate adverse effects on chondrocyte functions.[126] SOCS proteins are inhibitors of cytokines that signal via the JAK/signal transducer and activator of transcription (STAT) pathway in multiple cell types, including T cells, macrophages, chondrocytes, synoviocytes, osteoclasts, and osteoblasts. de Andres et al.[127] examined the levels of SOCS proteins in cartilage specimens from a series of patients with OA and found that SOCS2 mRNA levels were markedly reduced in the OA chondrocytes compared with chondrocytes from normal cartilage.

Adipokines, including leptin, adiponectin, resistin, and visfatin, were identified originally as products of adipocytes and have roles in cartilage metabolism based on investigations in various experimental models.[128,129] Cytokines secreted by the white adipose tissue contribute to the low-grade inflammation in obesity-associated OA.[130] There is a balance between pro- and anticatabolic adipokines that are secreted by joint tissues, including the fat pad of the knee, suggesting complex interactions between systemic and local factors.[131]

Chemokines, which are small heparin-binding cytokines identified originally as chemotactic factors, are classified as C, CX3C, or CC molecules, indicating the presence of distinct N-terminal cysteine (C) residues. Chondrocytes, when activated by IL-1 and TNF, express several chemokines, including IL-8, monocyte chemoattractant protein (MCP)-1, MCP-4, macrophage inhibitory protein (MIP)-1α, MIP-1β, RANTES (regulated on activation normal T cell expressed and secreted), and growth-related oncogene (GRO)-α, as well as the receptors that enable responses to some of these chemokines (Table 3-5).[9,132] Normal and OA chondrocytes express the CC chemokines, MCP-1, MIP-1α, MIP-1β, and RANTES. RANTES increases expression of its own receptor, CCR5. MCP-1 and RANTES increase MMP-3, iNOS, IL-6, and MMP-1 expression, inhibit proteoglycan synthesis, and enhance proteoglycan release from the chondrocytes. The RANTES receptors CCR3 and CCR5, but not CCR1, are expressed in normal cartilage, whereas all three receptors are expressed in OA cartilage or after stimulation of normal chondrocytes by IL-1β.

**TABLE 3-5** Chemokines and Receptors in Chrondrocytes*

| Functional Name | Systematic Name | Chemokine Receptor |
|---|---|---|
| GROα | CXCL1 | CXCR1, CXCR2 |
| IL-8 | CXCL8 | CXCR1, CXCR2 |
| MCP-1 | CCL2 | CCR2 |
| MIP-1α | CCL3 | CCR1, CCR5 |
| MIP-1β | CCL4 | CCR5 |
| RANTES | CCL5 | CCR1, CCR3, CCR5 |
| SDF-1 | CXCL12 | CXCR4 |

*Chemokines are classified according to the positions of the first two cysteines (C) of the four conserved N-terminal cysteines: CC chemokine ligand (CCL), first two cysteines are adjacent; CXC chemokine ligand (CXCL), first two cysteines are separated by amino acid X other than cysteine; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor.

GROα, Growth-related oncogene α; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MIP-1, macrophage inhibitory protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; SDF-1, stromal-derived growth factor-1.

Stromal cell–derived factor 1 (SDF-1) can be detected in OA and RA synovial fluids, and its receptor, CXCR4, is expressed by chondrocytes but not by synovial fibroblasts, suggesting a direct influence of this chemokine on cartilage damage.[132] In addition to recruiting leukocytes to sites of inflammation in arthritic joints and mediating synovial fibroblast responses and actions, chemokines are capable of modulating chondrocyte functions that are associated with cartilage degradation.

## Cytokine Signaling Pathways Involved in Cartilage Metabolism

Although the receptors for IL-1 and TNF and associated adapter molecules are distinct, they share the capacity to activate some of the same signaling pathways (Figure 3-8). The major pathways induced by catabolic cytokines involve signal transduction by the stress-activated protein kinases, c-Jun N-terminal kinases (JNKs) and p38 kinases, and the NF-κB and PI3K pathways.[1,2,79,99,133] The JAK/STAT signaling pathway is important for signaling by gp130 cytokines, including IL-6 and OSM.[134] Specific adapter molecules involved in the pathways induced by TNF receptors, which are members of the TNF receptor superfamily, are different from the adapter molecules used by IL-1 signaling pathways. The TNF receptor pathway uses TNF receptor–associated factor 2 (TRAF2), TRAF6, and the receptor interacting protein kinase, whereas the IL-1 receptor pathway uses TRAF6, IL-1 receptor–associated kinase (IRAK), and evolutionarily conserved signaling intermediate in Toll pathways (ECSIT) as adapter molecules. Signaling through TNF-RI associated with TNF receptor–associated death domain (TRADD) activates apoptosis, whereas TNF-RII signaling through TRAF2 activates JNK and NF-κB.

In contrast to ERK1 and ERK2, p38 and JNK signaling pathways are weakly activated by growth factors. Studies in chondrocytes in vitro have shown that the p38 and JNK cascades mediate the induction of proteinases and proinflammatory genes by IL-1 and TNF. These pathways also may be activated in chondrocytes by mechanical stress and

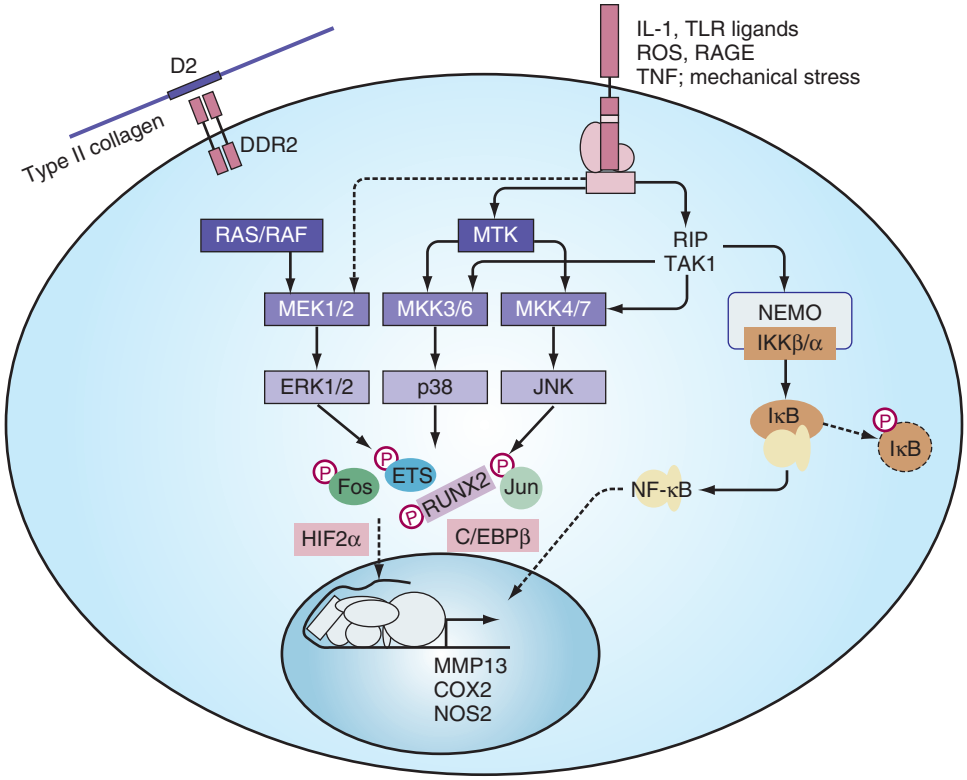



**Figure 3-8** Intra-cellular signaling pathways that regulate gene transcription in chondrocytes. Binding of the receptor tyrosine kinase, discoidin domain receptor (DDR) 2 to native type II collagen results in activation of RAS/RAF/MEK/ERK signaling in a manner independent of integrin- or cytokine-induced signaling. Interleukin (IL)-1, Toll-like receptor (TLR) ligand, reactive oxygen species (ROS), and receptor for advanced glycation end products (RAGEs) interact with the cell through distinct receptors that transduce phosphorylation events and initiate various protein kinase cascades. Binding of IL-1 to the type I IL-1 receptor (IL-1R1) leads to recruitment of the IL-1R accessory protein (IL-1RAcP). Cytoplasmic Toll/IL-1 receptor (TIR) domains of the receptor recruit MyD88 via its TIR, and the MyD88 death domain (DD) recruits IL-1 receptor–associated kinases (IRAK and IRAK2) to the receptor complex before being rapidly phosphorylated and degraded. The IRAKs mediate tumor necrosis factor receptor–associated factor 6 (TRAF6) oligomerization, initiating various protein kinase cascades. The major pathways involve activation of (1) the stress-activated protein kinases, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK), which lead to activation of activator protein-1 (AP-1) (cFos/cJun), activating transcription factor-2 (ATF-2), E26 transformation-specific (ETS) factors, hypoxia-inducible factor (HIF)2α, Runx2, and CCAAT enhancer binding protein (C/EBP)β, among other transcription factors; and (2) inhibitor of κB (IκB) kinases α and β (IKK-α and IKK-β), which lead to activation of nuclear factor-κB (NF-κB). Tumor necrosis factor (TNF) also stimulates these pathways, but mainly via TRAF2. Other signaling pathways may influence the target gene responses, such as growth factor–induced or chemokine-induced phosphatidylinositol 3-kinase (PI3K) via the serine/threonine kinase, Akt/protein kinase B, and gp130 cytokine–induced Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. Responses of the target genes depend on the presence of DNA sequences within the respective promoters that bind to various transcription factors. COX, Cyclooxygenase; ERK, extra-cellular signal-regulated kinase; ETS, E26 transformation-specific; MMP, matrix metalloproteinase; NOS, nitric oxide synthase; TAK1, TGF-β–activated kinase 1. *(Modified from Goldring MB: Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis.* Ther Adv Musculoskelet Dis *4:269–285, 2012.)*

cartilage matrix products via integrins and other receptor-mediated events. Upregulation of IL-1 and TNF expression via mechanotransduction pathways suggests their involvement as secondary mediators in a feedback mechanism. At least four isoforms of p38 MAPK exist with different substrate specificities and differential effects on essential chondrocyte functions.

JNKs are serine threonine protein kinases that phosphorylate Jun family members, components of activator protein 1 (AP-1) transcription factors, and they exist in humans as three JNK isoforms: JNK1, JNK2, and JNK3. A potent JNK1/2 inhibitor, SP600125, which blocks inflammation and joint damage in animal models of RA, and other JNK isoform–specific inhibitors are useful tools for analyzing chondrocyte function in vitro and in vivo. Activated JNK is detected in OA, but not in normal cartilage, and JNK inhibition attenuates cytokine-induced chondrocyte responses.

Members of the NF-κB family orchestrate mechanical, inflammatory, and oxidative stress-activated processes in chondrocytes. NF-κBs are released from inhibitory IκBs or modified by the catalytic activities of the IκB kinase (IKK)α and IKKβ subunits of the IKK signalosome complex, permitting translocation of active NF-κB to the nucleus.[99] In response to a host of pro-inflammatory stimuli, IKKβ is the dominant IκBα kinase in vivo, whose activation is essential for the nuclear entry of canonical NF-κB heterodimers. NF-κB mediates the expression of cytokines and chemokines induced by fibronectin fragments, and inhibition of nuclear translocation or DNA-binding activity of NF-κB heterodimers of p65/p50 blocks pro-inflammatory and catabolic effects of IL-1 and TNF on chondrocytes. Chondrocyte phenotype appears to be subject to differential control by IKKβ-driven canonical NF-κB, which drives cartilage catabolism, and kinase-independent IKKα, which promotes hypertrophic conversion.[46]

The IKKβ-driven, canonical NF-κB pathway signaling also has effects on downstream transcriptional regulators, including HIF-2α, β-catenin, Runx2, and the E26 transformation-specific (ETS) factor, Elf3, thus linking inflammatory and oxidative stress responses with phenotypic and functional changes in chondrocytes.[39,40,135] In addition, NF-κB signaling plays a central role in disease progression and perpetuation, mediating a cascade of inflammatory responses triggered by advanced glycation end products, TLR ligands, or released ECM products, including fibronectin fragments, which lead to the continued expression of MMPs, aggrecanases, inflammatory cytokines, and chemokines and to an abnormal differentiation status.

## ROLE OF THE CHONDROCYTE IN CARTILAGE REPAIR

### Aging of Articular Cartilage

It is important, but often difficult, to distinguish among the effects of aging itself and diseases such as OA that become more common with increasing age.[136] In both cases, biochemical alterations in matrix composition are reflected in changes in cartilage structure.[137] The thickness of articular cartilage, as shown by MRI, decreases with increasing age. Heterogeneous depletion of glycosaminoglycans at the cartilage surface and fatigue fracture of superficial collagen bundles may contribute to the mild splitting and fraying of superficial cartilage, which is termed *fibrillation*. If fibrillation progresses into deeper layers of cartilage, abnormal multicellular clusters of chondrocytes that stain intensely for glycosaminoglycans are found at the base of clefts. These changes include decreased size and aggregation of aggrecan and increased collagen denaturation, resulting in loss of compressive stiffness and tensile strength.

Zonal differences in tensile strength and compressive resistance are related to differences in matrix composition and can be observed to change during the aging of adult articular cartilage and in response to traumatic damage. The territorial, or pericellular, matrix and the interterritorial matrix differ in the amounts and types of matrix proteins. The chondrocytes are normally surrounded by a 2 μm pericellular matrix, composed of a highly branched filamentous network of collagen VI tetramers, that serves as a scaffold for decorin, biglycan, perlecan, chondroadherin, hyaluronan, fibrillin-1, and PRELP.[15] The interterritorial region, in contrast, contains primarily a collagen II/XI fibril network, which binds decorin, fibromodulin, collagen IX, and COMP, and large numbers of intact aggrecan molecules attached via link protein to long chains of hyaluronic acid. In the deep zone, the interterritorial region most remote from the cells contains a larger number of degraded aggrecan molecules that lack the G3 domain.

Proteoglycans in aged cartilage have a wide range of sizes, with small forms resulting from low substitution of glycosaminoglycan residues and shorter lengths compared with glycosaminoglycans in young articular cartilage. Unsubstituted proteoglycan core proteins of aggrecan and biglycan are detectable in articular cartilage from elderly subjects. Hyaluronan content increases in aged cartilage, but with reduced mean chain length, and link protein seems to be fragmented. Collagen fibrils become thinner with age and are less densely packed. Nonenzymatic glycation results in the formation and accumulation of the advanced glycation end product pentosidine in long-lived proteins, including cartilage collagen and aggrecan. Such biochemical changes may result in part from changes in chondrocyte synthetic function and from increased susceptibility of the matrix to degradation. Increased and more extensive collagen degradation can be observed in cartilage from older, healthy persons, and, similar to cartilage in early OA, the damage is concentrated closer to the articular surface and co-localizes with MMP-13 activity. (See Chapter 98 for a detailed discussion of the pathogenesis of OA.)

### Aging Chondrocyte

Chondrocyte function, including mitotic and synthetic activity, deteriorates with age.[136] Degradative changes are generally due to the actions of proteinases and are, at least in part, the cumulative consequences of adverse conditions, such as mechanical insults or inflammation, to which the chondrocyte is exposed throughout life. Deficiencies in cartilage matrix proteins also may disrupt chondrocyte-matrix interactions that are important to cell survival. The decline in chondrocyte number may be attributed to increased cell death with age. Although programmed cell death, or apoptosis, increases with age in adult rats and mice, this may be due to skeletal growth that occurs throughout life in these animals. In human adult cartilage, apoptotic cell removal does not seem to be common, however. Replicative senescence, detected as β-galactosidase activity and decreased telomere length, has been proposed to contribute to age-related changes in the proliferative potential of adult articular chondrocytes.[138,139] Furthermore, TGF-β, FGFs, IGF-I, and other anabolic factors that support cartilage matrix biosynthesis are expressed at declining levels with aging, or their activities are downregulated.[136]

Chondrocytes use autophagy as an efficient housekeeping program to maintain cellular function and homeostasis by removing damaged or malfunctioning cellular structures, eliminating exogenous cellular aggressors, and providing alternative sources of energy during endoplasmic reticulum (ER) stress, hypoxia, starvation, and other adverse events.[140,141] The loss of autophagy in articular cartilage during aging is associated with cell death and increasing OA severity because the chondrocyte is less resistant to mechanical or inflammatory stress.[142] Autophagy occurs by lysosome- or endosome-mediated mechanisms.

Also compromised during aging is the unfolded protein response (UPR), which is a nonlysosomal pathway for ubiquitin/proteasome degradation of unfolded proteins eliminated from the ER.[143] The UPR enables chondrocyte survival in response to ER stress during growth plate development[144] but also during inflammatory, mechanical, and oxidative stress in articular cartilage via C/enhancer binding protein (EBP) homologous protein (CHOP) and X-box protein 1 (XBP1).[143] Also related to autophagy are the classic cell survival signals, PI3K and its downstream target serine-threonine kinase (AKT).[79] In addition, the impairment of chondrocyte mitochondrial function has emerged as a mechanism involved in impaired autophagy and deregulated bioenergetics during aging and OA development.[119]

## Markers of Cartilage Matrix Degradation and Turnover

With increasing knowledge of the composition of the cartilage matrix, molecular markers in body fluids have been identified for monitoring changes in cartilage metabolism and for assessing joint damage in persons with arthritis.[11] Molecules originating from the articular cartilage, including aggrecan fragments containing chondroitin sulfate and keratan sulfate, type II collagen fragments, collagen pyridinoline cross-links, and COMP, usually are released as degradation products as a result of catabolic processes. Specific monoclonal antibodies have been developed for analyzing OA and RA body fluids for products of proteoglycan or collagen degradation (catabolic epitopes) or synthesis of newly synthesized matrix components (anabolic neoepitopes), which represent attempts to repair the damaged matrix. Different monoclonal antibodies can distinguish subtle biochemical differences in chondroitin sulfate or keratan sulfate chains that result from degraded versus newly synthesized proteoglycans. Such epitopes can be detected in the synovial fluids and sera of patients with OA and RA, and the synovial fluid to serum ratio has been suggested as a potential diagnostic indicator. The degradation of aggrecan in cartilage has been characterized using antibodies 846, 3B3(−), and 7D4, which detect chondroitin sulfate neoepitopes; 5D4, which detects keratan sulfate epitopes; and the VIDIPEN and NITEGE antibodies, which recognize aggrecanase and MMP cleavage sites within the interglobular G1 domain of aggrecan.[101,106]

Similarly, it is possible to monitor the synthesis of type II collagen by measuring serum and synovial fluid levels of the carboxyl-terminal propeptide (CPII), and urinary excretion of hydroxylysyl pyridinoline cross-links may indicate collagen degradation. Specific antibodies that recognize epitopes on denatured type II collagen at the collagenase cleavage site are promising diagnostic reagents. These include the C12C antibody (previously known as *Col2-3/4C Long mono*), which has been used to detect cleavage of the triple helix of type II collagen in experimental models and in OA and RA cartilage. The ratios of these markers to the synthetic marker, CPII, are associated with a greater likelihood of radiologic progression in persons with OA. These biomarker assays have been used as research tools and are currently being developed and validated as diagnostic tools for monitoring cartilage degradation or repair in OA and RA patient populations and for assessment of treatment (see Chapters 71 and 100).[11] Although a single marker may be insufficient, eventually it may be possible to identify a combination of biomarkers that discriminate between different stages of OA in different patient populations.

## Repair of Articular Cartilage

Articular cartilage has a poor capacity for regeneration, and pharmacologic enhancement of cartilage repair would have considerable potential in the treatment of arthritides and intra-articular fractures. The extent of intrinsic repair of a cartilage defect depends on the depth of the lesion and whether the defect penetrates the subchondral bone plate.[4] Repair of superficial defects occurs if the chondrocytes remain viable. Because of the avascularity of cartilage, it differs from most other tissues in its response to injury. The vascular-dependent inflammatory and reparative phases of the classic healing response are unavailable. Partial defects generally do not regenerate because resident chondrocytes cannot migrate into the defect, and there is no vascular access for progenitor cells. Deep cartilage defects with disruption of the subchondral bone plate initiate vascular responses, however, including bleeding, fibrin clot formation, and inflammation, which permit cell invasion from the blood or underlying bone marrow. The lesion becomes filled by granulation tissue, which eventually is replaced by fibrocartilage, but rarely by true hyaline cartilage. Current procedures for cartilage repair include joint lavage, tissue debridement, microfracture of the subchondral bone, and transplantation of autologous or allogeneic osteochondral grafts, in addition to the ultimate therapy of total joint replacement.[4] These procedures may lead to the formation of fibrous tissue, chondrocyte death, and further cartilage degeneration and have variable success rates.

Transplantation of cultured autologous chondrocytes has been used successfully to repair small, full-thickness lesions in knee cartilage in young adults with sports injuries. Evidence of successful repair has been shown by turnover and remodeling of the initial fibrocartilaginous matrix formed by transplanted chondrocytes as the result of enzyme degradation and new synthesis of type II collagen. The donor site, although not load bearing, may undergo significant morbidity and osteoarthritic changes. Such procedures, however, show little difference in efficacy compared with microfracture of subchondral bone.[4]

Major challenges for cartilage repair include restoration of the three-dimensional collagen structure and integration of the newly synthesized matrix with resident tissue.[4] Novel approaches using autologous chondrocytes genetically engineered ex vivo to express anabolic factors have been explored to promote differentiation before implantation in the defect.[145] Because IGF-1 and TGF-β/BMP and FGF family members have roles in chondrogenesis and articular cartilage maintenance, they have been used as additive factors in tissue engineering strategies. The introduction of these factors into joints directly or by in vivo or ex vivo gene delivery or via injectable or implantable carriers has been investigated for the repair of small defects in animal models. Gene transfer of combinations of anabolic factors and inhibitory cytokines combined with cartilage engineering approaches may be a long-term goal for repair of extensive defects in patients with RA or late-phase OA and for prevention of further damage.

The predominant research strategy aimed at engineering functional cartilage with the identical properties of the native tissue has been to increase the accumulation of a cartilaginous matrix by exposing cells to anabolic agents such as those previously discussed, either through exogenous addition of recombinant proteins or genetic manipulation of the cells to overexpress them.[146] This work has led to development of scaffold materials, identification of viable cell sources and culture conditions, including growth factor delivery and mechanical stimulation, as well as approaches for delivering cells alone or in a scaffold to the injury site.[147] However, we are far from achieving a construct with the mechanical stability and lubricating properties of native cartilage. Successful cartilage tissue engineering strategies

must also include retention of key matrix components within the construct over an extended life span and under inflammatory conditions and integration with the host tissue. Furthermore, the restoration of the pericellular matrix will be essential for any successful tissue engineering strategy.[15]

Recent investigations have focused on scaffolds with the mechanical and material properties of the osteochondral unit that will integrate with the host tissue and attract migration of chondrocytes or osteochondral progenitors from the subchondral bone.[147] Although these strategies for the future based on in vitro and in vivo studies are promising, many challenges remain, especially for cell-mediated cartilage repair. Among the cell sources are MSCs, which can be obtained from autologous adipose tissue, bone marrow, synovium, or muscle; they can be expanded ex vivo in conditions that promote chondrogenesis, incorporated into scaffolds, and/or used as gene delivery vehicles to the site of cartilage damage.[145] Although adult articular chondrocytes have little intrinsic repair capacity, the potential of a small population of cartilage-derived stem or progenitor cells to be stimulated in situ is the subject of current investigations.[148]

## CONCLUSION

As the single cellular component in adult articular cartilage, chondrocytes are responsible for maintaining the ECM components in a low-turnover state. The composition and organization of matrix macromolecules, unique to this tissue, are determined during chondrocyte differentiation in embryonic and postnatal development of cartilage. Adult chondrocytes exist in a hypoxic environment within articular cartilage. They are inactive metabolically, partially as a result of the absence of blood vessels and nerves, and display a rounded structure that reflects their quiescent state. Chondrocyte culture models have been developed with the aim of maintaining differentiated phenotypes, characterized by the major collagen and proteoglycan constituents, type II collagen and aggrecan. Chondrocytes interact with specific ECM components via cell surface receptors, including integrins, annexins, syndecans, DDR2, and CD44.

Studies in vitro and in vivo have shown that adult articular chondrocytes are capable of responding to biologic and mechanical stimuli that are anabolic or catabolic. Anabolic factors include members of the TGF-β/BMP and FGF families and IGF-I. Catabolic factors include pro-inflammatory cytokines, chemokines, DAMPs/alarmins, and adipokines, which stimulate the synthesis of matrix-degrading proteinases, such as MMPs and aggrecanases, in chondrocytes and increase intra-cellular events associated with inflammatory, mechanical, and oxidative stress. Many of the signaling pathways and transcription factors that mediate the responses of chondrocytes to these factors have been elucidated, but how they orchestrate specific chondrocyte functions is complex and is not fully understood. Under physiologic conditions, the adult articular chondrocyte maintains a stable equilibrium of low-turnover replacement of matrix components. Adult chondrocytes have a poor capacity for mediating effective repair of extensive cartilage lesions, and this capacity declines with age. Age-related changes in chondrocyte function decrease the ability of cells to maintain the tissue, and there is decreased responsiveness to anabolic growth factors. Further understanding of how the adult articular chondrocyte functions within its unique environment would aid in the development of rational strategies for maintaining homeostasis and protecting against cartilage damage.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 4



# Biology, Physiology, and Morphology of Bone

Georg Schett

**KEY POINTS**

- Intramembranous or endochondral ossification generates bone tissue.
- Bones consist of a dense cortical shell and sponge-like trabecular network.
- Bone formation depends on metabolically active osteoblasts synthesizing matrix proteins.
- Resorption of bone is mediated by multinucleated osteoclasts.
- The most abundant cell type in bone is the osteocyte.
- Bone is continuously rebuilt, a process known as *bone remodeling*.
- The immune system, in particular T lymphocytes, influences bone remodeling.
- Neuroendocrine loops exert systemic control on bone remodeling.

## STRUCTURE AND COMPOSITION OF BONE

Bone is a specialized connective tissue that assists in (1) locomotion, by providing the insertion site of the muscles; (2) protection of the internal organs and the bone marrow; and (3) metabolic function, such as storage and provision of calcium to the body. Bone consists of cells and the extracellular matrix, which is composed of type I collagen fibers and a number of noncollagenous proteins. The specific composition of the bone matrix allows its mineralization, which is a specific feature of bone.

The two major types of bones are flat bones, which are built by intramembranous ossification, and long bones, which emerge from endochondral ossification. Intramembranous bone formation is based on the condensation of mesenchymal stem cells, which directly differentiate into bone-forming osteoblasts. In contrast, during endochondral ossification of the long bones, the mesenchymal stem cells first differentiate into chondrocytes that will later be replaced by osteoblasts. Long bones consist of the (1) epiphyses, which are protrusions at the ends of the long bones; (2) diaphysis, constituting its shaft; and (3) metaphyses, which are located between the epiphysis and the diaphysis (Figure 4-1). The metaphysis is separated from the epiphysis by the growth plate, a proliferative cartilage layer that is essential for the longitudinal growth of bones. After the growth is completed, this cartilage layer is entirely remodeled into bone.

The external shape of bones is formed by a dense cortical shell (cortical or compact bone), which is particularly strong along the diaphysis, where the bone marrow is located. The cortical bone shell becomes progressively thinner toward the metaphyses and epiphyses, where most of the trabecular bone is located. Trabecular bone (also called *cancellous bone*) is a sponge-like network consisting of myriads of highly interconnected bony trabeculae. The outer and the inner surfaces of cortical bone are covered by layers of osteogenic cells, termed the *periosteum* and the *endosteum*, which are involved in the growth of width by bone apposition at the periosteal sites and bone resorption at the endosteal sites.

Although cortical and trabecular bone is composed of the same cells and the same matrix components, there is a substantial difference between these two forms of skeletal tissue. Cortical bone almost exclusively consists of mineralized tissue (up to 90%), allowing it to fulfill its mechanical requirements. In contrast, only 20% of trabecular bone is mineralized tissue, with the bone marrow, blood vessels, and a network of mesenchymal stem cells constituting the remainder of the bone. As a consequence, trabecular bone shares a vast surface with the nonmineralized tissue, which is the basis for the metabolic function of bone, necessitating a high level of communication between the bone surface and the nonmineralized tissue.

### Bone Matrix

The key protein component of bone is type I collagen. Collagen fibers follow specific directions, forming the basis for the lamellar structure of bone. This lamellar structure, which can be visualized when examining bone in the polarized light, allows dense packaging, resulting in optimal resistance to mechanical load. The lamellar collagen structures can be assembled in parallel (e.g., along the cortical bone surfaces and inside the bony trabeculae) or concentrically around blood vessels embedded in the Haversian channels of the cortical bone. Upon rapid deposition of new bone, such as during fracture healing, this lamellar structure is missing, and the bone is then called *woven bone*. Woven bone is consecutively remodeled into lamellar bone, which is also considered "mature" bone. The composition of the collagen backbone also assists in the deposition of spindle- or plate-shaped hydroxyapatite crystals, which contain

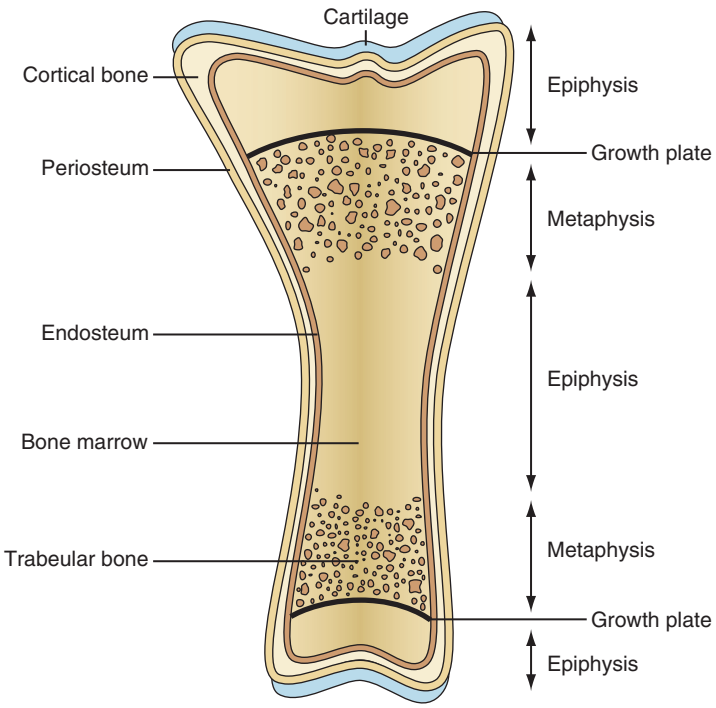



**Figure 4-1** Long bones consist of the epiphyses separated by growth plates from the metaphyses, which contain most of the trabecular bone. The outer lining of bone is the dense cortical bone, which is covered by the periosteum (outer surface) and the endosteum (inner surface). The latter connects bone to the bone marrow. The bony end plates are covered by the articular cartilage, consisting of a mineralized deep zone and a nonmineralized surface zone.

calcium phosphate, thus allowing the calcification of the bone matrix.

In addition to type I collagen, other so-called noncollagenous proteins also exist in bone. Some of them, such as osteocalcin, osteopontin, and fetuin, are mineralization inhibitors, which serve to balance the degree of mineralization of the skeletal tissue. Aside from their intrinsic function in bone, noncollagenous proteins also exert important metabolic functions, such as the control of energy metabolism by osteocalcin.

## Bone Cells: Osteoblasts

Osteoblasts are the bone-forming cells that derive from the mesenchymal stem cells of the bone marrow, which also form chondrocytes, myocytes, and adipocytes. Osteoblasts are cuboid-shaped cells that form clusters covering the bone surface. They are metabolically highly active, synthesizing the collagenous and noncollagenous bone matrix proteins that are excreted and then deposited between the osteoblasts and the bone surface. This newly built matrix, which is not yet calcified, is termed the *osteoid*. The lag phase between osteoid deposition and its mineralization is approximately 10 days. Osteoblast differentiation depends on the expression of two key transcription factors, Runx2 and its target Osterix 1, which confer the differentiation of these cells into osteoblasts in response to external stimuli.[1] Prostaglandin $E_2$ ($PGE_2$), insulin-like growth factor (IGF)-1, parathyroid hormone (PTH), bone morphogenic proteins (BMPs), and Wingless and Int-1 (Wnt) proteins are key stimuli for osteoblast differentiation.[2,3] For instance, $PGE_2$ is an important anabolic factor for bone and induces the expression of bone sialoprotein and alkaline phosphatase in mesenchymal cells. BMPs and transforming growth factor (TGF)-β, which shares structural similarities with BMPs, foster osteoblast differentiation by activating intra-cellular Smad proteins. Finally, Wnt proteins, a family of highly conserved signaling molecules, are potent stimulators of osteoblast differentiation. Wnt proteins bind to surface receptors on mesenchymal cells such as Frizzled and LRP5, eliciting activation and nuclear translocation of the transcription factor β-catenin, which induces the transcription of genes involved in osteoblast differentiation. Wnt proteins thereby act not only in close synergy with BMPs but also cross-talk to the receptor activator of nuclear factor-κB ligand (RANKL)–osteoprotegerin (OPG) system, which is involved in the differentiation and function of bone-resorbing osteoclasts.

## Bone Cells: Osteocytes

Osteocytes are by far the most abundant cell type within bone. One cubic millimeter of bone contains up to 25,000 osteocytes that are well connected with each other and the bone surface by small tubes (canaliculi), constituting a large and dense communication network inside the bone with similarities to the nervous system. The surface of this network of lacunae, which contains the osteocytes, and the canaliculi, which contains the interconnecting filaments of the osteocytes, covers an area of 1000 to 4000 square meters. Osteocytes are derived from osteoblasts, which are subsequently entrapped in the bone matrix.[4] Osteocytes, however, also start to express genes that are specific for these cells and are not found in other cells, such as osteoblasts. One of the

most interesting products of the osteocyte is sclerostin, a secreted molecule that binds lipoprotein receptor–related proteins (LRPs) and blocks Wnt-stimulated bone formation.[5] Consistent with its function as an inhibitor of bone formation, overexpression of sclerostin leads to low bone mass, whereas deletion of sclerostin leads to increased bone density and strength. This effect has recently been successfully used as a therapeutic strategy to increase bone mass by inhibiting sclerostin by a specific antibody.[6] Loss-of-function mutations in the human *SOST* gene that encodes sclerostin entail increased bone mass, a disease termed *sclerosteosis*.[7] Several local and systemic factors have been suggested as possible regulators of sclerostin expression by osteocytes. For instance, intermittent administration of PTH, which is associated with strong anabolic effects on the bone, potently inhibits sclerostin expression.

## Bone Cells: Osteoclasts

Osteoclasts are multinucleated cells that contain up to 20 nuclei and are unique in their ability to resorb bone.[8,9] They are directly attached to the bone surface and build resorption lacunae (Howship lacunae). Apart from their multiple nuclei, another characteristic of the osteoclast is the *ruffled border*, a highly folded plasma membrane facing the bone matrix and designed to secrete and resorb proteins and ions into the space between the osteoclast and bone surface (Figure 4-2). The space between this ruffled border and the bone surface is the place where bone resorption occurs. It is sealed by a ring of contractible proteins and tight junctions because it represents one of the few regions of the human body where a highly acidic milieu is found. Bone degradation by osteoclasts consists of two major steps: first, demineralization of inorganic bone components, and second, removal of organic bone matrix. To demineralize bone, osteoclasts secrete hydrochloric acid through proton pumps into the resorption lacunae. This proton pump requires energy that is provided by an adenosine triphosphatase (ATPase), allowing the enrichment of protons in the resorption compartment, which, in fact, represents an extra-cellular lysosome. In addition to protons and chloride, osteoclasts release matrix-degrading enzymes, including tartrate-resistant acid phosphatase (TRAP), lysosomal cathepsin K, and other cathepsins. Cathepsin K can effectively degrade collagens and other bone matrix proteins. Consequently, inhibitors of cathepsin K block osteoclast function and slow down bone resorption.

Osteoclasts originate from hematopoietic monocytic precursor cells and, upon the influence of specific signals, undergo a series of differentiation steps to become mature osteoclasts. Essential signals for osteoclast differentiation are macrophage colony-stimulating factor (M-CSF) and RANKL. During this differentiation and maturation process, osteoclasts acquire specific markers such as TRAP, fuse to multinucleated giant cells, and polarize on contact to bone. Osteoclastogenesis depends on an adequate microenvironment, which provides essential signals such as M-CSF and RANKL and certain cytokines such as tumor necrosis factor (TNF), which further enhance osteoclast differentiation. Mesenchymal cells such as preosteoblasts express M-CSF and RANKL and can induce osteoclast formation, highlighting the close interaction between bone formation and bone resorption.

RANKL, a TNF superfamily member, is a surface molecule expressed by osteocytes, preosteoblasts, and activated T cells.[10-12] Under steady-state conditions, its expression is induced in cells of the osteoblastic lineage in response to osteotropic factors such as vitamin D, PTH, and

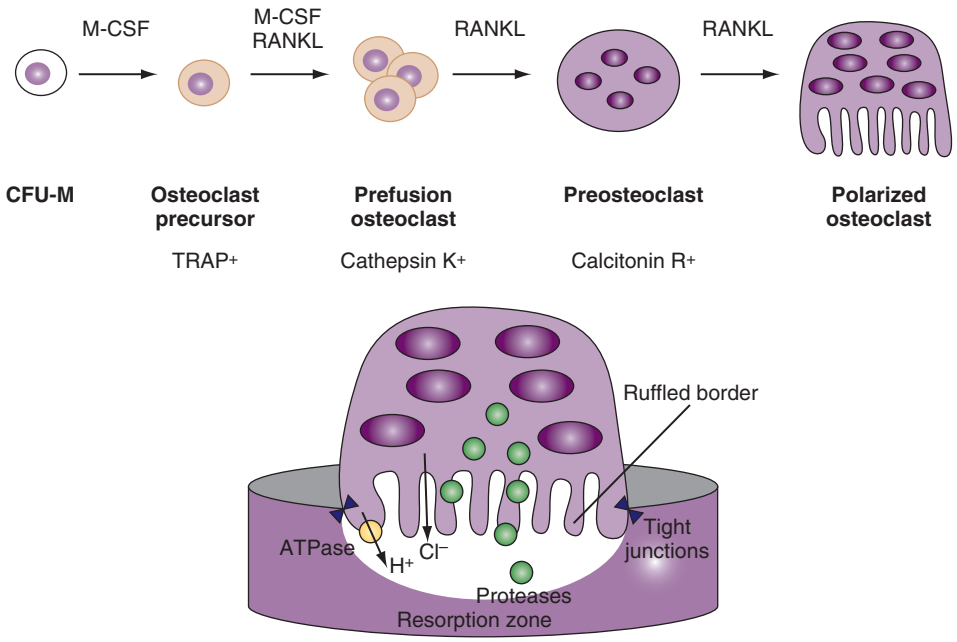



**Figure 4-2** Osteoclasts form from mononuclear precursors (colony-forming unit macrophages [CFU-M]), which differentiate into mononuclear osteoclast precursors. These cells fuse and build a polykaryon, forming a preosteoclast. Final differentiation is characterized by cell polarization and acquisition of a ruffled border. ATPase, Adenosine triphosphatase; M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-κB ligand; TRAP, tartrate-resistant acid phosphatase.

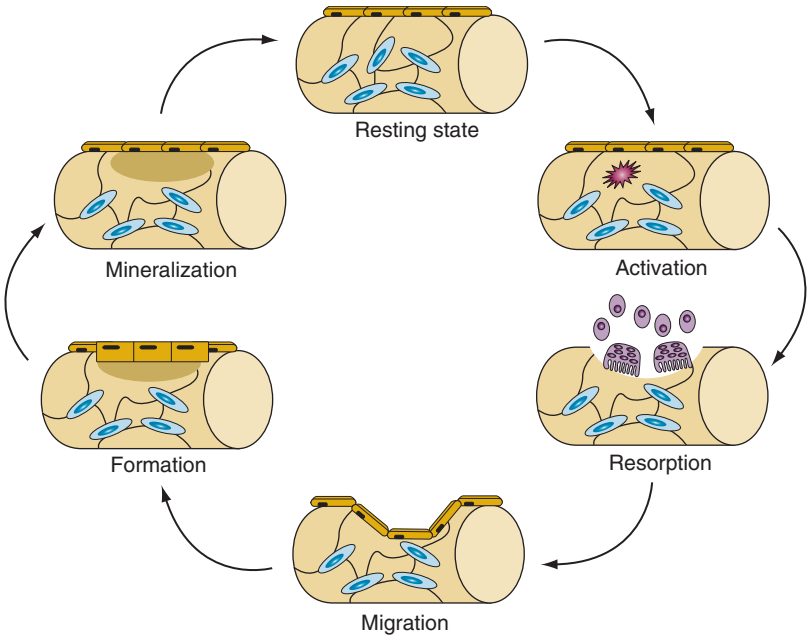



**Figure 4-3** Sequence of bone remodeling with *activation* characterized by sensing of damage by osteocytes, *resorption* by osteoclast differentiation and removal of bone matrix, *migration* of bone-lining mesenchymal cells into the lacuna, *formation* of new matrix by osteoblasts (cuboid cells), and *mineralization* of the newly synthesized matrix.

prostaglandins, whereby the nuclear factor peroxisome proliferator-activated receptor–beta represents an essential checkpoint in the regulation of RANKL expression.[13] Moreover, inflammatory cytokines such as TNF, interleukin (IL)-1, and IL-17 can induce RANKL expression.[14-17] RANKL is essential for the final differentiation steps of osteoclasts, as well as for their bone-resorbing capacity, by engaging its receptor RANK on monocytic osteoclast-precursor cells. The interaction of RANKL with its receptor RANK is modulated by OPG, a secreted glycoprotein, which has been identified as a soluble factor that strongly suppresses osteoclast differentiation both in vitro and in vivo. Interestingly, OPG expression is induced by estrogens, which explains the increase in osteoclast numbers and enhanced bone resorption during menopause. In accordance, RANKL-deficient mice display severe osteopetrosis as a result of the lack of osteoclasts. With regard to the central role of the RANKL-RANK-OPG signaling system in bone resorption, researchers are increasingly interested in the therapeutic targeting of this system in human disease, and recent clinical trials involving post-menopausal osteoporosis have revealed the potent anti-resorptive effect of a neutralizing RANKL antibody (denosumab).[18] Beyond RANKL-RANK interactions, other important pro-osteoclastogenic signaling pathways are based on the triggering receptor expressed on myeloid cells (TREM) 2, which interacts with the tyrosine kinase DAP12 and the osteoclast-associated immunoglobulin-like receptor (OSCAR). Both molecules are strong enhancers of osteoclastogenesis.[19]

## Bone Remodeling Process

Developmental bone growth, postdevelopmental maintenance and repair of bone, and provision of calcium from the bone depend on a dynamic process called *bone remodeling* (Figure 4-3). Factors yet unknown, which may likely be of mechanical nature and sensed by the osteocytes, initiate bone remodeling at a specific site. The death of osteocytes and the resulting metabolic changes leading to a lack of silencers for bone turnover, like sclerostin, may govern this activation process. It is followed by a resorptive phase dominated by osteoclast-mediated degradation of the bone matrix, resulting in a resorption lacuna. The naked bone surface inside this lacuna is subsequently populated by mesenchymal cells immigrating from the neighboring bone surface, which start differentiating into osteoblasts and produce the new bone matrix (also termed *osteoid*). This matrix then mineralizes, and the bone again returns to its resting state. This entire bone remodeling process takes about 3 to 6 months. Adults continuously remodel their skeleton, and this process occurs even faster during childhood and adolescence. In adults the entire skeleton is remodeled in 7 to 10 years, which indicates that we fully replace our skeleton several times during our lifetime. Most of the bone remodeling happens in the trabecular bone, which promotes the building of an optimal inner microstructure adapted to the individual mechanical demands. Trabecular bone is the leading structure in the vertebral bodies (constituting up to two-thirds of the bone substance) and in long bones such as the femurs (constituting about 50% of the bone substance).

Normal physiologic circumstances ensure a balance between bone formation and bone resorption to maintain skeletal homeostasis. This bone remodeling process requires a tight mutual regulation of bone resorption by osteoclasts and bone formation by osteoblasts, a phenomenon called *coupling*. Coupling is regulated on at least three different levels: (1) by a direct interaction between osteoblasts and osteoclasts; (2) by local interactions between the immune

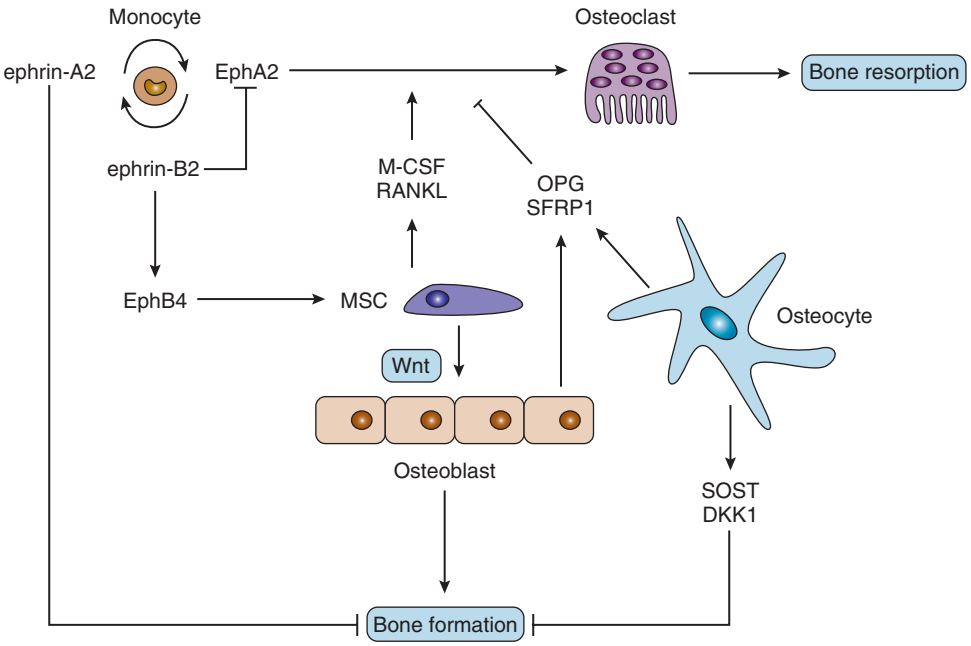



**Figure 4-4** Mesenchymal stem cells (MSCs) and preosteoblasts generated macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL), supporting osteoclast differentiation. In contrast, mature osteoblasts suppress osteoclast differentiation by expression of osteoprotegerin (OPG) and secreted frizzled-related protein 1 (SFRP1). The ephrin-A2 system is an autocrine stimulator for osteoclasts and also blocks bone formation. In contrast, the binding of ephrin-B2 to its receptor ephrin-B4 stimulates osteoblast differentiation. Osteocyte-derived mediators such as sclerostin (SOST) and dickkopf-1 (DKK1) suppress bone formation by inhibiting the Wnt pathway.

system and bone cells; and (3) by neuroendocrine systemic control of bone metabolism.

## Direct Interactions between Osteoblasts and Osteoclasts

A proper coupling between bone formation and bone destruction is essential to maintain bone integrity (Figure 4-4). This coupling process involves two main mechanisms; the first is the expression of the essential pro-osteoclastogenic cytokines by the osteoblast lineage, and the second involves the ephrin ligand/ephrin receptor bidirectional signaling.[20,21] Preosteoblasts are the main pro-osteoclastogenic cells in normal physiologic conditions and provide the first level of coupling between bone formation and bone resorption. In response to Wnt signaling, osteoblasts slowly lose their supportive activity for osteoclasts when they mature toward more mineralizing cells and then become the bone-embedded osteocytes. They then secrete antiosteoclastogenic molecules such as OPG and the Wnt inhibitors sclerostin, dickkopf-1, and secreted frizzled-related protein 1 (SFRP1), which either block osteoclast differentiation (OPG) or also inhibit the further differentiation of osteoblasts. A second level of coupling involves the expression of ephrin ligands on the surface of osteoclast progenitors that can bind to ephrin receptors and activate their tyrosine kinase activity. Two ephrin ligands regulate bone remodeling. The first, ephrin-B2, binds to the receptor EphB4 on osteoblast progenitors, increasing their differentiation and stimulating bone formation. The second, ephrin-A2, acts in an autocrine manner by binding to the EphA2 receptor on the osteoclasts, promoting their differentiation in a paracrine manner on the osteoblasts, thus inhibiting their differentiation.

## Bone Remodeling by the Immune System

Aside from the reciprocal regulation of osteoblasts and osteoclasts, bone remodeling is also controlled by the immune system. Insights into the control of bone by the immune system have led to a new research field known as *osteoimmunology*, which has defined new pathways involved in immune regulation of post-menopausal osteoporosis and bone loss during inflammatory diseases.[22-24] Importantly, T cells influence the differentiation of osteoclasts, and hence activated T cells express RANKL, which does not only stimulate osteoclastogenesis but also promotes the survival of dendritic cells.[25] RANKL expression has been found on various subsets of proliferative T cells (CD8 and CD4 and Th1 and Th2), as well as on FoxP3-expressing regulatory T cells (Tregs). Despite consistent expression of RANKL on various T cell lineages, individual T cells exert substantially different functions on the osteoclast, allowing a fine regulation of bone remodeling by the immune system. For instance, Th17 cells stimulate osteoclast differentiation via the production of IL-17.[17] Moreover, a subset of T cells expressing the IL-23 cytokine receptor is involved in bone remodeling, specifically at the insertion sites of the tendons.[26] In contrast, other T cell lineages express strong inhibitors of osteoclastogenesis, such as interferon (IFN)-γ in the case of Th1 cells, IL-4 in the case of Th2 cells, or cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) in the case of regulatory T cells, and thereby provide protection from bone loss.[27-30]

### Systemic Control of Bone Remodeling by Neuroendocrine Mechanisms

Bone remodeling is regulated not only locally but also by various systemic hormonal pathways, including the sex hormone and the growth hormone (GH)/IGF axes. In addition, two major systemic neuroendocrine regulators of bone homeostasis co-regulate bone, fat, and energy metabolism.[31,32] The two central players of this systemic loop seem to be osteocalcin and leptin. Osteocalcin is a hormone produced by mature osteoblasts and acts on the β cells of the pancreas to stimulate proliferation and thus insulin production in response to leptin.[32] In addition, osteocalcin can directly stimulate adipocytes to regulate insulin sensitivity. Leptin is a peptide hormone produced by adipocytes of the white adipose tissues. Leptin deficiency causes obesity and increased bone mass. Although the increased fat mass is certainly linked to the role of leptin in controlling appetite, the effect of leptin on bone and fat can be dissociated. Indeed, bone formation is negatively regulated by leptin through a hypothalamic pathway: the β-adrenergic sympathetic nervous system that mediates decreased osteoblast proliferation via the induction of clock genes in osteoblasts.[33,34] The question of how leptin regulates bone formation is debated. However, two potential hypothalamic relays have been identified. The first relay is the NPY peptide, a repressor of bone formation, which is silenced by leptin.[35] The second downstream modulator of leptin is neuromedin U, which inhibits clock gene expression and increases osteoblast proliferation.[36] These observations suggest that disturbance of the metabolic loops linking adipogenesis, osteogenesis, and insulin production have profound consequences on bone homeostasis.

## CONCLUSION

Bone is continuously remodeled by bone-resorbing osteoclasts and bone-forming osteoblasts. This remodeling process allows the optimal adaptation of the bone architecture to individual demands and tight control of calcium homeostasis. Local factors control the bone remodeling process on the basis of osteoclast-osteoblast interactions, as well as by systemic immune and neuroendocrine factors controlling the bone-resorbing and bone-forming cells.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 5



# Muscle: Anatomy, Physiology, and Biochemistry

Mark S. Miller • Bradley M. Palmer • Michael J. Toth • David M. Warshaw

**KEY POINTS**

- The structure and function of skeletal muscle and its neural recruitment pattern can change rapidly in response to activity level (i.e., plasticity).
- The smallest functional unit of muscle, the sarcomere, is composed of an almost crystalline array of filamentous proteins that convert metabolic energy into force and movement.
- Muscles are attached to the skeleton through collagenous tendons.
- Skeletal muscle contraction is controlled by the central nervous system through depolarization of specific efferent neurons called *motor neurons.*
- Motor neurons innervate and depolarize muscle fibers through cholinergic synapses called *neuromuscular junctions.*
- Afferent neurons provide the central nervous system with sensory information required for effective control of movement and posture.
- Force is transmitted to the exterior through two sets of protein cell adhesion complexes: integrins and dystroglycans.

Approximately 660 skeletal muscles support and move the body under the control of the central nervous system. These muscles constitute up to 40% of adult human body mass. Most skeletal muscles are fastened by collagenous tendons across joints in the skeleton. The transduction of chemical energy into mechanical work by muscle cells leads to muscle shortening and consequent movement. A high degree of specialization in this tissue is evident from the intricate architecture and kinetics of intra-cellular membrane systems, the contractile proteins, and the molecular components that transmit force extra-cellularly to the basement membrane and tendons. Muscle cells normally exhibit wide variations in activity level and are able to adapt in size, isoenzyme composition, membrane organization, and energetics. In pathologic states, they often become deconditioned. These examples of plasticity can be surprisingly swift and extensive. This chapter outlines the structure and function of muscle and its relationship to associated connective tissue. It also introduces the basis for the highly adaptive response to altered functional demands and diseases. Two excellent Web sites can be accessed for further information in these areas.[1,2]

## STRUCTURE

### Muscle Tissue

Parallel, aligned bundles of skeletal muscle fibers make up approximately 85% of muscle tissue and consist of a variety of signaling and contractile proteins (Table 5-1). Nerves, blood supply, and connective tissue structures that provide support, elasticity, and force transmission to the skeleton (see later discussion) constitute the remaining volume. Muscle fibers range in length from a few millimeters to 30 cm and in diameter from 10 to 500 μm, with a typical length of 3 cm and diameter of 100 μm. This elongated shape is determined by the organization of the contractile proteins that occupy most of the sarcoplasm. Each muscle has a limited range of shortening that is amplified into large motions by lever systems of the skeleton, usually operating at a mechanical disadvantage. Variations in geometric arrangements of the fibers—parallel, convergent (fan-shaped), pennate (feather-like), sphincter (circular), or fusiform (thick in the middle with tapered ends)—determine some of the mechanical properties. For example, a muscle with fibers aligned parallel to the force-generating axis will have more basic contractile units (i.e., sarcomeres, as discussed later) in series than a similarly sized pennate muscle, thus allowing the parallel muscle to contract more quickly, but with less force, than the pennate muscle. Muscles designed for strength (e.g., gastrocnemius) are typically pennate, whereas those designed for speed (e.g., biceps) tend to have parallel fibers. Muscles are commonly arranged around joints as antagonistic pairs facilitating bidirectional motion. When one muscle (the agonist) contracts, another (its antagonist) is relaxed and passively extended. Their roles reverse to actively generate the opposite motion, unless it occurs passively by the force of gravity.

An extensive network of areolar connective tissue, forming the endomysium, surrounds each muscle fiber. Fine nerve branches and small capillaries, which are necessary for the exchange of nutrients and metabolic waste products, penetrate this layer. The endomysium is continuous with the perimysium, a connective tissue network that ensheathes small parallel bundles of muscle fibers known as *fasciculi*, *intrafusal fibers*, *larger nerves*, and *blood vessels*. The epimysium encompasses the entire muscle. All three layers of connective tissue contain collagen—mostly types I, III, IV, and V—with types IV and V predominating in the basement membranes surrounding each skeletal muscle fiber. The

**TABLE 5-1** Signaling and Contractile Proteins of Skeletal Muscle

| Protein | Molecular Weight (kDa) | Subunits (kDa) | Location | Function |
|---|---|---|---|---|
| Acetylcholine receptor | 250 | 5 × 50 | Postsynaptic membrane of neuromuscular junction | Neuromuscular signal transmission |
| Annexins | 38 | — | F-actin–binding protein | Membrane repair |
| Dihydropyridine receptor | 380 | 1 × 160<br>1 × 130<br>1 × 60<br>1 × 30 | T-tubule membrane | Voltage sensor |
| Dysferlin | 230 | — | Periphery of myofibers | Membrane repair |
| Ryanodine receptor | 1800 | 4 × 450 | Terminal cisternae of SR | SR $Ca^{2+}$ release channel |
| $Ca^{2+}$ ATPase | 110 | — | Longitudinal SR | Uptake of $Ca^{2+}$ into the SR |
| Calsequestrin | 63 | — | Lumen of SR terminal cisternae | Binding and storage of $Ca^{2+}$ |
| Troponin | 70 | 1 × 18<br>1 × 21<br>1 × 31 | Thin filament | Regulation of contraction |
| Tropomyosin | 70 | 2 × 35 | Thin filament | Regulation of contraction |
| Myosin | 510 | 2 × 220<br>2 × 15<br>2 × 20 | Thick filament | Chemomechanical energy transduction |
| Actin | 42 | — | Thin filament | Chemomechanical energy transduction |
| MM creatine phosphokinase | 40 | — | M line | ATP buffer, structural protein |
| α-Actinin | 190 | 2 × 95 | Z line | Structural protein |
| Titin | 3000 | — | From Z line to M line | Structural protein |
| Nebulin | 600 | — | Thin filaments, in the I band | Structural protein |
| Dystrophin | 400 | — | Subsarcolemma | Structural integrity of sarcolemma |

ATP, Adenosine triphosphate; ATPase, adenosine triphosphatase; SR, sarcoplasmic reticulum.

$\alpha1_2\alpha2$-chain composition of the collagen IV isoform is the most prevalent and provides the mechanical stability and flexibility of the basil lamina.[3,4] The perimysium and endomysium merge at the junction between muscle fibers and tendons, aponeuroses, and fasciae. These layers give the attachment sites great tensile strength and distribute axial force into shear forces over a larger surface area.

## Fiber Types

Muscles adapt to their specific functions. In any given muscle, part of this adaptation arises from its composition and organization of fiber types. Human skeletal muscle fibers can be classified according to their myosin heavy chain (MHC) isoform (I, IIA, or IIX). MHC molecules break down adenosine triphosphate (ATP) to produce the energy necessary for muscle contraction. The rate of ATP breakdown, or the adenosine triphosphatase (ATPase) rate, of the MHC isoforms is I < IIA < IIX, leading to contractions that are relatively slow in MHC I fibers, fast in MHC IIA fibers, and very fast in MHC IIX fibers. ATP synthesis primarily occurs through aerobic respiration (i.e., oxygen is required) in MHC I (slow oxidative) fibers. ATP synthesis is aided by the nature of MHC I fibers in that they have more mitochondria, an increased capillary blood supply, and more myoglobin compared with MHC IIX (fast glycolytic) fibers, which use anaerobic respiration (i.e., oxygen is not required) to restore their ATP levels. MHC I fibers produce less power than MHC IIX fibers but are more resistant to fatigue. MHC IIA (fast oxidative-glycolytic) fibers can use both aerobic and anaerobic respiration and fall in between I and IIX fibers in terms of mitochondria, blood supply, myoglobin, power output, and fatigability. Although most human skeletal muscles contain a mixture of fiber types, MHC I fibers are more prominent in muscles used for posture and endurance, and MHC IIX fibers dominate in muscles used for quick movements of short duration. Notably, individual fibers can contain a mixture of MHC isoforms, leading to six different fiber types in humans ("pure": MHC I, IIA, and IIX; "mixed": MHC I/IIA, IIA/IIX, and I/IIA/IIX), which allow for a wide range of contractile properties. A list of various attributes of MHC isoforms can be found in Table 5-2.

During development, fiber-type specificity may be partially determined before innervation.[5] Although the biologic events and signals responsible for designating functional specialization in muscle fibers are not fully understood, classic cross-innervation experiments demonstrated that innervation can dynamically specify and modify the type of muscle fiber.[6] After cross-innervation, the functional and histologic properties listed in Table 5-2 shift toward the target fiber type over a few weeks' time, indicating the ability of muscles to adapt and remodel in accordance with the pattern of neuronal activity.

**TABLE 5-2** Classification of Muscle Fiber Types by Myosin Heavy Chain Isoform

| General Features | MHC I | MHC IIA | MHC IIX |
|---|---|---|---|
| Mitochondria | Many | Intermediate | Few |
| Capillary blood supply | Extensive | Moderate | Moderate |
| SR membrane | Sparse | Extensive | Extensive |
| Z line | Wide | Moderate | Narrow |
| **Protein Isoforms** | | | |
| Myosin essential light chain | Slow and fast | Fast | Fast |
| Myosin regulatory light chain | Slow and fast | Fast | Fast |
| Myosin binding protein–C | Slow | Fast | Fast |
| Thin filament regulatory proteins | Slow | Fast | Fast |
| **Mechanical Properties** | | | |
| SR calcium ATPase rate | Slow | Fast | Fast |
| Actomyosin ATPase rate | Slow | Fast | Very fast |
| Contraction time | Slow | Fast | Very fast |
| Shortening velocity | Slow | Fast | Very fast |
| Power production | Low | Moderate | High |
| Resistance to fatigue | High | Moderate | Low |
| **Metabolic Profile** | | | |
| Oxidative capacity | High | Moderate | Low |
| Glycolytic capacity | Moderate | High | High |
| Glycogen | Low | High | High |
| Myoglobin | High | Moderate | Low |

ATPase, Adenosine triphosphatase; MHC, myosin heavy chain; SR, sarcoplasmic reticulum.

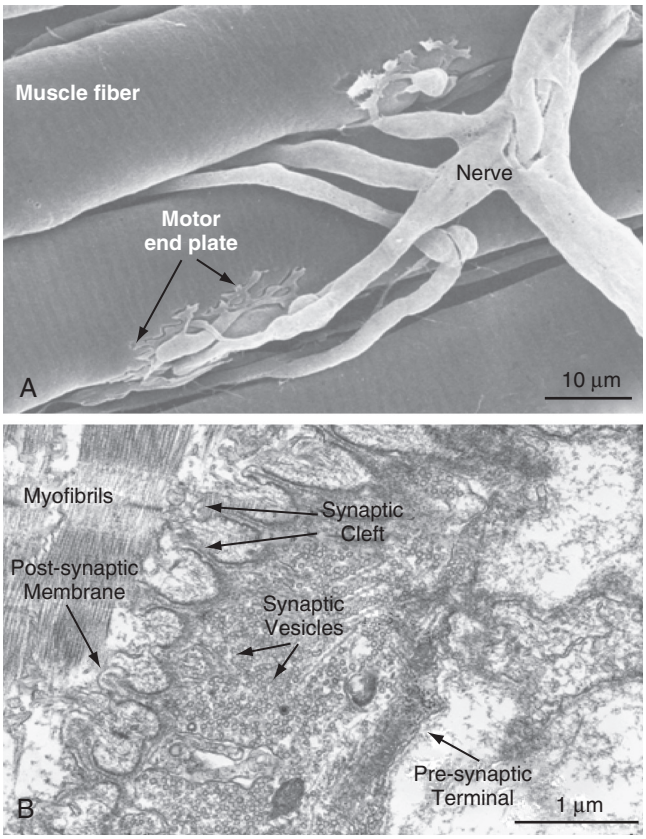



**Figure 5-1** Neuromuscular junction. **A,** Scanning electron micrograph of an α motoneuron innervating several muscle fibers in its motor unit. Calibration bar = 10 µm. **B,** Transmission electron micrograph. Calibration bar = 1 µm. *(**A,** From Bloom W, Fawcett DW:* A textbook of histology, *ed 10, Philadelphia, 1975, WB Saunders. **B,** Courtesy Dr. Clara Franzini-Armstrong, University of Pennsylvania, Philadelphia.)*

## EVENTS DURING MUSCLE CONTRACTION

### Neural Control

Voluntary control of muscle activation is a complex process. Afferent neurons emanating from sensory organs, such as cutaneous mechanoreceptors and thermoreceptors, pain receptors, joint receptors, and tendon organ and muscle spindles, provide the central nervous system (CNS) with stimuli in the form of action potentials that, with or without additional stimuli from the brain, provide the necessary information for feedback control of effector organs via efferent neurons.[7] Efferent neurons are specifically called *motor neurons* if their axons innervate muscle. Many times, more afferent than efferent neurons afford effective feedback control of movement and posture. The afferent and efferent neurons are accompanied by Schwann cells, which are glial cells residing in the peripheral nervous system.[8] Neurons are termed *myelinated* if Schwann cells wrap around the axon at regularly spaced intervals. The points of bare axon between these Schwann cells are called *nodes of Ranvier*. Myelination enhances the velocity of action potential propagation by compelling a saltatory conduction of the action potential between neighboring nodes. Schwann cells may also fully or nearly fully cover the axon, thus rendering the neuron unmyelinated and relatively slow in action potential propagation. Three groups of myelinated motor neurons (α, β, and γ) are distinguished by diameter, propagation velocity, and target fiber type. Skeletal muscle fibers typically are innervated at several neuromuscular junctions along their length by branches of an α motoneuron (the largest and fastest) or a β motoneuron (Figure 5-1). Muscle spindles are innervated by β or γ motoneurons, in addition to the afferent system, for sensing muscle length and force. A single motor neuron and the muscle fibers it innervates constitute a motor unit. When a motor neuron is excited, all fibers in the motor unit are triggered to contract simultaneously. A motor unit responsible for fine movement contains few muscle fibers, but motor units for gross movement generally contain many fibers. The level of muscle activation is controlled from the CNS by the number of motor units recruited and the stimulus rate.[9] Stimulus rate can be so infrequent as to elicit single muscle twitches, such as occur with the monosynaptic stretch reflex involving patellar tendon stretch and quadriceps activation, or conversely the stimulus rate can be so frequent that individual twitches effectively fuse, causing nearly continuous activation of muscle force.[10]

## Neuromuscular Transmission

At the neuromuscular junction, the axon tapers, loses its myelin sheath, and ends as a pre-synaptic terminal crowded with vesicles that contain the neurotransmitter acetylcholine. The postsynaptic membrane of the muscle is indented into folds that increase its surface area and the number of nicotinic acetylcholine receptors bound therein (see Figure 5-1). The junctional cleft is a 20 to 40 nm-wide space between the pre-synaptic and postsynaptic membranes.[11] When the motor neuron action potential reaches the pre-synaptic terminal, local voltage-gated $Ca^{2+}$ channels open and extra-cellular $Ca^{2+}$ streams into the terminal. Within milliseconds of $Ca^{2+}$ influx, the acetylcholine-loaded vesicles fuse with the pres-ynaptic membrane.[12] Exocytosed acetylcholine rapidly diffuses across the junctional cleft and binds to the nicotinic acetylcholine receptors, which in turn open $Na^+$ and $K^+$ channels of the postsynaptic membrane. The membrane is locally depolarized. An action potential is initiated and propagates along the muscle membrane (sarcolemma) at velocities up to 5 m/sec.

## Excitation-Contraction Coupling

A network of tubules invaginate the sarcolemma and run deep into the muscle fiber. This transverse tubule network (T-tubules) pervades the fiber at regular intervals, coinciding with sarcomere boundaries along the length of the muscle, and surrounds the contractile apparatus with connected longitudinal and lateral segments (Figure 5-2). The lumen of this network is open to the extra-cellular space and contains high $Na^+$ and low $K^+$ concentrations of interstitial fluid.[13] Action potentials at the surface membrane invade the entire T-tubular system. A specialized type of endoplasmic reticulum forms an entirely intra-cellular membrane system termed the *sarcoplasmic reticulum* (SR). Prevalent structures containing a T-tubule flanked by two terminal cisternae of the SR to form junctional complexes are termed *triads* (see Figure 5-2). Terminal cisternae contain oligomers of the $Ca^{2+}$-binding protein calsequestrin that provide the fiber with an internal reservoir of calcium ions. $Ca^{2+}$ channels, termed *dihydropyridine receptors* (DHPRs), are localized in the T-tubule membranes facing the cytoplasmic domain of SR $Ca^{2+}$ release channels, also called *ryanodine receptors* (RyRs), in the terminal cisternae membranes.[14] These membrane proteins are further characterized in Table 5-1.

When an action potential depolarizes the T-tubular membrane, the DHPRs, primarily voltage sensors in skeletal muscle, transfer a signal from the T-tubules to the RyRs by direct interprotein coupling. $Ca^{2+}$ is then released cooperatively through the RyRs from the SR into the myoplasm, where it activates the contractile machinery.[15] This sequence of events is termed *excitation-contraction coupling*.

Mutations in the α subunit of the DHPR in dysgenic mice lead to paralysis, because in these mutants, depolarization of the skeletal muscle membrane does not initiate release of $Ca^{2+}$ from the SR. Excitation-contraction coupling can be restored in cultured cells from these mice by transfection with complementary deoxyribonucleic acid (DNA) encoding for the DHPR,[16] and transfections using chimeric constructs[17] have pinpointed the domain within the DHPR that specifies skeletal- or cardiac-type excitation-contraction coupling.[18] Isoforms of the RyRs also help determine the characteristics of coupling between T-tubules and the SR.[19] Channelopathies in human skeletal and heart muscle have been linked to DHPR mutations.[20,21] Human malignant hyperthermia occurs in persons with mutant RyRs that become trapped in the open state after exposure to halothane anesthetic agents.[22]

## Contractile Apparatus

The specific locations and functions of the contractile proteins are listed in Table 5-1. Myofibrils (Figure 5-3D) are long, 1 μm diameter cylindrical organelles that contain the contractile protein arrays responsible for work production, generation of force, and shortening. Each myofibril is a column of sarcomeres, the basic contractile units, that are approximately 2.5 μm in length and are delimited by Z lines (Figure 5-3D and E) containing the densely packed structural protein α-actinin. The contractile and structural proteins within each sarcomere form a highly ordered, nearly

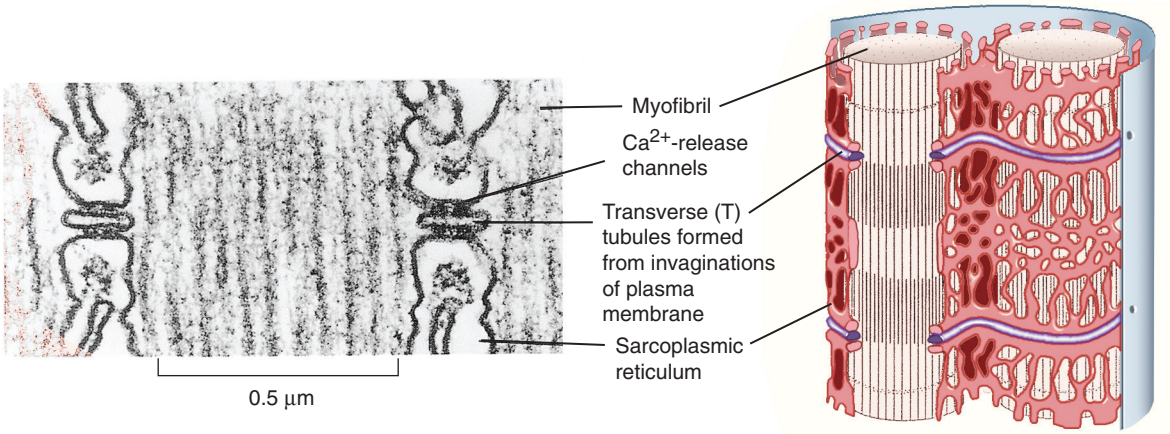



**Figure 5-2** Membrane systems that relay the excitation signal from the sarcolemma to the cell interior. In the electron micrograph, two T-tubules are cut in cross-section. Electron densities spanning the gap between T-tubules and sarcoplasmic reticulum membranes are the ryanodine receptors, which are channels that release calcium into the myoplasm. *(From Alberts B, Bray D, Lewish J, et al:* Molecular biology of the cell, *ed 2, New York, 1989, Garland Publishers. Micrograph courtesy Dr. Clara Franzini-Armstrong, University of Pennsylvania, Philadelphia.)*

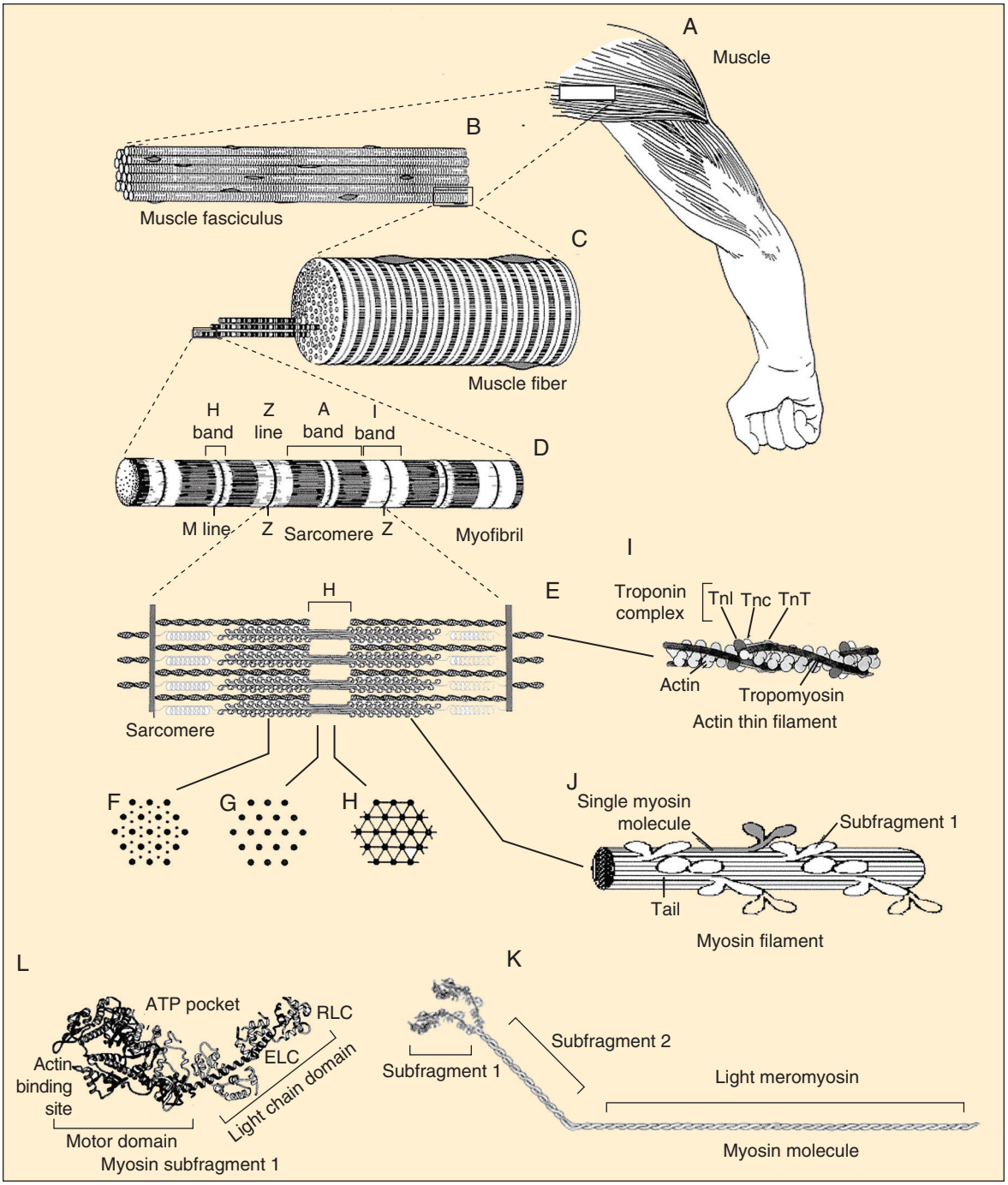



**Figure 5-3** Components of the contractile apparatus at successively increasing magnification from the whole muscle **(A)** to the molecular level (**I** through **L**). The myofibril **(D)** shows the banding pattern created by the lateral alignment of myofilaments (**I** and **J**) within the sarcomeres (**D** and **E**). Diagrams **F** to **H** show the cross-sectional structure of the filament lattice at various points within the sarcomere. Myosin is shown at the single two-headed molecule level **(K),** and the crystal structure of the globular motor domain is shown (**L** and subfragment 1) with the essential and regulatory light chains. ATP, Adenosine triphosphate; ELC, essential light chain; RLC, regulatory light chain. *(**A** through **K**, Modified from Bloom W, Fawcett DW: A textbook of histology, ed 11, Philadelphia 1986, WB Saunders; and **L** from Rayment I, Rypniewski WR, Schmidt-Base K, et al: Three-dimensional structure of myosin subfragment-1: a molecular motor.* Science *261:50–58, 1993.)*

crystalline lattice of interdigitating thick and thin myofilaments[23] (Figure 5-3E, I, and J). Myofilaments are remarkably uniform in both length and lateral registration, even during contraction,[24] resulting in the cross-striated histologic appearance of skeletal and cardiac muscles. This highly periodic organization has facilitated biophysical studies of muscle by sophisticated structural[23] and spectroscopic techniques.[25,26]

Thick filaments (1.6 μm long) that contain the motor protein myosin are located in the center of the sarcomere in the optically anisotropic A band (Figure 5-3D). These thick filaments are organized into a hexagonal lattice

stabilized by M protein[27] and muscle-specific creatine phosphokinase[28] in the M line (Figure 5-3D and E). Myosin (Figure 5-3K) is a highly asymmetric 470 kDa protein containing two 120 kDa globular $NH_2$-terminal heads, termed *cross-bridges* or *subfragment-1* (S1) (Figure 5-3L), and an α-helical coiled-coil rod, light meromyosin (Figure 5-3K). Two light chains, essential and regulatory, ranging from 15 to 22 kDa, are associated with the heavy chain in each S1 (Figure 5-3L). The rod portions of approximately 300 myosin molecules polymerize in a three-stranded helix to form the backbone of each thick filament (Figure 5-3J). The cross-bridges that protrude from these backbones contain ATPase and actin-binding sites responsible for the conversion of chemical energy into mechanical work. Besides their role in muscle contraction, at least 20 classes of nonmuscle myosins accomplish diverse tasks in cell motility such as chemotaxis, cytokinesis, pinocytosis, targeted vesicle transport, and signal transduction.[29] Thus myosin is the target for mutations, leading to a number of inherited muscle and neurologic diseases.[30,31]

Thin filaments (Figure 5-3I) are double-stranded helical polymers of actin that extend 1.1 μm from each side of the Z line and occupy the optically isotropic I band (Figure 5-3D and E). A regulatory complex containing one tropomyosin molecule and three troponin subunits (TnC, TnT, and TnI) is associated with each successive group of seven actin monomers along the thin filament (Figure 5-3I).[23] In the region where the thick and thin filaments overlap, the thin filaments are positioned within the hexagonal lattice equidistant from three thick filaments (Figure 5-3F). Both sets of filaments are polarized. In an active muscle, an interaction between the two filaments causes a concerted translation of the thin filaments toward the M line that shortens the sarcomere, and thus the muscle fiber and the entire muscle (Figure 5-3A through D). Actin is ubiquitous in the cytoskeleton of eukaryotic cells and, like myosin, fulfills many roles in determining cell shapes and motions.[32,33] Control of the actin cytoskeleton and diseases due to mutations in actin-binding proteins are being intensively investigated.[34]

Two of the largest identified muscle proteins, titin and nebulin, function in assembly and maintenance of the sarcomeric structure. Individual titin molecules (~3000 kDa) are associated with the thick filament and extend from the M line to the Z line.[35] Titin contains repeating fibronectin-like immunoglobulin and unusual proline-rich domains that confer molecular elasticity on the resting sarcomere.[36] Nebulin (~800 kDa) is associated with the Z line and thin filaments.[35] Protein connections from the contractile apparatus through the sarcolemma to the extra-cellular matrix are described later in this chapter. The cytoskeleton of muscle fibers also contains cytoplasmic actin, microtubules, and intermediate filaments.[37]

## Force Generation and Shortening

At rest, the thin filament regulatory proteins, troponin and tropomyosin, inhibit contraction (Figure 5-3I). During a twitch, $Ca^{2+}$ released from the SR binds to TnC, relieving this inhibition and thus allowing cross-bridges to attach to actin. A contraction results from cyclic interaction between actin and myosin (the cross-bridge cycle) that produces a relative sliding force between thin and thick filaments.[38] The energy source is the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate ($P_i$).

A simplified model of the chemomechanical events in the cross-bridge cycle is illustrated in Figure 5-4. Motor proteins, including myosin, can now be studied by

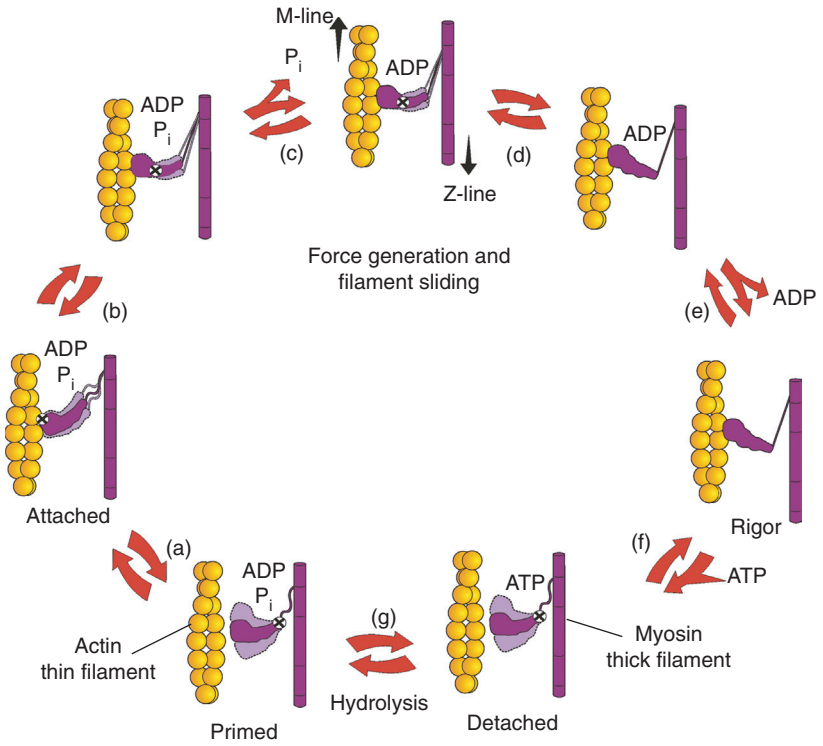



**Figure 5-4** The actomyosin cross-bridge cycle. Myosin molecules normally have two globular head regions (cross-bridges), but for clarity only one is shown. The ⊗ within the globular domain of myosin represents a hinge point with maximum flexibility. Each head binds with two actin monomers. The sequence of reactions consists of attachment (a); the force-generating transition (b); $P_i$ release (c); force generation and filament sliding (d); ADP release (e); ATP binding and detachment (f); and ATP hydrolysis (g). The *lighter shaded* heads near detached and force-generating myosin heads indicate mobility of cross-bridges in these states. ADP, Adenosine diphosphate; ATP, adenosine triphosphate; $P_i$, inorganic phosphate.

single-molecule biophysical techniques, which provide unprecedented detail on their dynamics.[39] When $Ca^{2+}$ is present, a complex of myosin, ADP, and $P_i$ attaches to the thin filament (step a), and structural change within the myosin S1 initiates force production and the release of $P_i$ (steps b and c).[40,41] The conformational change in the cross-bridge that leads to generation of force is a tilting motion of the light chain region.[42,43] Filament sliding that leads to shortening of the sarcomere occurs during a strain-dependent transition between two ADP states (step d). After ADP is released (step e), ATP binds to the active site and dissociates myosin from actin (step f). Myosin then hydrolyzes ATP (step g) to form the ternary myosin–ADP-$P_i$ complex, which can reattach to actin for the next cycle.

If the mechanical load on the muscle is high, the contractile apparatus produces a force without changing length (an isometric contraction). If the load is moderate, the thin filaments slide actively toward the center of the sarcomere, resulting in shortening of the entire muscle. The width of the muscle increases during shortening, so the volume stays constant. Work production (concomitant force and sliding) is associated with an increase in the ATPase rate. Thermodynamic efficiency (mechanical power divided by energy liberated by ATPase activity) approaches 50%—a remarkable figure given that manufactured combustion engines seldom achieve efficiencies greater than 20%.

### Relaxation

The twitch is terminated by reversal of all steps in the activation. $Ca^{2+}$ released from the SR is taken up again by $Ca^{2+}$-ATPase pumps located in longitudinal membranes of the SR. The myoplasmic $Ca^{2+}$ concentration then decreases, and $Ca^{2+}$ dissociates from TnC, deactivating the thin filament. When the number of attached cross-bridges declines below a certain threshold, tropomyosin inhibits further cross-bridge attachment, and tension declines to the resting level. $Ca^{2+}$ diffuses within the longitudinal SR to calsequestrin sites in the terminal cisternae, ready to be released in the next twitch. Myosin continues to hydrolyze ATP at a low rate in relaxed muscle, accounting for a sizable proportion of basal metabolism.

## TRANSMISSION OF FORCE TO THE EXTERIOR

### Cell-Matrix Adhesions

The muscle cell is closely connected to the basal lamina along its entire surface. Several transmembrane macromolecular complexes link the myofibrils and actin cytoskeleton to laminins and collagen in the extra-cellular matrix. Attachment complexes for muscle, analogous to the focal adhesions of motile and epithelial cells and to the adhesion plaques and intercalated disks in cardiac muscle, contain filamentous actin, vinculin, talin, and integrin (primarily the α7β1 isoform), which is the transmembrane link to laminin (Figure 5-5). In muscle, the main laminin isoforms are laminin-2 (α2β1γ1) and laminin-4 (α2β2γ1), which collectively are termed *merosin*. In addition to providing mechanical coupling between the cytoskeleton and the extra-cellular matrix, the laminin-integrin system may

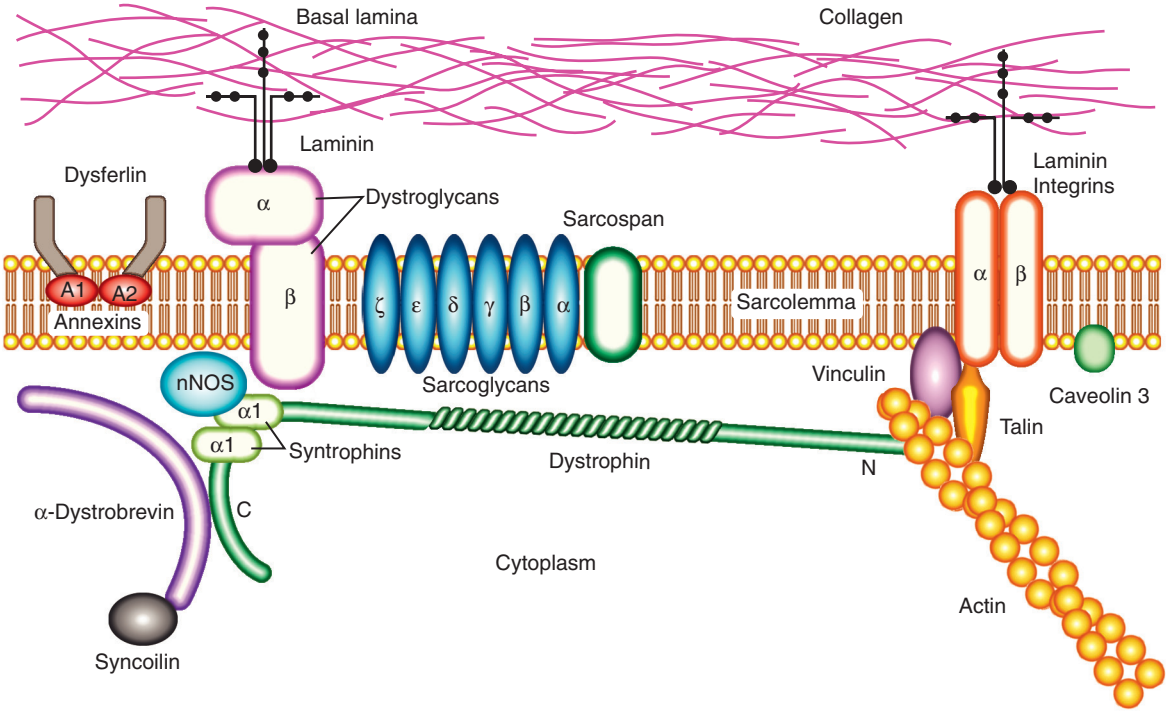



**Figure 5-5** Connections between the muscle cytoskeleton and the extra-cellular matrix. Actin is linked through integrins to the matrix, as in many cell types. Dystrophin forms an extra link through the dystroglycan-sarcoglycan complex of glycosylated proteins. The helical section of dystrophin is homologous to spectrin and may form homodimers or oligomers. Dystrophin links two intricate systems linking the sarcolemma to the basal lamina. The carboxy (COOH)-terminus of dystrophin is associated with the sarcoglycans, dystroglycans, dystrobrevin, syncoilin, neuronal nitric oxide synthase (nNOS), and the syntrophins. The $NH_3$-terminus links actin, vinculin, and the integrins with laminin and the basil lamina. These two adhesion systems provide a supportive substructure to maintain the integrity of the sarcolemma. The annexins and dysferlin have a role in muscle regeneration.

**TABLE 5-3** Classification of Muscular Dystrophies

| Disease | Genetic Locus | Inheritance | Protein | Outcome |
|---|---|---|---|---|
| Duchenne/Becker | | XR | Dystrophin | Lethal |
| Emery-Dreifuss | | XR | Emerin, lamins A and C | 40% lethality |
| **Limb-Girdle Muscular Dystrophies** | | | | |
| LGMD 1A | 5q31 | AD | Myotilin | With LGMD, less-severe forms can emerge during the first three decades, leading to loss of ambulation after 30 yr of age; the most severe forms start at 3-5 yr of age and progress rapidly |
| LGMD 1B | 1q11-q21 | AD | Laminin A/C | |
| LGMD 1C | 3p35 | AD | Caveolin | |
| LGMD 1D | 6q23 | AD | — | |
| LGMD 1E | 7q | AD | — | |
| LGMD 1F | 7q32 | AD | — | |
| LGMD 1G | 4p21 | AD | — | |
| LGMD 2A | 15q15.1-q21.1 | AR | Calpain 3 | |
| LGMD 2B | 2p13 | AR | Dysferlin | |
| LGMD 2C | 13q12 | AR | γ-Sarcoglycan | |
| LGMD 2D | 17q12-q21.33 | AR | α-Sarcoglycan | |
| LGMD 2E | 4q12 | AR | β-Sarcoglycan | |
| LGMD 2F | 5q33-q34 | AR | δ-Sarcoglycan | |
| LGMD 2G | 17q11-q12 | AR | Telethonin | |
| LGMD 2H | 9q31-q34.1 | AR | E3-Ubiquitin ligase (TRIM32) | |
| LGMD 2I | 19q13.3 | AR | Fukutin-related protein | |
| LGMD 2J | 2q24.3 | AR | Titin | |
| LGMD 2K | 9q34 | AR | Protein O-mannosyltransferase | |
| **CMDs with CNS Involvement** | | | | |
| Fukuyama CMD | 9q31 | AR | Fukutin | LE, 11–16 yr |
| Walker-Warburg CMD | 1p32 | AR | O-Mannosyltransferase | LE, <3 yr |
| Muscle-eye-brain CMD | 1p32-34 | AR | O-MNAGAT | LE, 10–30 yr |
| **CMDs without CNS Involvement** | | | | |
| Merosin-deficient classic type | 6q2 | AR | Merosin (laminin $A_2$) | Many patients never walk; others have an LGMD pattern |
| Merosin-positive classic type | 4p16.3 | AR | Selenoprotein N1, collagen VI $\alpha_2$ | Course stabilizes in late childhood; many continue to walk into adulthood |
| Integrin-deficient CMD | 12q13 | AR | Integrin α7 | Presents early in infancy with hypotonia and delayed milestones |
| **Other Dystrophies** | | | | |
| Facioscapulohumeral | 4q35 | AD | — | 20% wheelchair bound |
| Oculopharyngeal | 14q11.2-q13 | AD/AR | Polyadenylate binding protein nuclear 1 | Onset: ~48 yr, 100% symptomatic by age 70 yr |
| Myotonic dystrophy | 19q13.3 | AD | DMPK, CCHC-type zinc finger and CNBP | Onset: 50% show signs by age 20 yr; variable severity |

AD, Autosomal dominant; AR, autosomal recessive; CCHC, cysteine and histidine amino acid sequence in this class of zinc finger; CMD, congenital muscular dystrophy; CNBP, cellular nucleic acid–binding protein; CNS, central nervous system; DMPK, dystrophia myotonica-protein kinase; LE, life expectancy; LGMD, limb-girdle muscular dystrophy; O-MNAGAT, O-mannose β-1,2-*N*-acetylglucosaminyl transferase; XR, X chromosome–related.

provide a signaling pathway to regulate localized protein expression.[44] Defects in the expression of many of the cytoskeletal proteins lead to various forms of muscular dystrophy, as summarized in Table 5-3.[45]

A specialized linkage between the cytoskeleton and the basal lamina in muscle, complementary to the integrin focal adhesion system, is the dystrophin-glycoprotein complex (see Figure 5-5). Dystrophin, a 427 kDa peripheral cytoskeletal protein, has been postulated to function as a mechanical link between the cytoskeleton and the cell membrane or as a shock absorber, or to contribute mechanical strength to the membrane. Its absence or truncation causes Duchenne and Becker muscular dystrophies.[46] The N-terminus of dystrophin binds to actin via a region with sequence homology to the actin-binding domain of α-actinin. This end of the dystrophin molecule may be linked to the basal lamina via the same proteins described previously for focal adhesion-like complexes (see Figure 5-5). The C-terminus binds to a transmembrane dystroglycan-sarcoglycan complex, which in turn binds to the laminins. Muscular dystrophies of varied severity are associated with loss of these components (see Table 5-3).[47] Dystroglycans are also required for early embryonic development of muscle, possibly organizing laminin localization and assembly.[48,49] Utrophin, a smaller dystrophin-related protein (395 kDa), may also link the actin cytoskeleton to the dystroglycans, especially near the neuromuscular junction and in nonmuscle cells. Overexpression of this protein or truncated dystrophin constructs are promising avenues for gene therapy in persons with Duchenne muscular dystrophy.[50] The unusual intricacy of cell-matrix connection systems in muscle may relate to the high forces generated during contraction.

## Myotendinous Junction

The force of muscle contraction is transmitted to the skeleton via the tendons, which are composed of collagens type I and III, blood vessels, lymphatic ducts, and fibroblasts. At

the ends of the muscle fibers, myofibrils are separated by invaginations of sarcolemma filled with long bundles of collagen arising from the tendon. These membrane folds increase the surface area for bearing the mechanical load by approximately 30-fold. Instead of terminating in the Z disks, actin filaments insert into a subsarcolemmal matrix containing α-actinin, vinculin, talin, and integrin. The force is transmitted through laminin to the collagen of the tendon.

## ENERGETICS

Metabolic pathways in muscle cells are specialized for the variable and at times extreme rates of ATP splitting by the contractile apparatus and membrane ionic pumps. Among the dozens of metabolic enzymes present, only the most important ones with regard to normal muscle function are mentioned here.

### Buffering of Adenosine Triphosphate Concentration

The ATP content (~5 mM) is sufficient for only a few seconds of contraction, and thus rapid and effective buffering of ATP during contraction is essential for the maintenance of activity. ADP formed by the hydrolysis of ATP is rephosphorylated by transfer of a phosphate group from creatine phosphate (20 mM in a resting cell) by creatine phosphokinase located within the M line of the sarcomere, in the myoplasm, and between the inner and outer membranes of the mitochondria. Adenylate kinase, known in muscle as *myokinase*, catalyzes the transfer of a phosphate group between two ADP molecules, forming ATP and adenosine monophosphate (AMP). The by-products of the rapid enzymatic reactions that maintain ATP concentration are therefore creatine, $P_i$, and AMP. Some of the AMP is converted to inosine monophosphate by adenylate deaminase. A creatine phosphate shuttle has been proposed to enhance energy flux.[51] According to this hypothesis, creatine phosphate is split within the contractile apparatus and creatine is predominantly rephosphorylated in the mitochondria.

### Glycolysis

Muscles use some combination of glucose and fatty acids (and ketone bodies in some situations) as fuel, depending on their metabolic state (fasted vs. fed) and activity status (rested vs. exercise). The muscle compartment contains most of the body storage of glycogen, which is converted to glucose-6-phosphate for local use. Muscle fibers lack glucose-6-phosphatase and thus do not export glucose. During intense activity, especially in anaerobic conditions, the rate of glycolysis and the production of pyruvate exceed the rate of pyruvate consumption by the citric acid cycle. Excess pyruvate is reduced to lactate by lactate dehydrogenase, which has tissue-specific isoforms. The lactate-dehydrogenase reaction also produces nicotinamide adenine dinucleotide ($NAD^+$), which is necessary for glycolysis, but otherwise lactate is not useful within the muscle. Lactate is freely permeable through the sarcolemma, and a local increase in the extra-cellular lactate concentration or acidification produces exertional pain ("the burn"). Lactate is transported through the blood to the liver, where it is converted back to pyruvate and then to glucose, which is released into the blood for use by other tissues, such as muscle and brain. This sequence of steps, termed the *Cori cycle*, transfers some of the high metabolic load to the liver and "buys time" until oxidative metabolism is available.

### Oxidative Phosphorylation

In aerobic conditions, pyruvate enters the mitochondria, where it is converted to acetyl-coenzyme A (CoA). Acetyl-CoA can enter the tricarboxylic acid cycle, where it is oxidized to carbon dioxide and water, generating reduced NAD (NADH). Fatty acids also contribute to the mitochondrial acetyl-CoA pool through the process of beta-oxidation. The reducing equivalents NADH and reduced form of flavin adenine dinucleotide ($FADH_2$) are subsequently oxidized by the electron transport chain, and an $H^+$ gradient is established across the mitochondrial membrane. This gradient is used to catalyze the phosphorylation of ADP to ATP by the mitochondrial ATP synthase. For example, when glycolysis and oxidative phosphorylation are combined, up to 38 ATP molecules can be generated by the oxidation of each molecule of glucose. This process is energetically much more favorable than the production of lactate, but it can occur only when molecular oxygen is available. Myoglobin is an iron-heme complex protein that facilitates oxygen transport within muscle cells. Tissue hydrostatic pressure in a contracting muscle often exceeds arterial perfusion pressure, so the strongest contractions are anaerobic. The content of oxidative enzymes, myoglobin, and mitochondria determines the predominant type of energy metabolism and varies in different muscle types, as was discussed previously (see Table 5-2).

## FATIGUE AND RECOVERY

During intense or prolonged activity, muscle fatigue is caused by alterations of metabolite levels that suppress the generation of force at the contractile apparatus, during excitation-contraction coupling, or both.[52] Markedly increased myoplasmic $P_i$ and $H^+$ concentrations and decreased creatine phosphate levels have been detected by magnetic resonance spectroscopy.[53] When the creatine phosphate level declines, maintained activity depends on glycogenolysis until glycogen stores are depleted. During prolonged intense activity, the respiratory and circulatory systems are unable to meet the oxygen demands of the tissue. Production of force declines well before the ATP concentration is compromised.

The chemomechanical link between $P_i$ release and the generation of force (see Figure 5-4) implies that increased myoplasmic $P_i$ in fatigued muscle reduces the magnitude of force simply by mass action.[40] Decreased pH in the muscle, caused in part by accumulation of lactate, and insufficient availability of acetylcholine at the neuromuscular junction, leading to failure of synaptic transmission, also contribute to decreased work production. Because the respiratory and circulatory systems do not supply sufficient oxygen to support metabolism during intense activity, an oxygen debt is incurred. Blood flow and oxygen uptake continue at an enhanced level after the period of exercise to reclaim this

energy. Rephosphorylation of creatine can take place within a few minutes, but glycogen resynthesis requires several hours. Recovery processes also involve restoration of ionic gradients across membrane-bound compartments and require consumption of further energy.

## PLASTICITY

The strength and endurance of a muscle are altered dramatically within weeks after changes in the demands for its use, its mobility, or its hormonal or metabolic environment. The effects of this adaptive response should be considered in any clinical situation that causes a substantial shift in these factors and with regard to the long-term quality of life of the patient.

### Adaptation to Muscle Use/Disuse

Muscle is a use-dependent tissue, meaning that its functional characteristics are closely tied to the number and type of activity patterns that it experiences. Increased muscle use through physical exercise leads to adaptations in muscle fibers that include alterations in specific contractile, regulatory, structural, and metabolic proteins, as well as changes in neural recruitment patterns. The type (aerobic vs. resistance), frequency, intensity, and duration of a training stimulus and the external load influence the adaptive response.[54] Resistance training causes cross-sectional hypertrophy of primarily fast type II fibers (see Table 5-2) by increasing the size of existing fibers without inducing hyperplasia. This hypertrophy is driven primarily by increased myofilament protein, which occupies the vast majority of fiber volume (~80%), but other myocellular components (e.g., mitochondria) are also increased so as to maintain the relative volume fraction of each cellular component constant with hypertrophy. Aerobic training, on the other hand, enhances the oxidative capacity and volume density of mitochondria in oxidative type I and IIA fibers and generally does not induce hypertrophy. In this context, resistance training can be seen as altering function by increasing the overall quantity of the muscle, whereas aerobic training alters the functional quality of the muscle to elicit greater endurance to repetitive contraction.

When physical activity is reduced—for instance, with bed rest during hospitalization—the cross-section of the fibers decreases, causing muscle weakness, and endurance is reduced. Of note, these changes are worsened when accompanied by acute and chronic disease,[55] to the extent that up to 10% of muscle protein content can be lost per week.[56] After significant periods of muscle disuse, loss of muscle strength and endurance can progress to the point at which patients are unable to accomplish simple activities of daily living. Although younger, healthy persons tend to regain muscle size and function readily with exercise rehabilitation, the extent to which older persons and those with chronic disease can recover from muscle disuse appears to be impaired.

### Hormonal Control

Hormones, acting in an endocrine or paracrine-autocrine fashion, also have the capacity to alter muscle structure and function. The most prominent hormonal controller of muscle is insulin, which regulates muscle anabolism in response to feeding by suppressing protein breakdown and facilitating protein synthesis via stimulation of amino acid uptake into muscle. In addition to insulin, insulin-like growth factor-I, which largely mediates the effects of growth hormone on muscle, stimulates the hypertrophy of existing muscle fibers by stimulating muscle protein synthesis and inhibiting protein breakdown, and it may contribute to muscle growth/regeneration through effects on muscle satellite cells.[57,58] In men, testosterone has clear anabolic effects on muscle, and conditions that reduce circulating testosterone levels lead to muscle atrophy and weakness. The role of testosterone in women is less certain. Estrogen in women is widely believed to have effects on muscle size and strength, although experimental evidence to support this contention is lacking, and the effects of estrogen on other metabolic processes in muscle appear to be relatively minor at concentrations observed during normal menstrual cycles and in the post-menopausal period.

In many acute and chronic illnesses, changes in the hormonal milieu can affect muscle. The most predominant factors thought to elicit these changes are cytokines and other inflammatory modulators,[59] although alterations in classic stress hormones, such as cortisol and glucagon,[60] also contribute to muscle catabolism. In most cases, the effect of these catabolic hormones is to direct amino acid substrates toward the liver to necessarily support the acute phase protein response. In addition, the type of persistent low-grade inflammation that occurs with aging and in many chronic diseases may have similar detrimental effects on muscle when compounded over extended periods. Whether modulation of these catabolic hormonal factors has beneficial effects in maintaining muscle size and functionality under physiologic and pathophysiologic conditions, however, is not certain.

## AGING

*Sarcopenia*, defined as loss of skeletal muscle mass and function with age, manifests as an inability to perform simple tasks of everyday life and contributes to disability, a greater risk for falls and fractures, an increase in all-cause mortality, and, in general, a poor quality of life. Healthy persons exhibit approximately a 30% decrease in total muscle mass between the ages of 30 and 80 years. Although it is universally accepted that whole muscle force production is reduced with age,[61-64] disagreement exists about whether this effect is due solely to reduction in muscle mass or if loss of force production per unit muscle size is also a factor. Most single-fiber studies that have measured isometric force production per unit fiber cross-sectional area (i.e., accounting for age-related reductions in muscle size) show that aging decreases force-producing capabilities.[65-72] In addition, whole muscle[61,62,73-75] and single fiber[65,67-69,71,72,76] studies typically find decreases in contractile velocity with age, and this decrease leads to further reduction in the muscle performance of elderly persons, especially for movements requiring high-velocity contractions. Altogether, these results suggest that aging alters the fundamental contractile properties of skeletal muscle fibers, which decrease, at least in part, whole muscle performance. Age-related changes in

motor unit recruitment, activation of agonist and antagonist muscles, and fibrosis can exacerbate single fiber changes and further reduce whole muscle performance.

## CONCLUSION

The complex functional capacity of muscle to produce finely tuned and coordinated movements is ultimately expressed as the transduction of chemical to mechanical energy by actomyosin. A twitch is initiated by the following mechanisms: an action potential propagated from the CNS along an α motoneuron, neuromuscular chemical transmission, direct protein–protein communication at the T-tubule–sarcoplasmic reticulum junction, $Ca^{2+}$ diffusion in the myoplasm, and $Ca^{2+}$ binding to thin filament regulatory proteins. Because the CNS controls activity through recruitment of motor units, gradation and coordination of movement depend critically on the pattern of connections between α motoneurons and muscle fibers and on variations of properties among motor units. Development, maintenance, and aging of the muscular system involve a complex series of genetic programs and cellular interactions that are beginning to be understood at the molecular level. Adaptation of motor unit properties is evident not only in training regimens but also in reduced activity caused by pain or joint immobilization and in compromised metabolic, hormonal, or nutritional conditions. Hence the plasticity of muscle influences the clinical course of many diseases. In addition to its importance in pathophysiology, muscle serves as an excellent substrate for understanding the molecular basis of cell development, protein structure-function relationships, cell signaling, and energy transduction processes.



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CHAPTER 6



# Biomechanics

Kenton Kaufman • KaiNan An

**KEY POINTS**

**Kinematics is the study of the geometric and time-dependent aspects of motion without analyzing the forces causing the motion.**

**The general unconstrained movement in three-dimensional space requires the description of three translations and three rotations to fully describe joint motion.**

**Kinetics is the study of the forces that cause motion of a rigid body. These forces can be classified as either external forces or internal forces.**

**External forces represent the action of objects contacting the body, gravitational forces, or force due to inertia of the body.**

**Internal forces are the body's responses to external forces, which consist of muscle, ligament, and joint contact forces.**

**Because of the relatively smaller mechanical advantages, large muscle and tendon forces and thus internal joint forces are expected when performing any activities.**

**The anatomic structure responsible for joint constraint can be divided into passive and active elements. Passive elements, consisting of the capsulo-ligamentous structures and bony articulating surfaces, provide the static constraints of the joint. The active elements include muscle-tendon units, which provide dynamic joint constraints.**

Biomechanics combines the field of engineering mechanics with the fields of biology and physiology. Biomechanics applies mechanical principles to the human body in order to understand the mechanical influences on bone and joint health. Forces that load the joints are generated by muscles and transmitted by tendons. Bones must withstand these forces. Developments in the field of biomechanics have improved our understanding of normal and pathologic gait, mechanics of neuromuscular control, and mechanics of growth and form. This knowledge has contributed to the development of medical diagnostic and treatment procedures. It has provided the basis for the design and manufacture of medical implants and orthotic devices and has enhanced rehabilitation therapy practices. Biomechanics has also been used to improve human performance in the workplace and in athletic competition.

Mechanics is a branch of physics that is concerned with the motion and deformation of bodies that are acted on by mechanical forces. Mechanics is one of the oldest physical sciences, dating back to Aristotle (384-322 BC), who conducted an organized analytical analysis of animal movement. Leonardo da Vinci (1452-1519) investigated the mechanics of the human body, and his detailed anatomic sketches represent the birth of anatomy as a discipline and of mechanics as the science governing human motion. Although da Vinci wrote extensively on body mechanics, the man generally credited to be the father of modern biomechanics is Giovanni Alphoso Borelli (1608-1679). His book *De Motu Animalian* provided a quantitative graphical solution to a musculoskeletal biomechanics problem (Figure 6-1).[1]

Engineering mechanics is the discipline devoted to the solution of mechanical problems through the integrated application of mathematical, scientific, and engineering principles. With roots in physics and mathematics, engineering mechanics is the basis of all engineering mechanical sciences. Engineering mechanics is an applied mechanics branch of the physical sciences. The broad field of applied mechanics can be further divided into three main parts: rigid body mechanics, deformable body mechanics, and fluid mechanics. In general, a material can be characterized as either a solid or fluid. Solid materials can be considered to be rigid or deformable. A rigid body is one that cannot be deformed. In reality, every object undergoes deformation to some extent when acted upon by external forces. However, this is a definition of convenience that is used to simplify complex problems. For example, during the study of movement in gait analysis, the bones are considered to be rigid bodies when compared with the soft tissues joining the bones. External loads applied to a rigid body result in internal loads, stresses, and deformations. The mechanics of deformable bodies deal with the relationships between externally applied loads and their internal effects. The mechanics of deformable bodies have strong ties with the field of materials science and are more complex when compared with the analyses required in rigid body mechanics. The purpose of this chapter is to give you only a brief idea of biomechanics. We will focus on rigid body mechanics.

Basic biomechanics relies heavily on Newtonian mechanics. These laws were introduced by Sir Isaac Newton and form the basis for analyses in statics and dynamics. Statics analyzes the forces that occur in rigid bodies that are in static equilibrium. Dynamics is the study of bodies in motion. The general field of dynamics consists of two major areas: kinematics and kinetics. Ultimately, proper joint constraint and stability enables limb function in characteristic ways.

## KINEMATICS

Kinematics is the study of the geometric and time-dependent aspects of motion without analyzing the forces causing the

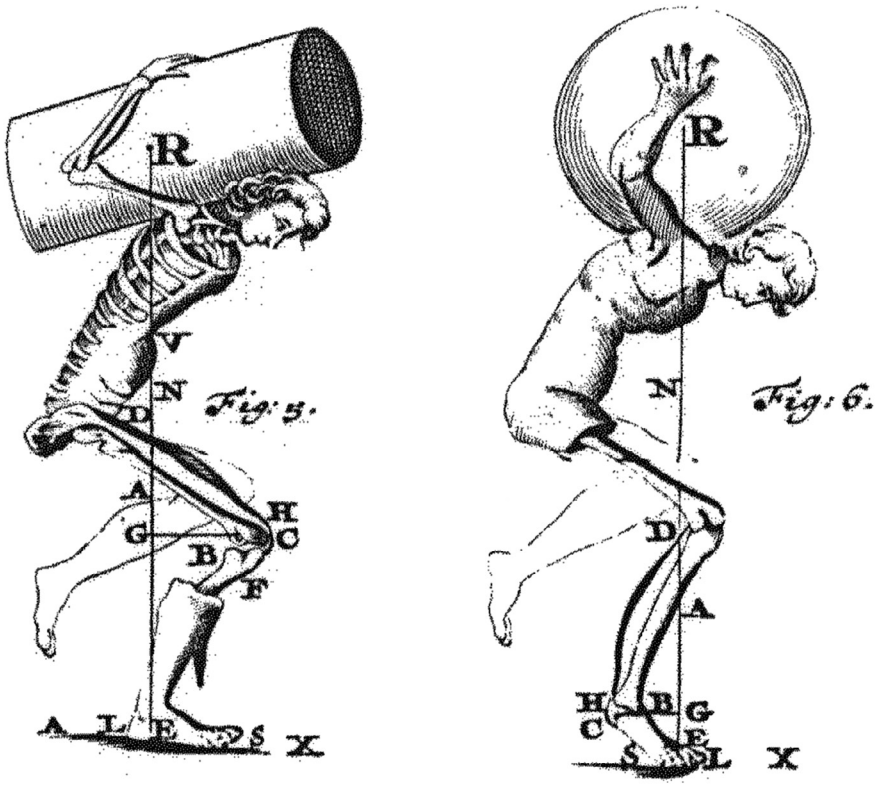



**Figure 6-1** Borelli's quantitative graphical solution to a musculoskeletal biomechanics problem. *(From Borelli GA:* De motu anumalium, *Batavis, 1685, Lugduni.)*

motion. Kinematic analysis is used to relate displacement, velocity, acceleration, and time. To study kinematics in an organized manner, it is common to classify the motion as translational, rotational, or general. Translational motion occurs when a straight line drawn between two points on the body remains in the same direction during the motion. Translational motion can be either rectilinear motion (if the paths are straight lines) or curvilinear (if the paths are curved lines). Rotational motion occurs when the points on the body move in a circular path around an axis of rotation. The angular motion occurs about a central line known as the *axis of rotation*, which lies perpendicular to the plane of motion. The third class of motion is called *general motion*, or *displacement*, which occurs if a body undergoes both translational and rotational motion simultaneously.

Kinematics can be analyzed in two-dimensional (2-D) or three-dimensional (3-D) space. When all points of a rigid body move parallel to a plane, the motion is referred to as *planar motion*, which can be thought of as 2-D motion. The more general type of rigid body motion is 3-D motion. Six independent parameters are required to describe 3-D motion. These parameters are called *degrees of freedom*, or the number of independent coordinates in a coordinate system that is required to completely specify the position of an object in space. A rigid body in space has a maximum of six degrees of freedom, consisting of three translations (expressed by linear coordinates) and three rotations (expressed by angular coordinates). The general movement of an object is defined by a vector quantity that is a combination of both linear and angular displacement. Velocity is the time-related change of displacement. Linear velocity is expressed in units of length per time (e.g., m/sec). Angular velocity is expressed in units of angular measure per time (e.g., rad/sec). Because velocities reflect vector quantities, both magnitude and direction must be specified. Acceleration is the time-rate of change of velocity. Linear acceleration is expressed in units of length per time squared (e.g., $m/sec^2$). Angular acceleration is the time rate of change in angular velocity (e.g., $rad/sec^2$). Accelerations are also vector quantities, and both magnitude and direction must be specified.

Kinematic techniques have been used to study body movements in both 2-D and 3-D space. The human body is typically modeled as a number of interconnected rigid body segments (Figure 6-2). A coordinate system is affixed to each rigid body segment to establish an anatomic coordinate system. External markers are used to define orthogonal coordinate systems whose axes define the position of these body segments. Joint motion is then described as the relative motion of the distal body segment with respect to the proximal body segment. Limb segments are assumed to undergo angular displacement during human movement. However, more sophisticated analyses will also quantify the linear displacement that limb segments may undergo. These measures of relative segmental angles have been used to describe human walking and other activities of daily living.[2-7]

For example, knee motion in the sagittal plane can be characterized throughout the gait cycle (Figure 6-3). At heel strike, the knee is nearly fully extended (with knee

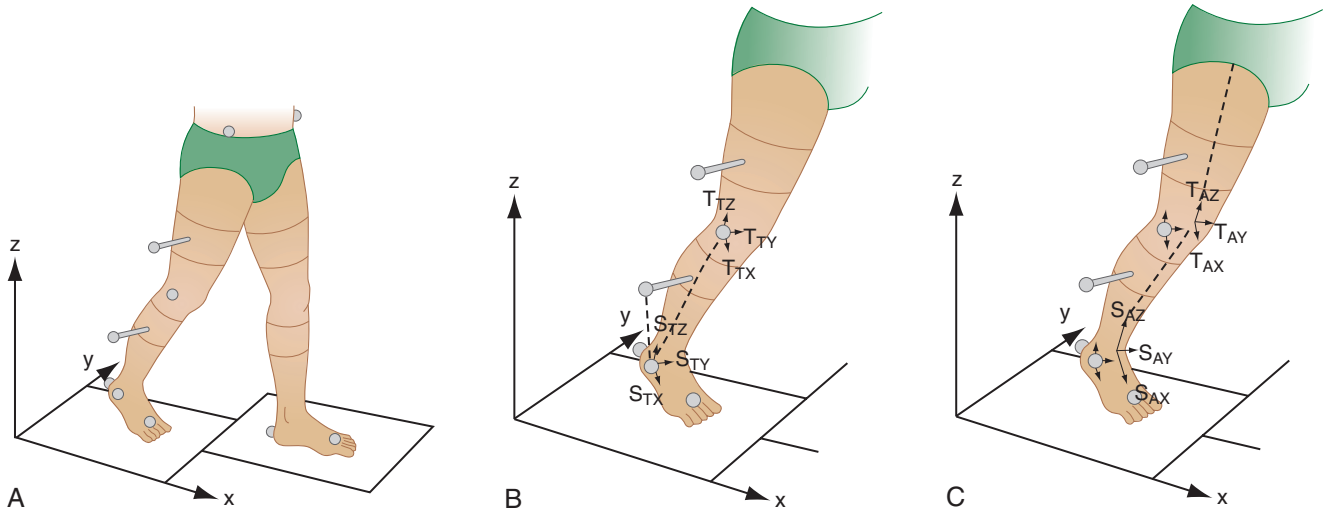



**Figure 6-2** The kinematic technique used to study body movement in three-dimensional space. Body-fixed reflective markers are used to establish anatomic coordinate systems. **A,** Video camera measurement systems calculate the location of external markers placed on the body segments and aligned with specific bony landmarks. **B,** A body-fixed external coordinate system is then computed from three or more markers on each body segment. Body-fixed external coordinate systems are shown for the thigh ($T_T$) and shank ($S_T$). The coordinate system is shown for the three orthogonal directions (e.g., x, y, and z). The body-fixed thigh coordinate system is designated $T_{Tx}$, $T_{Ty}$, and $T_{Tz}$. **C,** Using a subject-specific calibration relates the external coordinate system with an anatomic coordinate system through the identification of anatomic landmarks (e.g., the medial and lateral femoral condyles and medial lateral malleoli). The anatomic coordinate system for the thigh is then designated $T_{Ax}$, $T_{Ay}$, and $T_{Az}$. *(From Kaufman KR: Objective assessment of posture and gait. In Bronstein AM, Brandt T, Marjorie H, editors:* Clinical disorders of balance, posture, and gait, *Oxford, England, 2004, A Hodder Arnold Publication.)*

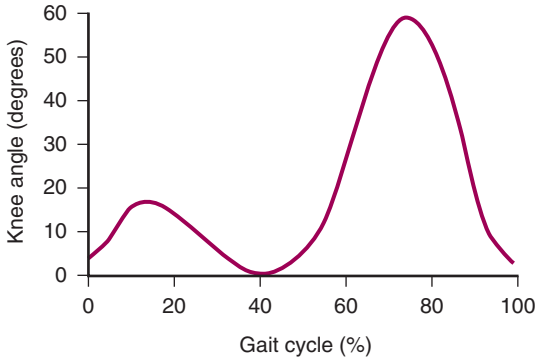



**Figure 6-3** Knee sagittal plane motion throughout the gait cycle. A positive value indicates knee flexion. For the first 60% of the gait cycle, the leg is in stance. The leg is in swing phase for the remainder of the gait cycle.

flexion of only five degrees). During midstance, the knee flexes to about 15 degrees, which occurs at 15% of the gait cycle. The knee joint is brought back into extension by midstance. At 50% of the gait cycle, opposite foot contact occurs. The weight is shifted to the opposite limb and the knee begins flexing. Toe-off occurs at 60% of the gait cycle. Peak knee flexion of 60 degrees occurs during the early portion of the swing phase. The knee motion can be described as two flexion waves, each starting in relative extension, progressing into flexion, and then returning again to extension. The first flexion wave, or stance phase knee flexion, acts as a shock absorber to aid weight acceptance. This curve peaks in early stance at opposite foot-off. The mechanical source for this shock absorber is the eccentrically contracting quadriceps muscles. The second flexion wave is necessary to clear the foot in the early swing phase. The knee is rapidly flexed beginning just after heel rise to a maximum in the swing phase just as the swinging foot passes the opposite limb.

The complexity of kinematic analysis increases substantially when one moves from planar analysis to 3-D analysis. The complexity of the analysis arises from a technical difficulty—that is, large rigid body rotations cannot be treated as vectors and hence do not obey the vectorial principles of transformation, independence, and interchangeability of operations. For finite spatial rotation, the sequence of rotations is extremely important and must be specified for a unique description of joint motion.[8,9] For the same amount of rotation, different final orientations will result from different sequences of rotation (Figure 6-4). However, with proper selection and definition of the axes of rotation between two bony segments, it is possible to make finite rotation sequence independent or communitive.[8,9] The concept of Eulerian angles has been adopted in the field of orthopedic biomechanics to unify the definition of finite spatial rotation. In the selection of reference axes, one axis is fixed to the stationary segment and another axis is fixed to the moving segment (Figure 6-5). In the knee joint, for example, the flexion/extension angle $\Phi$ occurs about a medial-laterally directed axis defined by a line connecting the medial and lateral femoral condyles. The axial rotation angle, $\Psi$, is measured about an axis defined by the line along the shaft of the tibia. The third axis, also defined as the floating axis, is orthogonal to the other two axes and defines abduction/adduction ($\Theta$). These rotations match the Eulerian angle description and are thought to be performed in such a way as to bring the moving segment from the reference orientation into the current orientation. The advantage of using this system for description of the spatial rotation of anatomic joints is that the angular rotations do not have to be referred back to the neutral position of the joint because the rotation sequence can be independent,

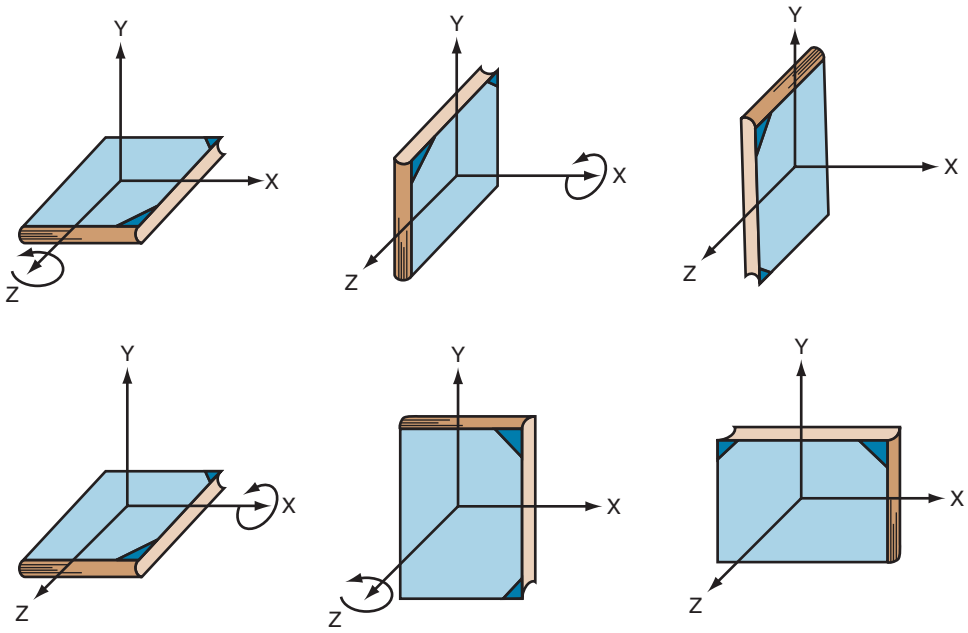



**Figure 6-4** Sequence dependence of rigid body motion. The object undergoes two rotations about the X-axis and Z-axis. The sequence of these rotations differs in the top and bottom rows. The result is that the final orientation of the object is different.

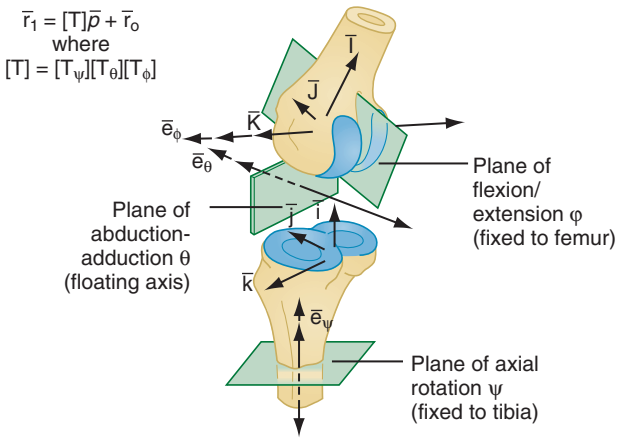



**Figure 6-5** Description of knee joint motion using the Eulerian angle system. An axis fixed to the distal femur defines flexion/extension motion, Φ. An axis, $e_\psi$, fixed to the proximal tibia along its anatomic axis defines internal external rotation, Ψ. A floating axis, $e_\theta$, which is orthogonal to the other two axes, is used to measure abduction-adduction, θ. The rotation matrix [T] between the proximal and distal joint segment is then given by the multiplication of the three rotation matrices associated with the rotations around the three axes (i.e., $[T] = [T_\psi][T_\theta][T_\Phi]$). *(From Chao EYS: Justification of triaxial goniometer for the measurement of joint rotation.* J Biomech *13:989–1006, 1980.)*

and thus the measurement can be easily obtained and related to anatomic structures.

A complete analysis of total joint movement (i.e., six degrees of freedom) can be obtained using markers embedded in the bone[10,11] or dual fluoroscopic imaging techniques.[12,13] This general unconstrained movement in 3-D space requires the description of three translations and three rotations to fully describe joint motion. The most commonly used analytic method for description of six-degrees-of-freedom displacement of a rigid body is the screw displacement axis or helical axis.[14-16]

## KINETICS

Kinetics is the study of the forces that cause motion of a rigid body. When unbalanced forces or moments are acting on a rigid body, it is under a nonequilibrium, or dynamic, condition, resulting in motion. Understanding the kinetics of human movement provides a fundamental understanding of the musculoskeletal system. Before one can begin to analyze the forces during human movement, some basic definitions are necessary and some basic assumptions must be made.

The key quantities in kinetics are *force*, *moment*, and *torque*. A force represents an interaction between two bodies. According to Newton's Second Law, force is any action that tends to change the state of rest or state of motion of a body to which it is applied. Forces can be contact forces (as when bodies touch each other) or field forces (as when bodies are separated by a distance, such as gravitational, electric, or magnetic forces). Forces are represented by *vectors*, which are composed of four components: magnitude, direction, sense, and position (also called *point of application*). The vector may be resolved into several component forces, usually along specified mutually perpendicular coordinate axes. Conversely, forces can be summed using vectorial addition.

A moment represents the turning, twisting, or rotational effect of a force. A moment is a vector. A moment is defined as the product of the force and the perpendicular distance between the line of action of the force and the axis of rotation of the motion that the force produces (Figure 6-6). Its magnitude is the force times the perpendicular distance to the axis of rotation. The direction of the moment is along the axis of rotation (or potential rotation) and thus perpendicular to the plane in which the twisting force is applied. The moment arm—that is, the distance used to calculate the moment—is the shortest distance from the force action line to the actual or potential pivot point of the system,

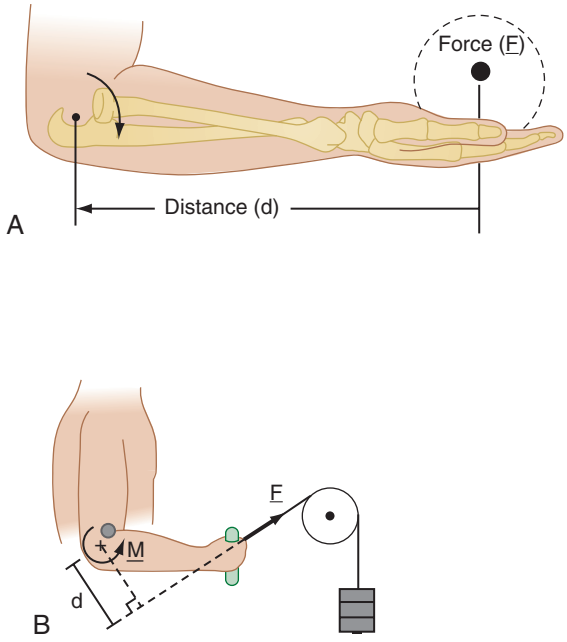



**Figure 6-6** The moment (M) of a force (F) about a point is equal to the force times the moment arm (d), which is the perpendicular distance between the point and the line of action of the force. The moment arm may **(A)** or may not **(B)** be the distance along the limb segment to the axis of rotation. The moment arm is always the shortest distance between the line of force application and the axis of rotation.

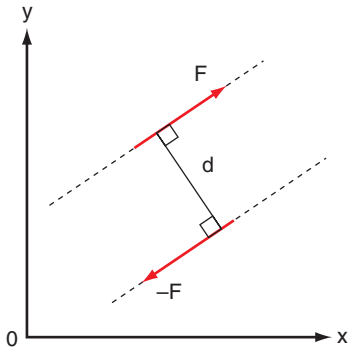



**Figure 6-7** A torque, or force couple, is created by two equal, noncollinear, and parallel but oppositely directed forces, F and –F. The magnitude of the torque is Fd, where *d* is the perpendicular distance between the two forces.

regardless of the state of motion. Skeletal motions are the result of moments applied by muscles that cross the joints on which they act. The moment of a force about an axis measures the tendency of the force to impart to the body a motion of rotation about a fixed axis.

A torque is a special type of moment that results when a pair of forces that have equal magnitude, parallel lines of action, and opposite senses act on a body (Figure 6-7). The magnitude of the torque is Fd, where *d* is the perpendicular distance between the two forces. The resultant force is zero because the two forces are equal and oppose each other.

Kinetics can be used to analyze forces affecting the musculoskeletal system. These forces can be classified as either external or internal. External forces represent the action of objects contacting the body, gravitational forces, or force due to inertia of the body. Internal forces are the body's responses to external forces. Internal forces consist of muscle, ligament, and joint contact forces. In general, the limb segments are assumed to be rigid, which simplifies the analysis because it is assumed that the structure does not deform under load. Further, joints are assumed to be frictionless hinges.

Statics is the study of forces acting on a body at rest. When performing a force analysis, the body or part of a body at equilibrium may be isolated from the environment, and the environment is replaced by forces acting on the system, resulting in what is called a *free-body diagram*. Because both forces and moments are vectors, they must sum to zero in each of the three perpendicular directions (reference system). Consider a person standing quietly (Figure 6-8). The person's weight (force of gravity) tends to pull the person toward the ground (Figure 6-8A). The person does not move downward because the ground is pushing up with a total force equal in magnitude to the individual's weight (Newton's Third Law). If the person has his or her weight distributed symmetrically, then his or her weight is evenly shared by each lower extremity (Figure 6-8A). The load under each foot can be represented by resultant ground reaction force (GRF) vectors, with one-half of the body weight supported by each foot. With one-half of body weight supported by each foot, the point of application of the resultant GRF passes approximately midway between the subject's two feet (Figure 6-8B). No motion occurs because the external forces (i.e., body weight and the GRF) are balanced—they are equal and opposite in magnitude. When the person leans to one side (Figure 6-8C), the GRF shifts in the direction that the person leans. Thus overall body posture can affect the GRF location. If the person leans more, he or she will become unstable (i.e., the downward body weight vector will fall outside the base of support). To remain in static equilibrium, the subject requires additional support (Figure 6-8D). The horizontal force applied to the upper body by the wall is balanced with an equal and opposite horizontal GRF component. Also, the two equal and opposite vertical forces are no longer aligned (i.e., collinear). They form a force couple that would tend to rotate the body in a counterclockwise direction. A second clockwise force couple formed by the two equal and opposite horizontal forces balances the counterclockwise force couple. As a result, the subject remains in both translational and rotational static equilibrium.

When unbalanced forces are present or no one is acting on a rigid body, it is under a nonequilibrium or dynamic condition, resulting in motion. Newton's Second Law of Motion links the kinematics of a body to its kinetics. The Second Law states, "If the resultant force acting on a body is not zero, the body will have an acceleration proportional to the magnitude of the resultant and in the direction of this resultant force." Analysis of the motion of the limb segments requires a set of governing equations and assumptions. The equations assume that each limb segment is a rigid body moving in 3-D space. Therefore, six scalar equations of motion define the general 3-D forces and motion of each limb segment:

$$\Sigma F = ma$$

$$\Sigma M = I\alpha$$

where ΣF is the sum of forces in each of three orthogonal directions, ΣM is the sum of moments, *I* is the moment of

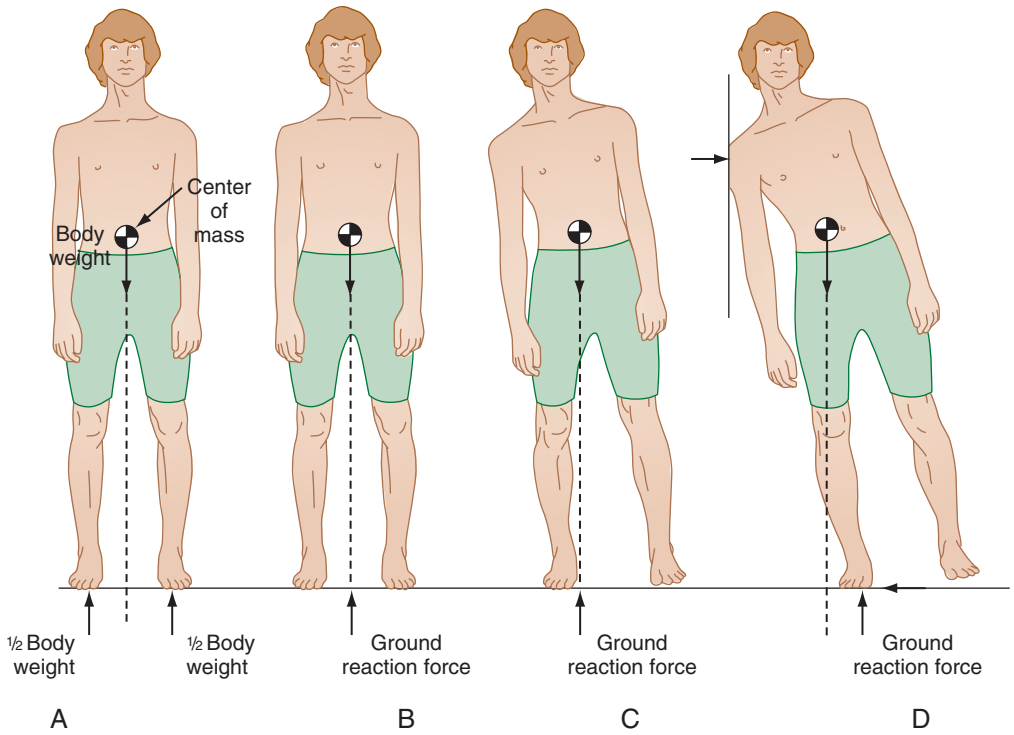



**Figure 6-8** Static force equilibrium. **A,** When a person stands quietly with the body weight evenly distributed on both feet, that person can be considered to be in static equilibrium. **B,** The loads under each foot can be combined into a single ground reaction force (GRF) equal to the sum of the forces under the two feet and located directly under the center of mass (COM), with a force equal to body weight. The GRF is located approximately midway between the two feet. **C,** As body weight is shifted laterally, the GRF also shifts in order to remain under the COM. **D,** When the body weight vector is shifted beyond the base of support (i.e., the lateral margin of the foot), an additional lateral force vector is required to maintain static equilibrium. *(From Davis RB, Kaufman KR: Kinetics of normal walking. In Rose J, Gamble JG, editors:* Human walking, *ed 3, Philadelphia, 2006, Lippincott Williams & Wilkins.)*

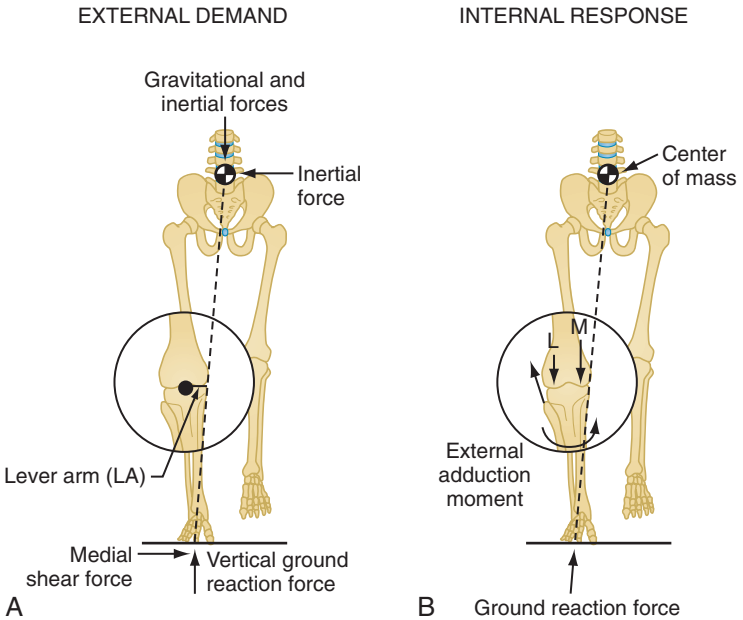



**Figure 6-9** Schematic of a person in single support during gait. **A,** External demand. The body weight and vertical ground reaction force (GRF) force couple tend to rotate the body clockwise. The medial shear GRF and the corresponding lateral inertial force produce a force couple that tends to rotate the body counter-clockwise. Thus the body is in dynamic equilibrium. **B,** Internal response. The resultant GRF passes medial to the knee joint, creating an external knee adduction moment. The external moment is balanced internally in the knee by creating a larger force on the medial tibial plateau (M) than on the lateral tibial plateau (L). Once again, the knee is in dynamic equilibrium.

inertia of the body, a is the linear acceleration of the body, $m$ is the mass of the body, and $\alpha$ is the angular acceleration of the body. Newton's Second Law makes it possible to calculate the forces acting on the musculoskeletal systems from measurements of segment motion and mass. During normal ambulation, there is a medial and vertical GRF that balances the body weight and lateral inertial force. The vertical GRF component generally passes lateral to the body's center of mass during gait (Figure 6-9A). The combined resultant GRF passes medial to the knee joint center, creating a load imbalance in the medial and lateral compartments of the knee. The medial and lateral

compartments of the knee carry more load than the lateral compartment of the knee (Figure 6-9B), and thus a higher degree of osteoarthritis (OA) typically develops in the medial compartment than in the lateral compartment. The resultant GRF vector creates a knee external adduction moment. Medial tibiofemoral OA is the most common form of OA, and it has become apparent that medial knee OA is at least partially mechanically driven. The peak external knee adduction moment has been implicated in the progression of radiographic OA[17] and has been identified as a marker of disease severity.[18,19]

## JOINT BIOMECHANICS

Diarthrodial joints connect long bones to allow force transmission and joint rotation. The type of joint articulating motion depends on the shape of the joint surfaces. For example, the hip is a congruent ball and socket joint, the elbow is a congruent hinge joint, the knee and proximal interphalangeal joint are bicondylar joints, and the carpometacarpal joint of the thumb is a saddle joint. In general, the joint articulating motion can be described in terms of sliding, spinning, and rolling (Figure 6-10). Sliding and spinning motions involve the relative translation of one surface against the other. Rolling has the least relative motion between articulating surfaces. The translational speeds range from 0.06 m/sec between the femoral head surface and acetabular surface during normal walking to 0.6 m/sec between the humeral head surface and glenoid surface during baseball pitching.[20]

Although diarthrodial joints experience an enormous amount of loading and motion, the cartilage surfaces undergo little wear and tear during normal conditions because of the special lubrication qualities of synovial fluid and the biphasic structure of the cartilage. Synovial fluid, which is secreted by the synovium into the joint space, contains mainly hyaluronate. Biomechanically, hyaluronate is a highly viscous liquid, with the shear stress depending on the rate of shear strain applied. As the shear rate increases, the viscosity of synovial fluid decreases. However, in the patient with rheumatoid arthritis, because of enzymatic degradation of the hyaluronate molecules, the viscosity of the rheumatoid synovial fluid does not have a normal shear rate dependency and is believed to be less effective in lubrication.[20]

Two lubrication mechanisms in the synovial joint provide minimal friction and cartilage wear: fluid film lubrication and boundary lubrication. During joint rotation, the sliding speed of the articulating surfaces and the viscosity of the synovial fluid create a thin film that is capable of supporting the load, resulting in fluid film lubrication. When the joint is loaded, the two articulating surfaces approach each other, and a squeeze film is generated to support the load. The pressure developed in the lubricating fluid carries the load

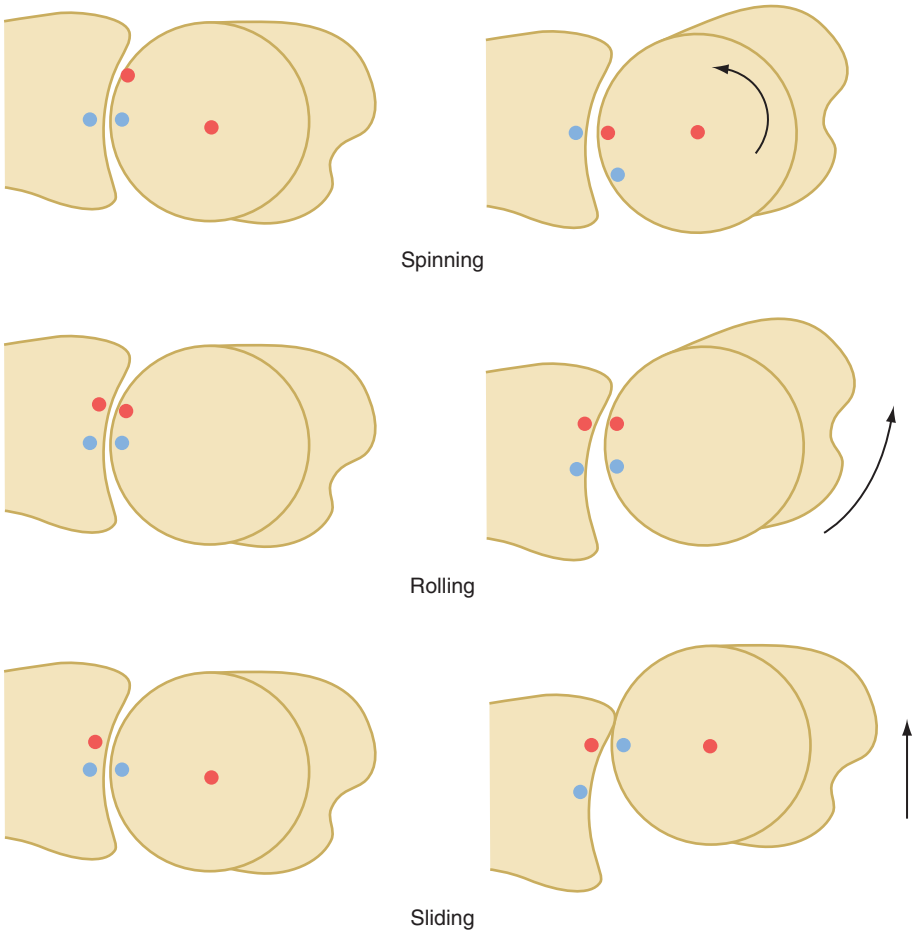



**Figure 6-10** The motions of joint articulation: spinning, rolling, and sliding. *(From Morrey BF, Itoi E, An KN: Biomechanics of the shoulder. In Rockwood CA, Matsen, F, editors:* The shoulder, *ed 2, vol 1, Philadelphia, 1998, WB Saunders, pp 233–276.)*

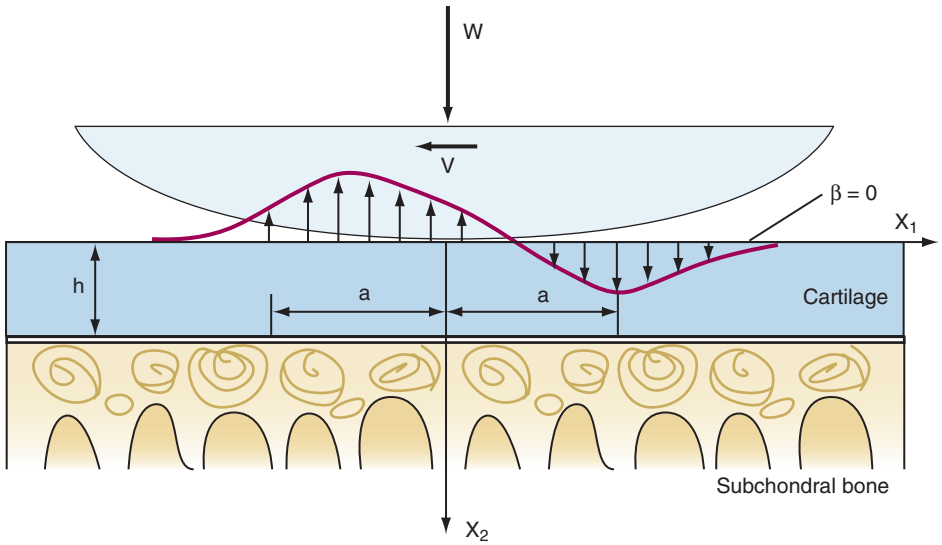



**Figure 6-11** Hydrodynamic lubrication. Surfaces moving relative to each other have an interposed viscous fluid that increases the pressure within the fluid to support the weight and keep the two surfaces separated. The pattern of predicted fluid exudation and imbibition over the articular surface (thickness = h) resulting from a frictionless, free-draining (β = 0) moving indenter is shown. V is the speed of the horizontal translation, and W is the resulting line load of a surface pressure distribution PA(×1) acting over a spread of |×1|<a. *(From Mow VC, Soslowsky LJ: Friction, lubrication, and wear of diarthrodial joints. In Mow VC, Hayes WC, editors:* Basic orthopaedic biomechanics, *New York, 1991, Raven Press, pp 245–292.)*

applied to the joint. The thin film of lubricant also produces a greater bearing surface. In addition, the cartilage is a biphasic porous medium. Water is bounded in the intrafibrillar space of the collagen matrix by proteoglycans. Intricate interaction of the mechanical stress, the electric charges, and the hydrodynamics result in a special fluid efflux pattern known as *elastohydrodynamic lubrication* (Figure 6-11). The fluid exudation ahead of and imbibition behind the moving contact point of the articulating surface further facilitates the lubricant film.[20]

On occasion, heavy loading exceeds the capacity that the lubricant film can support. In this situation, the cartilage surfaces are in direct contact, and lubrication is provided by the mechanism of boundary lubrication.[20] Boundary lubrication is accomplished by a monolayer of glycoprotein called *lubricin*, a superficial zone protein. In the pathologic condition of OA, the structural properties of cartilage such as porosity and permeability are altered. The fluid efflux and the synovial fluid film are diminished, and boundary lubrication is the remaining mechanism of joint lubrication.

## JOINT CONSTRAINT AND STABILITY

The human body is able to perform complex motions because the joints allow multiple degrees of freedom between articulating bones. Because of the variations in anatomic structures of the joints, various limbs have different movement characteristics and load transmissions. Adequate joint stability that balances motion and loads is necessary for a joint to function properly. Chronic disease or traumatic injury may cause damage to joint tissue, thus compromising the constraints and stability of the joint. To improve the diagnosis and treatment of such joint disorders, it is essential to understand the basic mechanics of the constraint, which provides stability. The anatomic structure responsible for joint constraint can be divided into passive and active elements. Passive elements, consisting of the capsulo-ligamentous structures and bony articulating surfaces, provide static constraints of the joint. The active elements include muscle-tendon units, which provide dynamic constraints of the joint.

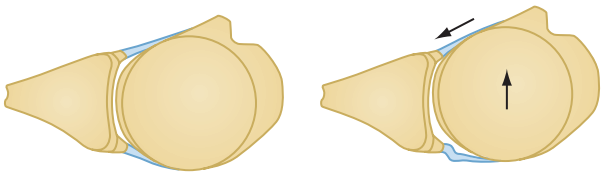


**Figure 6-12** When the joint is loaded or displaced, the capsulo-ligamentous tissues are stretched and the passive tensions develop. The amount of tension developed depends on the amount of deformation, as well as the material properties of the soft tissue. The location of the capsulo-ligamentous attachment on the bone and the direction of bony movement regulate not only the amount of deformation but also the direction of passive tension in stabilizing the joint.

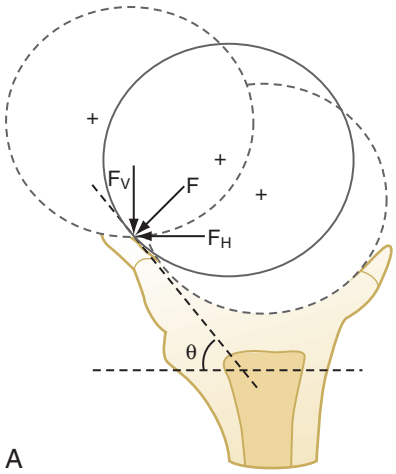
The contribution of capsulo-ligamentous structures to joint stability and constraint are determined by the change in length, the line of action, and the tissue material properties (Figure 6-12). For a given loading and displacement of the joint, the greater the elongation of the soft tissue, the higher the passive tension generated by that tissue. However, the specific contribution of such passive tension to joint constraint further depends in turn on the relative line of action of the capsulo-ligament structures. Therefore, the relative locations of the origins and insertions of the ligaments may determine the contribution of the ligamentous tension in resisting joint displacement in three orthogonal directions. Surgical alternations of the soft tissue attachment to the bone would therefore change the joint constraints.

Lengthening and shortening of the capsulo-ligament structures during joint loading and movement determine the tissue deformation. The tissue material properties, along with the amount of deformation, will determine the structure passive tension. For the same amount of deformation,

the stiffer the tissue, the higher the tension will be, and the softer the tissue, the lower the tension. Alterations of the material properties due to physiologic and pathologic conditions definitely alter the joint constraint and the articulation. For example, the tight posterior capsule of a baseball pitcher limits the range of physiologic motion. On the other hand, the softening of capsulo-ligamentous structures during pregnancy and in persons with rheumatologic diseases could potentially lead to abnormal motion and contact stress on the joint surface. These alterations of soft tissue material properties must be considered as potentially critical factors in initiating early joint cartilage degeneration and may lead to the vicious cycle that perpetuates arthritis.

Experimentally, the significance of various passive anatomic structures to joint stability has been assessed using two methods: the stiffness test and the laxity test. The stiffness test is commonly used to determine the relative contributions of individual anatomic elements to joint constraint. The laxity test demonstrates the outcome of joint instability when one or more constraining units are compromised. In the stiffness test, the joint is displaced under controlled testing and the corresponding load is monitored. Based on the joint load–displacement curves, it is obvious that the load required to displace the joint is higher when all the ligaments are intact than when a ligament is lacerated. The difference in the constraining load between these two curves at the same joint displacement represents the contribution of that particular sectioned ligament to the joint constraint. Because the capsulo-ligamentous structures are passive tissues, as long as the joint displacement is controlled and experimentally reproduced, the relative contributions of individual tissues will not be dependent on the sectioning sequence of soft tissue. Contrary to the stiffness test, during which displacement is varied and load is measured, in the laxity test, a specific load is applied to the joint and the extent of joint displacement is measured. Joint laxity is documented with changes in displacement as anatomic elements are removed. The joint laxity as observed and measured provides more of a clinical scenario of the joint instability experienced by a patient who has sustained a similar soft tissue injury.

In addition to soft tissue constraints, the joint articulating surface is an element that provides joint constraint and stability. Theoretically, its significance to joint stability is determined by both the geometric shapes of the joint surfaces and the amount of compressive force encountered between those two surfaces (Figure 6-13A). When two curved surfaces articulate, a vertically applied force will induce either a transverse displacement or a transverse constraining force. The greater the congruency between the curved articulating surfaces, the greater the induced horizontal joint constraint force. This mechanism is sometimes referred to as *concavity-compression*. In the shoulder joint, for example, the relative translations between the glenoid and humeral head and the forces resisting translation were recorded. The stability ratio, defined as the peak translational force divided by the applied compressive force, was higher in the hanging-arm position than in glenohumeral abduction (Figure 6-13B). The highest stability ratio was detected in the inferior and superior directions, and the anterior direction was associated with the lowest stability ratio. Resection of the glenoid labrum resulted in an average



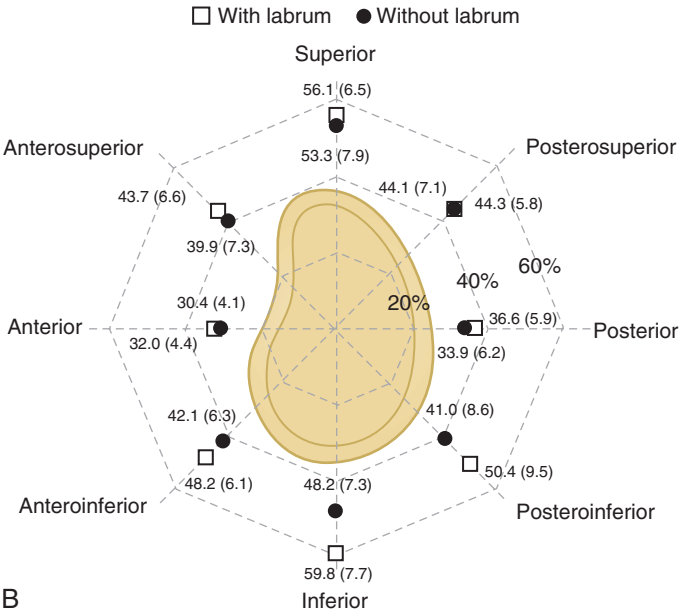




**Figure 6-13** Contributions of the articulating surface to joint stability. **A,** Transverse displacement of bone in the curved joint requires elevation against the articulating surfaces. With compressive $F_V$ applied, any transverse movement will encounter a resistance ($F_H$). If either the mating joint surface is flat (i.e., $\theta = 0$) or a compressive force, $F_V$ is not available, there will be no transverse constraint ($F_H = 0$). **B,** The stability ratio, defined as transverse constraint force $F_H$ divided by the applied compressive force $F_V$, was measured in the glenohumeral joint with the labrum intact or absent. This measure indicates the stability through the joint-surface interaction, independent of the magnitude of the compressive force applied.

decrease in the stability ratio of 10%. In the treatment of traumatic recurrent anterior shoulder instability, patients with a large osseous glenoid defect are at risk for recurrent instability after arthroscopic Bankart repair. Bone grafting performed in shoulders with a large osseous glenoid defect to restore the articulating surface provides excellent clinical outcomes.

To benefit from the concavity-compression joint constraint, a compressive or oppositional force across the joint surface is required. This joint compressive force is usually generated by muscle contraction. The relative location and direction of the resultant joint force to the available articulating surfaces will determine the potential stability of the

joint. For example, in the glenohumeral joint, if the resultant force is well located at the center of the glenoid articulating surface, the joint is considered stable. When the resultant force is directed outside the available joint surfaces, joint dislocation is most likely. However, when the resultant force is located within the joint surfaces but more toward the rim of the glenoid surface, the potential exists for joint subluxation. The interaction of muscle force resulting in a resultant joint constraint force is illustrated later.

Intra-articular or intracapsular pressure is the other component of passive and static joint constraint (Figure 6-14). The joint is usually surrounded by a capsule and sealed with synovial tissue. When the capsule is intact, any distraction force stretches and deforms the capsulo-ligamentous structure and will generate negative intra-articular pressure. This negative intra-articular pressure will counteract further displacement. When the joint capsule is vented, such negative pressure can no longer develop and cannot constrain the joint. Negative intracapsular pressure is therefore a constraint of all joints. However, it is especially important for joints under gravitational distraction, such as the shoulder joint.

Active muscle contraction is also important for joint stability. Proper coordination of muscle contraction provides dynamic joint constraint. On the other hand, deficiency of muscle innervation or a tendon defect can result in unfavorable joint constraint and lead to joint instability. Importantly, muscle force can act as either a joint stabilizer or a joint dislocator. The contribution of muscles to joint constraint is similar to that of passive capsulo-ligamentous tension. It is determined by the line of action of the passive and active tension of the muscle. Based on the relative position of the bony structure and the direction of the joint displacement, the same muscle-tendon structure can contribute differently to joint constraint. When the joint is spanned by multiple muscles, the coordination of muscle contraction will ultimately determine the dynamic joint stability. For example, the contributions of muscle contractions to the articulating surfaces of the shoulder can be described in terms of the normal and shear directions (Figure 6-15). The shear component has direct influence on the transverse translation of the bony element at the joint. In contrast, the normal force component of the muscle provides the compressive force to bring the surfaces together. A glenohumeral joint with a lax capsule and loose ligaments might be dynamically stabilized if the glenoid concavity is maintained and the function of the external and internal rotators, which are efficient stabilizers, is enhanced. On the other hand, in a patient with a rotator cuff tear, the pull of the major movers such as deltoids during arm elevation or

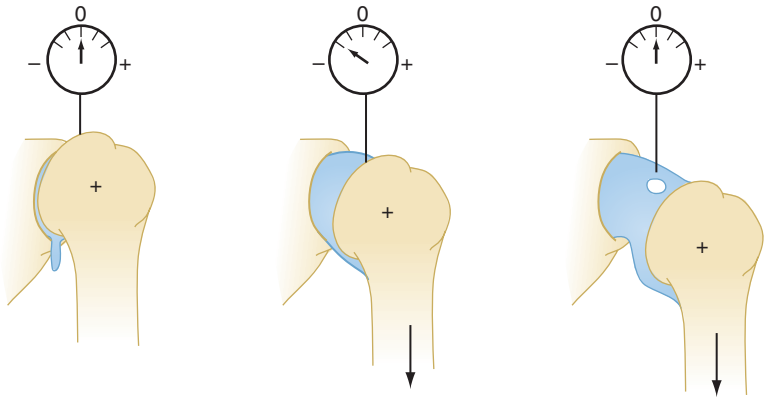



**Figure 6-14** Effect of negative intra-articular pressure on shoulder joint stability. In the normal situation, traction on the arm causes an increase in negative intraarticular pressure, and joint distraction is constrained. On the contrary, if the capsule is vented and air or fluid is introduced into the joint, traction on the arm no longer affects the intraarticular pressure; instead, the joint will be subluxed inferiorly.

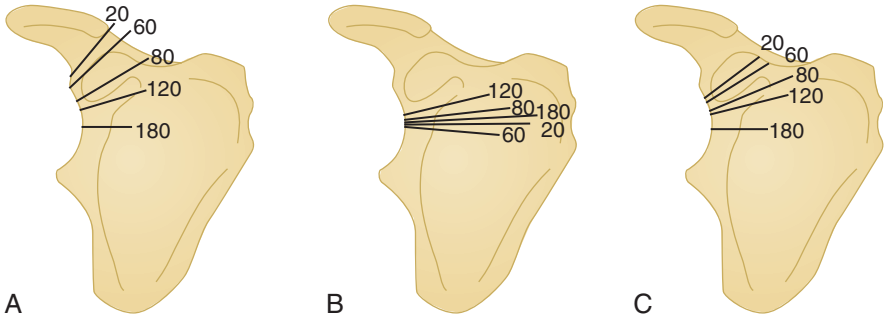



**Figure 6-15** Coordination of the muscle contractions to accomplish a given task is demonstrated by the situation when the deltoid and rotator cuff muscles work together to elevate the shoulder joint. **A,** For patients with a rotator cuff tear, the shoulder elevation is accomplished by the deltoid muscle alone. The resultant joint force is directed superiorly because of the line of action of the deltoid muscle. In this situation the joint is not stable and superior subluxation and migration results. **B,** On the other hand, when elevating the shoulder with the rotator cuff muscle alone, the resultant joint force points toward the center of the glenoid surface, which makes the joint very stable. However, the magnitude of the joint resultant forces are relatively high, which may not be ideal for the cartilage tissue. **C,** Ideally, shoulder elevation is accomplished with a combination of the deltoid muscles as the prime mover to provide strength and power, along with the rotator cuff muscles as the sterling in directing the joint resultant force to keep the joint in a more stable condition.

forward rotation will result in a resultant joint force in the superior and anterior directions, with resulting superior impingement or anterior instability.

In general, muscle contraction will generate moments about all three axes of rotation of a given joint. However, when the constraints from other periarticular soft tissues are not available, the muscle will be primarily responsible for maintaining stability during the joint's rotation. If a muscle crosses multiple joints, the effect of muscle recruitment depends on the moment arms of all the joints it spans. The concept of biarticular muscle is of extreme clinical importance. For example, when considering rehabilitation after reconstructive surgery of the anterior cruciate ligament, the goal is to strengthen the muscles without excessive tension being placed on the ligament. Quadriceps loading, especially with the knee in a near extended position, would result in an anterior joint shear force and anterior displacement of the tibia with respect to the femur. This shear force would induce tension in the anterior cruciate ligament. The concept of kinetic-chain exercise has been used extensively to address this problem. During closed-kinetic-chain–type exercises such as the squat and the leg press, the foot is fixed and knee motion is accompanied by motion at the hip and ankle joints. The ground reaction force causes external flexion moments at both the knee and hip joints. The antagonistic muscle action of hamstrings at the knee joint is recruited to balance the hip flexion moment. Co-contraction of quadriceps and hamstrings at the knee joint reduces the anterior joint shear force and the tension in the anterior cruciate ligament.[21]

## MECHANICAL LOADING ON TENDON

Tendons transmit the muscle contraction force to bone in order to cause limb movement. The force transmitted is primarily tensile force. In addition, specific portions of tendons also experience compression and shear force. Compression occurs when the tendon path is altered around a bony structure or pulley system. As a tendon is subjected to local compression, the mechanical loading does not distribute evenly within the tendon. The pressure decreases in deeper layers while the tension increases to the outer part. The overlapping of pressure and tension in the transition zone results in shear force inside the tendon. Furthermore, during tendon gliding against the surrounding tissues, shear force is generated on the contact surfaces of the tendon and surrounding tissues.

A tendon gliding through a pulley is analogous to a belt wrapped around a fixed mechanical pulley (Figure 6-16). As the tendon moves proximally, the tensions in the tendon proximal and distal to the pulley ($F_p$ and $F_d$) are related to the angle ($\theta$) of the tendon segments wrapped around the pulley or arc of contact and the friction coefficient ($\mu$).[22] Thus,

$$\text{Friction} = F_p - F_d = F_d(e^{\mu\theta} - 1)$$

This simple relationship clearly demonstrates the importance of the angle of contact and the friction coefficient. It explains why avoiding awkward joint postures is important ergonomically to reduce repetitive injury of soft tissue.

The three modes on mechanical loading (namely, tension, compression, and gliding) can be demonstrated by

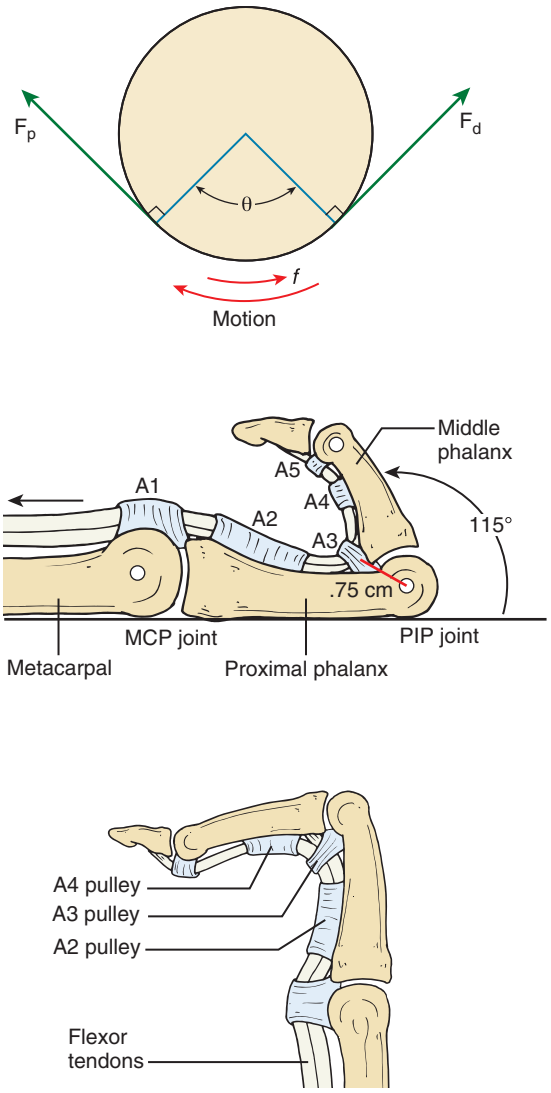



**Figure 6-16** To move the finger, the tendon glides through a pulley, which is analogous to a belt wrapped around a mechanical pulley. The tendon force must overcome the gliding resistance of the tendon and generate sufficient force to move the external load. The friction, *f*, of a tendon against the surrounding tissue is in the opposite direction of the tendon motion. The sliding resistance (friction) is the difference between the tension on the distal and proximal ends across the pulley ($F_d$ and $F_p$ respectively). MCP, Metacarpophalangeal; PIP, proximal interphalangeal.

three scenarios at the shoulder joint (Figure 6-17). The short head of the biceps brachii tendon is an example of the first scenario, in which the tendon mainly experiences tensile force alone. The second scenario, in which the tendon encounters tensile force and compression together, occurs internally at the bone insertion site of the supraspinatus tendon. The insertional area of the tendon encounters tensile force, as well as compression resulting from the transverse contact of the tendon with the attached bone. The third scenario occurs when the tendon is simultaneously subjected to tension, compression, and gliding. The long head of the biceps brachii tendon, where it passes through the bicipital groove, demonstrates an example of the third scenario. The supraspinatus tendon also fits the scenario, as well as the second scenario. Subacromial impingement of the supraspinatus tendon generates high

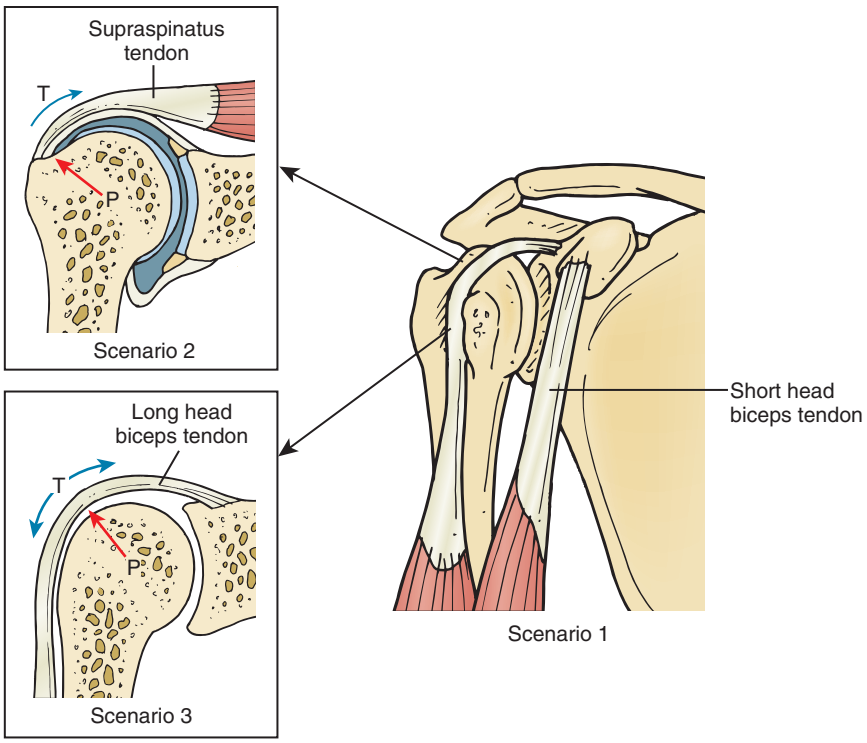



**Figure 6-17** Supraspinatus tendon and long head and short head biceps tendons. Scenario 1: tensile force only. Scenario 2: tensile force and compression. Scenario 3: tensile force and compression with gliding.

compressive stress that varies along the supraspinatus tendon, depending on humeroscapular elevation. The mode of mechanical tendon loading is potentially correlated to the incidence of tendon degeneration. The tendons in the first scenario rarely experience degenerative tendinopathy. Tendons commonly affected with tendinopathy are represented in the other two scenarios, in which the affected areas normally sustain compression and shear force with or without gliding in addition to tensile force.

In summary, knowledge of biomechanical principles can help explain many commonly encountered situations in the musculoskeletal system that contribute to musculoskeletal disease.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 7



# Regenerative Medicine and Tissue Engineering

Frank P. Luyten • Cosimo De Bari • Francesco Dell'Accio

**KEY POINTS**

- **Because tissue repair mimics the cellular and molecular cascade of embryonic tissue formation, investigating the role of developmental pathways in joint and skeletal disorders/diseases could identify novel therapeutic targets.**
- **Tissue repair and regeneration are partially determined by genetic factors.**
- **Tissue engineering has adopted the concept of biomimetics of in vivo tissue development. *Developmental engineering* is the term used to describe novel methodology for the rational and accurate design of robust, well-controlled manufacturing processes of "biological spare parts."**
- **Cellular therapeutics and their combination products are complex with regard to their mechanism of action and manufacturing and have evolved into advanced therapy medicinal products with a specific regulatory path.**
- **Recent advances in regenerative medicine and tissue engineering relevant to rheumatology have entered clinical practice and include the biologic repair of joint surface defects and bone healing.**

Destruction of joint tissues is ultimately the disabling outcome of most forms of inflammatory or degenerative arthritides. The need for repair and regeneration of joints and joint-associated tissues is becoming more relevant as the dramatic advances of targeted treatments and improved disease management have allowed much more efficient control of inflammation and joint destruction.

In view of this situation, other aspects of joint biology deserve more attention—notably, and most importantly, the mechanisms driving tissue response and repair.[1] Indeed, to restore the balance between tissue destruction and tissue repair (Figure 7-1), we should be looking at the more complete picture, that is, the "systems biology" of the joint as the organ of the rheumatologist. Introducing regenerative medicine provides a significant opportunity to restore joint homeostasis and thus possibly provide a cure. Targeting repair has entered our discipline, and investigating the potential to activate and enhance joint tissue repair mechanisms has become a prime goal.

Regenerative medicine and tissue engineering (TE) seek to repair or regenerate damaged tissues and organs, regardless of the cause of the damage, without leaving scar tissue, thereby restoring both the structure and function of the damaged tissues/organs. Nature demonstrates that this goal is achievable, because successful wound healing and fracture repair are processes that happen routinely after birth. We also know, as demonstrated in fetal surgery, that scarless repair is partially dependent on age and context. Thus it is attractive to envision that, with an in-depth understanding of the repair processes at the cellular and molecular level, we may be able to intervene quickly at the time of injury and guide the healing process more appropriately, thereby preventing scar formation.

Postnatal tissue healing mimics developmental processes of tissue formation. For example, it appears that the process of rebuilding an adult limb, such as seen in the axolotl, but also during fracture healing in higher species, has many similarities with how the limb forms in the first place in the embryo, and particularly requires signaling mechanisms to specify the final pattern. Thus both limb formation and limb regeneration are likely to use the same molecular pathways.[2] The remarkable advances in developmental biology during the past several decades have now provided the knowledge platform to advance into novel regenerative approaches in postnatal life. These advances not only include our understanding of the mechanisms of body axis formation and organogenesis but also impressive progress in stem cell biology, including the regulation of stemness, stem cell niches, lineage specification and cell differentiation, and the critical molecular pathways involved. We are now in a position to enter a new era in regenerative medicine and TE.[3,4] In this chapter we will review the approaches that seek to repair damaged and diseased tissues and, in particular, synovial joints and skeletal structures.

When we seek to repair tissues, two mechanistic approaches are possible. The first approach consists of enhancing intrinsic repair mechanisms with stimulation of cell proliferation, differentiation and tissue metabolic activity, and recruitment of endogenous progenitor populations into the damaged tissue. The second approach becomes necessary when intrinsic repair is not feasible or insufficient and entails extrinsic repair, that is, TE approaches via manufacturing of cellular and/or combination products that can contribute mostly locally to tissue repair (Figure 7-2).

## INTRINSIC REPAIR MECHANISMS

### Genetic Basis of Tissue Repair

For many years it has been commonly accepted that "cartilage…when destroyed, it is never recovered."[5] This statement probably still holds true for established defects

(e.g., defects that have failed to heal and have become chronically symptomatic, and therefore come to the attention of the clinician); however, prospective imaging and arthroscopy studies have revealed that even in adult humans, acute cartilage defects are much more prevalent than suspected, even in asymptomatic subjects, and have some capacity for spontaneous healing (reviewed in reference 6).[6] Such studies showed that roughly half of adults without joint symptoms have chondral defects[7,8] and that about a third of such lesions improve spontaneously, a third remain stable, and the remainder worsen.[7,8] The outcome appears to depend on a number of risk factors including age, co-existing osteoarthritis (OA), high body mass, female sex, abnormal bone geometry, and the presence of bone marrow lesions.[9,10]

A prospective, magnetic resonance imaging (MRI)-based study in a sib-paired cohort[11] identified a very high (>80%) heritability of the rate of progression of chondral defects. Nonetheless, in contrast with the remarkable progress in the identification of genetic markers predisposing to OA,[12,13] no quantitative trait linkage and association analysis study is available yet in humans with regard to the capacity of repairing cartilage defects.

Although the evidence for the heritability of cartilage repair capacity in humans is still circumstantial, findings of animal studies are more convincing. Comparing the capacity of different inbred mouse strains to heal full-thickness cartilage defects, Eltawil et al.[14] demonstrated that different inbred mouse strains have a dramatically different capacity to heal cartilage defects and to avoid the onset of post-traumatic OA. This finding supports the concept that the repair capacity has a genetic component. The development of such an animal model lends itself to genetic analysis. For instance, Rai et al.[14a] took advantage of the different healing capacity of two strains of mice: LG/J, which can efficiently heal joint surface defects and experimental wounds to the cartilage of the pinna of the ear, and SM/J, which are poor healers. A set of recombinant inbred lines were obtained by crossing the two strains. Thus it was found that both the capacity of healing joint surface defects and the capacity to heal ear wounds were highly heritable and that they correlated with each other. This finding suggests that the capacity to heal cartilage is inheritable by itself, whether the cartilage is on the surface of a diarthrodial joints or in the pinna of the ear, and is not just dependent on the resistance to develop OA or on factors extrinsic to cartilage such as bone shape/quality or synovitis. The fact

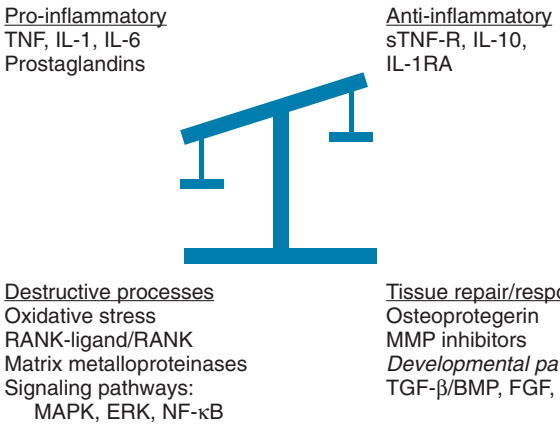



**Figure 7-1** The "systems biology" view of chronic arthritis. The severity and outcome of disease is determined by the balance between inflammation/destructive processes and anti-inflammatory signals with repair attempts. BMP, Bone morphogenetic protein; ERK, extra-cellular-signal regulated kinase; FGF, fibroblast growth factor; IL, interleukin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; RANK, receptor activator of nuclear factor-κB; sTNFrec, soluble tumor necrosis factor receptor; TGF, transforming growth factor; TNF, tumor necrosis factor.

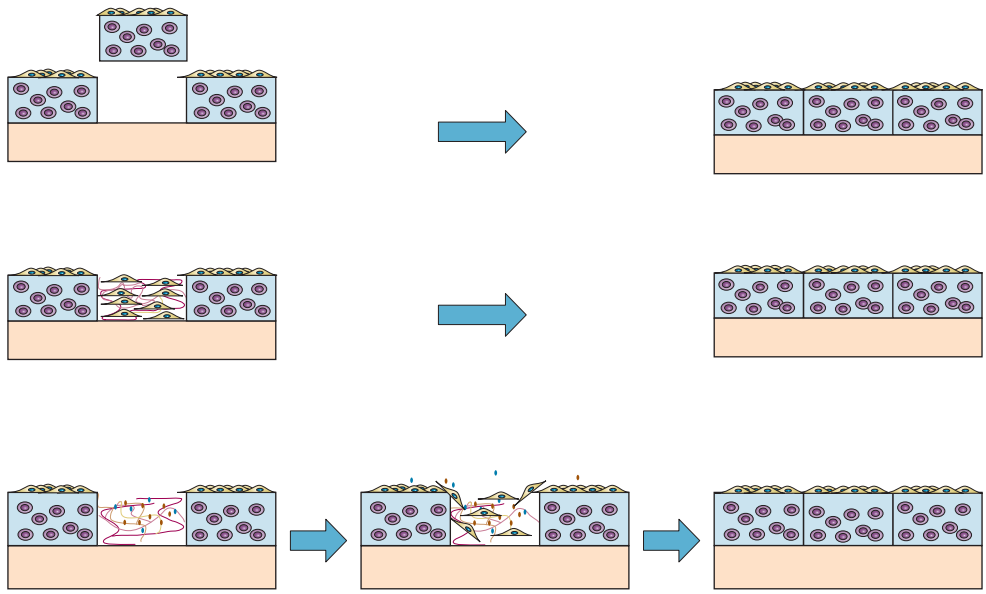


**Figure 7-2** In traditional tissue engineering *(top)*, the repair tissue is generated and matures in the laboratory and is inserted in the defect directly. No maturation is expected to happen in vivo. In cell-based approaches *(middle)*, nondifferentiated or partially differentiated in vitro expanded cells are implanted. Differentiation and remodeling is expected to happen in vivo. In cell-free approaches *(bottom)*, only biomaterials and bioactive molecules are implanted, which, in vivo, attract resident progenitor cells and induce their patterning and differentiation.

that homeostatic mechanisms affect the rate of OA progression suggests that the same genes associated with the predisposition to OA might affect also the capacity to repair; however, this assumption has never been rigorously tested experimentally. It must be remembered that other independent aspects come into play in the determination of OA progression, including joint shape,[17,18] body mass index, inflammation, and several other aspects that are also under genetic control.[19] Therefore allelic variants that affect such parameters will also affect OA predisposition, independently from repair mechanisms intrinsic to the joint. Nevertheless, it is interesting that signaling molecules belonging to the same pathways have been associated with both OA and regeneration. For instance, loss of function alleles of the WNT inhibitor FRZB are associated with OA predisposition,[20-22] and a single nucleotide polymorphism of the WNT ligand WNT-3A has been associated with the heritability of cartilage repair capacity in mice.[23]

## Signaling Pathways

Because postnatal tissue repair mimics tissue formation during development in many aspects, it is likely that developmental pathways involved in the formation of the joint and joint-associated tissues could be potential targets to enhance postnatal tissue repair.

Typical developmental pathways critically involved in the formation of skeletal tissues and structures such as the synovial joints are the transforming growth factor (TGF)-β superfamily, including the bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs), the Wnt family of proteins, the fibroblast growth factors (FGFs), the hedgehog proteins, parathyroid hormone (PTH)/PTH-related protein (PTHrP), and Notch signaling. In this chapter we select a few examples of how these signaling pathways are involved in joint formation and their role in postnatal joint repair, with a specific focus on some of these pathways that have led to advances in early clinical development in arthritic diseases.

### Transforming Growth Factor-β/Bone Morphogenetic Protein Signaling

Recent data have highlighted the relevance and critical role of the members of the TGF-β superfamily (TGF-β, BMPs, and GDFs) in the biology of articular cartilage, bone, joints and joint-associated tissues, both during development and in postnatal tissue homeostasis, repair, and tissue response to injury and ageing. TGF-β has been shown to be involved in the maintenance and ageing of articular cartilage and OA.[24] BMPs have been reported to play major roles in articular cartilage metabolism, with BMP-7/osteogenic protein-1 (OP-1) being of particular interest.[25,26] In addition, the data indicate that modulation of TGF-β/BMP downstream receptor-Smad signaling plays an essential role in both the regulation of chondrocyte differentiation and the development and progression of OA.[27] Therefore, given the overwhelming evidence of the regenerative potential of this family of growth and differentiation factors in preclinical models, it is not surprising that their therapeutic use is being explored in clinical studies and in indications such as long bone healing and OA. In addition, intense research is ongoing to identify modulators of the TGF-β/BMP receptor/Smad signaling pathways through peptide technology (peptidomimetics) or small molecule screens.[28] Because the synovial joints allow for local treatments, it is expected that some of the newly identified compounds will first be tested in local applications, such as joint surface repair and monoarticular OA. BMP devices are already in the clinic for orthopedic applications such as spine fusion and the healing of nonunions.[29,30]

### Fibroblast Growth Factor Signaling

Extensive investigations have identified FGFR3 signaling as a key regulator of chondrocyte and osteoblast function, both during development and postnatally. In particular, absence of signaling through FGFR3 in the joints of *Fgfr3*$^{-/-}$ mice leads to premature cartilage degeneration and early signs of OA.[31] One of the key ligands of FGFR3 signaling appears to be FGF18.[32] In the postnatal joint, FGF18 has significant anabolic effects on cartilage metabolism. Intra-articular injection of FGF18 induced a dose-dependent increase in cartilage formation and a reduction in cartilage degeneration scores in the medial tibial plateau of rats with OA.[33] Importantly, this effect was seen only in joints affected by OA, not in normal rat joints, suggesting a specific response to tissue injury. At the molecular level, this joint-protective effect may be due partially to its interaction with other signaling pathways such as BMP signaling by repressing noggin, a BMP antagonist.[34] These findings, combined with the increased understanding of FGF18 signaling in postnatal cartilage and bone biology, led to the development of a local treatment with FGF18 (sprifermin) and the design of clinical trials (clinicaltrials.gov) in both patients with cartilage lesions and patients with OA of the knee.

### Wnt Signaling

Overwhelming evidence indicates a critical role for Wnt signaling in cartilage and bone biology, with a specific relevance to osteoporosis and OA (for a review, see Luyten et al.[35]). Although Wnt signaling is essential for the development and homeostasis of synovial joints,[36-40] genetic studies in humans[20] and experimental data[22,41,42] demonstrate that excessive/uncontrolled activation of Wnt/β catenin signaling leads to reprogramming of articular chondrocytes toward catabolism or loss of their stable phenotype with subsequent loss of articular cartilage tissue structure and function. In particular, loss of function allelic variants of the Wnt inhibitor FRZB were associated with increased predisposition to develop OA.[20] Similarly, disruption of the *Frzb* gene in mice resulted in increased activity of the WNT signaling pathway and consequent increased bone stiffness and enhanced cartilage damage.[22] Importantly, upon induction of experimental OA, *Frzb*$^{-/-}$ mice showed greater cartilage loss than their wild-type counterparts.[43] Increased cartilage damage in *Frzb*$^{-/-}$ mice was associated with higher levels of β-catenin dependent Wnt signaling and with higher expression levels of matrix metalloproteinase (MMP)-3. It was also demonstrated that FRZB can directly inhibit MMP-3, probably through the netrin domain, indicating the potential complexity of the underlying mechanisms of observed phenomena.[44]

Tightly regulated Wnt signaling is critical for the homeostasis of synovial joints.[38,40,45,46] Diarra et al.[47] demonstrated that in human and experimental murine arthritis, the inflammatory cytokine tumor necrosis factor drives the excessive production of the Wnt antagonist DKK1. The consequent excessive inhibition of the Wnt pathway was found to be responsible for bone resorption (erosions), typical of this arthritis model, as well as of rheumatoid arthritis (RA). Accordingly, blocking DKK1, and therefore de-repressing Wnt signaling, resulted in reversal of bone damage.[47] In the same model, direct activation of WNT signaling using the Wnt agonist R-Spondin 1 reversed not only the bone damage (also achieved with DKK1 blockade), but also the cartilage damage.[48] Therefore, both uncontrolled suppression and activation of the WNT signaling may lead to catabolism and cartilage loss. It appears that a tight control, both in terms of timing and magnitude, is essential not only to preserve homeostasis but even to induce tissue regeneration. For instance, Yuasa et al.[46] demonstrated how controlled and temporary activation of β catenin signaling in adult mice led to an initial reduction of extra-cellular matrix (ECM), followed by chondrocyte proliferation and thickening of the articular cartilage.[46] Even more strikingly, although more remote from the context of joint disease, Kawakami et al.[49] demonstrated that although WNT signaling is essential for the capacity of some species (i.e., axolotl or Xenopus tadpoles) to regenerate entire limbs, it was sufficient to slightly modify the spatiotemporal distribution of WNT-β catenin activation to afford the chick embryo with the capacity to regenerate an entire limb.

In the clinical context of joint disease and regeneration, the complexity of Wnt signaling and its potential downstream effects may offer the opportunity to target catabolic effects while preserving the homeostatic ones. Nalesso et al.[50] demonstrated that WNT-3A drives chondrocyte proliferation through activation of the β catenin pathway and loss of differentiation through activation of CaMKII. Therefore, targeting CaMKII specifically allowed enhancement of differentiation without affecting proliferation. Better understanding of the downstream signaling mechanism and its regulation will be essential to target only the pathogenic effect of these pleiotropic signaling pathways in cartilage, bone, and the osteochondral junction.

### Growth Hormone/Insulin-like Growth Factor Axis

Other targets besides skeletal developmental pathways are activated by signaling molecules that also play critical roles in postnatal joint tissue homeostasis and turnover. These proteins/pathways can be regarded as potential postnatal "anabolic" agents that contribute to the restoration of joint homeostasis. In this regard, the growth hormone (GH)/insulin-like growth factor (IGF) axis appears to be of interest. IGF-1 has been reported to be critical in the maintenance of the homeostasis of articular cartilage explants ex vivo.[51] Further evidence of its anabolic effect in in vivo models has led to the early clinical development of intra-articular treatments with IGF-1 in knee OA, although reports of clinical trials are awaited. Furthermore, it was reported that systemic administration of GH in horses may be beneficial to joint/articular cartilage biology because it increases IGF-1 levels in synovial fluid.[52] Improved formulations of GH have been explored to improve the duration and effect size in synovial joints.[53] Some suggestions exist of a relationship between levels of IGF-1 and OA, further suggesting a potential benefit of targeting the GH/IGF-1 axis, particularly in OA. However, data thus far are inconclusive, and further systematic analysis of the hypothalamic-pituitary axis, including GH, IGF-1, and somatostatin, is required.[54]

As for all growth factor technology, in particular for the major signaling pathways previously mentioned, further studies are needed to address critical issues such as the relevant genetic background of the patients, stage of the disease process, and tissue specificity to restrict an effect to the targeted tissues, thereby avoiding systemic adverse effects.

## Stem Cell Niches

Stem cells persist in adult life to safeguard tissue maintenance and regeneration. They have the capacity to self-renew (that is, to produce more stem cells), thereby preserving a constant pool of stem cells, and they have the capacity to differentiate to replace the mature cells that are lost to physiologic turnover, injury, disease, or ageing. Self-renewal and differentiation are regulated by stem cell intrinsic factors and signals from the surrounding microenvironment in which the stem cells reside, called the "stem cell niche."

Stem cell niches have been described for a number of tissue types such as the hair follicle, intestine, and bone marrow.[55,56] In bone marrow the niches of hematopoietic stem cells (HSCs) have received considerable attention, and the understanding of HSCs has led to refinements of bone marrow transplantation in hematology. The HSC niches include the endosteal niche, where HSCs are in close contact with osteoblasts residing at the bone surface of the trabeculae, and the perivascular niche, where the HSCs are found close to the sinusoids in the bone marrow.[44]

Mesenchymal stromal/stem cells (MSCs), which are derived from bone marrow and connective tissues, including the synovium, have the ability to undergo lineage specification into chondrogenic and osteogenic cells in vitro, and to some extent also in vivo when combined with proper biomaterials and/or growth factors. Therefore, MSCs are of interest for their potential clinical use for joint tissue repair.

The joint environment is rich in progenitor/stem cells, but our understanding of their anatomic location in apposite niches and the molecular regulation of their functions remains limited. The lack of specific cell-surface markers has hindered the direct identification of the so-called MSCs in their native tissues. In bone marrow, MSCs collect in perivascular areas, where they are in close proximity to HSCs and contribute to maintaining their quiescence.[57]

Evidence is growing that pericytes may be the native cells of the ex vivo MSCs.[58] Pericytes are located on the abluminal side of small blood vessels and are in close connection with the endothelial cells of the vessels. The proximity to vessels would allow MSCs to enter the bloodstream and migrate to sites of injury,[59] but it is not clear to what degree this happens and if this phenomenon has clinical relevance. However, the notion that cells with MSC

characteristics are derived from pericytes is challenged by the retrieval of MSC-like cells in articular cartilage,[60-62] notoriously an avascular tissue. Thus pericytes may not be the only source of MSCs, and such a relationship is likely to be tissue specific and context dependent. It also must be noted that the term *pericyte* is an anatomic description of a cell that is abluminal and that its developmental origin is not clear. It is likely that, rather than having a common ancestor, pericytes have distinct ontogeny and biologic properties that are related to their tissues of residence. Cell lineage tracing experiments, which are increasingly feasible with modern technologies and detection systems, will shed light on these unresolved issues related to the in vivo nature of the native MSCs in the natural environment of their intact tissues.

Recently a double nucleoside labeling scheme in a mouse model of joint surface injury allowed the identification in the adult synovium in vivo of a population of label-retaining slow-cycling stromal cells with an MSC-like phenotype, which proliferated and differentiated into chondrocytes after injury, hence displaying typical properties of MSCs.[63] In synovium, MSCs are located in two niches: a niche in the lining layer and a perivascular niche in the sublining tissue, with the latter apparently distinct from pericytes. In these two niches, MSCs could have distinct functions, but a hierarchy remains to be demonstrated (Figure 7-3). Furthermore, MSCs in synovium is a common denomination but could reflect a co-existence of functionally distinct cell subsets.[63] The developmental origins of MSCs in the adult synovium are not known. They could in part derive from the embryonic joint interzone, as lineage tracing studies suggest.[40,64] Of note, some of the cells of the synovial lining and the superficial layer of the articular cartilage not only share a common origin from the embryonic joint interzone[40,64] but also contain cells displaying similar features, such as the ability to produce lubricin, a critical component of synovial fluid that participates in the lubrication of synovial joints.[65] Mesenchymal progenitor cells are thought to reside in the superficial zone of the articular cartilage because they display functional properties of stem/progenitor cells after isolation and culture expansion.[62] However, thus far there is no evidence that cells residing in the superficial layer are professional stem/progenitor cells with the specific function to remodel and regenerate the articular cartilage, thereby explaining the poor intrinsic repair capacity of the cartilage.

An understanding of the native MSCs and how signals at the niche sites are orchestrated toward joint homeostasis, remodeling, and repair will be essential to provide guidance regarding clinical applications that use cell-based approaches. The restoration of a functional niche will safeguard durable repair by ensuring lifelong replacement of mature cells. An exciting prospect is the pharmacologic targeting of MSCs and niche repair signals to promote joint surface repair and influence outcomes of joint disorders such as OA, with the ultimate goal of restoring joint homeostasis.

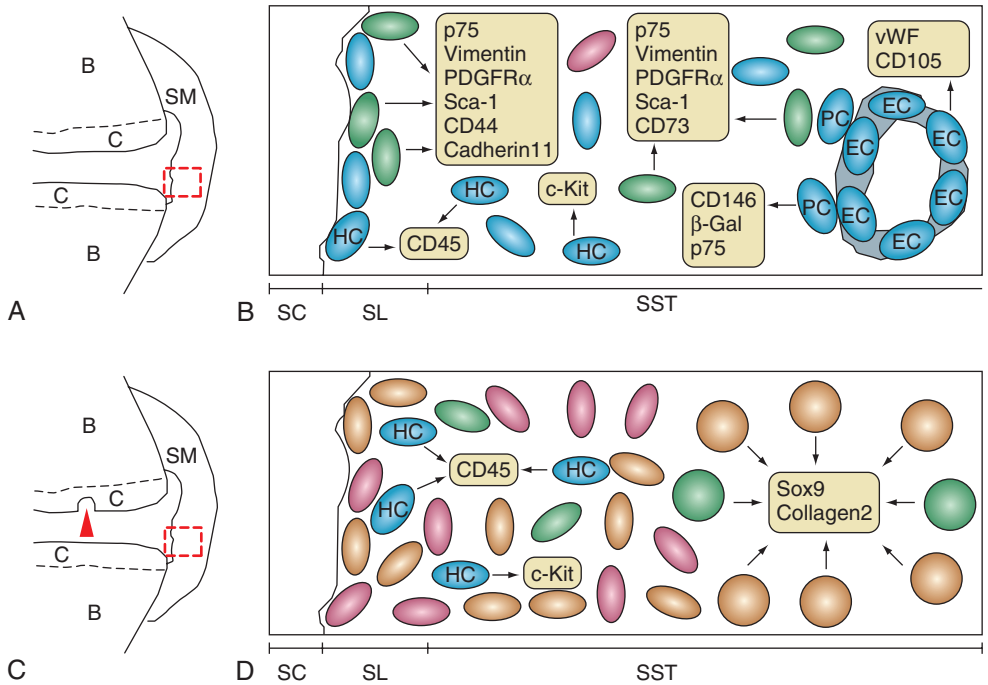



**Figure 7-3** Schematic representations of mesenchymal stem cells (MSCs) and their niches in synovium identified in mice using a double nucleoside analogue cell-labeling scheme.[76] **A**, Schematic drawing of an uninjured control synovial joint. **B**, Details of the dashed box in **A**, showing cell populations in the synovium of uninjured joints. Iododeoxyuridine (IdU)-retaining cells *(green)* were located in both the synovial lining (SL) and the subsynovial tissue (SST). Subsets of IdU-positive cells displayed an MSC phenotype. IdU-negative cells *(blue)* included hematopoietic lineage cells (HC), endothelial cells (EC), pericytes (PC), and other cell types of unknown phenotype. **C**, Schematic drawing of a synovial joint 12 days after articular cartilage injury in mice *(arrowhead)*. **D**, Details of the dashed box in **C**, showing cell populations in the synovium. Proliferating cells were detected in both the synovial lining and the subsynovial tissue and were either double positive for IdU and chlorodeoxyuridine (CldU; *orange*) or single positive for CldU *(red)*. Subsets of cells positive for IdU and CldU and cells positive only for IdU *(green)* expressed chondrocyte lineage markers. The boxed areas in **B** and **D** show cell phenotypes. B, Bone; C, cartilage; p75, low-affinity nerve growth factor receptor; PDGFR, platelet-derived growth factor receptor; SC, synovial cavity; SM, synovial membrane; vWF, von Willebrand factor. *(From Kurth TB, Dell'Accio F, Crouch V, et al: Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo,* Arthritis Rheum *63:1289–1300, 2011.)*

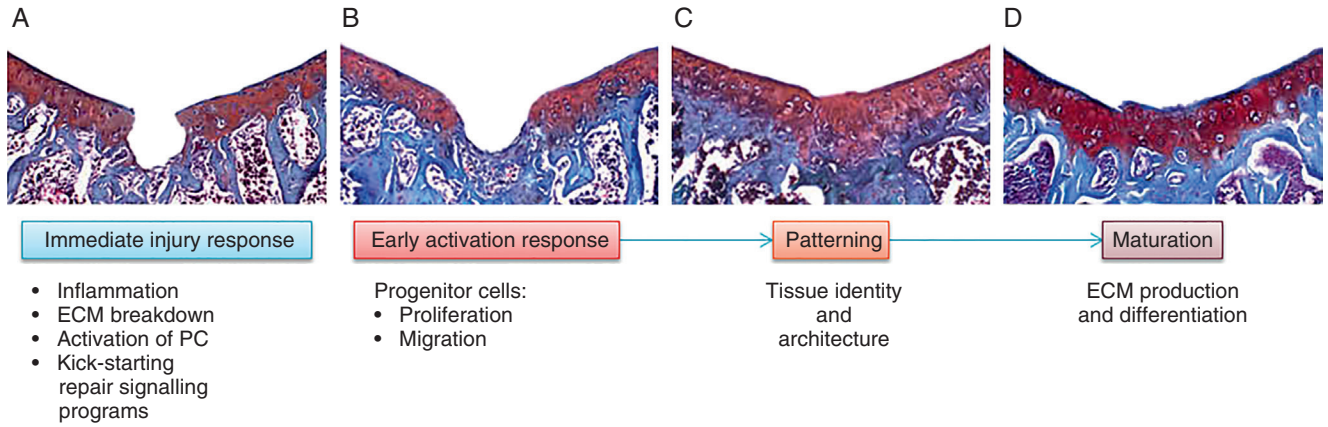



**Figure 7-4** Minutes after injury, tissues, including the articular cartilage, deploy a molecular response **(A)**, which activates the migration of progenitor cells **(B)**, the formation of a patterned repair tissues **(C)**, and its maturation **(D)**. ECM, Extra-cellular matrix; PC, progenitor cell.

## Targeting Homeostasis of the Joint

In the resting condition, articular cartilage has an extremely low rate of turnover. For instance, the half-life of type II collagen has been estimated as being around 117 years,[66] and articular chondrocytes are all essentially in the G0 of the cell cycle. After acute traumatic injury, however, chondrocytes deploy strong adaptive responses that ultimately result in a coordinated sequence of activation and chemotaxis of progenitors within the cartilage itself and in other joint tissues, along with cell proliferation and matrix turnover. In a number of cases, such adaptive responses are sufficient to repair damage and re-establish homeostasis.[15,16] However, if the injury exceeds the adaptive capacity or if the adaptive responses are hampered in some way, cartilage loss leads to OA and ultimately joint failure. In OA the "injury" (i.e., excessive mechanical load) is continuous, and no immediate loss of tissue occurs. In such a situation the homeostatic responses are more subtle and are heralded by the upregulation of the transcription factor SOX9 and its direct targets, type II collagen and aggrecan,[67-69] as well as a limited degree of chondrocyte proliferation. It is interesting to notice that such types of homeostatic responses persist even at late stages of OA.[70-74]

### Cellular and Molecular Mechanisms of the Homeostatic Response

The sequence of events that leads to an adaptive response in cartilage is difficult to study in OA, which is a slow-progressing disease, but such study has been facilitated by the development of in vitro and in vivo models of acute injury. The first evidence of adaptive responses consisting of chondrocyte proliferation and new deposition of ECM dates back nearly one century (reviewed in reference 6). More accurate molecular analyses have identified several other components that are amenable for targeting. These components include an immediate molecular response, the activation and attraction of progenitors, breakdown of the residual mature ECM, the filling of the defect, tissue patterning, and, finally, tissue maturation—which, in humans, can take several years. In the entire joint, inflammation is an integral part of a natural response to injury, providing molecular signals and cascades that are likely to have distinct context and tissue-specific beneficial or detrimental effects. Studies aimed at teasing apart the "good" from the "bad" pathways will help modulate repair processes and restore/maintain joint homeostasis.

In therapeutic terms, therefore, it may be possible to target any of these phases, but it is likely that a stratified approach will be necessary to ensure efficacy and consistency. Such an approach will start with the understanding of which step of the homeostatic response has failed in groups of individual patients so that the appropriate approach will be targeted to the appropriate patients (Figure 7-4).

**Immediate Molecular Response.** Scarification of the articular cartilage in vitro or in vivo induces a strong early molecular response dominated by the activation of signaling pathways known to play important roles in embryonic skeletogenesis.[14,75-80] Although this response is very rapid and transient, it primes a cascade of downstream steps. Probably the best known pathways involved in this phase are the WNT, FGF, and TGF-β/BMP pathways, which are discussed elsewhere in this chapter. It is important to say here, however, that although proof of concept for efficacy with each of these pathways has been provided in some way in different models, the often disparate functions that these pleiotropic signaling molecules have in each different cell type remain a challenge. Tissue-targeted treatments will be of importance, including the use of novel smart delivery systems to confine the signaling domain of such powerful morphogens and avoid off-target effects.

**Activation and Attraction of Mesenchymal Progenitors.** It has been suggested that mesenchymal progenitors are implicated in the repair of joint surface defects, including those derived from bone marrow,[81] the synovial membrane, and the cartilage itself.[61-63,82-84] However, the origin and lineage of the cells that contribute structurally to cartilage regeneration is still uncertain and the object of intense research. Yet the understanding of the pathophysiologic mechanisms driving stem cell commitment and differentiation is paving the way to promising therapeutic interventions that exploit homeostatic mechanisms. In 2012, Johnson et al.[15] identified a small compound, kartogenin, which, by interacting with filamin A, regulates the

activity of the CBFβ-RUNX1 transcriptional program in bone marrow–derived mesenchymal cells, inducing their chondrogenic differentiation. The delivery of kartogenin to mice subjected to instability-induced OA improved pain and resulted not only in the arrest of disease progression but even in regeneration of the cartilage tissue to some degree. Although kartogenin could induce chondrocytic differentiation of bone marrow stromal cells in vitro, it is not clear whether this effect on bone marrow stromal cells contributed to the chondroprotective effect of kartogenin in vivo. In fact, recent data show that kartogenin could support harmonious skeletal growth, joint morphogenesis, and the resorption of the interdigital mesenchyme, which are otherwise stunted in ex vivo cultures of embryonic mouse limbs.[85] These effects were associated with the activation of several signaling pathways and in particular of the TGF-β pathway. The effect of kartogenin is likely to be more complex than the simple capacity to induce chondrogenic differentiation of MSCs.

**Patterning and Differentiation.** After the joint surface defect is filled with mesenchymal cells, the repair tissue needs patterning to acquire the final architectural structure, with a properly layered cartilage and osteochondral junction. Interestingly, both morphologically and molecularly, the repair tissue in an osteochondral defect resembles the epiphysis of a developing skeletal element, with flat cells at the surface, then chondrocytes in different stages of maturation, and hypertrophic chondrocytes at the boundary with the bone front.[86] With time the bone front advances and, for successful repair, must stop to leave a layer of permanent stable articular cartilage. Failure of this patterning process results in failure of repair both in animal models[87,88] and also in humans, where excessive advancement of the bone front can lead to intra-articular osteophytes after surgical procedures such as bone marrow stimulation by microfracture.[89] Clearly such sophisticated coordination of differentiation requires a tight control and positional information so that each cell "knows where it is and what to do" within the repair tissue.

PTHrP signaling during embryonic development of the skeletal elements contributes to the positional information and regulates the rate of differentiation of the chondrocytes through the epiphysis, preventing precocious or ectopic hypertrophic differentiation and endochondral bone formation.[90-93] The PTH/PTHrP receptor PPR is hardly expressed in normal cartilage but is strongly upregulated in the intermediate layers of repair cartilage, just above the bottom layer of hypertrophic chondrocytes[86] and in osteoarthritic cartilage. Because hypertrophic differentiation of articular chondrocytes is not only a feature of OA but also drives cartilage breakdown[94,95] and PTH/PTHrP signaling is known to delay hypertrophy, Sampson et al.[16] tested whether a truncated form of human recombinant PTH currently used in the clinic for osteoporosis could improve the outcome of OA in mice. Indeed, human recombinant PTH could not only stop cartilage breakdown but also induced a regenerative effect. Unfortunately, the doses required to achieve this impressive effect in mice were very high and induced osteopetrosis. A restriction of the signaling domains to the tissue of interest is therefore required before this approach can be used in the clinic. Another caveat is that this study cannot discriminate between the effects on cartilage and those on the bone. In this context, it is noteworthy that strontium ranelate, another compound used as an anabolic agent in osteoporosis, was also able to reduce the rate of joint space narrowing and to improve symptoms in patients with OA.[96] Similar results were replicated in a rat and a dog model of instability-induced OA.[97,98]

## EXTRINSIC REPAIR: CURRENT THERAPEUTIC INTERVENTIONS

Cell-based therapies and TE for joint repair have entered clinical practice with long-term follow-up data. At an earlier stage of development, the use of embryonic stem cells, induced pluripotent stem cells, or adult stem cells (such as MSCs) have shown promising pre-clinical and early clinical safety and efficacy data.

The mechanisms through which the cellular therapies and combination products contribute to tissue repair are multiple and involve direct engraftment, proliferation, and differentiation to tissue-specific cell types but also include paracrine actions such as the secretion of growth and differentiation factors that enhance the local tissue response.[99] In addition, adult stem cells may have immunomodulatory properties, which have been clinically explored in graft-versus-host disease and autoimmune diseases.

### Joint Surface Defects

A variety of articular cartilage repair techniques have been developed, typically for the treatment of (sub)acute focal joint surface defects that are often associated with excessive joint loading, either as a result of high-impact trauma or cumulative low-impact repetitive overload. Joint surface defects are classified as chondral or osteochondral. Joint surface lesions can extend to the subchondral bone (osteochondral defects) or be limited to the cartilage (chondral defects). Most chondral defects do not reach to the bone (partial thickness), and only about 5% are full thickness[100]; nevertheless, because most partial-thickness lesions are debrided at the time of surgery to full thickness, including the removal of the calcified layer (Figure 7-5), repair strategies focus on the treatment of full-thickness defects. The best established cell-based technology for the treatment of these lesions is autologous chondrocyte transplantation and its several variations.

#### Autologous Chondrocyte Transplantation

Regeneration or repair of symptomatic articular cartilage defects has been at the forefront of regenerative medicine ever since 1994 when Brittberg et al.[101] reported a remarkably good clinical and structural outcome using a procedure called autologous chondrocyte transplantation/implantation (ACI). Briefly, cell populations were prepared by enzymatic release from a biopsy specimen of articular cartilage taken from an unloaded area in the symptomatic joint. The chondrocytes were subsequently expanded in vitro, and after six to eight population doublings, they were reimplanted in the joint surface defect under a periosteal flap taken from the tibia from the same patient. This procedure was then followed by a long rehabilitation period to reach its optimal outcome at 18 to 24 months. This high-profile publication

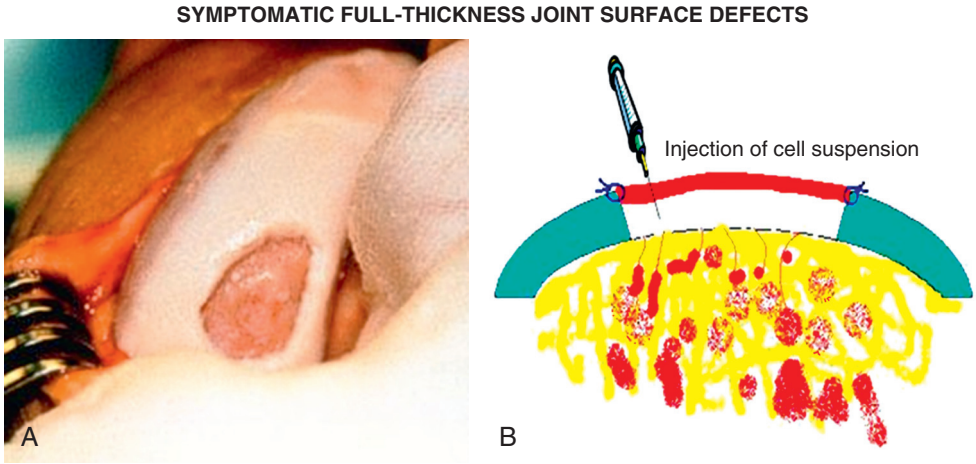



**Figure 7-5** **A,** View at open knee surgery of a large full-thickness articular cartilage defect. Note the sharp borders of the lesion after surgical debridement. **B,** A cell suspension, such as autologous chondrocytes, is injected beneath a periosteal flap or membrane sutured on the joint surface and sealed with fibrin glue to prevent leakage.

attracted considerable interest and triggered a wave of basic, translational, and clinical research in the field. It also evolved quite quickly in 1997 in the marketing of Carticel, an autologous cell product for the repair of symptomatic condylar defects of the knee, by Genzyme Corporation (Cambridge, Mass.).

Progress has been made since then with studies aiming to improve and standardize the autologous chondrocyte preparation (the cell product), the development of other delivery systems for the chondrocytes and the replacement of the periosteal flap with a membrane of diverse composition, and a series of clinical studies. After a number of mostly open studies with diverse outcomes (for a review see van Osch et al.[102]), a study by Knutsen et al.[103]—the first multicenter, prospective, randomized trial—failed to demonstrate that ACI was superior to microfracture, a technique mostly considered to be the standard of care for small (<2 to 3 $cm^2$) symptomatic joint surface defects. Microfracture is a bone marrow stimulation procedure and is based on the puncturing of the subchondral bone plate into the bone marrow, the generation of a blood clot containing precursor cell populations derived from the subchondral bone marrow, and the spontaneous transformation of the repair clot into a fibrocartilaginous repair tissue.[104] Microfracture is clearly associated with a clinical benefit and a filling of the joint surface defect with repair tissue, but it is generally accepted that this repair is not durable, resulting in a consistent decline in clinical outcome over the long term.[105,106] It is the aim of ACI to repair the cartilage defect with better quality tissue, mostly hyaline cartilage with tissue characteristics matching those of the neighboring tissue, thereby resulting in improved long-term outcomes (Figure 7-6). Although the study by Knutsen et al.[103] did not indicate clinical superiority of ACI over microfracture, there were some indications that good structural repair was associated with durable clinical outcome.[107]

Subsequently, progress was made by improving the manufacturing process of the chondrocyte preparation. Indeed, in Europe, an autologous chondrocyte cell product called ChondroCelect (TiGenix, Leuven, Belgium) was developed and obtained the first cellular product central registration

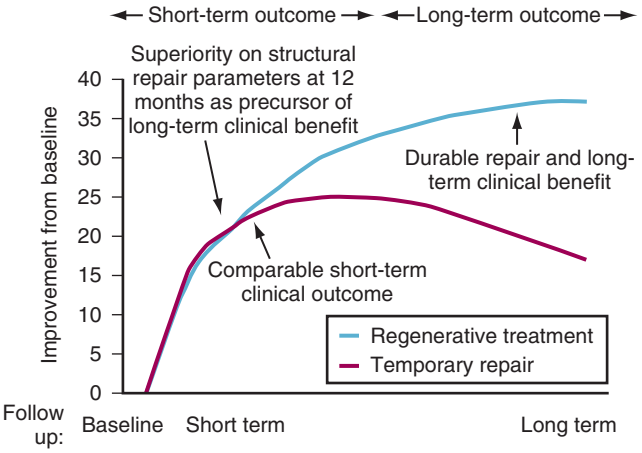



**Figure 7-6** The expected difference between repair of a joint surface lesion by a mechanism of scarring versus true tissue regeneration. Both treatment approaches (e.g., microfracture and autologous chondrocyte transplantation [ACI]) show clinically equal benefit at the short term, but repair tissue closer to the native tissue is seen with a regenerative approach (e.g., as seen in ACI). Long-term outcomes are more durable using regenerative approaches, in some cases leading to cure.

by the European Medicines Agency under the new advanced therapy medicinal product regulation. In a prospective randomized multicenter study, ChondroCelect, defined and characterized as a cell population capable of making stable cartilage in surrogate in vivo models and quality controlled through use of biomarkers, was shown to result in structurally superior repair tissue at 12 months after implantation compared with microfracture, with a superior clinical outcome at 3 years, but noninferiority in the overall population at 5 years.[108] The 5-year data demonstrate a clinically relevant superior outcome in the subpopulation of patients with relatively recent lesions (<3 years). This prospective, well-designed landmark clinical trial supports the concept that an improved understanding of the underlying biology and the consequent optimization of the manufacturing process lead to improved clinical outcome. In addition, stratification of patients will have a major impact on

the potential for regenerative medicine approaches to move forward successfully to proper clinical use in daily practice.

The surgical delivery of these therapies is another important factor that requires standardization. In this regard, ACI is becoming a more easily controllable arthroscopic procedure. A recent study that used an arthroscopic approach reported a statistically better clinical outcome for matrix-applied autologous chondrocytes after 2 years when compared with microfracture.[109] Interestingly, in this case, structural repair as assessed by MRI and biopsy was not different from microfracture at 2 years.

Although still open for debate with respect to long-term clinical durability, there is a consensus that ACI, and variations thereof, can be considered a regenerative treatment with good structural and clinical outcome. Long-term clinical outcomes have been reported and indicate a good durability of the treatment in a substantial number of patients over periods exceeding 10 years.[110]

Importantly, the data indicate that the outcome is superior to standard care if the "proper" patients are treated. Positive predictors of good outcome include the age of the patient, the location of the defect, early intervention (<3 years after becoming symptomatic), good quality of chondrocytes, well-trained surgeons, adherence to rehabilitation protocols, and no signs of OA (as defined by the Kellgren II grading scale on standard radiographs).[111] Cost-effectiveness will certainly improve if the treatment is also shown to prevent the progression toward (early) OA, but these data are not yet available.

### Stem Cell–Based Approaches

The next wave of development is the use of progenitor cell populations, or the so-called MSCs, in particular in an allogeneic approach.

MSCs are easy to isolate, expandable in culture, and chondrogenic, and therefore they are attractive alternatives to chondrocytes in an ACI procedure. These properties of MSCs would allow upscaling and generation of large quality-controlled batches, thus circumventing the limitations and patient-to-patient variability of autologous cell protocols. For these reasons, the use of MSCs for joint surface repair is intensively sought[112-114] and has been explored in humans.[115] These studies have shown promising results, with cartilage formation and endochondral bone formation restoring the bone front. However, uplift of the bone front at the expense of the articular cartilage has been commonly observed[87] and has its clinical equivalent in intralesional osteophyte formation in patients after microfracture.[116] This "side effect" appears to be less frequent in patients treated with ACI,[117] suggesting that an imprinted memory of articular chondrocytes could preserve the normal thickness of the repair cartilage and limit the advancement of the bone front. This finding points to a need, when using MSCs, for strategies to enhance stable cartilage properties and reduce fibrous tissue and hypertrophic cartilage formation.

Another important area of investigation is the tissue source of MSCs. Adult bone marrow MSCs are leading candidates for use in the repair of cartilage defects and other skeletal tissues such as bone defects.[118] However, MSCs from bone marrow appear to have a high propensity to cartilage hypertrophy and bone formation[119,120] and may not be the ideal chondroprogenitors for the repair of articular cartilage. In this regard, superiority of MSCs from synovial membrane for cartilage formation in vitro when compared with MSCs from other tissue sources, including bone marrow and periosteum, has been reported.[82,121,122] Promising data using synovium-derived progenitors have been reported in vivo, but at this point the long-term outcome of these approaches is unclear.[123,124]

The differences in chondrogenic potency of MSC populations could find their origins in the distinctive molecular programs of embryonic formation of their native tissues, because lineage tracing studies in mice demonstrated a common developmental origin of articular cartilage and synovial membrane from the Gdf5-positive cells of the embryonic joint interzone.[40,64]

The solution to the challenge of stable-cartilage formation, displaying also the very specific articular cartilage characteristics, may come from the use of chondroprogenitors isolated from the articular cartilage itself, which display the ability to maintain chondrogenic potency upon extensive culture expansion.[125] A proof-of-principle pilot study in a goat model in vivo demonstrated the apparent noninferiority of these chondroprogenitors to form a cartilage-like repair tissue in a chondral defect when compared with full-depth chondrocytes.[126] Human studies will be needed to demonstrate noninferiority of MSCs or any other stem/progenitor cell populations in clinical and structural outcomes when compared with the gold-standard articular cartilage–derived chondrocytes in ACI-like procedures.

The use of MSCs in joint resurfacing in animal models and humans reveals a variable structural outcome, ranging from hyaline-like cartilage to fibrous tissue. This finding has prompted interventions to support tissue maturation by using cartilage-promoting growth factors along with smart biomaterials.[127] However, the combination of cells, biomaterials, and morphogens would make the assessment of efficacy and toxicity more complex and the path from animal experimentation to clinical application arduous.

The use of allogeneic MSCs has shown an acceptable safety profile.[128] However, the pre-clinical data on the immunogenicity of MSCs are conflicting, and the acquired differentiated phenotype of the implanted stem cells may result in the loss of the immunologic privilege, with consequent rejection of the cells.[129,130]

Nonetheless, the use of allogeneic stem cells would allow manufacturing of large batches of stem cell products with specific clinical indications, off the shelf. Such an approach increases consistency and decreases costs of cell therapies, potentially accelerating the translational process toward routine clinical application.

### Osteochondral Repair

Whereas regeneration strategies for chondral lesions have been tested in high-quality clinical trials, the management and repair of osteochondral defects, with damage to the articular cartilage and reaching deep into the subchondral bone, remains a significant problem (for a review see Nukaravapu and Dorcemus[131]). Indeed, both the articular cartilage and subchondral bone need to be repaired with

restoration of a new calcified layer and tidemark, fully integrated and aligned with the neighboring tissue. The major challenge lies in the development of biologically educated regenerative treatments for osteochondral defects without resorting to prostheses. Prosthetic joint resurfacing has a limited life span, and revisions can be surgically challenging and are not ideal in younger patients with higher functional expectations.

The most common clinically used methods to treat osteochondral defects include debridement, bone marrow stimulation techniques such as microfracture, and the use of osteochondral allografts. Although they have shown some short-term positive results, the approaches taken are suboptimal and not curative and face serious drawbacks. Most of these treatments address only symptoms, and the use of allografts for larger osteochondral defects involves risks for immune rejection, disease transmission, and issues related to donor site morbidity and tissue availability. Currently, for osteochondral lesions bigger than 2.5 $cm^2$, the most commonly used clinical restorative method is ACI, using surgical variations with a so-called "sandwich approach." However, the results are mixed and are neither robust nor predictable.

Several scaffold strategies have been developed and evaluated for osteochondral repair requiring bone, cartilage, and bone-cartilage interface regeneration. Indeed, one of the main aims in the development of repair strategies for osteochondral applications is to closely mimic the native osteochondral tissue gradient structure. The challenge is to create distinct but seamlessly integrated layers intended to repair articular cartilage, calcified cartilage, and bone.[132] Therefore, osteochondral TE research has been evolving from cell-based therapies to single-layer scaffold repair, followed by biphasic constructs, and ultimately to multiphasic constructs. Classically, biphasic materials entailing a cartilaginous layer and bone part have been described in several studies and have shown some success.[125,133] Among biphasic scaffolds, hybrids can be prepared by filling soft hydrogels into hard sponges. As an example of this approach, Wang et al.[134] successfully developed a composite construct consisting of a polylactide-co-glycolide (PLGA) sponge filled with fibrin gel, bone marrow MSCs, and TGF-β1 for full-thickness cartilage defect restoration in vivo. In vitro and in vivo studies in a rabbit model revealed that the PLGA/fibrin gel/MSCs/TGF-β1 constructs are an appealing candidate for osteochondral restoration.[134] This type of biphasic composite construct paved the way for the use of multilayered scaffolds for osteochondral defects.

Following this progression, increasing interest is being devoted to gradient scaffolds with continuous interfaces to more faithfully mimic the natural hierarchical architecture. A clinical pilot study was performed in which a nanostructured, three-layered, collagen-hydroxyapatite construct was applied for the treatment of chondral and osteochondral lesions of the knee joint. This system was able to induce in situ regeneration via stem cells coming from the surrounding bone marrow in an innovative cell-free approach translatable to clinical practice.[135] In a sheep model, similar trilayered magnesium-hydroxyapatite-collagen type I composites were able to promote bone and cartilage tissue restoration by inducing the selective differentiation of resident progenitor cells, such as bone marrow–derived and/or synovium-derived cells, to osteogenic and chondrogenic lineages. Thus this approach indicates the potential of a suitable hybrid scaffold matrix that can direct and coordinate the process of bone and hyaline-like cartilage regeneration.[135] Other studies, also using biomimetic multilayered scaffolds, focused more on the development of a tidemark (intermediate layer). For example, hydroxyapatite/collagen bio-hybrid composites, prepared via a biologically inspired mineralization process, supported selective cell differentiation toward both osteogenic and chondrogenic lineages. Finally, continuous gradients in both material and bioactive signals take us one step further in scaffold complexity to achieve a high degree of mimicry. Continuous gradients can be added into hydrogels with incorporation of drug delivery systems (e.g., drug-loaded microspheres). As an elegant example of this approach, Wang et al.[134] incorporated recombinant human (rh) BMP-2 and rhIGF-1, microencapsulated in PLGA and silk microspheres, to form continuous concentration gradients in alginate gels and silk sponges. The combination of growth factors in different carrier systems enabled spatial control of the growth factors' distribution and temporal control of their release.

Although the use of tailored scaffolds associated with suitable factors and proper stem cell technology appears to be a more advanced TE strategy, the complexity intrinsic to such scaffolds presents a considerable set of technological, regulatory, and financial challenges on the way to routine clinical application.

## Bone Regeneration

The most promising field to explore the full potential of regenerative medicine approaches in musculoskeletal medicine is bone regeneration. Bone already has a remarkable potential to heal postnatally, which indicates that all the necessary tools are available to obtain full tissue repair, without any tissue scarring, and thus also to obtain tissue integration and remodeling. We should thus understand how nature heals bone fractures postnatally and subsequently mimic these repair processes when bone healing goes wrong, such as in delayed healing and nonunions, or in the repair of large bone defects such as avascular necrosis or after resection of bone tumors.

The standard of care in bone repair is the use of autologous bone grafts, which are typically obtained from the iliac crest. However, important developments have moved the boundaries in the field of bone engineering and include the development and improvement of biomaterials and new technologies in the design and production/manufacturing of these new biomaterials. It is not within the scope of this chapter, but suffice it to say that the spectrum of the smart biomaterials for bone engineering is vast, and recent progress has resulted in the production of resorbable and osteoconductive products, with some even displaying osteoinductive properties.[136] These latter characteristics have been obtained by coating biomaterials with bioactive factors, including growth factors such as the BMPs.[30] BMP devices have not only led to more robust and predictable outcomes in spine fusion but most importantly have demonstrated that recapitulation of embryonic tissue formation processes can lead to successful bone healing postnatally, as well as in long bone healing.

Despite all this progress, we still are not capable of healing large long bone defects in patients. In view of this, we and others believe that a combined implant incorporating smart and resorbable biomaterials, stem/progenitor cell populations, and growth factors will be required.[137] Failure of bone healing is typically associated with the lack of vascularization and the proper precursor cell populations not being available. Therefore, the generation of tissue-engineered viable implants will need to provide tools to enhance vascularization of the implant and promote cell survival to come to full fruition. Thus far, the results of the so-called combination products (scaffold and cells, eventually enriched with growth factors) have been somewhat disappointing. Lack of an underlying scientific basis for the design and manufacturing of these implants is probably responsible for a large part of this disappointment. Therefore, a bio-inspired model called developmental engineering was proposed and extensively described.[138,139] Recent publications indicate that this approach may be more successful than the traditional cells on the scaffold trial-and-error approach.[120] Most importantly, good prospective multicenter clinical trials are ongoing (see the World Health Organization International Clinical Trials Registry Platform and the European Union Drug Regulating Authorities Clinical Trials [EUDRACT] database) to better establish the clinical relevance of the different bone TE strategies. They include the direct percutaneous injection of expanded bone marrow MSCs in long bone nonunions, both autologous and allogenic, and the implantation of bone marrow–expanded MSCs with a bone substitute (hydroxy apatite/tricalcium phosphate carrier) in long bone nonunions. The development of some of the more complex TE techniques is raising further challenges, also with regard to the manufacturing of those products.

## Regeneration of Other Joint-Related Structures

Lesions of the articular cartilage are often associated with damage of other joint structures such as menisci, ligaments, and tendons, which need to be addressed to restore joint homeostasis. An attractive holistic approach to joint regeneration could be intra-articular delivery of MSCs. The injection of bone marrow MSCs in a hyaluronan suspension in a goat model of OA obtained by medial meniscectomy and anterior cruciate ligament resection resulted in a meniscus-like neo-tissue formation that provided protection from OA progression.[140] Similar data were obtained with intra-articular injection of synovial membrane–derived MSCs that promoted meniscus regeneration in a rat model.[141,142] Recently, a proof-of-concept phase I/II clinical trial assessed the safety and efficacy of intra-articular injection of autologous adipose-derived MSCs for knee OA. After a phase I study consisting of three dose-escalation cohorts with three patients each, phase II included nine patients receiving the high dose ($1.0 \times 10^8$ MSCs). The primary outcomes were the safety and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) at 6 months; secondary outcomes included clinical, radiologic, arthroscopic, and histologic evaluations. The intra-articular injection of MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events and reduced cartilage defects by regeneration of hyaline-like articular cartilage.[143] Reports from other ongoing clinical trials are awaited.

### Menisci

The menisci have a poor healing potential largely because their vascularity is restricted to the external third of the tissue.[144] A subtotal or total meniscectomy is now rarely performed in view of the high risk of OA development, but a partial meniscectomy can be necessary in symptomatic patients. The use of a cell-free biomaterial to replace part of the meniscus relies on repopulation of the scaffold by the host cells from the joint environment. The collagen meniscus implant (ReGen Biologics, Franklin Lakes, N.J.) was the first regenerative method applied to meniscal tissue in clinical practice.[145-147] Noncontrolled case series have reported pain relief and functional improvement at 10-year follow-up.[148] Despite the wide clinical use, no randomized controlled trial supports the use of meniscal implants in routine clinical practice. The Actifit (Orteq Ltd, London) meniscus implant is a polyurethane-polycaprolactone–based synthetic meniscal substitute for partial meniscal defects,[149] but evidence about its safety and effectiveness is awaited.

Meniscal transplantation is a valid short-term option in selected patients with symptomatic, totally meniscectomized, aligned knees.[150] Limitations of this procedure include tissue availability, graft sizing, and risks of immune reaction and disease transmission. Fixation of the allograft also remains a significant challenge.

Regenerative medicine holds the potential for restoring anatomy and function of meniscal fibrocartilage. Several cell types have been assessed in meniscal TE, including meniscal cells from the meniscectomized meniscus itself; articular, costal, and nasal chondrocytes; MSCs; and embryonic stem cells (reviewed in reference 151). They are seeded onto biomaterials, both natural and synthetic, which are anticipated to be biocompatible, biodegradable, bio-instructive (promoting cell differentiation and cell migration if cell free), biomimetic (mimicking architecture and biomechanics of the native meniscus), resistant to mechanical forces, porous (allowing diffusion of nutrients), and user friendly for the surgeon. Natural materials such as small intestine submucosa[152] and acellular porcine meniscal tissue[153] have high biocompatibility but do not allow varying structure geometry and adequate initial mechanical properties. Isolated tissue components such as collagens and proteoglycans allow generation of custom-made scaffolds with high biocompatibility.[154] However, these scaffolds usually have low biomechanical properties and are characterized by rapid biodegradation, and they are not sufficiently long to be completely replaced by the newly formed tissue.[151] Synthetic polymers (e.g., polyglycolic acid [PGA] and polylactic acid [PLA]) can be manufactured in custom-made shapes and with custom-made porosity and biomechanical properties, and the use of biopolymers such as silk fibrous protein has been explored to improve biocompatibility and biodegradability.[155] A possible solution is to couple the high biocompatibility of natural polymers with the superior mechanical strength and ease of being tailored of the synthetic polymers.[156]

A fascinating strategy entails the use of hydrogel materials,[157] because their semiliquid nature allows engineering

anatomic geometries derived from medical imaging such as computed tomography.[158]

MSCs seeded onto a hyaluronan-collagen–based scaffold were recently used to repair a critical-size meniscal defect in an orthotopic rabbit model.[159] Although menisci repaired with the engineered tissue demonstrated a significantly better regeneration than did those repaired with the cell-free scaffold, the cell-loaded implants did not restore a normal meniscus.[159] Uncultured bone marrow cells in hyaluronan-collagen composite matrices stimulated the development of integrated meniscus-like repair tissue,[160] suggesting the feasibility of a one-step approach for partial meniscus TE.

Total meniscus TE offers an alternative to allografts to overcome the problems of availability, graft sizing, and immune reaction. The implantation of a PGA-PLGA scaffold seeded with allogeneic meniscal cells in a rabbit meniscectomy model resulted in a tissue histologically resembling normal meniscus.[161] Similar promising results were reported with a hyaluronic acid/polycaprolactone material seeded with expanded autologous articular chondrocytes in a sheep orthotopic model.[156] Mandal et al.[155] reported the development of a TE meniscus based on a tri-layered silk fibrous protein scaffold seeded with human culture-expanded fibroblasts in the outer part and chondrocytes in the inner part. The same authors reported the use of such trilayered scaffold seeded with human culture-expanded MSCs, showing native-like tissue structure and compressive properties in vitro.[162]

Despite the extensive pre-clinical and clinical investigations, none of the current strategies has demonstrated regeneration of a functional, durable meniscal tissue and full re-establishment of knee homeostasis. Advances in cell biology, biomaterial science, and bioengineering (e.g., bioreactors) have the potential to drive meniscus regeneration into more clinically relevant and feasible strategies.

### Tendons

Common tendon injuries are tears of the rotator cuff, Achilles tendon, and flexor tendons of the hand. At present, they are treated with surgical repair and/or conservative approaches. Unfortunately, current treatment strategies fail to restore the functional, structural, and biochemical properties of the repaired tendons compared with native tendons. Tendon repair has been attempted with platelet-rich plasma, an autologous source of concentrated growth factors relevant to wound repair.[163] Numerous growth factors have been shown to play a role in tendon healing, but the application of growth factors such as IGF-1, TGF-β, or GDF-5 (BMP-14) to clinical tendon repair remains challenging.[164]

In cases of severe tendon injury, surgical treatments may be used to repair or replace the damaged tendon with autografts, allografts, xenografts, or prosthetic devices. The unsatisfactory clinical outcomes with high failure rates[165] have prompted the development of TE strategies. With regard to the choice of cell types to use, the harvest of autologous tenocytes may cause secondary tendon defects, and thus dermal fibroblasts have been considered because they are easily accessible and do not cause major donor site morbidity.[166] In a recent clinical trial, an injection of dermal fibroblasts suspended in autologous plasma improved the healing of refractory patellar tendinopathy.[167] MSCs are another promising cell source for tendon TE. In a rabbit model, PGA sheets seeded with bone marrow MSCs enhanced mechanical strength when compared with a cell-free PGA scaffold.[168] Stem cells have also been identified in tendons.[169] Tendon-derived stem cells in fibrin glue, compared with fibrin glue alone, promoted superior tendon repair histologically and biomechanically.[170] Despite their potential advantages, the isolation of autologous cells would present the same donor site morbidities as tenocytes.

Three major categories of scaffolds are used in tendon TE: native tendon matrices, synthetic polymers, and derivatives of naturally occurring proteins. Cutting-edge processing protocols allow decellularized tendon scaffolds to maintain similar biomechanical properties compared with native tendon tissues.[171] Moreover, a number of ECM proteins and growth factors are preserved in acellular tendon,[172] suggesting potential bio-functionality of these scaffolds. Synthetic polymers such as PGA and poly-L-lactic acid have been used but have limitations, including the absence of biochemical motifs for cell attachment and the inability to regulate cell activity.[173] Scaffolds made from natural proteins and their derivatives may address these issues. Because tendon ECM is mainly composed of type I collagen, scaffolds based on collagen derivatives are highly biocompatible and exhibit superior biofunctionality by supporting cell adhesion and cell proliferation better than polyester materials.

A greater understanding of the molecular mechanisms underpinning tendon development and natural healing coupled with the advances in biomaterials and nanotechnology that allow a closer replication of the native tissues and delivery of growth factors with high spatiotemporal resolution and specificity[174] will allow tissue engineers to recapitulate tendon morphogenesis for repair. The insertion of tendons into bone remains a challenge.

Researchers are also investigating the possibility of regeneration by triggering/enhancing the natural healing response. This goal could be achieved by using off-the-shelf biomaterials capable of delivering growth factors at specified times to recruit endogenous cells and direct repair to restore native tissue anatomy and function.[175] The application of biophysical modalities such as low-intensity pulsed ultrasound[176] is also promising for promotion of intrinsic healing and improving the mechanical properties of healing tendon-bone insertions.

## REGENERATIVE MEDICINE AND TISSUE ENGINEERING IN ARTHRITIS

As we start to understand intrinsic tissue response mechanisms and attempts to counteract destructive processes by enhancing tissue repair, new therapeutic targets have been identified and targeted with "classical" pharmaceutical approaches such as the development of protein therapies and small molecules interfering with critical repair mechanisms. However, these approaches may still be far from sufficient and "too targeted," and therefore more comprehensive approaches may be needed. For this reason, cellular therapies and combination products have been explored. Indeed, cellular therapies will affect local processes in many ways as cell populations are manufactured so they deliver a

vast array of secreted signals, also called their *secretome*, which is likely to influence local disease processes and enhance repair. Conversely, it becomes clear that the microenvironment will affect the cellular products and influence what they secrete and how they interact with the environment, including their engraftment, proliferation, differentiation, and tissue integration and remodeling.[177] Therefore, for regenerative medicine and cellular therapies to be successful, particularly in disease, we need to assess and quantify the microenvironment and mutual interactions between the cell-based product and the microenvironment. This approach points toward the importance of personalized medicine, and indeed, for regenerative medicine approaches to become cost-effective and successful, identifying the patients at risk and predicting responders to treatment will be of utmost importance.

Different cellular therapeutic approaches have been investigated in chronic arthritis, with most data available on the use of adult MSCs. Proof-of-principle studies have been established for distinct indications. For inflammatory diseases such as RA and other autoimmune systemic diseases, the immune suppressive and immune modulatory effects of cellular therapies can contribute to the control of disease activity,[178] but this application is outside of the scope of this chapter.

Cellular therapies to enhance tissue repair have been explored quite extensively in pre-clinical animal models of post-traumatic OA.[140] The use of MSCs has been explored in patients with OA.[115,179-183] The mechanisms through which they can influence the disease processes in OA appear diverse as previously discussed and include the so-called trophic effects, immune suppressive effects, and anti-inflammatory effects, as well as cell engraftment and contributing to the local de novo tissue formation, leading to meniscal and cartilage repair.[141,184] Because cellular products can potentially have all these effects, properly manufactured cell-based therapies might restore joint homeostasis thereby preventing disease progression in OA.

## CONCLUSION

Regenerative medicine approaches are providing new and exciting opportunities in medicine in general and in the field of musculoskeletal disorders and diseases in particular. In orthopedics, cell-based tissue repair has entered the routine armamentarium and daily clinical practice. The development of injectable or arthroscopically implantable "regenerative biologics" will soon introduce regenerative medicine to the therapeutic options of rheumatologists. These options will include but are not limited to the repair of damaged joint surfaces, both chondral and osteochondral defects, the regeneration of difficult to heal fractured bones, the repair of damaged ligamentous structures, and the fabrication of a variety of "off the shelf" skeletal tissue structures, such as *viable* pieces of bones, ligaments, menisci, and other joint tissues. These regenerative treatments will not only be of use for post-traumatic damaged joints and skeletal tissues but also for (post)inflammatory and osteoarthritic joints.

Ultimately, the implantation of a *biological* prosthesis with the potential of full tissue integration and remodeling is a dream target, and proof of principle exists that such a prosthesis may be within reach.[185,186] To achieve this goal in a more robust and predictable fashion, we need to change our strategy, using a bio-inspired engineering approach.[138,139] We proposed the term "developmental engineering" to describe a methodology for rational and accurate design of robust, well-controlled manufacturing processes. A close interaction between the efforts of biologists and engineers will speed up TE processes.

The stage is set, and it is up to the biomedical TE community to break the boundaries toward the manufacturing of biological spare parts of the body. It is anticipated that the musculoskeletal field, and thus patients with skeletal disorders and diseases, will benefit from this approach sooner rather than later.

In spite of the advances in the field of tissue repair/regeneration in musculoskeletal conditions, these technologies are not yet in common use, and the National Health Services of several countries do not provide reimbursement. This situation is largely related to the high direct costs associated with such procedures, and cost-effectiveness remains a critical issue. A Swedish study has shown the health economic benefit of regenerative treatments when the reduction of disability, absenteeism, and other indirect costs are taken into account.[187] For those treatments to become affordable, however, a number of technological gaps still need to be filled. Some of the emerging approaches include (1) the use of heterologous cell sources such as stem cells; (2) the harnessing of the intrinsic reparative mechanisms and homeostatic pathways through the use of bioactive molecules and/or acellular devices; and (3) the use of physical intervention, such as joint distraction,[188] which, by temporarily reducing the mechanical injury, would allow reparative mechanisms to take place.

At this stage we have obtained convincing evidence that regenerative musculoskeletal medicine has the potential for long-term, possibly lifelong efficacy in patients. The challenges have been identified and described. The growing knowledge in the molecular and cellular basis of the processes that drive tissue destruction and repair, disease progression, and homeostasis is paving the path by supplying an increasing number of targets for both pharmacologic interventions, potential biomarkers, and a new generation of advanced therapeutic medicinal products.



The full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 8



# Proteinases and Matrix Degradation

Yasunori Okada

**KEY POINTS**

- Proteinases are generally classified into aspartic proteinases, cysteine proteinases, serine proteinases, and metalloproteinases according to their catalytic mechanism.
- Because of the optimal acidic pH and intra-cellular localization within lysosomes, most of the aspartic proteinases and cysteine proteinases are involved in intra-cellular degradation of extra-cellular matrix (ECM) components.
- Serine proteinases and metalloproteinases are neutral proteinases and play a central role in extra-cellular degradation of ECM macromolecules.
- ECM-degrading metalloproteinases are composed mainly of the MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) gene families.
- Most endogenous proteinase inhibitors are proteinase class specific, whereas α2 macroglobulin inhibits the activities of all proteinases.
- The activities of ECM-degrading proteinases at the local tissues are regulated by the balance between the proteinases and their inhibitors, which may be determined by production rates of proteinases and inhibitors, their secretion, activation of proenzymes, and pericellular anchoring and recycling systems of the activated proteinases.
- ProMMPs (precursors of MMPs) are activated via the extra-cellular, intra-cellular, and pericellular pathways depending on the MMP species.
- Aggrecan and type II collagen, two major ECM components, may be degraded in articular cartilage by differential or complementary actions of the MMP and ADAMTS species in arthritides.
- In rheumatoid arthritis, articular cartilage is destroyed by proteinases accumulated in synovial fluid, direct contact with proteolytic synovium and pannus tissue, and proteinases derived from chondrocytes, whereas bone is resorbed by osteoclasts mainly by the action of cathepsin K and MMP-9 under acidic and hypercalcemic conditions in subosteoclastic compartments.
- In osteoarthritis, chondrocyte-derived metalloproteinases, including the MMP and ADAMTS species, contribute primarily to the breakdown of articular cartilage.

Extra-cellular matrix (ECM) plays critical roles in normal development and function of the organism by interacting with cells and supporting tissue structures. The in vivo cellular functions regulated by cell-ECM interaction include proliferation, differentiation, apoptosis, and motility. The proteolytic turnover and remodeling of ECM are transient and highly controlled under physiologic conditions, and excessive degradation of ECM components by proteinases causes tissue destruction in many pathologic conditions. In rheumatoid arthritis and osteoarthritis, ECM-degrading proteinases are elevated without sufficient endogenous inhibitors, and they are believed to play central roles in the destruction of articular cartilage and bone on the basis of a local imbalance between proteinases and inhibitors. This chapter provides up-to-date information about the ECM-degrading proteinases and their inhibitors.

## EXTRA-CELLULAR MATRIX–DEGRADING PROTEINASES

ECM is degraded by endopeptidases (i.e., proteinases) that act internally on polypeptide chains; little evidence is present for the roles of exopeptidases that cleave one or a few amino acids from the N- or C-terminus. Proteinases comprise aspartic proteinases, cysteine proteinases, serine proteinases, and metalloproteinases, which are classified according to catalytic mechanism. Proteinases from each of the four classes are involved in the degradation of ECM macromolecules.

### Aspartic Proteinases

Most aspartic proteinases have two aspartic acid residues in their catalytic sites, where the nucleophile that attacks the scissile peptide bond is an activated water molecule. Mammalian aspartic proteinases include the digestive enzymes (pepsin and chymosin), the intra-cellular cathepsin D and cathepsin E, and rennin. Among the proteinases belonging to this group, cathepsin D is the major aspartic proteinase involved in ECM degradation (Table 8-1). It exhibits proteolytic activity against most substrates such as aggrecan and collagen telopeptides with optimal pH between pH 3.5 and 5. Because of the acidic optimal pH and intra-cellular localization within lysosomes, cathepsin D is probably responsible for intra-cellular degradation of phagocytosed

**TABLE 8-1** Proteinases That May Be Involved in Degradation of Extra-cellular Matrix

| Enzyme | Source | Inhibitor |
|---|---|---|
| **Aspartic Proteinases** | | |
| Cathepsin D | Lysosome | Pepstatin |
| **Cysteine Proteinases** | | |
| Cathepsin B | Lysosome | Cystatins |
| Cathepsin L | Lysosome | Cystatins |
| Cathepsin S | Lysosome | Cystatins |
| Cathepsin K | Lysosome | Cystatins |
| Calpain | Cytosol | Calpastatin |
| **Serine Proteinases** | | |
| Neutrophil elastase | Neutrophils | α1 PI |
| Cathepsin G | Neutrophils | α1 Antichymotrypsin |
| Proteinase 3 | Neutrophils | α1 PI, elafin |
| Plasmin | Plasma | Aprotinin |
| Plasma kallikrein | Plasma | Aprotinin |
| Tissue kallikrein | Glandular tissues | Aprotinin; kallistatin |
| tPA | Endothelial cells; chondrocytes | PAI-1; PAI-2 |
| uPA | Fibroblasts; chondrocytes | PAI-1; PAI-2; PN-1 |
| Tryptase | Mast cells | Trypstatin |
| Chymase | Mast cells | α1 PI |
| **Metalloproteinases*** | | |
| MMPs | Tissue cells; inflammatory cells | TIMP-1, 2, 3, and 4; RECK for MMP-2, 7, 9, and 14 |
| ADAMTSs | Tissue cells | TIMP-3 |
| ADAMs | Tissue cells; inflammatory cells | TIMP-3; RECK for ADAM10 |

*For details of ADAMs, ADAMTSs, and MMPs, see Tables 8-2, 8-3, and 8-4.

ADAMs, A disintegrin and metalloproteinases; ADAMTSs, a disintegrin and metalloproteinases with thrombospondin motifs; MMPs, matrix metalloproteinases; PAI, plasminogen activator inhibitor; PI, proteinase inhibitor, PN, proteinase nexin; RECK, reversion-inducing, cysteine-rich protein with Kazal motifs; TIMP, tissue inhibitor of metalloproteinases; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

ECM fragments that previously were degraded in the extra-cellular spaces. However, a study on cartilage explant cultures using the aspartic proteinase inhibitor suggests the possibility that cathepsin D secreted extra-cellularly contributes to the degradation of aggrecan in articular cartilage.[1]

## Cysteine Proteinases

Cysteine proteinases are endopeptidases in which the nucleophile of the catalytic site is the sulfhydryl group of a cysteine residue. The ECM-degrading cysteine proteinases include lysosomal cathepsins B, L, S, and K and the calpains (see Table 8-1). Cathepsins B and L digest the telopeptide regions of fibrillar types I and II collagen, the nonhelical regions of types IX and XI collagen, and aggrecan at acidic pH. Cathepsin S has a similar spectrum of substrates within a broad range of pH values. Cathepsin K, also called *cathepsin O, O2*, or *X*, is a collagenolytic cathepsin that efficiently cleaves type I collagen at the triple helical regions at pH values between 4.5 and 6.6.[2] The proteinase also degrades gelatin and osteonectin. On the basis of data that cathepsin K is highly expressed by human osteoclasts[3] and that inactivating mutations or deletion of the gene result in an osteopetrotic phenotype in humans and animals, cathepsin K is believed to play a key role in bone resorption in joint diseases (see later discussion). Because cathepsins B, L, S, and K are expressed in synovium or articular cartilage or both in rheumatoid arthritis and osteoarthritis, they may also be involved in the cartilage destruction through degradation of the ECM macromolecules.[4]

Calpains are $Ca^{2+}$-dependent, papain-like cysteine proteinases that are ubiquitously distributed among mammalian cells. The best-characterized members of the calpain superfamily are μ-calpain and m-calpain, which also are called *conventional* (μ-calpain) and *classic* (m-calpain) *calpains*.[5] Calpains are involved in various pathologic conditions such as muscle dystrophy by acting intra-cellularly. They are present in the extra-cellular spaces and in osteoarthritic synovial fluid, and they can degrade aggrecan.

## Serine Proteinases

Serine proteinases require the hydroxyl group of a serine residue acting as the nucleophile that attacks the peptide bond. Serine proteinases include the largest number of proteinases, classified to about 40 families. Most serine proteinases can degrade ECM macromolecules. The major ECM-degrading serine proteinases in joint tissues are subsequently described (see Table 8-1).

### Neutrophil Elastase and Cathepsin G

Neutrophil elastase and cathepsin G are serine proteinases that are synthesized as precursors in promyelocytes in bone marrow and subsequently stored in the azurophil granules of polymorphonuclear leukocytes as active enzymes. Mature leukocytes do not synthesize elastase, but they mobilize azurophil granules to the cell surface and release the proteinases in response to various stimuli. Monocytes have low levels of elastase but lose the enzyme during the differentiation into macrophages. Neutrophil elastase and cathepsin G are basic glycoproteins with isoelectric points larger than 9 (neutrophil elastase) and about 12 (cathepsin G). They can be readily trapped in cartilage matrix that has a negative charge.

Neutrophil elastase and cathepsin G cleave elastin; the telopeptide region of fibrillar collagen types I, II, and III; other collagen types IV, VI, VIII, IX, X, and XI; and other ECM components such as fibronectin, laminin, and aggrecan at neutral pH. These serine proteinases also can be involved indirectly in the breakdown of ECM by activating the zymogen of matrix metalloproteinases (proMMPs)[6] and by inactivating endogenous proteinase inhibitors such as α2 antiplasmin, α1 antichymotrypsin, and tissue inhibitors of metalloproteinases (TIMPs).

### Mast Cell Chymase and Tryptase

Chymase and tryptase are packaged in secretory granules together with histamine and other mediators in mast cells, which are infiltrated in rheumatoid synovium. Chymase is a chymotrypsin-like proteinase with a broad spectrum of activity against ECM components such as type VI collagen[7] and aggrecan. It also activates proMMPs such as proMMP-1, 3, and 9.[6] Although prochymase is activated intracellularly and stored in the granules, the activity in the granules is limited at low pH and becomes fully active when released extra-cellularly. Tryptase is a trypsin-like proteinase that degrades type VI collagen[7] and fibronectin; it also activates proMMP-3.[6]

### Plasmin and Plasminogen Activators

Plasminogen is synthesized in liver and secreted to plasma. It can bind to fibrin and to cells, and after activation by plasminogen activators, plasmin readily digests fibrin. Membrane-bound plasmin also degrades many ECM components including proteoglycan, fibronectin, type IV collagen, and laminin.[8] Other important functions of plasmin are to initiate the activation of proMMPs,[6] activate latent cell-associated transforming growth factor (TGF)-β1, and act as proenzyme convertase. Plasmin is generated through activation of plasminogen mainly by plasminogen activators, principally two serine proteinases, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA).

The tPA is synthesized as a proenzyme of 70 kDa and is secreted into the circulating blood primarily by endothelial cells, fibroblasts, chondrocytes, and tumor cells.[8] In addition to being a major activator of plasminogen for fibrinolysis, tPA plays a key role in the clearance of fibrin from the circulation.

The uPA molecule was first purified from urine as a proenzyme of 54 kDa.[8] It is converted to the active form of two chains of 30 kDa and 24 kDa linked by a disulfide bond. Another fully active form of 33 kDa is generated by plasmin. Although the expression of uPA is limited to certain cells such as renal tubules and bladder urothelium under physiologic conditions, it is more widely expressed in various cells, including invasive cancer cells, migrating keratinocytes, and activated leukocytes in pathologic situations. Pro-uPA and two-chain uPA bind to a specific uPA receptor, a single-chain glycoprotein with a glycosylphosphatidylinositol (GPI) moiety expressed on fibroblasts, macrophages, and tumor cells. Receptor-bound uPA preferentially activates cell membrane–bound plasminogen into plasmin. Cell membrane–bound plasmin can activate receptor-bound pro-uPA. Among its specificities, uPA has a limited action on fibronectin.

### Kallikreins

Two types of kallikreins, plasma and tissue kallikreins, have been identified. Plasma kallikrein, with two disulfide-linked chains (36 kDa and 52 kDa), is generated from prokallikrein of 88 kDa by coagulation factor XIIa or by kallikrein itself. It activates kininogens to bradykinin and activates proMMP-1 and proMMP-3.[6] Tissue kallikrein, which is synthesized in glandular tissues, releases Lys-bradykinin from kininogen and activates proMMP-8.[6]

## Metalloproteinases

Similar to aspartic proteinases, metalloproteinases are endopeptidases in which the nucleophilic attack on a peptide bond is mediated by a water molecule. A divalent metal cation, usually zinc, activates the water molecule. Among the metalloproteinases, MMPs (matrix metalloproteinases), which are also designated matrixins (a subfamily of the metzincin superfamily), are key ECM-degrading, zinc-dependent endopeptidases (Table 8-2). However, accumulated evidence indicates that some members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family, which is an MMP-related gene family, also are involved in the degradation of ECM, especially cartilage proteoglycan (Table 8-3). Only a few members of the ADAM (a disintegrin and metalloproteinase) family have limited activity to ECM components (Table 8-4).

### Matrix Metalloproteinases

The human MMP family comprises 23 different members that have MMP designations (numbered according to a sequential numbering system) and common names coined by the authors of the published reports (see Table 8-2). On the basis of the biochemical properties provided by the domain structures and on their substrate specificity, these family members are classified into two major subgroups: secreted-type MMPs and membrane-anchored MMPs. MMP-4, 5, and 6 are excluded from the list because they are identical to other known MMPs (i.e., MMP-3 and 2). MMP-18 and 22 also are missing in Table 8-2 because they are assigned to *Xenopus* collagenase-4 and chicken MMP.

Most secreted-type MMPs, including collagenases, stromelysins, are composed of three basic domains—the propeptide, catalytic, and hemopexin-like domains—that are preceded by hydrophobic signal peptides (Figure 8-1). The N-terminal propeptide domain has one unpaired cysteine in the conserved sequence of PRCGXPD. The cysteine residue in the sequence interacts with the essential zinc atom in the catalytic domain to prevent it from binding the catalytic water molecule, maintaining the proenzyme in an inactive state. The catalytic domain has the zinc-binding motif HEXGHXXGXXH, in which three histidines bind to the catalytic zinc atom. The four-blade, C-terminal hemopexin-like domain, which is connected to the catalytic domain by a proline-rich hinge region, interacts with ECM components and can play a role in determining the substrate specificity in some MMPs. Gelatinases have these domains with additional insertions of collagen-binding type II repeats of fibronectin in the catalytic domain (see Figure 8-1), which provides them with collagen-binding properties. Matrilysins are the shortest, lacking the hemopexin-like domains. Furin-activated MMPs contain insertions of a basic motif with a cleavage site by proprotein convertases including furin at the end of the propeptide domains (see Figure 8-1).

Membrane-anchored MMPs include three different types of MMPs: type I transmembrane-type MMPs, GPI-linked

**TABLE 8-2** Substrates of Human Matrix Metalloproteinases

| Enzymes | ECM Substrates | Non-ECM Substrates |
|---|---|---|
| **Secreted-Type MMPs** | | |
| **Collagenases** | | |
| MMP-1 (Interstitial collagenase) | Collagens I, II, III, VII, X; gelatins; aggrecan; link protein; entactin; tenascin; perlecan | α2 Macroglobulin; α1 PI; α1 antichymotrypsin; IGF-BP-2, -3, and -5; pro-IL-1β; CTGF |
| MMP-8 (Neutrophil collagenase) | Collagens I, II, and III; gelatins; aggrecan; link protein | α1 PI |
| MMP-13 (Collagenase-3) | Collagens I, II, III, IV, IX, X, XIV; aggrecan; Fn; tenascin | CTGF; pro-TGF-β; α1 antichymotrypsin |
| **Gelatinases** | | |
| MMP-2 (Gelatinase A) | Gelatins; collagens IV, V, VII, XI; Ln; Fn; elastin; aggrecan; link protein | Pro-TGF-β; FGF receptor I; MCP-3; IGF-BP-5; pro-IL-1β; galectin-3; plasminogen |
| MMP-9 (Gelatinase B) | Gelatins; collagens III, IV, V; aggrecan; elastin; entactin; link protein | Pro-TGF-β; IL-2 receptor α; Kit-L; IGF-BP-3; pro-IL-1β; α1 PI; galectin-3; ICAM-1; plasminogen |
| **Stromelysins** | | |
| MMP-3 (Stromelysin-1) | Aggrecan; decorin; gelatins; Fn; Ln; collagens III, IV, IX, X; tenascin; link protein; perlecan | IGF-BP-3; pro-IL-1β; HB-EGF; CTGF; E-cadherin; α1 antichymotrypsin; α1 PI; α2 macroglobulin; plasminogen; uPA; proMMP-1, 7, 8, 9, 13 |
| MMP-10 (Stromelysin-2) | Aggrecan; Fn; Ln; collagens III, IV, V; link protein | ProMMP-1, 8, 10 |
| **Matrilysins** | | |
| MMP-7 (Matrilysin-1) | Aggrecan; gelatins; Fn; Ln; elastin; entactin; collagen IV; tenascin; link protein | Pro-α-defensin; Fas-L; β4 integrin; E-cadherin; pro-TNF; CTGF; HB-EGF; RANKL; IGF-BP-3; plasminogen |
| MMP-26 (Matrilysin-2) | Gelatin; collagen IV; Fn; fibrinogen | α1 PI; proMMP-9 |
| **Furin-Activated MMPs** | | |
| MMP-11 (Stromelysin-3) | Fn; Ln; aggrecan; gelatins | α1 PI; α2 macroglobulin; IGF-BP-1 |
| MMP-28 (Epilysin) | Unknown | Casein |
| **Other Secreted-Type MMPs** | | |
| MMP-12 (Metalloelastase) | Elastin; Fn; collagen V; osteonectin | Plasminogen; apolipoprotein A |
| MMP-19 (RASI-1) | Collagen IV; gelatin; Fn; tenascin; aggrecan; COMP; Ln; nidogen | IGF-BP-3 |
| MMP-20 (Enamelysin) | Amelogenin; aggrecan; gelatin; COMP | Unknown |
| MMP-21 | Unknown | Unknown |
| MMP-27 | Unknown | Unknown |
| **Membrane-Anchored MMPs** | | |
| **Type I Transmembrane-Type MMPs** | | |
| MMP-14 (MT1-MMP) | Collagens I, II, III; gelatins; aggrecan; Fn; Ln; fibrin; Ln-5 | ProMMP-2, 13; CD44; tissue transglutaminase |
| MMP-15 (MT2-MMP) | Fn; tenascin; nidogen; aggrecan; perlecan; Ln | ProMMP-2; tissue transglutaminase |
| MMP-16 (MT3-MMP) | Collagen III; Fn; gelatin | ProMMP-2; tissue transglutaminase |
| MMP-24 (MT5-MMP) | PG | ProMMP-2 |
| **GPI-Linked MMPs** | | |
| MMP-17 (MT4-MMP) | Gelatin; fibrinogen | Unknown |
| MMP-25 (MT6-MMP) | Gelatin; collagen IV; fibrin; Fn; Ln | ProMMP-2 |
| **Type II Transmembrane-Type MMP** | | |
| MMP-23 | Gelatin | Unknown |

COMP, Cartilage oligomeric matrix protein; CTGF, connective tissue growth factor; ECM, extra-cellular matrix; FGF, fibroblast growth factor; Fn, fibronectin; GPI, glycosylphosphatidylinositol; HB-EGF, heparin-binding epidermal growth factor; IGF-BP, insulin-like growth factor binding protein; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; Ln, laminin; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; PG, proteoglycan; PI, proteinase inhibitor; RANKL, receptor activator for nuclear factor-κB ligand; TGF, transforming growth factor; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator.

MMPs, and type II transmembrane-type MMPs. MMP-14, MMP-15, MMP-16, and MMP-24 (MT1-, MT2-, MT3-, and MT5-MMPs, respectively) have type I transmembrane domains in the C-terminal region, but MMP-17 (MT4-MMP) and MMP-25 (MT6-MMP) contain GPI anchors in the C-terminal region without transmembrane domains (see Figure 8-1). MMP-23 is unique in that it has type II transmembrane domain, a cysteine array, and an immunoglobulin-like domain instead of a hemopexin-like domain (see Figure 8-1).

## Secreted-Type Matrix Metalloproteinases

**Collagenases (MMP-1, MMP-8, and MMP-13).** The collagenases include MMP-1 (interstitial collagenase, collagenase-1), MMP-8 (neutrophil collagenase, collagenase-2), and

**TABLE 8-3** Members of the ADAMTS Family

| ADAMTS | Other Names | Demonstration of Proteinase Activity | Functions and Biochemical Features | Tissue and Cell Expression |
|---|---|---|---|---|
| ADAMTS1 | C3-C5; METH1; KIAA1346 | + | Digestion of aggrecan and versican; binding to heparin | Kidney; heart; cartilage |
| ADAMTS2 | Procollagen N-proteinase; hPCPNI; PCINP | + | Processing of collagen I and II N-propeptides | Skin; tendon |
| ADAMTS3 | KIAA0366 | + | Processing of collagen N-propeptides | Brain |
| ADAMTS4 | KIAA0688; aggrecanase-1; ADMP-1 | + | Digestion of aggrecan, brevican, and versican | Brain; heart; cartilage |
| ADAMTS5 | ADAMTS11; aggrecanase-2; ADMP-2 | + | Digestion of aggrecan | Uterus; placenta; cartilage |
| ADAMTS6 | — | – | — | Placenta |
| ADAMTS7 | — | – | — | Various tissues |
| ADAMTS8 | METH-2 | + | Digestion of aggrecan; inhibition of angiogenesis | Lung; heart |
| ADAMTS9 | KIAA1312 | + | Digestion of aggrecan | Cartilage |
| ADAMTS10 | — | – | — | — |
| ADAMTS12 | — | – | — | Lung (fetus) |
| ADAMTS13 | VWFCP; C9orf8 | + | Cleavage of von Willebrand factor | Liver; prostate; brain |
| ADAMTS14 | — | + | Processing of collagen N-propeptides | Brain; uterus |
| ADAMTS15 | — | + | Digestion of aggrecan | Liver (fetus); kidney (fetus) |
| ADAMTS16 | — | + | Digestion of aggrecan | Prostate; brain; uterus |
| ADAMTS17 | FLJ32769; LOC123271 | – | — | Prostate; brain; liver |
| ADAMTS18 | ADAMTS21; HGNC:16662 | + | Digestion of aggrecan | Prostate; brain |
| ADAMTS19 | — | – | — | Lung (fetus) |
| ADAMTS20 | — | + | Digestion of versican (and aggrecan) | Brain; testis |

*Proteinase activities are shown in 13 members of the ADAMTS family, but not in 6 other members.
ADAMTS, A disintegrin and metalloproteinases with thrombospondin motifs.

MMP-13 (collagenase-3). These MMPs attack triple helical regions of interstitial collagen types I, II, and III at a specific single site after the Gly residue of the partial sequences Gly-(Ile or Leu)-(Ala or Leu), located about three-fourths of the distance from the N-terminus. This cleavage generates fragments approximately three-fourths and one-fourth of the size of the collagen molecules. A biochemical study has disclosed the molecular mechanism of the cleavage: MMP-1 unwinds the triple helical structure by interacting with the α2(I) chain of type I collagen and cleaves the three α chains in succession.[9] MMP-13 is unique in that it cleaves α chains of type II collagen at two sites of the $Gly^{906}$-$Leu^{907}$ $Gly^{909}$-$Gln^{910}$ bonds.[10] All of these collagenases degrade the interstitial collagens, but their specific activities against the collagens are different; MMP-1, 8, and 13 preferentially digest types III, I, and II collagen.[10,11] Although rodents such as mice were originally thought to have only two collagenases (*MMP-8* and *MMP-13*) and to lack the *MMP-1* gene, rodent homologues of the human *MMP-1* gene were cloned and named mouse collagenase A and B (*Mcol-A* and *Mcol-B*).[12]

In addition to the interstitial fibrillar collagens, MMP-1, 8, and 13 degrade many other ECM macromolecules. MMP-1 digests entactin, collagen X, gelatins, perlecan, aggrecan, and cartilage link protein (see Table 8-2). MMP-8 digests aggrecan, gelatins, and cartilage link protein (see Table 8-2). MMP-13 hydrolyzes aggrecan; types IV, IX, X, and XIV collagens; fibronectin; and tenascin. Non-ECM substrates of MMP-1, 8, and 13 include α2 macroglobulin, α1 anti-proteinase inhibitor, α1 antichymotrypsin, insulin-like growth factor binding protein (IGF-BP)-2 and IGF-BP-3, connective tissue growth factor (CTGF), and pro-TGF-β (see Table 8-2).

**Gelatinases (MMP-2 and MMP-9).** MMP-2 (gelatinase A) and MMP-9 (gelatinase B) belong to the gelatinase subgroup. Both MMPs readily digest gelatins and cleave types IV and V collagen.[13,14] Elastin, aggrecan, and cartilage link protein also are substrates of the gelatinases. Although MMP-2 and 9 share such substrates, they have different activities on several ECM macromolecules. MMP-2, but not MMP-9, digests fibronectin and laminin,[13] and type III collagen and α2 chains of type I collagen are degraded only by MMP-9.[14] The gelatinases also process directly TGF-β into an active ligand (see Table 8-2). MMP-2 and 9 cleave fibroblast growth factor receptor type I and interleukin (IL)-2 receptor type α (see Table 8-2). MMP-9 also releases soluble Kit-ligand.[15] MMP-2 processes monocyte chemoattractant protein (MCP)-3 into an MCP-3 fragment deleting the N-terminal four amino acids, which can bind to CC-chemokine receptors and act as a general chemokine antagonist.[16]

**Stromelysins (MMP-3 and MMP-10).** The subgroup of stromelysins consists of MMP-3 (stromelysin-1) and MMP-10 (stromelysin-2). They share 78% identity in

**TABLE 8-4** Members of the Human ADAM Family

| ADAM | Other Names | Proteinase-Type (P) or Non–Proteinase-Type (NP) | Functions and Biochemical Features | Tissue and Cell Expression |
|---|---|---|---|---|
| ADAM2 | PH-30β; Fertilin-β | NP | Sperm/egg binding/fusion; binding to integrin α$\beta$1, α6β, and α9β | Sperm |
| ADAM7 | EAP I; GP-83 | NP | Binding to integrin α4β1, α4β7, and α9β1 | Testis |
| ADAM8 | MS2 (CD156) | P | Neutrophil infiltration; shedding of CD23 | Macrophages; neutrophils |
| ADAM9 | MDC9; MCMP; Meltrin-γ | P | Shedding of HB-EGF, TNF-p75 receptor, and APP; digestion of fibronectin and gelatin; binding to integrin α2β1, α6β1, α6β4, α9β1, and αVβ5 | Various tissues |
| ADAM10 | MDAM; Kuzbanian | P | Shedding of TNF, Delta, Delta-like 1, Jagged, N-cadherin, E-cadherin, VE-cadherin, Ephrin A2, Ephrin A5, Fas-l, IL-6R, APP, L1,CD44, and HB-EGF; digestion of collagen IV, gelatin, and myelin basic protein; presence of RRKR sequence | Kidney; brain; chondrocytes |
| ADAM11 | MDC | NP | Tumor suppressor gene (?) | Brain |
| ADAM12 | Meltrin-α; MCMP; MLTN; MLTNA | P | Muscle formation; presence of RRKR sequence; binding to integrin α4β1 and α9β1; digestion of IGF-BP-3 and -5; shedding of HB-EGF and epiregulin; digestion of collagen IV, gelatin, and fibronectin | Osteoblasts; muscle cells; chondrocytes; placenta |
| ADAM15 | Metargidin; MDC15; AD56; CR II-7 | P | Expression in arteriosclerosis; binding to integrin αvβ3, α5β1, and α9β1; digestion of collagen IV and gelatin; shedding of CD23 | Smooth muscle cells; chondrocytes; endothelial cells; osteoclasts |
| ADAM17 | TACE; cSVP | P | Shedding of TNF, TGF-β, TNF-p75 receptor, RANKL, amphiregulin, epiregulin, HB-EGF, APP, L-selectin, and CD44; presence of RRKR sequence; binding to integrin α5β1 | Macrophages; various tissues; carcinoma tissue |
| ADAM18 | tMDC III | NP | — | Testis |
| ADAM19 | Meltrin-β; FKSG34 | P | Formation of neuron; shedding of neuregulin, and RANKL; binding to integrin α4β1 and α5β1 | Testis |
| ADAM20 | — | P | Formation of sperm | Testis |
| ADAM21 | — | P | — | Testis |
| ADAM22 | MDC2 | NP | — | Brain |
| ADAM23 | MDC3 | NP | Binding to integrin αvβ3 | Brain; heart |
| ADAM28 | e-MDC II; MDC-Lm; MDC-Ls | P | Digestion of VWF, IGF-BP-3, and CTGF; shedding of CD23; binding to integrin α4β1, α4β7, and α9β1 | Epididymis; lung; stomach; pancreas |
| ADAM29 | svph1 | NP | — | Testis |
| ADAM30 | svph4 | P | — | Testis |
| ADAM32 | AJ131563 | NP | — | Testis |
| ADAM33 | — | P | Mutation in bronchial asthma patients; shedding of APP and KL-1; digestion of insulin B chain; binding to integrin α4β1, α5β1, and α9β1 | Lung (fibroblasts, smooth muscle cells) |
| ADAMDEC1 | — | P | — | Lymphatic system; gastrointestinal system |

ADAM, A disintegrin and metalloproteinase; ADAMDEC1, ADAM-like decysin 1; APP, amyloid precursor protein; CTGF, connective tissue growth factor; HB-EGF, heparin-binding epidermal growth factor; IGF-BP, insulin-like growth factor binding protein; IL-6R, interleukin-6 receptor; KL-1, kit ligand-1; RANKL, receptor activator of nuclear factor-κB ligand; TGF, transforming growth factor; TNF, tumor necrosis factor; VWF, von Willebrand factor.

amino acid sequence and have similar enzymatic properties.[17] The enzymes hydrolyze numerous ECM macromolecules including aggrecan, fibronectin, laminin, and collagen IV (see Table 8-2).[18] Types III, IX, and X collagen and telopeptides of types I, II, and XI collagen also are digested by MMP-3.[19] In addition to the ECM components, MMP-3 is active on IGF-BP-3, IL-1β, heparin-binding epidermal growth factor (HB-EGF), CTGF, E-cadherin, α1 antichymotrypsin, and α1 proteinase inhibitor (see Table 8-2). MMP-3 also activates many proMMPs.[6] A similar activator function has been identified for MMP-10.[20]

**Matrilysins (MMP-7 and MMP-26).** Matrilysins include MMP-7 (matrilysin-1) and MMP-26 (matrilysin-2), which are the smallest of the MMPs, having only the propeptide and catalytic domains. The substrate specificity of MMP-7 is similar to that of stromelysins, digesting numerous ECM

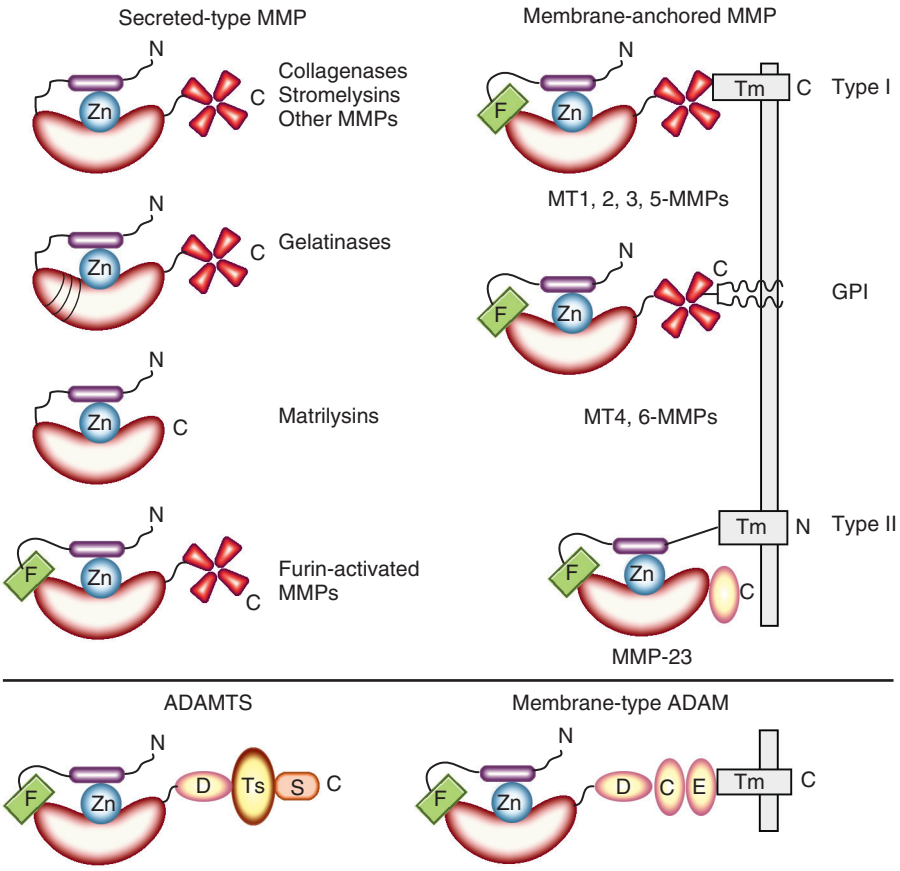



**Figure 8-1** Domain structures of two types of matrix metalloproteinases (MMPs) (secreted-type MMP and membrane-anchored MMP) and two types of a disintegrin and metalloproteinases (ADAMs) (a disintegrin and metalloproteinase with thrombospondin motifs [ADAMTS] and membrane-type ADAM). The typical domain structure of most secreted-type MMPs (collagenases, stromelysins, and other MMPs) is composed of a prodomain, catalytic domain, hinge, and hemopexin-like domain. Gelatinases (MMP-2 and MMP-9) have additional insertions of collagen-binding type II repeats of fibronectin in the catalytic domain, whereas matrilysins (MMP-7 and MMP-26) lack a hemopexin-like domain. Furin-activated MMPs (MMP-11 and MMP-28) contain an RKRR sequence (furin recognition site = F) at the end of the propeptide. Membrane-anchored MMPs are composed of type I transmembrane-type MMPs (MT1-, MT2-, MT3-, and MT5-MMPs); glycosylphosphatidylinositol (GPI)-linked MMPs (MT4- and MT6-MMPs); and type II transmembrane-type MMP (MMP-23). All of them have furin recognition sites. ADAMTS has a prodomain, a furin recognition site (F), a catalytic domain, a hinge, a disintegrin domain (D), thrombospondin motifs (Ts), and a spacer domain (S). Membrane-type ADAM is composed of a prodomain, furin recognition site (F), catalytic domain, hinge, disintegrin domain (D), cysteine-rich domain (C), EGF-like domain (E), and transmembrane domain (Tm). Zn, Active site zinc.

components, including aggrecan; gelatins; fibronectin; laminin; elastin; entactin; types III, IV, V, IX, X, and XI collagen; fibrin/fibrinogen; vitronectin; tenascin; and link protein (see Table 8-2). Although these substrates overlap with the substrates of other MMPs, the specific activity of MMP-7 to most substrates is highest among the MMPs.[21,22] Non-ECM molecules such as α-defensin, Fas ligand, β4 integrin, E-cadherin, plasminogen, tumor necrosis factor (TNF), and CTGF also are the substrates for MMP-7 (see Table 8-2). MMP-26 degrades gelatin, type IV collagen, fibronectin, fibrinogen, and α1 proteinase inhibitor,[23,24,25] but information about other substrates is still limited.

**Furin-Activated Matrix Metalloproteinases (MMP-11 and MMP-28).** MMP-11 (stromelysin-3) and MMP-28 (epilysin) contain an RKRR sequence at the end of the propeptide, which is a unique motif for intra_cellular processing of proproteins to mature molecules by furin and other proprotein convertases. ProMMP-11 is activated intra-cellularly by the action of furin.[26] MMP-11 shows only weak proteolytic activity against gelatin, laminin, fibronectin, and aggrecan,[27] but it has respectable catalytic action in digesting α1 proteinase inhibitor, α2 macroglobulin, and IGF-BP-1 (see Table 8-2).[28,29] MMP-28 can degrade casein, but its natural substrates are unknown.[30]

**Other Secreted-Type Matrix Metalloproteinases (MMP-12, MMP-19, MMP-20, MMP-21, and MMP-27).** MMP-12 (metalloelastase),[31] MMP-19 (RASI-1),[32] MMP-20 (enamelysin),[33] MMP-21,[25] and MMP-27 have structural characteristics similar to those of collagenases and stromelysins. These MMPs are not classified into the previously mentioned subgroups, however, because their substrates and other biochemical characters are not fully examined at present. Overall, information on the substrate specificity of MMP-12,[31] 19,[34,35] and 20[33,35] suggests that they are stromelysin-like proteinases. MMP-12[31] digests elastin, fibronectin, collagen V, osteonectin, and plasminogen (see Table 8-2). MMP-19, which was originally reported as MMP-18 but renamed as MMP-19, cleaves type IV collagen, laminin, fibronectin, gelatin, tenascin, entactin, fibrin/fibrinogen, aggrecan, and cartilage oligomeric matrix protein (COMP; see Table 8-2).[34,35] MMP-20 also digests amelogenin, aggrecan, and COMP.[35] Substrates of MMP-21 and 27 are unknown, however.

### Membrane-Anchored Matrix Metalloproteinases

Type I transmembrane-type MMPs include MMP-14 (MT1-MMP),[36] MMP-15 (MT2-MMP),[37] MMP-16 (MT3-MMP),[38] and MMP-24 (MT5-MMP).[39] All of these MT-MMPs can activate proMMP-2, but MMP-14 may play a major role in the activation of proMMP-2 in various tissues (see later discussion). Besides the activator function, however, MMP-14 digests the triple helical portions of interstitial collagen types I, II, and III and other ECM components, including fibronectin, laminin, aggrecan, and gelatin (see Table 8-2).[40] MMP-15 also digests fibronectin, tenascin, nidogen, aggrecan, perlecan, and laminin.[41] MMP-16 cleaves type III collagen, fibronectin, and gelatins.[42] MMP-17 (MT4-MMP)[43] and MMP-25 (MT6-MMP)[44] are GPI-linked MMPs. MMP-17 and MMP-25 can digest gelatin and fibrin/fibrinogen (see Table 8-2).[44–46] MMP-23 (cysteine array–MMP, MIFR) is a type II transmembrane-type MMP[47]; almost identical genes are cloned, called *MMP-23A* and *MMP-23B*. MMP-23 digests gelatin,[48] but no information about other substrates is available (see Table 8-2). A unique aspect of MMP-23 is that this MMP is expressed in only female and male reproductive organs such as endometrium, ovary, testis, and prostate,[48] but its functions are not well established.

### ADAM and ADAMTS Families

Two ADAM (a disintegrin and metalloproteinase) gene families exist: membrane-type ADAM with transmembrane domain (ADAM) and secreted-type ADAM with thrombospondin motifs (ADAMTS; see Figure 8-1). The active sites in the catalytic domains of most members of both gene families contain a common sequence of HEXGHXXGXXHD with the "Met-turn," which also is present in MMP members.

The ADAMTS family includes 19 members. Although information about substrates and biologic functions is still limited, ADAMTS1-5, 8-9, 14-16, 18, and 20 all are ECM-degrading proteinases (see Table 8-3). ADAMTS1,[49] 4,[50] 5,[51] 9, and 15 can preferentially cleave aggrecan at the five Glu-X bonds, including the $Glu^{373}$-$Ala^{374}$ bond (the aggrecanase site). Because of aggrecan-degrading activity, ADAMTS4 and ADAMTS5 are named aggrecanase-1 and aggrecanase-2[50,51]; versican also is digested by these proteinases,[52] and brevican is cleaved by ADAMTS4 (see Table 8-3).[53] The C-terminus–truncated ADAMTS4 also degrades fibromodulin and decorin.[54] ADAMTS16, 18, and 20 also appear to have weak aggrecanase activity. ADAMTS2 and 3 process the N-terminal propeptides of type I and II collagens and are named procollagen N-proteinase. Activity of procollagen N-proteinase also is known with ADAMTS14. ADAMTS13 is a von Willebrand factor–cleaving proteinase, and its mutation causes thrombotic thrombocytopenic purpura. Proteinase activities of other ADAMTS species are still unknown.

The human genome contains 25 *ADAM* genes, including 4 pseudogenes, and thus the human ADAM family is composed of 21 members (Table 8-4). Among the ADAMs, ADAM8-10, 12, 15, 17, 19-21, 28, 30, 33, and ADAM-like decysin 1 (ADAMDEC1) exhibit proteolytic activity (i.e., they are proteinase-type ADAMs; see Table 8-4). Although ADAM10, 12, and 15 degrade type IV collagen, the main substrates of these ADAMs are various membrane proteins, which include precursors of cytokines and growth factors such as TNF, HB-EGF and neuregulin, IGF-BPs, receptors such as p75 TNF receptor, IL-1 receptor II, and other membrane proteins related to development such as Notch ligand and ephrin (see Table 8-4).[55-60] According to these data, a major function of the ADAMs is shedding of the membrane proteins. ADAM17 cleaves a proform of TNF at the physiologic processing site into the soluble form of TNF and is called *TNF-converting enzyme*. ADAM17 also is involved in release of L-selectin, TGF-α, and p75 TNF receptor.[55] ADAM9, 12, and 17 can shed HB-EGF from its precursor. ADAM12 and 28 cleave IGF-BP-3 and IGF-BP-5.[60,61] CD23 is shed by ADAM8, 15, and 28.[62] Other functions of ADAMs include binding to integrins, cell-cell interaction, cell migration, and signal transduction (see Table 8-4).[63]

## ENDOGENOUS PROTEINASE INHIBITORS

Endogenous proteinase inhibitors control the activities of proteinases in vivo. The inhibitors are derived from plasma or cells in the local tissues. Plasma contains several proteinase inhibitors, and about 10% of all the plasma proteins are proteinase inhibitors. Most are proteinase class specific, but α2 macroglobulin inhibits the activities of proteinases from all four groups. Major endogenous inhibitors of the ECM-degrading proteinases are listed in Table 8-5.

### α2 Macroglobulin

The α2 macroglobulin molecule is a large plasma glycoprotein of 725 kDa that consists of four identical subunits of 185 kDa that are linked in pairs by disulfide bonds. The pairs assemble noncovalently. Almost all active proteinases, regardless of the proteinase classes, bind and attack the so-called bait region, located near the center of the subunit. After cleaving within the bait region, the proteinase is physically trapped within the molecule by inducing a conformational change of the inhibitor, resulting in a proteinase/α2 macroglobulin complex. Although the proteinase in the complex remains active against small substrates, it is trapped by the arms of the α2 macroglobulin from degrading larger proteins. Besides the function as a proteinase inhibitor, α2 macroglobulin may act as a carrier protein because it also binds to numerous growth factors and cytokines such as platelet-derived growth factor, basic fibroblast growth factor, TGF-β, insulin, and IL-1β.

The α2 macroglobulin molecule is synthesized mainly in liver but also locally by macrophages, fibroblasts, and adrenocortical cells. Concentration of the inhibitor in plasma is 250 mg/dL. Because of its large molecular weight, it is not present in non-inflammatory synovial fluid. During synovial inflammation, α2 macroglobulin penetrates into the joint cavity. Rheumatoid synovial fluid has about the same concentration of the inhibitor as plasma.

### Inhibitors of Serine Proteinases

The primary inhibitors of serine proteinases include the members of the serpin (serine proteinase inhibitor) gene family, Kunitz-type inhibitors, and others (see Table 8-5). The serpins are glycoproteins of 50 to 100 kDa and share

**TABLE 8-5** Endogenous Inhibitors of Extra-cellular Matrix–Degrading Proteinases

| Inhibitor | Molecular Mass (kDa) | Source | Target Enzyme |
|---|---|---|---|
| α2 Macroglobulin | 725 | Plasma (liver); macrophages; fibroblasts | Most proteinases from all classes |
| **Inhibitors of Serine Proteinase** | | | |
| **Serpins** | | | |
| α1 Proteinase inhibitor | 52 | Plasma; macrophages | Neutrophil elastase, cathepsin G, proteinase 3 |
| α1 Antichymotrypsin | 58 | Plasma | Cathepsin G; chymotrypsin; chymase; tissue kallikrein |
| α2 Antiplasmin | 67 | Plasma | Plasmin |
| Proteinase nexin-1 | 45 | Fibroblasts | Thrombin; uPA; tPA; plasmin; trypsin; trypsin-like serine proteinase |
| PAI-1 | 45 | Endothelial cells; fibroblasts; platelets; plasma | tPA; uPA |
| PAI-2 | 47 | Plasma; macrophages | uPA; tPA |
| Protein C inhibitor | 57 | Plasma; urine | Active protein C; tPA; uPA; tissue kallikrein |
| C1-inhibitor | 96 | Plasma | Plasma kallikrein; C1 esterase |
| Kallistatin | 92 | Plasma; liver; stomach; kidney; pancreas | Tissue kallikrein |
| **Kunins** | | | |
| Aprotinin | 7 | Mast cells | Plasmin; kallikrein |
| Trypstatin | 6 | Mast cells | Tryptase |
| Proteinase nexin-2 (β-amyloid protein precursor) | 100 | Fibroblasts | EGF binding protein, NGF-γ, trypsin, chymotrypsin, factor XIa |
| **Others** | | | |
| SLPI | 15 | Bronchial secretions; seminal plasma; cartilage | Neutrophil elastase; cathepsin G; chymotrypsin; trypsin |
| Elafin | 7 | Horny layers of skin | Neutrophil elastase; proteinase 3 |
| **Inhibitors of Cysteine Proteinase** | | | |
| Stefin A | 11 | Cytosol | Cysteine proteinases |
| Stefin B | 11 | Cytosol | Cysteine proteinases |
| Cystatin C | 13 | Body fluids | Cysteine proteinases |
| Cystatin S | 13 | Seminal plasma; tears; saliva | Cysteine proteinases |
| Kininogens | 50-78/108-120 | Plasma | Cysteine proteinases |
| Calpastatin | 120 | Cytosol | Calpains |
| **Metalloproteinase Inhibitors** | | | |
| TIMP-1 | 28 | Connective tissue cells; macrophages | MMPs |
| TIMP-2 | 22 | Connective tissue cells; macrophages | MMPs |
| TIMP-3 | 21/24* | Fibroblasts; synovial cells | MMPs; ADAMs; ADAMTS |
| TIMP-4 | 21 | Heart; brain; testis | MMPs |
| RECK | 110 | Many tissue cells; fibroblasts | MMP-2; MMP-7; MMP-14 (MT1-MMP); ADAM10 |

*Glycosylated form.

ADAM, A disintegrin and metalloproteinase; EGF, epidermal growth factor; MMP, matrix metalloproteinase; NGF, nerve growth factor; PA, plasminogen activator; PAI, plasminogen activator inhibitor; RECK, reversion-inducing, cysteine-rich protein with Kazal motifs; SLPI, secretory leukocyte proteinase inhibitor; TIMP, tissue inhibitor of metalloproteinases; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator.

homology with human α1 proteinase inhibitor.[64] The major serpins involved in the regulation of ECM-degrading serine proteinases are α1 proteinase inhibitor, α1 antichymotrypsin, α2 antiplasmin, plasminogen activator inhibitors (PAI-1 and PAI-2), protein C inhibitor (PAI-3), C1-inhibitor, kallistatin, and proteinase nexin-1 (PN-1). The main proteinases inhibited by these molecules are listed in Table 8-5. Although PAI-1 and PAI-2 inhibit tPA and uPA, the inhibition by PAI-1 is more effective to tPA and that of PAI-2 is more efficient to uPA.

Kunitz-type inhibitors include aprotinin, trypstatin, and PN-2, which is identical to a β-amyloid protein precursor. Secretory leukocyte proteinase inhibitor, which inhibits neutrophil elastase and cathepsin G, is present in many secretory and inflammatory fluids and in cartilage. Elafin is a serine proteinase inhibitor with 38% identity with the second domain of secretory leukocyte proteinase inhibitor; it inhibits neutrophil elastase and proteinase 3.

## Inhibitors of Cysteine Proteinases

The members of the cystatin superfamily and calpastatin belong to the family of inhibitors of ECM-degrading cysteine proteinases (see Table 8-5). Cystatins that inhibit

lysosomal cysteine proteinases consist of three groups. Subgroup 1 comprises stefins A and B. Each has a molecular mass of 11 kDa, and the stefins reside within cells. Subgroup 2 comprises cystatin C and S, each with a molecular mass of 13 kDa. They occur at relatively high concentrations in cerebrospinal fluid and saliva. Subgroup 3 comprises the kininogens. Kininogens that participate in blood coagulation and inflammation also are inhibitors of cysteine proteinases. Calpains are not inhibited by cystatins but are inhibited by calpastatin (120 kDa), which is a cytosolic-specific inhibitor of calpain.

### Tissue Inhibitors of Metalloproteinases

TIMPs are a gene family consisting of four different members with approximately 40% to 50% sequence identity (i.e., *TIMP-1*, *TIMP-2*, *TIMP-3*, and *TIMP-4*) that have molecular masses ranging from 21 to 28 kDa in humans.[65-69] Virtually all TIMPs inhibit the activities of MMPs by binding in a 1:1 molar ratio to form tight, noncovalent complexes,[65] except that TIMP-1 does not efficiently inhibit MT-MMPs.[41,42,70] TIMPs contain 12 highly conserved cysteine residues that form six intrachain disulfide bonds, which are essential for maintaining the correct ternary structure of the molecule[67,71] and stable inhibitor activity.[65] The TIMP molecules have two structurally distinct subdomains: an N-terminal subdomain that consists of loops 1 through 3 and a C-terminal subdomain that consists of loops 4 through 6. The N-terminal subdomain of each TIMP molecule contains the inhibitory activity for MMPs.[65] Studies on the crystal structures of the MMP/TIMP complexes show that the wedge-shaped TIMPs bind with their edge into the entire length of the active-site cleft of their cognate MMPs.[67] High affinity and efficient inhibitor activity of TIMP-2 to MMP-14 (MT1-MMP) are explained by the interaction between a quite long hairpin loop of TIMP-2 and a loop over the rim of the active-site cleft of MMP-14.[72]

TIMP-1 and TIMP-2 are unique in that they make the complexes with proMMP-9 and proMMP-2 (i.e., the proMMP-9/TIMP-1 and proMMP-2/TIMP-2 complexes). Similar complex formation also is known between TIMP-4 and proMMP-2. Because the complexes are made through the interaction between their C-termini,[65] TIMPs in the complexes retain inhibitor activity against MMPs. The activation of proMMP-9 and proMMP-2 is suppressed in the complex forms; the complex formation may be a safety device for these gelatinases.[14] The proMMP-2/TIMP-2 complex is useful for the efficient activation of proMMP-2 by MMP-14 on the cell membranes because MMP-14 captures proMMP-2 to the cell membranes through the trimolecular complex formation between the catalytic domain of MMP-14 and the N-terminal domain of TIMP-2 (see later discussion).[73,74]

Besides the inhibition and interactions of TIMPs to MMPs, TIMP-3, among the TIMPs, most efficiently inhibits the activities of ADAM10, 12, 17, 28, and 33,[75] although ADAM8, 9, and 19 are not inhibited by TIMPs. Because TIMP-3 also efficiently inhibits the aggrecan-degrading activity of ADAMTS4 and 5, TIMP-3 may be a common tissue inhibitor of the ADAM members. The N-terminal subdomain of TIMP-3 is critical to the inhibition of the activities of ADAM members and MMPs, but the inhibition mechanism seems to be different.[76] TIMPs are multifunctional proteins with more diverse actions than MMP/ADAM inhibitors, including growth factor activity, antiangiogenic activity, and regulatory activity of apoptosis.[69]

Another new MMP inhibitor is *RECK* (*re*version-inducing, *c*ysteine-rich protein with *K*azal motifs).[77] RECK is a GPI-linked glycoprotein harboring three inhibitor-like domains and inhibits the activities of at least MMP-2, 7, 9, 14, and ADAM10. Although this inhibitor seems to play a key role in the angiogenic processes in vivo, its biochemical mechanism as an inhibitor to the MMPs and ADAM10 and its functions in pathologic conditions such as arthritides remain unknown.

## REGULATION OF PROTEINASE ACTIVITY

The activities of ECM-degrading proteinases in tissues are regulated by the balance between the proteinases and their inhibitors. The balance at the local tissues depends on several factors, including production rates of proteinases and inhibitors, their secretion, activation of proenzymes, and pericellular anchoring and recycling systems of the activated proteinases. Production levels of the proteinases and inhibitors within the cells are controlled mainly by their gene expression. Activation processes of proMMPs and membrane anchoring of their activities have been established by extensive experimental work.

### Gene Expression of Proteinases and Inhibitors

#### Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases

Normal cells except for inflammatory cells produce only limited levels of MMPs or TIMPs in the tissues under physiologic conditions, but their expression is stimulated by many factors under pathologic conditions. Neutrophils and macrophages synthesize MMP-8 and MMP-9 during the differentiation and store them within the granules of the differentiated cells. Tumor cells express many MMPs such as MMP-1, 7, 9, 10, and MMP-14 (MT1-MMP), as well as TIMP-1, predominantly by oncogenic transformation. The gene expression of *MMPs* and *TIMPs* in the tissue cells other than inflammatory cells and tumor cells is regulated by numerous factors, however, including cytokines, growth factors, and chemical and physical stimuli.

Much information is available for regulators of *MMP-1* and *MMP-3*, which are coordinately expressed in many cell types after stimulation with cytokines and growth factors, factors acting at the cell surface, and chemical agents (Table 8-6). The induced production of MMP-1 and MMP-3 is suppressed by retinoic acid, TGF-β, and glucocorticoid. The gene expression of *MMP-7* and *MMP-9* is regulated by similar factors, but the regulation is stricter and fewer factors modulate the expression (see Table 8-6). *MMP-14* expression is upregulated by phorbormyristate acetate (12-*O*-tetradecanoylphorbol-13-acetate), concanavalin A, basic fibroblast growth factor, and TNF, and it is downregulated by glucocorticoids in various cells. TNF and IL-1α stimulate osteoarthritic chondrocytes to express the

**TABLE 8-6** Factors That Modulate Synthesis of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases

| Enzyme or TIMP | Stimulating Factor | Suppressive Factor |
|---|---|---|
| MMP-1 | **Cytokines and growth factors:** IL-1; TNF; EGF; PDGF; bFGF; VEGF; NGF; TGF-α; IFN-α; IFN-β; IFN-γ; leukoregulin; relaxin<br>**Factors acting at cell surface:** calcium ionophore A23187; cell fusion; collagen; concanavalin A; integrin receptor antibody; crystals of urate, hydroxyapatite, and calcium pyrophosphate; SPARC (osteonectin/BM 40); iron; extra-cellular matrix metalloproteinase inducer (EMMPRIN/CD147/basigin/M6 antigen); phagocytosis<br>**Chemical agents:** cAMP; colchicine; cytochalasins B and D; LPS; pentoxifylline; TPA; calmodulin inhibitors; serotonin; 1,25-$(OH)_2$ vitamin $D_3$; platelet-activating factor; serum amyloid A; β-microglobulin<br>**Physical factors:** heat shock; ultraviolet irradiation<br>**Others:** viral transformation; oncogenes; autocrine agents; aging of fibroblasts | Retinoic acids; glucocorticoids; estrogen; progesterone; TGF-β; transmembrane neural cell adhesion molecule; cAMP; IFN-γ; adenovirus E1A |
| MMP-2 | TGF-β; concanavalin A; H-ras transformation; extra-cellular matrix metalloproteinase inducer (EMMPRIN/CD147/basigin/M6 antigen) | Adenovirus E1A |
| MMP-3 | IL-1; TNF; EGF; concanavalin A; SPARC (osteonectin/BM 40); LPS; TPA; extra-cellular matrix metalloproteinase inducer (EMMPRIN/CD147/basigin/M6 antigen); viral transformation; oncogenes; integrin receptor antibody; heat shock; calcium ionophore A23187; cytochalasin B | Retinoic acids; glucocorticoids; estrogen; progesterone; TGF-β; adenovirus E1A |
| MMP-7 | IL-1; TNF; EGF; TPA; LPS | Unknown |
| MMP-8 | TNF; TPA; IL-1 | Unknown |
| MMP-9 | IL-1; TNF; EGF; TGF-β; TPA; H-ras; v-Src; SPARC (osteonectin/BM40) | Retinoic acids; adenovirus E1A |
| MMP-10 | TPA; A23187; TGF-β; EGF | Unknown |
| MMP-11 | Retinoic acids | bFGF |
| MMP-13 | bFGF; TNF; TGF-β | Unknown |
| MMP-14 (MT1-MMP) | Concanavalin A; TPA; bFGF; TNF; IL-1α | Glucocorticoids |
| TIMP-1 | IL-1; IL-6; IL-11; TPA; TGF-β; TNF; retinoic acids; LPS; progesterone; estrogen; oncogenic transformation; viral infection | Extra-cellular matrix; cytochalasins |
| TIMP-2 | Progesterone | TGF-β; LPS |
| TIMP-3 | EGF; TGF-β; TPA; TNF; glucocorticoids; oncostatin M | Unknown |

*Factors regulating gene expression of other MMPs excluded from this table and TIMP-4 are unknown.

bFGF, Basic fibroblast growth factor; cAMP, cyclic adenosine monophosphate; EGF, epidermal growth factor; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VEGF, vascular endothelial growth factor.

*MMP-14* gene.[78] In contrast to these MMPs, MMP-2 and TIMP-2 are unique in that factors capable of enhancing the production of MMP-1, MMP-3, and TIMP-1 are inactive.

*TIMP-1* expression is enhanced or suppressed in response to many factors, including cytokines, growth factors, and oncogenic transformation (see Table 8-6). Effects of these stimulatory factors are common to the gene expression of *MMPs*, but they are regulated independently. TGF-β, retinoic acid, progesterone, and estrogen enhance *TIMP-1* expression in fibroblasts, but they suppress the expression of *MMP-1* and *3*. Although information about stimulating and suppressive factors of *TIMP-1*, *2*, and *3* is available (see Table 8-6), factors controlling the gene expression of *TIMP-4* are not well known. Previous studies have identified the elements in the promoters of *MMPs* and *TIMPs*, which are related to or responsible for the stimulation or suppression of the gene expression with various factors. Regulation of the gene expression is generally explained by the structural characteristics of the promoters.

## Serine Proteinases and Their Inhibitors

Neutrophil elastase, cathepsin G, chymase, and tryptase are stored within the secretory granules and secreted into the extra-cellular milieu after activation of neutrophils and mast cells. The expression of these serine proteinases is controlled mainly by the cellular differentiation. Precursors of plasmin and plasma kallikrein are constitutively synthesized predominantly in the liver, circulate in blood as zymogen forms (i.e., plasminogen and prekallikrein), and reach the inflamed tissues by being released from blood vessels. The proteinase activities in the tissues are controlled mainly through activation of the proenzymes by activators. The uPA and tPA molecules, activators of plasminogen, are synthesized by tissue cells, and their gene expression is regulated by many factors (Table 8-7). The uPA synthesis is upregulated in many normal cell types and in transformed cells by agents that increase intra-cellular cyclic adenosine monophosphate (cAMP) levels (e.g., calcitonin, vasopressin, cholera toxin, and cAMP analogues); growth factors (e.g., EGF, platelet-derived growth factor, and vascular endothelial growth factor); cytokines (IL-1 and TNF); and phorbol esters, whereas glucocorticoid decreases the expression.[8] The expression of *tPA* is regulated by similar factors (see Table 8-7). In endothelial cells, proteinases are enhancers; thrombin and plasmin stimulate the production of tPA.[8] *PAI-1* and *2* also are regulated by common factors, many of which also enhance the production of uPA and tPA (see

**TABLE 8-7** Factors That Regulate Expression of Plasminogen Activators and Their Inhibitors

| Enzyme or Inhibitor | Stimulatory Factor | Suppressive Factor |
|---|---|---|
| uPA | TPA; IL-1; IFN-γ; EGF; PDGF; bFGF; VEGF; TGF-β; cholera toxin; cAMP; estrogen; calcitonin; vasopressin; disruption of E-cadherin–dependent cell-cell adhesion | Glucocorticoids; TGF-β |
| tPA | TPA; EGF; bFGF; VEGF; retinoic acids; glucocorticoids; cAMP; thrombin; plasmin; follicle-stimulating hormone; luteinizing hormone; gonadotropin-releasing hormone | TNF |
| PAI-1 | IL-1; TNF; TGF-β; bFGF; VEGF; TPA; glucocorticoids | cAMP |
| PAI-2 | TPA; LPS; TNF; colony-stimulating factor; cholera toxin; dengue virus | Glucocorticoids |
| PN-1 | TPA; EGF; thrombin | Unknown |

bFGF, Fibroblast growth factor; cAMP, cyclic adenosine monophosphate; EGF, epidermal growth factor; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; PN, proteinase nexin; TGF, transforming growth factor; TNF, tumor necrosis factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

Table 8-7). Most serpins are constitutively produced in the liver and secreted to plasma.

### Lysosomal Cysteine and Aspartic Proteinases

The expression of lysosomal cysteine proteinases, *cathepsins B, L,* and *K,* is generally constitutive, but cellular transformation is often associated with increased synthesis of cathepsins B and L. Cathepsin B transcription varies with cell type and the state of differentiation of tumor cells; it is increased in chondrocytes by IL-1. Malignant transformation, tumor promoters, and growth factors stimulate the synthesis of cathepsin L. *Cathepsin K* gene expression in monocyte-macrophage lineage depends on the cellular differentiation to osteoclasts, but all-*trans* retinoic acid upregulates the expression in rabbit osteoclasts. Lysosomal aspartic proteinase, *cathepsin D,* is constitutively expressed in almost all cells, although estradiol, calcitriol, and retinoic acid can regulate the expression.

## Activation Mechanisms of the Zymogens of Matrix Metalloproteinases

All of the MMPs are synthesized as inactive zymogens (proMMPs), and activation of proMMPs is prerequisite to their functioning in vivo. ProMMPs are kept inactive by an interaction between a cysteine-sulfhydryl group in the conserved propeptide sequence PRCGXPD and the zinc ion bound to the catalytic domain, preventing the formation of a water-zinc complex that is essential to the enzymatic reaction. Activation requires proteolytic removal of the propeptide domain. There are three pathways of proMMP activation—extra-cellular, intra-cellular, and pericellular (Figure 8-2).

### Extra-cellular Activation

Extra-cellular activation, which is applicable to many secreted MMPs (e.g., proMMP-1, 3, 7, 8-10, 12, and 13), is initiated through the disruption of the Cys-$Zn^{2+}$ interaction by treatment with nonproteolytic agents or proteinases and completed by autocatalytic processing.[6,69] Nonproteolytic activators used in vitro include thiol-modifying reagents (e.g., mercurial compounds, iodoacetamide, *N*-ethylmaleimide, and oxidized glutathione), hypochlorous acid, sodium dodecyl sulfate, chaotropic agents, and physical factors (heat and acid exposure).[6] Most of these factors, especially 4-aminophenylmercuric acetate (APMA), enable proMMP molecules to generate a short-lived intermediate, which is formed by removal of a part of propeptide by an intramolecular reaction.[6] The fully activated form is made by an intermolecular autocatalysis cleaving three amino acids downstream from the conserved sequence PRCGXPD and leading to generation of active MMPs, starting with Tyr or Phe at the N-terminus. Such a process may not be essential, however, for proMMP-9 activation by APMA because a fully active form retaining the PRCGVPD sequence is generated by the cleavage of the $Ala^{74}$-$Met^{75}$ bond upstream of the conserved sequence.[14] Concerning proMMP-9 activation during cerebral ischemia in vivo, nitric oxide is reported to activate proMMP-9 by *S*-nitrosylation.[79]

A similar stepwise activation is proposed for the proteolytic activation of proMMPs. Proteinases initially attack the proteinase-susceptible bait regions in the propeptides and generate proteolytically active intermediates through destabilization of the Cys-$Zn^{2+}$ interaction.[6] In the second step, the final activation site is autolytically catalyzed by the active intermediate instead of the trigger proteinases, and active MMPs without propeptides are made. In many cases, the bait region sequences in the propeptide dictate which proteinases can become an activator of a particular MMP.[6] Potential activators of proMMPs are listed in Table 8-8. Plasmin may play a major role in the activation of proMMP-3 and proMMP-10 in vivo because treatment of these proMMPs with plasmin leads to full activation in vitro.[80] ProMMP-1 activation by plasmin alone results in only about 25% of the potential MMP-1 activity, however, and full activation requires the subsequent cleavage of the $Gln^{80}$-$Phe^{81}$ bond by active MMP-3, 7, or 10.[6,22] MMP-3 and 10 can directly activate proMMP-7,[22] proMMP-8, proMMP-9,[14,20] and proMMP-13[11] into fully active forms. This intermolecular activation cascade of MMPs may be important to in vivo activation of proMMPs.

### Intra-cellular Activation

Because proMT-MMPs, proMMP-11, proMMP-23, and proMMP-28 have basic motifs containing an RXKR sequence at the end of the propeptide domains, proprotein convertases such as furin, a processing enzyme in the *trans*-Golgi apparatus, are considered to activate these proMMPs intra-cellularly (see Figure 8-2). Intra-cellular activation of

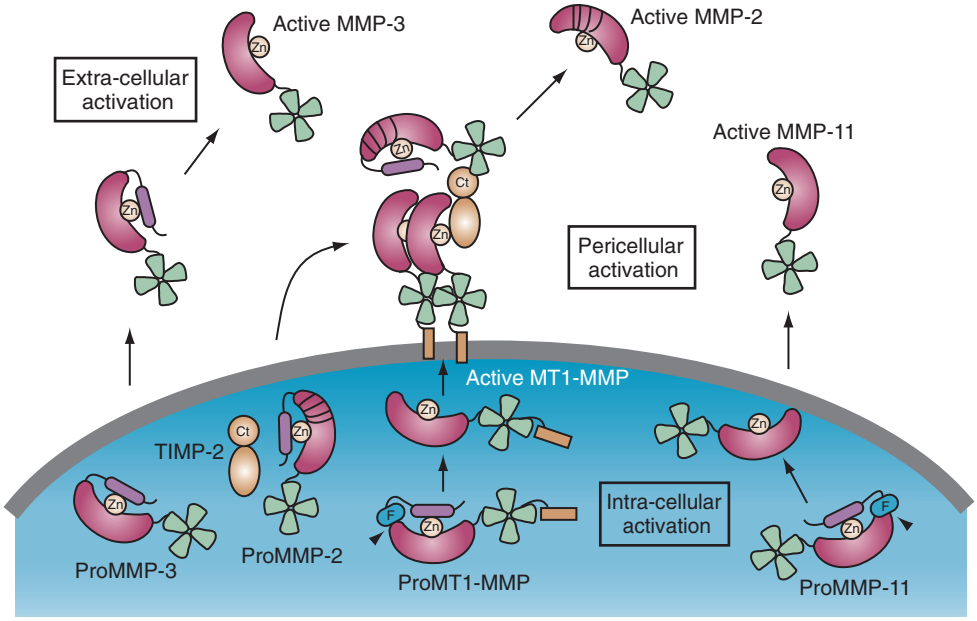



**Figure 8-2** Activation mechanisms of zymogen of matrix metalloproteinases (proMMPs). Most secreted-type proMMPs such as proMMP-3 are activated extra-cellularly by many proteinases (extra-cellular activation). Furin-activated secreted proMMPs, including proMMP-11, and proMT-MMPs, such as proMT1-MMP (proMMP-14), are intra-cellularly activated through removal of the propeptides *(arrowheads)* by the action of proprotein convertases such as furin (intra-cellular activation). ProMMP-2 is activated on the cell membrane by MT1-MMP (MMP-14); this activation requires the trimolecular complex of MT1-MMP/TIMP-2/proMMP-2 and dimerization of MT1-MMP (pericellular activation). Ct, C-terminal domain of TIMP-2; F, furin recognition site; TIMP, tissue inhibitor of metalloproteinase; Zn, active site zinc.

**TABLE 8-8** Activators of Pro-Matrix Metalloproteinases

| ProMMP | Activator |
|---|---|
| ProMMP-1 | Trypsin (partial); plasmin (partial); plasma kallikrein (partial); chymase (partial); MMP-3; MMP-7; MMP-10; MMP-11 |
| ProMMP-2 | MT1-MMP; MT2-MMP; MT3-MMP; MT5-MMP |
| ProMMP-3 | Plasmin; plasma kallikrein; trypsin; tryptase; chymase; cathepsin G; chymotrypsin; neutrophil elastase; thermolysin |
| ProMMP-7 | MMP-3; MMP-10 (partial); trypsin; plasmin (partial); neutrophil elastase (partial) |
| ProMMP-8 | MMP-3; MMP-10; tissue kallikrein; neutrophil elastase; cathepsin G; trypsin |
| ProMMP-9 | MMP-3; MMP-2; MMP-7; MMP-10 (partial); MMP-13; trypsin; chymotrypsin; cathepsin G; tissue kallikrein |
| ProMMP-10 | Plasmin; trypsin; chymotrypsin |
| ProMMP-11 | Furin |
| ProMMP-13 | MMP-2; MMP-3; MT1-MMP; plasmin |
| ProMMP-14 (ProMT1-MMP) | Furin |

Pro-MMP, Pro-matrix metalloproteinase.

proMMP-11 and proMMP-14 (proMT1-MMP) by furin is shown.[26,81] After the activation, MMP-11 is secreted from the cells and MMP-14 is expressed on the cell membranes. Because other proMT-MMPs, proMMP-23, and proMMP-28 also have the motif, furin is presumably responsible for the intra-cellular activation of these proMMPs.

### Pericellular Activation

ProMMP-2 is unique in that it is activated pericellularly by MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP), MMP-24 (MT5-MMP), and MMP-25 (MT6-MMP) but not by ordinary MMP-activatable endopeptidases.[13] MMP-17 (MT4-MMP) does not activate proMMP-2. This pericellular activation has been extensively studied with MMP-14 and found to occur in a two-step manner. MMP-14 cleaves the $Asn^{37}$-$Leu^{38}$ bond in the propeptide of proMMP-2, generating an intermediate form that is converted to a fully activated enzyme by an intermolecular autocatalytic mechanism.[70] TIMP-2 is essential to the efficient pericellular activation of proMMP-2 by MMP-14. The N-terminal inhibitor and C-terminal tail domains of TIMP-2 bind to the catalytic domain of MMP-14 and the C-terminal hemopexin-like domain of proMMP-2, forming a trimolecular complex of MMP-14/TIMP-2/proMMP-2 on the cell membranes (see Figure 8-2). Capturing proMMP-2 by the trimolecular complex formation on the cell membranes assists proMMP-2 activation by increasing a local concentration of proMMP-2 and presenting it to the near, noninhibited MMP-14.[73] Dimerization of MMP-14 through an interaction of the C-terminal hemopexin-like domain is required for the efficient activation of proMMP-2 by MMP-14.[82] MMP-14 initiates proMMP-2 activation by attacking a part of the proMMP-2 propeptide, and another already activated MMP-2 finally activates proMMP-2 by removing a residual portion of the propeptide. Integrins such as $\alpha v\beta 3$ may be involved in the process as an additional receptor for transferring activated MMP-2 to the integrin.[83] MMP-15, 16, or 24 can activate proMMP-2 in the transfected cells,[37-39,84]

but the activation mechanisms for these MT-MMPs are not well understood. MMP-14 also activates proMMP-13 on the cell surface,[85] and this activation does not seem to require TIMP-2.[86]

Pericellular activation of proMMP-7 has been discovered through screening proMMP-7–binding molecules by a yeast two-hybrid system.[87] ProMMP-7 is captured on the cell membrane by the interaction of the proMMP-7 propeptide with the C-terminal extra-cellular loop of CD151, a member of the transmembrane 4 superfamily, and is pericellularly activated.[87] This new pericellular activation of proMMP-7 requires a substrate of MMP-7. Since α chains of α3β1 and α6β4 integrins interact with CD151, these integrins also may be involved in the activation. Although the precise molecular mechanisms of this pericellular activation system, including the activator itself, are still unclear, proMMP-7 and CD151 are overexpressed in osteoarthritic chondrocytes, and proMMP-7 is activated by the interaction with CD151 in articular cartilage of osteoarthritis.[88]

### Pericellular Docking and Recycling of Matrix Metalloproteinases

Discovery of membrane-anchored MMPs (i.e., MT1-, MT2-, MT3-, MT4-, MT5-, and MT6-MMPs and MMP-23) and subsequent studies of proMMP-2 activation by MMP-14 (MT1-MMP) have established pericellular actions of these MMPs, including MMP-2. Secreted MMPs were originally thought to digest ECM macromolecules extra-cellularly after the activation, but more recent studies have indicated the possibility that they also may function on the cell membranes through their cell surface docking.[74,89,90] Besides the proMMP-2/TIMP-2/MMP-14 system, several secreted MMPs are reported to interact with cell membrane proteins, which include α2 chains of integrin α2β1 and CD147 (EMMPRIN) for MMP-1,[91] αvβ3 integrin and caveolin-1 for MMP-2,[83,92] CD44 heparan sulfate proteoglycan and cholesterol sulfate on the cell surface for MMP-7,[93,94] and CD44 for MMP-9.[95] Because all of these cell membrane proteins bind to active forms of the MMPs, their proteolytic activities can be used on the cell surfaces to digest ECM and non-ECM molecules located close to the cell membranes. On the other hand, MT1-MMP and secreted MMPs such as MMP-2 and 9 are internalized via clathrin-coated pits for recycling and low-density lipoprotein receptor–related protein-1 for catabolism in lysosomes, respectively. Pericellular docking and recycling indicate that pericellular activities of the MMPs are dynamically regulated. The importance of pericellular regulation of MT-MMPs and secreted MMPs by pericellular docking and clearing via internalization in arthritic tissues should be shown by further work.

## JOINT DESTRUCTION AND PROTEINASES

### Degradation of Extra-cellular Matrix in Articular Cartilage

In most joint diseases such as rheumatoid arthritis and osteoarthritis, articular cartilage is the major target tissue for destruction, and excessive degradation of cartilage ECM by proteinases is a key process in the destruction. Histologically, depletion of proteoglycans from articular cartilage (degradation of proteoglycans) is a common initial change in these joint diseases, and subsequently collagen fibrils are degraded, leading to fibrillation and laceration secondary to destruction of the arcade structures of collagen fibrils in the articular cartilage (Figure 8-3).

Aggrecan, a major proteoglycan in cartilage, is susceptible to degradation by many proteinases, including MMPs, ADAMTS species, neutrophil elastase, cathepsin G, and cathepsin B. Because most of these proteinases mainly cleave the peptide bonds located in the interglobular G1-G2 domain, the major glycosaminoglycan-bearing aggrecan fragments are detached from the hyaluronan attachment site (G1 domain) after the cleavage and released from the cartilage matrix. The two major aggrecan fragments with the N-terminal sequences starting from $Phe^{342}$ or $Ala^{374}$ of the core protein are detected in joint fluids from patients with various inflammatory arthritides and osteoarthritis.[96] Many MMPs, including MMP-1, 2, 3, 7, 8, 9, 13, and 14, preferentially cleave the $Asn^{341}$-$Phe^{342}$ bond (the MMP site).[21] ADAMTS species including ADAMTS1,[49] 4,[50] 5,[51] 8, 9, and 15 clip the $Glu^{373}$-$Ala^{374}$ bond (the aggrecanase site), in addition to other sites in the G2-G3 domains. Members of the MMP and ADAMTS families may play central roles in the aggrecan degradation in arthritides. On the basis of the data that synthetic MMP inhibitors do not efficiently prevent aggrecan degradation in articular cartilage, the focus has been on ADAMTS species as

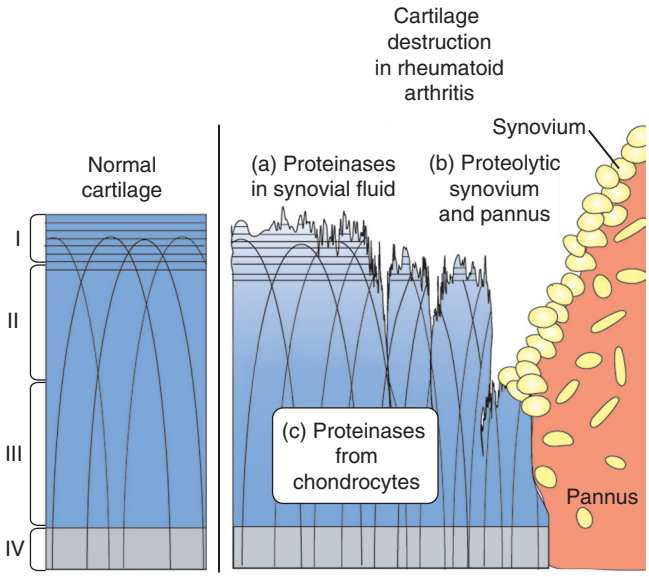



**Figure 8-3** Structure of normal articular cartilage and its destruction by proteinases in rheumatoid arthritis. Normal articular cartilage is divided into four zones (I, II, III, and IV). Collagen fibrils are aligned parallel to the articular surface in the superficial zone, and they blend with radial fibers and form plates or sheets that sweep vertically through the middle zone, showing arcade structures that originate from the calcified zone (IV). In persons with rheumatoid arthritis, synovial tissue cells and inflammatory cells produce various proteinases, most of which are secreted into synovial fluid. These proteinases in synovial fluid attack the surface of the articular cartilage from the synovial fluid *(a)*. At the periphery of the articular surface, proteolytic synovium degrades cartilage by direct contact, and pannus tissue covers and invades cartilage *(b)*. Chondrocytes, which secrete proteinases by stimulation with various cytokines and growth factors, also are implicated in cartilage destruction *(c)*.

aggrecan-degrading proteinases in arthritides. It is not settled, however, whether MMP and ADAMTS species play differential or complementary roles in the aggrecan degradation. Decorin, a leucine-rich repeat proteoglycan, also is digested by MMP-2, 3, and 7,[97] as well as ADAMTS4.[54] Information is limited about the proteinases responsible for the degradation of other proteoglycans, including fibromodulin, lumican, biglycan, PRELP (arginine-rich end leucine-rich repeat protein), chondroadherin, and syndecan present in articular cartilage, although fibromodulin is cleaved by C-terminus–truncated ADAMTS4.[54]

Fibrillar interstitial collagens (i.e., types I, II, and III collagens) are extremely resistant to most proteinases because of their triple-helical structures. In general, classic collagenases including MMP-1, 8, and 13 are responsible for the degradation of these collagens. Among them, MMP-13 may be most important for the degradation of cartilage collagen because it preferentially digests type II collagen.[11] MMP-14 degrades types I, II, and III collagens.[40] Type III collagen also is susceptible to degradation by MMP-3,[80] 9,[14] 16,[42] and neutrophil elastase. Cleavage of the telopeptides by the telopeptidase activity of MMP-3,[19] MMP-9,[14] neutrophil elastase, cathepsin G, and cysteine proteinase cathepsins is important for depolymerization of the cross-linked collagens. When the collagen molecules are cleaved, the helical structures are unwound at 37° C (i.e., body temperature) and become denatured into gelatins, which are digested into smaller peptides by gelatinases (MMP-2 and MMP-9)[13,14] and other nonspecific tissue proteinases. Type V collagen is readily digested by MMP-2[13] and MMP-9.[14] In contrast, type VI collagen is resistant to most MMPs, including MMP-1, 2, 3, 7, 9, 10, and 14, but is susceptible to neutrophil elastase, cathepsin G, chymase, and tryptase.[7] Type IX collagen is degraded by MMP-3.[19] Type X collagen is susceptible to MMP-1 and MMP-2, and type XI collagen is degraded by MMP-2.

Fibronectin is degraded by many MMPs, including MMP-2, 3, 7, 10, 11, 13-16, 19, and other serine proteinases. Link protein also is susceptible to many proteinases such as MMP-1, 2, 3, 7-10, neutrophil elastase, and cathepsin G. COMP is digested by MMP-19 and MMP-20.[35] Proteinases capable of digesting cartilage matrix protein and cartilage intermediate layer protein are unknown, however.

## Cartilage Destruction by Proteinases in Rheumatoid Arthritis

Articular cartilage in persons with rheumatoid arthritis is destroyed by proteinases in three pathways: (1) destruction from surfaces of articular cartilage by proteinases present in synovial fluid, (2) destruction through direct contact of proteolytic synovium or pannus tissue or both to articular cartilage, and (3) intrinsic destruction by proteinases derived from chondrocytes (see Figure 8-3).

Rheumatoid arthritis is characterized by chronic proliferative synovitis, which shows hyperplasia of the synovial lining cells, inflammatory cell infiltration, and angiogenesis in the sublining cell layer. Hyperplastic synovial lining cells overproduce MMP-1, 3, 9, 14, ADAMTS4, TIMP-1, and TIMP-3.[98-100] Sublining fibroblasts produce MMP-2 and TIMP-2. Polymorphonuclear leukocytes infiltrated in the synovium and joint cavity contain MMP-8 in the specific granules and MMP-9 and neutrophil elastase, cathepsin G, and proteinase 3 in the azurophil granules. They are released from cells during phagocytosis of tissue debris and immune complexes. Other inflammatory cells in the synovium include macrophages, lymphocytes, and mast cells. Macrophages produce MMP-1, MMP-9, TIMP-1, and TIMP-2, and uPA and cathepsins B, L, and D also are secreted from activated macrophages. T lymphocytes in the synovium synthesize MMP-9. Chymase and tryptase are degranulated from mast cells in response to activation by immune complexes. Endothelial cells express many MMPs, including MMP-1, 2, 3, 9, and 14; tPA; and their inhibitors. These proteinases may be involved in tissue remodeling during angiogenesis in the synovium instead of cartilage destruction.

All of these proteinases and inhibitors produced by synovial tissue cells and inflammatory cells seem to be secreted into the synovial fluid and attack the surfaces of articular cartilage when active proteinases overwhelm inhibitors. MMP-1, 2, 3, 8, 9, TIMP-1, and TIMP-2 are detectable in rheumatoid synovial fluids, and the molar ratios of MMPs to TIMPs correlate with metalloproteinase activity, which is detectable in rheumatoid synovial fluids.[101] MMPs are thought to be in favor of proteinase in rheumatoid synovial fluids. The cartilage in the central part of the articular surface shows surface irregularity (fibrillation) and proteoglycan depletion without being covered by pannus tissue even in the early stage of rheumatoid arthritis (Figure 8-4). This cartilage degradation may be ascribed to the proteolytic damage by the action of the proteinases present in synovial fluid (see Figure 8-3). Among the MMPs detected in synovial fluids, MMP-3 has the highest concentration and serum MMP-3 can be used to monitor the activity of rheumatoid synovitis.[102-104]

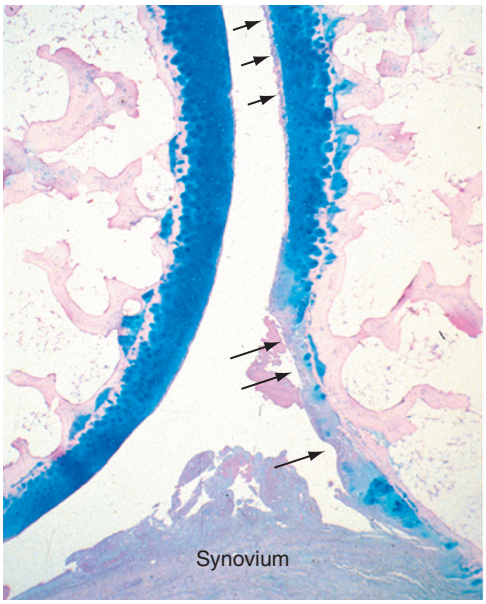



**Figure 8-4** Destruction of articular cartilage of the proximal interphalangeal joint obtained by autopsy from a patient with early-stage rheumatoid arthritis. Articular cartilage shows fibrillation and proteoglycan depletion at the central part of the articular surface *(short arrows)* and marked destruction at the marginal area, which contacts synovium *(long arrows)*. Alcian blue staining was performed for proteoglycans.

Articular cartilage at the margins of the articular surface, to which synovial tissue can directly attach, is progressively degraded even in the early stage (see Figure 8-4). Because rheumatoid synovial lining cells exhibit strong gelatinolytic activity, which is probably generated through activation of proMMP-2 by the action of MMP-14,[98] direct contact of the proteolytically active synovial tissue to articular cartilage is a destruction pathway of the cartilage (see Figure 8-3). Although rheumatoid synovium contains high concentrations of active proteinases, the synovium can avoid the attack by MMPs because type VI collagen, a major component in the lining cell layer,[105] is resistant to the activities of MMP-1, 2, 3, and 14.[40,105] Pannus tissue is a connective tissue growing from the marginal transitional zone on the surface of the partially degraded articular cartilage. It is unknown whether pannus tissue formation is a sign of active destruction or repair of the articular cartilage, but the balance of data favors the former. Immunolocalization of MMP-1 and phagocytosis of collagen fibrils by pannus cells at sites of pannus-cartilage junction may suggest a role of the tissue in the cartilage destruction.

In addition to the extrinsic pathway for the cartilage damage, cartilage may be destroyed by proteinases derived from chondrocytes (see Figure 8-3). Chondrocytes under stimulation are capable of expressing various proteinases, including MMP-1, 2, 3, 7, 9, 13, 14, 16, ADAM9, 10, 17, ADAMTS4, and other classes of proteinases. In rheumatoid arthritic cartilage, MMP-1, 2, 3, 7, 9, 13, and14 are expressed in the chondrocytes located in the proteoglycan-depleted zone. When large areas of the cartilage surface are ulcerated after degradation of cartilage ECM, death of chondrocytes occurs, leading to further progressive cartilage destruction.

Based on the data that MMPs play a central role in degradation of ECM molecules in rheumatoid arthritis and cancers, a number of MMP inhibitors were developed and used in clinical trials.[106-109] However, all of them failed because of no major clinical benefit and/or adverse effects. MMP inhibitors for the treatment of patients with rheumatoid arthritis and osteoarthritis were withdrawn from the phase III trials, mainly because of a lack of efficacy.[107] In clinical trials for patients with cancer, most of the inhibitors had no definite benefit, and prolonged or high-dose treatment with broad-spectrum MMP inhibitors caused unexpected symptoms in the musculoskeletal system, such as inflammatory polyarthritis,[110] which are called musculoskeletal syndrome.[111] The failure occurred mainly because the trials in the early stage were carried out with active site-directed broad-spectrum inhibitors, which cross-react with other MMPs and ADAMs/ADAMTSs. Thus one future strategy may be development of allosteric or exosite inhibitors that would exhibit higher specificity than conventional inhibitors targeting the active site.[109] However, another favorable type of inhibitor may be human antibodies to MMPs and/or ADAMTS4/5, which would be highly specific to the target proteinase.

## Bone Resorption in Rheumatoid Arthritis

Bone is resorbed by osteoclasts even in the early stage of rheumatoid arthritis. This phenomenon is commonly observed at the bare zone, where pannus-like granulation tissue invades the bone marrow and destroys subchondral bone. Activated osteoclasts attach to only mineralized bone matrix, and this cell-matrix contact is carried out between αvβ3 integrin of osteoclasts and Arg-Gly-Asp (RGD) sequence of osteopontin in the matrix. ECM degradation of the mineralized bone is possible only after demineralization of the bone matrix by proton secreted by osteoclasts because proteinases cannot permeate the matrix components in the mineralized tissues. Matrix degradation by osteoclasts is performed in the subosteoclastic compartments, which have acidic (pH 4 to 5) and hypercalcemic (40 to 50 mM $Ca^{2+}$) conditions.[112]

The major component of the ECM proteins in mature bone is insoluble, highly cross-linked type I collagen, although type III and V collagens also are present. Other minor components in bone matrix are leucine-rich repeat proteoglycans (decorin and biglycan) and glycoproteins such as osteopontin, osteonectin (SPARC), osteocalcin (bone Gla-protein), and thrombospondin. Among collagenolytic cysteine proteinases, including cathepsins B, K, L, and S, cathepsin K is the most important for bone resorption because of its collagenolytic activity with a broad pH optimum and selective expression in osteoclasts and giant cells of giant cell tumors. Mutations of human cathepsin K are responsible for pyknodysostosis, an autosomal recessive osteochondrodysplasia characterized by osteopetrosis and short stature.[113] *Cathepsin K*–deficient mice have a similar phenotype. Despite the importance of cathepsin K in bone resorption, osteoclastic bone resorption cannot be completely inhibited by cysteine proteinase inhibitors; it is inhibited to a similar degree by MMP inhibitors.[112] MMP-9 is highly expressed in osteoclasts in normal and rheumatoid bones[114] and giant cells of giant cell tumors. MMP-9 has telopeptidase activity against soluble and insoluble type I collagen and strong gelatinolytic activity.[14,114] ProMMP-9 is activated by acid exposure, and when activated, it is proteolytically active under acidic and hypercalcemic conditions.[114] *MMP-9*–deficient mice show a transient disturbance of growth plate development. Cathepsin K and MMP-9 may be involved in bone resorption in persons with rheumatoid arthritis. Although MMP-14 is reportedly expressed in osteoclasts in persons with rheumatoid arthritis,[115] evidence of the direct involvement in osteoclastic bone resorption is limited. MMP-7 may be involved in the solubilization of receptor activator of nuclear factor-κB ligand (RANKL) in a murine prostate cancer–induced osteolysis model,[116] but no data are available regarding the osteoclastic bone resorption in rheumatoid arthritis.

For treatment of osteoporosis and/or destructive bone diseases, bisphosphonates that reduce bone turnover by inhibiting bone-resorbing activity of mature osteoclasts are commonly utilized. Denosumab, a human monoclonal antibody against RANKL, has been approved by the U.S. Food and Drug Administration and used for treatment of osteoporosis in persons with rheumatoid arthritis. Phase II and III clinical trials of cathepsin K inhibitors are currently underway for treatment of post-menopausal osteoporosis. Because the reduction of osteoclast-stimulated bone formation is less compared with anti–bone resorptive drugs such as bisphosphonates,[117] these inhibitors might be useful for treatment of osteoporosis in persons with rheumatoid arthritis.

## Cartilage Destruction by Proteinases in Osteoarthritis

Prominent synovial inflammatory changes can be absent during early stages of osteoarthritis, but elevated production of enzymes by the chondrocytes themselves contributes to the breakdown of cartilage. Many MMPs, including MMP-1,[118] 2,[78,119] 3,[120] 7,[121] 8,[118] 9,[119] 13,[10,118] and 14,[78] are expressed in osteoarthritic cartilage. MMP-3, 7, and 14 are immunolocalized to chondrocytes in the proteoglycan-depleted zone of osteoarthritic cartilage, and the levels of their staining correlate directly with the histologic Mankin score.[78,120,121] Among the classic collagenolytic MMPs (i.e., MMP-1, 8, and 13), MMP-13 may be most important for degradation of the cartilage collagen because of preferential digestion of type II collagen over type I and III collagens.[10,11] Because MMP-14 efficiently activates proMMP-2 within the osteoarthritic cartilage,[78] and because it can activate proMMP-13,[86] MMP-14 may play a key role in cartilage degradation through activation of proMMP-2 and proMMP-13 and its own proteolytic activity against cartilage ECM. When considering intermolecular activation cascade for proMMPs by active MMPs, another key MMP in osteoarthritic cartilage tissue is MMP-3, which can activate proMMP-1, 7, 8, 9, and 13. MMP-3 not only digests many cartilage ECM components such as aggrecan, type IX collagen, and link protein but also activates those proMMPs. ProMMP-7 is activated pericellularly after being anchored via the complex formation with CD151 in osteoarthritic chondrocytes.[88] Because CD151 immunoreactivity directly correlates with the Mankin score and the degree of chondrocyte cloning and MMP-7 not only digests cartilage ECM but also sheds precursors of growth factors such as HB-EGF, MMP-7 may be involved in cartilage destruction, chondrocyte cloning, or both.[88]

Other proteinases implicated in cartilage destruction in osteoarthritis are members of the ADAMTS family. Chondrocytes are known to express ADAMTS1, 4, and 5, the latter two of which most efficiently degrade aggrecan. On the basis of the data that the cartilage destruction in experimental osteoarthritis is prevented in *ADAMTS5* knockout mice, but not in *ADAMTS4* knockout mice, ADAMTS5 is considered to play a key role in the cartilage destruction in mice.[122] In human osteoarthritic cartilage, however, ADAMTS4 is overexpressed, whereas ADAMTS5 expression is constitutive.[123] The expression of *ADAMTS4*, but not *ADAMTS5*, is stimulated with IL-1 and TNF in human osteoarthritic chondrocytes.[124] The differential role of ADAMTS4 and ADAMTS5 in human osteoarthritic cartilage remains elusive. Members of the ADAM family (i.e., *ADAM10*, *12*, *15*, and *17*) are expressed in osteoarthritic cartilage. ADAM12 may be involved in cartilage repair because ADAM12 is overexpressed by clustered chondrocytes and plays a role in chondrocyte proliferation by promoting the availability of IGF-I through cleavage of IGF-BP-5.[125] On the basis of data from *ADAM15* knockout mice, ADAM15 is considered to have a chondroprotective role in cartilage.[126] RECK is overexpressed by clustered chondrocytes in human osteoarthritic cartilage and may play a role in chondrocyte cloning (cluster formation) through suppression of chondrocyte migration and promotion of chondrocyte proliferation.[127]

The references for this chapter can also be found on ExpertConsult.com.

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PART 2 CELLS INVOLVED IN AUTOIMMUNE DISEASES AND INFLAMMATION

CHAPTER 9



# Dendritic Cells: General Overview and Role in Autoimmunity

Antoine Tanne • Nina Bhardwaj

## KEY POINTS

- Dendritic cells (DCs), together with monocytes and macrophages, comprise the mononuclear phagocyte system.
- DCs are professional antigen-presenting cells. They are abundant at body surfaces and within tissues, where they sense and sample the environment for self- and non–self-antigens.
- Three major subsets of DCs—plasmacytoid DCs, conventional DCs, and monocyte-derived DCs—are characterized by distinct origins, receptors, and functions.
- Upon antigen capture, DCs undergo a process of "maturation" exemplified by enhanced antigen processing, induction of major histocompatibility complex molecules, co-stimulatory molecules (CD80/86), and cytokine production. DCs migrate to primary and secondary lymphoid organs, where they present processed antigens to naïve T cells to induce immunity or tolerance.
- Mature DCs acquire the ability to differentiate naïve T cells into T helper (Th) 1, Th2, or Th17 cells, T follicular helper cells, or regulatory T cells. Maturing DCs also express cytokines that enable the activation of B cells and natural killer cells and promote the recruitment of other innate immune effector cells.
- DCs are innate immune phagocytic cells that sense the environment through membrane and cytosolic pattern recognition receptors. DCs contribute to the maintenance of tolerance in the thymus and in the periphery, although their functions are only partially understood.
- There is a fine equilibrium in DC regulation between tolerogenic and inflammatory states, which can contribute to autoimmune diseases or to tolerance of tumors.
- DCs, as one the main regulators of the immune response, can either be targeted or used as adjuvants to contain immunopathologies such as autoimmune diseases and cancers, respectively.

The first morphologic description of dendritic cells (DCs) was provided in 1973 by Drs. Ralph Steinmann and Zanvil Cohn.[1] Five years later, Dr. Steinmann proposed that DCs "will prove to be a critical accessory cell required in the generation of many immune responses."[2] Although not generally appreciated by the broader scientific community until many years later, DCs remain one of the most exciting discoveries in immunology in the 20th century. In 2011, jointly with Drs. Bruce Beutler and Jules Hofmann, who contributed to the discovery and the characterization of the role of Toll-like receptors (TLRs) in the regulation of innate immunity, Dr. Steinman received the Nobel Prize in Physiology or Medicine for the discovery of DCs and their role in the regulation of innate and adaptive immunology.

It is now well accepted that DCs are central players in immunity and in the maintenance of tolerance. DCs represent a sparsely distributed population of bone marrow–derived mononuclear cells that are found in most tissues of the body. They are professional antigen-presenting cells (APCs) that, in their immature state, have the ability to recognize and capture self- and non–self-antigens through expression of several pattern recognition receptors (PRRs).[3] DCs are activated upon encountering pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs), which leads to a process of "maturation" that involves changes in the DC phenotype, antigen acquisition capacity, migration, and an ability to traffic to draining lymph nodes, where they prime cellular and humoral adaptive immune responses.[4] Upon maturation, DCs will process antigens (self and nonself) and then present them to antigen-specific adaptive immune cells (T cells and B cells) to induce immune responses to contain diseases such as cancer or infections, but also to maintain tolerance to self-antigens. Although most autoimmune cells are deleted in the thymus through a process of negative selection, others must be tolerized through active and sustained mechanisms.

This chapter summarizes our current understanding of the functions of DCs, their potential role in tolerance and autoimmunity, and their potential therapeutic use to contain immunopathologies. See Figure 9-1.

## DENDRITIC CELL SUBSETS

DCs have a broad tropism and have been identified in almost every tissue, including skin, intestine, heart, and primary and secondary lymphoid organs, as well as more recently in the brain parenchyma in mice.[5] All DCs share the key ability to present antigens to T cells; however, DC subsets differ considerably in ontogeny, tissue localization, cytokine profile, and immunologic functions.[6,7] Despite the identification and characterization of many different DC subsets in rodent and human tissue, the absence of specific

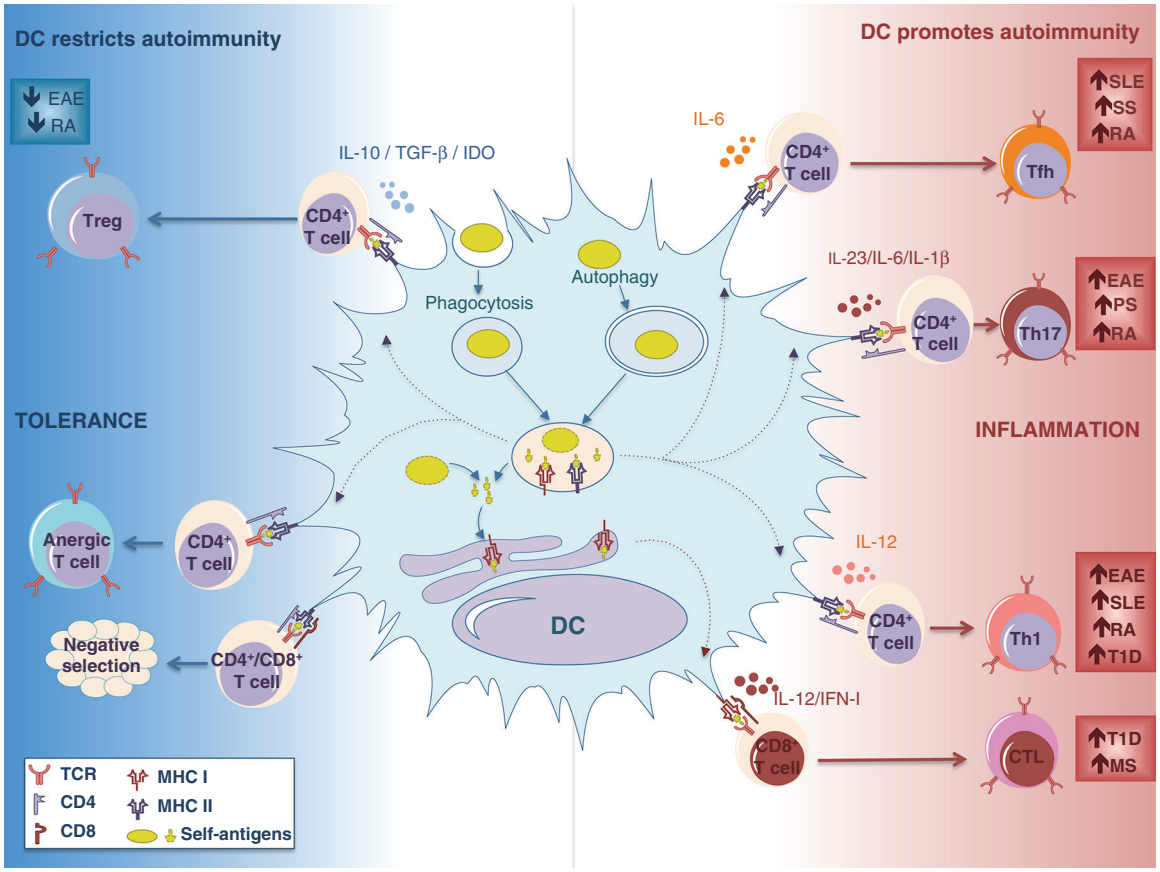



**Figure 9-1** Dendritic cells (DCs) play key roles in the regulation of tolerance and immunogenic response to autoantigens. DCs process autoantigens through phagocytosis and autophagy and present them on class I major histocompatibility complex (MHC I) or class II MHC (MHC II) molecules to CD4 T helper cells or CD8 cytotoxic T cells through processes including cross-presentation. DC-mediated presentation of self-antigen might promote or inhibit autoimmune responses, depending on the inflammatory context and the expression of cell-intrinsic immune regulators such as immune check points. The left part of the figure shows the presentation of self-antigens to T cells in the context of inhibitory factors, such as transforming growth factor–β (TGF-β), interleukin (IL)-10, or indoleamine-2,3-dioxygenase (IDO), which can promote their development into regulatory T (Treg) cells. Alternatively, DCs can induce T cell tolerance through anergy and can play a role in the negative selection of T cells in the thymus. In contrast, if DCs take up and present self-antigens to T cells in the context of pro-inflammatory mediators (such as IL-6, IL-12, IL-23, and interferon [IFN]-I), they can promote the development of an immunogenic response mediated by self-reactive effector $CD4^+$ T cells and cytotoxic T lymphocytes (CTLs). These self-reactive T cells can contribute to pathologic autoimmune responses, such as experimental autoimmune encephalomyelitis (EAE) in mice, multiple sclerosis (MS), Sjögren's syndrome (SS), systemic lupus erythematous (SLE), psoriasis (PS), rheumatoid arthritis (RA), and type 1 diabetes (T1D). IFN-I, Type I interferon; TCR, T cell receptor; Tfh, T follicular helper; Th, T helper. *(Modified from Ganguly D, Haak S, Sisirak V, Reizis B: The role of dendritic cells in autoimmunity.* Nat Rev Immunol *13:566–577, 2013.)*

DC precursors in bone marrow, as well as the absence of specific distinguishing phenotypic markers, has generated some skepticism about the relevance or existence of a DC lineage. However, recent studies infer the existence of such a lineage through the identification of specific transcription factors and cytokines that regulate DC development[8] and the isolation of DC progenitors that specifically give rise to DCs and no other immune cells.[6,7] Unlike B cells and T cells, which can be recognized by expression of surface immunoglobulin or T cell receptors (TCRs), respectively, DC subsets are heterogeneous based on their surface markers, although almost all DCs express class II major histocompatibility complex (MHC) antigens and different levels of the integrin αX (ITGAX), also known as CD11c.[6,7]

DCs are commonly divided into two main subsets: the conventional DCs (cDCs) and nonclassical DCs, which include plasmacytoid DCs (pDCs), monocyte-derived DCs (moDCs), and inflammatory monocyte–derived DCs (infDCs). This division of DCs into cDC, pDC, moDC, and infDC subsets likely presents an oversimplified view of DC heterogeneity. The heterogeneous nature of DCs presents a challenge for identifying developmental lineages; however, it is probably a reflection of the specialized functional role of DC subsets in different tissues and antigenic challenges, such as inflammation versus tolerogenic conditions.

## Conventional Dendritic Cells

Conventional DCs, or myeloid DCs, herein referred to as cDCs, comprise different subsets based on location, function, and phenotype. cDCs are APCs with high phagocytic activity as immature cells and high antigen presentation as mature cells, with the ability to secrete large quantities of cytokines. They have strongly evolved to constantly sense and respond to their environment and to appropriately interact with T cells. cDCs express myeloid markers (CD11c, CD33, and CD13) and high levels of class I MHC, class II MHC, and co-stimulatory molecules (CD80 and CD86).

cDCs are specialized in antigen processing; contrary to other phagocytes such as macrophages, which rapidly degrade antigens after their uptake, DCs process their cargo, allowing them to preserve and control the presentation of peptide epitopes on their MHC molecules. cDCs, in contrast to most other immune and nonimmune cells, can present endogenous and exogenous antigens in the context of both class I and II MHC. Presentation by class I MHC molecules primes $CD8^+$ T cells, whereas presentation by class II MHC molecules triggers $CD4^+$ T cells. Upon their phagocytosis, exogenous antigens are taken up into endosomal compartments, where proteases break down protein antigens into peptides for presentation on class II MHC molecules by DCs. Typically, antigenic peptides presented on class I MHC molecules are synthesized within the cytoplasm, where they are further processed before being shuttled to the endoplasmic reticulum to engage newly synthesized class I MHC molecules to then be exocytosed. The noncanonical presentation of exogenous noncytosolic antigens on class I MHC relies on vacuolar and cytosolic "cross-presentation pathways."[9] DCs can also present endogenous cytosolic antigens on class II MHC through autophagy, a process that allows for recycling of cytosolic compartment constituents through autophagosomes.[10]

cDCs are present in the circulatory system and can be found in virtually every peripheral tissue, as well as in lymphoid organs. To ensure the stimulation of naïve T cells, cDCs express chemokine receptors, such as CCR7. This expression allows directional migration toward T cell zones either within their respective lymphoid organs of residence or toward remote tissue-draining lymph nodes via afferent lymphatic and endothelial venules. Finally, cDCs are specialized in T cell cross-talk and are essential to activate naïve T cell and memory T cell responses, in addition to regulating follicular helper T cell (Tfh) responses, the latter being required for B cell differentiation. In lymph nodes, cDCs can regulate T cell responses both in steady state conditions (when they are typically not fully mature to induce tolerance or anergy) and during infection (after maturation, when they can prime a host T cell response). Depending on their maturation state and the profile of cytokines they secrete upon stimulation, they will prime naïve T cells toward various types of T helper (Th) cells, including Th1, Th2, Th17, Th22, Th9, and T regulatory (Treg) cells, which regulate specific aspects of the immune response to the antigen.[6,7,11,12]

cDCs can be subcategorized into three components according to their localization: (1) peripheral tissue resident, such as skin; (2) circulating blood cDCs; and (3) secondary lymphoid organ resident. Although many tissues have been studied in murine models, the skin remains the best described tissue in humans and will be our model to introduce the different DC subsets. Understanding of DC subsets is critical not only in any future development of vaccines or immunotherapeutic treatments, but also in the understanding of autoimmune disease pathogenesis, because each subset induces distinct responses.

## Tissue Resident cDCs

The skin is composed of two layers, the epidermis and the dermis, separated by a basal membrane. In healthy skin, three main DC subsets can be identified: The epidermis contains only Langerhans cells (LCs), whereas the dermis is highly vascularized and contains both tissue and circulating moDCs ($CD14^+$) and cDC subsets ($CD14^+$, $CD1a^+$, $CD1c^+$, and $CD141^+$). Upon skin infection or inflammation, nonconventional DCs (pDCs and infDCs) are locally recruited.

**Langerhans Cells.** LCs are self-renewing epidermal myeloid cells located in the epidermis, where they form a network between the keratinocytes. LCs are also observed in squamous stratified epithelia, such as the bronchus, and in oral and genital mucous membranes. They can migrate and mature into DCs. Compared to cDCs, LCs express shared common markers, such as CD11c, CD32, CD45, FcεR1, including CD1a and the C-type lectin receptor (CLR) langerin (CD207). Langerin delivers antigen through receptor-mediated endocytosis to Birbeck granules; these granules are structures connected to the endosomal network that are implicated in canonical and noncanonical antigen processing and presentation. In the steady state, similar to tissue resident macrophages, LCs are continuously replaced from a resident precursor pool, which is established early in development.[13] LC maintenance is dependent on factors produced by keratinocytes, including interleukin (IL)-34 in mice.[14,15] In inflammatory conditions, however, LCs appear to be repopulated by blood precursors.[12] LCs also present antigens in a CD1a-restricted manner.[16] The CD1 family of molecules is associated with presentation of glycolipids to specific subsets of T cells, such as the Dendritic epithelial T Cells (DETC) in the epidermis, which express γδ-TCR. CD1a antigen presentation is independent of endosomal localization and acidification.[17] LCs constitutively express E-cadherin (CD324), a homotypic adhesion molecule that anchors LCs to neighboring keratinocytes[12] and is released during LC maturation. Under steady-state conditions, immature LCs migrate to the lymph node and induce T cell tolerance.[18] In mice, LCs may induce the differentiation of Th1, Th2, Th17, and even Th22 cells.[19,20]

**Dermal Dendritic Cells.** In the steady state, dermal DCs are composed of $CD14^+$ DCs, a less characterized subset of $CD1a^+$ DCs, and two subsets of blood-derived circulating cDCs ($CD1c^+$ and $CD141^+$). Upon inflammation, infDCs and pDCs also accumulate locally.

**$CD14^+$ Dendritic Cells.** The $CD14^+$ dermal DCs were initially described as "interstitial/dermal type" DCs. They have an intermediate phenotype between DCs and monocytes/macrophages. They express macrophage and monocyte markers, such as CD163, CD11b (ITGAM), CXCR3CR1, and a broad spectrum of CLRs, including DEC-205 (CD205), LOX-1, CLEC6, DCIR (CLEC4a), DECTIN-1 (CLEC7a), and DC-SIGN (CD209).[21,22] $CD14^+$ dermal DCs have been shown to have a variety of functions, depending on the context in which they are evaluated. They can regulate B cell responses by differentiation of CD40-activated B cells into immunoglobulin (Ig) M–producing plasma cells through cytokine secretion (IL-12 and IL-6) and through direct cell-cell interaction.[23] $CD14^+$ DCs also promote antibody responses through skewing of $CD4^+$ T cells into Tfh cells, which induce naïve B cells to switch isotype and secrete large quantities of immunoglobulin.[24] $CD14^+$ DCs can skew $CD8^+$ T cells into TC2 cells in

a manner dependent upon the expression of Ig-like transcript inhibitory receptors ILT-2 and ILT-4. Finally, $CD14^+$ DCs, in the steady state, can induce Treg differentiation and promote tolerance.[25] Similar types of DCs can be generated in vitro from monocytes differentiated with IL-10 and vitamin D3, making it possible to better define the functions of this DC subset.[26]

**$CD1a^+$ Dermal DCs.** $CD1a^+$ dermal DCs express blood DC antigen-1 (BDCA-1 or CD1c), are present in the upper layer of the dermis, and can be found as $CD1a^+$ migratory DCs in the draining lymph nodes.[27] Phenotypically, they are similar to LCs but lack expression of langerin and E-cadherin. Upon stimulation, $CD1a^+$ DCs produce IL-15 and few pro-inflammatory cytokines.[28] They can induce Th2 responses and cytotoxic T lymphocyte (CTL) responses, although with less potency than LCs or $CD14^+$ DCs.[27]

## Circulating Dendritic Cells

Blood contains both cDCs and pDCs. cDCs can be found in healthy peripheral tissue, and when inflammation occurs, they are recruited into inflammatory sites, after which they migrate into secondary lymphoid tissue through afferent lymph. The physiologic roles of circulating blood cDCs include blood and tissue surveillance for pathogens and regulation of tolerance. cDCs in blood can be further subdivided into $CD1c^+$ ($BDCA\text{-}1^+$), $CD16^+$, and $BDCA\text{-}3^+$ populations, which differentiate from blood precursors. In addition, monocytes have been shown to differentiate into DCs, which can accumulate locally with pDCs in inflamed sites.

**$CD1c^+$/$BDCA\text{-}1^+$ Dendritic Cells.** $CD1c^+$ DCs originate from bone marrow, circulate in the blood, and migrate to peripheral tissues and secondary lymphoid organs (e.g., spleen and tonsil) as resting interstitial DCs.[6] They are characterized by the expression of CD1c, CD1a, CD11c, CD13, CD33, CD172 (SIRP1-α), CD45-RO and CD11b (ITGAM). They are sometime referred to the human double positive (hDP) DCs[3,8] and are hypothesized to be similar to mouse double positive (mDP) DCs expressing $CD11b^+$, CD172 ($SIRP1\text{-}\alpha^+$) but which are $CD8\alpha^+$.[8] $CD1c^+$ DCs possess a wide range of receptors such as TLRs and CLRs such as Dectin-1 (CLEC7A) and Dectin-2 (CLEC6A). IL-12p70 is efficiently produced by stimulated blood $CD1c^+$ DCs, whereas skin $CD1c^+$ DCs have a limited ability to produce IL-12 and CXCL10 (Interferon gamma-induced protein 10 [IP10]).[29] $CD1c^+$ DCs can promote Th1 or Th17 responses through IL-12p70 or IL-23 production. Similar to their murine counterparts, they have the capacity to present antigens to $CD4^+$ T cells but have limited ability to cross-present antigens to $CD8^+$ T cells. Whether they induce Treg differentiation and possibly regulate Th2 differentiation, as is done by their murine counterparts, has yet to be definitely established.[30]

**$CD141^{++}$/$BDCA\text{-}3^+$ Dendritic Cells.** CD141 expressing DCs are found in lymph nodes, tonsils, bone marrow, and spleen, where they localize to T cell areas. In the blood, they represent the minor subset of circulating cDCs. They do not express CD14 and have low levels of CD11c and CD11b. Sometimes referred to as human single-positive (hSP) DCs, they are considered to be the counterpart of mouse $CD11b^-$, $CD103^+$, $CD8\alpha^+$ DCs.[31,32] $CD141^{++}$ DCs express high levels of the chemokine receptor XCR1, TLR1, TLR2, TLR3, TLR7, and TLR8, as well as CLRs such as CLEC9A (DNRG-1) and BDCA-3 (CD141). They are characterized by production of IL-12p70 and IFN-β and have a superior capacity to induce Th1 cell responses when compared with $CD1c^+$ DCs. $CD141^{++}$ DCs can recognize and process dead or necrotic cells expressing β-actin via CLEC9A,[33,34] leading to cross-presentation of antigens to $CD8^+$ T cells. Because cross-presentation is essential for eradication of cancers, viruses, and other pathogens, $CD141^{++}$ DCs present a promising target in vaccination strategies. The selective expression of chemokine receptor XCR1 by $CD141^{++}$ DCs may promote their recruitment into sites of inflammation by activated natural killer (NK) cells and $CD8^+$ T cells, which produce XCL1, a ligand for XCR1.[35] Upon stimulation with TLR3 or -7/8 agonists $CD141^{++}$ DCs produce TNF, CXCL10 and high levels of anti-viral cytokine IFN-λ.

## Inflammatory Monocyte-Derived Dendritic Cells

Monocytes, which are precursors to macrophages, can differentiate into DCs (moDCs) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4. They acquire DC morphology, produce inflammatory cytokines, and skew naïve T cell responses in vitro. TLR agonists such as lipopolysaccharide (LPS) or LPS-expressing bacteria can mediate differentiation of monocytes to moDCs.[36] Recently, inflammatory subsets of $CD11c^{++}$, class II $MHC^{++}$ moDCs have been described, including $CD14^+$ $CD1c^+$ human infDCs and inflammatory dendritic epidermal cells (IDEC); $CD14^-$ $CD1c^-$ TNF and INOS producing (TIP) DCs and 6-sulfo LacNAc–expressing (SLAN) DCs. However, those remain hard to segregate from SLAN monocytes, and their actual classification as DCs remains questionable. Immature SLAN DCs express FcγRII (CD16), which allows them to strongly interact with immune complexes (ICs). They tend to accumulate in skin lesions of cutaneous and systemic lupus erythematous (SLE) and in autoimmune diseases in which IC-containing autologous antigens SLAN DCs can contribute to the pathogenesis by stimulating disease activity.[37,38] All inflammatory DCs drive Th17 polarization by production of IL-6, IL-1β, TGF-β, and IL-23 to regulate Th17 skewing.[39]

## Plasmacytoid DCs

pDCs represent a subset of DCs that, in the steady state, resemble plasma cells and do not have dendrites. pDCs differ from cDCs in that they do not express CD11c but express CD4, CD123 (IL-3R), CD303 (CLEC4C/BDCA2), and the neuropilin CD304 (BDCA-4). pDCs are circulating DCs that can also be found in lymphoid tissues and rarely in healthy nonlymphoid tissue. In lymph nodes and tonsils, they accumulate in the T cell zone. When inflammation occurs, pDCs are rapidly recruited to tissue sites. pDCs have both pro-inflammatory and tolerogenic functions,[40] depending on their maturation status. They selectively express TLR7 and TLR9 that detect nucleic acids (single-stranded RNA [ssRNA] and single-stranded DNA [ssDNA]) derived from viruses, bacteria, and dead cells, or within

immune complexes in autoimmune conditions. TLR7 and TLR9 can trigger downstream signaling cascades, resulting in secretion of type I interferons (IFN-I) (IFN-α and IFN-β) and IFN-III (IFN-λ). In the steady state, pDCs have poor capacity to stimulate $CD4^+$ T cells because of their low level of expression of class II MHC. Upon activation, pDCs show similar characteristics to cDCs (e.g., dendritic structure and a high level of expression of class II MHC and co-stimulatory molecules) but remain less efficient at priming naïve T cells.[41] pDCs can prime naïve T cells toward the Th1 profile via production of IFN-α and IL-12 upon viral/TLR stimulation[42] but can also induce Tregs in an indoleamine-2,3,-dioxygenase (IDO)–dependent manner, possibly to contain immune activation, as in SLE or chronic human immunodeficiency virus (HIV) infection.[43] pDCs also seem to be able to cross-present antigens, particularly viral antigens.[44] In contrast to cDCs, pDCs contain "lymphoid" messenger (m)RNA transcripts for pre-T α chains, germline IgK, and Spi-B. pDCs have been shown to accumulate in inflamed tissues (as in early stages of psoriasis and SLE) through their expression of ChemR23 and CXCR4, and their failure to maintain tolerance to self–nucleic acid is linked to those autoimmune diseases.[45]

### Lymphoid Tissue–Associated Dendritic Cells

DC subsets are present in tonsils, lymph nodes, and spleen and comprise migratory cDCs, such as the $CD1c^+$ and $CD141^+$ subsets.[27] Certain specific DC subsets, such as intestinal DCs, can be found in the lamina propria, Peyer's patches, solitary lymphoid tissue, and mesenteric lymph nodes, where they process and sample luminal and self-antigens for presentation. $CD103^+$ DCs reside in intestinal lymph nodes and efficiently induce expression of gut homing receptors CCR9 and α4β7 by responding T cells and B cells through a retinoic acid receptor–dependent mechanism.[46] $CD103^+$ DCs can also induce the generation of Tregs from naïve $CD4^+$ T cells mediated through retinoic acid and TGF-β.[47,48] The function of secondary lymphoid tissue DC subsets in human autoimmune disease is still under investigation. In Crohn's disease, $CD103^+$ DCs maintain their ability to induce CCR9 expression by T cells, similar to healthy control DCs in small intestine,[49] although it remains to be determined whether they maintain their tolerogenic phenotype.

## DENDRITIC CELL DEVELOPMENT

DC development is complex, and although the ontogeny and functional specialization of different human DC subsets are beginning to be unraveled, the translation to human DCs remains complicated.

DC subsets develop from a distinct hematopoietic lineage from other members of the mononuclear phagocyte system (MPS). They are derived from the common DC progenitor (CDP).[50] Whereas the common monocyte progenitors (CMoPs) can generate monocytes, macrophages, and monocyte-derived DCs, the CDP has irreversibly lost the potential to generate monocytes and macrophages and can differentiate only into pDCs and cDCs.[51] Recent fate-mapping studies[52] and cellular bar-coding studies[53] have confirmed that cDCs represent an independent hematopoietic lineage. Once in the periphery, cDCs have a short life span but retain a high degree of plasticity, allowing the generation of multiple professional subsets that regulate either tolerogenic or immunogenic responses based on their final maturation and differentiation. Mutations in the *GATA2* gene generate a complete absence of multilymphoid progenitors (MLPs), which leads to the total absence of cDCs, monocytes, pDCs, and B and NK cells, whereas the granulocyte lineage is not affected.[54]

Human DC ontogeny is less well understood. *IRF8* gene mutations affect the myeloid lineage and lead to defects in cDCs, monocytes, granulocytes, and pDCs that resemble the phenotype induced by the hypomorphic mutation BXH-2 IRF8 in mice.[55,56] In this case, LC development is not affected in mice or human cells. These phenotypes suggest that similar to the mouse, the human cDC lineage develops from a myeloid and lymphoid common progenitor dependent on *GATA2* and then from an *IRF8*-regulated myeloid-dependent progenitor. The lack of DC subset–restricted markers complicates the identification of early human DC precursors in human blood. It has been proposed that human circulating cDCs ($CD1c^+$ and $CD141^+$) are not unipotent and can be considered forms of circulating precursors that can undergo final differentiation and specialization in situ.[6] In contrast, pDCs are unipotent when they leave the bone marrow, because both circulating and lymph node–derived pDCs do not proliferate.[6] Among the human MPS, DCs are distinguished from monocytes and macrophages by their structure, their high levels of class II MHC expression, their ability to migrate, and their ability to prime naïve T cells. The transcriptional profiling of the MPS components has confirmed that resting cDCs ($CD1c^+$ and $CD141^+$) and pDCs cluster together independently of monocytes and macrophages, which strongly suggests that the DC ontogeny model proposed in mice can be translated to humans.[57] On the other hand, moDCs and infDCs are associated with the monocyte lineage. Similarly to tissue macrophages, LCs seem to develop from an independent lineage related to embryonic tissue precursors. Considering lineage ontology, it is therefore questionable whether their classification as DCs is relevant or whether they should rather be classified as a specific type of tissue macrophage.

Recently Guilliams and collaborators[58] proposed a new system of nomenclature for the MPS. Three main developmental pathways have been proposed. The macrophage lineage is related to cells that develop from early embryonic precursors, such as tissue macrophages (e.g., alveolar and peritoneal), microglial cells, Kupffer cells, and LCs. Adult hematopoietic stem cells are then proposed to split into two lineages: the monocytic lineage derived from the cMoP, which give rise to monocytes and monocyte-derived cells, and the DC lineage, which derives from the CDP and differentiates into pre-cDC and pre-pDC. Pre-cDCs give rise to cDC1 dermal and lymphoid $CD141^+$ and to cDC2, which regroups the human lymphoid and dermal $CD1c^+$. Pre-pDCs give rise to pDCs.

### Dendritic Cell Maturation

DC maturation is one of the critical features of DC biology. This complex process relies on the acquisition of key

functional properties, such as environmental sensing, antigen uptake, processing and presentation, migration, and T cell stimulation.[59] The DC process of maturation is related to their environment and is therefore heterogeneous. It confers specific and distinctive functional properties to different DC subsets. It is important to consider DC maturation in the steady state versus upon inflammatory stimulation.

In the steady state, cDCs are in a quiescent state that is characterized by a low surface expression of MHC molecules and co-stimulatory molecules, such as CD80, CD86, and CD40. The program of maturation can be activated upon sterile and nonsterile inflammation and vaccination and to maintain homeostasis. DCs acquire the ability to induce clonal expansion and differentiation of antigen-specific naïve T cells into effector T cells. Immunogenic conditioning of cDCs induces class I MHC and class II MHC and T cell co-stimulatory molecule expression, such as CD80 and CD86. Additional changes include the regulation of endocytic properties, the induction of CCR7-dependent migration to follicular T cell–enriched zones in lymphoid organs, and the polarization of DCs toward a tolerogenic or immunogenic phenotype related to the profile of cytokines that they produce to induce the differentiation of naïve antigen-specific T cells, either Th1, Th2, Th17, Treg, or Tfh, or the activation of other immune cells.[3] Based on their environmental conditioning, DCs regulate the adaptation of T cell polarization to the specific nature of the stimuli. DC maturation relies on the sensing and sampling of the environment. The process requires the integration of multiple autocrine, exocrine, and paracrine signals through the recognition of cytokines, chemokines, growth factors (e.g., GM-CSF), and/or hormones, which can be produced by cells such as the MPS, lymphocytes, epithelial cells, and neurons. Although these stimuli may contribute to an autoimmune process, we will focus on recognition of DAMPs and PAMPs in the maturation of DCs as model stimuli.

## Phagocytosis and Autophagy

Phagocytosis is an active cellular process that allows MPS cells to sense, engulf, internalize, and process endogenous or exogenous antigens present in the extra-cellular environment. Autophagy is the process by which cells can process endogenously or exogenously derived intra-cellular components, including intra-cellular microbes (xenophagy) and organelles such as mitochondria (mitophagy). Autophagy and phagocytosis both contribute to maintaining homeostasis and to containing infection. Both phagosomes and autophagosomes will progressively acidify and terminate their maturation by fusing with lysosome to allow the degradation of their cargo. Depending on the cell type, the stringency of this process varies; the phagolysosomes of the DCs are less degrading than those of the macrophages or neutrophils.[60,61] Autophagy and phagocytosis contribute to inform myeloid cells about the nature of the targets that they are engulfing, including the nature of the cargo, whether it is dead or alive, and whether it is a threat. The integration of multiple signals allows the proper maturation of the MPS and DCs to regulate the local innate and adaptive immune response.

## Homeostatic Maturation

In the steady state, a fraction of cDCs undergo "homeostatic maturation."[62] They upregulate expression of class II MHC molecules at the cell surface and migrate to the lymphoid follicular T cell zones in a CCR7-dependent manner.[63] This homeostatic maturation program confers tolerogenic properties to cDCs.[64,65] As we will describe later, tolerogenic cDCs are thought to present self-antigen and to promote a tolerogenic environment to regulate peripheral tolerance to eradicate or inhibit self-reactive T cells that have escaped central tolerance. Thus steady-state cDC maturation is likely to play an important role in peripheral tolerance.

## Pattern-Recognition Receptors and Dendritic Cell Maturation

DCs sense their environment through a broad panel of PRRs. PRRs are highly conserved innate immune receptors that detect PAMPs or endogenous signals such as DAMPs. These PRRs are extremely diverse, detecting a wide range of molecular patterns, including proteins, lipids, carbohydrates, nucleic acids, and mineral crystals.[66] PRRs include the TLRs, the C-type lectins, the Nod-like receptors (NLRs), the retinoic acid–inducible gene (RIG)-I–like receptors (RLRs), the scavenger receptors (SRs), the integrins, and others such as the Ig Fc receptors (FcRs). These different receptor signal pathways can interact, and their integration allows a fine-tuning of the immune response adapted to specific conditions. Triggering of PRRs such as TLRs or C-type lectins on DCs is thought to be critical for their functional maturation and the priming of T cell responses to infection, therefore coupling innate and adaptive immunity.

**Dendritic Cell Maturation and Toll-like Receptors.** TLRs are highly conserved nonphagocytic PRRs that recognize many different specific molecular patterns, including PAMPs, such as microbial proteins, lipopeptides, glycopeptides, and nucleic acids, but they can also interact with some endogenous motifs such as nucleic acid.[67] In fact, inappropriate DC activation by these endogenous TLR agonists may contribute to autoimmune disease development, such as rheumatoid arthritis (RA), psoriasis, and SLE. Furthermore, in the steady state, TLR-mediated recognition of commensal microorganisms in the digestive tract contributes to tissue homeostasis via DC homeostatic maturation to maintain tolerance by retaining the integrity of the intestinal barrier. In fact, the knockout for certain TLR pathways in mice can increase susceptibility to the development of colitis.[26,68,69] TLRs can be grouped into two subfamilies based on their subcellular localization: the TLRs expressed on the cell surface (in humans and mice, TLR1, TLR2, TLR5, and TLR6, and restricted to mice, TLR11, TLR12, and TLR13) and those localized to specialized endosomal compartments (TLR3, TLR7, TLR8, and TLR9). Finally, TLR4 can localize to both sites.[70] Activation of DCs in response to binding of TLR agonists is controlled by signaling through the Toll/IL-1 receptor (TIR) domain present in the cytoplasmic domain of TLR. TLR ligands can induce DC maturation and regulate cellular processes, such as metabolism, life span,[71] cytoskeletal and organelle dynamics,[72] autophagy,[73] and intra-cellular sorting and processing

of endocytosed antigens.[74] Each DC subset expresses distinct panels of TLRs, which modulate their functional specialization. In response to infection or other abnormal conditions, pDCs are key producers of the type I IFNs. Human pDCs express high levels of nucleic acid–specific TLRs such as TLR7 and TLR9. TLR7 and TLR9 detect ssRNA and unmethylated cytosine-phosphatidyl-guanine (CpG) DNA, respectively, which are motifs usually found in both bacteria and viruses. TLR8 also recognizes bacterial ssRNA. Human $CD1c^+$ cDCs express mostly TLR1 through TLR8 and TLR10, whereas the $CD141^+$ cDCs express TLR1, TLR2, TLR3, TLR7, and TLR8.[75]

Overall, with regard to their TLR expression pattern, pDCs and $CD141^+$ cDCs appear to be more predisposed for the detection of intra-cellular pathogens, either directly or after phagocytosis and processing of material from infected cells, whereas $CD1c^+$ and moDCs might be more prone to detect extra-cellular pathogenic components. TLR stimulation by self- and non–self-antigens can regulate cytokines such as TNF, IL-12, IL-6, IL-23, and IL-17, which have an important role in the polarization of T helper cell subsets during priming by DCs, and thus the functional consequences of DC activation.

**C-Type Lectin Receptors.** C-Type lectins are another family of PRR that recognize sugar motifs in a calcium-dependent manner through their carbohydrate recognition functional domain. We will focus on the transmembrane CLRs, which are expressed at the surface of DCs and can recognize carbohydrate ligands of self, viral, bacterial, and fungal origins. The repertoire of CLR expression varies among the different DC subsets and is tightly regulated by the interactions with the environment. Some CLRs are almost restricted to specific DC subsets such as langerin on LCs. CLRs are involved in endocytosis, phagocytosis, antigen processing, and presentation through regular class II MHC or by class I MHC cross-presentation. Some of the CLRs regulate the immunomodulatory-transduction cascades through intra-cellular motifs such as the immunoreceptor tyrosine-based inhibitory motif (ITIM), which can induce the Src-homology phosphatase (SHP) cascade or the immunoreceptor tyrosine-based activation motif (ITAM), which signal through spleen tyrosine kinase (SYK) pathways.[76] Several CLRs, such as DEC205, mannose receptor (MR), and DC immunoreceptor (DCIR), have been shown to contribute to the cross-presentation of endocytosed antigens.

In most cases, CLRs fine-tune the immunogenic profile of DCs by regulating DC functions or modulating other pathways such as TLR signaling. It has been suggested that CLRs such as DEC205 contribute to immune tolerance in the absence of pro-inflammatory priming of DCs. However, various CLR-deficient mice show limited effects on tolerance induction and susceptibility to autoimmune diseases. In fact, only the genetic deletion of DCIR1, one of the murine homologues of DCIR (CLEC4A2), was associated with spontaneous induction of autoimmune disease (autoantibodies and RA-like syndromes) and immune homeostasis disruption.[77] DCIR1-deficient mice also have a higher susceptibility to collagen-induced arthritis. DCIR and DCIR1 contain ITIM domains, which are proposed to induce inhibitory pathways through SHP activation. Interestingly, SHP1 hypomorphic mutations in mice lead to autoimmune disease that is related to improper immune stimulation by commensal flora, probably mediated by either TLRs, CLRs, or other PRRs, which can be negatively regulated by SHP1 pathway induction.[78] Finally, activation of DCIR can negatively modulate TLR-induced inflammation.[79,80]

DC-specific intercellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN or CD209) recognizes endogenous and exogenous mannosylated and fucosylated motifs. In humans, DC-SIGN contributes to the modulation of the immune response.[81,82] DC-SIGN can regulate the host pathogen interaction with the tick *Ixodes scapularis* by modulating TLR-dependent inflammatory cytokine production and reprofiling cytokines produced by the DCs in such a way that tolerance is induced and parasitism is favored. DC-SIGN also interacts with commensal bacteria such as lactobacilli.[83,84] DC-SIGN recognition of endogenous antigens has been associated with maintenance of tolerance in the central nervous system through recognition of fucosylated and glycosylated myelin.[85] Finally, in RA synovia, the numbers of cells expressing DC-SIGN on macrophages and DCs and intercellular adhesion molecule (ICAM)-3 on naïve T cells were increased in the affected joints, with interaction leading to increased secretion of matrix metalloproteinases, which promotes local tissue lysis and induces inflammation.[86]

BDCA-2 expressed by pDC pairs with FcεRγ, which contains an ITAM. Activation of BDCA-2 by antibody ligation has a tolerogenic effect and results in downregulation of TLR9-mediated response in pDCs.[87,88] Persons with SLE have reduced expression of BDCA-2 on pDCs, and the lack of negative regulation through BDCA-2 signaling may account for excessive IFN-α production upon TLR stimulation.[89] BDCA-2 and DEC-205 share similar immunomodulatory properties and have been targeted by recombinant antigens bound to antibodies in order to modulate DC function by inducing antigen-specific tolerance or immune stimulation with the addition of a maturation signal.[90]

Some other lectins can directly induce an immune response. Dectin-1 recognizes β-1,3 glucan expressed by fungal pathogens. Dectin-1 induces SYK-dependent signaling pathways that can regulate TLR cross-talk. Activation of Dectin-1 leads to the generation of Th1 and Th17 cells,[91] and it can participate in the induction of disease in the SKJ mouse model of inducible RA; however, the function of dectin-1 in human RA remains speculative.[92]

Finally, many CLRs recognize ligands expressed by apoptotic or necrotic cells and participate in the uptake of these dead cells by DCs, although most of the clearing process is mediated by neutrophils and macrophages. Clearance of apoptotic cells in a steady-state condition is a non-inflammatory process and prevents accumulation of self-antigens, thus preventing autoimmune disease. The CLR contribution to apoptotic cell removal must be highly redundant because their silencing in mice does not seem to significantly increase the risk of developing autoimmune syndromes. On the other hand, some CLRs are implicated in the recognition of necrotic dead cells and induce DC activation and immunogenic responses. DNRG1 (CLEC9a) is expressed on $CD141^+$ DC and recognizes F-actin, which is expressed at the surface of necrotic cells. This interaction

induces immunogenic responses and promotes the cross-presentation of processed necrotic antigens on class I MHC.[93,94] In the same way, macrophage-inducible C-type lectin (MINCLE), which is another CLR that recognizes necrotic cells, can induce inflammatory responses.[95]

Because CLRs are involved in immune homeostasis, their dysregulation might contribute to the predisposition to autoimmune disease. This supposition is supported by associations of single-nucleotide polymorphisms of CLR and key components of their signaling pathways with a number of autoimmune diseases, including CLEC16A with type 1 diabetes, multiple sclerosis, juvenile RA, and inflammatory bowel disease; DCIR with RA; and CARD9 with the spondyloarthropathies, ankylosing spondylitis, and inflammatory bowel disease.

**Nod-like and Retinoic Acid-Inducible Gene I–like Receptors.** NLRs and RLRs are cytoplasmic receptors that modulate nuclear factor-κB (NF-κB) or inflammasome signaling pathways through the recruitment and the activation of caspase reaction cascades.[96] The inflammasome is a complicated multiprotein complex that can be activated through PRRs by PAMPs or DAMPs and induces the activation of key inflammatory cytokines, such as IL-1β and IL-18, and regulates an inflammatory cell death pathway known as pyroptosis.[97]

RLRs recognize genomic or replication intermediate viral double-stranded RNA (dsRNA). The RLR family is composed of RIG-I, melanoma differenciation-associated protein 5 (MDA5), and the RIG-I like RNA helicase DHX-58 (LGP2). RIG-I and MDA5 directly activate a signaling cascade, leading to expression of type I IFN genes via TNF receptor–associated factor (TRAF)-3 and IFN response factor (IRF)-3/7. LGP2 is a modulatory co-receptor of RIG-I and MDA5 and can negatively modulate immune response to dsRNA. RLRs are key immunomodulatory PRRs in the regulation of anti-viral immunity, rather than in homeostasis, and may contribute to the development of inflammatory syndromes related to primary viral infections.[97]

NLR family proteins have a conserved structure but different functional domains. NOD1 and NOD2 sense various bacterial PAMPs in the cytoplasm and induce inflammatory cytokines through RIP-K2 (Receptor-interacting serine/threonine-protein kinase 2)-dependent of NF-κB and mitogen-associated protein kinases (MAPKs). NACHT-LRR-PYD–containing protein-3 (NALP3) and the NLR family apoptosis inhibitory protein-5 (NAIP5) regulate inflammasome activation through PRR and possibly RLR. IL-1β, IL-18, and IL-33 are the key inflammatory cytokines whose production is dependent initially on PRR-mediated transcriptional upregulation and translation of their proforms and subsequently into their active forms through activation of caspase-1 as a result of inflammasome activation.[97] Therefore the inflammasome is a key regulator of local inflammation, which can be activated directly or indirectly by various ligands. The NALP3 inflammasome can be induced by exogenous ligands such as viral RNA and by host endogenous ligands, including monosodium urate (MSU) and adenosine triphosphate (ATP). The inflammasome is associated with different autoinflammatory diseases, including RA.[98] Gout, an acute and chronic inflammation of joints, is associated with the deposition of MSU, which most likely induces inflammation through NALP3 inflammasomes.[99] NLRs have also been implicated in various inflammatory diseases, graft-versus-host syndrome, and autoimmune diseases. For example, the Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and neonatal-onset multisystem inflammatory disease (NOMID) have been shown to be associated with mutations in NLRP3, causing constitutive activation of the NLRP3 inflammasome.[98] Deletion of *ATG16L1*, an autophagy-related gene, leads to hyperactivation of inflammasomes and subsequent secretion of bioactive IL-1β and IL-18, which contribute to intestinal inflammation. *NOD2* regulates Th17 responses for clearance of bacteria through the selective induction of IL-23 and IL-1β, which is abrogated in DCs from persons with Crohn's disease who carry a mutation in *NOD2*.[100] Therefore inappropriate responses to exogenous stimuli, such as bacteria from the microflora, may prime DCs into an inflammatory state, which contributes to the development of autoinflammatory or autoimmune diseases.

**Fc Receptors.** FcRs, which are expressed by many immune cells, are important in regulation of immune responses to ICs composed of antigen bound to antibody and sometimes to components of the complement system. DCs express activating receptors FcγRI, FcγRIIA, FcγRIIIA, and inhibitory receptor FcγRIIB. Infected cells or pathogens coated with IgG activate FcγR-mediated clearance by antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis and/or indirectly through the release of cytokines. This process is mediated by ITAM phosphorylation and subsequent activation of SYK and the initiation of the downstream signaling cascade resulting in DC activation. In contrast, inhibitory FcγRIIB has an ITIM domain in its cytoplasmic tail, resulting in inhibition of phagocytosis and cytokine secretion. FcγRIIB also prevents spontaneous IC-mediated DC maturation by the low levels of ICs that can be identified in the serum of healthy persons.[101] In a small subset of patients with well-controlled RA who were able to stop taking disease-modifying drugs, peripheral blood monocyte-derived DCs expressed high levels of FcγRIIB, which inhibited TLR4-mediated DC activation.[102] In addition, ICs taken up by FcγRIIB are degraded inefficiently and are presented on the cell surface in native conformation, where they can negatively regulate B cell function to prevent autoreactivity.[103] An FcγRIIB gene polymorphism has been identified in persons with SLE and RA, and DCs from patients with RA who have an FcγRIIB variant manifested an increase in IC-mediated inflammation in vitro.[104]

FcγRs also alter CR3-mediated phagocytosis and signaling upon co-stimulation in vitro. CR3-mediated phagocytosis is inhibited upon co-stimulation of CR3 with FcγRI, whereas it is enhanced by FcγRIIA co-stimulation. Patients with SLE have been reported to have a high FcγRI and a low FcγRIIA expression, which can lead to inefficient CR3-mediated phagocytosis of apoptotic cell uptake, which affects homeostatic regulation.[105]

**Apoptotic Cell Recognition Receptors.** DCs also contribute to tissue homeostasis through clearance of apoptotic cells in the periphery by a process named *efferocytosis*. Contrary to necrotic or other inflammatory cell death, the uptake of apoptotic cells or apoptotic microparticles maintains DCs in an immature phenotype and induces

immunomodulatory effects.[106] These DCs downregulate co-stimulatory molecules (CD80/86) and produce TGF-β, a cytokine necessary for differentiation of naïve T cells to Tregs.[107] In a steady state, apoptotic cells are a source of self-antigen presentation by DCs and may contribute to tolerogenic DC homeostatic maturation and peripheral tolerance regulation.[108,109] Inefficient efferocytosis leads to the accumulation of apoptotic cells, which then evolve into secondary necrotic cells, which have been implicated in the development of SLE. Recognition and engulfment of apoptotic cells involve multiple ligands, including "eat me" signals, bridging molecules, and phagocytic receptors. One of the principal "eat me" signals is translocation of phosphatidylserine (PS) to the outer leaflet of the plasma membrane of apoptotic cells. Apoptotic cells and microparticles are recognized by a plethora of receptors that recognize molecular patterns enriched on apoptotic cells, such as certain sugars and phosphatidylserine. These receptors include integrins, scavenger receptors, milk fat globular-EGF factor-8 protein (MEGF-8), stabilin, low-density lipoprotein receptor, receptor tyrosine kinases, and proteins from the immunoglobulin family. The Tyro 3 subfamily of receptor tyrosine kinases—Tyro 3, Axl, and Mer (TAM)—bind through intermediate adaptor proteins to apoptotic cell membranes to trigger suppressors of cytokine synthesis (SOCS)-1 and SOCS-3 expression in DCs, which inhibit the TLR-induced and cytokine-induced signaling cascades and therefore immunogenic DC maturation.[110] Consistent with this view, TAM triple gene–deficient mice possess hyperactive DCs and develop systemic autoimmunity.[111] The uptake of apoptotic cells triggers TGF-β release, which leads to DC-mediated Treg induction. The DC-specific loss of TGF-β activation of integrin αvβ8 resulted in the failure of Treg development initiated by DCs in vitro and caused autoimmune colitis in mice.[48] The scavenger receptor SCARF1 regulates efferocytosis, and SCARF1-deficient mice recently have been associated with the development of SLE-like syndrome in mice, possibly because of the accumulation of apoptotic debris.[112] A common feature of patients with lupus is the presence of dying cells in both lymph nodes and inflamed tissues. It is not clear whether this phenomenon is due to increased cell death or innate or acquired inability to clear apoptotic cells, which normally occurs very rapidly. Increased cell death might be the consequence of oxidative stress or infection, both of which are thought to trigger autoimmune disease. Regardless of the mechanism, cell death accumulation increases the presence of autoantigens available for presentation by DCs. Apoptotic cells may eventually undergo secondary necrosis. Necrotic cells attract phagocytes by secreting hyaluronic acid, uric acid, and high-mobility group box-1 protein (HMGB1), which are known inducers of the inflammasome and can contribute to inflammatory priming and development of autoimmune disease.[113]

## Antigen Presentation

DCs are specialized in antigen processing and can efficiently present endogenous and exogenous antigens in both class I and II MHC contexts. As opposed to macrophages, cDCs degrade their engulfed cargo slowly and can control lysosomal degradation, potentially to preserve peptides for T cell recognition.[114] This activity is influenced by the maturation and activation status of the DC, which promotes phagolysosome maturation, peptide processing, peptide macropinocytosis, and DC metabolism to promote antigen processing and presentation of antigens by class I MHC and class II MHC, as well as lipid presentation by CD1 molecules.

### Class I Major Histocompatibility Complex Antigen Presentation

Class I MHC molecules are expressed in almost all cell types and present peptides derived from proteasome-digested cytosolic proteins, including endogenous, viral, or bacterial proteins, to $CD8^+$ T cells. Those antigenic peptides are predominantly generated from ubiquitinated nascent, misfolded, and neosynthesized defective proteins, defective ribosomal products (DRiPs), and cytoplasmic proteins. Peptides are transported to the endoplasmic reticulum (ER) through transporter associated with antigen processing (TAP) proteins, where long peptides are further trimmed to 8-mer or 9-mer peptides for loading onto class I MHC molecules. The trimeric complex of class I MHC heavy chains, β2-microglobulin, and peptide allows for optimal folding, glycosylation, and delivery to the cell surface.[115]

As professional APCs, DC subsets express specific forms of immunoproteasomes that generate a different spectrum of peptides from the standard proteasome and broaden the repertoire of antigens presented to cytolytic $CD8^+$ T cells. Transformed or infected cells also display peptides from modified self or exogenous proteins on their class I MHC molecules in order to be recognized by the TCR of activated cytolytic T cells for degradation. However, antigen-specific naïve $CD8^+$ T cells cannot directly eliminate transformed or infected cells; they must be activated by APCs such as DCs. To activate $CD8^+$ T cells, DCs must process antigens from infected or transformed cells, and even self-antigens, and present them through canonical class I MHC presentation or though "cross-presentation," which is described in the next section.

**Class I Cross-Presentation.** DCs can acquire antigens exogenously and process them for presentation by class I MHC molecules, which is referred to as *cross-presentation*.[9,32] This property is atypical because class I MHC molecules exclusively present endogenous proteins in most cells. Although the precise mechanism of cross-presentation remains controversial, it is well established that DCs use this process to activate $CD8^+$ T cells. DCs acquire antigens by endocytosis in the form of apoptotic cells, necrotic cells, antibody-opsonized cells, immune complexes, heat shock proteins, and exosomes, and even by nibbling of live cells.[9,32] In the same way, autophagosomes are another source of peptides for class I MHC molecules. The process of autophagy normally clears ubiquitinated cytoplasmic protein aggregates.[61] TLR activation or exposure to stress also induces DC aggresome-like structures (DALIS) containing polyubiquitinated protein, and peptides derived from DALIS can be presented by class I MHC molecules.[116]

Two main intra-cellular pathways traffic exogenous antigens from phagolysosomal compartments to the ER for cross-presentation. The cytosolic cross-presentation

pathway relies on antigen processing by the proteasome coupled to transport to the ER by TAP1 and TAP2 transporters. This pathway is sensitive to proteasome inhibitors. In contrast, the vacuolar pathway is resistant but is sensitive to inhibitors of lysosomal proteolysis.[9,32] Vacuolar cross-presentation is also less susceptible to TAP gene knockout in mice. The mechanism of transfer of antigens from phagosomes to class I MHC loading compartments has yet to be elucidated; however, it has been observed that high pH and low proteolytic activity favor the atypical class I MHC cross-presentation of phagocytosed antigens.[114,117] Recently, progress has been made in better understanding the cross-talk between the different cellular compartments that regulate the cross-presentation. Nair-Gupta and collaborators[118] have identified an important role in DCs for communication between the endosomal recycling compartment (ERC), which is a key reservoir of class I MHC molecules, and the phagosome in cross-presentation. Trafficking from the ERC to phagosomes is controlled by the TLR-MyD88-IKK2 pathway, which stabilizes membrane interactions between the phagosome and the ERC, ensuring that ERC-deployed class I MHC are specifically routed to phagosomes engaged in TLR signaling. On the other hand, the class I MHC peptide loading complex (PLC) is recruited from another subcellular compartment, the ER and Golgi intermediate compartment (ERGIC), in a TLR-independent manner. The highly coordinated trafficking from ERC and ERGIC delivers essential components for cross-presentation to phagosomes during infection, and control of the ERC pathway by TLR signals favors phagosomes that contain microbial proteins for cross-presentation in the context of TLR-primed T cell co-stimulation.[118]

### Class II Major Histocompatibility Complex Antigen Presentation

The class II MHC pathway is constitutively expressed only by APCs. Class II MHC α/β heterodimers rely on a specialized type II transmembrane chaperone protein, the invariant chain (Ii), for stable assembly in the ER. Ii stabilizes the class II MHC complex and contains an endosomal sorting and retention signal.[119] Class II MHC molecules are then transported and concentrated in multivesicular and multilamellar late endosomal compartments called class II MHC–containing compartments (MIICs). Antigens are endocytosed or autophagocytosed and retained within late endosome or phagosomes before fusing with lysosomes to form phagolysosomes. Concomitant TLR signals induce activation of the vacuolar proton pump that enhances lysosomal acidification and antigen proteolysis into antigenic peptides within phagolysosomes.[120] Acidification of this compartment allows optimal activity of cathepsins. Cathepsin S degrades the cytoplasmic tail of Ii, leaving a short peptide, the class II MHC–associated invariant-chain peptide (CLIP), bound to the peptide-binding groove and thus protected from proteases. CLIP is replaced by an antigenic peptide through the action of the catalyst-chaperone protein HLA-DM, which accelerates the rate of CLIP release, and the loaded class II MHC molecules are thought to be transported through cytoskeletal tubular structures that are directed toward the site of T cell interaction at the plasma membrane.[121]

TLRs regulate phagosome maturation,[74] enhance lysosomal acidification,[120] and increase antigen uptake transiently.[72] Class II MHC surface expression and turnover rates are regulated by cytoplasmic domain ubiquitination in DCs.[122] This mechanism explains the expression of low levels of class II MHC molecules by immature DCs and their increase in half-life after maturation, sustaining antigen presentation after migration into secondary lymphoid organs.

RA is characterized by autoantibodies against citrullinated proteins. A possible mechanism of generation of the immune response against citrullinated proteins has been described in mice.[123] It has been shown that two distinct conformers of MHC-peptide complexes are known for the same peptide; these conformers are referred to as type A for stable conformation and type B for less stable conformation. Peptides generated from intact protein are loaded onto class II MHC complex in the late endosome/lysosome. In this compartment, HLA-DM catalyzes peptide loading and acts as a conformational catalyst, and hence only more stable type A conformers are loaded. In comparison, the same peptide may be loaded exogenously at the cell surface and in the early endosome in exchange for poorly binding peptides occupying class II MHC molecules in the absence of HLA-DM. These mechanisms of loading give rise to both stable type A and flexible type B conformers. Hence only type A T cells are primed with native protein, and exogenous peptide gives rise to priming of both type A and B T cells. In the thymus, T cells recognizing the type A conformation for a peptide are deleted, whereas type B T cells escape thymic negative selection.[123] Thus self-reactive type B T cells are present in the naïve peripheral repertoire and have potential for autoimmune activation. This was recently shown in nonautoimmune-prone mice after immunization with unmodified hen egg lysozyme (HEL); T cells specifically reactive to citrullinated epitopes of HEL were present among the responding repertoire to immunization. The citrullinated HEL epitopes were processed and loaded onto class II MHC complexes and were presented to T cells by DCs.[124] Hence naturally occurring self-reactive T cells, which have evaded thymic negative selection, may recognize citrullinated peptides loaded as flexible type B conformers onto class II MHC molecules and subsequently provide help for B cell–mediated antibody responses against citrullinated proteins.

**Class II Cross-Presentation.** There is also a cross-presentation mechanism that allows the presentation of intra-cellular self-antigens on class II MHC to activate $CD4^+$ T cell responses. This process relies on autophagy, wherein cytoplasmic antigens are acquired and loaded onto class II MHC molecules in the autophagolysosome.[125]

### Lipid Presentation

CD1 molecules present lipid antigen to T cells. The CD1 family of class I MHC–like glycoproteins includes CD1d on the surfaces of DCs and CD1e, which remains in the endoplasmic reticulum. CD1a, CD1b, CD1c, and CD1d-restricted T cells express CD4 or CD8 or lack both CD4 and CD8 (double negative). By contrast, most CD1d-restricted T cells express a semi-invariant TCR and markers of NK cells and are identified as invariant NK T (iNKT) cells.[126]

Similar to class I MHC and class II MHC molecules, CD1 molecules assemble in the ER, where they noncovalently bind the chaperones calnexin, calreticulin, ERp57, and β2-microglobulin.[127] Lipid antigens are generated by lysosomal hydrolases of internalized phagosomes, membrane-bound lipids in clathrin-coated vesicles, or internalized apoptotic bodies or exosomes and are loaded onto CD1 molecules in the lysosomal lumen.

## T Cell Activation

cDCs interact with naïve T cells upon arrival in the lymphoid organs and with effector memory T cells at inflammatory sites. Exposure to the environment induces DC-derived signals that determine the outcomes of these interactions, which include the following: antigen presentation in the context of MHC molecules (signal 1), co-stimulatory molecules, including CD80 and CD86 (signal 2), and cytokines (signal 3). Some cell surface molecules are inhibitory, such as PDL1, which interacts with T cell PD1, or CD80/CD86, which interacts with T cell cytotoxic T lymphocyte–associated antigen 4 (CTLA-4). Signal 3—provided in cis by APCs to antigen-specific T cells—includes IL-12, IL-23, TNF, IL-6, IL-1β, and type I IFN produced by DCs. Because cytokine secretion varies according to PRR activation, PAMPs and DAMPs are important factors that can skew the adaptive immune response.

T cell activation depends on the intensity and length of DC–T cell interactions, mediated by the immunologic synapse (IS), which is a region of spatially and temporally organized motifs of membrane proteins and cytosolic molecules formed at the interface between a T cell and a DC. Stable long-lasting contacts of APCs with T cells are dependent on co-stimulatory molecules and integrin molecules ICAM-1 and ICAM-3. These integrins form a ring in the outer part of the IS, in a region adjacent to the corresponding area containing lymphocyte function–associated antigen-1 (LFA-1).[128] It is hypothesized that the stability of the IS confers temporal inhibition of DC apoptosis, which would enhance activation of clonal T cells in the lymph node.[127]

$CD4^+$ Th cells play a vital role in the induction of humoral and cellular immunity, providing signals for the activation of B cells and $CD8^+$ T cells. Controlled by the activated DCs, $CD4^+$ T cells can differentiate into Th1 cells, producing IFN-γ and IL-2; Th2 cells, producing IL-4, IL-5, and IL-13; Tfh cells, expressing inducible T cell co-stimulator and producing IL-21; Th17 cells, producing IL-17; or Tregs, producing TGF-β or IL-10.

DCs promote a Th1 phenotype by acting on both NK cells and naïve $CD4^+$ T cells. Collaboration of IFN-γ and IL-12 induces and sustains Th1 differentiation. The Th2 response is promoted by induction of OX40 ligand (OX40L) on DCs, which is mediated by release of thymic stromal lymphopoietin (TSLP) by epithelial cells, mast cells, and basophils.[129] Absence or depletion of IL-12 production by DCs also skews the T cell response to Th2. In turn, IL-10 secretion by Th2 cells can negatively regulate DC function. DCs make a deficient response to TSLP stimulation in Crohn's disease, an inflammatory bowel disease associated with Th1 and Th17 immunity.[130]

Pro-inflammatory Th17 cells expressing IL-17 and the RAR-related orphan receptor γ (RORγt) transcription factor were described in RA synovium.[131] Upon TLR stimulation, innate immune cells, including DCs, secrete IL-6 and IL-1β. The presence of IL-6 and TGF-β induces Th17 differentiation, subsequent production of IL-21, and expression of IL-23R. IL-21 amplifies Th17 differentiation; however, IL-23, which is produced by DCs, is needed for the maintenance of Th17 cells.[132]

IL-1β is a critical amplifier of Th17 differentiation, especially in humans. IL-1β and IL-6 also promote the reprogramming of forkhead box $P3^+$ ($FoxP3^+$) Tregs to Th17.[133] This scenario is relevant to the development of autoimmune disease, because Tregs are selected on the basis of intermediate affinity for self-peptides in the thymus.[134]

Although Th1 cells were the major subset of T cells identified in RA synovium, identification of Th17 cells and the cytokines IL-17 and IL-23 within affected tissues and/or fluids has implicated both Th1 and Th17 cells in the pathogenesis of both RA and spondyloarthropathy.[132,135] Synovial tissue DCs express IL-12p70 and IL-23p19, providing a mechanism by which Th1 and Th17 cell production is locally perpetuated.[136]

Tfh cells are induced by IL-6 and IL-21, which induce expression of CXC chemokine receptor 5 (CXCR5) to enable the migration of T cells into B cell follicles. They express B cell lymphoma 6 (Bcl-6), a master transcription factor for Tfh cell differentiation. Tfh cells also produce IL-21, which can induce affinity maturation and somatic hypermutation and increase memory B cell and long-lived plasma cell activity in germinal centers (GCs). However, abnormally activated Tfh or the lack of tolerogenic regulatory Tfh give rise to an immune reaction against autoantigens, which may occur in autoimmune diseases such as SLE and RA.[137]

pDCs, which are potent producers of type I IFN, also present viral and tumor antigens to initiate both $CD4^+$ and $CD8^+$ T cell responses, thereby providing an important link between innate and adaptive immunity.[138,139] Increased production of IFN-α may activate $CD8^+$ T cells, which are increased in kidneys in persons with lupus nephritis.[140,141] In addition, pDCs can induce Treg generation by induction of IDO (discussed later).

## B Cell Activation

B cells express both antigen-specific B cell receptors (BCRs) and various TLRs, thus allowing them to play a role in innate and adaptive immunity. Although DCs present processed antigens to naïve T cells, B cells recognize antigen in its unprocessed native state. Two mechanisms of resistance to intra-cellular antigen degradation in DCs are described: (1) the internalization of antigen-containing immune complexes into nondegradative intra-cellular compartments through FcγRIIB, followed by receptor recycling to the cell surface and presentation to the BCR[103]; and (2) accumulation of intact antigen in neutral endosomes by DC-SIGN (carbohydrate-containing antigen).

When DCs and B cells take up the same antigen, DCs can activate B cells in an antigen-specific $CD4^+$ T cell–dependent manner. This action results in B cell activation and isotype switching to IgG, IgA, and IgE, as well as

memory B cell generation in response to T-dependent antigens.

DCs can also stimulate B cell proliferation by expression of B cell–activating factor (BAFF) and its closely related tumor necrosis family member APRIL (a proliferation-inducing ligand).[142] Furthermore, inflammatory cytokines secreted by DCs can also affect B cell activation. IFN-α and IL-6, or ICAM-1, expressed by activated pDCs, regulate B cells to differentiate into plasma cells for T cell–independent antibody production.[143] IFN-α can enhance antibody secretion in vivo[144] and can induce cDCs to produce BAFF and APRIL, which triggers isotype switching independently of CD40 ligation.[145]

### Natural Killer Cell Activation

DCs can induce activation and/or proliferation of NK cells, resulting in increased NK cell cytolytic activity and/or IFN-γ production.[146] Both cell-cell contact–dependent interactions and soluble cytokine signals are involved. NKG2D ligands are cell contact–dependent signals expressed by DCs in response to stressful stimuli. LFA-1/ICAM-1 interactions have also been implicated in NK cell activation by DCs. Type I IFN augments NK cell cytotoxicity; IL-12 induces IFN-γ secretion and cell proliferation; IL-15 determines cell differentiation and survival; and others such as IL-18 and IL-2 are mediators in DC-mediated NK cell activation.

Reciprocally, NK cell–mediated activation of DCs depends mainly on cytokines, principally TNF and IFN-γ, whereas cell contact–dependent interactions are required for DC elimination. NK cells can lyse immature DCs (imDCs) through NKp30—a process referred to as *DC editing*.[147] A TNF-related apoptosis-inducing ligand (TRAIL)–mediated mechanism and CD94-NKG2A–mediated signals also induce DC lysis.

## DENDRITIC CELLS AND TOLERANCE

Self-tolerance is the essential process by which the immune system can discriminate self from nonself and remain anergic to the body's constituents (self-/endogenous antigens) while dealing with nonself/exogenous antigens such as invading microorganisms (pathogenic antigens). Central tolerance occurs in the thymus with the negative selection of thymocytes that react to self-antigens. Peripheral tolerance is the process by which autoreactive T cells that have escaped thymic selection can be anergized or deleted. It also includes the induction of Treg cells. Finally, the MPS, including DCs, regulate tissue homeostasis, which is key to maintaining peripheral tolerance.

### Central Tolerance

Central (or thymic) tolerance defects are important and are probably essential contributors to spontaneous autoimmune disease.[148] T cells are selected in the thymus according to their affinity for self-MHC molecules that are loaded with endogenous self-antigens displayed by thymic cortical epithelial cells. The T cells that are reactive to self-antigen above a threshold of affinity for self-antigen–MHC complexes expressed and presented by medullary APCs are then deleted by negative selection. In the thymus, medullary thymic epithelial cells (mTECs) are primarily responsible for negative selection because they express many self-antigens. mTECS express the transcription factor autoimmune regulator (AIRE), which drives the expression of self-antigens for presentation to thymocytes. It has been proposed that thymic DCs acquire those self-antigens either directly or indirectly from mTECs by phagocytosis of apoptotic mTECs, through exosomes trafficking between the two cell types, or through membrane exchanges by trogocytosis. Thymic lymphoid DCs, by cross-presenting those self-antigens, can supplement self-antigen presentation mediated by mTECs and contribute to the negative selection. Furthermore, the interaction between mTECs and thymic DCs leads to the generation of tolerogenic Tregs specific to autoantigens. In addition to thymic cDCs, peripheral migratory DCs such as pDCs can migrate in steady state from tissue to present self-antigens in order to induce clonal deletion of T cells and induce Treg generation.

### Peripheral Tolerance

Peripheral tolerance mechanisms provide a second level of control to restrain potentially autoreactive T cells that escape thymus negative selection and migrate to the periphery. Central tolerance is stochastic because it relies on the affinity threshold between TCRs and self-antigens; some autoreactive T cells can therefore escape. As for central tolerance, the direct function of DCs in peripheral tolerance remains incompletely understood. Studies targeting specific DC subsets in vivo in the mouse by injection with self-antigenic peptides fused to recombinant Fab fragment of antibodies against innate immune endocytic receptors, such as the CLR DEC205 (CD205,Ly75) or DCIR2/33D1,[149,150,151] have shown that DEC205 targeting generates tolerance in autoimmune murine models, such as experimental autoimmune encephalomyelitis (EAE), diabetes, and arthritis.[152,153]

A genetic approach restricting antigen expression on DC in mice showed that steady-state, high-level presentation caused drastic and stable unresponsiveness of antigen-specific $CD8^+$ T cells.[64] The peripheral CD8–T cell inhibition was mediated by the upregulation of the inhibitory co-receptors programmed cell death protein 1 (PD-1) and CTLA-4. Thus DCs can induce peripheral T cell tolerance to immune-dominant antigens that are expressed at high levels; however, the relevancy of these models to endogenous self-antigens and negative selection remains uncertain.

DCs have been also associated with the peripheral tolerance by facilitating the homeostasis of peripheral Tregs. DCs induce Treg cells in the presence of strong anti-inflammatory stimuli such as TGF-β and retinoic acid (RA),[154-157] and can support the proliferation of $Foxp3^+$ Treg cells after their depletion in vivo.[158] Migratory cDCs, but not resident lymphoid cDCs, induce the development of induced Treg cells specific for a particular self-antigen and can induce a specific tolerogenic response that could protect against induced autoimmune syndromes such as EAE or RA.[159,160] Finally, pDCs and cDCs produce tolerogenic factors, such as IDO, that negatively regulate T cells

by starving them of tryptophan, an essential amino acid required for cell growth and viability, and promoting the generation of FoxP3$^+$ Tregs with timing that ensures regulated expansion of activated T cells and other innate inflammatory pathways.[161,162] In the same way, DCs can produce IL-27, which in a CD39-dependent manner regulates the inflammasome and Th1 and Th17 T cell activation.[163]

Similar to central negative selection mediated by the mTECs, CD8$^+$ autoreactive T cells can be inhibited or deleted by radio-resistant lymph node stromal cells (LNSCs) via direct presentation of class I MHC self-antigens to autoreactive CD8 T cells[164] in a process relying on cell contact, such as by trogocytosis, and also on the production of exosomes.[164] The interaction with CD4$^+$ T cells induces their anergy and affects their survival.

It is unclear if actual tolerogenic subsets of DC exist or if their tolerogenic function is conditioned by their functional state because genetic or epigenetic evidence for tolerogenic DC subsets is still lacking. Deficiencies in certain signaling molecules have been shown to regulate some aspects of the tolerogenic function of DCs. These pathways primarily act through affecting the size of the DC compartment or the extent of DC activation. NF-κB activity is a key regulator of the DC functional state. Tolerance at some point relies on the activation of this pathway because NF-κB1 and p50 (active forms of NF-κB) deficiency in DCs favors autoimmune responses by deregulating the immunogenicity and life span of DCs.

A deficiency in DCs of A20, a ubiquitin-editing enzyme implicated in the degradation of various signaling molecules that activate NF-κB signaling, or the disruption of CD31, which is a co-inhibitory receptor of the NF-κB pathway, induces immunogenic DC development and autoimmune disease.[165,166] CD31 regulates the NF-κB pathway through the activation of the protein tyrosine phosphatase-1 (also known as Src homology region 2 domain-containing phosphatase-1 [SHP1]).[167] Similarly, the deficiency of SHP1, the kinase lyn, signal transducer and activator of transcription 3 (STAT3), or SOCS-1, which all negatively regulate the NF-κB pathway, also lead to auto-inflammation or autoimmunity.[148] In fact, inhibition of NF-κB and Notch1 by miR-23b promotes murine tolerogenic DCs in vitro, and in the absence of RelB (one of the members of the NF-κB transcription factor family), antigen-exposed DCs induce antigen-specific Tregs and suppress induced autoimmune syndrome after adoptive transfer into wild-type hosts.[168]

The mammalian target of rapamycin (mTOR) has also been implicated in DC-induced tolerance. DCs generated in the presence of rapamycin, an inhibitor of mTOR, induced antigen-specific Tregs and suppressed rejection after adoptive transfer into allograft recipients.[168a] The wingless type (Wnt)/β-catenin is a third major regulatory pathway involved in DC function.[169] β-Catenin was shown to be constitutively active in intestinal DCs and to regulate the balance between Foxp3$^+$ Tregs and Th17 effector cells in the lamina propria through induction of retinal dehydrogenase. Finally, enhanced and prolonged extra-cellular regulating kinase (ERK) signaling has also been shown to be important for suppression of IL-12 and induction of IL-10 secretion by DCs, which promote tolerance.[170]

In the steady state, DC tolerogenic function remains elusive, because constitutive DC ablation causes only a small reduction in T cell numbers without any obvious functional consequences upon autoreactive T cell activation.[171] Another study has claimed that constitutive ablation of DCs leads to autoimmune manifestations[172]; however, this study did not distinguish between T cell hyperproliferation and autoimmune disease induction. It is more likely that the autoimmune diseases emanated from a myeloproliferative syndrome caused by Flt-3-L accumulation in the absence of DCs.[173-175]

## AUTOIMMUNITY

In autoimmune diseases, clearance of self-antigens and/or tolerance against self-determinants are impaired. Activated autoreactive T cells, B cells, innate immune cells, and parenchymal cells participate in the process of tissue damage. DC antigen presentation can promote the priming and/or the effector differentiation of autoreactive T cells, either because of inappropriate activation signals or because of a cell-intrinsic breakdown of negative regulation, as previously described. Locally they act as both APCs and effector cells, producing pro-inflammatory molecules and regulating other effector cells. In different autoimmune conditions, cDCs and pDCs are enriched in inflamed tissues, particularly in a perivascular distribution. Moreover, DCs infiltrate tissues at very early stages of disease and contribute to the recruitment and organization of other immune cells. For example, in mice, DC infiltration is an early feature of islet cell autoimmunity in diabetes mellitus and contributes to local lymphoid tissue formation in the pancreas.[176,177] Organization of tissue into lymph node–like structures, including lymphoid follicles, is a common feature of tissues affected by autoimmune inflammation,[178,179] which contain follicular DCs (FDCs) and nurse-like fibroblast-like synoviocytes, supporting chronic production of autoantibodies.[180]

Here we discuss prototypic autoimmune diseases associated with rheumatoid disorders and the role that DCs play in their initiation and progression.

### Dendritic Cells in Psoriasis

Psoriasis is a chronic inflammatory skin disease that is characterized by the thickening of the epidermis as a result of the hyperproliferation and abnormal differentiation of keratinocytes. The causes of psoriasis and psoriatic arthritis are not yet fully understood; it is thought that primary infection by bacteria may initiate the development of the chronic autoimmune disease. In psoriatic plaques, the local inflammation is driven by activated keratinocytes that express anti-microbial peptides, chemokines, and growth factors and drive the recruitment of inflammatory innate immune effectors.

Dermal DC infiltration is increased in psoriatic skin and is associated with proliferation of autoreactive T cells, inducing a Th1 response.[181] A subset of dermal infDCs, known as Tip-DC, produces inducible nitric oxide synthase (iNOS) and TNF, promoting a pro-inflammatory environment. In addition, it has been shown that effector Th17 and potentially skin γδ T cells that produce IL-17 and IL-22 have a major role in psoriatic inflammation.[182,183] In

psoriatic lesions, a vicious cycle develops in which activated T cells produce inflammatory cytokines such as TNF and IFN-γ, which in turn activate keratinocytes to produce a wide variety of cytokines and chemokines, resulting in DC activation. The latter mechanism leads to a larger recruitment of inflammatory T cells. Although the trigger remains unknown, considering the effector T cell component of the disease, it is highly suspected that APCs play a key role in psoriasis development.

One experimental model of psoriasis-like inflammation has emerged after repeated application of Aldara, a cream containing the TLR7 ligand imiquimod, to mouse skin.[184] The contribution of TLR7 ligand in this model remains unclear because experimental psoriasis also seems to be induced by the vehicle independently of imiquimod and TLR7 stimulation but in an inflammasome-dependent manner.[185] Because both TLR7 triggering and inflammasome activation induce a strong inflammatory response, they may have a redundant or synergistic function in driving experimental psoriasis in mice. In another model, after treatment with imiquimod, mice in which MYD88 expression is restricted to $CD11c^+$ cells developed identical psoriatic symptoms as wild-type mice, which highlights that $CD11c^+$[186] DCs are sufficient to prime psoriasis development. In addition, it was shown that DC depletion in CD11c–diphtheria toxin receptor (DTR) mice protects against the development of psoriasis plaques in mice. In this study, it was proposed that IL-36 produced by skin cells induced DCs to produce IL-23 and to drive Th17 cell differentiation.[187] In fact, it has been shown that the selective expression of TLR7 signaling in specific DC subsets differentially contributes to the disease development. Restricted expression of TLR7 to $CD207^+$ (langerin) cDCs such as LCs resulted in attenuated disease and a more tolerogenic environment, which suggests that it is the $CD207^-$ dermal cDCs and moDCs that are essential to prime the imiquimod-induced psoriasis in mice. Dermal $CD207^-$ DCs secrete IL-23, which promotes dermal T cell activation and production of IL-17 and IL-22.[188]

Upon treatment with systemic type I IFN, flares of psoriasis have been observed, suggesting that type I IFN contributes to psoriasis development. Type I IFN–producing pDCs were therefore also proposed to have a major role in the initiation of psoriasis.[189] pDCs are recruited to the skin through the production of chemerin in inflamed skin and the chemokine-like receptor 1 (CMKLR1/ CHEMR23).[190] Activated keratinocytes produce anti-microbial peptide LL37, which has been shown to bind self–extra-cellular nucleic acids (DNA and RNA) that are generated in the skin during normal cell turnover or during injury and to trigger type I IFN in psoriasis.[191] LL37–nucleic acid particles can induce type I IFN production by pDCs and activate cDCs.[191-193] Surprisingly, pDC ablation did not affect the severity of the experimental psoriasis in mice,[188] which suggests that pDCS and type I IFN may instead contribute to the chronic aspect of the disease.

## Dendritic Cells in Systemic Lupus Erythematosus

SLE is often considered to be a systemic antibody-driven autoimmune disorder in which primarily nuclear components, such as DNA, histones, and riboproteins, are targeted. It is induced by the loss of tolerance to self-antigens, the accumulation of activated autoreactive T and B cells, and the abnormal production of cytokines. In SLE, microenvironmental and genetic factors may induce inappropriate self-antigen presentation by APCs such as DCs, leading to the abnormal activation of autoreactive T and B cell activation.

SLE is characterized by an increase of cell death and a significant decrease of dead cell clearance, which lead to the accumulation of autoantigens. In addition, it has been shown that phagocytic cells have a decreased ability to interact with and process dead cells.[194,195] Although the accumulation of dead cells contributes to disease development, it is not sufficient to induce autoimmune disease because vaccination with apoptotic cells/blebs does not induce adaptive immunity in the steady-state condition of non–lupus-prone mice, whereas necrotic and apoptotic cell exposure can lead to lupus development in SLE-prone murine experimental models.[196,197]

Neutrophil extra-cellular trap (NET)osis is a neutrophil specialized form of controlled cell death. During NETosis, the neutrophils extrude fibrillary networks composed of DNA, DNA-binding proteins such as histones, and also the content of their granules, such as anti-microbial peptides, neutrophil elastase, myeloperoxidase, and cathepsin G. NETosis is induced upon infection but also upon sterile inflammation, such as by TNF monosodiumurate and cytokines (IL-8, IL-1α, and IFN-I). SLE has been associated with a deficiency in clearance of the NETs. Some patients have a reduced DNAse-I activity, which is a key enzyme to digest extra-cellular DNA and degrade NETs. Those patients have significantly higher titers of autoantibodies targeted against dsDNA, similar to DNAse I–deficient mice that develop SLE-like syndromes.[198-201] Furthermore, DAMPs such as anti-microbial peptides and alarmins such as LL37 and HMGB1 tend to accumulate in NETs from SLE and contribute to disease development. An abundance of protein modifications exist, such as methylation, acetylation, citrullination, poly-ADP-ribosylation, and ubiquitinylation, in the cellular waste of nondegraded NETs and apoptotic cells; these modifications may give rise to neoantigens, which can be recognized as modified self.[202] It has been speculated that APCs such as DCs may cross-present these antigens to induce autoimmunity.

It is suspected that APCs are one of the key regulators of SLE development. Recent studies of the profile of maturation of the DC subset in patients with SLE have shown that both cDCs and pDCs have a more inflammatory activated phenotype and more efficiently prime T cell responses.[194] pDCs and inflammatory cDCs accumulate in nephritic kidneys and skin lesions of patients with SLE, but the role of DCs in SLE remains poorly understood. In the C57/BL6 murine background, the DC-restricted loss of Fas (CD95), a receptor of the family of TNFR implicated in apoptosis regulation, induces disease manifestations.[203] In fact, the most common experimental model for SLE are MRL mice, which express the lymphoprolipherative-deficient allele of the gene Fas (MRL-FasLpr). The constitutive deletion of DCs in the MRL mice decreases autoreactive T cell proliferation and plasmablast differentiation, which improves outcome of the disease.[204] Similarly, DC function has been

suggested by the DC-restricted deletion of negative regulators, which leads to the loss of tolerance and SLE-like disease in vivo. The DC-specific ablation of B lymphocyte–induced maturation protein-1 (BLIMP-1 or PRDM-1), which is a transcriptional repressor that controls the differentiation of B and T cells, induces an SLE-like syndrome in female mice.[205] Interestingly, human polymorphisms of the *PRDM1* gene have been associated with SLE, and SLE incidence is higher in women. In mice, the DC-restricted deficiency of PRDM1 results in higher IL-6 production and Tfh cell differentiation that enhances B cell germinal center formation and induces humoral autoreactivity. In humans, both heterozygote or homozygote loss of PRDM1 in DCs induces the same phenotype in vitro, clearly suggesting that abnormal activation of DCs correlates with a loss of tolerance and a higher susceptibility to SLE.

Type I IFN is known to be associated with the exacerbation of SLE disease parameters. A leukocytic interferon-stimulated gene (ISG) expression signature in patients with SLE correlates with disease outcome.[206] Type I IFNs might promote SLE by inducing the maturation of monocytes, the stimulation of autoantibody production by B cells, and NETosis. Indeed, inhibition of type I IFN signaling in mice improves SLE development, such as in the New Zealand black/New Zealand white (NZB/NZW)–derived SLE-prone experimental model.[207,208] pDCs are recognized as the type I IFN professional producer, and these cells have been proposed to be an important source of aberrant type I IFN secretion and major drivers of SLE progression. Patients with SLE have reduced numbers of pDCs in the blood and an increased accumulation of pDCs in tissue lesions.[209]

pDCs express a specific profile of endosomal nucleic acid–sensing PRR such as TLR7, TLR8, and TLR9, whereas cDCs express TLR8. pDCs can be activated by self–nucleic acids bound to antibodies, or with DNA- or RNA-binding proteins such as HMGB1 or LL37, which accumulate in NETs.[210] These complexes induce pDCs to secrete type I IFN, which in turn can drive a vicious cycle by activating MPS and driving immune activation in patients with SLE.[211,212] The exact contribution of PRR remains poorly understood because TLR7 silencing in SLE-prone mouse models improves disease progression, whereas TLR9 and TLR8 silencing have an opposite effect and exacerbate the disease development.[213,214] Recently, it has been proposed that TLR8 and TLR9 restrain TLR7 silencing in cDCs and B cells, respectively.[215] Other studies seem to suggest that B cells and DCs contribute differentially via TLR signaling to SLE syndrome, with B cells being required for renal disease and autoantibody production, whereas DCs contribute to other aspects. Considering the Myd88 signaling activated by the TLR7 and TLR9, it seems that the B cells are contributing to SLE development, whereas DCs only contribute to some specific aspect of the disease, such as skin lesions.[216] Thus, in addition to other cell types such as B cells, pDCs and cDCs might represent a key component of SLE pathogenesis and may represent attractive therapeutic targets.

## Dendritic Cells in Rheumatoid Arthritis

RA is a chronic autoimmune disease characterized by severe inflammation of the synovial joints, causing cartilage breakdown and bone destruction. The precise cause of RA remains poorly understood, although autoreactive T cells contribute to disease pathogenesis. DCs are thought to initiate the inflammatory disease through presentation of autoantigens, as suggested by animal models of RA in which DCs have been depleted, or through pro-inflammatory and regulatory effector functions.

For instance, joint DCs and monocyte-derived DCs isolated from patients can present human cartilage glycoprotein 39 (HCgp39) and epitopes from synovial fluid (SF) to antigen-specific T cells, respectively.[217] Further evidence for the contributing role of DCs to disease initiation and perpetuation in RA and the spondyloarthropathies derives from the substantial accumulation of immature and mature DC subsets, including cDCs and pDCs perivascularly in inflamed synovium, in close association with T and B cell follicles.[218] This accumulation of DCs contains the most significant numbers of mDCs and pDCs identified in any human inflammatory site.[136,218,219] DCs identified in RA synovial tissue tend to be activated because they have heightened levels of MHC, CD86,[220] and RelB.[221] They also express the receptor activator of NF-κB (RANK) and its ligand (RANKL),[222] and finally, they produce more pro-inflammatory cytokines ex vivo (IL-1, IL-6, and TNF) in response to stimulation with ICs or TLR agonists.[223]

TLR8 is expressed by inflammatory cells such as DCs and myeloid cells in RA and systemic arthritis, such as Sjögren's syndrome and Still's disease. The recent generation of transgenic mice expressing the human TLR8 has highlighted the contribution of this PRR in the development of complex spontaneous autoimmune diseases, including systemic arthritis.[224] These DCs may have migrated into the joints in response to locally produced cytokines and chemokines (CCL19 to CCL21) or may differentiate locally from myeloid progenitors in response to growth factors contained within SF.[220,225] Ectopic expression of CCL19 may also induce the formation of lymphoid tissue similar to that seen in rheumatoid synovial tissue[226] and may therefore play a key role in RA development. In the peripheral blood of persons with RA and juvenile RA, cDCs were found to be reduced relative to healthy peripheral blood, and this reduction correlated with disease severity,[227-229] supporting the idea that migration to synovium is increased by inflammatory chemotactic activity. Furthermore, cDCs in persons with RA appear to be threefold to fourfold enriched in fresh SF compared with the blood and are more differentiated than are blood DC precursors.[230]

SF DCs and blood DCs stimulate resting allogeneic T cells with equivalent efficacy; however, SF DCs are markedly more efficient stimulators of autologous T cells in the absence of exogenous antigen, suggesting that their MHC molecules are loaded with relevant RA autoantigens.[231] RA was initially believed to be exclusively Th1 driven. However, identification of Th17 cells and the cytokine IL-17 within affected tissues and/or fluids now implicates both Th1 and Th17 cells in its pathogenesis.[131] DCs are required for the differentiation of both subsets from naïve precursors. Th1 cells, which produce IFN-γ, activate macrophages to produce pro-inflammatory cytokines (IL-1, IL-6, and TNF) and induce immunoglobulin class switching to complement-fixing antibodies. IL-17 recruits leukocytes and stimulates IL-1 and TNF production by human macrophages, which all together stimulate activation of synovial fibroblasts.

Th17 cells mediate bone destruction in RA by stimulating osteoclastogenesis through RANK-RANKL interactions.[232] Subsets of synovial DCs express the cytokines essential for full differentiation of Th1 and Th17 cells (IL-12 and IL-23, respectively), providing a mechanism by which their production is locally facilitated or perpetuated. Activated DCs can also produce the chemoattractant molecule CCL5 to promote the recruitment of inflammatory Th1 and Th17 T cells.[233] Anti-citrullinated protein antibodies (ACPAs) and rheumatoid factor antibodies are presumed to bind to FcRs on macrophages and DCs, thereby inducing their activation and production of pro-inflammatory cytokines.[234] It is possible that ectopic lymphoid organs that resemble GCs and support autoantibody production are local sources of these antibodies.[235]

Finally, pDCs have also been found in rheumatoid synovium, and through production of IFN-α, they may enhance autoantibody production.[236] The number of $CD304^+$ pDCs expressing type I IFN and IL-18 was found to exceed that of the $CD1a^+$ subset of cDCs expressing IL-12 and IL-23 in RA synovium, particularly in patients positive for rheumatoid factor and ACPAs.[136] pDCs also displayed immunoreactivity for IDO[237] and may contribute to tolerance deregulation.

Altogether, human data strongly suggest multiple roles for DCs in rheumatoid synovium, local inflammation, joint destruction, and immune regulation. These data complement murine studies of inflammatory arthritis, which establish the important role of DCs in priming, inflammatory responses, and regulation of arthritic autoimmune responses.

### Dendritic Cells in Sjögren's Syndrome

Sjögren's syndrome (SS) is a chronic disease of autoimmune origin that typically affects middle-aged women. Although SS is considered a rather benign disease, there is a risk that non-Hodgkin's lymphoma may develop in persons with SS. SS is considered an autoimmune disorder because glandular and extraglandular epithelia are the target of lymphocyte infiltration and tissue damage. SS affects secretory organs, mainly the salivary and lacrimal glands, and induces oral and ocular dryness. It is also associated with a broad range of clinical manifestations related to a more diffuse autoimmune syndrome.[238] Genetic, environmental (bacterial and viral infection), and hormonal factors seem to be responsible for the disease onset. SS and other autoimmune diseases have been associated with the autoimmune/inflammatory syndrome induced by adjuvants (ASIA), which is observed as a negative side effect of vaccination.[239]

In SS, tissue-infiltrating cells are mainly $CD4^+$ T cells and some $CD8^+$ T cells, B cells, and some plasma cells.[240-242] SS-related autoantibodies include SSA/Ro, SSB/La, antinuclear antibodies, and rheumatoid factor. GC formation with follicular lymphoid DC networks has been shown in the salivary glands of persons with SS,[243-245] possibly recruiting lymphocytes to the site. Follicular DCs have also been implicated as accessory cells for B cell function.[246] Recently, it has been shown that salivary gland epithelial cells induce Tfh cell differentiation in the glands of patients with SS.[247] The function of DCs in this process remains unclear, but like salivary gland epithelial cells, DCs express membrane-bound molecules such as inducible T cell co-stimulator ligand, which play a pivotal role in Tfh cell differentiation.[248] Mouse models of SS showed that DC infiltration precedes lymphocyte infiltration into the submandibular gland, suggesting the initiating role of DCs in disease development. Increased numbers of mature DCs were detected in a mouse model and in humans with SS.[249] Decreased levels of pDCs in the blood of persons with SS is associated with their recruitment and detection into salivary glands of persons with SS but not of healthy control subjects. Given the role of pDCs and the increased levels of anti-nuclear antibodies and of type I interferon in the salivary glands,[250-252] several studies have investigated the role of Epstein-Barr, hepatitis C, human T-lymphotropic virus type I, and coxsackie virus in patients with SS,[253-255] but no evidence has been found to support their direct involvement. Interestingly, IFN-related genes are induced in SS salivary gland tissues. In addition, it seems that some noncoding RNAs, such as long interspersed nuclear element 1 retroviral elements, tend to be overexpressed and correlate with the accumulation of type I IFN. These noncoding RNAs have been shown to be immunogenic and can be sensed as DAMPs or endogenous activator of TLR7, TLR8, and TLR9. In peripheral blood, in line with previous observations in lupus and other systemic autoimmune diseases, overexpression of IFN-inducible genes as a result of peripheral type I IFN activity in association with disease activity has also been detected. Altogether, these data implicate a role for DC subsets in SS development or exacerbation.

## CONCLUSIONS AND FUTURE DIRECTIONS

DCs are a heterogeneous population of bone marrow–derived mononuclear cells that are found in an immature state in virtually all tissues in the body. As professional APCs, DCs are key effectors of innate immunity that stimulate naïve T cells, as well as B cells and NK cells, and initiate immune responses. In this way they contribute to protection against infections, maintenance of homeostasis, and also maintenance and regulation of tolerance. Their roles in tolerance regulation suggest that they might be key effectors in the development of autoimmune diseases and inflammatory diseases and also in graft tolerance and rejection. In fact, DCs are involved in the progression and maintenance of autoimmune diseases and can modify both innate and adaptive immune responses to derive autoimmune pathology. In this way the function of the different DC subsets needs to be intensively investigated to be able to specifically target the subsets implicated in specific phases of development of autoimmune disease.

### Inflammation Resolution

One of the challenges in autoimmune diseases and chronic inflammatory diseases is to contain detrimental aspects of the immune response. Several approaches have been tried using nonsteroidal drugs, glucocorticoids, and disease-modifying agents of rheumatoid diseases.[256] Anti-cytokine therapies that block TNF or its receptors provide beneficial effects in RA, psoriasis, ankylosing spondylitis, irritable bowel disease, and Crohn's disease. Other cytokines have also been targeted, such as IL-1β and IL-6 in RA and

juvenile arthritis, IL-12 p40 (subunits of IL-12 and IL-23) in psoriasis, and IL-17 in psoriasis and autoimmune uveitis. IL-21 is another product of Th17 T cells that is implicated in B cell regulation. IL-21–blocking therapies are under development to be used against autoimmune and auto-inflammatory diseases. New treatments are also in development to target cytokine and growth factor receptor pathways. The inhibition of Janus kinase (1 and 3) interferes with activation, growth, and survival of immune cells and is being investigated through inhibitors to treat specific cases of RA.[257] Other drugs are under development to target other MAPK pathways, such as p38 MAPK, SYK, and phosphatidylinositol 3-kinases.

## Immune Response Activation and Co-stimulation

The interactions between CD80/86 on APCs and CD28 on T cells results in powerful co-stimulation. CTLA-4 is a negative regulator expressed at the surface of T cells. CTLA-4–Ig fusion protein, which binds CD80/CD86 at the surface of APCs, is actively being used to block the CD28-mediated co-stimulatory pathway in order to inhibit the activation of CD28-expressing T cells and contain the T cell–mediated autoimmune response. The agonist CTLA-4–Ig has been used successfully in RA, whereas anti–CTLA-4 antibodies are approved to treat advanced melanoma. It is possible that other co-stimulatory pathways may be amenable to manipulation in the treatment of autoimmune diseases, such as PD-1 and others. A final approach is to target immune cells with antibodies to induce their depletion; in this way, an antibody against CD52, which is expressed on lymphoid and myeloid cells, or against CD19 and CD20, which are expressed by B cells, may ultimately show effects in certain conditions.

## Vaccines and Cellular Immunotherapy

Autoimmune disease could be targeted by vaccines that restore specific tolerogenic responses and promote the negative selection of autoimmune T effectors targeting autoantigens. More research is required to identify immunogenic epitopes implicated in autoimmune diseases, to develop specific tolerogenic adjuvants, and to engineer new vehicles to target APCs, such as DCs, that can mediate tolerogenic response. Different vaccine approaches could be considered, such as generating tolerogenic DC vaccines against autoimmune epitopes in order to induce Tregs, regulate B cell responses, or restore peripheral negative selection. In that light, tolerogenic DCs pulsed with peptides from citrullinated proteins are being used to actively tolerize T cells in persons with RA.

In the era of immunotherapies, substantial progress has been made in understanding innate and adaptive immune responses and how they could be regulated and better targeted to contain immunopathologies, for example, to reduce graft rejection and graft-versus-host disease and improve autoimmune disease outcome.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 10

# Mononuclear Phagocytes



Siamon Gordon • Annette Plüddemann

**KEY POINTS**

- Mononuclear phagocytes, which include monocytes, macrophages, dendritic cells, and osteoclasts, play an important role in tissue homeostasis and in cellular and humoral innate and adaptive immunity.
- Macrophage populations in many tissues derive from yolk sac and fetal liver, which persist and proliferate in the adult.
- Resident tissue macrophages display marked phenotypic heterogeneity, depending on their local microenvironment.
- Postnatally, distinct subsets of bone marrow–derived monocytes are recruited to inflammatory tissue sites.
- Microbes and cytokines (e.g., interferon-γ, interleukin [IL]-4, and IL-10) regulate macrophage activation.
- Macrophages express a range of opsonic and non-opsonic receptors to recognize and take up foreign and altered host components.
- Toll-like membrane, nucleotide oligomerization domain (NOD)-like, and cytosolic nucleic acid receptors induce alterations in macrophage gene expression and secretion of inflammatory mediators.
- Macrophages and their products contribute to tissue damage and repair and to chronic inflammation and autoimmunity.

Mononuclear phagocytes are widely distributed, biosynthetically active cells originating in the yolk sac and fetal liver during development. These cells persist in many tissues as resident macrophage populations throughout adult life. Postnatally, the bone marrow contributes circulating monocytes, which enter tissues constitutively and in response to inflammatory stimuli, giving rise to macrophages, dendritic cells (DCs), and osteoclasts. In connective tissue and bone, these cells play a major role in homeostasis, growth, and remodeling as mature macrophages (Figure 10-1) and osteoclasts. Myeloid DCs represent a distinct form of differentiation and are specialized to maintain immune tolerance or to induce humoral and cellular immunity through B and T lymphocytes. Through their recognition, antigen-presenting, regulatory, and effector mechanisms, mononuclear phagocytes contribute to a range of inflammatory, infectious, autoimmune, metabolic, and degenerative rheumatic diseases, providing targets for therapeutic intervention.

Growth of knowledge in mononuclear phagocyte biology has developed in close association with understanding of the pathogenesis and treatment of chronic arthritis. Relevant examples include the development of steroidal and nonsteroidal anti-inflammatory agents and of anti–tumor necrosis factor (TNF) monoclonal antibodies.[1] Genetic lesions in macrophage colony-stimulating factor (M-CSF) cause osteoclast deficiency and osteopetrosis in mouse models, whereas human mutations in cytosolic nucleotide oligomerization domain (NOD)-like receptors (NLRs) result in interleukin (IL)-1β overproduction and hyperinflammatory syndromes[2] that are often associated with persistent joint disease. Immune complex deposition and complement activation combine with Toll-like receptor (TLR) recognition to induce effector pathways of tissue destruction and repair.

This chapter reviews general aspects of mononuclear phagocyte differentiation, recruitment, and activation, integrating the properties of different cellular subtypes. Studies on the biology of macrophages, DCs, and osteoclasts have diverged to the extent that common features have been overlooked in the understandable search for specificity. This review is an attempt, in part, to reintegrate these differentiated sublineages. General features especially relevant to physiologic and pathologic consequences of their presence in bone, joints, and connective tissues are discussed, and gaps in our knowledge are pointed out. The emphasis is on studies in humans, where available, with reference to murine models where applicable. The subject area encompasses innate and acquired immunity, autoimmunity, and *osteoimmunology*,[3] a reflection of local specialization of mononuclear phagocyte and lymphocyte biology. Relevant topics discussed elsewhere in this textbook include innate immunity (see Chapter 17), cytokines and chemokines (see Chapter 26), osteoclast functions (see Chapter 4), and anti-TNF therapy (see Chapter 63). For further details in connection with macrophage[4-8] and DC biology,[9-11] see reviews cited in this chapter.

## OVERVIEW

Recent studies in the mouse have demonstrated that resident macrophage populations in many adult tissues, including skin, brain, liver, and lung, derive from the yolk sac and fetal liver, persisting and turning over slowly as required. The bone marrow, which develops around birth, generates circulating monocytes that can also give rise to selected tissue macrophages—for example, in the gut—as well as newly recruited, "elicited" macrophages in response to a range of inflammatory and metabolic agents. Resident and elicited macrophages differ in turnover and phenotype, but both types display marked microheterogeneity in particular

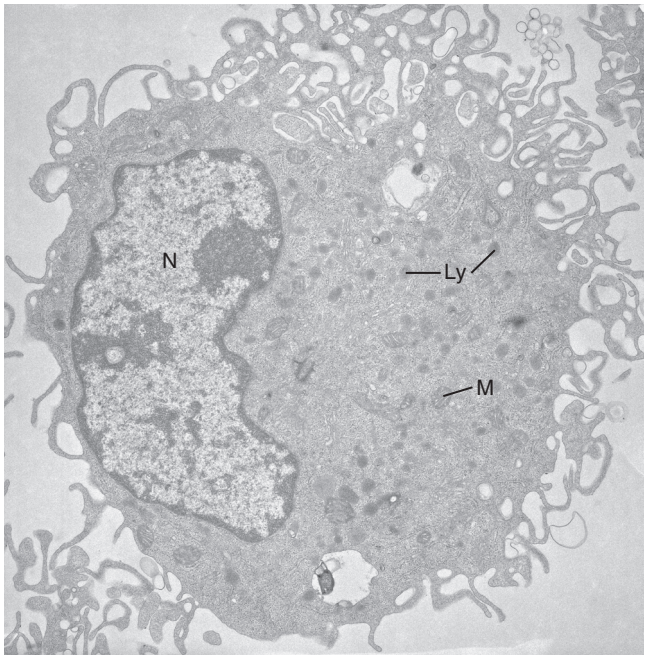



**Figure 10-1** Thin section of a bone marrow–derived mouse macrophage. Cells were cultured for 7 days and were fixed and processed for conventional electron microscopy. Ly, Lysosome; M, mitochondrion; N, nucleus. *(Courtesy Chantal de Chastellier, Centre d'Immunologie de Marseille-Luminy, Marseille, France.)*

tissue environments, such as brain and lung.[8] Similarly, and with some overlap in properties, myeloid DCs are present as heterogeneous resident sentinel cells at mucosal and cutaneous surfaces,[10-12] undergoing a complex maturation process as they migrate to lymphoid organs after capture of antigens. Monocyte-derived DCs are recruited to many tissues during infection as a result of antigenic stimulation. Plasmacytoid DCs may represent a distinct sublineage that is specialized to produce high levels of type I interferon (IFN) in response to viral stimuli. Poorly defined monocytic precursors in the blood give rise to multinucleated osteoclasts, which are specialized to resorb bone.[13]

Circulating monocytes are themselves heterogeneous, using distinct chemokine and adhesion receptors to give rise to different tissue mononuclear phagocytes.[14] These cells differ in life span depending on the recruitment stimuli and on local factors in their environment, and they express diverse plasma membrane receptors, making it possible for them to interact with many different cell types and with microbial and modified host components. Phagocytosis is a hallmark of the ability of macrophages and DCs to engulf particulates, including foreign materials, bacteria,[15] and dying host cells generated by apoptosis or necrosis.[16]

These particulates are recognized by non-opsonic receptors, including scavenger and lectin-like receptors, or after opsonization with antibodies or complement or both that enhance uptake via Fc and complement receptors. In addition, other humoral proteins, such as pentraxins, interact with their target ligands,[17] bridging them to less well-defined macrophage receptors, which regulate early cellular responses during innate immunity. TLRs play an important role in sensing the nature of the captured cargo, often interacting with the extensive repertoire of non-TLR receptors (NTRs). An extended family of NLRs senses various cytosolic ligands, resulting in a complex assembly of proteins (inflammasomes), caspase activation, and release of IL-1β.[18] Recent studies have defined additional complex sensors of nucleic acids in the cytoplasm.[19]

Uptake of particulate and soluble antigen, directly or by "cross-priming,"[20] induces DC maturation, processing, and association with class II major histocompatibility complex (MHC) molecules, as well as presentation of peptides to naïve CD4 T lymphocytes. Endogenously generated or foreign peptides generated during biosynthesis of virus glycoproteins associate with class I MHC molecules for surface recognition by cytotoxic T cells (mainly CD8). DCs and possibly macrophages regulate T cell activation or tolerance through additional co-stimulatory surface antigens and cytokines, depending in part on concomitant TLR stimulation. Activated T lymphocytes and their products, such as IFN-γ, IL-4/IL-13, and IL-10, regulate the effector functions of mononuclear phagocytes.

Digestion of macromolecules, which occurs in the vacuolar compartment, involves dynamic membrane traffic and interactions with the cytoskeleton. Complex intra-cellular pathways transduce surface and vacuole-derived stimuli to initiate formation of intra-cellular protein signaling complexes. Transcription factors translocate to the nucleus and form activation or inhibitory chromatin-binding complexes that regulate gene expression and biosynthesis of secretory products, such as TNF. Low-molecular-weight metabolites are generated by nontranscriptional mechanisms, yielding inflammatory mediators and anti-microbial activities. Osteoclasts are able to secrete hydrochloric acid and potent proteolytic enzymes into sealed-off localized areas of bone, to which they adhere tightly through specialized podosomes and actin rings.

The secretory activities of mononuclear phagocytes influence a range of cellular and extra-cellular targets to maintain tissue homeostasis but also are responsible for tissue destruction. Their trophic actions,[21] through cellular contact with other stromal cells and extra-cellular matrix and secretion, regulate tissue catabolism, cell growth, angiogenesis, and repair. In addition to local effects, macrophages contribute to systemic integration of pro-inflammatory and anti-inflammatory effects, acting on the central nervous system, endocrine organs, liver, and energy stores.

Overall, the activation phenotype of mononuclear phagocytes is modulated by extrinsic factors (e.g., cytokines and hormones), balance of surface receptors with activating/inhibitory cytoplasmic motifs, cytosolic regulators such as suppressors of cytokine synthesis (SOCS)[22] proteins, phosphorylation/dephosphorylation of signaling molecules, assembly of transcription factor complexes on chromatin, and epigenetic mechanisms such as histone and DNA methylation and acetylation. Microarray analysis of macrophages has made it possible to distinguish characteristic signatures of gene expression after innate activation via TLRs, deactivation via glucocorticoids, or modulation via cytokines. As a result, the phenotype of tissue macrophages is markedly heterogeneous, making for complexity of function in different sites in health and disease, but also providing opportunities for novel state-specific or tissue-specific targeting by drugs.

## LIFE HISTORY AND HETEROGENEITY (MACROPHAGES, DENDRITIC CELLS, AND OSTEOCLASTS)

In the adult, all three sublineages of mononuclear phagocytes originate from $CD34^+$ committed progenitor cells in the bone marrow. These cells diverge from lymphoid cells and subsequently from polymorphonuclear leukocytes, although many genes are still expressed but not translated in both neutrophils and monocytes/macrophages. Monocyte-derived DCs also express many genes in common with macrophages, although they can be distinguished by microarray analysis. In vitro, bone marrow precursors and blood monocytes can be stimulated by growth factors[23] to generate macrophages (M-CSF or granulocyte-macrophage colony-stimulating factor [GM-CSF]), DCs (GM-CSF, with or without IL-4), or osteoclasts (M-CSF and receptor activator of nuclear factor-κB [NF-κB] [RANK] ligand). Some of these growth factors also are essential in vivo (e.g., osteopetrotic, M-CSF–deficient mice lack many but not all populations of tissue macrophages and osteoclasts).[24,25] Monocytopoiesis is less well understood in humans and may depend on M-CSF and GM-CSF. Trophic interactions between hematopoietic precursors and stromal cells in the bone marrow (which are mesenchymal and hematopoietic in origin) are mediated by cell contact, via surface receptors,[21] and by soluble factors (e.g., c-Kit ligand and IL-1). Transcription factors that are essential for monocyte/macrophage production and related cells include Pu-1, other Ets family members, Maf,[26] Mi, which is implicated in microphthalmia, and GATA6, which is associated with resident peritoneal cells.[27,28] DCs express a unique transcription factor, ZDC.[29]

Although bone marrow precursors proliferate vigorously as they differentiate in the presence of M-CSF or GM-CSF, monocytes become refractory to these growth stimuli. Local macrophage proliferation can be induced by IL-4 during Th2 inflammation.[30] Restriction of DNA synthesis is associated with chromatin condensation, whereas RNA and protein synthesis persists and can be modulated by a variety of stimuli, as will be discussed further.

Tissue macrophages, DCs, and osteoclasts all derive from circulating monocytes, although these already may display heterogeneity (Table 10-1). Recent studies in transgenic mouse models and in humans have described progenitor cells and precursor-product relationships in monocyte-macrophages, as well as DC differentiation.[12] Cells are recruited constitutively to peripheral sites in the steady state. A subset of patrolling monocytes may not leave the vasculature in the steady state. Additional monocytes can be recruited to local sites in response to infectious, inflammatory, and metabolic stimuli. Such "elicited" cells display distinct properties from the "resident" cells, which, in the case of macrophages and DCs, also display marked diversity, depending on their location. A considerable body of evidence indicates that different subsets of macrophages and DCs originate from distinct monocyte populations in peripheral blood.[31-34] Apart from marker antigens (e.g., CD14, the receptor for lipopolysaccharide (LPS)-binding proteins, and Gr-1, a mouse Ly-6 antigen expressed by polymorphonuclear neutrophils and by some monocytes), levels of chemokine receptors for fractalkine (CX3CR1) and CCR2 (MCP1) seem to distinguish monocyte subsets that give rise to inflammatory and resident macrophages (see Figure 10-2). Resident macrophages and immature DCs are present in many lymphohematopoietic and nonlymphoid organs; selected markers and properties are also listed in Table 10-1. The CD68 antigen, a late endosome mucin-like glycoprotein related to the lysosome-associated membrane protein (LAMP) family, is the most broadly expressed

**TABLE 10-1** Selected Properties of Mononuclear Phagocytes and Related Cells

| Properties | Functions |
|---|---|
| A. Monocytes<br>Low level in blood (~1%-4%)<br>CD68 dim | |
| *Subpopulations:*<br>Classic (~90%) $CD14^{hi}$ $CD16^{var}$<br>Nonclassic (~10%) $CD14^{mod/dim}$ $CD16^{hi}$ | <br>Phagocytosis, secretion, inflammation<br>Patrolling, intravascular |
| *Migration/adhesion:*<br>$CCR2^+$ (classic)<br>$CX_3CR_1$, variable<br>$CD11b^+$<br>$CD11c^+$<br>LFA-1 (nonclassic) | Recruitment by inflammation, infection, immune stimulation |
| *Recognition, endocytosis, phagocytosis:*<br>Opsonic receptors (e.g., FcR, CR)<br>Non-opsonic receptors (e.g., TLR, CD36, lectins)<br>Cytosolic sensors | Clearance<br>Humoral immunity<br>Innate immunity, homeostasis<br>Inflammasome activation |
| *Biosynthesis: varied secretion:*<br>Myeloperoxidase, lysozyme<br>Respiratory burst<br>Cytokines<br>Leukotrienes<br>Tissue factor | Link to acquired immunity<br>Anti-microbial<br>Microbial killing<br>Cell activation, regulation<br>Inflammation<br>Procoagulant |
| *Differentiation/modulation:*<br>Precursor for macrophages and DC | <br>Inflammation, T cell activation |

*Continued*

**TABLE 10-1** Selected Properties of Mononuclear Phagocytes and Related Cells—cont'd

| Properties | Functions |
|---|---|
| B. Macrophages | |
| Widely distributed: lymphohemopoietic tissues as resident and recruited populations | Local and systemic homeostasis<br>Trophic functions—inflammation and repair |
| Variable life span | |
| Heterogeneous phenotype | |
| Sessile (mainly) | |
| Markers $CD68^{++}$, MerTK, CD64, RON | |
| Expressed by mouse resident peritoneal macrophages, CD169 splenic marginal zone metallophils, lymph node subcapsular sinus macrophages | |
| *Endocytosis and phagocytosis:* | Clearance, digestion, host defense |
| Opsonic (Fc, CR) | Humoral immunity |
| Non-opsonic recognition SR (e.g., SR-A, MARCO, lectins, e.g., CD206, Dectin-1) | Defense and homeostasis |
| CD163, CD71, folate receptors | Uptake hemoglobin—haptoglobin, transferrin, B12 |
| Sensing (TLR, cytosolic) | Innate immunity |
| Regulatory receptors (e.g., TREMs, F4/80 [EMR1], mouse, EMR2 [human], GPCR) | Potentiate responses |
| *Biosynthesis and secretion:* | Trophic and cytocidal |
| Pro- and anti-inflammatory cytokines | Inflammation, repair |
| IL-1, TNF, IL-6, IL-12, IL-18, IL-10, TGF-β | Innate immunity |
| Neutral proteinases, enzymes | Tissue injury |
| Low molecular weight mediators: RB, nitric oxide, prostanoids | Defense and inflammation |
| *Modulation:* | |
| Classic (M1) and alternative activation (M2) spectrum | Regulation of cellular immunity |
| *Immune interactions:* | Activation/suppression of primed T cells |
| Expression of class II MHC and co-stimulatory molecules | Adaptive immune regulation |
| C. Dendritic Cells | |
| Minor population in blood, lymph tissues | Sentinel cells |
| Short to medium-lived | Bridge innate to adaptive immunity or tolerance |
| Subsets described, varied antigen markers (e.g., class II MHC, DC-SIGN [CD209], CD11c, CD206/DEC205) | |
| Motile | Migration |
| Chemokine receptors (e.g., CCR7) | Recruitment |
| Transient adhesion | |
| Endocytosis and phagocytosis | Antigen capture |
| *Two-stage maturation/activation:* | Induced by LPS, other ligands (e.g., TLR) |
| Loss endocytosis, macropinocytosis, phagocytosis | Processing, direct and cross-presentation to naïve lymphocytes |
| Intra-cellular class II MHC redistributed to surface | |
| Limited digestion, capture peptides HLA DM | |
| Specialized endosomal compartment (multivesicular bodies, Birbeck granules) | |
| CD1d expression | Lipid antigen presentation |
| Cytosolic sensors | Nucleic acid recognition |
| Co-stimulatory molecules | Activation and inhibition of APC functions |
| TLR, PRR-dependent cytokine production (e.g., IL-12, IL-18) | Activation, regulation of tissue activation |
| D. Plasmacytoid Dendritic Cells | |
| Minor, blood, tissue | |
| Short-lived, labile | |
| Mixed phenotype markers (e.g., CD123 [IL-3R]; TLR3, 7, 9; Siglec H [mouse], RLR, helicases) | |
| *Biosynthesis, secretion:* | |
| Type I $interferon^{+}$ | Anti-viral responses |
| E. Osteoclasts | |
| Multinucleated, CD68, RON, calcitonin receptor | Markers, adhesion, and regulation |
| Tartrate-resistant acid phosphatase, vacuolar $H^{+}$ATPase, proteinase K | Resorption of living bone<br>Polarized secretion |

*Note:* Marker expression varies, depending on cell localization, maturation, and activation. Some markers also are present on other myeloid cells (e.g., polymorphonuclear neutrophils) and on selected endothelial cells. Structures and functions of receptor antigens are described elsewhere in this chapter. For further details see Schraml BU, Reis e Sousa C: Defining dendritic cells. *Curr Opin Immunol* 32:13–20, 2015.

APC, Antigen-presenting cell; ATPase, adenosine triphosphatase; CCR, CC chemokine receptor; CR, complement receptor; DC, dendritic cell; EMR, epidermal growth factor (EGF)-like module containing mucin-like hormone receptor; FcR, Fc receptor; GPR, G-protein-coupled receptor; HLA DM, human leukocyte antigen DM; IL, interleukin; LFA-1, leukocyte function–associated antigen 1; MARCO, macrophage receptor with collagenous structure; MHC, major histocompatibility complex; PRR, pattern recognition receptor; RB, respiratory burst (NADPH oxidase/superoxide, etc.); RLR, Rig-I-like receptor; RON, Recepteur d'Origine Nantais; SR, scavenger receptor; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TREM, triggering receptor expressed on myeloid cells.

Modified from Collin M, Hughes A, Plüddemann A, Gordon S: Monocytes, macrophages and dendritic cells. In Greer JP et al, editors: *Wintrobe's clinical haematology*, Philadelphia, 2014, Lippincott Williams and Wilkins.

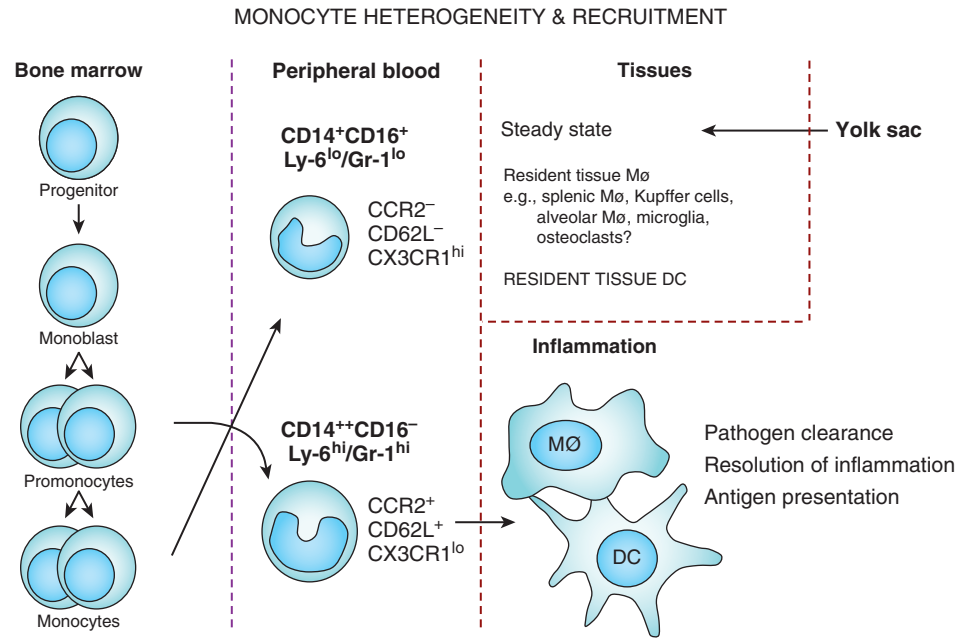



**Figure 10-2** Differentiation and distribution of mononuclear phagocytes. Distinct subpopulations of yolk sac–derived and circulating monocytes are thought to give rise to resident tissue macrophages (MØ), dendritic cells (DCs), and osteoclasts compared with cells recruited by an inflammatory stimulus. Further phenotypic heterogeneity arises from microenvironmental stimuli, such as cytokines and microbial products. *(For additional details see Ginhoux F, Jung S: Monocytes and macrophages: developmental pathways and tissue homeostasis.* Nat Rev Immunol *14:392–404, 2014; Hoeffel G et al: C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages.* Immunity *42:665–678, 2015; and Lee J, et al: Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow.* J Exp Med *212:385–399, 2015.)*

marker for all mononuclear phagocytes, although its function is still obscure.

Osteoclasts and resident macrophages are found on the surface of bone and display distinct phenotypes. Although antigenic markers for human and mouse mononuclear phagocytes are useful for phenotypic analysis, no single marker is definitive in their classification, and it is impossible, in most tissues, to distinguish locally activated resident cells from "elicited," newly recruited, and locally activated monocytes. This problem is due in part to rapid modulation of antigen expression in blood monocytes when they enter a particular tissue microenvironment. Species differences in anatomy and in marker expression are a further confounding factor.

Apart from the expression of chemokine receptors,[35] adhesion molecules play a role in selective monocyte "homing," but their differential expression by macrophages, DCs, and osteoclasts is less well defined than for lymphocyte subpopulations. Adhesion molecules include various heterodimeric integrins implicated in adhesion to endothelium, to extra-cellular matrix, and to bone. There is considerable scope for characterization of additional receptors and markers; one example is the EGF-TM7 family of leukocyte receptors, illustrated in Figure 10-3.[36] Although the F4/80 antigen has been extremely useful as a differentiation marker in the mouse, the human counterpart (EMR1) has not been of comparable value, showing a predilection for eosinophils. EMR2, a related human myeloid cell receptor, is a useful marker, although it is expressed by many tissue mononuclear phagocytes, as well as other myeloid leukocytes; it binds chondroitin sulfate proteoglycans that are broadly present in connective tissue and has been implicated in leukocyte adhesion, migration, and activation.[37]

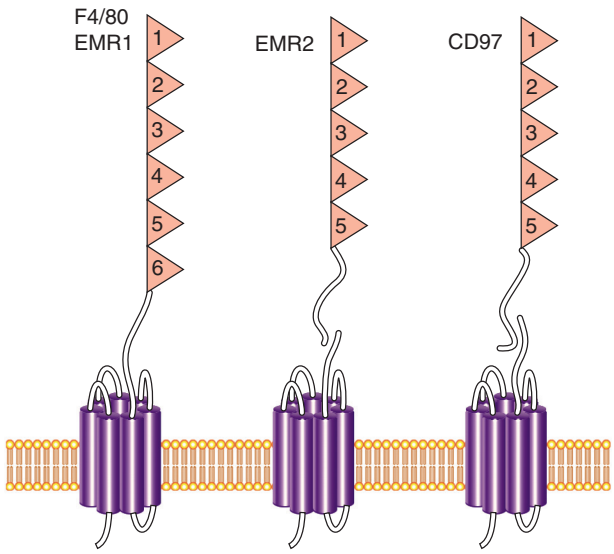



**Figure 10-3** Myeloid cell antigens of the EGF-TM7 family. These G protein–coupled receptor (GPCR)-related receptors have a large extracellular domain consisting of multiple epidermal growth factor (EGF) modules. The F4/80 antigen, an excellent marker for mouse macrophages, has been implicated in peripheral tolerance.[110] The human orthologue EGF-like module containing mucin-like hormone receptor (EMR)1 is a marker for human eosinophils. EMR2 is not expressed in the mouse but is present on human monocytes, macrophages, immature myeloid dendritic cells, and activated polymorphonuclear neutrophils. It is a useful surface marker for macrophages in tissues, including rheumatoid arthritic joints. CD97, expressed on myeloid cells and selected non-myeloid cells, is a receptor for the complement regulatory protein, CD55.[111] EMR2 and CD97 bind chondroitin sulfate B.[37]

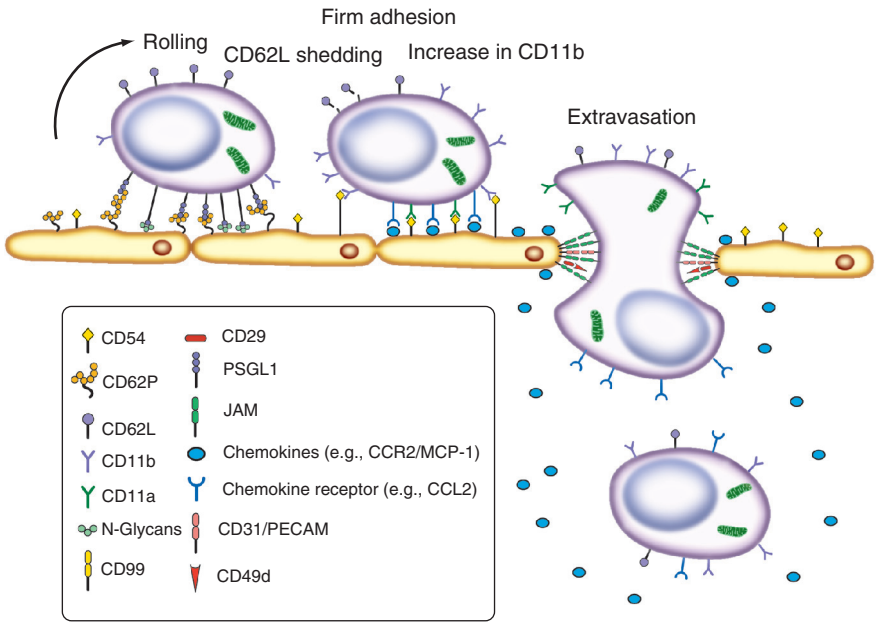



**Figure 10-4** Stages and molecular interactions implicated in monocyte recruitment by inflammatory stimuli. Diapedesis of monocytes shares features with polymorphonuclear neutrophils.

In joints, a resident synovial macrophage population is present, and recruited monocytes and macrophages are prominent in inflammatory, autoimmune, and infectious diseases, together with DCs and other myeloid and lymphoid cells. These resident and recruited mononuclear phagocytes have not yet been attributed to particular monocyte subsets. These may not be the same monocyte subpopulations that give rise to resident and recruited mononuclear phagocytes in other tissues and in response to different diseases.

## MOBILIZATION OF MONONUCLEAR PHAGOCYTES

Similar to other white blood cells, mononuclear phagocytes are distributed in intravascular and extravascular compartments, sharing common mechanisms of mobilization but also displaying distinct features among themselves and compared with other cell types. Exit from the bone marrow is controlled constitutively and on demand, but this process is not well understood, except for the role of chemokine receptors, such as CCR2 (ligands MCP1 through MCP4).[38] It is not known what determines differentiation into the mature macrophages that form part of the stromal microenvironment in bone marrow; one possibility is re-entry of circulating monocytes from blood to bone, as for osteoclasts. Apart from the circulating pool of monocytes, many mature macrophages adopt a sinusoidal distribution in liver (Kupffer cells) and in selected lymphoid and endocrine organs.[39] These cells are distinct from, but share endocytic properties with, sinusoidal endothelial cells. The constitutive exit through vascular endothelium to become tissue macrophages and DCs is not understood, whereas induced mobilization is well characterized, sharing many features with polymorphonuclear neutrophils.

Figure 10-4 summarizes stages and molecules implicated in monocyte egress. Although most monocytes exit the microvasculature by diapedesis between endothelial cells, evidence of an alternative transcytotic mechanism is present, as for lymphocytes. The roles of L-selectin, β2 and other integrins, the immunoglobulin superfamily molecule CD31, and CD99 have been established by analysis of genetic deficiencies in humans and mice[40] and by the use of monoclonal antibodies against these[41] and other defined adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and very late activation antigen-4 (VLA-4). Endothelial cell ligands implicated in monocyte adhesion include fractalkine, a tethered chemokine; other chemokines may be presented by glycosaminoglycans.

The subsequent migration and fate of mononuclear phagocytes in tissues differ strikingly. Although many resident macrophage and DC subpopulations in tissues are well characterized,[8,42] the origins of several functionally specialized cell types related to this overall lineage remain unclear.[43] Macrophages become sessile but can be induced by inflammatory stimuli to migrate to draining lymph nodes and remain there, without re-entering the circulation. Immature DCs respond to antigenic and inflammatory stimuli by migrating to draining lymphoid tissues, thereby transporting antigens for presentation to lymphocytes. The subcapsular sinus macrophages of lymph nodes have been implicated in trapping of DC antigen transported by afferent lymph[44] (Figure 10-5). DC maturation is accompanied by major changes in DC properties (Figure 10-6 and Table 10-2). DCs become highly motile and express a range of chemokine and adhesion receptors. Entry of myeloid DCs and tissue macrophages into lymphatics is less well understood but may involve interactions with the mannose receptor. As a result, DCs can present exogenous antigens and self-antigens to CD4 T lymphocytes to activate or tolerize their responses.

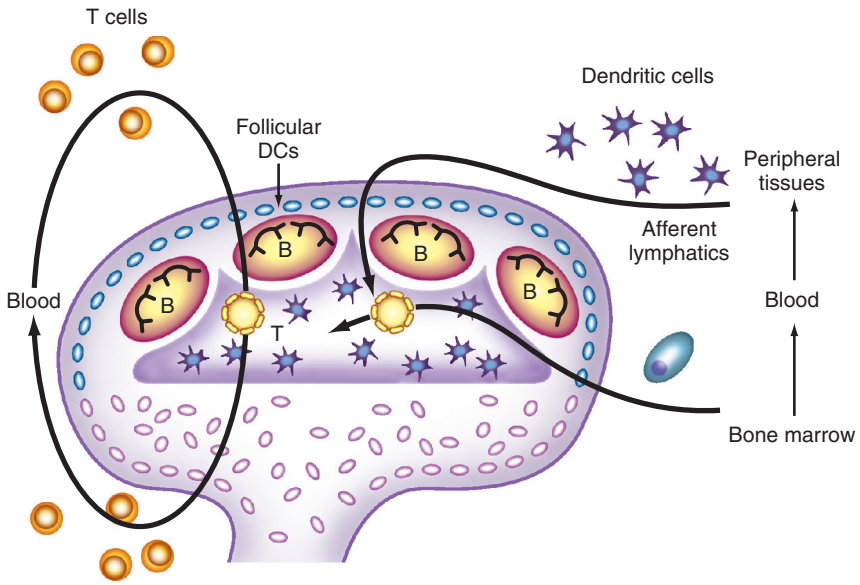



**Figure 10-5** Positioning of dendritic cells (DCs) within lymphoid tissues. Yolk sac–derived precursors enter skin and mucosal peripheral tissues and differentiate into Langerhans cells within epithelia and into other immature DCs. When established, Langerhans cells turn over independently from bone marrow–derived cells[50] (e.g., after ultraviolet irradiation). Upon exposure to stimuli (e.g., foreign antigens and local infections) and constitutively (after uptake of apoptotic cells, such as in the gastrointestinal tract), DCs undergo maturation, upregulate CCR7, and enter afferent lymphatics and secondary lymphoid tissues, where they interact with CD4 T lymphocytes. CD4 T lymphocytes become activated, interacting with B cells or CD8 T lymphocytes, and re-enter blood, homing to peripheral sites. DCs also are able to interact with innate lymphocytes and natural killer cells. Follicular DCs in B cell areas have a distinct, poorly defined bone marrow origin, express novel antigenic markers, and are able to capture immune complexes through complement activation. Plasmacytoid DCs have a distinct interfollicular location and express markers of a myeloid and a lymphoid nature. Subcapsular macrophages resemble splenic marginal metallophilic macrophages and contribute to antigen capture. *(Courtesy R. Steinman.)*

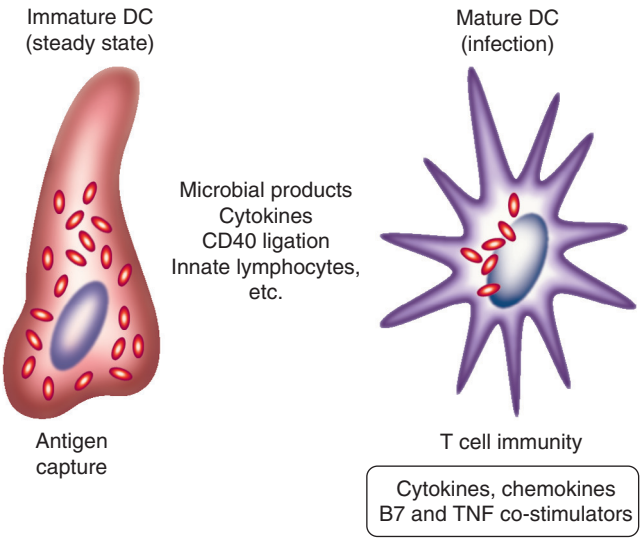



**Figure 10-6** Dendritic cell (DC) maturation. In the steady state, immature DCs are actively endocytic, capturing antigens efficiently through pattern recognition receptors. In response to a range of exogenous and endogenous stimuli, they translocate major histocompatibility complex class II to the surface, express a range of co-stimulatory molecules and secretory products, and become efficient antigen-presenting cells. As a result, DCs control adaptive immunity or induce tolerance. TNF, Tumor necrosis factor. *(Courtesy R. Steinman.)*

**TABLE 10-2** Maturation Markers for Human Monocyte–Derived Dendritic Cells

| | Immature | Mature |
|---|---|---|
| **Enhancer/Co-Stimulatory Molecules** | | |
| CD80 (B7-1) | Low | High |
| CD86 (B7-2) | Low | High |
| CD83 | | De novo |
| EMR2 (CD312) | High | Moderate/low |
| **Antigen Uptake** | | |
| FcγRII | High | Low |
| **Signaling** | | |
| CD40 | Low | High |
| CXCR4 | Low | High |
| CCR5 | High | Low |
| CCR6 | High | Low |
| CCR7 | Low | High |
| **Antigen Presentation** | | |
| HLA-DR (class II) | Moderate | High |
| HLA-DQ (class II MHC) | Moderate | High |
| CD1a | High | Low |
| **Others** | | |
| DC-SIGN (CD209) | High | Low |
| CD14 | High/moderate | Negative |
| CD123 (IL-3R) | Low | High |

*Note:* In addition to the listed antigens and antigen-presenting cell functions, dendritic cell production of and response to other growth factors, chemokines and cytokines, microbial products, and immune complexes varies during maturation and activation in vitro. Validation in vivo is incomplete.

DC-SIGN, Dendritic cell–specific intra-cellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin; EMR2, epidermal growth factor (EGF)-like module-containing mucin-like hormone receptor-like 2; HLA, human leukocyte antigen; IL, interleukin; MHC, major histocompatibility complex.

Although in vitro systems are widely used to generate DCs by cultivation of monocytes or bone marrow precursors in cytokine-supplemented media (GM-CSF, with or without IL-4), their properties may not correspond to those of DC populations in vivo.

Mononuclear phagocytes use integrins and receptors for extra-cellular matrix such as CD44, thus regulating the dynamics of their adhesion and migration. Osteoclast adhesion to bone depends on αvβ3 and possibly other adhesion molecules implicated in podosome attachment. Plasma membrane receptors such as CD97 and EMR2, members of the EGF-TM7 family, and triggering receptor expressed on myeloid cells (TREM)-1,[45] an immunoglobulin superfamily molecule with an immunotyrosine-based activation motif (ITAM) (see later discussion), regulate myeloid cell effector functions and adhesion and migration within extravascular tissue compartments.

Monocytes recruited to tissues by poorly degradable foreign materials or by selected microbial pathogens or parasites form granulomas, that is, organized structures rich in macrophages, incorporating other myeloid and lymphoid cells, fibroblasts, and extra-cellular matrix. Pathogen-induced granuloma formation[46] depends on adhesion molecules such as CR3, a β2 integrin, TNF, and chemokine receptors such as CCR4; granuloma macrophages express abundant secretory products such as lysozyme[47] and pro-inflammatory cytokines. Characteristic morphologic evidence of macrophage differentiation in granulomas includes epithelioid cell and multinucleated giant cell formation, such as with *Mycobacterium tuberculosis* infection. Giant cells arise by monocyte recruitment and macrophage fusion, rather than by impaired cytokinesis.[48,49] Cytokines such as IL-4 and IL-13, acting through a common receptor chain, and GM-CSF promote macrophage fusion. Foreign surfaces including biomaterials also can play a role in granuloma formation, and chemokine receptors have been implicated in the induction of foreign body–induced giant cells.

The surface molecules involved in these examples of macrophage homokaryon formation and the functional significance of induced fusion are still poorly understood. By contrast, multinucleation in osteoclasts is a physiologic process, dependent on M-CSF and RANK ligand, and several plasma membrane (CD44, CD9, TREM-2, and DC-STAMP) and intra-cellular (c-src and c-fos) molecules have been implicated in this differentiation process. Multinucleation is thought to favor efficient localized resorption of bone. Osteoclasts become polarized for secretion and display highly active plasma membrane ruffling. Interactions with osteoblasts; local cytokines, such as osteoprotegerin; and circulating hormones, including calcitonin, parathormone, and vitamin D metabolites, regulate their gene expression and function, as is discussed in Chapter 4. Vitamin D receptors also modulate the function of macrophages and DCs.

The turnover of different mononuclear phagocytes varies, depending on their activation status and tissue localization.[50] Resident macrophages can live for weeks or months, whereas inflammation reduces survival to hours or days. DCs are relatively short-lived cells; osteoclast turnover in vivo has not been studied in detail. The role of apoptosis in mononuclear phagocyte turnover is poorly characterized, in contrast to that in polymorphonuclear neutrophils, but cell survival is regulated by growth factors such as M-CSF and by interactions with neighboring cells and pathogens.

## RECOGNITION

In the past decade, emphasis on the problem of immune recognition has shifted to a considerable extent from somatically rearranged receptors for peptide on lymphocytes to germline-encoded receptors on myeloid antigen-presenting cells (APCs).[51-54] Previous studies on myeloid cells focused on well-known opsonic receptors for antibody (Fc receptors [FcRs]) and complement (complement receptor). The concept of direct pattern recognition receptors for conserved structures on microbes provided an impetus to studies on innate immunity, reinforced by the discovery of TLRs. More recently, investigation of numerous NTRs, including a range of lectin-like recognition molecules and of scavenger receptors (SRs), markedly enhanced knowledge of the recognition repertoire.[18] Finally came the discovery of cytosolic recognition proteins, the NLR, retinoic acid inducible gene (RIG)-I families, and AIM2,[55] which are implicated in genetic and microbially induced hyperinflammatory syndromes, Crohn's disease, and nucleic acid sensing.

These molecules are mainly, although not exclusively, expressed by macrophages and DCs and play an important role in immune responses to foreign or modified host ligands, resulting in inflammation and autoimmunity. Given the complexity of microbial and cellular particulates compared with discrete soluble ligands, different receptors collaborate and synergize with one another to activate or inhibit subsequent APC responses. The original distinction drawn between pattern recognition receptors for exogenous, so-called pathogen-associated molecular patterns and for endogenous, host-derived ligands has been eroded to some extent because individual receptors are able to bind both types of ligand,[56] although their distinct cellular expression and signaling responses may account for discrimination by host APCs. In particular, recognition and uptake of apoptotic cells result in downregulation of macrophage effector molecules, as opposed to the pro-inflammatory effects induced by microbial ligands. The signaling pathways induced by other, modified host-derived ligands, such as oxidized lipoproteins and hyaluronates, are not well characterized.

This section summarizes selected receptor structures, indicates some of their ligands, and addresses briefly signaling and antigen-processing pathways. Further details are provided in Chapter 17, which also illustrates interactions between humoral and cellular arms of the innate response.

### Toll-Like Receptors

Key features of TLRs are illustrated elsewhere (see Chapter 17).[57,58] TLRs consist of homodimers or heterodimers of transmembrane glycoproteins containing extra-cellular leucine-rich repeats (LRRs) and cytoplasmic Toll/IL-1 receptor (TIR) domains, similar to the intra-cellular domain of the IL-1 receptor, which contains extra-cellular immunoglobulin superfamily domains. TLRs are expressed on the surface of APCs or, in the case of TLR3, TLR7/8, and TLR9, on vacuolar membranes, contributing to pro-inflammatory

and immunogenic signaling after "sensing" of cargo. Isolated TLRs do not bind ligands directly but collaborate with humoral factors and with other plasma membrane glycoproteins, as in the well-studied case of TLR4, the glycosyl phosphoinositide (GPI)-anchored CD14 receptor for the plasma LPS-binding protein and the membrane-associated molecule MD2.

## Non–Toll-Like Receptors

NTRs constitute a variety of lectin-like,[53,59] scavenger, and other plasma membrane glycoprotein non-opsonic receptors; selected examples are illustrated in Figures 10-7 and 10-8. They include type 1 and 2 transmembrane molecules, with C-type lectin or lectin-like, collagenous, or immunoglobulin superfamily domains. In addition, macrophages express sialic acid–binding receptors, which are members of the SIGLEC family,[60] as do plasmacytoid dendritic cells (pDC). Table 10-3 summarizes known ligands and potential functions relevant to their role in homeostasis and immunity. Apart from direct recognition of selected self, modified self, and foreign structures, they play an important role in cell-cell and cell-matrix interactions; in phagocytosis and endocytosis; and in less well-understood processes, such as targeted delivery of antigens to peripheral lymphoid organs.[61]

Receptors that promote rapid clearance of apoptotic cells, directly or after opsonization by complement and other extra-cellular proteins, are shown in Figures 10-9 and 10-10. More recent research has shown collaboration of different receptors at the cell surface,[62] regulating cellular responses. Other regulatory surface receptors include transmembrane molecules with tyrosine-based activating (ITAM) or inhibitory (ITIM) cytoplasmic motifs—for example, C-type lectin-like molecules, immunoglobulin superfamily members such as FcRs, discussed further subsequently, and receptor-ligand pairs that regulate macrophage activation (CD200/CD200R)[63] and phagocytosis (SIRPα and CD47; Figure 10-11).[64]

## Complement Receptors

Complement receptors are heterogeneous receptors expressed by APCs (Figure 10-12) that mediate direct and lectin- and antibody-dependent binding of activated complement components and play a role in cell migration and phagocytosis and immune regulation.[65] The discovery of a novel complement receptor selectively expressed by Kupffer cells suggests that mononuclear phagocyte heterogeneity in complement receptor function provides evidence of tissue specificity for some macrophage subsets.[66] Genetic deficiencies in complement receptor expression and their ligands have provided insights into the pathogenesis of autoimmune disease and of rheumatic joint injury.[67] Complement deposition on follicular DCs has been implicated in the activation of B cell responses. Complement regulatory proteins are expressed by many cell types and by APCs and play an important role in limiting cell activation. In addition, complement receptors collaborate with other receptors to modulate myeloid cell responses. Their signaling pathways are less well defined than those of FcRs.

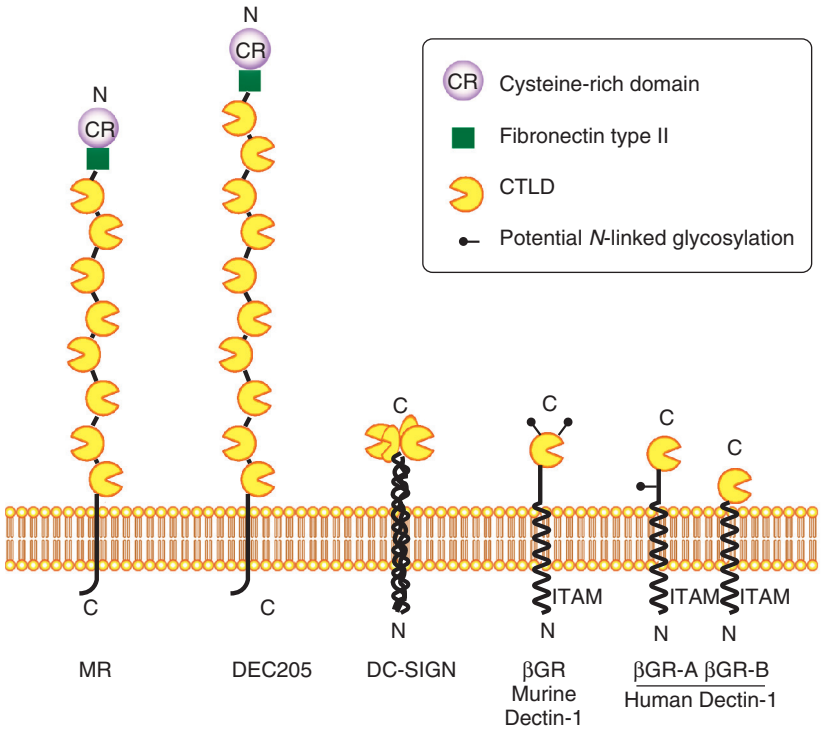



**Figure 10-7** Selected lectin-like receptors expressed by macrophages and dendritic cells (DCs; see Table 10-3 for ligands). These receptors play an important role in non-opsonic adhesion, cell-cell interactions, endocytosis, and phagocytosis. The cysteine-rich (CR) domain of the mannose receptor (MR)[53,54] has been postulated to play a role in transport of glycoconjugates to peripheral lymphoid organs for clearance or antigen-dependent activation of B lymphocytes.[61] DEC205 has been used to show efficient targeting of antigens to DCs.[112] The immunoreceptor tyrosine-based activation motif (ITAM)-like motif of Dectin-1, the β-glucan receptor (βGR), is essential for myeloid cell and Th17 lymphoid responses to fungal particles, in collaboration with Toll-like receptor[59,62,91,113] and nucleotide oligomerization domain (NOD)-like receptor. CTLD, C-type lectin domain.

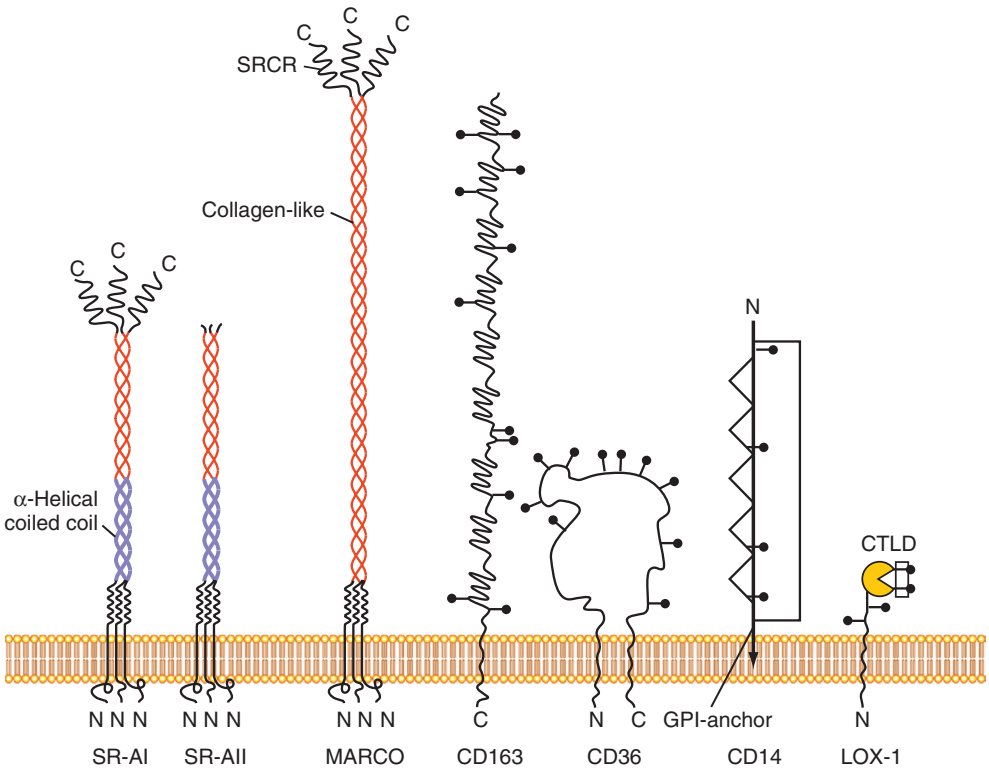



**Figure 10-8** Selected scavenger receptors. These non–Toll-like receptors (NTLRs) consist of diverse structures and bind a range of microbial and endogenous ligands (see Table 10-3).[114,115] They differ in regulation and expression, providing useful markers of macrophage activation (CD14, macrophage receptor with collagenous structure [MARCO]),[15,115,116] heterogeneity (CD163, the scavenger receptor [SR] for clearance of haptoglobin-hemoglobin complexes),[98] and lipid homeostasis (SR-A, CD36, and lectin-like oxidized LDL receptor-1 [LOX-1]).[114] Scavenger receptors such as MARCO can collaborate with TLRs in antigen-presenting cell responses to microbes.[117] CTLD, C-type lectin domain; GPI, glycosyl phosphoinositide; SRCR, scavenger receptor cysteine-rich.

**TABLE 10-3** Ligands for Selected Non-Opsonic, Non–Toll-like Receptors

| Class | Receptor | Microbial Ligands | Endogenous Ligands | Function |
|---|---|---|---|---|
| Scavenger receptors | SR-A I/II | Gram-positive/gram-negative bacteria | Apoptotic cells | Phagocytosis |
| | | Lipoteichoic acid<br>Lipid A<br>Neisserial surface proteins | Modified low-density lipoproteins<br>AGE-modified proteins<br>β-Amyloid | Endocytosis<br>Adhesion<br>Foam cell formation |
| | MARCO | Gram-positive/gram-negative bacteria<br>Neisserial surface proteins | Marginal zone B lymphocytes<br>Uteroglobin-related protein | Adhesion<br>Phagocytosis<br>Innate activation |
| | CD36 | Deacylated lipopeptide from gram-positive bacteria | Apoptotic cells (with thrombospondin and vitronectin receptor) | Uptake, exchange of lipids, macrophage fusion |
| | | *Plasmodium falciparum*–parasitized erythrocytes | HDL<br>Outer rod segments | Adhesion |
| Lectins | Dectin-1 | β-Glucan | T lymphocytes (noncarbohydrate) | Fungal uptake and immunomodulation |
| | DC-SIGN | Mannosyl/fucosyl glycoconjugates including viruses (e.g., human immunodeficiency virus-1) | ICAM 2/3 T lymphocytes | Adhesion<br>Endocytosis |
| | Mannose receptor CRD | Mannosyl/fucosyl glycoconjugates on bacteria, viruses (e.g., dengue), fungi, parasites | Lysosomal hydrolases<br>Thyroglobulin<br>Ribonuclease<br>β-Amylase | Adhesion<br>Endocytosis |
| | Cysteine-rich domain | | Sulfated carbohydrates in marginal zone (spleen) and subcapsular sinus (lymph node) | Antigen targeting |
| | Fibronectin type II domain | | Collagens | Adhesion |

*Note:* This table illustrates the dual recognition properties and diverse cellular functions of selected non–Toll-like receptors. For additional details, see references 53, 115, and 122-125.

AGE, Advanced glycation end product; CRD, carbohydrate recognition domains ($Ca^{++}$-dependent); DC-SIGN, dendritic cell–specific intra-cellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin; HDL, high-density lipoprotein; ICAM, intercellular adhesion molecule; MARCO, macrophage receptor with collagenous structure.

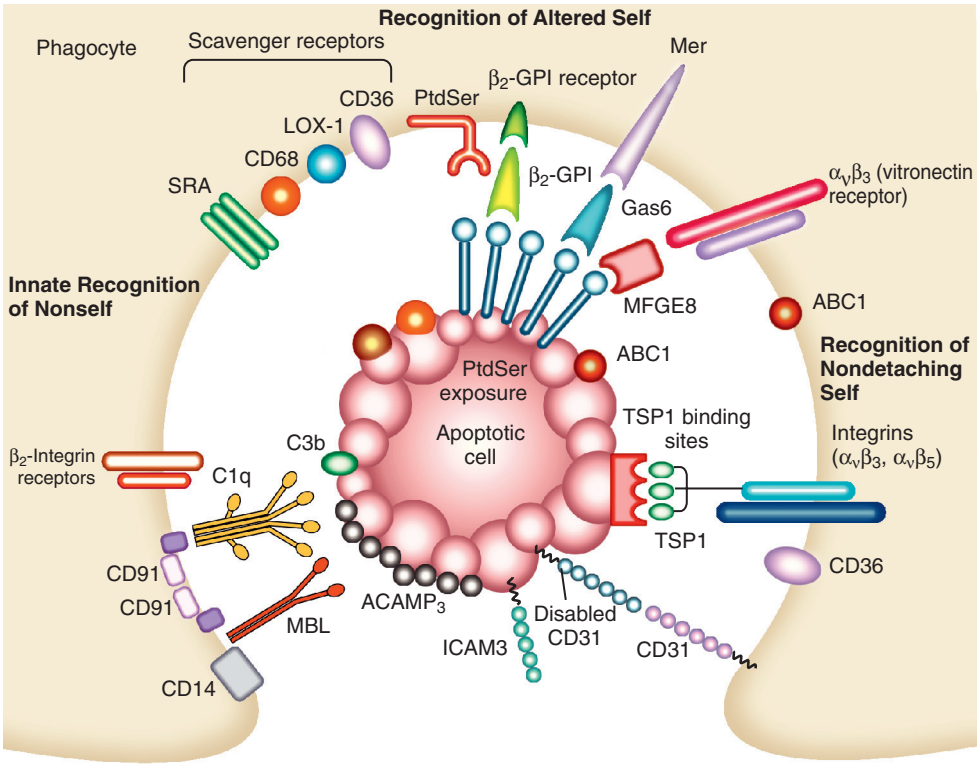



**Figure 10-9** Phagocytic receptors for apoptotic cell phagocytosis.[118] Macrophages (MØ) and immature myeloid dendritic cells (DCs) are the main immune cells involved in the clearance of apoptotic cells. They express broadly similar multiple receptors, which can bind directly or via opsonic soluble proteins (e.g., mannose-binding lectins [MBLs] to ligands). Phosphatidylserine (PtdSer) becomes exposed on the outer surface of the apoptotic cell, and a receptor for this ligand has been long sought. A new receptor (T cell immunoglobulin mucin (TIM)4 and related TIM1) has been discovered on resident MØ, with specificity for PtdSer. Other MØ populations use MFGE8 (a milk fat globulin protein secreted by MØ) as an opsonin. Discrimination of non-self and altered self may involve combinations of different phagocyte receptors. Apoptotic cell uptake results in an anti-inflammatory response by MØ (e.g., release of transforming growth factor-β and prostaglandin $E_2$) and has been implicated in cross-presentation by DCs. ABC1, Adenosine triphosphate-binding cassette 1; $ACAMP_3$, apoptotic cell–associated molecular pattern 3; GPI, glycosyl phosphoinositide; ICAM3, intercellular adhesion molecule-3; LOX-1, lectin-like oxidized LDL receptor-1; MFGE8, milk fat globule–epidermal growth factor 8 protein; SRA, scavenger receptor A; TSP1, thrombospondin 1. *(From Savill J, Dransfield I, Gregory C, et al: A blast from the past: clearance of apoptotic cells regulates immune responses.* Nat Rev Immunol *2:965–975, 2002.)*

## Fc Receptors

APCs express diverse receptors for monomeric and complexed immunoglobulins that regulate effector responses to antigens. Figure 10-13 illustrates the properties of human FcRs with activating or inhibitory motifs in their cytoplasmic tails. FcR polymorphisms have been implicated in autoimmune human diseases such as systemic lupus erythematosus (SLE). Studies on genetically manipulated mouse models have led to considerable insights into the role of individual FcRs in immunopathogenesis. FcRs are able to cooperate with chemokine and other receptors. The nature and role of Fc-dependent and Fc-independent interactions of antibody with APCs are under investigation and include lectin-like recognition of sialylated[68] and mannosyl[69] residues.

## Cytosolic Receptors

Identification of microbial peptidoglycan breakdown and other products as ligands for an intra-cellular family of leucine-rich repeat (LRR)-containing cytosolic sensors that regulate caspase activation and IL-1β secretion, especially in macrophages, provided a major impetus to this burgeoning field.[2,18] Other chapters describe examples of NLRs and their role in hyperinflammatory syndromes, as well as RIG-I–like and other nucleic acid sensing receptors. NLRs and other cytosolic receptors participate in signaling pathways implicated in TLR-induced functions and play a major role in inflammasome activation, caspase-1 activation, and IL-1 and IL-18 release.

RIG-I and melanoma differentiation–associated protein 5 (MDA-5) are members of the RIG-I–like receptor (RLR) RNA helicase family, which are found in macrophages and other myeloid cells, as well as in nonhematopoietic cells. They play a role in recognition of viral RNA, such as single-stranded RNA with 5′-triphosphate and/or double-stranded RNA (see later discussion). Although distinct, they share signaling features (e.g., recruitment by adapter IFN-β promoter stimulator 1 [IPS; also known as *MAVS*, *CARDIF*, or *VISA*] to the outer membrane of mitochondria) and activate transcription factors involved in type I IFN induction and anti-viral immunity. RLR recognition of RNA is predominant in macrophages, conventional (c)DCs, and fibroblasts, but plasmacytoid (p)DCs utilize TLR preferentially.[70] Gene deletion[71] or silencing has shown that RIG-I modulates the generation and differentiation of granulocytes in the absence of virus infection and that it is involved in

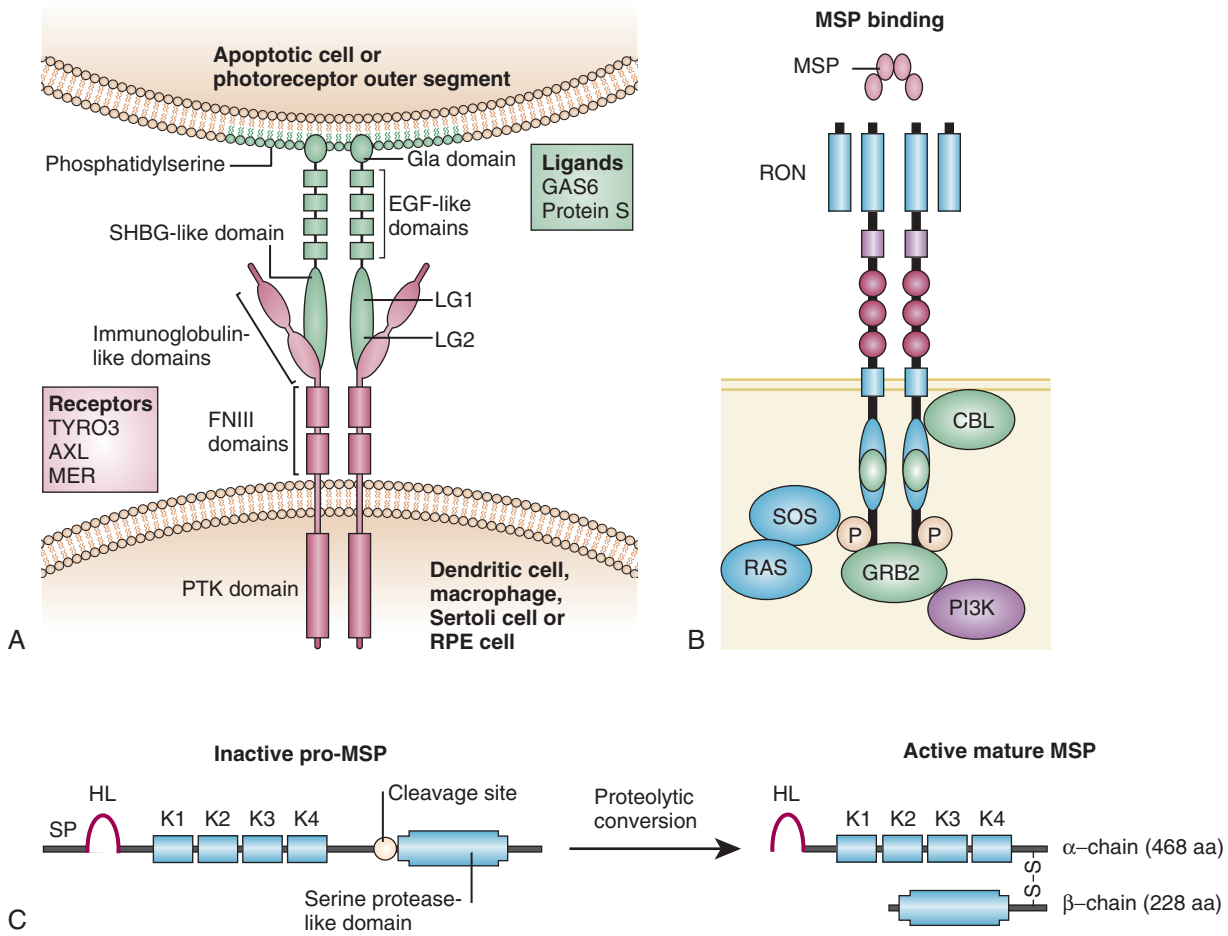



**Figure 10-10** Receptor protein tyrosine kinases expressed by mononuclear phagocytes. **A,** TYRO3, AXL, and MER are receptor protein tyrosine kinases (PTKs) that are expressed by dendritic cells (DCs), macrophages, and immature natural killer (NK) cells of the immune system, Sertoli cells of the testis, retinal pigment epithelial (RPE) cells of the eye, and several other cell types. TAM receptor dimers bind to their two ligands, growth-arrest–specific 6 (GAS6) and protein S, through interaction between the two N-terminal immunoglobulin-like domains of the receptors and the two C-terminal laminin G (LG) regions, which together make up the sex hormone binding globulin (SHBG) domain, of the ligands. (The solved x-ray crystal structure of the GAS6 SHBG domain bound to the immunoglobulin domains of AXL7 reveals that both ligand and receptor crystallize as dimers.) Via their N-terminal Gla domains, GAS6 and protein S then bind to phosphatidylserine that is displayed on the extra-cellular surface of the plasma membranes of apoptotic cells or on the outer segments of photoreceptors. **B,** Macrophage-stimulating protein (MSP)-induced Recepteur d'Origine Nantais (RON) dimerization is the classical mode of activation. RON is selectively expressed by resident peritoneal mouse macrophages and osteoclasts. **C,** Pro-MSP is cleaved at Arg554–Val555 to form a 50 kDa α chain and a 30 kDa β chain linked by a disulphide bond. The α chain contains a hairpin loop (HL) followed by four kringle domains (K1–K4). The β chain has a serine protease-like domain harboring a high-affinity binding site for RON4. CBL, Casitas B-lineage lymphoma E3 ubiquitin-protein ligase; EGF, epidermal growth factor; FNIII, fibronectin type III; GRB2, growth factor receptor-bound protein 2; PI3K, phosphatidylinositol 3-kinase; RAS, small GTPase encoded by ras gene; SOS, son of sevenless; SP, transcription factor binding site SP-1. (***A,*** *From Lemke G, Rothlin CV: Immunobiology of the TAM receptors.* Nat Rev Immunol *8:327–336, 2008.* ***C,*** *From Yao HP, Zhou YQ, Zhang R, Wang MH: MSP-RON signalling in cancer: pathogenesis and therapeutic potential.* Nat Rev Cancer *13:466–481, 2013.)*

phagocytosis of bacteria by LPS-stimulated macrophages, indicating broad function in innate immunity.[72]

AIM2 is a DNA-sensing cytosolic protein that triggers the NACHT domain, LRR domain, and pyrin domain (PYD)-containing protein (NALP)-independent inflammasome.[55] It contains an N-terminal pyrin domain and a C-terminal oligonucleotide binding domain. It binds double-stranded DNA, recruits the inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD (ASC), and becomes localized to ASC-containing speckles, forming a pyroptosome and inducing pyroptotic cell death mediated by caspase-1. RNA silencing impairs DNA-induced maturation of IL-1β in THP-1 human monocytic cells, abrogating caspase-1 activation by double-stranded DNA or *Vaccinia* infection. PYHIN proteins AIM2 and IFI16 form a new family of innate DNA sensors, termed *AIM2-like receptors* (ALRs).[73]

## Damage/Danger-Associated Molecular Patterns

Host-derived molecules that activate TLR pathways and inflammasomes such as NLRP3 include nuclear and cytosolic proteins (e.g., HMGB1 and S-100), matrix components (hyaluronate), adenosine triphosphate (ATP), and uric acid. They are released by cell stress, especially necrosis, and contribute to the initiation and perpetuation of sterile and infectious inflammation. Other receptors include receptor for advanced glycation end products (RAGE) and possibly scavenger receptors, although ligands are promiscuous

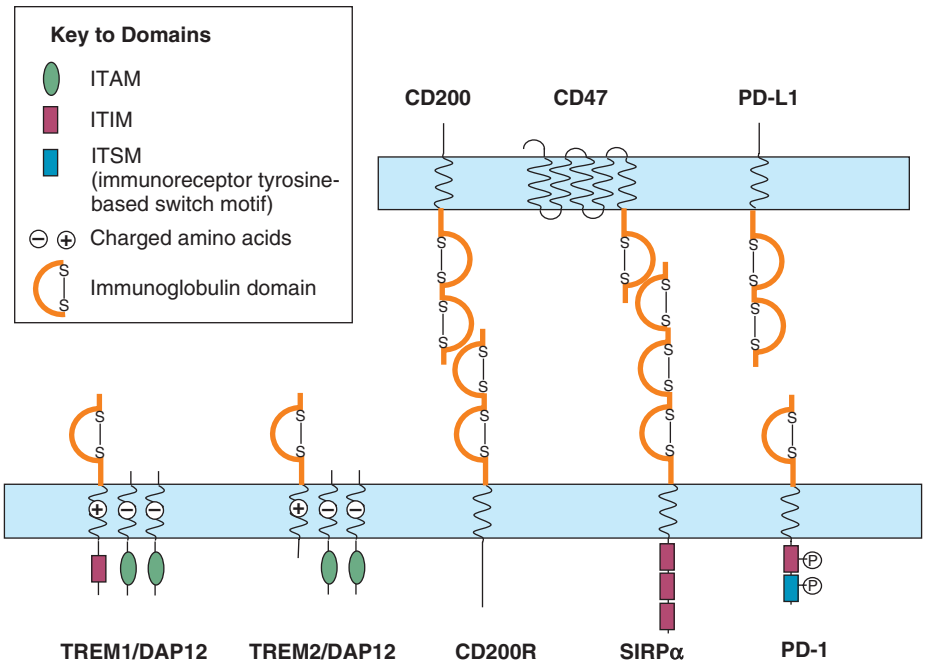



**Figure 10-11** Surface receptors that, singly or as paired receptors, modulate macrophage responses. Illustrated are immunoglobulin superfamily and associated transmembrane molecules using immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs) to generate activating or inhibitory signals. DAP12 can associate with a range of other membrane molecules as a signaling partner in macrophages, dendritic cells, and natural killer cells.[119] Note the charged amino acid residues in transmembrane domains. CD200 and CD47, which are broadly expressed in tissues, generate inhibitory signals in macrophages via CD200R[63] and signal regulatory protein α (SIRPα).[55] In CD200 knockout mice, macrophages show spontaneous activation.[109] Macrophages themselves are induced by Toll-like receptor stimuli to express CD200.[63] The extracellular region of programmed cell death 1 (PD-1) consists of a single immunoglobulin (Ig)V-like domain, and its cytoplasmic region contains an ITIM and an immunoreceptor tyrosine-based switch motif (ITSM). The expression of PD-1 can be induced not only in T cells but also in B cells and myeloid cells, suggesting PD-1 has broader roles in immune regulation. PD-L1 is a type I transmembrane protein with IgV- and IgC-like domains in the extracellular region. PD-L1 is constitutively expressed in T cells, B cells, macrophages, and dendritic cells and further upregulated after activation of these cells. TREM, Triggering receptor expressed on myeloid cells. *(From Okazaki T, Honjo T: The PD-1-PD-L pathway in immunological tolerance.* Trends Immunol *27:195–201, 2006).*

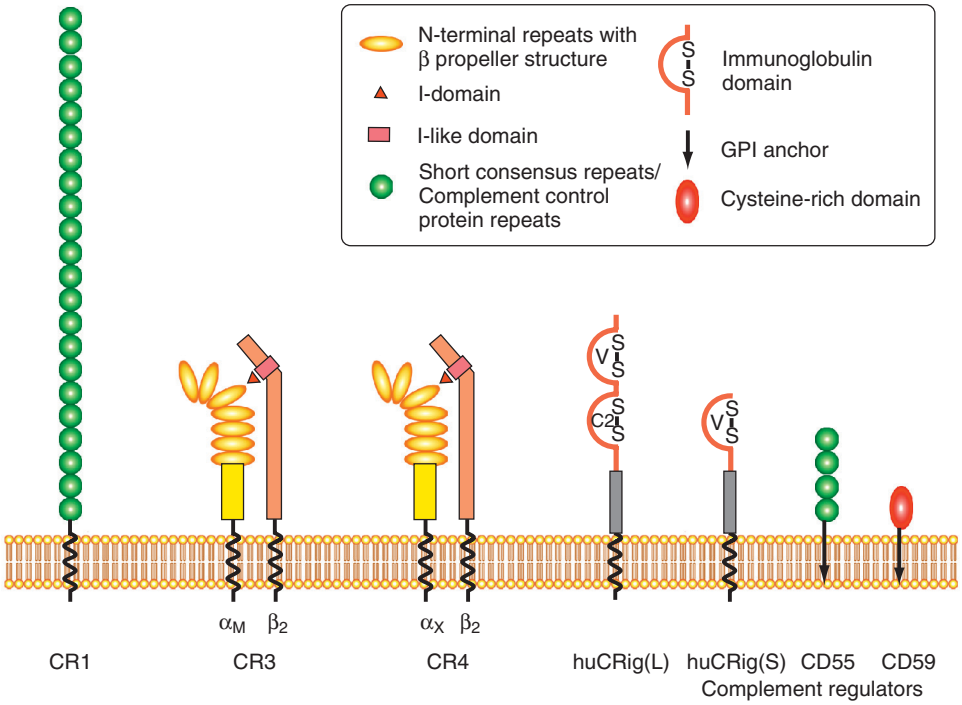



**Figure 10-12** Complement receptors and membrane regulators expressed by macrophages. CR1 is broadly expressed by nucleated cells, acting as a "sink" for activated complement; CR3 (CD11b/CD18), a phagocytic receptor for C3b-coated particles, and CR4 (CD11c/CD18) are β2 integrins, which, together with leukocyte function–associated antigen 1 (LFA-1) (CD11a/CD18), mediate adhesion of myeloid cells to endothelium and extra-cellular matrix and migration. huCRIg L and S are long and short forms of a complement-binding receptor on Kupffer cells, which mediate uptake of opsonized bacteria.[66] CD55 and CD59 are glycosyl phosphoinositide (GPI)-anchored regulators of complement activation.

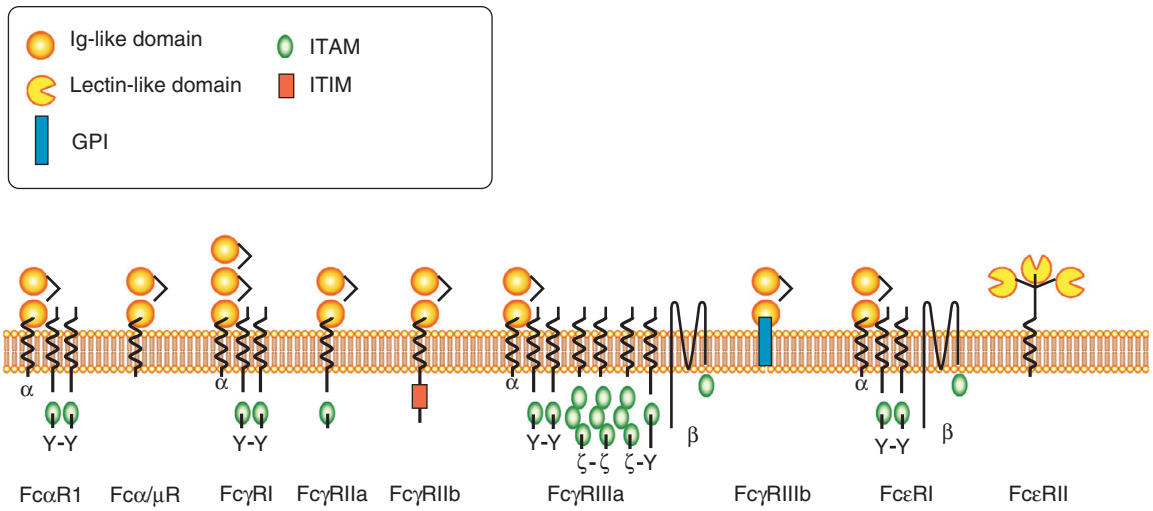



**Figure 10-13** Human Fc receptors. Myeloid cells express a range of classic Fc receptors that initiate a variety of cellular responses, including phagocytosis, antibody-dependent cell-mediated toxicity, antigen presentation, respiratory burst, and release of inflammatory mediators. Immunoglobulin subclasses are bound by extra-cellular domains; signaling via cytoplasmic immunotyrosine activation motif (ITAM) or immunotyrosine inhibition motif (ITIM) is mediated by associated membrane-spanning polypeptides. Activation and inhibitory receptors usually are co-expressed on the cell surface and function in concert, determining the magnitude of effector cell responses. A range of Fc receptor–like molecules (immunoglobulin superfamily [IGSF] extra-cellular domains) with similar ITAM/ITIM cytoplasmic motifs are mainly expressed by B or T/natural killer cells; they may regulate lymphocytic differentiation and responses.[120] GPI, Glycosyl phosphoinositide.

and often are poorly defined. The concept gained currency from work by Matzinger and colleagues.[74] Various hypotheses have been proposed for inflammasome activation, including $K^+$ efflux, membrane pore formation, lysosome disruption and cathepsin release, and reactive oxygen species (ROS), which may be necessary but is not sufficient for caspase activation (Figure 10-14).[75]

Figures 10-15 and 10-16 summarize features of intracellular nucleic acid recognition and signaling, including a model for cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS)-mediated cytosolic DNA sensing.

## RESPONSES AND MODULATION

This section summarizes some of the major cell biologic effects resulting in antigen processing and presentation by APCs. Other chapters address related topics, such as recognition of lipids by CD1 and intra-cellular trafficking of related molecules.

### Phagocytosis and Endocytosis: Antigen Processing

The vacuolar apparatus of APCs is illustrated schematically in Figure 10-17.[76,77] Internalization of the plasma membrane results in phagosome/endosome formation, with progressive acidification and digestion, depending on delivery of vesicles and their hydrolytic contents. Membrane and receptors are recruited, modified by maturation, and retrieved by recycling. Further fusion with Golgi-derived vesicles and primary lysosomes yields phagolysosomes and secondary lysosomes, reaching a pH of approximately 5.5 to 6.0. Depending on the bulk of plasma membrane internalized and the size of the particle, the uptake process involves cytoskeletal components[78] and small guanosine triphosphatases (GTPases) and docking machinery. Guanosine triphosphate (GTP) hydrolysis serves as an important mechanism for controlling intra-cellular membrane traffic and coupling to the cytoskeleton. Cytokines such as IFN-γ can have a major effect on activation and relocation of GTP-binding proteins, contributing to host cell–pathogen interactions.[79]

Proteomic analysis of isolated phagolysosomes has revealed more than 600 constituents, classified according to the source of the membrane and various putative functions.[80] Although most membrane constituents are derived from the plasma membrane, descriptions of contributions from the endoplasmic reticulum have elicited considerable interest, along with different estimates of their extent.[80,81] Another controversial aspect relates to the role of TLR engagement in enhancing the kinetics of the maturation process, a transcription-independent process.[82] Variations on this theme occur, depending on cell type and maturity, whether the extra-cellular cargo is microbial (living or dead) or host derived, and whether the process arises by autophagy (Figure 10-18)[83] rather than by heterophagy. The role of autophagy in cellular resistance to intra-cellular pathogens, such as M. *tuberculosis*, is receiving increasing consideration.[84,85]

Immature DCs are actively endocytic and phagocytic but poor APCs, whereas mature DCs downregulate uptake but acquire highly efficient APC function.[20] Changes associated with DC maturation and antigen presentation are illustrated in Figure 10-19 and Table 10-2. Pathogenic intracellular organisms vary in their subversion of the aforementioned process, interfering with different stages such as cytosolic signaling mechanisms, fusion, or acidification, and in selected cases, inducing a novel membrane composition.[86] Organisms can replicate in immature or mature compartments or can translocate their genome to the cytosol by acid-induced envelope fusion or by disruption of the vacuolar membrane.

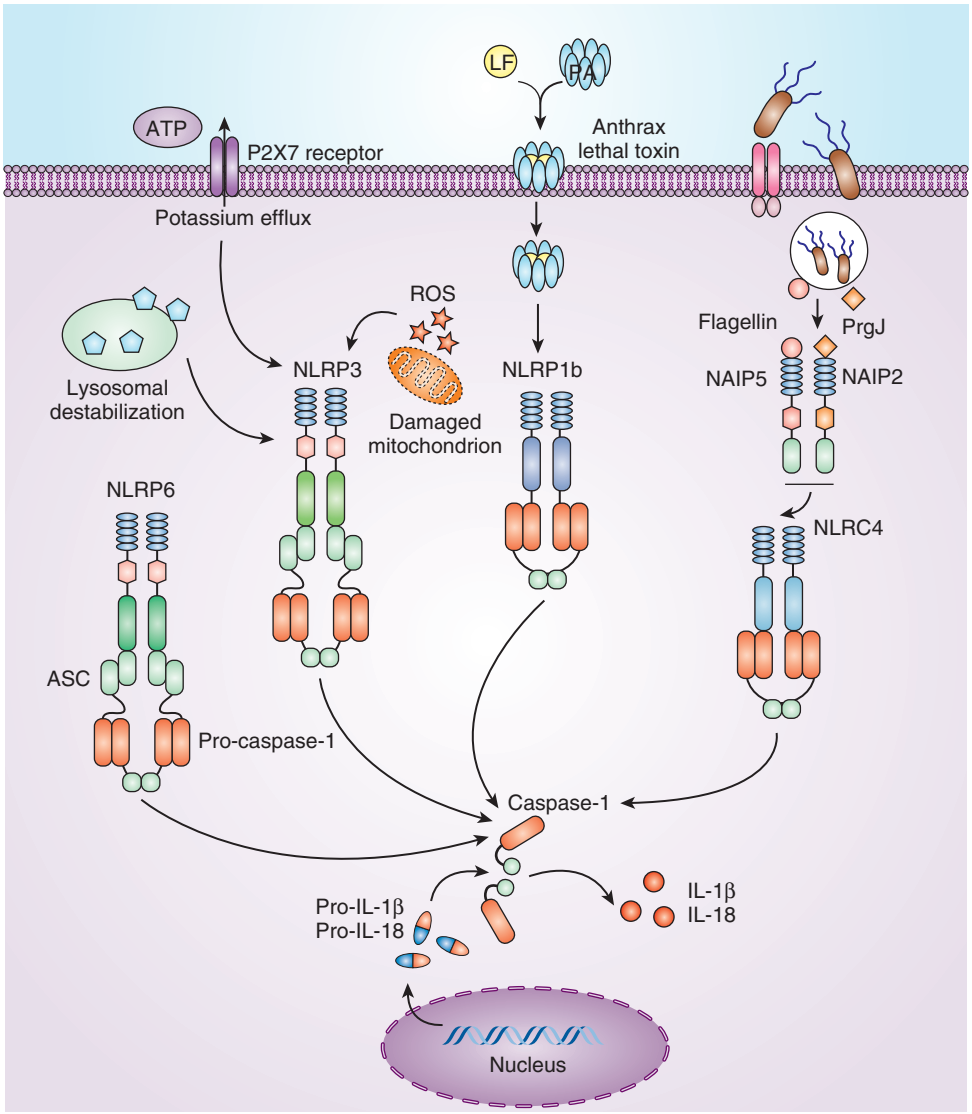



**Figure 10-14** Basic mechanisms of activation of the main nucleotide oligomerization domain (NOD)-like receptor (NLR) inflammasomes. NLRP3 is activated by three common cellular events elicited by different stimuli: potassium efflux; the generation of reactive oxygen species (ROS); and phagolysosomal destabilization and the release of endogenous mediators into the cytosol. NLRP1b is activated by anthrax lethal toxin. In the mouse, NLRC4 is activated by neuronal apoptosis inhibitory protein (NAIP) proteins bound to specific ligands. NAIP5 and NAIP6 bind to bacterial flagellin, whereas NAIP2 binds to the bacterial T3SS component PrgJ. These NAIP-ligand complexes subsequently bind and activate NLRC4. ATP, Adenosine triphosphate; IL, interleukin; LF, lethal factor; PA, protective antigen. *(From Rathinam VAK, Vanaja SK, Fitzgerald KS: Regulation of inflammasome signaling.* Nat Immunol *13:333–342, 2012.)*

## Signaling

The best-characterized signaling responses in macrophages include the TLR,[87] type I IFN,[88,89] and Fc receptor–induced pathways.[90] The various signaling cascades are complex, interact with one another, and result in phosphorylation/dephosphorylation, formation of cytosolic protein complexes, and activation of signaling proteins such as NF-κB, which enter the nucleus to regulate transcription. In the case of TLR-dependent sensing, a restricted number of adapters, such as myeloid differentiation primary-response gene 88 (MyD88), TIRAP-MAL, and Toll/IL-1R (TIR) domain-containing adaptor protein inducing IFN-β (TRIF), channel the flow of information into the cell. Emerging evidence suggests that NTR-induced signaling (e.g., by the β-glucan receptor [Dectin-1] cytoplasmic ITAM-like motif)[59] collaborates with TLR and NLR signaling pathways, with differential involvement of syk and CARD9[91] in effector responses in different mononuclear phagocytes. Distinct but interlinked pathways involve IFN regulatory factors as part of an amplification pathway with broader immunoregulatory functions than anti-viral responses alone. A more recently defined cytosolic specialized antiviral pathway involves interaction with mitochondrial components (Figure 10-15).[73] Cell surface or intra-cellular proteins serve as negative regulators, as exemplified by members of the SOCS family.

Figure 10-19 illustrates the pathway by which effector response–derived antigens are transported to the cytosol during synthesis, are processed in proteasomes, and become associated with class I MHC molecules for presentation at the cell surface. Effector response chaperones, such as heat

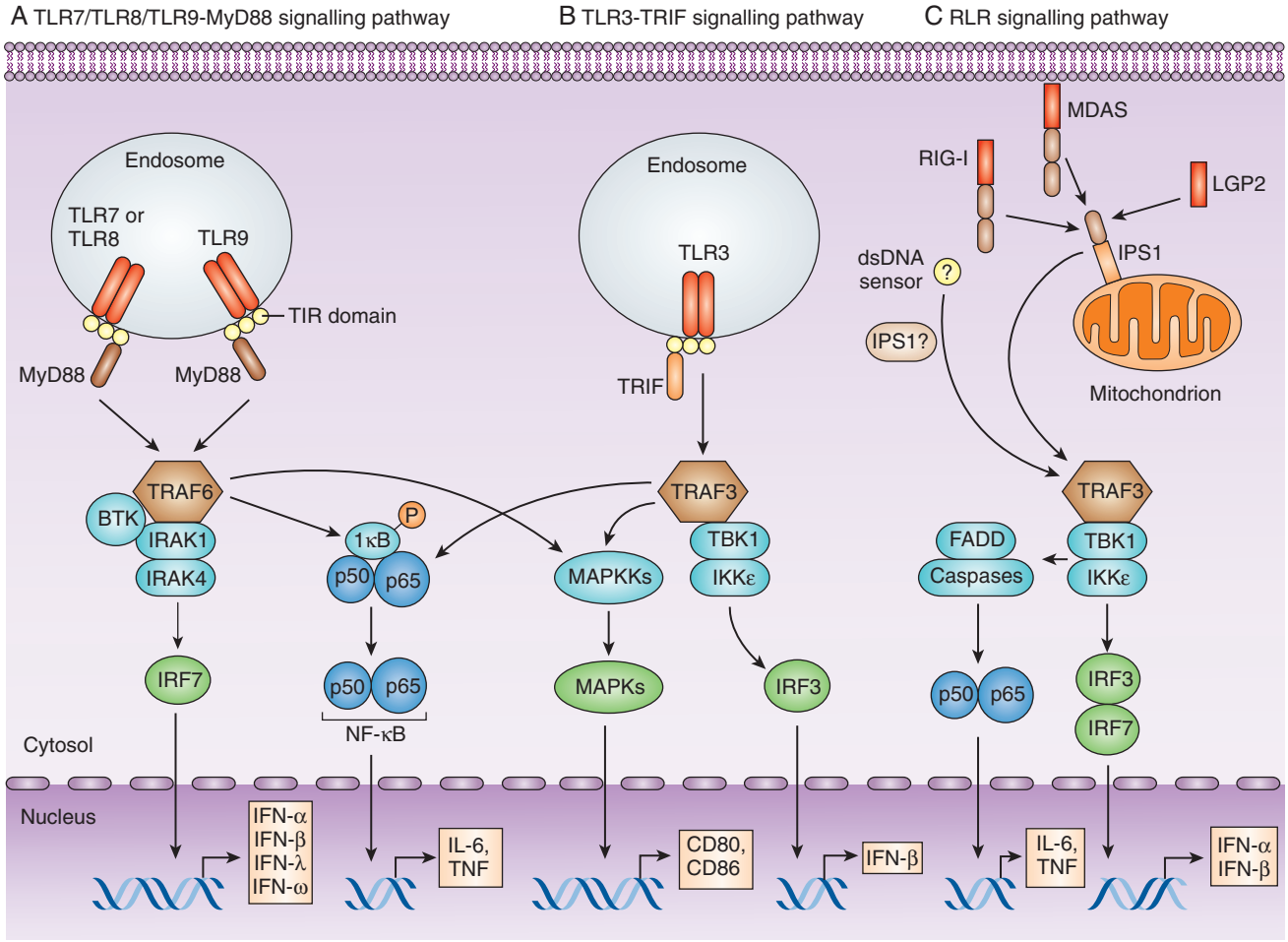



**Figure 10-15** Intra-cellular nucleic-acid sensors and signaling pathways. **A,** Endosomal Toll-like receptor 7 (TLR7), TLR8, and TLR9 recognize RNA and DNA ligands and signal through a key adaptor myeloid differentiation primary-response gene 88 (MyD88) in the cytosol. MyD88 in turn associates with a signal complex comprised of tumor-necrosis factor (TNF)-receptor–associated factor 6 (TRAF6), Bruton's tyrosine kinase (BTK), interleukin-1–receptor (IL-1R)-associated kinase 4 (IRAK4) and IRAK1, which leads to the activation of interferon-regulatory factor 7 (IRF7), nuclear factor-κB (NF-κB), and mitogen-activated protein kinases (MAPKs). Overall, this pathway potently induces the production of high levels of type I interferons (IFNs, such as IFN-α, IFN-β, IFN-λ, and IFN-ω), pro-inflammatory cytokines, and expression of co-stimulatory molecules. **B,** Alternatively, the binding of double-stranded RNA (dsRNA) to endosomal TLR3 triggers a signaling pathway mediated by Toll/IL-1R (TIR) domain-containing adaptor protein inducing IFN-β (TRIF), which involves TRAF3, TANK-binding kinase 1 (TBK1), and inhibitor of NF-κB kinase ε (IKK-ε). Subsequent IRF3 activation leads to IFN-β production. The TLR3–TRIF signaling pathway also activates NF-κB and MAPKs and results in the production of pro-inflammatory cytokines and the expression of co-stimulatory molecules (CD80 and CD86). **C,** The dsRNA receptors retinoic-acid–inducible gene I (RIG-I), melanoma differentiation–associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) constitute the third nucleic-acid sensor system, exclusively located in the cytoplasm of most cells. RIG-I, MDA5, and LGP2 signal through a common adaptor molecule IFN-β–promoter stimulator 1 (IPS1), which is associated with the mitochondria. By coupling with TRAF3, TBK1, and IKK-ε, IPS1 directly activates IRF3 and NF-κB through FAS-associated via death domain (FADD), caspase-8, and caspase-10. This pathway induces the production of type I IFNs and other cytokines. dsDNA-induced IFN production involves a yet-to-be defined sensor that signals through certain components of the RIG-I–like receptor (RLR) pathway, such as TBK1 and IRF3. IkB, Inhibitor of NF-κB; MAPKK, MAPK kinase. *(From Gilliet M, Cao W, Liu Y-J: Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases.* Nature Rev Immunol *8:594-606, 2008.)*

shock protein, may contribute to the surface delivery of antigens. The inflammasome is another example of a multiprotein assembly within the cytosol by which NLRs bring about IL-1 activation.

Several of the processes already described involve proteolytic enzymes, including cathepsins and caspases, and lipid-interacting chaperones. The ubiquitin pathway provides an important mechanism for cytosolic degradation of proteins. In addition, vacuolar $H^+$ adenosine triphosphatases (ATPases) contribute to acidification in different mononuclear phagocytes, which is especially relevant to osteoclast function. Comparison and further elucidation of the coupling between extra-cellular and intra-cellular signaling pathways in macrophages, DCs, and osteoclasts would be of great interest.

## Efferent Pathways: Gene Expression and Secretion

The complex responses of different mononuclear phagocytes to intrinsic and extrinsic stimuli have been defined by microarray studies and, in some cases, by protein and functional analyses. Although the major differentiation pathways (i.e., macrophages, immature and mature DCs, and osteoclasts) involve selective, stereotypic changes in gene expression, the extensive heterogeneity and plasticity of

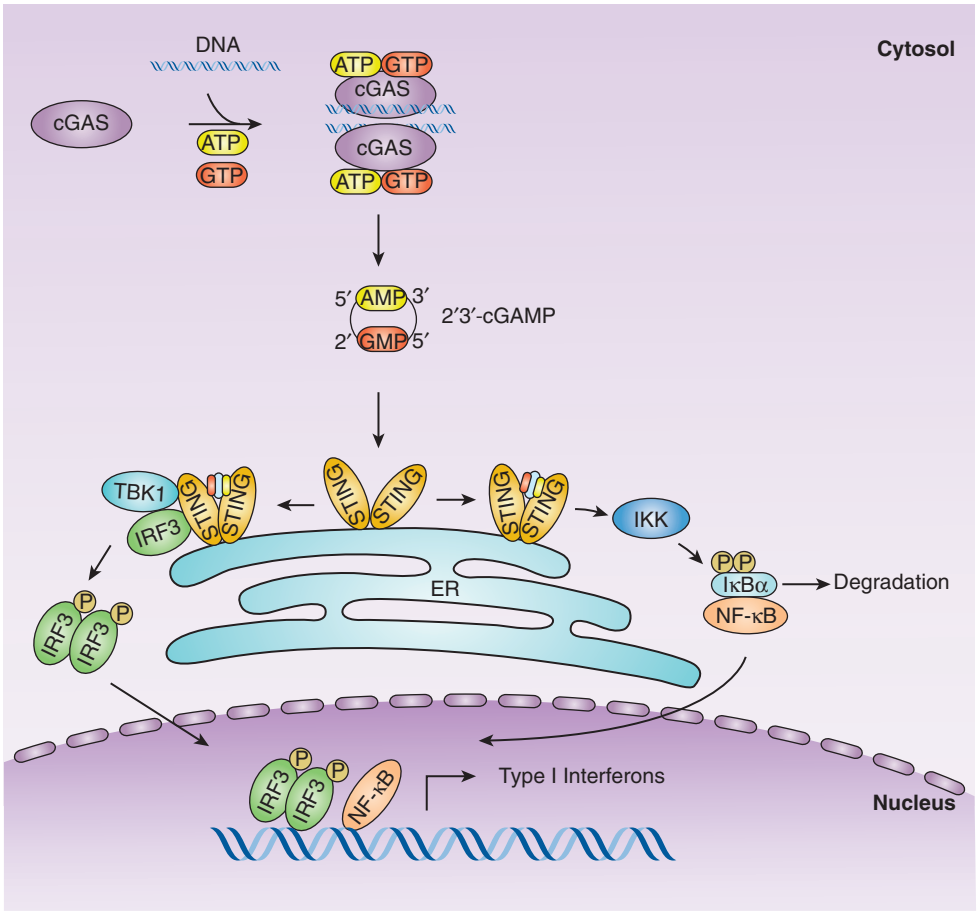



**Figure 10-16** Model for cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) (cGAMP) synthase (cGAS)-mediated cytosolic DNA. Cytosolic DNA activates cGAS to form a dimeric cGAS-DNA complex that synthesizes 2′3′-cGAMP from adenosine triphosphate (ATP) and guanosine triphosphate (GTP). As a high-affinity ligand for STING, 2′3′-cGAMP binds and activates STING through a series of structural changes. Activated STING then recruits TANK-binding kinase 1 (TBK1) to phosphorylate interferon-regulatory factor 3 (IRF3) and activate inhibitor of nuclear factor-κB (NF-κB) kinase (IKK) to phosphorylate IκBα, leading to IκBα degradation. IRF3 subsequently dimerizes and translocates into the nucleus along with NF-κB to induce the production of type I interferons and other cytokines. ER, Endoplasmic reticulum; STING, stimulator of interferon genes. *(From Cai X, Chiu YH, Chen ZJ: The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling.* Mol Cell *54:289–296, 2014.)*

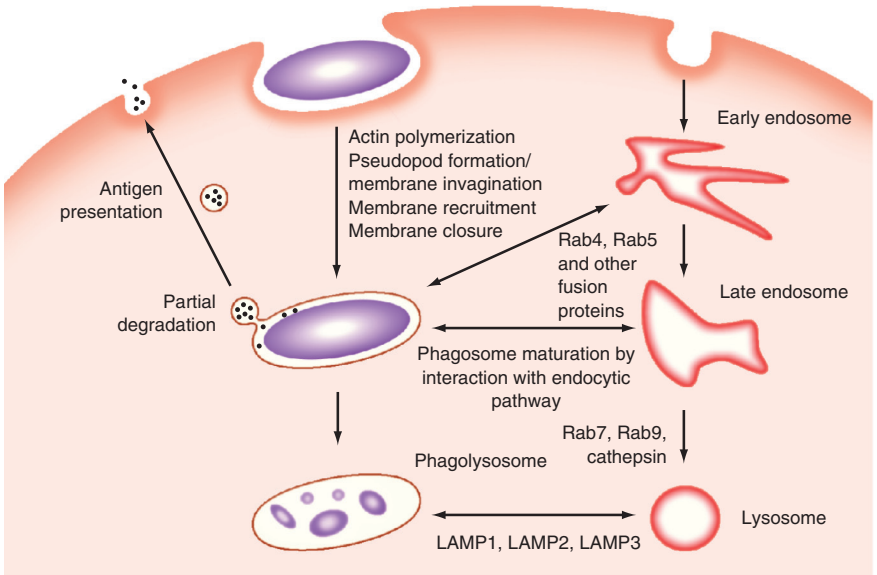



**Figure 10-17** Schematic presentation of phagocytosis and endocytosis. Particulates are taken up by actin-dependent sequential maturation processes involving membrane fusion and fission, which intersect with the endocytic pathway at several stages. Cytosolic small guanosine triphosphatases (GTPases) (Rabs) determine organelle-specific interactions. Membrane is recycled to the plasma membrane with processed antigen (see Figure 10-19). Progressive acidification and delivery of lysosomal hydrolases result in terminal degradation. Compartment membranes express marker proteins such as lysosome-associated membrane protein 1 (LAMP1); the pan-macrophage CD68 antigen is associated with late endosomes and lysosomes.

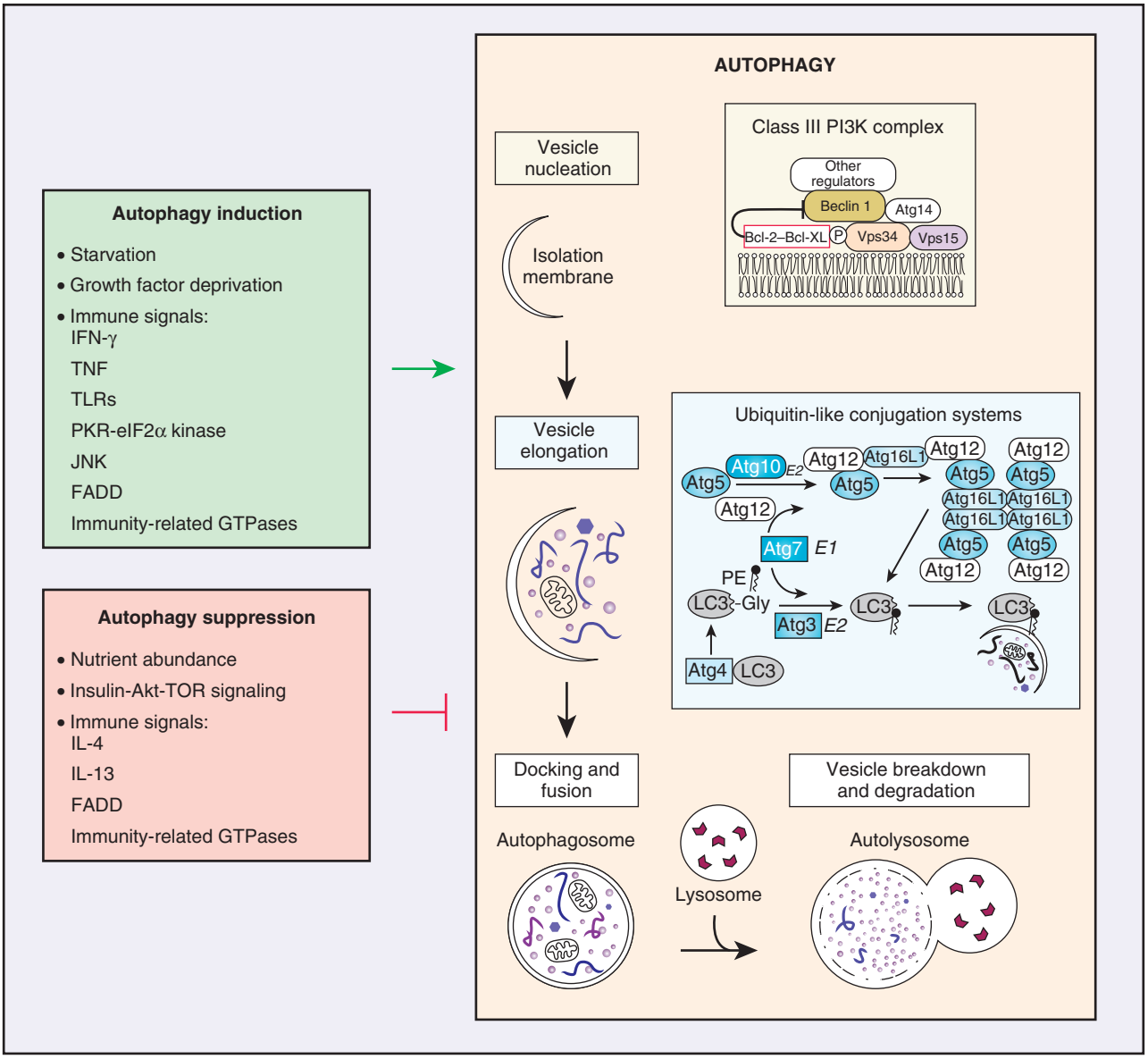



**Figure 10-18** The autophagy pathway and its regulation. The autophagy pathway proceeds through a series of stages, including nucleation of the autophagic vesicle, elongation and closure of the autophagosome membrane to envelop cytoplasmic constituents, docking of the autophagosome with the lysosome, and degradation of the cytoplasmic material inside the autophagosome. Vesicle nucleation depends on a class III PI3K complex that contains various proteins *(light yellow box at right)*, as well as additional proteins that regulate the activity of this complex (such as rubicon, UVRAG, Ambra-1 and Bif-1). Vesicle elongation and completion involves the activity of two ubiquitin-like conjugation systems *(light blue box at right)*. The autophagy pathway is positively regulated *(green box at left)* and negatively regulated *(red box at left)* by diverse environmental and immunological signals. E1 and E2, Ligases for ubiquitin-like conjugation systems; FADD, FAS-associated via death domain; Gly, glycine; GTPase, guanosine triphosphatase; IFN, interferon; IL, interleukin; Jnk, c-Jun N-terminal kinase; PE, phosphatidylethanolamine; PKR, protein kinase R; TLR, Toll-like receptor; TNF, tumor necrosis factor; TOR, target of rapamycin. *(From Virgin HW, Levine B: Autophagy genes in immunity.* Nat Immunol *10:461–470, 2009.)*

phenotypes characterizing these cells in situ have begun to be appreciated only more recently.[4] Macrophages are known to express numerous nuclear receptors,[92] which undergo dynamic changes depending on cellular differentiation and the microenvironment, as well as epigenetic histone modification[93,94] and microRNA expression. Exposure to particular cytokines, hormones, and other stimuli, such as microbial antigens and immune complexes, modulates gene expression profoundly and selectively and induces post-transcriptional and post-translational changes. Through alternative splicing, glycosylation, and poorly characterized protein modifications (e.g., methylation and acetylation of nuclear and cytoplasmic macromolecules), this exposure provides different mononuclear phagocytes with extensive diversity in adaptation and function.

Table 10-4 provides a partial list of macrophage secretory products that include low-molecular-weight metabolites (oxygen, nitrogen, and lipid-derived metabolites) implicated in inflammation and its resolution[95] and in anti-microbial responses. Other products include pro-inflammatory and anti-inflammatory cytokines and neutral proteinases. The cell biology of the secretory pathway in macrophages has only begun to be studied. The release of IL-1β after inflammasome activation is independent of a

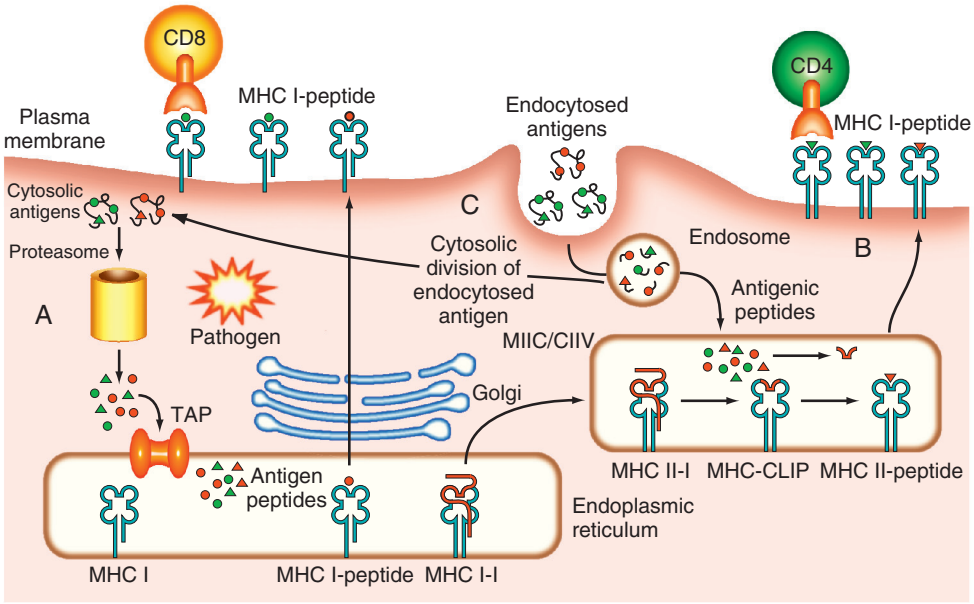



**Figure 10-19** Different antigen-processing pathways for the class I and class II major histocompatibility complex (MHC) molecules.[121] **A,** Class I MHC molecules present peptides that are derived primarily from endogenously synthesized proteins of self or pathogen origin. These proteins are degraded into peptides by the proteasome and are transported through the transporters of antigen-processing (TAP) molecules into the endoplasmic reticulum for loading on class I MHC molecules. **B,** Class II MHC molecules present proteins that enter the cell through the endocytic route. During maturation of class II MHC molecules, they are prevented from binding to endogenous antigens in the endoplasmic reticulum by association with the invariant chain (Ii). Invariant chain–class II MHC complexes (MHC II-Ii) move through the Golgi to the MHC II loading compartment/MHC II vesicles (MIIC/CIIV) compartment, where the invariant chain is degraded to class II–associated invariant-chain peptide (CLIP). CLIP is removed from the CLIP–MHC class II (MHC-CLIP) complexes and is exchanged for antigenic peptide. **C,** Dendritic cells can endocytose antigens from other cells and cross-present them to $CD8^+$ cytotoxic T lymphocytes. In most cases, these antigens also are processed into the class II MHC presentation pathway for recognition by $CD4^+$ T helper cells. *(From Heath WR, Carbone FR: Cross-presentation in viral immunity and self-tolerance.* Nat Rev Immunol *1:126–134, 2001.)*

canonical signaling sequence. The differential effect of T helper type 1 and 2 cytokines on nitric oxide production versus its destruction is thought to be relevant to microbicidal mechanisms versus repair.[4] The secretory activities of DCs and osteoclasts are relevant to their specialized functions (e.g., lymphocyte activation and bone remodeling) and depend on polarized secretion.

Table 10-5 shows a classification of characteristic effects induced by prototypic activating and inhibitory stimuli. It is convenient to distinguish the phenotype of mononuclear phagocytes stimulated by an innate stimulus, including exposure to microbial products such as LPS; by a classic immune-activating cytokine such as IFN-γ; and by IL-4/IL-13 induction of an alternative activation pathway.[4] Other stereotypic responses are induced by immune complexes that selectively modulate IL-12/IL-10 expression.[96] Innate and immune cytokine stimuli are able to potentiate one another.

Conversely, deactivating stimuli such as IL-10 and transforming growth factor (TGF)-β and glucocorticosteroids[97] induce their own distinctive patterns of gene expression. Upregulation of CD163, a hemoglobin-haptoglobin SR, is a striking example of glucocorticosteroid-enhanced endocytic function.[98] Stimulation of the vagus nerve attenuates macrophage activation by activating the JAK2/STAT3 signaling pathway.[99] Deactivation of macrophages plays a critical role in limiting pro-inflammatory activation induced by cytokines (e.g., IFN-γ) and opsonic (FcR) and non-opsonic stimulation (TLR, NLR, and inflammasomes), acting via a range of signal transduction pathways and naturally occurring antagonists. Deactivation of macrophages is a particular feature of phagocytic uptake of apoptotic cells (see Figure 10-9) thought to be mediated by TGF-β, prostaglandin $(PG)E_2$, and IL-10, which also inhibit the generation of reactive nitrogen and oxygen species. The resolvins and protectins derive from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) produced by the cyclooxygenase (COX)-2 pathway, especially in the presence of aspirin. They inhibit production and recruitment of leukocytes to sites of inflammation and counteract pain.[100]

Plasma membrane signals to deactivate macrophages arise from transligation of CD200 (expressed by many cells, including innate-activated macrophages) and its signaling immunoglobulin superfamily (IGSF) counterpart CD200R, expressed selectively by macrophages.[63] Another inhibitory receptor pair consists of signal regulatory protein α (SIRPα) and CD47[64]; cytosolic inhibitors include the SOCS proteins.[22] These extra-cellular mediators and poorly defined interactions with surface expressed molecules make the different APCs reciprocally interactive with other immune cells (helper and regulatory CD4 T cells, CD8 T cells, natural killer cells, and natural killer T cells) and with nonhematopoietic cells such as fibroblasts, epithelium, and neurons and with malignant cells.[101] Induced responses on macrophages include altered class II MHC and co-stimulatory molecule expression, marker antigens involved in cellular interactions, phagocytosis and antigen presentation, and altered secretion. As a result, different mononuclear phagocytes are able to modulate their interactions

**TABLE 10-4** Selected Products Secreted by Macrophages

| Proteins | Product | Comment |
|---|---|---|
| Enzymes | Lysozyme | Bulk product |
| | Urokinase-type plasminogen activator | Regulated by inflammation |
| | Collagenase | Regulated by inflammation |
| | Elastase | Regulated by inflammation |
| | Metalloproteinases | Also inhibitors |
| | Complement | All components and regulators |
| | Arginase | Alternative activation |
| | Angiotensin-converting enzyme | Induced glucocorticoids, granulomas |
| | Chitotriosidase | Gaucher's disease, lysosomal storage disease |
| Inhibitors | Acid hydrolases | All classes (mainly intra-cellular) |
| | TIMP | |
| Chemokines | Many C-C, C-X-C, CX3C (e.g., MCP, RANTES, IL-8) | Initiate short- and long-term recruitment of myeloid and lymphoid cells |
| Cytokines | IL-1β, TNF, IL-6, IL-10, IL-12, IL-18, IL-23 | Pro-inflammatory and anti-inflammatory |
| | Type I interferon | Also antagonists (e.g., IL-1Ra) |
| | | Autocrine and paracrine amplification |
| Apolipoproteins | Apolipoprotein E | Local source, bone marrow origin after adoptive transfer |
| Growth/differentiation factors | TGF-β | Also other family members (activins) |
| | M-CSF | Myeloid growth and differentiation |
| | GM-CSF | |
| | FGF | Fibrosis |
| | PDGF | Repair |
| | VEGF | Angiogenesis |
| Opsonins | Fibronectin, pentraxin (PTX3) | Additional uncharacterized receptor on macrophages |
| Soluble receptors | Mannose receptor | Soluble mannose receptor |
| Cationic peptides | Defensins | Subpopulations and species variation |
| Lipids | Procoagulant | Initiation of clotting |
| | Arachidonate metabolites | Pro-inflammatory and anti-inflammatory mediators |
| | Prostaglandins | |
| | Leukotrienes | |
| | Thromboxanes | |
| | Resolvins, maresins | |
| Metabolites | Reactive oxygen intermediates | Respiratory burst and signaling |
| | Reactive nitrogen intermediates | Anti-microbial and signaling |
| | Hemoglobin breakdown (bile pigments) | |
| | Iron, vitamin $B_{12}$ binding protein | |
| | Vitamin D metabolites | Cell regulation, including osteoclasts |

*Note:* For additional details, see references 126 and 127.

FGF, Fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MCP, monocyte chemotactic protein; M-CSF, macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; RANTES, regulation upon activation normal T cell expressed and presumably excreted; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

with CD4 T lymphocytes, with endothelial and other cell types, with extra-cellular matrix, and with bone to induce trophic and cytostatic/cytocidal and catabolic effects.

Human inborn errors or defects in macrophage responses to intra-cellular pathogens such as mycobacteria[102] have validated studies on cell activation in the mouse. The immunosuppressive effects of macrophages in immune tolerance to an allogeneic fetus have been ascribed to induction of indoleamine-2,3-dioxygenase, which catabolizes the essential amino acid, L-tryptophan.[103] Macrophage products act mainly locally but also can have profound systemic effects, regulating metabolic and regulatory responses within the host. These effector programs and products provide targets for pharmacologic intervention.

## MACROPHAGE PLASTICITY

The question of macrophage plasticity has become interesting because of the extensive heterogeneity of macrophages in different organs, the diversity in phenotypes associated with cell activation, and the power of contemporary gene expression analysis and its relevance to potential modulation by therapy. Although no simple conclusions can yet be drawn of its extent and mechanisms, we summarize aspects and limitations of current knowledge.

It is useful to distinguish differentiation of mononuclear phagocytes and the microheterogeneity of resident cells in different tissue microenvironments, from the biosynthetic and secretory adaptation of blood monocyte–derived mononuclear phagocytes to inflammatory, immune, and metabolic stimuli. Morphology, differential antigen expression and in situ analysis of RNA and protein expression have all contributed to our still limited understanding of cellular heterogeneity. Lineage-specific growth and differentiation factors, selective expression of transcription factors, and enhancers, epigenetic modifications and local and systemic modulators such as retinoids, lipids, and microbial products all contribute to cell heterogeneity. For example, in vitro

**TABLE 10-5** Immunomodulation of Macrophage Phenotype

| Stimulus | Category | Markers | Function |
|---|---|---|---|
| Microbial (bacterial) | Innate<br>Activation | Induction of MARCO<br>Co-stimulatory molecules<br>CD200 | Enhanced phagocytosis<br>Antigen presentation<br>Inhibition (CD200R) |
| IFN-γ | Classic activation | Induction of class II MHC<br><br>Potentiation of innate markers<br>TNF<br>iNOS induction<br>NADPH, respiratory burst<br>LGP47 induction<br><br>Downregulation of MR<br>Modulation of FcR expression<br>Proteasomal composition | Cell-mediated immunity/delayed-type hypersensitivity<br><br>Pro-inflammatory<br>Anti-microbial (NO), signaling<br>Host defense, inflammation<br>Association with phagosome/intra-cellular pathogen killing<br>Unknown<br><br>Antigen presentation |
| IL-4/IL-13 | Alternative activation<br>Upregulation | Enhanced MR<br>Induction of arginase<br>Induction of YM1<br>FIZZ1 (mouse)<br>Induction of CCL17 (MDC) and CCL22 (TARC)<br>Fusion, giant cell formation<br>CD23 (FcRε) | Endocytosis, clearance<br>Th2 responses, allergy, anti-parasitic, repair |
| IL-10<br>TGF-β | Deactivation | Downregulation of class II MHC and of pro-inflammatory NO and ROI | Immunoregulation<br>Immunoregulation, repair and Treg induction |
| Immune complexes | Modified activation | Selective IL-12 downregulation<br>IL-10 induction | |
| Glucocorticoids | Deactivation | CD163 induction<br><br>Monocyte recruitment downregulated<br>ACE induction<br>Stabilin induction | Anti-inflammatory, homeostatic, clearance of hemoglobin-haptoglobin complexes |

*Note:* For additional details, see references 4, 17, and 128.

ACE, Angiotensin-converting enzyme; FcR, Fc receptor; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; LGP47, lysosomal glycoprotein; MARCO, macrophage receptor with collagenous structure; MDC, macrophage-derived chemokine; MHC, major histocompatibility complex; MR, mannose receptor; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NO, nitric oxide; ROI, reactive oxygen intermediates; TARC, thymus and activation-regulated chemokine; TNF, tumor necrosis factor; Treg, regulatory T cell.

and in vivo studies of the actions of cytokines such as IFN-γ and IL-4/IL-13 and of sensors such as the TLRs and opsonic phagocytic receptors have provided paradigms of cell activation phenotypes, termed variously classic, alternative and innate, or M1/M2 activation. The characterization and limitations of this terminology have been extensively discussed in recent reviews.[104] It has been proposed that a spectrum of activation states is more appropriate than simple binary alternatives, and recommendations have been put forward to standardize and define experimental specificity.[105] A general conclusion is that potential markers should be used in combination to define a signature, rather than individually.

However, to gain insight into the question of plasticity, the following considerations must be kept in mind for further research into flexibility and manipulation of phenotypes by microarray, RNA sequencing, and in situ gene and protein analysis. How stable, complete, and reversible are the signatures obtained after single and multiple stimuli? How do they evolve in the course of a dynamic process, at different times, and at different locations? How can we distinguish changes in cell populations and at the single cell level? To what extent do species differences apply, for example, between humans and rodents? What epigenetic mechanisms are at play? What do we mean by terms such as priming and tolerance? Above all, is it possible to convert a particular macrophage phenotype from one state to another? Although it has been reported that one type of tissue macrophage can be changed to another by adoptive transfer and we know that differentiated macrophages can be derived from induced pluripotent precursors derived from other somatic cells,[106] a great deal more needs to be done to validate their correspondence to macrophages in vivo. It may be premature to conceive of direct switching of pro-inflammatory cells, for example, to anti-inflammatory, trophic, or regulatory macrophages or to induce tumor-promoting macrophages to become tumoricidal by simple pharmacologic means. Clearly the full complexity of phenotypic heterogeneity and its regulation requires a great deal of further systematic investigation.

## Relevance to Selected Rheumatic Diseases

Although the different mononuclear phagocytes have been addressed as part of a general host homeostatic system, there are good examples in human rheumatic diseases and in animal models of arthritis in which these cells play a role in the local initiation or effector phase of the disease. Primary defects in osteoclast differentiation and function contribute to osteopetrosis or, if overactive, osteoporosis.

We have referred to autoinflammatory syndromes and newly discovered cytosolic receptors. Gout might also fall in this category because uric acid crystals provide a metabolic stimulus for inflammasome activation. Macrophages play a role in the pathogenesis and complications of osteoarthritis, including the response to foreign implants. DCs and macrophages play a part in the induction and effector mechanisms of autoimmune arthritis, in concert with T lymphocytes and B lymphocytes and their products, immune complexes, and complement. Co-stimulatory receptors such as PD-1 and its ligands already provide potential targets for antibody therapy. The catabolic role of TNF, IL-1, neutral proteinases, reactive oxygen radicals, and arachidonate metabolites is well known. Less appreciated are the trophic interactions of macrophages with fibroblasts through production of TGF-β, growth and angiogenic factors, and modulation by alternative activation pathways.[107] Type I IFNs, produced by macrophages and other cells, play an important amplification role in autoimmune inflammation (e.g., SLE).

Although animal models may not mimic the natural diseases in humans, more recent examples suggest that APC membrane receptors involved in recognition of modified self and foreign microbial ligands could be relevant to arthritis; examples include streptococcal rheumatic disease and exacerbation of recurrent arthritis by zymosan in T cell transgenic mice, acting via Dectin-1, the APC receptor for β-glucans.[108] In addition, inhibitory plasma membrane receptor interactions (CD200/CD200R) limit macrophage activation and collagen-induced murine arthritis.[63,109] Impaired clearance of apoptotic cells by (uncharacterized) C1q receptors of mononuclear phagocytes may contribute to autoimmune diseases such as lupus.[67] Finally, macrophages and DCs could contribute to wider connective tissue disorders, such as scleroderma, because they are able to regulate fibroblast and matrix synthesis and turnover through metalloproteinases and TGF-β; dysregulation of these pathways has been neglected as a pathogenetic mechanism in these systemic diseases.

## ISSUES FOR FURTHER INVESTIGATION

As we take stock of how increasing knowledge of mononuclear phagocyte biology has affected pathogenesis and therapy of rheumatic diseases, additional questions arise regarding basic mechanisms and new selective targets. Treating macrophages, DCs, and osteoclasts as specialized forms of a unitary mononuclear phagocyte family helps to bring out their common and distinctive properties. Selective expression and signaling pathways of pattern recognition receptors by macrophages and DCs are still poorly characterized; regulation of TLR signaling has already received considerable attention as a possible drug target. Discrimination of microbial and host-derived ligands may account for mimicry and autoantigen cross-reactivity, but mechanisms for regulating homeostatic and tolerogenic responses are still unclear.

On the effector side, success with anti-TNF monoclonal antibodies suggests that targeting of individual effector molecules can succeed, especially when these are expressed at the cell surface and are proximal in cascade reactions, although further study is needed to account for resistance to therapy in a group of patients. The impact of anti-TNF therapy on macrophage functions and mechanisms for resistance to TNF blockade are poorly understood. The role of inhibitory/activating pairs of surface molecules as modulators of APC pathways is unexplored and is likely to move to center stage. One neglected aspect of differentiation and function for osteoclasts and granuloma macrophages is the mechanism of fusion and the possible functional significance of multinuclear giant cell formation. Basic knowledge is still lacking and may provide useful insights into the pathogenesis of metabolic bone diseases and enhanced perception of links with the immune system, both innate and adaptive.

Although powerful tools of messenger RNA and proteomic analysis and gene silencing by RNA interference inhibition have become available, the heterogeneity and complexity of mononuclear phagocytes in human tissues, especially at early stages of disease, need improved characterization in situ and at different sites. Much remains to be learned concerning trophic processes and repair and their relationship to the resolution of inflammation and fibrosis. These processes involve cell-cell interactions that are poorly understood but are increasingly amenable to investigation.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 11

# Neutrophils



Binita Shah • Nathalie Burg • Michael H. Pillinger

**KEY POINTS**

- **Neutrophils are myeloid-lineage cells characterized by the presence of granules that contain enzymes and other potentially toxic agents involved in host defense.**
- **Neutrophils are short-lived, terminally differentiated cells that exist primarily in the bloodstream, where they participate in host surveillance of foreign organisms.**
- **Neutrophils function in instances of acute inflammation and provide an essential defense against acute bacterial infections. Abnormalities of neutrophil function are uncommon and impair ability to respond to life-threatening infections.**
- **Key functions of neutrophils include phagocytosis and degradation of foreign organisms, such as bacteria, through activation of proteases and other antibiotic molecules, as well as generation of toxic oxygen radicals.**
- **Neutrophils play a role as major inflammatory cells in many rheumatic conditions and may be attracted into tissues by noninfectious stimuli such as activated complement components and lipid inflammatory mediators.**
- **Neutrophil extra-cellular traps are aggregates of extruded neutrophil chromatin and granule enzymes that may trap and destroy bacteria but also play a role in promoting inflammation and thrombosis.**

Neutrophils (polymorphonuclear neutrophils [PMNs]) belong to the family of polymorphonuclear leukocytes, which are hematopoietically derived cells that share the features of a multilobed nucleus and highly developed intracytoplasmic granules (hence the alternative term *granulocytes* for this family of cells). On the basis of the histocytochemical staining properties of their respective granules, three classes of polymorphonuclear leukocytes have been identified: neutrophils, eosinophils, and basophils. Whereas neutrophil granules stain with neutral dyes, the granules of eosinophils are most effectively stained with acidic dyes such as eosin, and basophil granules stain with basic dyes. In a standard polychromatic Wright stain of a peripheral blood smear, the cytoplasm of neutrophils, eosinophils, and basophils appears blue-pink, pink, and blue, respectively. These classes of polymorphonuclear leukocytes differ not only in appearance but also in biochemistry and function. Polymorphonuclear leukocytes constitute an important part of the organism's system of innate immunity; their responses to foreign organisms, antigens, or both are preprogrammed and do not depend on prior exposure to the foreign particle.

Neutrophils are the body's first line of defense against foreign invaders and constitute the major cell type involved in acute and some forms of chronic inflammation. The importance of neutrophils in bacterial defense is illustrated by patients who have hereditary defects in neutrophil function and are prone to repeated and often life-threatening infections. Neutrophils are the most prevalent leukocytes in the bloodstream, typically constituting more than 50% of all bloodstream leukocytes. During bacterial infection, the percentage of neutrophils may increase to 80% or more. In contrast, tissue concentrations of neutrophils during periods of homeostasis are quite low but increase locally in response to infection or other triggers. Neutrophils may be considered to be surveillance cells that sweep through the bloodstream, scanning for tissue infections or other inflammatory events. Unfortunately, the capacity of neutrophils to destroy foreign organisms is matched in some circumstances by a capacity for host tissue destruction. In this chapter we review neutrophil development, structure, and function, the role of neutrophils in protecting against infection, and the role and impact of neutrophils in the pathogenesis and pathophysiology of immune deficiency and autoimmune and autoinflammatory diseases.

## NEUTROPHIL DEVELOPMENT, MORPHOLOGY, AND CONTENT

### Neutrophil Myelopoiesis and Clearance

The abundance of neutrophils in the bloodstream is duplicated in the bone marrow, where 60% of hematopoietic capacity may be dedicated to neutrophil production. Daily, $10^{11}$ neutrophils are released into the bloodstream. Neutrophil development in the marrow takes about 14 days, originating with the hematopoietic stem cell. Stem cells fated to become neutrophils first differentiate into myeloblasts, which retain the capacity to develop into eosinophils, basophils, and neutrophils. Subsequent differentiation leads to the neutrophilic promyelocyte, a dedicated precursor of the neutrophil, and proceeds through the stages of neutrophilic myelocyte, metamyelocyte, band cell, and mature neutrophil. At the metamyelocyte stage, neutrophil mitosis ceases, whereas neutrophil development and organization of granules continue. Only the mature neutrophil demonstrates the classic feature of a multilobed nucleus. Neutrophils are

terminally differentiated; they neither divide nor, as a general rule, alter their gross phenotype after their release from the marrow. However, in the setting of certain infections, such as helminths, some neutrophils show a distinct profile of gene expression and a ring-form nucleus, in contrast to the typical multilobed cell seen in the peripheral blood.[1]

Given the origin of neutrophils from pluripotent stem cells, as well as the precise phases of their development, the mechanisms regulating neutrophil differentiation are of considerable interest. Although this process remains incompletely understood, studies have emphasized the role of a particular complement of transcription factors and cytokines that seem to direct the early cells toward neutrophil development. Several myeloid factors are necessary for the transcriptional regulation of neutrophils, including LEF-1, CCAAT enhancer binding proteins α and ε (C/EBPα and C/EBPε), and GFI-1. Principal among the cytokines regulating granulopoiesis is granulocyte colony-stimulating factor (G-CSF). G-CSF effects include induction of myeloid differentiation, proliferation of granulocyte precursors, and release of mature neutrophils from the marrow.[2] Biologic effects of G-CSF are mediated through its receptor (G-CSFR or CD114), a member of the class I cytokine receptor family. Although other hematopoietic cytokines contribute to granulopoiesis in vivo (including granulocyte-macrophage colony-stimulating factor [GM-CSF], interleukin [IL]-6, and IL-3), their individual presence is not essential as demonstrated by murine knockout experiments.

Once mature, neutrophils exit the bone marrow through the tight-fitting pores of the sinusoidal endothelium and enter the circulation, a process called *transcellular migration*.[3] Neutrophils released from the marrow have a bloodstream half-life of approximately 6 hours and a tissue half-life only marginally longer. Neutrophil life spans may be modulated by soluble signals; when exposed to stimuli such as tumor necrosis factor (TNF) and Fas (CD95) ligand, neutrophils undergo apoptosis or programmed cell death.[4,5] The high output and short half-life of neutrophils imply that neutrophil clearance mechanisms must exist. Recently, the stromal-derived factor 1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) signaling system has been implicated in neutrophil clearance. CXCR4, a G protein–coupled receptor, is expressed at low levels in the mature neutrophil. As they age, neutrophils alter their phenotype and upregulate CXCR4. This change supports homing to the bone marrow via the chemoattractant SDF-1 (also known as CXCL12). Once back in the marrow, senescent neutrophils are phagocytosed by stromal macrophages.[6] Senescent or apoptotic bloodstream neutrophils are also cleared by liver and spleen macrophages (i.e., the reticuloendothelial system). Although little is known about the molecular mechanisms underlying neutrophil clearance in the liver and spleen, upregulation of the adhesion molecule P-selectin on Kupffer cells appears to be relevant. It remains uncertain whether tissue neutrophils are cleared primarily via local macrophages or if they first traverse through the lymphatic drainage system.

## Neutrophil Morphology and Contents

Neutrophil nuclei tend to have more lobes (typically three to five) than do the nuclei of other polymorphs (Figures 11-1 and 11-2). The multilobed nature of the neutrophil nucleus reflects chromatin condensation, which suggested that neutrophils might be incapable of transcription; it is now known, however, that neutrophils retain the capacity for both constitutive and stimulated protein synthesis, albeit at a limited rate.

Neutrophil granules identifiable by classic histochemical staining comprise two classes (Figures 11-1 and 11-2). Special techniques are required to recognize and identify two additional classes of granules.

### Primary Granules

Neutrophil *primary granules* form first and, by virtue of their staining tendencies (i.e., affinity for the basic dye azure A), are also referred to as *azurophilic granules*.[7] These granules are oval or round and vary in size. They are similar, and functionally equivalent, to the lysosomes of other cells. Characteristic of azurophilic granules is the presence of myeloperoxidase (MPO), an enzyme that catalyzes the

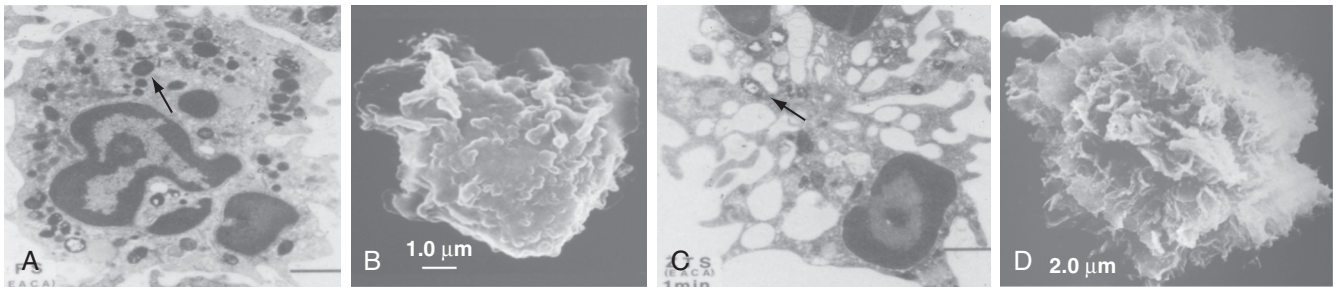



**Figure 11-1** Resting and stimulated neutrophil morphology. **A** and **B,** Transmission **(A)** and scanning **(B)** electron micrographs of resting neutrophils. In **A,** note the multilobed nucleus and the rich population of granules. At least two populations of granules may be discerned: The larger, darker granules represent the primary (azurophilic) granules, whereas the smaller, slightly paler granules are predominantly secondary (specific) granules and may include a population of gelatinase granules (the *arrow* indicates a primary granule). In **B,** note the relatively smooth surface area with some membrane surface irregularities. **C** and **D,** Transmission **(C)** and scanning **(D)** electron micrographs of neutrophils 1 minute after stimulation with zymosan. The cellular diameter is enlarged, and the overall surface (plasma) membrane area is greatly increased. Most of the membrane contributing to the increased surface area is supplied via the fusion of internal granule membranes with the plasma membrane. In **C,** this fusion is apparent as the depletion of granules, leading to an appearance of empty vesicles (the *arrow* indicates a partially depleted primary granule; clear circular areas represent fully depleted vesicles whose membranes are fused to the plasma membrane). In **D,** this fusion is apparent as the increase in plasma surface membrane extensions, known as *lamellipodia. (Courtesy G. Weissmann, NYU School of Medicine.)*

formation of hypochlorous acid from chloride in the presence of hydrogen peroxide ($H_2O_2$) (see the following Respiratory Burst). The presence of large amounts of this enzyme in azurophilic granules lends collections of neutrophils (i.e., pus) their typical greenish-yellow color. Consistent with their role as lysosomes, azurophilic granules also contain a variety of proteases and other enzymes including elastase, lysozyme, acid phosphatase, cathepsins, and enzymes directed at nucleic acids and sugars (Table 11-1). At the membrane level, however, they differ from true lysosomes in their lack of lysosome-associated membrane proteins (LAMPs)-1 and -2 and the mannose-6-phosphate receptor system.[8]

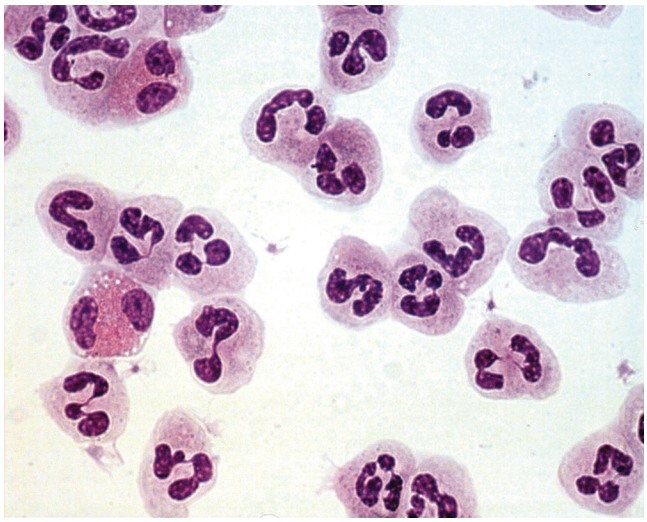


**Figure 11-2** Resting neutrophils and eosinophils under light microscopy. A blood smear stained with hematoxylin and eosin shows neutrophils and eosinophils; the three-lobulated nucleus (polymorphonuclear) cells are characteristic of neutrophil structure. Two eosinophils are distinguished by their bilobed nuclei and pink-stained granules (eosin stains basic structures). *(Courtesy K.A. Zarember, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health.)*

## Secondary Granules

In contrast to primary or azurophilic granules, neutrophil *secondary granules* constitute a population unique to neutrophils, a fact reflected in the alternatively employed nomenclature of *specific granules*. Specific granules possess an extensive array of membrane-associated proteins, including cytochromes, signaling molecules, and receptors. Specific granules constitute a reservoir of proteins destined for topologically external surfaces of phagocytic vacuoles and the plasma membrane (Table 11-1).[7] One particularly important family of proteinases found in neutrophil-specific granules are the matrix metalloproteinases (MMPs), including neutrophil collagenase–2 (MMP-8), gelatinase-B (MMP-9), stromelysin (MMP-3), and leukolysin (MMP-25). MMPs are stored as inactive proenzymes and undergo proteolytic activation after specific granule fusion with the phagocytic vacuole, along with interaction with azurophilic granule contents. MMP activation confers upon neutrophils the ability to alter and degrade integral membrane components of phagocytosed bacteria. Neutrophil MMP function is not limited to bacterial killing, however. For example, MMPs are also important for neutrophil extravasation and diapedesis (see the following discussion).[9]

Azurophilic and specific granules also contain antimicrobial proteins and peptides that are the cornerstone of innate immunity. A detailed description of the neutrophil's armamentarium against foreign invaders is beyond the scope of this chapter, but a few mechanisms warrant discussion. Elastase, mentioned previously, aids in the killing of Gram-negative bacteria via degradation of bacterial outer membrane protein A.[10] Elastase-deficient mice are more susceptible to infection with Gram-negative (but not Gram-positive) organisms than are wild-type mice. The defensins, stored in azurophilic granules, accumulate in mg/mL concentrations in phagocytic vacuoles (discussed later) and render target cell membranes (e.g., from bacteria) permeable. Bactericidal/permeability-inducing protein, also stored in azurophilic granules, acts in concert with the defensins;

**TABLE 11-1** Neutrophil Granule Contents

| | Secretory Vesicles | Gelatinase Granules | Specific Granules | Azurophilic Granules |
|---|---|---|---|---|
| Relative size | Smallest | Intermediate | Intermediate | Largest |
| Soluble components | Plasma proteins | Gelatinase<br>Acetyltransferase | Gelatinase<br>MMP-3<br>MMP-8<br>MMP-9<br>Lactoferrin<br>β2 Microglobulin | Myeloperoxidase<br>Glucuronidase<br>Elastase<br>Lysozyme<br>Proteinase 3<br>α1 Anti-trypsin<br>Defensins<br>Cathepsin<br>BPI |
| Membrane-associated components | FMLP receptor<br>CD11b/CD18<br>Cytochrome $b_{558}$<br>Alkaline phosphatase<br>Uroplasminogen activator<br>CD10, CD13, CD16, CD45<br>CR1<br>Decay-accelerating factor | FMLP receptor<br>CD11b/CD18<br>Deacylating enzyme | FMLP receptor<br>CD11b/CD18<br>Cytochrome $b_{558}$<br>CD66, CD67<br>Fibronectin receptor<br>TNF receptor | CD63, CD68 |

BPI, Bactericidal/permeability-increasing protein; FMLP, formyl-methionyl-leucyl-phenylalanine; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.

it potently neutralizes endotoxin and is cytotoxic to Gram-negative bacteria. Bactericidal/permeability-inducing protein also enhances the activity of secretory phospholipase $A_2$, which has activity against Gram-negative and Gram-positive bacteria. Lactoferrin, which is found in specific granules, deprives microorganisms of iron and has antiviral and antibacterial effects. Other granule-associated proteins such as cysteine-rich secretory protein 3 (CRISP3) and ficolin 1 have recently been described, although their functions are still unclear.

### Gelatinase Granules and Secretory Vesicles

Special techniques have confirmed the existence of two additional classes of vesicles. *Gelatinase granules* are identical in size to specific granules and share some common proteins. As their name implies, however, gelatinase granules are distinguished by their high concentrations of gelatinase, a latent enzyme with the capacity for tissue destruction.[11] *Secretory vesicles* are smaller and lighter than the other classes and do not seem to contain proteolytic enzymes.[12] Rather, secretory vesicles are noteworthy for an extensive complement of membrane-associated proteins, including receptors otherwise identified with the plasma membrane. These and other data suggest that the secretory vesicle is a reservoir of neutrophil plasma membrane and other membrane proteins (Table 11-1).

Neutrophil granule contents play important roles beyond their direct anti-microbial effects, including amplifying or dampening innate and adaptive immune responses. Lactoferrin released during phagocytosis inhibits proliferation of mixed lymphocyte cultures in vitro by decreasing release of IL-2, TNF, and IL-1β. On the other hand, proteinase 3 augments release of active TNF and IL-1β in monocyte/neutrophil co-cultures by releasing the membrane-bound forms of these cytokines.[13] Gelatinase B converts latent IL-1β into its active form and potentiates IL-8 activity by truncating this chemoattractant and increasing its release, consequently amplifying neutrophilic influx.[14,15] Neutrophil elastase may also play a pro-inflammatory role by virtue of its ability to cleave and disrupt phosphatidyl serine receptors on macrophages. Apoptotic cells undergo membrane alterations that lead to expression of phosphatidyl serine on their outer membrane surface, and interaction of phosphatidyl serine with its receptor leads to macrophage responses that downregulate inflammation through the generation of transforming growth factor (TGF)-β.[16] By disrupting these interactions, neutrophil elastase may permit inflammation to continue.

## NEUTROPHIL ACTIVATION AND SIGNAL TRANSDUCTION

For bloodstream neutrophils to destroy foreign targets in the periphery, they must first sense the presence of such targets at a distance. They must then attach to the activated endothelium of blood vessels through multiple interactions involving adhesion molecules and their receptors (rolling and adhesion). After passing through the endothelium of postcapillary venules (diapedesis), neutrophils migrate to the source of the signal (chemotaxis). Finally, neutrophils must encounter a target, engulf it, and destroy it. Collectively, the processes that allow neutrophils to respond this way are referred to as *neutrophil activation*. Because of the potential for tissue destruction, neutrophil activation must be carefully regulated. The internal responses through which a cell translates an encounter with a stimulus into a particular phenotypic response are termed *signal transduction* (Figure 11-3).[17]

### Stimuli and Receptors

Classic neutrophil chemoattractants include lipid mediators (e.g., leukotriene $B_4$ [$LTB_4$] and platelet-activating factor) and proteins/peptides (e.g., formylated peptides, the complement split product C5a, and IL-8). In vivo, chemoattractants are formed at sites of inflammation—either produced at the site by inflammatory cells (e.g., $LTB_4$ or IL-8) or liberated from already synthesized proteins, as in the case of C5a. The ability of formylated peptides such as *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) to stimulate neutrophils probably represents a particularly ancient arm of the innate immune response because prokaryotic cells but not eukaryotic (e.g., human) cells synthesize proteins whose first amino acid is a formylated methionine, allowing the more advanced organisms to recognize the more primitive ones. In addition to their attractant activities, chemoattractants also stimulate most other aspects of neutrophil activation. Their individual potencies for particular responses differ, however, suggesting that they serve overlapping but distinct functions in neutrophil activation.[18] CXC chemokines are a group of chemoattractants characterized by the presence of two N-terminal cysteines ("C") separated by any other amino acid ("X") at the carboxy terminus. CXC chemokines that play a role in neutrophil recruitment include IL-8 (CXCL8), KC (CXCL1), and macrophage inflammatory protein (MIP)-2 (CXCL2).

Bloodstream activation of neutrophils depends on the presence of specific surface receptors. Most chemoattractant receptors belong to a class known as *seven-transmembrane-domain receptors* (also called *serpentine seven receptors* or G *protein–coupled receptors* [GPCRs]); these receptors are composed of a single protein chain whose hydrophobic domains snake across the plasma membrane a total of seven times.[19] Binding of chemoattractants occurs in a pocket on the extra-cellular face of the plasma membrane, at or below the level of the lipid hydrophobic head groups. Receptors for soluble ligands other than chemoattractants have also been identified on neutrophils, including receptors for growth factors, colony-stimulating factors, and cytokines. Growth factor receptors are members of the protein tyrosine kinase receptor family, in which ligand interaction with two identical or related receptors brings them into proximity, causing their cross-phosphorylation and activation. Receptors for a variety of inflammatory stimuli become highly expressed only in mature neutrophils.[20] These receptors include CXC and CC chemokine receptors such as IL-8R-α and -β; CXCR-4 and CCR-1, -2, and -3; receptors for TNF and interferon (IFN)-α and -γ; and interleukin receptors IL1R, IL4R, IL6R, IL10R, and IL17R. Some nonchemoattractant ligands do not directly activate neutrophils but modulate their function. For example, pre-treatment of neutrophils with either insulin or GM-CSF results in amplification of

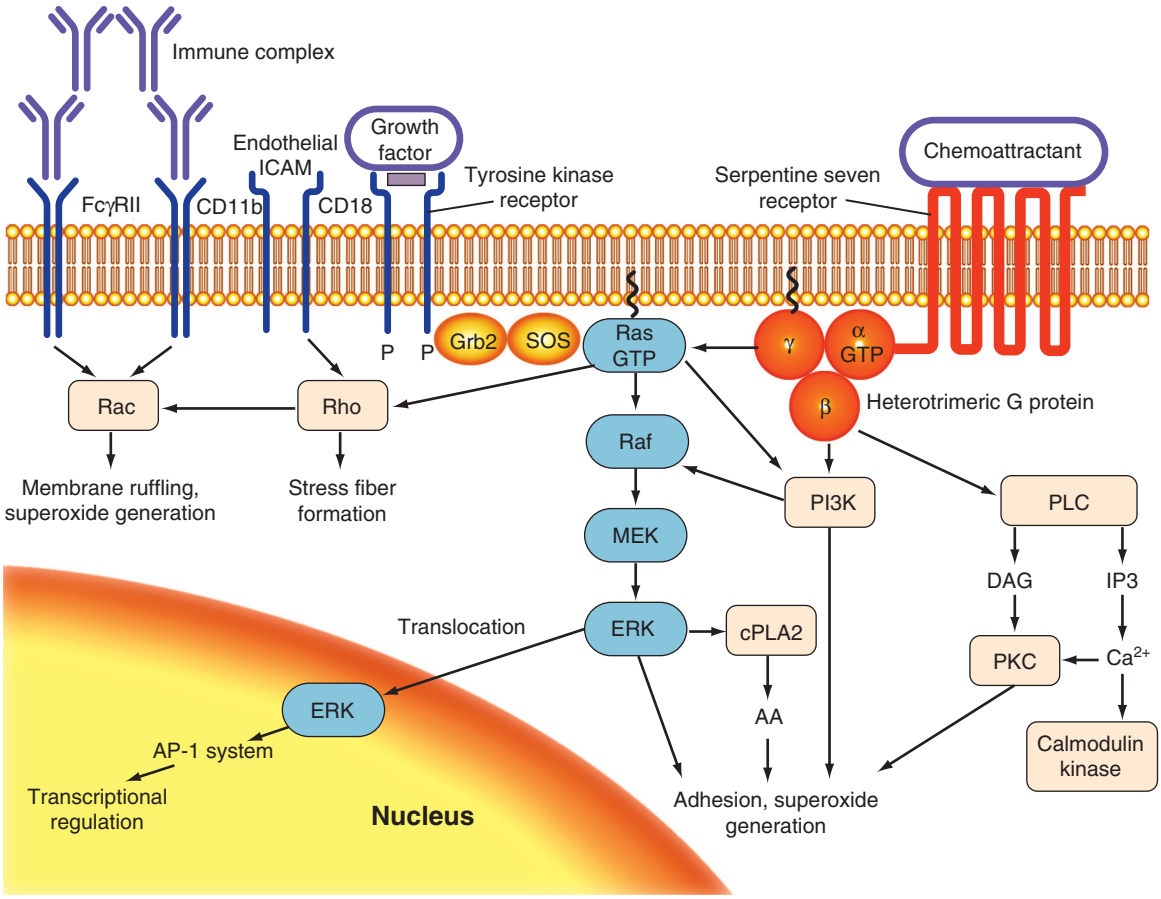



**Figure 11-3** Signaling pathways in neutrophil activation. Engagement of Fc, growth factor, and chemoattractant receptors and adhesion molecules initiate signaling pathways that result in pro-inflammatory neutrophil responses including cytoskeletal and morphologic changes, activation of adhesion molecules and the superoxide generating system (nicotinamide adenine dinucleotide phosphate oxidase), and regulation of transcription. Some of the well-established pathways participating in these responses are illustrated (see text for details). AA, Arachidonic acid; cPLA2, cytosolic phospholipase $A_2$; DAG, diacylglycerol; ERK, extra-cellular–signal regulated kinase; GTP, guanosine triphosphate; ICAM, intercellular adhesion molecule; IP3, inositol 1,4,5-triphosphate; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C.

subsequent neutrophil responses to chemoattractants, a process referred to as *priming*.

## Guanosine Triphosphate–Binding Proteins

Ligation of seven-transmembrane-domain receptors results in the activation of a class of intra-cellular effectors known as *heterotrimeric guanosine triphosphate (GTP)–binding proteins*, or G proteins. G proteins are comprised of α, β, and γ subunits, and individual G protein types are distinguished by the particular α, β, and γ subunits they employ. In neutrophils, the predominant G proteins are of the $G_i$ family. G protein γ subunits are modified by the addition of prenyl (polyisoprene) and carboxy-terminal methyl groups, which anchor them to the plasma membrane. All G proteins share the capacity, localized to their α subunits, to bind GTP and hydrolyze it to guanosine diphosphate (GDP). G proteins are active when they are GTP bound, but they are inactive in the GDP-bound form. Engagement of the appropriate serpentine-seven receptor promotes the binding of GTP on the α subunit. As a consequence of GTP binding, heterotrimeric G proteins dissociate into α and β/γ components, each with specific effector functions.

A monomeric class of low-molecular-weight (20 to 25 kDa) GTP-binding proteins (LMW-GBPs) is also important in neutrophil signal transduction. Because the first LMW-GBP described was the proto-oncogene Ras, these proteins also are referred to as *Ras-related* or *Ras superfamily proteins*, or simply *small guanosine triphosphatases (GTPases)*. LMW-GBPs combine, in one molecule, the prenyl and methyl modifications of the G protein γ subunit with the GTP-binding capacity of the α subunit.[21] At least four families of LMW-GBP have been described: the Ras family, whose members play roles in activation, as well as cell growth and division; the Rho family, which functions in cytoskeletal rearrangements; and the Rab and Arf families, which are crucial for vesicular and endomembrane trafficking.[22] All four classes of LMW-GBPs are represented in neutrophils.

## Second Messengers

Second messengers are small, diffusible molecules that are generated in response to stimuli and transmit signals from membrane receptors to downstream effector proteins. In the classic model of neutrophil activation, engagement of receptors results in the activation of phospholipase C,

which cleaves phosphatidylinositol triphosphate ($PIP_3$) into diacylglycerol (DAG) and inositol 1,4,5-triphosphate ($IP_3$). DAG and $IP_3$ mediate the influx of cytosolic calcium and the activation of protein kinase C (PKC), respectively. Other phospholipases present in the neutrophil include cytosolic phospholipase $A_2$ (cPLA2), which cleaves phosphatidylcholine and/or ethanolamine to generate arachidonic acid (AA), and phospholipase D, which cleaves phosphatidylcholine into phosphatidic acid and choline.[23] Although many second messengers are implicated in neutrophil activation, others may have inhibitory effects. For example, sphingosine and ceramide inhibit neutrophil phagocytosis.

In addition to lipids, other organic and inorganic second messenger molecules have been characterized. Intracellular concentrations of cyclic adenosine monophosphate (cAMP), a classic second messenger, increase rapidly in neutrophils exposed to both stimuli and inhibitors. In these settings cAMP is likely to provide a negative regulatory (off) signal because direct exposure to cAMP inhibits most neutrophil responses, probably through the activation of protein kinase A (PKA).[18] In contrast, increases in cyclic guanosine monophosphate (cGMP) have a modest enhancing effect on some neutrophil responses. Nitric oxide (NO), an important molecule in the regulation of host defense, is produced in neutrophils at low levels.[24] NO exerts a variety of second messenger effects, including inhibition of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, actin polymerization, and chemotaxis (discussed later). Conversely, excessive NO production has been implicated in many rheumatic diseases.[25]

## Kinases and Kinase Cascades

Multiple kinases—that is, proteins capable of enzymatically adding phosphate groups to target molecules—contribute to signaling in myeloid and nonmyeloid cells. PKC, which is now understood to be a family of kinases, was among the first kinases implicated in neutrophil activation in response to chemoattractants. The ability of phorbol myristate acetate (PMA), a synthetic activator of PKC, to stimulate neutrophil responses supports a role for PKC in neutrophil activation.[26] Conversely, inhibitors of PKC block stimulation of neutrophil functions.

The mitogen-activated protein kinases (MAPKs) are a family of serine threonine kinases, including the extracellular–signal regulated kinase (ERK), p38, and c-Jun amino terminal kinase (JNK) families. In neutrophils, chemoattractants and other stimuli activate ERK, p38, and JNK, with time courses consistent with neutrophil activation. A role for ERK activation in signaling for both neutrophil superoxide anion ($O_2^-$) and neutrophil adhesion and phagocytosis has been demonstrated.[18,27] Phosphatidylinositol 3–kinase (PI3K) is a set of related enzymes that are found in abundance in neutrophils and primarily catalyze the phosphorylation, not of proteins, but of the 3-position of phosphatidylinositol phospholipids. One of the main bioactive products of PI3K is $PIP_3$. Chemoattractants such as FMLP rapidly activate PI3K in neutrophils, where it participates in diverse neutrophil functions including $O_2^-$ generation, adhesion, and degranulation.[28] PI3K also may regulate neutrophil survival and apoptosis.

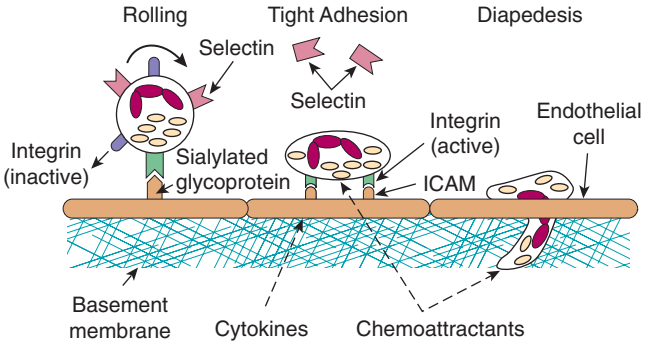



**Figure 11-4** Neutrophil adhesion to the vascular endothelium. *Left,* Rolling. An unstimulated neutrophil adheres with low affinity to the unstimulated endothelium of a postcapillary venule, a process mediated by the interaction of selectins (on neutrophil and endothelium) with sialylated glycoproteins and resulting in the rolling of neutrophils along the vessel wall. *Center,* Tight adhesion. Exposure of the neutrophil to chemoattractants results in activation of integrins (CD11a/CD18, CD11b/CD18); exposure of endothelium to cytokines results in the expression of intercellular adhesion molecules (ICAMs). These molecules interact, resulting in tight adhesion. Concurrently, selectins may be shed from the cell surfaces. *Right,* Diapedesis. A neutrophil undergoes diapedesis, passing across the endothelium and making its way through the basement membrane. Bloodstream neutrophils have the capacity to adhere to and move out of the vasculature in response to tissue signals for inflammation.

# NEUTROPHIL FUNCTION

## Adhesion

One of the earliest, crucial aspects of the inflammatory response is the ability of bloodstream neutrophils to adhere to vascular endothelium preparatory to movement into the tissues (Figure 11-4). Stimulated neutrophils also possess the ability to adhere to each other (homotypic aggregation), which can bring bloodstream neutrophils into proximity with neutrophils already adherent to the vessel or concentrate them at a site of inflammation. Several families of interacting adhesion molecules have been shown to exist on neutrophils and endothelial cells, including selectins, integrins, intercellular adhesion molecules (ICAMs), and sialylated glycoproteins.

### Selectins and Sialylated Glycoproteins

The selectin family includes three related molecules (L-selectin on leukocytes, E-selectin on endothelial cells, and P-selectin on activated platelets and endothelial cells). Selectins share a common structure of two or more complement regulatory domains, an epidermal growth factor–like domain, and a lectin domain. Each selectin binds to a specific sialylated glycoprotein on the surface of its interacting cell: E-selectin binds to the sialyl Lewis$^x$ antigen on neutrophils, P-selectin binds P-selectin glycoprotein-1 (PSGL-1) on neutrophils, and L-selectin binds PSGL-1 and glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1) on the endothelium. Selectin/sialylated glycoprotein interactions are of low affinity and transient, and leukocytes therefore repetitively bind to, and release from, the endothelial surface. The result is a pool of bloodstream neutrophils that is, at any given time, loosely marginated to the vascular surface and moving along it slowly

in a rolling, tumbleweed-like motion. Exposure of neutrophils and endothelium to appropriate stimuli (e.g., adrenergic discharge and corticosteroids) leads to shedding of selectins and neutrophil release (demargination), with apparent increases in the peripheral neutrophil count.

### Integrins and Intercellular Adhesion Molecules

The integrins are a large family of heterodimeric molecules generated by various combinations of α and β chains. Like the selectins, integrins require divalent cations ($Ca^{2+}$ or $Mg^{2+}$ or both) to engage their ligands. Neutrophils express three $\beta_2$-type integrins, each constructed from a distinct α subcomponent (CD11a, CD11b, or CD11c) and a common $\beta_2$ chain (CD18). Integrins primarily use ICAMs as their counterligands. CD11b/CD18 (also called *Mac-1* or *CR3*) binds to fibrinogen, factor X, heparin, and the complement component iC3b in addition to ICAMs; it is most strongly implicated in neutrophil/endothelial and neutrophil/neutrophil interactions. In contrast to the selectins, neutrophil CD11b/CD18 is constitutively expressed but inactive; stimulation of neutrophils results in changes in the CD11b/CD18 activation state and increases its affinity for ICAMs and other ligands.[29] Stimulation of endothelium with cytokines such as IL-1β results in increased expression of ICAM-1 and ICAM-2, providing a coordinate mechanism for the regulation of adhesion. In contrast to selectin-mediated adhesion, integrin/ICAM interactions are high affinity and persistent. Stimulation of rolling neutrophils therefore results in their tight adhesion to vessel walls and constitutes the first committed step in the movement of neutrophils into tissues. Additionally, engagement of integrins by their counterligands sends signals into the cell ("outside in" signaling) that regulate selective cell responses such as cytoskeletal reorganization, oxidant production, and degranulation. Outside-in signaling through CD11b/CD18 also coordinates with signaling through Fc receptor FcγRIII to regulate a number of different functions, including phagocytosis of particles opsonized by immunoglobulin (Ig)G and the complement component iC3b, along with neutrophil-dependent adhesion in immune complex–mediated vascular inflammation.[30] Cross-talk between neutrophils and endothelial cells has been shown to be a CD11b/CD18-dependent event: cross-linking of CD18 on neutrophils leads to increased endothelial permeability, probably through the release of neutrophil proteases.[31]

The function of CD11a/CD18 (also known as lymphocyte function–associated antigen [LFA]-1) on neutrophils has been controversial, but accumulating data indicate that this molecule may be necessary for neutrophil adhesion and emigration.[32] During neutrophil rolling on inflamed endothelium, neutrophil engagement by PSGL-1 leads to CD11a/CD18 activation, which in turn contributes to neutrophil arrest.[33] The function of CD11c/CD18 in neutrophils remains less clear.

## Diapedesis and Chemotaxis

### Diapedesis

The mechanism by which neutrophils pass through blood vessels *(diapedesis)* is not fully established. One report suggests a model in which neutrophils pass directly through pores generated within the endothelial cells themselves, but neutrophils are more generally thought to pass between endothelial cells by disrupting cell-cell junctions (Figure 11-5).[34] Diapedesis requires homotypic interactions between adhesion molecules found on neutrophils and endothelial cells known as *platelet–endothelial cell adhesion molecules* (PECAMs). These molecules are concentrated at endothelial cell junctions, and antibodies that block PECAM inhibit transmigration in vitro by limiting neutrophils to the apical surface of the endothelium. Transmigrating neutrophils undergo upregulation of α6β1, an integrin that mediates binding to laminin (a key component of the perivascular basement membrane). Antibodies to α6β1 generally block neutrophil transmigration but fail to do so in a PECAM knockout mouse, implicating α6β1/PECAM as crucial to the passage of neutrophils out of the vasculature.[35] CD47, otherwise known as *integrin-associated protein*, and CD99, expressed on neutrophils and endothelial junctions, have also been implicated in neutrophil passage through the endothelium.

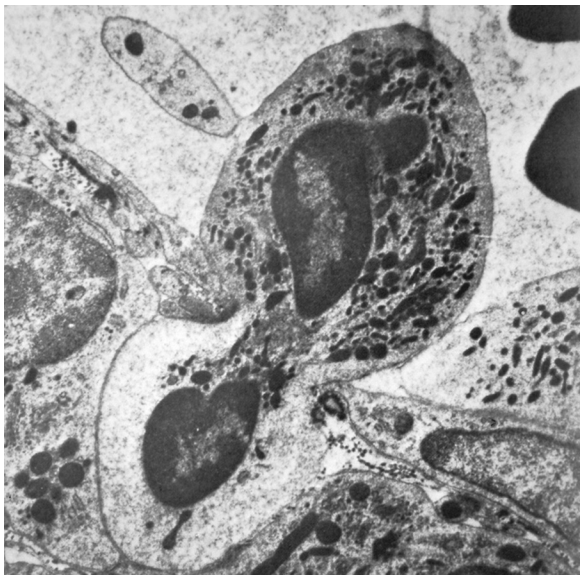


**Figure 11-5** Neutrophil diapedesis through the vascular endothelium. A neutrophil passaging between, or through, one or more endothelial cells is illustrated. The characteristic neutrophil multilobed nucleus and multiple granule types are visible. The leading edge of the neutrophil, passing through the endothelium, is relatively devoid of granule contents, suggesting that it represents the formation of a specialized structure for diapedesis, including the formation of F-actin cytoskeleton.

Once beyond the endothelium, neutrophils typically pause before traversing the basement membrane (basal lamina). Classic studies by Huber and Weiss suggest that neutrophils pass through the basement membrane via active disruption of its patency, without utilization of known proteases or oxygen radicals.[36] The disruptions are rapidly repaired by an unknown mechanism likely involving the endothelium.

### Chemotaxis

Chemotaxis in the direction of a molecular gradient is achieved by the extension of membrane ruffles (lamellipodia), followed by anchorage of the ruffles to the substrate

and withdrawal of the trailing edge of the cell in the direction of movement. These changes are accomplished primarily through rearrangement of the actin cytoskeleton after sensing chemotactic gradients that signal through GPCRs and PI3K. Actin is a 41 kDa protein that exists as a soluble, globular monomeric form (G-actin) and as an insoluble linear polymer (F-actin). F-actin may be assembled (extended) at one end (the barbed end) and disassembled at the other, under the control of regulatory molecules. During chemotaxis, F-actin formation and extension is concentrated at the leading edge of the neutrophil, permitting extension of the cell membrane (Figure 11-5). Chemoattractant receptors also concentrate at the leading edge, defining the cell's directional response to the gradient (the headlight phenomenon). As the neutrophil moves along, receptors that were formerly at the leading edge are swept to the tail and internalized.

## Phagocytosis and Degranulation

### Phagocytosis

Neutrophil phagocytosis of an encountered bacterium or other particle requires direct contact. Phagocytosis can be activated by direct neutrophil recognition of pathogen-associated molecular patterns (PAMPs), which are small molecule motifs found on or in bacteria or viruses (but not typically mammalian cells). PAMPs are recognized by Toll-like receptors (TLRs)[37] and other pattern recognition receptors (PRRs). Human neutrophils express all TLRs except for TLR3, and TLR activation stimulates human neutrophil phagocytosis by promoting structural and conformational changes.[38] However, neutrophils are generally poor at phagocytosing unmodified targets, particularly encapsulated bacteria. Phagocytosis is greatly enhanced by opsonization (from the Greek, "to prepare for the table"), that is, the modification of a target via its decoration with immunoglobulin, complement components, or both (Figure 11-6).

Neutrophils express two families of receptors for the Fc portion of complexed or aggregated IgG: low-affinity FcγRIIa and high-affinity FcγRIIIb. During some infections, or after in vitro stimulation with interferon or G-CSF, neutrophils also express the high-affinity receptor FcγRI, which binds monomeric IgG. FcγRIIa binds subclasses of IgG with varying efficiency depending on a polymorphism at amino acid position 131. FcγRIIIb polymorphisms of neutrophil antigens NA1 and NA2 also determine binding to IgG subclasses. Persons homozygous for the NA2 allele have a lower capacity to mediate phagocytosis than do persons homozygous for the NA1 allele. These differences have important implications for rheumatic diseases in which immune complexes play an important role.

Phagocytosis is an active process involving extension of the neutrophil membrane (filopodia and lamellipodia formation) and invagination of the neutrophil at the locus of the target. Engagement of FcγR and complement receptors results in the activation of different signaling pathways that play distinct roles in phagocytosis.[39] Whereas engagement of CR3 (CD11b/CD18) results in actin stress fiber formation and invagination, engagement of FcγRII results primarily in extension of membranes out from and around the target. Signaling by these receptors depends on the activation of distinct members of the Rho family of LMW-GBPs.

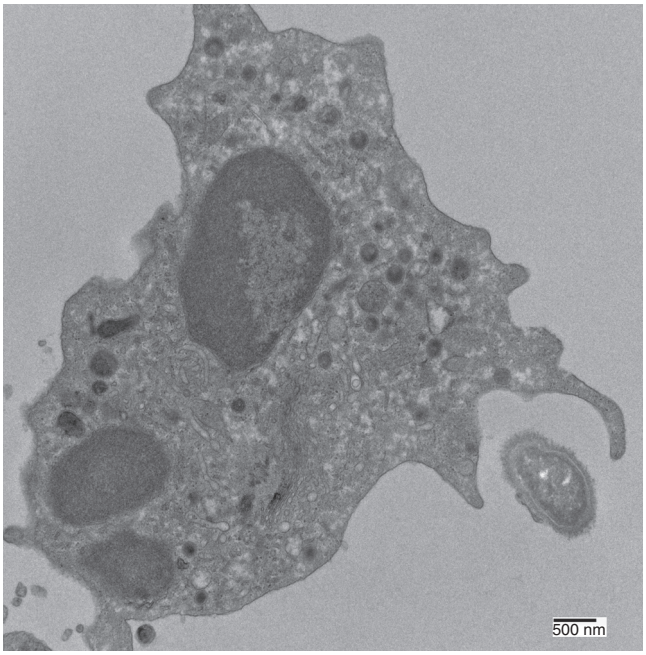



**Figure 11-6** A neutrophil engulfing bacteria. Neutrophils can internalize and kill many microbes, with each phagocytic event resulting in the formation of a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted. This transmission electron micrograph shows the precise moment in which a bacterium is being phagocytosed by a neutrophil. *(Courtesy K.A. Zarember, D.E. Greenberg, and K. Nagashima, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health.)*

### Degranulation

Upon engaging a target, neutrophils degranulate, a term reflecting two possible and distinct events. Vesicles can fuse with the plasma membrane, spilling their contents into the extra-cellular space (see Figure 11-1), or they can fuse with the phagocytic vacuole to form a phagolysosome. The former type of degranulation is regulated differentially from the latter and favors mobilization of lighter granules in response to stimuli (secretory vesicles > gelatinase granules > specific granules > azurophilic granules). In the latter type of degranulation (phagolysosome formation), the preferential fusion of azurophilic granules with the phagocytic vacuole results in the delivery of proteolytic proenzymes, MPO, and antibacterial proteins to the site of an ingested bacterium. Fusion of specific granules with the phagocytic vacuole permits the delivery of collagenase, activation of azurophilic granule enzymes, and the appropriate localization of cytochrome $b_{558}$, a requisite for NADPH oxidase (discussed later). Containment of potentially toxic substrates within the phagolysosome keeps host tissue damage and neutrophil autodestruction in check. However, control of granule content is imperfect and toxic molecules may still spill into the extra-cellular milieu. As discussed subsequently, however, in several of the rheumatic diseases, neutrophil activation plays an important role in abetting inflammation and host tissue damage.

## Respiratory Burst

In addition to the proteases and other antibacterial proteins contained in their granules, neutrophils have the capacity to kill bacteria through the generation of toxic oxygen metabolites such as NO, $O_2^-$, and $H_2O_2$. This process, mediated by the NADPH oxidase system and frequently referred to as the *oxidative or respiratory burst*, is extremely potent and requires tight regulation to prevent neutrophil autodestruction.[40] The central component of NADPH oxidase is cytochrome $b_{558}$, which is localized to the membranes of specific granules and consists of two subunits: a 22 kDa component (gp22$^{phox}$, for *ph*agocyte *ox*idase) and a 91 kDa component (gp91$^{phox}$). This cytochrome lacks independent activity, however, and three cytosolic proteins are also required for oxidase activation: a 47 kDa and a 67 kDa component (p47$^{phox}$ and p67$^{phox}$) and a LMW-GBP, p21$^{rac}$.[41] On neutrophil stimulation, the p47$^{phox}$ and p67$^{phox}$ components translocate to the membranes to form an active complex with the cytochrome (Figure 11-7).[41] Although p21$^{rac}$ also translocates in response to stimuli and appears to be necessary for oxidase system activity, the significance of its translocation is more controversial.[42] A fifth protein, p40$^{phox}$, associates with p47/p67, regulates the oxidase system, and plays a role in phagocytosis-induced superoxide production.[43]

When assembled and activated, the NADPH oxidase transfers electrons from NADPH to generate $O_2^-$:

$$2O_2 + NADPH \xrightarrow{\text{NADPH Oxidase}} 2O_2^- + NADP^+ + H^+$$

A subsequent, spontaneous dismutase reaction rapidly produces $H_2O_2$:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Although $O_2^-$ and $H_2O_2$ can kill organisms in vitro, they are short-lived and probably do not account for most of the bacterial killing capacity of the system under normal circumstances Indeed, many bacteria possess catalase, an enzyme that degrades $H_2O_2$. Instead, the production of $H_2O_2$ within the same space into which MPO has been released permits the generation of large quantities of hypochlorous acid, a powerful oxidant with potent killing capacity. Hypochlorous acid can interact further with proteins to form chloramines, which are less potent but longer-lived oxidants. Neutrophil oxidant production plays a key role in the body's defense against microorganisms.

The current view that oxidant production, via the production of hypochlorous acid by MPO, is the neutrophil's most powerful tool against microbes has been challenged, however. Mice lacking either NADPH oxidase or elastase and cathepsin G are susceptible to infection, implying that both arms of defense—oxidant production and protease-mediated microbial destruction—are equally crucial. Moreover, superoxide production in phagocytic vacuoles may indirectly promote protease function by causing the vacuolar pH to rise (secondary to the consumption of protons necessary to make $H_2O_2$), which, in turn, causes an influx of $K^+$. The resulting increase in ionicity liberates cationic proteases from the anionic proteoglycan matrix, freeing them to kill bacteria. In this new model, oxidants are not primarily destructive to microbes, but rather are necessary to assist proteolytic damage.[44]

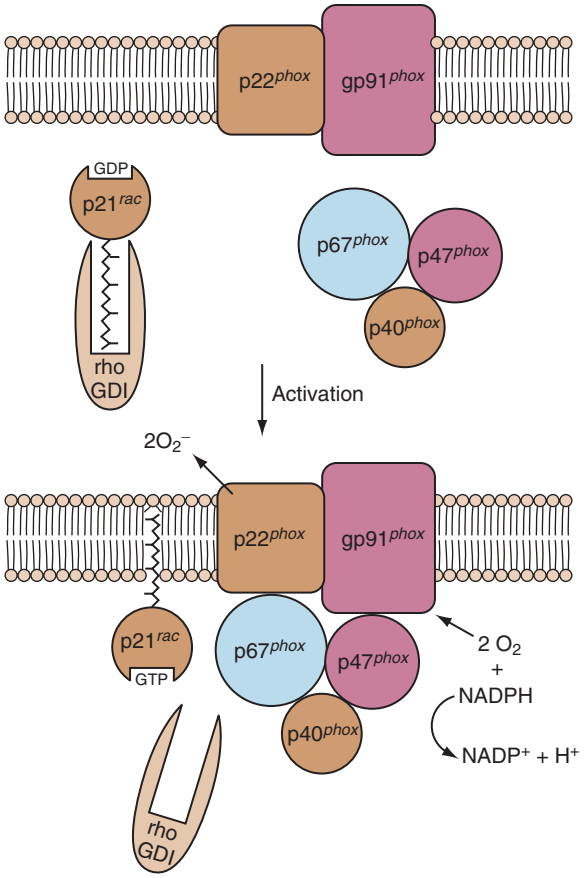



**Figure 11-7** Assembly of the neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system. *Top,* Basic components of the NADPH oxidase as they are distributed in a resting state. The cytochrome $b_{558}$, composed of the two subunits gp91$^{phox}$ and p22$^{phox}$, are membrane associated, whereas p47$^{phox}$, p67$^{phox}$, and the more recently identified p40$^{phox}$ exist as a complex in the cytoplasm. p21$^{rac}$ in an inactive, guanosine diphosphate (GDP)-bound form also resides in the cytoplasm, in association with a chaperone (Rho-GDI) that sheaths its hydrophobic tail to permit solubility. *Bottom,* Activation of the neutrophil leads to translocation of the cytosolic components of the oxidase to the neutrophil membrane, where they form an active complex with the cytochrome, resulting in the generation of oxygen. The potentially damaging oxidase system is carefully regulated through the segregation and assembly of its component parts.

## Nonphagocytic Mechanisms of Neutrophil Action

Recently discovered nonphagocytic mechanisms of neutrophil-mediated bacterial killing further augment host defense.

### Neutrophil Extra-cellular Traps

Neutrophil extra-cellular traps, or NETs, are extra-cellular meshes composed of chromatin and neutrophil granular proteins (Figure 11-8).[45] In some circumstances NETs are released upon cellular activation. They entrap bacteria while simultaneously providing a scaffold to promote high local concentrations of anti-microbial components, thus killing microbes extra-cellularly. NET formation can be suicidal to neutrophils, leading to chromatin decondensation, nuclear swelling, and membrane perforation (suicidal NETosis).[46] Chromatin decondensation depends on histone

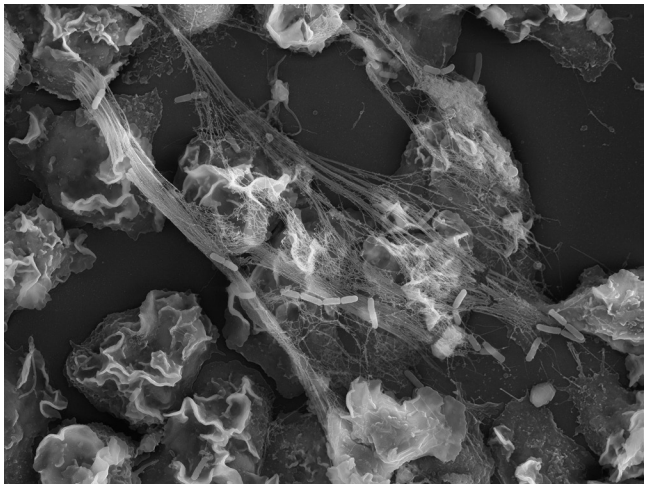


**Figure 11-8** Neutrophil extra-cellular traps (NETs). NETs are complex extra-cellular structures that are composed of chromatin, with specific proteins from the neutrophilic granules attached. NETs can trap Gram-negative bacteria, Gram-positive bacteria, and fungi. A scanning electron micrograph shows stimulated neutrophils forming NETs to trap *Shigella flexneri. (Courtesy V. Brinkmann and A. Zychlinsky, Max Planck Institute for Infection.)*

hypercitrullination catalyzed by peptidylarginine deiminase 4, as well as neutrophil elastase, which digests nucleosomal histones.[47,48] MPO binding of chromatin enhances nuclear decondensation and may also thereby promote suicidal NETosis.[49] However, not all NETosis leads to neutrophil destruction. In some cases neutrophils undergo "vital NETosis" in which they lose their nuclear material but retain the ability to chemotax and phagocytose microorganisms.[50] NETosis is also driven partly by reactive oxygen species, because neutrophils in which oxidant production is blocked are unable to undergo NETosis.[51,52]

## Microparticles

Neutrophil-derived microparticles are emancipated cytosol-entrapping vesicles of neutrophil plasma membrane. Like NETs, microparticles are previously unappreciated propagators of neutrophil-mediated outcomes (Figure 11-9). During activation, neutrophils release these heterogeneous vesicles, which range in diameter between 100 and 1000 nm and express surface markers of their parent cells. Microparticles can bind to target cells (including endothelial cells) in a receptor-dependent manner and, in so doing, transfer parent

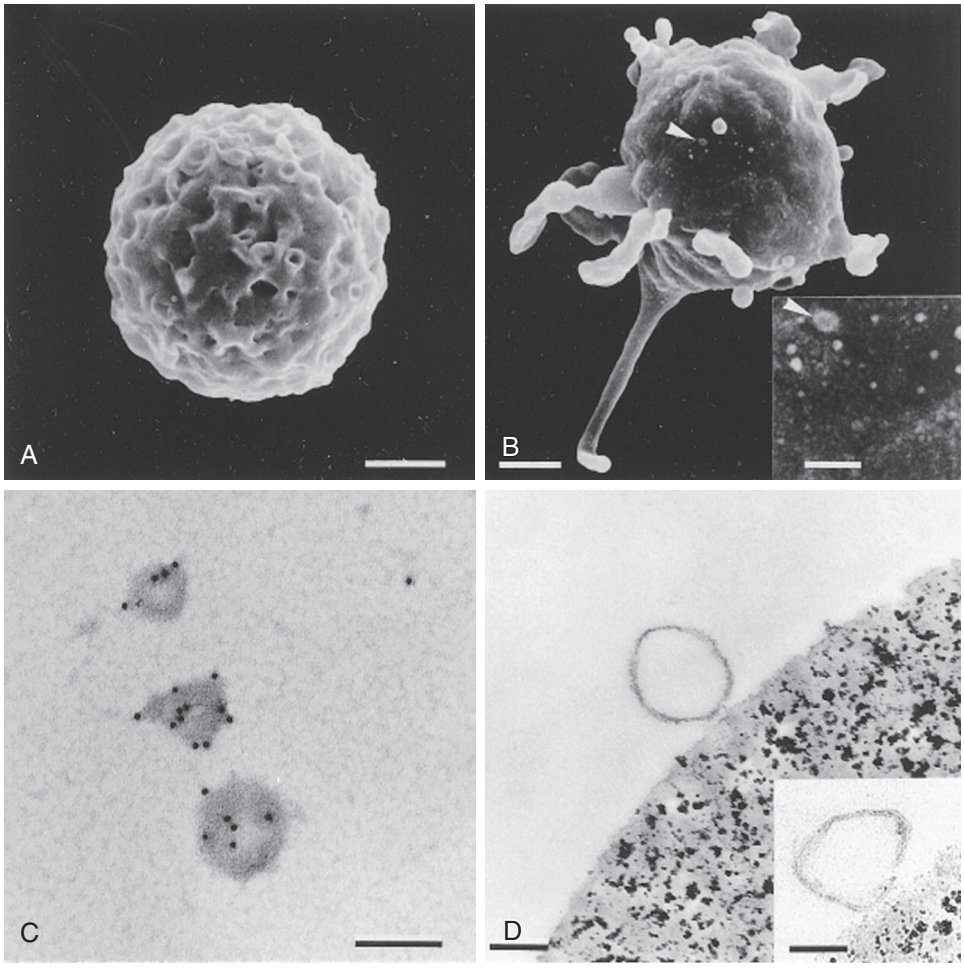



**Figure 11-9** Neutrophil microparticles. **A,** Scanning electron micrograph of a resting neutrophil (scale bar: 1.5 μm). **B,** Scanning electron micrograph of a stimulated neutrophil 0.1 μM N-formyl-methionyl-leucyl-phenylalanine (FMLP). Note the large pseudopodia and budding of small vesicles (70-300 nm in size) from the neutrophil cell membrane (*arrowhead*; inset, higher magnification showing microparticle at *arrowhead*). **C,** Transmission electron micrograph of immunogold-labeled microparticles, indicating the presence of antigens also found on the neutrophil cell surface (also seen in **D**) (scale bar: 150 nm). **D,** Thin sections of microparticles precipitated with Dynabeads (Thermo Fisher Scientific, Waltham, Mass.) demonstrate bilamellar structure. *(From Hess C, Sadallah S, Hefti A, et al: Ectosomes released by human neutrophils are specialized functional units.* J Immunol *163:4564–4573, 1999.)*

cell messenger RNA (mRNA) and microRNA (miRNA) to drive transcellular responses. Microparticle numbers increase in sepsis, as well as vascular disease, and they also appear to play a significant role in infection-mediated thrombosis.[53-56]

## Neutrophil Production of Pro-inflammatory Mediators

### Arachidonic Acid Metabolites

The capacity of stimulated neutrophils to liberate AA from membranes has implications for acute inflammation. Although AA has intrinsic chemoattractant and neutrophil-stimulatory properties, its metabolites are more crucial to inflammatory effects.[27,57,58] Best recognized among these metabolites are the leukotrienes (LTs). Neutrophils can produce $LTB_4$, a highly potent mediator for the chemoattraction of other neutrophils.[57] Intermediates of LT production (e.g., 5-hydroxyeicosatetraenoic acid) also are produced by neutrophils and may have stimulatory properties.[27]

The cyclo-oxygenase (COX) (endoperoxide synthase) pathway is the other major pathway of AA metabolism. AA metabolized by COX is converted into prostaglandin H, which undergoes further cell type-specific conversion to a variety of other prostaglandins.[59] The prostaglandin of most relevance to inflammation is prostaglandin $(PG)E_2$, whose numerous pro-inflammatory effects include increased vasodilation, vascular permeability, and pain. Interestingly, the direct effects of $PGE_2$ on neutrophils are largely inhibitory, probably through elevations of intra-cellular cAMP.[60] Although resting neutrophils exhibit little COX activity, persistent activation results in upregulation of COX-2, suggesting that neutrophils may contribute $PGE_2$ to both the inflammatory brew and the downregulation of their own activity.

### Cytokine Production

Although the relative amount of cytokine production by neutrophils is small, the large numbers of neutrophils present in infected or inflammatory sites suggest that overall neutrophil cytokine production may play a role in recruiting additional neutrophils to the target area. Cytokines produced by neutrophils include IL-8, IL-12, MIP-1α and β (CCL3 and CCL4), growth-related oncogene-α (GRO-α), oncostatin M, monocyte chemotactic protein (MCP)-1, and TGF-β. Multiple lines of investigation suggest that neutrophils may also be a source for IL-17, a potent cytokine that can amplify neutrophil migration and recruitment.[61]

Neutrophils do not produce IL-1β, TNF, or IL-6, the classical products of macrophages and synovial cells.[62] The transcriptional program of terminally differentiated neutrophils requires a selective combination of stimulants for the production of chemokines. In the presence of lipopolysaccharide, TNF drives neutrophil production of IL-8, GRO-α, and MIP-1, whereas IFN-γ drives CXCL9 and 10.[63] Other neutrophil-derived molecules have been identified as bridging factors between innate and adaptive immunity. Activated neutrophils release both B-lymphocyte stimulator (BLyS) and TNF-related apoptosis-inducing ligand (TRAIL).[64,65] Whereas BLyS stimulates B cell proliferation (through the TNF family receptors BAFF-R, TACI, and BCMA), TRAIL induces anti-tumor T cell effects and apoptosis.

TGF-β is a neutrophil chemoattractant at femtomolar concentrations, and its recruitment of neutrophils into an inflammatory space may lead to additional neutrophil cytokine production, including further production of TGF-β.[66] TGF-β also has potent anti-inflammatory effects and blocks neutrophil degranulation in response to lipopolysaccharide (LPS), FMLP, and TLR ligands.[16,67]

## Role of Neutrophils in Thrombosis

Recent evidence indicates that neutrophils play a key role in the activation of platelets and thrombin, underlining the interaction between inflammation and thrombosis. Neutrophils that accumulate at sites of inflammation do so in concert with aggregating platelets and further enhance platelet aggregation (Figure 11-10). Platelet-neutrophil complexes are elevated in patients with systemic lupus erythematosus and rheumatoid arthritis (RA), likely as a result of neutrophil activation.[68] On the other hand, in the setting of vascular injury and endothelial damage, platelets activated in response to exposed collagen express P-selectin, promoting neutrophil-platelet associations via neutrophil PSGL-1 counter-receptors. Subsequent activation of neutrophils leads to firmer bonds as a result of integrin CD11b/CD18 adhesion molecule activation.[69]

Neutrophil-platelet interactions result in surface expression of tissue factor that can initiate the coagulation cascade.[70] Neutrophil NETs can also play roles in promoting clotting, including disseminated intravascular coagulation. In those cases, extruded neutrophil chromatin serves as a platform for the extra-cellular co-localization of neutrophil elastase and the anti-thrombotic tissue factor pathway inhibitor (TFPI). Inactivation of TFPI by elastase then permits clotting to proceed.[71] In a flow restriction murine model of deep vein thrombosis, NETs were present in thrombi, and mice treated with deoxyribonuclease (DNAse) or rendered neutropenic were protected against clot formation.[72]

## Resolution of Neutrophil Infiltration

Inflammatory responses must eventually be resolved to avoid excessive tissue damage and initiate the healing process. Resolution of inflammation is an active and carefully regulated process. Pro-resolution signals include lipid derivatives (e.g., resolvins and lipoxins), annexin A1 and chemerin-derived peptides, and certain chemokines and cytokines.

### Resolvins

Resolvins differ according to the lipid from which they are derived. Resolvin E1 is produced from eicosapentaenoic acid and inhibits monocytes, macrophages, and dendritic cells (DCs), as well as neutrophils.[73,74] Resolvin D derives from docosahexaenoic acid and is also a potent inhibitor of neutrophil diapedesis and migration.[75] Maresin-1 (macrophage mediator in resolving inflammation-1) has properties similar to the D-resolvins.[76] Some prostaglandins such as $15d\text{-}PGJ_2$ also have anti-inflammatory properties.[77]

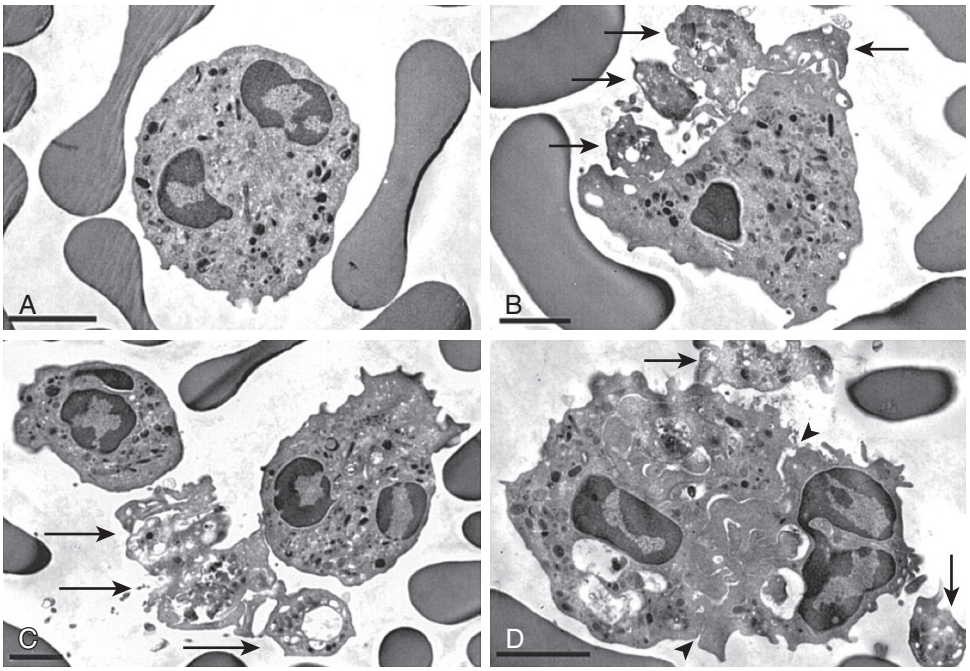



**Figure 11-10** Neutrophil-platelet interactions and neutrophil-neutrophil aggregation in blood specimens subjected to hydrodynamic shear, examined by transmission electron micrograph. **A,** A single neutrophil with few to no adherent platelets appears round (unactivated). **B,** A neutrophil bound to four platelets undergoes a shape change (i.e., it is activated by platelet contact). **C** and **D,** Neutrophil aggregates observed with platelets forming bridges between neutrophils **(C)** and on the periphery of homotypically aggregating neutrophils **(D)**. In **B, C,** and **D,** *arrows* mark platelets; in **D,** *arrowheads* mark the neutrophil-neutrophil contact region (scale bar in each panel: 2 µm). *(From Konstantopoulos K, Neelamegham S, Burns AR, et al: Venous levels of shear support neutrophil-platelet adhesion and neutrophil aggregation in blood via P-selectin and beta2-integrin.* Circulation *98:873–882, 1998.)*

### Lipoxins

The lipoxins are derived from AA and are potent anti-inflammatory molecules.[57] Lipoxin synthesis requires coordinated activity of neutrophil 5-lipoxygenase and a related enzyme (either 12-lipoxygenase or 15-lipoxygenase) from another cell type—either platelets or endothelial cells (Figure 11-11).[78] In contrast to LTs and prostaglandins, lipoxins are anti-inflammatory, suggesting that the accumulation of a mixed population of inflammatory cells may redirect arachidonate metabolism into the synthesis of anti-inflammatory molecules to promote the resolution of inflammation.[79] In some cases, apoptotic neutrophils at sites of inflammation are taken up by resident macrophages, promoting lipoxin A4 production.[80] Aspirin stimulates the generation of biologically active epilipoxins, suggesting a previously unappreciated mechanism for its anti-inflammatory action.[81]

### Other Inflammation-Resolving Molecules

After neutrophil activation, annexin A1 (lipocortin) is released in response to chemoattractants, downregulating transmigration and promoting neutrophil apoptosis and clearance.[82] Similar activities have been attributed to chemerin-derived peptides.[83] Activated neutrophils also produce IL-1 receptor antagonist (IL-1ra).[84] The efficacy of recombinant IL-1ra (anakinra) therapy in autoinflammatory diseases emphasizes its clinical importance in suppressing inflammation. An MMP-mediated mechanism of inflammatory resolution has also been identified. Macrophage-derived MMPs, such as MMP-1, MMP-3, and

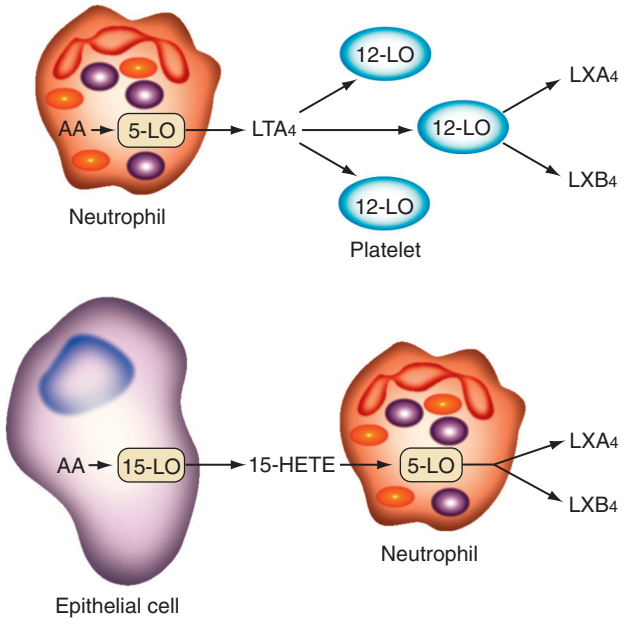



**Figure 11-11** Generation of the anti-inflammatory lipoxins $A_4$ and $B_4$ depends on the interaction between two different classes of inflammatory cells. *Top,* Lipoxin generation by neutrophils and platelets. Arachidonic acid (AA) generated by activated neutrophils is converted by neutrophil 5-lipoxygenase (5-LO) into leukotriene $A_4$ ($LTA_4$). $LTA_4$ may be converted by 12-LO in nearby platelets into lipoxin $A_4$ ($LXA_4$) and lipoxin $B_4$ ($LXB_4$). *Bottom,* Lipoxin generation by epithelial cells and neutrophils. AA generated by epithelial cells may be converted by 15-LO into 15-hydroxyeicosatetraenoic acid (15-HETE). In the setting of inflammation, 5-LO from adjacent neutrophils subsequently may convert 15-HETE into $LXA_4$ and $LXB_4$.

MMP-12, cleave CXC-chemokines, provoke the loss of their neutrophil-recruiting activity, and dampen the influx of cells.[85]

### Role of Apoptotic Neutrophils in Resolving Inflammation

Apoptotic neutrophils can inhibit live neutrophil chemotaxis and migration via release of lactoferrin and annexin A1.[86] Apoptotic neutrophils can also suppress granulopoiesis by inhibiting the pro-inflammatory consequences of IL-17/IL-23 axis activation. In this model, macrophages and DCs produce IL-23 at the site of insult, which in turn promotes IL-17 production by T cells (T helper [Th]17, γδ T cells, and natural killer [NK] T cells). IL-17 is a promoter of G-CSF production and a powerful neutrophil chemoattractant. Recruited neutrophils undergo apoptosis and are phagocytosed by macrophages, resulting in a decrease of IL-23. This decrease is followed by a reduction in IL-17 and G-CSF production, which downregulates granulopoiesis.[87]

An intriguing mechanism through which neutrophils might downregulate the action of protein inflammatory mediators has been defined. Apoptotic neutrophils (present during the resolving phase of inflammation) show increased expression of the chemokine receptor CCR5 on their surface (mediated by D and E resolvins), and this receptor can scavenge and reduce the soluble concentration of chemokines such as CCL3 and CCL5. These data emphasize again that neutrophils are not only inflammatory cells but may also play a direct role in the subsequent resolution of inflammation.[88]

## HERITABLE DISORDERS OF NEUTROPHIL FUNCTION

A variety of acquired conditions result in neutrophil dysfunction and/or depletion, including malignancies (myeloid leukemias), metabolic abnormalities (diabetes), and drugs (e.g., corticosteroids and chemotherapy). In addition, rare congenital disorders of neutrophils have been identified (Table 11-2). In general, patients with congenital impaired neutrophil function are prone to infection by bacteria (predominantly *Staphylococcus aureus*, *Pseudomonas* species, *Burkholderia*) and fungi (e.g., *Aspergillus* and *Candida*), but not viruses and parasites. The major sites of infection include skin, mucous membranes, and lungs, but any site may be affected, and spreading abscesses are common. Most of these diseases are potentially life threatening in the absence of available effective therapy.

### Diseases of Diminished Neutrophil Number

Severe congenital neutropenia (SCN, Kostmann's syndrome) results from marrow arrest of bone marrow myelopoiesis and leads to neutrophil counts persistently less than $0.5 \times 10^9$ cells/L. Monogenic autosomal dominant, autosomal recessive, sporadic, and polygenic subtypes have been identified.[89,90] Patients are prone from early infancy to severe bacterial infections including omphalitis, pneumonia, otitis, gingivitis, and perirectal infections. Because of the lack of acute inflammation, infections tend to spread extensively before being noted, and the mortality rate is high. Therapy consists of antibiotics and long-term treatment with recombinant G-CSF, which may help maintain normal or near-normal neutrophil counts. Patients with SCN are also at risk for acute myeloid leukemia (AML) and myelodysplastic syndrome, particularly those who respond poorly to G-CSF.[91] A milder form of neutropenia (benign congenital neutropenia) with higher neutrophil counts and fewer infections has also been observed. Another variant is cyclic neutropenia, which causes transient, recurrent neutropenia on a 21-day cycle. Studies suggest that defects in neutrophil elastase affect neutrophil survival in the marrow and may be responsible for severe congenital and cyclic neutropenia.[92] At least 52 different mutations in the *ELANE* gene, which encodes neutrophil elastase, have been described as the cause in around half of the patients.[93] Mutations in *HAX1* and glucose-6-phosphatase catalytic subunit 3 *(G6PC3)* genes account for a smaller proportion of cases of SCN. Other, less frequent deficiencies include X-linked neutropenia—caused by constitutively active mutations of the *WAS* gene—and defects in several myeloid transcription factors.

### Leukocyte Adhesion Deficiencies

Leukocyte adhesion disorders arise from defects in cell adhesion to extra-cellular matrix and vascular endothelium. Three distinct entities have been described in humans. Leukocyte adhesion deficiency type 1 (LAD1) results from an autosomal recessive defect in ITGB2, which encodes the CD18 chain of β2 integrins. Consequently, neutrophil β2 integrins fail to form, and bloodstream neutrophils are unable to adhere firmly to vascular endothelium and transmigrate to sites of infection.[94] Phagocytosis is also impaired. The clinical picture is similar to that of the neutropenias, with recurrent life-threatening infections. Peripheral neutrophil counts are typically elevated, however, reflecting the intravascular accumulation of cells in the face of their inability to exit the vessels. Complete LAD1 manifests in infancy and is characterized by omphalitis, recurrent life-threatening bacterial and fungal infections, gingivitis, and delayed wound healing. Absence of pus at infection sites is a hallmark of LAD1. Bone marrow transplantation is the only curative therapy. LAD2 results from an autosomal recessive defect in the glycosylation of sialyl Lewis$^x$ (SLex or CD15s), the neutrophil counter-ligand for endothelial selectins. Patients with LAD2 have neutrophils that are unable to roll along the endothelium and have symptoms similar to LAD1 but may also have mental retardation, short stature, distinctive facies, and the Bombay (hh) blood type.[95] A third variant, LAD3, has been described in which leukocytes have normal populations of surface integrins but lack the ability to signal these molecules into an active state.[96] Because integrin activation in this condition is also deficient in platelets, patients with LAD3 are at increased risk for infection and bleeding.

### Granule Defects

Chédiak-Higashi syndrome is an autosomal recessive disorder in which granule subtypes—in neutrophils, lymphocytes, melanocytes, Schwann cells, and others—undergo disordered fusion, resulting in giant, dysfunctional granules. The cause seems to be related to a defect in the gene for

**TABLE 11-2** Heritable Disorders of Neutrophil Function

| Disorder | Defect | Inheritance | Presentation | Therapy | Typical Prognosis |
|---|---|---|---|---|---|
| **Neutropenia** | | | | | |
| Severe congenital neutropenia (Kostmann's syndrome) | Maturation arrest (<0.5 × $10^9$ PMN/L) | AR (HAX1 mutations) | Bacterial infections (omphalitis, abscesses, gingivitis, UTIs) | RhG-CSF | Improved with treatment |
| Benign congenital neutropenia | Multiple etiologies (0.2-2 × $10^9$ PMN/L) | Variable | Mild infections | None | Good |
| Cyclic neutropenia | Stem cell defect, elastase gene deficiency (nadir every 21 days) | AD (ELA2 mutations) | Infection during nadirs | RhG-CSF | Improved with treatment |
| **Adhesion Deficiency** | | | | | |
| Leukocyte adhesion deficiency type 1 | Absent or abnormal CD18; deficiency in β2-integrin chain of leukocyte adhesion molecules | AR | Leukocytosis; recurrent infections (skin mucous, membranes, gastrointestinal tract) | Marrow transplant | Fair-poor |
| Leukocyte adhesion deficiency type 2 | Absent sialyl-Lewis$^x$ | AR | Neutrophilia; infection; retardation, short stature | | Poor |
| Leukocyte adhesion deficiency type 3 | Impaired activation of Rap1 GTPase | AR | Leukocytosis; recurrent infections; bleeding tendency | | Poor |
| **Chemotaxis Deficiency** | | | | | |
| Hyper-IgE syndrome | Chemotaxis defect | AD | Eczema; recurrent infections; elevated serum IgE levels | Skin care; antibiotics | Good |
| **Granule Disorders** | | | | | |
| Chédiak-Higashi syndrome | Defective lysosomal trafficking regulator gene | AR | Albinism; infection | Marrow transplant; antibiotics | Poor |
| Specific granule deficiency | Abnormal/reduced specific and azurophilic granules (lactoferrin deficiency) | AR? | Infection of skin, mucous membranes, lungs | | Fair-good |
| Myeloperoxidase deficiency | Myeloperoxidase absent | Variable (mostly AR) | None | Transfusion of HLA-identical leukocytes if severe | Excellent |
| p14 deficiency | Defective endosomal adapter protein gene | Recessive | Albinism; infection; short stature | None known to date | ? |
| **Oxidase Defects** | | | | | |
| Chronic granulomatous disease (multiple types) | gp91$^{phox}$ absent<br>p22$^{phox}$ absent<br>p47$^{phox}$ absent<br>p67$^{phox}$ absent<br>p40$^{phox}$ absent | X-linked 50%<br>AR 5%<br>AR 35%<br>AR 5%<br>AR 5% | Early childhood infections, especially skin and mucous membranes, abscesses | Interferon-γ | Improved with treatment |

AD, Autosomal, dominant; AR, autosomal, recessive; GTPase, guanosine triphosphatase; HLA, human leukocyte antigen; IgE, immunoglobulin E; PMN, polymorphonuclear neutrophil; RhG-CSF, recombinant human granulocyte colony-stimulating factor; UTIs, urinary tract infections.

lysosomal transport regulator (Lyst or CHS1).[97] Patients with Chédiak-Higashi syndrome present with partial oculocutaneous albinism, neutropenia, frequent infection, mild bleeding diathesis, and neurologic abnormalities. Approximately 85% of patients who survive childhood enter a so-called accelerated phase, a lymphoma-like infiltration of lymphocytes and histiocytes throughout the body that is generally fatal. Other diseases of neutrophil granules have less ominous prognoses. A novel immunodeficiency syndrome relating to lack of the endosomal adapter protein p14 (encoded by the *LAMTOR2* gene) has been described. Patients with this syndrome have congenital neutropenia with structurally abnormal neutrophil primary granules and abnormalities of B cells, cytotoxic T cells, and melanocytes. In addition to immunodeficiency, clinical findings include short stature and partial albinism.[98]

## Oxidase Deficiencies—Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) resembles other diseases of neutrophil dysfunction in that it results in severe, recurrent infections of the skin and mucous membranes. Osteomyelitis and intra-abdominal abscesses are common. As a result of the inability to kill organisms, accumulation of neutrophils at a site of infection generally results in granuloma formation, rather than clearance of the target. Cutaneous infections tend to show persistent drainage and

scarring. The presence of even partially responsive neutrophils results in a lower frequency of sepsis in these patients relative to patients with absolute neutropenia. CGD is typically a disease of early childhood, although some milder cases may be recognized later in life.

CGD encompasses a group of diseases. In each, a genetic defect in a different component of NADPH oxidase results in failure of neutrophils (and other phagocytes) to generate $O_2^-$, impairing both intra-cellular killing and the ability of neutrophils to generate NETs.[45,46] X-linked CGD affecting $gp91^{phox}$ (the *CYBB* gene) is the most common form and accounts for about 70% of cases, whereas the autosomal recessive forms are responsible for the remainder.[99,100]

Treatment for CGD consists of aggressive antibiotic prophylaxis and therapy, along with long-term therapy with recombinant human IFN-γ to improve neutrophil function. Clinical trials of gene therapy for X-linked CGD have thus far yielded equivocal results.[101]

### Defects of TLR Signaling

Defects in human TLR signaling including IRAK-4 and MyD88 deficiencies lead to impaired neutrophil function and increased susceptibility to bacterial infections.[102,103]

## NEUTROPHIL RELEVANCE TO RHEUMATIC DISEASE

### Neutrophil-Mediated Tissue Destruction

Despite sophisticated regulatory mechanisms, tissue destruction by neutrophils is common. Several mechanisms may permit the release of neutrophil proteases and oxygen radicals into the extra-cellular milieu. First, necrosis or destruction of neutrophils, or both, may liberate cellular contents indiscriminately. Second, studies have revealed that degranulation and $O_2^-$ generation may begin before complete closure of the phagocytic vacuole, releasing products either into the external environment (regurgitation during feeding) or against a target surface. Neutrophils may destroy host tissues that they have been misdirected to attack. Although serum and joint fluid contain anti-proteases and antioxidants, the "protected space" between a neutrophil and a surface (e.g., cartilage) may exclude these factors. Release of hypochlorous acid, MPO, and proteinases into the extra-cellular milieu may also inactivate protective compounds and act as an "anti-proteinase shield."[37]

### Neutrophil Fc Receptor Polymorphisms

Given that polymorphisms of FcγR determine phagocytic capacity of IgG isotypes, it is not surprising that they determine susceptibility to diseases in which autoantibodies play a key role. Phagocytes from persons with one FcγRIIa polymorphism (H131) allele are able to bind and phagocytose $IgG_2$, whereas phagocytes from persons with a different polymorphism (R131) cannot do so. In white European and African American populations, patients with lupus nephritis have a higher frequency of the FcγRIIa-R131 allele than do control groups; their relative inability to clear immune complexes may make them more susceptible to renal disease.[104] The significance of different Fc receptor polymorphisms may vary among rheumatologic diseases. Tse and colleagues[104a,104b] found no association between FcγRIIa polymorphisms and likelihood of developing anti-neutrophil cytoplasmic antibody (ANCA)–associated vasculitides, but they found an overrepresentation of homozygosity for the FcγRIIIb NA1 allele among patients with anti-myeloperoxidase antibodies. Few studies have been published on Fc polymorphisms and susceptibility or severity of RA. Authors of more recent studies found no association among FcγRIIa, IIb, or IIIb haplotypes and rheumatoid arthritis susceptibility but did find a correlation between patients with extra-articular disease and the homozygous R/R 131 genotype. In contrast, FcγRIIIa receptor expression is increased in persons with RA.[105]

### Gout

Gout is the quintessential neutrophilic rheumatic disease. Although the initiation of an acute gouty attack involves the phagocytosis of urate crystals by synovial macrophages and macrophage generation of cytokines such as IL-1 and IL-8, the hallmark of acute gout is the presence of enormous numbers of neutrophils (sometimes >100,000/$mm^3$) in the affected synovial fluid. Intra-articular urate crystals nonspecifically bind immunoglobulin and activate complement by the classic and alternative pathways. C5a liberated by this process attracts neutrophils to the joint space, where they phagocytose opsonized crystals via receptor-dependent mechanisms, resulting in further activation of the neutrophil and production of $LTB_4$, IL-8, and other mediators, promoting ingress of additional neutrophils. Naked urate crystals can also directly activate neutrophils. Neutrophils in the gouty joint may damage joint structures through discharge of contents directly into the joint fluid during crystal phagocytosis or directly against cartilage during attempted phagocytosis of urate crystals embedded in or adherent to cartilage. Interaction of phagocytosed urate crystals with lysosomal membranes additionally results in the dissolution of the latter, spilling lysosomal proteases into the cytoplasm and, eventually, into the extra-cellular space.[106] Perhaps surprisingly, recent studies implicate NETs as playing a role in the resolution, rather than the activation, of gouty inflammation. As increasing numbers of neutrophils are recruited to the synovial fluid, NETs aggregate and promote degradation of inflammatory mediators, and thereby contribute to breaking the inflammatory cycle.[107]

### Rheumatoid Arthritis

RA may be conceptualized as a two-compartment inflammatory disease. In the synovium, lymphocytes, fibroblasts, and macrophages predominate, but the joint space contains mainly neutrophils. The classic model suggests that rheumatoid factor and/or anti-citrullinated protein antibody (ACPA)–based immune complexes, produced in the pannus (inflamed synovium) and present in the joint space in high concentrations, can activate complement to recruit neutrophils into the joint space. The ability of rheumatoid fibroblast–like synoviocytes to secrete IL-1, IL-8, and other cytokines indicates that pannus itself can play an important role in attracting neutrophils out of the bloodstream and into the joint. Conversely, neutrophils in the RA joint may

contribute to the propagation of pannus. RA neutrophils produce pro-inflammatory cytokines that may act on synovium, including oncostatin M, MIP-1α, and IL-8.[108] In animal models, injection of lysates of neutrophil granules into joints produces a synovitis histologically indistinguishable from rheumatoid synovitis, an effect that can be reproduced by injection with purified active or inactive myeloperoxidase.[109]

Neutrophil proteinase 3 may enhance the pro-inflammatory impact of synovial macrophages by cleaving and releasing active IL-1 and TNF from the surface of the latter. Neutrophil defensins enhance phagocytosis by macrophages and stimulate the activation and degranulation of mast cells, an interesting observation in light of a report that mice deficient in mast cells are resistant to the development of erosive arthritis.[110] Neutrophil proteases also enhance the adherence of rheumatoid synovial fibroblasts to articular cartilage, and neutrophils may regulate synovial vascularization through the production of vascular endothelial growth factor, leading to endothelial proliferation. Studies by Lee and others confirm that neutrophils are necessary for the propagation of rheumatic disease in mouse models of RA. These studies implicate the ability of neutrophils to produce $LTB_4$, as well as the presence of FCγRIIA and C5a receptors on the neutrophil surface as necessary for arthritis development.[111,112] Several studies have raised the unexpected possibility that, under certain conditions of stimulation, neutrophils can serve as antigen-presenting cells. Neutrophils in rheumatoid arthritis synovial fluid synthesize and express large amounts of class II major histocompatibility complex.

## Systemic Lupus Erythematosus

Until recently, the role of the neutrophil in lupus was considered to be limited to its function as an inflammatory effector. However, the discovery of NETs led to renewed interest in the role of neutrophils in pathogenesis, not only through endothelial damage but also in the induction of autoimmunity. NETs express several autoantigens that are targets of lupus autoantibodies.[113] As previously mentioned, although peptidylarginine deiminase facilitates NET formation, its inhibition is protective against organ damage in a model of murine lupus.[114] NETs can be dismantled by DNase, and some patients with lupus cannot degrade NETs because of inhibitors of or antibodies to DNase. The inability to degrade NETs correlates with the presence of lupus nephritis.[115] Moreover, it was reported that patients with low NET-degrading activity in their sera have higher levels of autoantibodies and lower levels of C3 and C4 compared with patients who have high NET-degrading activity in their sera, because NETs that are not degraded bind and activate complement.[116] NETs may also contribute to the IFN-α signature in systemic lupus erythematosus (SLE) because they induce IFN-α production by plasmacytoid dendritic cells in a TLR9-dependent manner.[117]

## Vasculitis

Neutrophils may be identified, to a greater or lesser degree, in the lesions of virtually all kinds of vasculitis. The mechanisms of neutrophil accumulation may vary, however, with different mechanisms predominating in different conditions. The early observation that infusions of allospecies serum produced acute inflammation in skin and joints (serum sickness), together with the appreciation that subcutaneous re-challenge with previously administered antigen leads to intense local inflammation (the Arthus reaction), led to the development of a model in which immune complex deposition in blood vessels results in complement activation and an influx of neutrophils. Because immune complex formation is a hallmark of many small vessel vasculitides (e.g., essential mixed cryoglobulinemia, hypersensitivity vasculitis, and Henoch-Schönlein purpura), it is likely that immune complex deposition is crucial to the genesis of these diseases. In several of these conditions, neutrophil disruption and fragmentation—*clasis*—is a prominent pathologic finding, leading to their designation under the rubric *leukocytoclastic vasculitis*. In some rheumatic diseases in which vasculitis is a secondary phenomenon, such as RA and SLE, the role of immune complex deposition is implicit. It has been suggested that patients with lupus experience transient accumulations of neutrophils (leukoaggregation) in small vessels of the lungs and other tissues as a result of complement activation within these vessels or in the soluble phase.[118]

Induction of adhesion molecules on endothelial cells, neutrophils themselves, or both is an alternative mechanism through which neutrophil accumulation in vessels may be propagated. The Shwartzman phenomenon, in which reinjection of cellular material leads to vascular inflammation via a cytokine-dependent, immune complex–independent mechanism, is a model for this avenue to vasculitis. Adhesion molecule upregulation may be particularly relevant to vasculitides in which immune complex formation is not a hallmark. It is likely that many rheumatic diseases employ both immune complex–dependent and immune complex–independent mechanisms in the pathogenesis of neutrophil ingress into vascular structures. For example, in addition to the role of immune complexes, Belmont and colleagues have shown the induction of adhesion molecules in patients with SLE.[119]

Several vasculitides are noteworthy for the presence, in the serum of affected patients, of antibodies directed at cytoplasmic components of neutrophils (ANCA). In these diseases, partial neutrophil degranulation, resulting in exposure of ANCA antigens (e.g., MPO and proteinase 3), appears to be critical for disease pathogenesis. $ANCA^+$ vasculitides are discussed in detail in Chapter 89.

## Neutrophilic Dermatoses and Familial Mediterranean Fever

Sweet's syndrome is characterized by fever, neutrophilia, and painful erythematous papules, nodules, and plaques. The syndrome may be idiopathic, parainflammatory (i.e., associated with inflammatory bowel disease or infection), paraneoplastic (most commonly in the setting of leukemia), pregnancy related, or drug associated (usually after treatment with G-CSF). Clinically, Sweet's syndrome is a diagnosis of exclusion. Sweet's syndrome frequently appears after an upper respiratory tract infection and has a propensity to involve the face, neck, and upper extremities. When found on the legs, Sweet's syndrome lesions can be confused

with erythema nodosum. Histopathologic features are characterized by dense neutrophilic infiltrate in the superficial dermis and edema of the dermal papillae and papillary dermis. Leukocytoclasia may suggest leukocytoclastic vasculitis, although vascular damage is absent. It is typically accompanied by peripheral neutrophilia. Treatment with systemic corticosteroids usually induces a dramatic resolution of the lesions and the systemic symptoms. Although the cause of the disease is unclear, many investigators believe that Sweet's syndrome may represent a hypersensitivity reaction to microbial or tumor antigens. Antibiotics do not influence the course of the disease in most patients.

Pyoderma gangrenosum is characterized by painful ulcerating cutaneous lesions over the lower extremities, usually in patients with an underlying inflammatory illness. Inflammatory bowel disease, RA, and seronegative arthritis are the most common associations, although an association with malignancy has also been reported. Fifteen percent of patients have a benign monoclonal gammopathy, usually IgA. Like Sweet's syndrome, pyoderma gangrenosum is a diagnosis of exclusion, is characterized on biopsy specimen by neutrophilic infiltrate, and usually remits with systemic corticosteroids, although topical and intralesional injections of corticosteroids may be beneficial as well. Other rare neutrophilic dermatoses include rheumatoid neutrophilic dermatitis, described as symmetric erythematous nodules on extensor surfaces of joints; bowel-associated dermatosis-arthritis syndrome occurring after bowel bypass surgery for obesity; and neutrophilic eccrine hidradenitis, which is sometimes linked to acute myelogenous leukemia.

In familial Mediterranean fever (discussed in detail in Chapter 97), patients experience episodic inflammatory exacerbations characterized by large influxes of neutrophils. A defect in the regulatory protein pyrin seems to permit the inappropriate development of inflammation, leading to its categorization as an autoinflammatory disease. Pyrin is expressed primarily in myeloid cells, including neutrophils and eosinophils.

## Effects of Anti-rheumatic Agents on Neutrophil Functions

Many anti-rheumatic therapies currently in use have been documented to act at least partly at the level of the neutrophil. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most frequently used class of anti-rheumatic agents. By virtue of their ability to inhibit COX activity and prostaglandin production, moderate doses of NSAIDs have diverse effects on inflammation, including inhibition of vascular permeability and modulation of pain. At higher, clinically anti-inflammatory concentrations, NSAIDs inhibit chemoattractant-stimulated neutrophil CD11b/CD18-dependent adhesion and degranulation and NADPH oxidase activity.[120,121] It is unlikely, however, that these effects are due solely to COX inhibition because (1) as noted earlier, neutrophils exhibit little COX activity under normal circumstances, and (2) concentrations of NSAIDs required to inhibit neutrophil function exceed the concentrations required to inhibit COX. Salicylates may be unique as NSAIDs because of their ability to promote lipoxin formation to inhibit neutrophil cell signaling at high concentrations.[122]

### Glucocorticoids

Glucocorticoids exert potent effects on neutrophils, including inhibition of neutrophil phagocytic activity and adhesive function. The ability of steroids to increase peripheral blood neutrophil populations acutely—an effect known as *demargination*—is attributable to both a release of neutrophils from the bone marrow and the release (demargination) of neutrophils adherent to vessel walls. In addition, glucocorticoids lead to inhibition of phospholipase $A_2$ and LT and prostaglandin production. Glucocorticoids may also regulate the expression of COX-2 and stimulate the release of annexin A1, which inhibits arachidonate release from membranes. Effects of glucocorticoids on other cells may also reduce neutrophil responses indirectly through the suppression of cytokines at inflammatory sites.

### Disease-Modifying Anti-rheumatic Drugs

Several disease-modifying anti-rheumatic drugs have well-established effects on neutrophils. Methotrexate, which is widely used in persons with RA, has no direct neutrophil effect but is capable of producing indirect effects, probably by virtue of its ability to stimulate the release of adenosine from surrounding cells. Some data suggest that methotrexate-induced adenosine release might inhibit phagocytosis, $O_2^-$ production, and adhesion, and treatment of patients with methotrexate inhibits the capacity of neutrophils to generate $LTB_4$.[123] Sulfasalazine has been shown to inhibit neutrophil chemotaxis, degranulation, and $O_2^-$ generation; decrease $LTB_4$ production; and scavenge oxygen metabolites.[120]

### Colchicine

Colchicine, a standard agent in the treatment of gout and familial Mediterranean fever, inhibits microtubule formation and has pleiotropic effects on neutrophils, including inhibition of adhesion via decrements in selectin expression.[124] Colchicine has been observed to stimulate the expression of pyrin in neutrophils. Because pyrin deficiencies are implicated in familial Mediterranean fever, this observation suggests a previously unappreciated mechanism of action of colchicine in neutrophilic diseases.[125]

### Biologic Agents

The current era of biologic therapies has been ushered in through the introduction of agents designed to block the effects of TNF or IL-1β. As noted earlier, IL-1β and TNF directly affect neutrophil function, including priming for stimulus-induced responses such as $O_2^-$ production, cartilage destruction, and production of cytokines such as IL-8 and $LTB_4$. Nonetheless, studies examining the effects of anti-TNF treatment on neutrophil function measured ex vivo have not indicated extensive action. Treatment of patients with etanercept or adalimumab induced no effect on neutrophil ex vivo responses including chemotaxis, phagocytosis, and superoxide generation (although CD69 levels were reduced).[126,127] Reduction of neutrophil populations in RA joint effusions after anti-TNF therapy is more likely due to alteration of the inflammatory environment

rather than to direct effects on the neutrophils themselves. Similarly, recent studies indicate that anti-IL-6 therapy with tocilizumab may induce neutropenia in patients but has no direct effect on neutrophil function.[128]

## CONCLUSION

The complex machinery of neutrophil surveillance and response—the chemotactic, phagocytic, enzymatic and oxidative defenses that enable neutrophils to sense and destroy foreign organisms—provide robust host defense by aborting many forms of infection and keeping others at bay until more specific mechanisms can be mobilized. When improperly regulated, however, these same mechanisms become the basis of inflammatory and autoinflammatory disease and potentially lead to tissue destruction. Moreover, it is increasingly appreciated that neutrophils play critical roles in bridging the gap between innate and acquired immunity. In several rheumatic diseases, the neutrophil therefore serves not merely as an effector cell but as a direct participant in disease pathogenesis.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 12

# T Lymphocytes



Ralph C. Budd • Karen A. Fortner

**KEY POINTS**

- **T cells develop primarily in the thymus. The importance of the thymus is underscored by the complete absence of T cells in patients in whom a thymus has failed to develop (e.g., complete DiGeorge syndrome).**
- **Thymic selection consists of a positive phase in which T cells must recognize self–major histocompatibility complex (MHC) molecules and a negative phase in which thymocytes bearing high-affinity T cell receptors for self-MHC peptide are deleted through apoptosis.**
- **T cells emerge from the thymus as naïve T cells that are quiescent and, when activated, express low to negligible levels of most cytokines. Once they acquire a memory phenotype ($CD45RO^+$), they can produce high levels of cytokines.**
- **Naïve T cells can spontaneously undergo homeostatic proliferation to self-MHC peptides in peripheral lymphoid tissues to generate a critical number of T cells. This process requires IL-7 and IL-15.**
- **Th1 and Th17 cells accumulate in inflammatory synovium such as rheumatoid arthritis, whereas Th2 cells accumulate at sites of allergic responses such as asthma.**

The evolutionary pressures that have molded the immune response and promoted a highly diverse repertoire clearly derive from infectious agents. Two very different strategies exist. The more primitive innate immune response (see Chapter 17) uses a limited repertoire of nonpolymorphic receptors that recognize structural motifs common to many micro-organisms, such as small glycolipids and lipopeptides. The evolutionarily newer adaptive immune response (see Chapter 18) relies on generating myriad different receptors that can recognize a wide array of foreign compounds from infectious agents. Whereas the innate immune response allows a rapid focused response, adaptive immunity permits a broader, albeit slower, response, as well as immune memory.

T lymphocyte development constantly confronts the dilemma of generating pathogen-specific T cells to combat infection without provoking a response to the host. The price for generating an increasingly varied population of antigen receptors needed to recognize a wide spectrum of pathogens is the progressive risk of producing self-reactive lymphocytes that can provoke an autoimmune diathesis. T lymphocytes are thus subjected to a rigorous selection process during development in the thymus to delete self-reactive T cells (i.e., central tolerance). In addition, premature activation of mature peripheral T cells is prevented by requiring two signals for activation (i.e., peripheral tolerance). Finally, the expansion of T cells that occurs during either homeostatic proliferation in the periphery or in response to an infection is resolved by the active induction of cell death. The consequences of inefficient lymphocyte removal at any one of these junctures can be devastating to the health of the organism. This situation is vividly displayed in both humans and mice when naturally arising mutations in death receptors such as Fas (CD95) result in massive accumulation of lymphocytes and autoimmune sequelae. These sequelae are discussed in more detail in Chapter 28.

The activation of T lymphocytes yields a variety of effector functions that are pivotal to combating infections. Cytolytic T cells can kill infected cells through the release of granules containing perforin and granzyme that induce holes in cell membranes and cleave cellular substrates, respectively, or through the expression of ligands for death receptors such as Fas or tumor necrosis factor (TNF) receptor. Production of T cell cytokines such as interferon-γ (IFN-γ) can inhibit viral replication, whereas other cytokines such as interleukin (IL)-4, IL-5, and IL-21 are critical for optimal B cell growth and immunoglobulin production.[1] However, this same armamentarium, if not tightly regulated, can also precipitate damage to host tissues and provoke autoimmune responses, such as in the synovium of inflammatory arthritides, pancreatic islets in type 1 diabetes, and the central nervous system in multiple sclerosis. Damage in these cases need not be directly the result of recognition of target tissues by the T cells. T cells may be activated elsewhere and then migrate to the tissue and damage innocent bystander cells. T cells may also promote autoimmunity through the augmentation of B cell responses.

## T CELL DEVELOPMENT

T cells must traverse two stringent hurdles during their development. First, they must successfully rearrange the genes encoding the two chains of the T cell antigen receptor (TCR). Second, a T cell must survive thymic selection during which T cells that interact strongly with self-peptides are eliminated (i.e., negative selection). This process minimizes the chances of autoreactive T cells escaping to the periphery and is known as *central tolerance*.

The TCR is an 80 to 90 kDa disulfide-linked heterodimer composed of a 48 to 54 kDa α chain and a 37 to 42 kDa β chain. An alternate TCR composed of γ and δ

chains is expressed on 2% to 3% of peripheral blood T cells (discussed later). The TCR has an extra-cellular ligand binding pocket and a short cytoplasmic tail that by itself cannot signal. Consequently it is noncovalently associated with as many as five invariant chains of the CD3 complex, which relay information to the intra-cellular signaling machinery via immunoreceptor tyrosine-based activation motifs (ITAMs; discussed later). Not surprisingly, the structure of the TCR gene is similar to that of immunoglobulin genes in B cells (see details in Chapter 13). Each overcame the problem of how to encode approximately 10 million different T or B cell specificities within the human genome, which contains fewer than 30,000 genes. To economically package this diversity, the process of gene rearrangement and splicing evolved using machinery similar to what already existed to promote gene translocations. The β and δ chain genes of the TCR contain four segments known as the V (variable), D (diversity), J (joining), and C (constant) regions. The α and γ chains are similar but lack the J component. Each of the segments has several family members (approximately 50 to 100 V, 15 D, 6 to 60 J, and 1 to 2 C members). An orderly process occurs during TCR gene rearrangement in which a D segment is spliced adjacent to a J segment, which is subsequently spliced to a V segment. After transcription, the VDJ sequence is spliced to a C segment to produce a mature TCR messenger RNA (mRNA). Arithmetically, this random rearrangement of a single chain of the TCR locus can give rise to minimally 50V × 15D × 6J × 2C or about 9000 possible combinations. At each of the splice sites, which must occur in-frame to be functional, additional nucleotides not encoded by the genome (so-called N-region nucleotides) can be incorporated, adding further diversity to the rearranging gene. The combinations from the two TCR chains, plus N-region diversity, yield at least $10^8$ theoretical possible combinations. The cutting, rearranging, and splicing are directed by specific enzymes. Mutations in the genes for these processes can result in an arrest in lymphocyte development. For example, mutation in the gene encoding a DNA-dependent protein kinase required for receptor gene recombination results in a severe combined immunodeficiency known as *scid*.

Because the developing T cell has two copies of each chromosome, there are two chances to successfully rearrange each of the two TCR chains. As soon as successful rearrangement occurs, further β chain rearrangements on either the same or the other chromosome are suppressed, a process known as *allelic exclusion*, which limits the chance of dual TCR expression by an individual T cell. The high percentage of T cells that contain rearrangements of both β chain genes attests to the inefficiency of this complex event. Rearrangement of the α chain occurs later in thymocyte development in a similar fashion, although without apparent allelic exclusion, which can result in dual TCR expression by a single T cell.

Development of T cells occurs within a microenvironment provided by the thymic epithelial stroma. The thymic anlage is formed from embryonic ectoderm and endoderm and is then colonized by hematopoietic cells, which give rise to dendritic cells, macrophages, and developing T cells. The hematopoietic and epithelial components combine to form two histologically defined compartments—the cortex, which contains immature thymocytes, and the medulla, which contains mature thymocytes (Figure 12-1A). As few as 50 to 100 bone marrow–derived stem cells enter the thymus daily.

The stages of thymocyte development can be defined by the status of rearrangement and expression of the two genes that encode the α and β chains of the TCR and the expression of CD4 and CD8, proceeding in an orderly fashion from $CD4^-8^- \rightarrow CD4^+8^+ \rightarrow CD4^+8^-$ or $CD4^-8^+$ (Figure 12-1B). CD4 and CD8 define the helper and cytolytic subsets of mature T cells, respectively.

$CD4^-8^-$ thymocytes can be further subdivided based on their expression of CD25 (the high-affinity IL-2 receptor α chain) and CD44 (the hyaluronate receptor). Development proceeds in the following order: $CD25^-CD44^+ \rightarrow CD25^+CD44^+ \rightarrow CD25^+CD44^- \rightarrow CD25^-CD44^-$. These subpopulations correspond to discrete stages of thymocyte differentiation. $CD25^-44^+$ cells express low levels of CD4, and their TCR genes are in germline configuration. These cells downregulate CD4 and upregulate CD25 to give rise to $CD25^+CD44^+$ thymocytes, which now express surface CD2 and low levels of CD3ε. At the next stage ($CD25^+CD44^-$), a brief burst of proliferation occurs, followed by upregulation of the recombination-activating enzymes, RAG-1 and RAG-2, and the concomitant rearrangement of the genes of the TCR β chain. A small subpopulation of T cells rearranges and expresses a second pair of TCR genes known as γ and δ. Productive TCR β chain rearrangement results in downregulation of RAG and a second proliferative burst. Loss of CD25 then yields $CD25^-CD44^-$ thymocytes.

The TCR β chain cannot be stably expressed without an α chain. Because the TCR α chain has not yet rearranged, a surrogate invariant TCR pre–α chain is disulfide linked to the β chain. When associated with components of the CD3 complex, this allows a low level surface expression of a pre-TCR and progression to the next developmental stage. Failure to successfully rearrange the TCR β chain results in a developmental arrest at the transition from $CD25^+CD44^-$ to $CD25^-CD44^-$. This developmental arrest occurs in RAG-deficient mice, as well as in *Scid* mice and humans.[2]

A number of transcription factors, receptors, and signaling molecules are required for early T cell development (Figure 12-2). IKAROS encodes a transcription factor required for the development of cells of lymphoid origin. Notch-1, a molecule known to regulate cell fate decisions, is also required at the earliest stage of T cell lineage development.[3] Cytokines, including IL-7, promote the survival and expansion of the earliest thymocytes. In mice that are deficient for IL-7, its receptor components IL-7Rα or $\gamma_c$, or the cytokine receptor–associated signaling molecule JAK-3, thymocyte development is inhibited at the $CD25^-CD44^+$ stage. In humans, mutations in $\gamma_c$ or JAK-3 result in the most frequent form of SCID.[4] Pre-TCR signaling is required for the $CD25^+CD44^- \rightarrow CD25^-CD44^-$ transition. Thus loss of signaling components including Lck, SLP-76, and LAT-1 results in a block at this stage of T cell development. TCR signals are also required for differentiation of $CD4^+CD8^+$ to mature $CD4^+$ or $CD8^+$ cells. Humans deficient in ZAP-70 (see later discussion) have $CD4^+$ but not $CD8^+$ T cells in the thymus and periphery.[5]

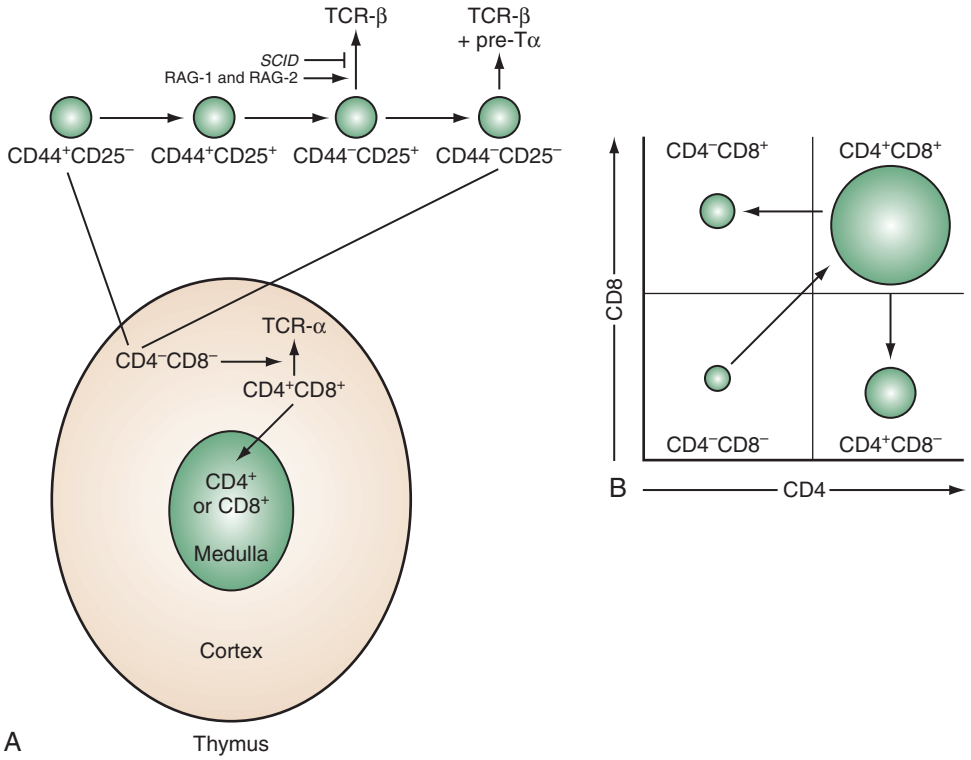



**Figure 12-1** Sequence of thymocyte development. **A,** The earliest thymocyte precursors lack expression of CD4 and CD8 ($CD4^-CD8^-$). These can be further divided into four subpopulations based on the sequential expression of CD44 and CD25. It is at the $CD44^-CD25^+$ stage that the T cell antigen receptor (TCR) β chain rearranges. The *SCID* mutation or deficiencies of the rearrangement enzymes RAG-1 and RAG-2 result in inability to rearrange the β chain and maturational arrest at this stage. The thymocytes that successfully rearrange the β chain express it associated with a surrogate α chain known as pre-Tα. Concomitant with a proliferative burst, development can then progress to the $CD4^+CD8^+$ stage in the cortex where the TCR α chain rearranges and pairs with the β chain to express a mature TCR complex. These cells then undergo thymic positive and negative selection (as diagrammed in Figure 3B). Successful completion of this rigorous selection process results in mature $CD4^+$ or $CD8^+$ T cells in the medulla, which eventually emigrate to peripheral lymphoid sites. **B,** Schematic two-color flow cytometry showing subpopulations of thymocytes defined by CD4 and CD8 expression in their relative proportions.

$CD25^-CD44^-$ cells upregulate expression of CD4 and CD8 to become $CD4^+8^+$, at which point the α chain of the TCR rearranges. Unlike the β chain, allelic exclusion of the α chain is not apparent. Rearrangement of the α chain can occur simultaneously on both chromosomes, and if one attempt is unsuccessful, repeat rearrangements to other Vα segments are possible. Reports exist of dual TCR expression by as many as 30% of mature T cells in which the same T cell expresses different α chains paired with the same β chain.[6] However, in most cases of dual TCR α chains, one is downregulated during positive selection by Lck and Cbl, through ubiquitination, endocytosis, and degradation.

Although the structure of immunoglobulin and TCR are quite similar, they recognize fundamentally different antigens. Immunoglobulins recognize intact antigens in isolation, either soluble or membrane bound, and are often sensitive to the tertiary structure. The TCRαβ recognizes linear stretches of antigen peptide fragments bound within the grooves of either class I or class II major histocompatibility complex (MHC) molecules (Figure 12-3A). Thymic selection molds the repertoire of emerging TCRs so they recognize peptides within the groove of self-MHC molecules, ensuring the *self-MHC restriction* of T cell responses. The MHC structure is described in detail in Chapters 18, 19, and 21. Pockets within the MHC groove bind particular residues along the peptide sequence of 7-9 amino acids for class I MHC and 9-15 amino acids for class II MHC molecules. As a result, certain amino acids will make strong contact with the MHC groove, whereas others will contact the TCR.

The contact between the TCR and MHC/peptide has been revealed by crystal structure to be remarkably flat, rather than a deep lock and key structure that one might imagine.[7] The TCR axis is tipped about 30 degrees to the long axis of the class I MHC molecule and is slightly more skewed to class II MHC. The affinity of the TCR for antigen/MHC is in the micromolar range, which is a lower affinity than many antibody-antigen affinities, and several logs less than many enzyme-substrate affinities. This finding has led to the notion that TCR interactions with antigen/MHC are brief and that successful activation of the T cell requires multiple interactions, resulting in a cumulative signal.

Once the T cell has successfully rearranged and expressed a TCR in association with the CD3 complex, it encounters the second major hurdle in T cell development, *thymic selection*. Selection has two phases, positive and negative, and the outcome is based largely upon the intensity of TCR signaling in response to interactions with MHC/self-peptides expressed on thymic epithelium and dendritic cells. TCR signals that are either too weak (death by neglect) or too intense *(negative selection)* result in elimination by apoptosis, whereas those with intermediate signaling intensity

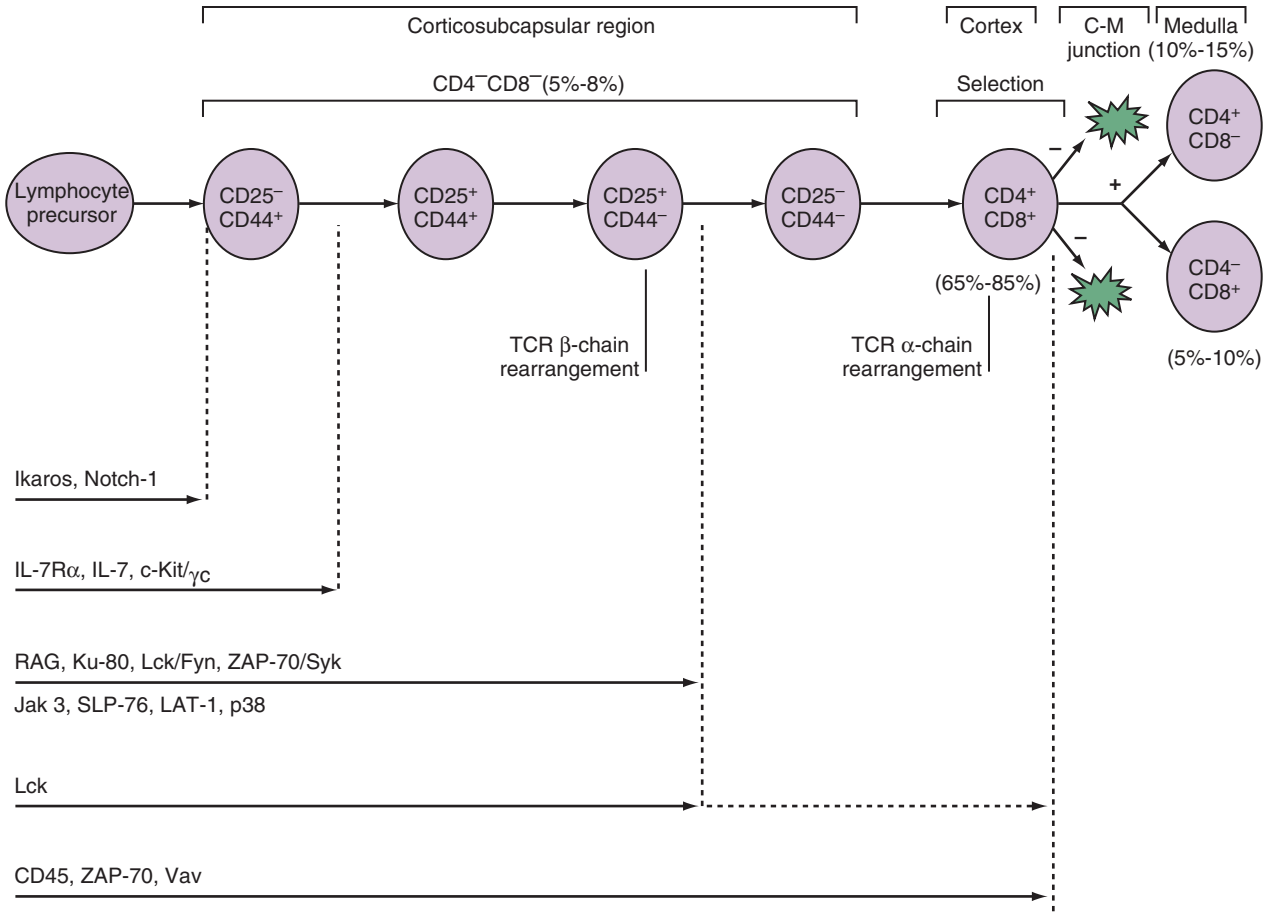



**Figure 12-2** Sequence of αβ T cell development in the thymus. The earliest thymocyte precursors lack expression of CD4 and CD8 ($CD4^-CD8^-$). These can be further divided into four subpopulations based on the sequential expression of CD25 and CD44. At the $CD25^+CD44^-$ stage, the T cell antigen receptor (TCR) β chain rearranges and associates with a surrogate α chain known as pre-Tα. Concomitant with a proliferative burst, thymocytes progress to the $CD4^+CD8^+$ stage, rearrange the TCR α chain, and express a mature TCR complex. These cells then undergo thymic positive and negative selection (as diagrammed in Figure 12-3B). The thymocytes that survive this rigorous selection process differentiate into mature $CD4^+$ or $CD8^+$ T cells. Also shown are the various signaling molecules that are involved at specific stages of thymic development.

survive *(positive selection)* (Figure 12-3B). Successful positive selection at the $CD4^+8^+$ stage is coincident with upregulation of surface TCR, the activation markers CD5 and CD69, and the survival factor Bcl-2. T cells bearing a TCR that recognizes class I MHC maintain CD8 expression, downregulate CD4, and become $CD4^-8^+$. T cells expressing a TCR that recognizes class I MHC become $CD4^+8^-$.

An enigma for thymic selection has been how to present the myriad self-proteins to developing thymocytes so that self-reactive thymocytes are effectively eliminated by negative selection, particularly including antigens with tissue or developmentally restricted expression. A solution was found with the discovery of the autoimmune regulator *(AIRE)* gene. AIRE is a transcription factor expressed by the medullary epithelium of the thymus that induces transcription of a wide array of organ-specific genes, such as insulin, that might otherwise be sequestered from developing thymocytes.[8] This effectively creates a self-transcriptome within the thymus against which autoreactive T cells can be deleted. Gene knockout mice of *Aire*, and humans bearing *AIRE* mutations, manifest various autoimmune sequelae in a syndrome known as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED).[9]

Not surprisingly, a variety of signaling molecules activated by TCR engagement are important to thymic selection. Lck, the RasΠRaf-1ΠMEK1ΠERK kinase cascade, the kinase ZAP-70, and the phosphatases CD45 and calcineurin are involved with positive selection. Among these, the Ras ΠERK pathway is particularly important because dominant negative variants of these molecules can disrupt positive selection. Conversely, an activator of Ras known as GRP1 assists with the positive selection of thymocytes that express weakly selecting signals. These molecules are discussed in more detail in the section on TCR signaling. By contrast, although a number of molecules may promote negative selection—among them the mitogen-activated protein (MAP) kinases JNK and p38—there appears to be sufficient redundancy so that only rarely does elimination of any one of these molecules alter the efficient deletion of thymocytes. The few exceptions include CD40, CD40L, CD30, or the proapoptotic Bcl-2 family member, Bim, where preservation of at least some thymocytes bearing self-reactive TCR could be observed in mice deficient in these molecules.[10]

The survivors of these stringent processes of TCR gene rearrangement and thymic selection represent less than 3%

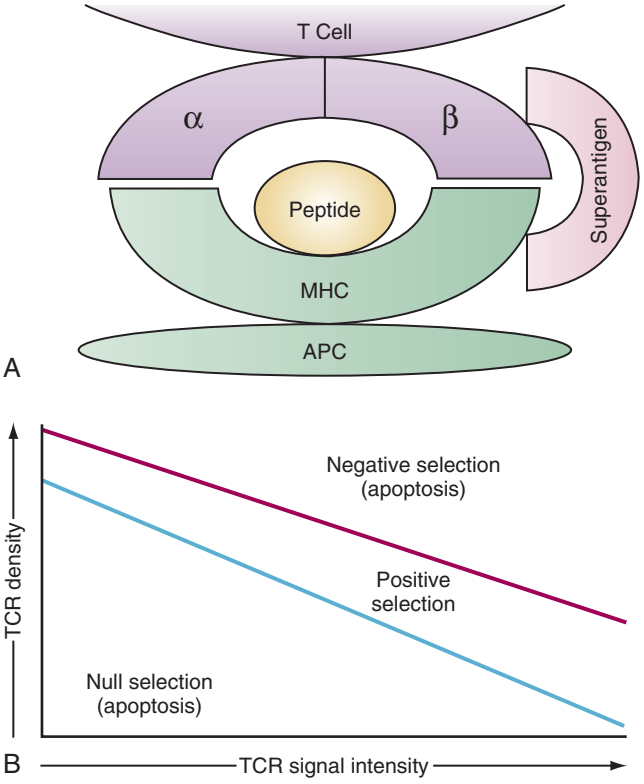



**Figure 12-3** T cell antigen receptor (TCR) interaction with the major histocompatibility complex (MHC)/peptide complex. **A,** Polymorphic residues within the variable region of the α and β chains of the TCR make contact with determinants on the MHC molecule on an antigen presenting cell (APC), as well as with the peptide fragment that sits in the MHC binding groove. **B,** A schematic diagram illustrating that during thymocyte development, the TCRs that confer either a very low signal intensity (null selection) or high intensity (negative selection) each lead to apoptosis. Only the thymocytes whose TCRs can engage MHC/peptides and confer moderate intensity survive by positive selection.

of total immature thymocytes, which is reflected by the presence of a high rate of cell death in developing thymocytes. This process can be visualized by the measurement of DNA degradation, a hallmark of apoptosis, as shown in Figure 12-4. The survivors become either $CD4^+$ helper or $CD8^+$ cytolytic T lymphocyte cells (CTLs) and reside in the thymic medulla for 12 to 14 days before emigrating to the periphery. The decision to become a $CD4^+$ versus $CD8^+$ T cell involves further developmental signals, provided in part by Notch-1, which is required for progression to $CD8^+$ but not $CD4^+$ thymocytes. This scenario parallels the observation that long TCR interactions are required for CD4 progression, whereas shorter TCR engagement favors CD8 progression.[11]

## Abnormalities of Human T Cell Development

Given the vast number of events in T cell development, it is not surprising that a multiplicity of causes can underlie human T cell immunodeficiencies.[12] The influence of the thymic stroma on thymocyte ontogeny is underscored in the DiGeorge anomaly in which development of the pharyngeal pouches is disrupted and the thymic rudiment fails to form, resulting in the failure of normal T cell development. Less severe T cell deficiencies are associated with a failure to express class I and/or class II MHC (the "bare lymphocyte syndrome"), which are directly involved with interactions required to induce the positive selection of, respectively, $CD8^+$ and $CD4^+$ mature T cells.

Metabolic disorders can affect thymocytes more directly. The absence of functional adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) results in the buildup of metabolic byproducts that are toxic to developing T and B lymphocytes, ultimately producing forms of severe combined immunodeficiency disorders (SCIDs).

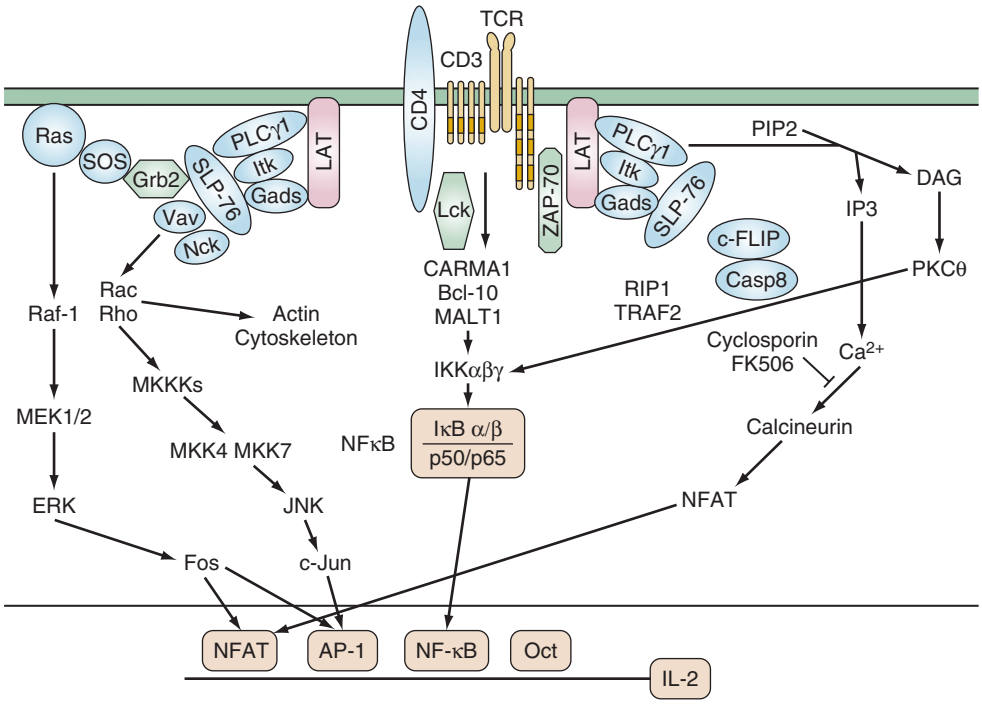



**Figure 12-4** T cell antigen receptor (TCR) signal pathways. The schema shows the principle signal pathways resulting from TCR activation and how they impinge on the regulatory region of the *IL2* gene. See the text for details.

## PERIPHERAL MIGRATION AND HOMEOSTATIC PROLIFERATION OF T CELLS

The migration of naïve T cells to peripheral lymphoid structures or their infiltration into other tissues requires the coordinated regulation of an array of cell adhesion molecules. T cell recirculation is essential for host surveillance and is carefully regulated by a specific array of homing receptors. Entry from the circulation to tissues occurs via two main anatomic sites: the flat endothelium of the blood vessels and specialized postcapillary venules known as high endothelial venules (HEVs). A three-step model has been described for lymphocyte migration: rolling, adhesion, and migration.[13] L-selectin expressed by naïve T cells binds via lectin domains to carbohydrate moieties of GlyCAM-1 and CD34 (collectively known as peripheral node addressin), which are expressed on endothelial cells, particularly HEV. The weak binding of CD62L to its ligand mediates a weak adhesion to the vessel wall, which, combined with the force of blood flow, results in rolling of the T cell along the endothelium. The increased cell contact facilitates the interaction of a second adhesion molecule on lymphocytes, the integrin LFA-1 (CD11a/CD18), along with its ligands, ICAM-1 (CD54) and ICAM-2 (CD102). This interaction results in the arrest of rolling and firm attachment. Migration into the extra-cellular matrix of tissues may involve additional lymphocyte cell surface molecules such as the hyaluronate receptor (CD44) or the integrin α4β7 (CD49d/β7), which binds the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on endothelium of Peyer's patches and other endothelial cells.

Other cytokines known as chemokines may contribute to lymphocyte homing. Chemokines are structurally and functionally related to proteins that bear an affinity for heparan sulfate proteoglycan and promote migration of various cell types.[14] The chemokines RANTES, MIP-1α, MIP-1β, MCP-1, and IL-8 are produced by a number of cell types including endothelium, activated T cells, and monocytes and are present at inflammatory sites such as rheumatoid synovium (see Chapter 69).

Once mature T cells have reached the peripheral lymphoid tissues of lymph node and spleen, they undergo a low level of turnover known as *homeostatic proliferation* in response to self-peptide/MHC complexes, IL-7, and IL-15.[15] This low level of turnover serves to maintain the number of peripheral T cells and can become accelerated in states of lymphopenia, such as after chemotherapy or irradiation.[16] Because homeostatic proliferation is driven by self-MHC/peptides and is thus inherently autoimmune, its acceleration could precipitate an autoimmune syndrome. In this regard, it is of interest that one of the standard murine models of autoimmunity is day 3 thymectomy, which results in lymphopenia and autoimmunity of various organs.[17] In addition, NOD diabetic mice have chronic lymphopenia that contributes to their diabetes,[18] and evidence of augmented homeostatic proliferation has been suggested to occur in rheumatoid arthritis (RA).[19]

### Activation of T Cells

T cell activation initiates an intra-cellular signaling cascade that ultimately results in proliferation, effector function, or death, depending on the intensity of the TCR signal and associated signals. To guard against premature or excessive activation, T cells have a requirement of two independent signals for full activation. Signal 1 is an antigen-specific signal provided by the binding of the TCR to antigenic peptide complexed with MHC. Signal 2 is mediated by either cytokines or the engagement of co-stimulatory molecules such as B7.1 (CD80) and B7.2 (CD86) on the antigen-presenting cell (APC). Receiving only signal 1 without co-stimulation results in T cell unresponsiveness or anergy, a process known as *peripheral tolerance*.

### TCR and Tyrosine Kinases

TCR αβ and γδ have very short cytoplasmic domains and by themselves are unable to transduce signals. The molecules of the noncovalently associated CD3 complex couple the TCR to intra-cellular signaling machinery (see Figure 12-4). The CD3 complex contains nonpolymorphic members known as CD3ε, CD3γ, CD3δ, as well as ζ and η chains that are alternatively spliced forms of the same gene and are not genetically linked to the CD3 complex. Although the functional stoichiometry of the TCR complex is not completely defined, current data indicate that each TCR heterodimer is associated with three dimers: CD3εγ, CD3εδ, and ζζ or ζ. CD3ε, -γ, and -δ have an immunoglobulin-like extra-cellular domain, a transmembrane region, and a modest cytoplasmic domain, whereas ζ contains a longer cytoplasmic tail. The transmembrane domains of ζ and the CD3 chains contain a negatively charged residue that interacts with positively charged amino acids in the transmembrane domain of the TCR.

None of the proteins in the TCR complex has intrinsic enzymatic activity. Instead, the cytoplasmic domains of the invariant CD3 chains contain conserved activation domains that are required for coupling the TCR to intra-cellular signaling molecules. These ITAMs contain a minimal functional consensus sequence of paired tyrosines (Y) and leucines (L): (D/E)XXYXXL$(X)_{6\text{-}8}$YXXL. ITAMs are substrates for cytoplasmic protein tyrosine kinases (PTKs), and upon phosphorylation they recruit additional molecules to the TCR complex.[20] Each ζ chain contains three ITAMs, whereas there is one in each of the CD3ε, -γ, and -δ chains. Thus each TCR complex can contain as many as 10 ITAMs.

Activation of PTKs is one of the earliest signaling events after TCR stimulation. Four families of PTKs are known to be involved in TCR signaling: Src, Csk, Tec, and Syk. The Src family members Lck and $Fyn^T$ have a central role in TCR signaling and are expressed exclusively in lymphoid cells. Src PTKs contain multiple structural domains, including (1) N-terminal myristoylation and palmitoylation sites that allow association with the plasma membrane; (2) an Src homology (SH)3 domain that associates with proline-rich sequences; (3) an SH2 domain that binds proteins that contain phosphotyrosine; and (4) a carboxy-terminal negative regulatory site. Their catalytic activity is regulated by the balance between the actions of kinases and phosphatases. Activity is repressed by phosphorylation of a conserved carboxy-terminal tyrosine, and dephosphorylation by the phosphatase CD45 is critical for the initiation of TCR-mediated signal transduction. In addition, autophosphorylation of other tyrosines within the kinase domain enhances

catalytic activity. Lck is physically and functionally associated with CD4 and CD8; 50% to 90% of total Lck molecules is associated with CD4, and 10% to 25% is associated with CD8. CD4 and CD8 physically associate with the TCR/CD3 complex during antigen stimulation as a result of their interaction with class II MHC and class I molecules, respectively, and thus they enhance TCR-mediated signals by recruiting Lck to the TCR complex. Lck phosphorylates the CD3 chains, TCRζ, ZAP-70, phospholipase C-γ1 (PLC-γ1), Vav, and Shc. Fyn binds TCRζ and CD3ε and, although its substrates are less well defined, T cells lacking Fyn have diminished response to TCR signals.[21] Vitamin D, the deficiency of which is linked to various autoimmune disorders, strongly upregulates PLC-γ1 after TCR activation and enhances signaling efficiency.[22] In addition, the SH2 and SH3 domains of Src PTKs can mediate their association with molecules that contain phosphotyrosine and proline, respectively.

Somewhat less information is known about the Csk and Tec PTKs. Csk negatively regulates TCR signaling by phosphorylating the carboxy-terminal tyrosine of Lck and Fyn. Dephosphorylation of this negative regulatory tyrosine is mediated by the transmembrane tyrosine phosphatase CD45. CD45 activity is essential for TCR signaling because CD45-deficient T cells fail to activate by TCR stimulation. The Tec family member Itk is preferentially expressed in T cells. T cells from Itk-deficient mice have diminished response to TCR stimulation. The mechanism by which Itk regulates TCR signaling has not been determined, although recent studies have shown that Itk is an important component of the pathway leading to increased intra-cellular $Ca^{2+}$.

Phosphorylation of the ITAM motifs on the CD3 complex recruits the Syk kinase family member ZAP-70 by its tandem SH2 domains. ZAP-70 is expressed exclusively in T cells and is required for TCR signaling. After TCR stimulation, ZAP-70 is activated by phosphorylation of tyrosine 493 by Lck.[23] Loss-of-function hypomorphic alleles of ZAP-70 result in reduced TCR signaling and a propensity for autoimmune phenomena such as rheumatoid factor production.[24]

## Adaptor Proteins

Phosphorylation of tyrosine residues in ITAMs and PTKs after TCR stimulation creates docking sites for adaptor proteins. Adaptor proteins contain no known enzymatic or transcriptional activities but mediate protein-protein interactions or protein-lipid interactions. They function to bring proteins in proximity to their substrates and regulators, as well as sequester signaling molecules to specific subcellular locations. The protein complexes that are formed can function as either positive or negative regulators of TCR signaling, depending on the molecules they contain.

Two critical adaptor proteins for linking proximal and distal TCR signaling events are SH2-domain–containing leukocyte protein of 76 kD (SLP-76) and Linker for activation of T cells (LAT; see Figure 12-4). Loss of these adaptor proteins has profound consequences for T cell development. Mice deficient for LAT or SLP-76 manifest a block in T cell development at the $CD4^{-}8^{-}$ $CD25^{+}CD44^{+}$ stage. LAT is constitutively localized to lipid rafts and, after TCR stimulation, is phosphorylated on tyrosine residues by ZAP-70. Phosphorylated LAT then recruits proteins that contain SH2 domain, including PLCγ1, the p85 subunit of phosphoinositide-3 kinase, IL-2–inducible kinase (Itk), and the adaptors Grb2 and Gads. Because the SH3 domain of Gads is constitutively associated with SLP-76, this brings SLP-76 to the complex, where it is phosphorylated by ZAP-70. SLP-76 contains three protein-binding motifs: tyrosine phosphorylation sites, a proline-rich region, and an SH2 domain. The N-terminus of SLP-76 contains tyrosine residues that associate with the SH2 domains of Vav, the adaptor Nck, and Itk. Vav is a 95 kDa protein that acts as a guanine nucleotide exchange factor for the Rho/Rac/cdc42 family of small G proteins. The complex of LAT, SLP-76/Gads, PLCγ1, and associated molecules results in the full activation of PLCγ1 and activation of Ras/Rho guanosine triphosphatases (GTPases) and the actin cytoskeleton.

In addition to acting as positive regulators for TCR signaling, adaptors can also mediate negative regulation. As described previously, the activity of the Src family kinases is regulated by the interaction of kinases (Csk) and phosphatases (CD45) specific for inhibitory C-terminal phosphotyrosine, which is determined by the subcellular localization of these regulatory molecules. A second mechanism by which adaptor proteins can negatively regulate TCR stability is through regulation of protein stability. Proteins can be targeted for degradation by the conjugation of the 76–amino acid protein ubiquitin to lysine residues via a series of enzymatic reactions by E1, E2, and E3 ubiquitin ligases. Cbl-b, c-Cbl, ITCH, and GRAIL are E3 ligases, and mice lacking these proteins manifest T cell hyperproliferation and autoimmune phenotypes. For example, Cbl-b binds and ubiquitinates ZAP70, resulting in its degradation and reduced TCR signaling.[25]

## Downstream Transcription Factors

The aforementioned signaling events couple TCR stimulation to downstream pathways that culminate in changes in gene transcription that are required for proliferation and effector function (see Figure 12-4). One of the best-characterized genes induced after T cell activation is the T cell growth factor IL-2. Transcription of the *IL2* gene is regulated in part by the transcription factors AP-1, nuclear factor of activated T cells (NFAT), and nuclear factor (NF)-κB, all of which are activated after TCR stimulation. Proximal signaling events lead to the activation of Ras and PLCγ.[26] Ras initiates a cascade of kinases including Raf-1, MEK, and the MAP kinase ERK, which leads to the production of the transcription factor Fos. Ligation of the co-stimulatory molecule CD28 results in the activation of another member of the MAP kinase family, c-Jun N-terminal kinase (JNK), and phosphorylation of the transcription factor c-Jun. c-Jun and Fos associate to form AP-1. PLCγ hydrolyzes membrane inositol phospholipids to generate phosphoinositide second messengers including inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol. $IP_3$ stimulates the mobilization of calcium from intra-cellular stores. Diacylglycerol activates protein kinase C (especially PCKθ in T cells) and, along with CARMA, connects with the NF-κB pathway.[26]

Increased intra-cellular calcium is central to many forms of cellular activation. Calcium activates the calcium/calmodulin-dependent serine phosphatase, calcineurin,

which dephosphorylates NFAT.[27] Dephosphorylated NFAT translocates to the nucleus and, together with AP-1, forms a trimolecular transcription factor for the *IL2* gene. The immunosuppressive agents cyclosporin-A and FK-506 specifically inhibit the calcium-dependent activation of calcineurin, thereby blocking activation of NFAT and the transcription of NFAT-dependent cytokines such as IL-2, IL-3, IL-4, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Recently it has been appreciated that differences in the amplitude and duration of calcium signals mediate different functional outcomes. Although high spikes of calcium are easily measured in lymphocytes during the first 10 minutes after antigen stimulation, sustained low-level calcium spikes over a few hours are necessary for full activation. These latter more subtle calcium fluxes appear to be controlled by cyclic adenosine phosphate (ADP)-ribose and ryanodine receptors.[28] Selective inhibitors exist for these molecules, opening the potential for new specific blockers of T cell activation.

A surprising discovery was the observation that caspase activity, particularly of caspase-8, is required to initiate T cell proliferation and activation of NF-κB.[29,30] Previously the role of caspases had been confined to apoptosis. However, it is now appreciated that after TCR ligation, caspase-8 is activated and forms a complex that includes the NF-κB adaptor proteins CARMA1, Bcl-10, and MALT1.[31]

T cell development, proliferation, differentiation, and apoptosis are regulated by small RNA noncoding molecules called microRNAs (miRNAs). These miRNAs bind to their target mRNAs, resulting in mRNA degradation and reduced protein expression. miRNAs modulate TCR signal strength and apoptosis during thymocyte development and have a critical role in the differentiation of $CD4^+$ T cell subsets, particularly T regulatory cells (Tregs).[32] Known targets for miRNAs include IL-2, IL-10, FOXP3, FasL, Jak1, Bcl-2, and Bim. miRNAs also regulate ERK and JNK signaling. Dysregulation of miRNA expression profiles is linked to autoimmune diseases, including RA and systemic lupus erythematosus (SLE).[32] The therapeutic potential of miRNA modulation has been tested in a murine model of lupus, where restoration of miRNA was shown to prevent progression of SLE.[32]

## Co-Stimulation

Signal 2 is mediated either by growth factor cytokines or through a co-stimulatory molecule, of which the prototype is CD28 interacting with CD80 (B7-1) or (CD86) (B7-2). CD28 is a disulfide-linked homodimer constitutively expressed on the surface of T cells. Virtually all murine T cells express CD28, whereas in human T cells, nearly all $CD4^+$ and 50% of $CD8^+$ cells express CD28. The $CD28^-$ subset of T cells appears to represent a population that has undergone chronic activation and can manifest suppressive activity. Increased levels of $CD28^-$ T cells have been reported in several inflammatory and infectious conditions, including granulomatosis with polyangiitis (GPA), RA, cytomegalovirus (CMV), and mononucleosis.[33-35] The cytoplasmic domain of CD28 has no known enzymatic activity but does contain two SH3 and one SH2 binding sites. CD28 interacts with $PI_3$ kinase and GRB2 and promotes JNK activation, as previously noted. CD28 ligation alone does not transmit a proliferative response to T cells, but in conjunction with TCR engagement, it augments IL-2 production at the level of both transcription and translation. It also increases the production of other cytokines, including IL-4, IL-5, IL-13, IFN-γ, and TNF, as well as the chemokines IL-8 and RANTES. An additional member of the CD28 superfamily, inducible T cell co-stimulator (ICOS), is expressed on activated T cells and binds to B7-related protein (B7RP1) on APCs.[36] It is believed to be important particularly to $CD4^+$ Th2 cells. Several products are in development to interrupt ICOS signaling and are in early phases of testing for SLE.

The ligands for CD28, CD80 (B7-1), and CD86 (B7-2) are expressed in a restricted distribution on B cells, dendritic cells, monocytes, and activated T cells. CD80 and CD86 have similar structures but share only 25% amino acid homology. They each contain rather short cytoplasmic tails that may signal directly and bind to CD28 with different avidities. Increased levels of soluble co-stimulatory molecules CTLA-4, CD28, CD80, and CD86 have been reported in patients with SLE and correlate with disease activity.[37] Recent studies with biologic therapies have revealed alterations in co-stimulatory molecules. For example, B cell depletion with anti-CD20 (rituximab) can lead to reduced CD4 T cell expression of CD40L, a B cell co-stimulatory molecule.[38]

## The Immunologic Synapse

Antigen-specific interaction between the T cell and APC results in the formation of a specialized contact region called the immunologic synapse or supramolecular activation cluster (SMAC)[39] (Figure 12-5). Synapse formation is an active, dynamic process that requires specific antigen to drive synapse formation; TCR:MHC interaction alone is not sufficient. The synapse also overcomes the obstacles resulting from interactions of tall molecules (e.g., ICAM-1, LFA-1, and CD45) to promote T cell/APC contact that is mediated by short molecules (e.g., TCR, MHC, CD4, and CD8). Two stages of assembly have been described. During the nascent stage, cell adhesion molecules such as ICAM-1 on APC and LFA-1 on T cells make contact in a central zone, surrounded by an annulus of close contact between MHC and TCR.[39] Within minutes the engaged TCR migrates to the central area, resulting in a mature synapse in which the initial relationships are reversed—the central area (CSMAC) now contains TCR, CD2, CD28, and CD4 and is enriched for Lck, Fyn, and PKC-θ. Surrounding the central domain is a peripheral ring (PSMAC) that contains CD45, LFA-1, and associated talin. T cell activation leads to compartmentalization of activated TCR and TCR signaling molecules to plasma membrane microdomains called "rafts."[40] Rafts are composed primarily of glycosphingolipids and cholesterol and are enriched in signaling molecules, actin, and actin-binding proteins. Src family kinases, Ras-like G proteins, LAT, and phosphatidylinositol-anchored membrane proteins have all been shown to localize to raft domains.

Full T cell activation requires engagement of a minimum of approximately 100 to 200 MHC/peptide molecules on an APC, which can serially stimulate 2000 to 8000 TCRs. It has been estimated that naïve T cells also require a sustained signal for 15 to 20 hours to commit to proliferation.

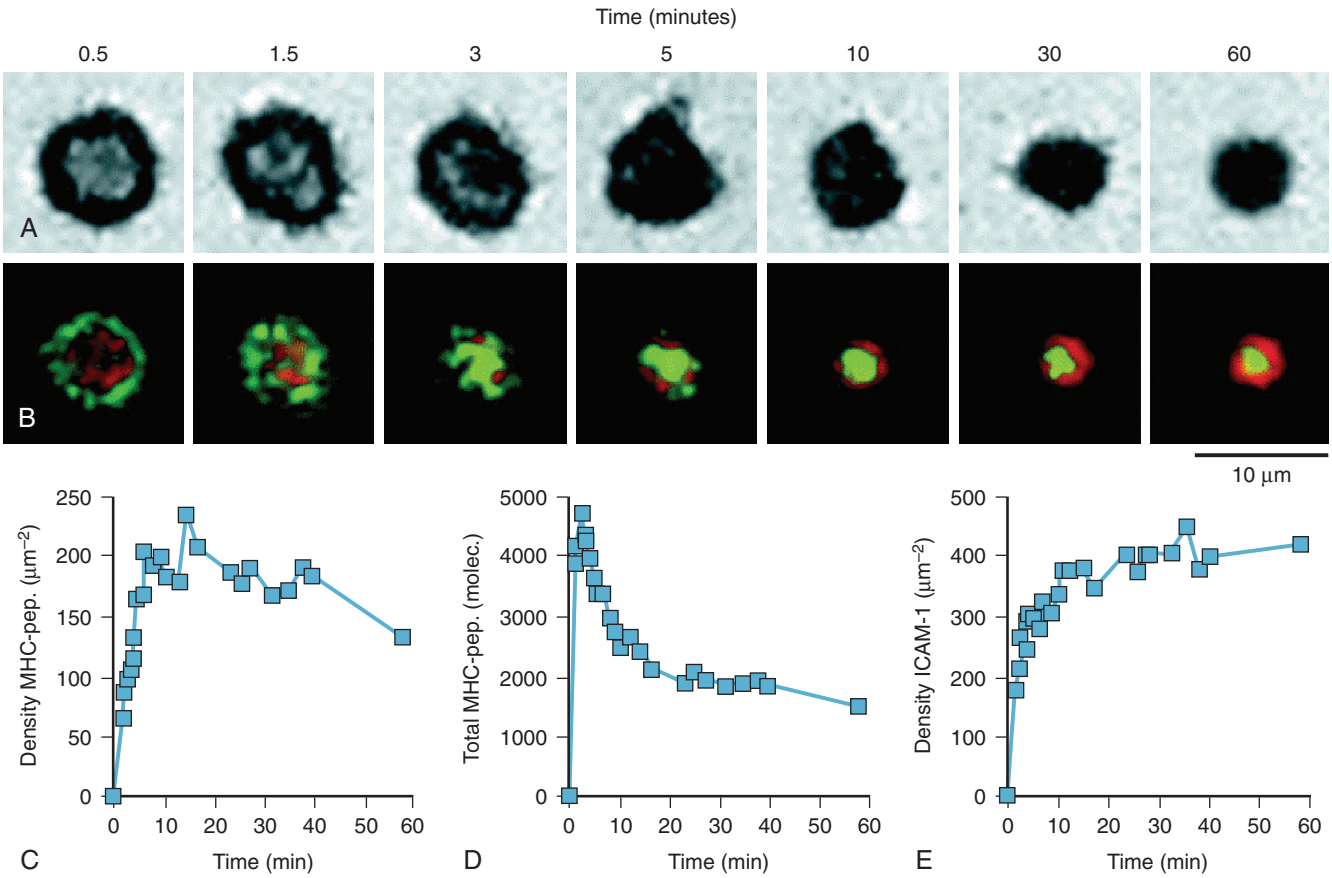



**Figure 12-5** Formation of the immunological synapse. **A,** Contact areas of T cells shown over the time points are indicated as dark gray against a light background. **B,** Images containing Oregon green $E^k$-antigen (mouse cytochrome peptide 88-103) and Cy5 intercellular adhesion molecule-1 (ICAM-1). **C,** Density of accumulated $E^k$-MCC88-103. **D,** Total accumulated $E^k$-MCC88-103. **E,** Density of accumulated ICAM-1. MHC, Major histocompatibility complex. *(From Grakoui A, Bromley SK, Sumen C, et al: The immunological synapse: a molecular machine controlling T cell activation.* Science *285:221-227, 1999.)*

T cells face a number of obstacles to achieving full activation, including the small physical size of the TCR and MHC molecules compared with other cell surface molecules, the low affinity of TCR for MHC/peptide complex, and the low number of MHC molecules present on the APCs that contain the antigenic peptide. The immunologic synapse may provide the mechanism for overcoming these barriers and achieving the duration of TCR stimulation necessary to commit the cell to proliferation.[39] The spatial organization of the synapse juxtaposes the membranes of the APC and T cell, facilitating the interaction of the TCR and MHC/peptide complex. The available MHC/peptide complexes and TCRs are concentrated at the site of contact via actin cytoskeleton–mediated transport. The multistep process of mature synapse formation may also enable the T cell to discriminate among the potential antigen-containing MHC/peptide complexes it encounters on the APC cell surface. It has been shown that co-stimulatory signals may contribute to synapse formation by initiating the transport of membrane rafts containing the kinases and adaptor molecules required for TCR signaling to the site of contact.[41]

It was recently observed that certain $CD4^+$ T cells can respond to the citrullinated peptides[42] and that such citrullination of peptides is induced by autophagy, a reprocessing of organelles during cellular stress. In addition, class II human leukocyte antigen (HLA)-DR4 molecules, a haplotype associated with RA, are very efficient at presenting citrullinated peptides to $CD4^+$ T cells.[42] This may begin to explain the mystery of why HLA-DR4 is associated with RA.

## Tolerance and Control of Autoreactive T Cells

The immune system is constantly confronted by the problem of how to ensure that T cells are activated only under conditions in which a true need exists for a response to a foreign pathogen and not merely a self-component. As with many biologic filters, thymic negative selection is not 100% efficient, and not all self-reactive T cells are eliminated. Hence, a variety of fail-safe mechanisms are engaged to suppress the ability of these errant T cells to undergo premature clonal expansion. This process is partly regulated by the requirement of two distinct signals from separate molecules to be coordinately triggered in order for T cell activation and proliferation to proceed. If only one of the signals is received, the T cell will not proliferate and will actually enter a nonresponsive state known as tolerance or anergy.

The anergy that results from the absence of a CD28 co-stimulatory signal manifests as a failure to fully couple the TCR signal to the Ras/MAP kinase pathway and

consequent AP-1 transcriptional activity. An additional method of provoking an incomplete TCR signal and unresponsiveness is to make amino acid substitutions in the recognized peptide antigen. These so-called "altered peptide ligands" cause a suboptimal phosphorylation of TCRζ and consequent inefficient recruitment of ZAP-70.

Additional members of the CD28 family serve as inhibitory molecules, many of which have been targeted for therapeutic intervention of autoimmune disorders. Cytolytic T lymphocyte–associated protein 4 (CTLA-4) also binds to CD80 and CD86 with 20-fold higher affinity than CD28. Unlike CD28, CTLA-4 is expressed only transiently after T cell activation and confers an inhibitory signal for T cell proliferation.[43] In this capacity, CTLA-4 functions to limit T cell clonal expansion induced by CD28. The consequences of the loss of this negative regulation are striking. The genetic deletion of the *Ctla4* gene in mice results in enormous uncontrolled T cell expansion and an autoimmune diathesis.[44] *Ctla4* was also identified as a genetic risk factor in RA.[45] CTLA4-Ig (abatacept) has been licensed in the United States for treatment of RA and has been used in clinical trials for a variety of autoimmune disorders.

Programmed cell death 1 (PD-1) is another member of the CD28 family that possesses an immunoreceptor tyrosine–based inhibiting motif (ITIM) within its cytoplasmatic tail that associates with the tyrosine phosphatase SHP2.[46] PD-1 does not bind the CD28/CTLA-4 ligands, CD80 or CD86, but rather binds to PD-L1 and PD-L2. PD-L1 is expressed by both lymphoid (e.g., regulatory $CD4^+CD25^+$ T cells, inflammatory macrophages) and nonlymphoid tissues such as heart, placenta, lung, and pancreas. PD-L2 expression is more confined to macrophages and dendritic cells.[47] Ligation of PD-1 on T cells leads to cell cycle arrest. Treatment of mice with a blocking antibody to PD-L1 caused virus-specific $CD8^+$ T cells to undergo marked expansion.[46,47] The fact that many tumors also express PD-L1 enhances the interest in reversing suppression of immune responses during infection and tumorigenesis. The finding that lupus-like arthritis and glomerulonephritis develop in PD-1–deficient mice enhances interest in manipulating PD-1 for therapeutic purposes.[48] The clinical trials of anti–PD-1 and anti–PD-L1 therapy for a variety of tumors will warrant careful observations for potential autoimmune adverse effects.

Chronic exposure to certain inflammatory cytokines, most notably TNF, can also induce anergy. It has been appreciated for some time that T cells from rheumatoid synovium manifest profound deficiencies of proliferation and cytokine production,[49] and TNF is one of the major cytokines detectable in rheumatoid synovial fluid. Further studies demonstrated that chronic exposure of T cell clones to TNF for 10 to 12 days suppressed proliferative and cytokine responses to antigen by as much as 70%.[50] Furthermore, a single administration of anti-TNF receptor monoclonal antibody to patients with RA rapidly restored the response of peripheral T cells to mitogens and recall antigens.[50] Similar observations have been made in TCR transgenic mice after TNF exposure.[51] The observation that chronic TNF exposure inhibited calcium responses after TCR ligation[51] supports the view that TNF may uncouple TCR signaling. Conceivably, other members of the TNF family may invoke similar T cell anergy.

An additional negative regulator for T cells is the B lymphocyte–induced maturation protein 1 (Blimp-1), which was initially identified in B lymphocytes. Blimp-1–deficient mice manifest augmented levels of peripheral effector T cells and experience severe colitis as early as 6 weeks of age.[52] Blimp-1 mRNA expression increases with TCR stimulation, and Blimp-1–deficient T cells manifested enhanced proliferation and produced more IL-2 and IFN-γ after activation.[52]

Another layer of regulation occurs via a phenotypically defined subpopulation of $CD4^+CD25^+$ $FoxP3^+$ Tregs that has the ability to inhibit antigen-induced proliferation.[53] This subset is expressed in the periphery at a low frequency and appears to be at least partly thymic-dependent. The latter point may be of interest because it suggests that the absence of Tregs after day 3 thymectomy may be involved with the subsequent development of autoimmune disease in these animals.[54] Indeed, diminished levels of $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells have been observed in other autoimmune syndromes, and the transfer of Tregs to autoimmune mice has shown some alleviation of symptoms. The production of TGF-β and IL-10, and possibly expression of CTLA-4, appear to be critical to the suppressive activity of Tregs.[55] This area of research is very active because of the potential therapeutic implications for autoimmune diseases and their possible role in generating IL-17–producing $CD4^+$ T cells (Th17; see later discussion).[56] In this regard, recent studies to treat type 1 diabetes by increasing Treg number and function using anti-CD3 antibody or IL-2 are promising.[57] In addition, a recent genetic analysis of risk loci for RA identified the greatest enrichment of trimethylation of histone H3 at lysine 4 of RA risk factors in Tregs, out of 34 cell types studies, further supporting a role for Tregs in the pathogenesis of RA.[58]

## T CELL SUBSETS

### $CD4^+$ Helper and $CD8^+$ Cytolytic T Cells

αβ T cells can be subdivided into two main subsets based on their recognition of peptides presented by class I or class II MHC molecules and their respective expression of CD8 or CD4. $CD4^+$ and $CD8^+$ T cells have different functions and recognize antigens derived from different cellular compartments. The peptides presented by class I MHC molecules are produced by the proteasome[59] and can be derived from either self-proteins or intra-cellular foreign proteins, as might occur during viral infection. Class II MHC–bound peptides are derived largely from extra-cellular infectious agents or self–cell surface proteins that have been engulfed and degraded in the lysosomal complex.

$CD4^+$ T cells express a variety of cytokines and cell surface molecules that are important to B cell proliferation and immunoglobulin production and $CD8^+$ T cell function. After antigen stimulation, $CD4^+$ T cells differentiate into different classes of effector T cells that are identified by their cytokine profiles, including T helper 1 (Th1) Th2, Th17, follicular helper T cells (Tfh; described later), and Treg cells, which were previously described (Figure 12-6). The CD4 molecule is structurally related to immunoglobulins and has an affinity for nonpolymorphic residues on the MHC class II molecule. In this capacity CD4 presumably

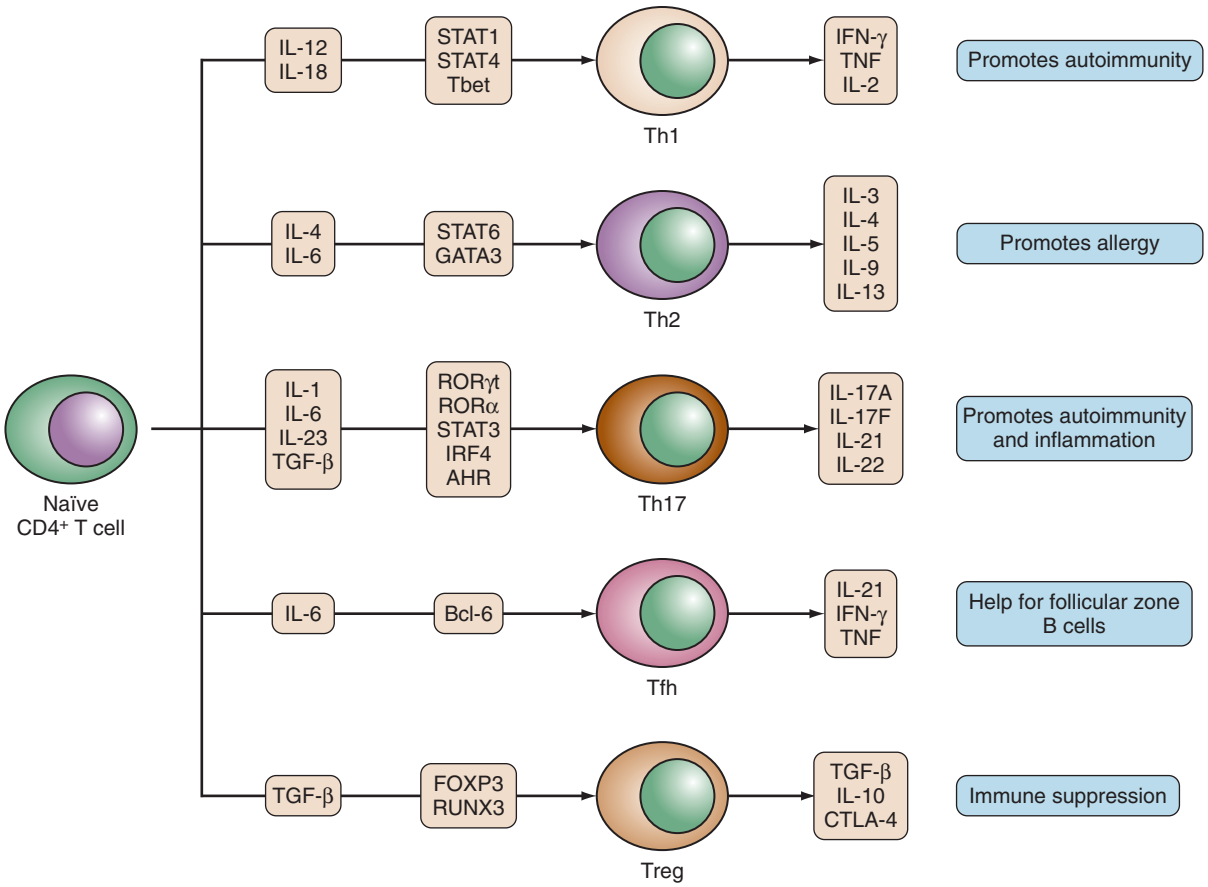



**Figure 12-6** T helper subsets. Naïve CD4 T cells can be polarized into producing particular patterns of cytokines depending upon the cytokine environment in which they develop and the expression of certain transcription factors.

increases the efficiency with which $CD4^+$ T cells recognize antigen in the context of class II MHC molecules, whose expression is restricted to B cells, macrophages, dendritic cells, and a few other tissues during states of inflammation. In addition, the cytoplasmic tail of CD4 binds to Lck and promotes signaling by the TCR as described earlier. However, ligation of CD4 prior to engagement of the TCR renders the T cell susceptible to apoptosis upon subsequent engagement of the TCR.[60] This phenomenon is clinically important in HIV infections in which the gp120 molecule of HIV binds to CD4 and primes the T cell to undergo cell death when later triggered by the TCR. Accelerated apoptosis of $CD4^+$ T cells has been demonstrated in patients with acquired immunodeficiency syndrome (AIDS).[61]

$CD8^+$ T cells are very efficient killers of pathogen-infected cells. Given the ubiquitous expression of class I MHC molecules, mature CTLs can recognize viral infections in a wide array of cells, in distinction to the more restricted distribution of class II molecules. CTLs lyse target cells through the secretion of perforin and granzymes, which induce holes in cell membranes and cleave cellular substrates, respectively, and through the expression of Fas-Ligand and TNF, which induce apoptosis. Similar to CD4, CD8 manifests an affinity for class I MHC molecules, enhances the signaling of CTLs, and also binds Lck by its cytoplasmic tail.

## T Cells in the Innate Immune Response

In addition to the broad array of antigens recognized by αβ T cells, there is growing appreciation that the immune system also contains small subpopulations of T cells that may be specialized to recognize conserved structures that are either uniquely expressed by prokaryotic pathogens or on stressed host cells. These T cells are discussed in detail in Chapter 17. Such common antigenic motifs include bacterial lipoproteins and lipopolysaccharides recognized by Toll-like receptor (TLR)2 and TLR4, respectively, double-stranded RNA from RNA viruses that binds TLR3, and methylated cytosine residues in bacterial CpG sequences, or anti-DNA/DNA complexes that bind to TLR9.[62] TLR expressed by APCs can trigger the release of cytokines and co-stimulatory molecules for T cells. Another family of molecules that likely binds bacterial components is CD1. CD1 structurally resembles class I MHC but contains a deeper and more hydrophobic binding pocket that can accommodate certain lipopeptides and glycolipids. By using such molecular strategies to focus on common and nonpolymorphic molecules, this innate immune response can respond quickly during the early phase of infections. Although it may represent the remnants of an evolutionarily more primitive immune response, it nevertheless provides a very vital early defense system. Among T cells, this function is provided by γδ and natural killer (NK) T cells.

## γδ T Cells

γδ T cells were identified after the serendipitous discovery of rearranged genes while searching for the TCR α chain gene; we did not have pre-existing knowledge of their presence and biologic function.[63] Structurally, the γ chain locus contains at least 14 Vγ region genes, of which six are pseudogenes, each capable of rearranging to any of five Jγ regions and two Cγ regions. The δ-chain genes are nested within the α-chain gene locus between Vα and Jα. There are about six Vδ regions, two Dδ and two Jδ regions, and a single Cδ gene. Transcription of rearranged γ and δ genes begins prior to αβ genes and is apparent on days 15 to 17 of mouse thymus development, after which it declines in the adult thymus. In addition to the ordered appearance of TCR-γδ before TCR-αβ, there is also a highly ordered expression of γ and δ V-region genes during early thymic development, which results in successive waves of oligoclonal γδ T cells migrating to the periphery. The reason for this remarkable regimentation remains unclear.

γδ T cells manifest a number of differences from αβ T cells. γδ T cells are often anatomically sequestered to epithelial barriers or sites of inflammation and frequently manifest cytotoxicity toward a broad array of targets.[64] In contrast to αβ T cells, γδ T cells can respond to antigen directly, without evidence of MHC restriction,[65] or conversely, they can react to MHC molecules without peptide.[66] γδ T cells exit the thymus with a memory-like phenotype and can therefore respond rapidly to their first encounter with antigen. Unlike $CD4^+$ T cells that differentiate into effector subsets after TCR and cytokine signaling, mature γδ T cell subsets already manifest a distinct cytokine profile and includes production of IL-17, IFN-γ, and IL-4.

Human γδ T cell clones, particularly those expressing the Vδ2 gene and derived from peripheral blood of healthy individuals, frequently react to nonprotein components of mycobacteria.[67] These components have been identified as nucleotide triphosphates,[68] prenyl pyrophosphate,[69] and alkylamines.[70] These molecules are, respectively, subunits in DNA and RNA, substrates in lipid metabolism for the synthesis of farnesyl pyrophosphate, and products of pathogenic organisms. These phosphate-containing nonpeptides can be found in both microbial and mammalian cells. This finding suggests that γδ cells may recognize a class of antigens shared by a number of pathogens, as well as by damaged or transformed mammalian cells, and may provide insight into the role of γδ cells in infection and their accumulation at sites of inflammation. Another subpopulation of γδ T cells expressing the Vδ1 gene is typically found in the intestine and in inflamed synovial fluid and reacts to the class I MHC–like molecules known as MICA and MICB.[71] Unlike classical class I MHC molecules that are expressed ubiquitously and continuously, MICA and MICB expression appears to be restricted to gut epithelium and occurs only during times of stress, similar to that of a heat shock response.

The contribution of γδ T cells to defense against infection has been examined in mice using a number of pathogens including *Listeria*, *Leishmania*, *Mycobacterium*, *Plasmodium*, and *Salmonella*. All of these studies have shown a moderately protective role for γδ T cells. The cumulative evidence suggests that γδ T cells may not react directly to components of micro-organisms but rather indirectly via the ability of microbial products to stimulate the innate immune response. An example is the γδ T cells in synovial fluid from Lyme arthritis, which are activated by *Borrelia burgdorferi* lipidated hexapeptides of the outer surface proteins that stimulate TLR2.

γδ T cells accumulate at inflammatory sites in autoimmune disorders such as RA,[72] celiac disease,[73] and sarcoidosis.[74] The reason for this accumulation remains an enigma. However, evidence shows that γδ cells can be highly cytolytic toward a variety of tissues, including $CD4^+$ T cells,[75] in part because of their high and sustained expression of surface Fas-ligand.[76] In this regard, deletion of γδ T cells in various autoimmune models often results in exacerbation of disease.[77,78] The presence of γδ T cells can strongly bias the cytokine profiles of the infiltrating $CD4^+$ cells, in some instances toward Th1 profiles,[79] and in others toward Th2.[80]

## Natural Killer T Cells

A minor subpopulation of T cells bearing the NK determinant manifests a curiously restricted TCR repertoire. NK T cells are found within the $CD4^+$ and $CD4^-8^-$ T cell subsets and, in both mouse and human, express a very limited number of TCR-Vβ chains and an invariant α chain (Vα14 in mice and Vα24 in humans).[81] Furthermore, most NK T cells are restricted in their response to a monomorphic class I MHC–like molecule, CD1d. Crystallographic analysis of CD1d has shown that it contains a deeper groove than traditional MHC molecules and is highly hydrophobic, conferring a preference for binding lipid moieties. Originally the sea sponge sphingolipid, α galactosylceramide, was the only known CD1d ligand. Now both endogenous and bacterial (*Sphingomonas* and *B. burgdorferi)* sources of CD1d-binding sphingolipids have been identified.[82,83] This may represent another type of innate T cell response whereby bacterial lipids or lipopeptides may be presented to NK T cells to provoke a rapid early immune response.

The potential importance of NK T cells in autoimmune disease stems from their rapid production of high levels of certain cytokines, particularly IL-4 and IFN-γ.[81] In this capacity, the IL-4 response may be important for modulating inflammatory responses dominated by Th1 infiltrates. This phenomenon has been noted in the nonobese diabetic (NOD) mouse model of diabetes, which has reduced levels of NK T cells.[84] Adoptive transfer of NK T cells into NOD mice blocks the onset of diabetes.[85] A study extended this observation to human type 1 diabetes. The NK T cells of diabetic individuals produced more IFN-γ and less IL-4 than did the NK T cells of their unaffected siblings.[86] NK T cells have also been reported to be the predominant $CD4^+$ T cell in the airways of patients with asthma.[87] Thus, this minor population of T cells may play a pivotal role in early innate responses to certain infections and also in the regulation of inflammatory sites.

## Naïve Versus Memory T Cells

$CD4^+$ and $CD8^+$ T cells emigrate from the thymus bearing a naïve phenotype. Naïve T cells produce IL-2 but only low levels of other cytokines and as such provide little B cell help. Naïve T cells express high levels of Bcl-2 and can survive for extended periods without antigen but require the

presence of MHC molecules. Naïve T cells circulate from the blood to lymphoid tissues of the spleen and lymph nodes, where antigen, APCs, T cells, and B cells are concentrated. Particularly important in this environment as APCs are dendritic cells, which can migrate from other areas of the body such as the skin and are highly efficient at processing and presenting antigen to T cells (see Chapter 9). Dendritic cells express high and constitutive levels of class II MHC and co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) that are critical to promote proliferation of naïve T cells. In this capacity dendritic cells are particularly adept at promoting clonal expansion of antigen-specific T cells. The development of antigen peptide/MHC tetramer technology has led to direct quantitation of antigen-specific CD8 T cells using flow cytometry. Viral-specific CD8 T cells have an incredible ability to expand after infection, from levels that are undetectable to nearly 50% of the CD8 population, representing a nearly 1000-fold increase in only a few days.[88]

During the process of clonal expansion of naïve T cells and their differentiation into effector and eventually memory T cells, as many as 100 genes are induced. These genes are manifested primarily as increased expression of certain surface molecules involved with cell adhesion and migration (CD44, ICAM-1, LFA-1, α4β1 and α4β7 integrins, and the chemokine receptor CXCR3), activation (CD45 change from high molecular weight CD45RA to lower molecular weight CD45RO isotype), cytokine production (increased production of IFN-γ, IL-3, IL-4, and IL-5) and death receptors (e.g., Fas/CD95) (Table 12-1). More transiently induced are CD69, the survival factor Bcl-xL, and the high-affinity growth factor IL-2 receptor α chain (CD25). Survival of effector T cells to the memory stage is partly dependent upon the cytokines IL-7 and IL-15.[89]

The concept of immune memory has existed since the first successful vaccinations by Jenner for smallpox. A useful memory T cell marker in the murine system is CD44, the hyaluronate receptor. Surface CD44 is low on mature single positive T cells as they emerged from the thymus, but its expression is upregulated upon the first encounter with antigen stimulation in the periphery. Expression of the IL-7 receptor identifies a subset of effector T cells that are destined to become memory T cells. Several other markers have been shown to change upon primary antigenic stimulation. Most notable for human T cells is CD45, in which an isoform known as CD45RA is expressed on naïve T cells, whereas CD45RO expression characterizes memory T cells (Table 12-1). Using these markers, it has been possible to identify a variety of differences between naïve and memory T cells. Activation of memory T cells appears to be more efficient than that of naïve T cells and appears not be absolutely dependent upon co-stimulation. Memory T cells are also able to migrate to nonlymphoid tissues such as lung, skin, liver, and joints.[90] Particularly interesting have been recent reports that the metabolic state of an effector T cell may profoundly affect which T cells survive to the memory state. After T cell activation they rapidly activate glycolysis, which provides many of the precursors need for the synthesis of nucleic acids, amino acids, and lipids needed for proliferation and effector T cell function. However, the survival of T cells to the memory stage requires the return to a relatively nonglycolytic state of fatty acid metabolism and mitochondrial oxidative phosphorylation. Thus, improved survival of memory T cells was conferred by agents such as rapamycin and metformin, which inhibit anabolic glycolytic metabolism and promote catabolic fatty acid metabolism.[91]

## T Helper Subsets

CD4 T cells can be further subdivided on the basis of their cytokine profiles (see Figure 12-6). Th1 cells participate in cell-mediated inflammatory reactions, activate macrophages, and produce IL-2, TNF, and IFN-γ. Th2 cells produce IL-4, IL-5, IL-6, and IL-10. IL-4 and IL-5 are important B cell growth factors.[92] In addition, IL-4 promotes B cell secretion of immunoglobulin (Ig)$G_1$ and IgE, whereas IFN-γ drives $IgG_{2a}$ production. Because Th1 and Th2 cells mediate different functions, the type of response generated can influence susceptibility to disease. These

**TABLE 12-1** Surface Markers on Naïve and Memory T Cells

| Molecule | Other Designation | Molecular Weight (kDa) | Characteristic | EXPRESSION | |
|---|---|---|---|---|---|
| | | | | Memory | Naïve |
| CD58 | LFA-3 | 45-66 | Ligand for CD2 | ++ | + |
| CD2 | T11 | 50 | Alternative activation pathway | +++ | ++ |
| CD11a/CD18 | LFA-1 | 180-195 | Receptor for ICAM-1, ICAM-2, ICAM-3 | +++ | ++ |
| CD29 | | 130 | β chain of β1 (VLA) integrins | ++++ | + |
| CD45RO | | 220 | Isoform of CD45 | ++++ | – |
| CD45RA | | 80-95 | Isoform of CD45 | – | ++++ |
| CD44 | Pgp-1 | 90 | Receptor for hyaluronic acid | +++ | ++ |
| CD54 | ICAM-1 | 120 | Counterreceptor for LFA-1 | + | – |
| CD26 | | 40 | Dipeptidyl peptidase IV | + | – |
| CD7 | | Multichain complex | T cell lineage marker | +/– | ++ |
| CD3 | | | Part of TCR complex | + | + |

CD, Cluster of differentiation; ICAM, intercellular adhesion molecule; LFA, leukocyte function–associated antigen; TCR, T cell antigen receptor; VLA, very late activation antigen.

patterns have been best characterized during chronic infections. In general, a Th1 response helps eradicate intracellular micro-organisms, such as *Leishmania major* and *Brucella abortus*,[93] whereas a Th2 cell response can better control extra-cellular pathogens, such as the helminth, *Nippostrongylus braciliensis*.[94] The cytokine profiles of Th1 and Th2 cells are mutually inhibitory, such that the Th1 cytokine IFN-γ or IL-12 from APC suppresses Th2 responses and augment Th1 cytokine gene expression, whereas the Th2 cytokines IL-4, or IL-6 from APCs, promote the opposite pattern. Polarization of the cytokine environment also occurs at the sites of inflammation in many autoimmune syndromes. Th2 skewing has been observed in models of SLE where increased levels of immunoglobulins and autoantibodies are typical, as well as in chronic allergic conditions such as asthma.[95] Frequently, however, the infiltrating lymphocytes exhibit a bias toward Th1 cytokines. This bias occurs with brain-infiltrating lymphocytes in multiple sclerosis and its animal model experimental allergic encephalomyelitis (EAE),[96] β islet lymphocytes in diabetes,[97] and synovial lymphocytes in inflammatory arthritides.[98] Unlike the beneficial effects of Th1 responses during infections, these same cytokines can be quite deleterious in autoimmune disorders. Thus therapies based on inhibition of certain Th1 cytokines have been of considerable interest and often ameliorative, such as TNF inhibitor treatment of RA.[99] Several cytokines can have pleiotropic effects, and predicting the effects of modulating their levels can be complex. For example, despite the tendency of IL-6 to promote a Th2 cytokine profile, nonetheless, blocking IL-6 is beneficial in RA.[100]

A subset of IL-17–producing $CD4^+$ T cells (Th17) has emerged as being critical for promoting a variety of autoimmune disorders. Th17 cells are regulated by the transcription factor RORγt and can be broadly divided into two groups: first, host protective cells that express both IL-17 and IL-10 and are activated by TGF-β and IL-6, and second, a highly inflammatory population that expresses IL-17, IL-22, and IFN-γ and is activated by IL-23 and IL-1β.[101,102] IL-22 has important functions at mucosal surfaces in host defense, as well as in wound repair. It is produced primarily by Th17 $CD4^+$ T helper cells and innate lymphocytes but acts only on nonhematopoietic stromal cells, particularly epithelial cells, keratinocytes, and hepatocytes.[102] In addition to strengthening epithelial barrier functions, excessive IL-22 can evoke pathologic conditions, such as psoriasis-like skin inflammation.[101] Injections of IL-23 into skin produced increased IL-17 in the epidermis and inflammatory lesions that resembled psoriasis.[103] Th17 $CD4^+$ cells are increased in human psoriatic plaques,[103] in RA synovial fluid, and in multiple sclerosis.[104] Genome-wide association studies (GWAS) have linked genes involved with Th17 cells in RA and Crohn's disease.[102] In addition, inhibitors of Th17 cells have been described that are likely to be used clinically.[105] An additional CD4 T helper subset is found in B cell follicles of secondary lymphoid organs because of their expression of the B cell follicle homing receptor CXCR5.[106] These Tfhs facilitate B cell activation and germinal center formation through expression of CD40L, IL-4, and IL-21. Dysregulation of Tfh function can result in autoantibody production and systemic autoimmunity.[107] Finally, $CD4^+FoxP3^+$ Tregs have already been described earlier.

## Molecular Mimicry

Perhaps the oldest concept in autoimmune mechanisms is that of molecular mimicry, the notion that the response of the immune system to a foreign substance may provoke cross-reactivity to a self-protein. This concept is best established in rheumatic heart disease, where a B cell antibody response to a group A streptococcal cell wall component can precipitate a cross-reactivity to cardiac myosin. Similarly for T cells, investigators used the peptide sequence of myelin basic protein (MBP) recognized by specific T cell clones from patients with multiple sclerosis to search a database of infectious agents. Some of the candidate sequences obtained were able to stimulate the MBP-reactive T cell clones,[108] which suggested for the first time that T cells responding to an infectious agent might manifest cross-reactivity to self-peptides. Another example is one of the outer surface proteins of *B. burgdorferi* known as OspA, which may trigger a cross-reactive T cell response.[109] A T cell immunodominant peptide of OspA was identified in a subset of HLA-DR4 patients who are more resistant to antibiotic treatment and manifest an antibody and a T cell response to OspA.[110] Using a sequence algorithm to identify homologous peptides that bind the DR4 pocket, a sequence in LFA-1 was identified that bound DR4 and stimulated a T cell response from these OspA-reactive patients.[109] The technology of peptide/MHC tetramers previously discussed will enable investigators to determine whether a given subpopulation of T cells within an inflammatory synovium might manifest dual specificity for both a foreign pathogen and a cross-reacting self-protein.

## Death of T cells

The rapid removal of the effector T cells after clearance of the infection is as important as the initial clonal expansion of responding T cells for the health of the organism. Failure to clear activated lymphocytes increases the risk of cross-reactivity with self-antigens and a sustained autoimmune reaction. To ensure that resolution of an immune response occurs rapidly, a number of processes promote active cell death of clonally expanded T cells. One means to control T cell proliferation is through limited availability of growth factors. Upon activation, T cells express receptors for various growth cytokines for approximately 7 to 10 days but only produce cytokines for a more limited period, which results in an unstable situation in which T cells tend to outgrow the availability of growth cytokines. For example, T cells expressing IL-2R in the absence of IL-2 will rapidly undergo programmed cell death. Another method is restimulation of TCR on actively dividing T cells, which triggers a death cascade known as activation-induced cell death (AICD).

The discovery of a family of death receptors expressed by T cells elucidated an additional regulatory process. These molecules are described more extensively in Chapter 28, Cell Survival and Death in Rheumatic Diseases, and will only be discussed here as they relate to T cell function. The best described of these is Fas (CD95). Both Fas-deficient mice and humans bearing Fas mutations (Canale-Smith syndrome)[111] manifest a profound lymphadenopathy accompanied by an autoimmune diathesis, which underscores the

importance of efficiently removing T cells after their activation. Nearly all cells have some level of surface Fas, whereas expression of its ligand (FasL) is restricted primarily to activated T cells and B cells. Consequently, regulation of Fas-mediated apoptosis is to a large extent under the governance of the immune system. FasL expression has also been reported for certain components of the eye, the Sertoli cells of the testis, and perhaps some tumors, providing "immune privileged" sites within which immune responses are difficult to initiate.[112] Expression of FasL by these nonlymphoid cells is thought to prevent immune responses at sites where such inflammation might cause tissue damage. During T cell activation, expression of FasL is rapidly induced, and these T cells readily kill Fas-sensitive target cells.

The sequence of T cell activation followed by cell death is graphically displayed after the administration to mice of bacterially or virally derived compounds called *superantigens*. Superantigens activate T cells by directly cross-linking class II MHC molecules with particular β-chain V families of the TCR (see Figure 12-2A). The superantigen staphylococcal enterotoxin B (SEB) strongly activates $V\beta8^+$ T cells.[113] This activation initiates a rapid expansion of $V\beta8^+$ T cells over 2 to 3 days followed by an equally rapid loss of these cells, such that by day 7 very few $V\beta8^+$ T cells remain. A similar process of T cell activation occurs in the human disease toxic shock syndrome in which a related staphylococcal toxin TSST stimulates the expansion of $V\beta2^+$ T cells.[114] The devastating illness that results from this profound activation of a large proportion of T cells underscores the need to rapidly eliminate activated T cells. At least some of the damage in toxic shock syndrome likely results from the extensive T cell expression of FasL and TNF, particularly in certain tissues such as the liver. Hepatocytes are exquisitely sensitive to damage by these ligands. Activation of T cells leads to homing to the liver, and administration of antigen to TCR transgenic mice can yield a syndrome resembling autoimmune hepatitis.[115] Thus certain autoimmune disorders may result from the death or damage of "innocent bystander" cells as a consequence of the migration of activated T cells to an organ and nonspecific damage due to the expression of FasL family members. Proapoptotic members of the Bcl-2 family, Bim, Bad, and Bax, appear to regulate death in vivo from cytokine withdrawal or after acute foreign antigen stimulation, as with certain infections.[116] These molecules are related to the cell survival molecule Bcl-2 but are more truncated, containing only the BH3 domain of Bcl-2, hence their designation as the "BH3-only" family. They function as sentinels within the cell in that they are attached to various cytoskeletal proteins and organelles and sense cellular damage. If damage occurs they are released from these sequestered areas and migrate to the mitochondria to inhibit the survival function of Bcl-2.[116] By contrast, Fas serves to eliminate T cells after chronic TCR stimulation, such as that which occurs in homeostatic proliferation or chronic infections.[117,118]

## T Cells at Sites of Inflammation

The observation that T cells infiltrate target organs in autoimmune diseases such as RA, type 1 diabetes mellitus, and multiple sclerosis quickly led to an analysis of T cell subsets and, more recently, to a more detailed study of the T cell repertoire based on TCR expression. In many of these disorders, the known class II HLA association is paralleled by a predominant, though by no means exclusive, infiltration of $CD4^+$ T cells bearing a broad TCR repertoire.[119] Many of these $CD4^+$ T cells also manifest a Th1-like cytokine profile, as discussed earlier.[96-98] Evidence for the importance of these $CD4^+$ cells derives from numerous studies showing the efficacy of CD4 depletion in animal models of these disorders.[120] A parallel situation has often occurred in humans with these autoimmune diseases who concurrently become infected with HIV. The CD4 depletion occurring during AIDS can actually ameliorate RA.[121] However, the resulting CD8 predominance during HIV infection has also frequently resulted in exacerbation of psoriatic arthritis and Sjögren's syndrome, suggesting the $CD8^+$ subset of T cells may be important in these disorders.[121] Given the aforementioned effect of the metabolic state of T cells on their survival, it will be highly informative in future studies to examine the metabolic state of T cells at sites of autoimmune tissue inflammation versus that observed in secondary lymphoid tissues during infections. Conceivably the cytokine environment of inflamed tissues may confer a metabolic state that favors T cell survival compared with states of infection.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 13

# B Cells



Yong-Rui Zou • Christine Grimaldi • Betty Diamond

**KEY POINTS**

- **Immunoglobulins (Igs) are key to B cell function because they serve as both an antigen receptor and a major secreted product. The variable region binds antigen and is generated by random rearrangement of gene segments to give rise to numerous specificities. The constant region (Fc) defines the isotype and mediates effector functions.**
- **Surface Ig is the major component of the B cell receptor complex, which regulates B cell selection, survival, and activation. Secreted Ig mediates antigen neutralization and opsonization with uptake by phagocytic cells, complement activation, and cellular activation or inhibition through engagement of receptors for the Fc region of Ig.**
- **B cells are generated from hematopoietic precursors in the bone marrow and undergo several stages of maturation and selection before becoming immunocompetent, naïve B cells that reside in peripheral lymphoid organs. After antigen activation, B cells differentiate to memory cells and Ig-secreting plasma cells.**
- **Follicular B cells respond to protein antigens in a T cell–dependent fashion and are the major source of B cell memory. B1 and marginal zone B cells are less dependent on T cell help and display limited heterogeneity of the B cell receptor.**
- **Autoreactive B cells are generated in all individuals. Multiple checkpoints extinguish autoreactive B cells during early and later stages of B cell development. One or more of these checkpoints is breached in autoimmune-prone individuals, leading to the maturation and activation of autoreactive B cells.**

The immune system is composed of numerous cells that are required to generate innate and adaptive immune responses. Adaptive responses are characterized by immunologic memory generated during the first exposure to an antigen, thereby permitting a rapid response to the antigen after subsequent exposure.

B cells are lymphocytes that recognize antigens through a molecule called the B cell receptor (BCR). The BCR is a surface immunoglobulin (Ig) molecule that recognizes the antigen and is associated with two additional proteins, which transduce the signal. Upon encountering its antigen, a B cell begins a process of activation that leads to antibody secretion and memory formation regulated by interplay with antigen-activated T cells, dendritic cells (DCs), soluble factors, and in some cases follicular dendritic cells (FDCs). Both T and B lymphocytes can differentiate from naïve to memory cells, but only B cells have the capacity to fine-tune their antigen receptor structure to increase its specificity and affinity, giving rise to more effective antibodies. Beyond immunoglobulin secretion, B cells regulate the immune response by cytokine secretion and antigen presentation to T cells in the context of class II major histocompatibility complex (MHC) molecules.

Importantly, much of the knowledge of B cell biology has been generated in mouse models. However, in this chapter, human B cell biology is described whenever possible.

## IMMUNOGLOBULINS: STRUCTURE AND FUNCTION

The hallmark of a B cell is the expression of the Ig molecules. There are two forms of Igs, membrane-bound Igs and secreted Igs, that are generated through alternative messenger RNA (mRNA) splicing. Cell surface Ig, also termed BCR, contributes to B cell maturation and survival and initiates an activation cascade after contact with antigen. Secreted Igs, referred to as *antibodies*, are produced by B cells after antigen activation to protect the host through neutralization of the eliciting antigen.

Structurally, Igs are composed of four polypeptide chains: two identical light (L) chains with a molecular weight of approximately 25 kDa and two identical heavy (H) chains of 50 to 65 kDa. Each of the chains contains a folding motif that is highly conserved among proteins of the immune system, the "Ig domain." These domains constitute the backbone of the Ig molecule and help permit pairing of the polypeptide chains (Figure 13-1). The quaternary structure of an Ig molecule assumes a Y-shaped conformation that contains two functional moieties: two identical antigen-binding regions or variable regions, which are the arms of the "Y," and a constant region, which is the base of the "Y."[1]

This definition of functional moieties derives from early studies analyzing proteolytic fragments of Ig molecules. Cleavage with papain generates two identical fragments that retain antigen-binding capacity and hence are named Fab, as well as a distinct crystallizable fragment (Fc) that mediates immune effector functions but is unable to interact with antigen.[2]

The antigen-binding regions are formed by pairing of the variable domain of the L chain ($V_L$) to the variable domain of the H chain ($V_H$). In contrast to the remainder of the molecule, great diversity exists in the amino acid sequence

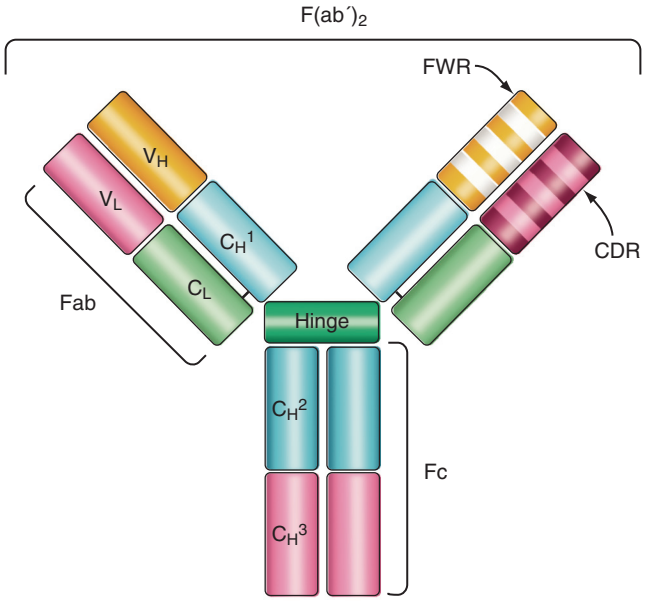



**Figure 13-1** Schematic of the antibody molecule. An antibody monomer consists of two heavy (H)-chain molecules covalently linked to two light (L)-chain molecules. The variable region is composed of the $V_H$ and $V_L$ domains of the H and L chains, respectively. Within the $V_H$ and $V_L$ domains are four framework regions (FWRs) and three complementary-determining regions (CDRs), which together make up the antigen-binding pocket. Papain digestion generates the Fab portion, which consists of $V_H$, $C_H1$, $V_L$, and $C_L$ domains, and pepsin digestion generates two covalently linked Fabs, known as the F(ab′)$_2$. The Fc region of the H-chain constant region, which mediates immune effector functions, consists of the hinge domain (only in immunoglobulin [Ig]G, IgA, and IgD), which increases flexibility and $C_H2$ and $C_H3$ domains.

of the variable domains, which allows for a broad repertoire of Ig molecules that can recognize a wide array of antigens. Within the variable region of the Ig molecule are discrete regions, known as *complementary determining regions* (CDRs) that make direct contact with antigen. The amino acid sequences of the CDR are highly variable and are flanked by more conserved amino acid sequences called *framework regions*. The H- and L-chain molecules each contain three CDRs and four framework regions (see Figure 13-1). The minimal antigenic determinant recognized by the variable regions of the H and L chains is known as an epitope, which may be a continuous or discontinuous region on a protein, carbohydrate, lipid, or nucleic acid. The presence of two identical variable regions in a single Ig molecule confers the capacity to interact with repetitive antigenic determinants present in multivalent antigens (i.e., polysaccharides) or enhances avidity by binding two separate antigen molecules containing the same antigenic determinant.[1]

The constant region directs the Ig effector functions that mediate the killing and removal of invading organisms and both the activation and homeostasis of the immune system. Strictly speaking, the constant region is formed by the constant domain of the L chain ($C_L$), which is paired to the first constant domain of the heavy chain ($C_H1$), and the remaining constant domains of the two heavy chains ($C_H2$ and $C_H3$ and $C_H4$ in IgM), paired to each other. However, the functions associated with the constant region are mediated by the constant domains of the H chain.

## Immunoglobulin Heavy Chain Constant Region

The specific binding interactions that occur between the Ig variable region and antigen may be sufficient to block microbial infectivity or neutralize toxins. However, the ability to eliminate pathogens is mediated by the Fc portion of the molecule. The Fc regions of antigen-antibody complexes are made accessible to serum factors that constitute the complement cascade or to cytotoxic and phagocytic cells that mediate the destruction and removal of pathogens. In mice and humans, there are five different types of H-chain constant regions, or isotypes, designated IgM (μ), IgD (δ), IgG (γ), IgA (α), and IgE (ε)[3]; each is encoded by a distinct constant region gene segment present in the H-chain locus of chromosome 4 in humans or chromosome 12 in mice. Each isotype is capable of specific effector functions, and each cellular receptor for Ig binds specific isotypes and initiates a distinct intra-cellular signaling cascade. The number of $C_H$ domains, the presence of a hinge region to increase flexibility between Fab regions, the serum half-life, the ability to form polymers, complement activation, and Fc receptor binding vary among isotypes. Characteristics of the different Ig H-chain isotypes are presented in Table 13-1.[1,3,4] BCRs of different isotypes may also deliver different intra-cellular signals when activated by antigen. It should be noted that the interplay between antibodies and the cells that bear the Fc receptors extends beyond pathogen clearance and shapes the immune response by mediating activation or inhibition of specific cell types[5] and by mediating cell death.[6]

### Immunoglobulin M

IgM is the first isotype expressed in developing B cells and the first antibody secreted during a primary immune response. It is found predominantly in serum but is also present in mucosal secretions and breast milk. Because the process that increases antibody affinity for a particular antigen (affinity maturation) has not yet been initiated during the early stages of a primary immune response, IgM antibodies usually exhibit low affinity. Their low affinity is balanced by the fact that much of the secreted IgM that exists is in pentameric form, generating multiple binding sites, providing high avidity for antigen, and assisting with the binding of large, multimeric antigens. IgM also exists as a monomer and a hexamer, but only the pentameric form is linked by the polypeptide, called the *joining* (J) chain. The J chain allows the active transport of IgM to mucosal secretions.[7]

Many of the biologic functions of IgM are mediated by its ability to activate the classic complement pathway.[1] The complement cascade consists of a series of enzymes that, upon activation, mediate the removal and lysis of invading organisms. Deposition of antibody molecules or complement components on the surface of the antigen assists with phagocytosis. Proteins such as antibody and complement that enhance phagocytosis are called *opsonins*. Once the complement cascade has been activated, monocytes, macrophages, or neutrophils engulf opsonized particles through specific receptors present on phagocytic cells such as CD21, which recognizes fragments of the C3 complement component. Activation of the complement pathway also results in

**TABLE 13-1** Properties of Human Immunoglobulin Isotypes

| Characteristic | IgM | IgG | IgA | IgE | IgD |
|---|---|---|---|---|---|
| Structure | Pentamer, hexamer | Monomer | Dimer ($IgA_2$), monomer ($IgA_1$) | Monomer | Monomer |
| $C_H$ domains | 4 | 3 | 3 | 4 | 3 |
| Serum values (mg/mL) | 0.7-1.7 | 9.5-12.5 | 1.5-2.6 | 0.0003 | 0.04 |
| Serum half-life (days) | 5-10 | 7-24 | 11-14 | 1-5 | 2-8 |
| Complement activation (classic) | Yes | Yes | No | No | No |
| FcR-mediated phagocytosis | No | Yes | Yes | No | No |
| Antibody-dependent cell mediated cytotoxicity | No | Yes | No | No | No |
| Placental transfer | No | Yes | No | No | No |
| Presence in mucosal secretions | Yes | No | Yes | No | No |
| Main biologic characteristic | Primary antibody response | Secondary antibody responses | Secreted immunoglobulin | Allergy and parasite reactivity | Marker for naïve B cells |

$C_H$, Constant domain of the heavy chain; FcR, Fc receptor; Ig, immunoglobulin.

the generation of the membrane attack complex, which is composed of late complement components and directly lyses C3-opsonized pathogens. Because activation of the classic complement pathway requires exposed Fc regions to be spatially close, multimeric IgM is a potent activator of the classic complement pathway once it has bound its antigen. For example, hexameric IgM is between 20 and 100 times more potent as an inducer of complement activation than is monomeric IgM.[8] C1q is an early complement factor that binds to IgM, as well as IgG. Immune complexes containing C1q can downregulate immune responses because C1q binds LAIR-1, a negative regulator that is present on the cell surface of myeloid cells and lymphocytes. LAIR-1, when associated with C1q, can suppresses inflammation by preventing DC maturation and activation induced by toll-like receptors (TLRs).

### Immunoglobulin G

IgG is the most common isotype found in serum, constituting about 70% of the circulating antibody. IgG antibodies are usually of higher affinity than IgM antibodies and predominate in a recall immune response. Four subclasses of IgG exist in humans: IgG1, IgG2, IgG3, and IgG4. IgG1 and IgG3 arise in response to viral and protein antigens. IgG2 is the main antibody present in response to polysaccharide antigens, and IgG4 participates in responses to nematodes and is observed in patients with IgG4-related systemic disease.[9]

All IgG subclasses exist as monomers and have a high structural similarity; however, minor differences result in distinct biologic effects. IgG3 and IgG1 are potent activators of the classic complement pathway, and IgG2 can initiate the alternative complement pathway (see Chapter 23).

All IgG subclasses engage specific Fcγ receptors (FcγRs) present on DCs, macrophages, neutrophils, and natural killer (NK) cells. The FcγRs on phagocytic cells, when cross-linked, mediate the removal of immune complexes from circulation and initiate antibody-dependent, cell-mediated cytotoxicity, resulting in the release of granules that contain perforin, a pore-forming protein, and enzymes known as *granzymes* that induce programmed cell death (apoptosis) of target cells.[10,11] FcγR engagement also allows the internalization and subsequent presentation of antigens in the context of class II MHC molecules.

Because IgG antibodies are the only ones that cross the placental barrier, they are critical for the survival of newborns. The transport of IgG from the maternal circulation into the fetal blood supply is mediated by the neonatal FcR (FcRn).[12] FcRn is also responsible for the long half-life of IgG in serum by blocking IgG catabolism.[13] FcRIIb is an important inhibitory receptor expressed on myeloid cells and B cells. Its function is discussed in more detail in the later Surface Co-Receptors section.

### Immunoglobulin A

Despite its relative low concentration in serum, more IgA is produced than all other isotypes combined. Most IgA exists as secretory IgA (SIgA) in mucosal cavities and in milk and colostrum, and only a small fraction is present in serum. Two subclasses of IgA exist in humans: IgA1 and IgA2. IgA1 is mainly produced as a monomer. In contrast, polymeric IgA2 is produced along mucosal surfaces.[14]

Polymeric IgA exists mainly as a dimer and includes a J chain (the same chain that links pentameric IgM). It is captured by the polymeric immunoglobulin receptor (pIgR) that is expressed on the basolateral surface of the epithelial cells and then transcytosed to the apical side. Release of IgA into mucosal secretions requires cleavage of the pIgR; a fragment known as the secretory component (SC) remains associated with SIgA and protects it from the action of proteases and increases its solubility in mucus, where it neutralizes toxins and inhibits the adherence of SIgA-coated micro-organisms to the mucosal surface.[15]

People with IgA deficiency have reduced levels of both serum and secreted IgA and are prone to respiratory tract and diarrheal infections, as well as an increased incidence of autoimmune disorders.[16] The presence of FcαR on the surface of neutrophils and macrophages has been suggested

to play a regulatory role in the immune system; it is possible that autoimmune manifestations in IgA-deficient patients arise from the absence of immune regulation mediated by FcαR.[17]

### Immunoglobulin E

IgE is involved in protection against parasitic infections but also triggers immune responses associated with allergic reactions. Only a small amount of IgE is detectable in serum, where it exists as a monomer.[18] Mast cells and basophils express a high-affinity IgE Fc receptor (FcεRI) that binds free IgE. Cross-linking of the FcεR by antigen binding to the IgE induces degranulation and release of histamine, proteases, lipid mediators such as prostaglandin $D_2$, and leukotrienes, many of which are associated with anaphylaxis.

### Immunoglobulin D

The role of IgD in the humoral response has been the subject of multiple speculations. IgD is found predominantly as a membrane-associated Ig on mature naïve B cells. Soluble IgD is scarce in serum; however, IgD-producing plasma cells are found in tonsils and tissue associated with the respiratory tract. High levels of secreted IgD can be found in persons with immunodeficiencies.[19]

## Light Chains

Two distinct L-chain polypeptides exist, designated κ and λ. L chains contain a variable and a single constant domain. Even though two L-chain isotypes exist, there is no known function associated with the L-chain constant region. The κ chain is used more often than the λ chain in human (65%) and mouse (95%) Ig molecules.[20]

## Immunoglobulin Variable Region

The recognition of a virtually unlimited number of antigens requires a mechanism to generate Ig molecules with similarly broad specificities. The molecular basis of this process has been known for several years.[21] The Ig molecule is encoded by gene segments within distinct genetic loci residing on separate chromosomes (Figure 13-2); the H-chain locus is on human chromosome 14,[22] the κ-chain locus is on chromosome 2, and the λ-chain locus is on chromosome 22.[23]

The H-chain variable region is encoded by a variable ($V_H$), diversity ($D_H$), and a joining ($J_H$) segment. The L chain is encoded by either $V_κ$ and $J_κ$ or $V_λ$ and $J_λ$ segments; it does not contain D segments. The human H-chain locus contains from 38 to 46 $V_H$, 23 $D_H$, and 9 $J_H$ functional genes (these numbers represent a typical haplotype but vary among individuals). The κ-chain locus contains approximately 31 to 35 $V_κ$ genes and 5 $J_κ$ functional genes; the λ-chain locus contains 29 to 32 $V_λ$ genes and 4 or 5 $J_λ$ functional genes.[24]

### Generation of Immunoglobulin Diversity

In a developing B cell, different $V_H$, $D_H$, and $J_H$ or $V_L$ and $J_L$ gene segments are randomly combined to generate a large number of different Ig molecules (Figure 13-2). This process,

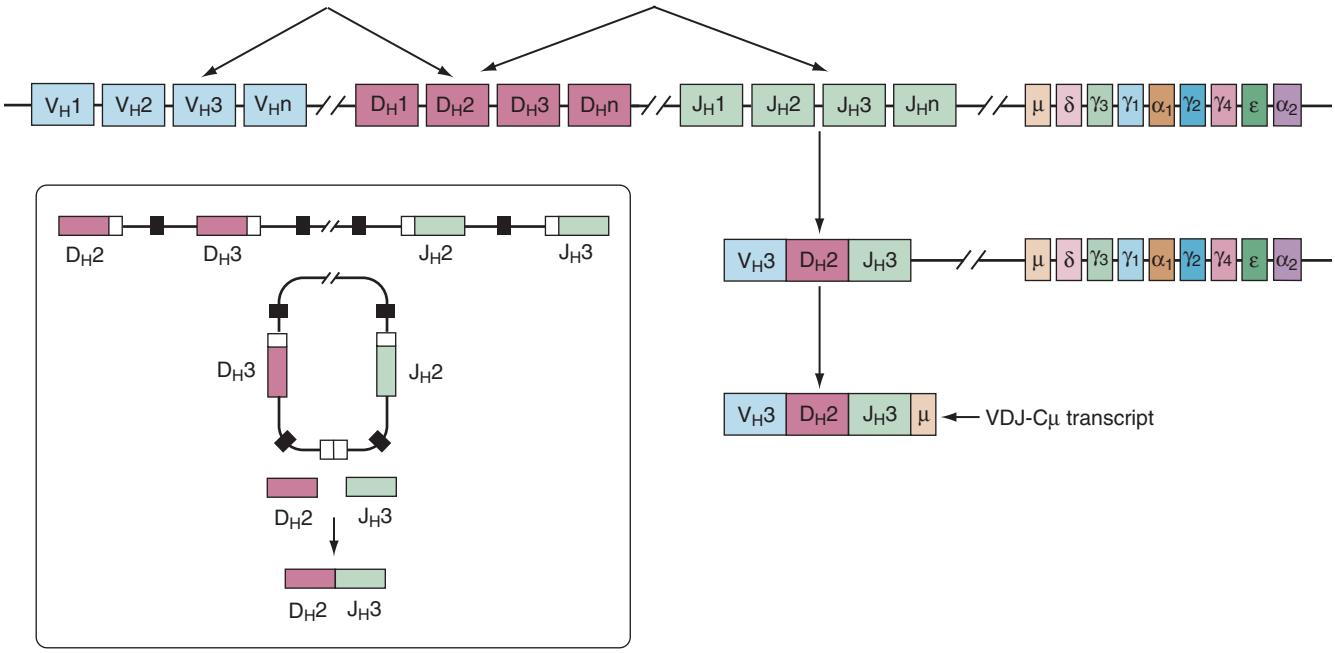



**Figure 13-2** V(D)J recombination at the immunoglobulin gene locus. V(D)J recombination at the heavy (H)-chain locus is depicted at the top. A single $V_H$ gene segment randomly recombines with a $D_H$ and $J_H$ gene segment residing on the same chromosome. After $V_HD_HJ_H$ recombination, a transcript containing the immunoglobulin (Ig)M H-chain constant region gene (Cμ) is generated. The *inset* represents an example of V(D)J recombination occurring between a single $D_H$ and $J_H$ gene segment. The *white squares* represent the heptamer, and the *black squares* represent the nonamer recombination recognition sequences. After recognition and cleavage of these sequences, the coding junctions of the rearranged $D_H$ and $J_H$ gene segments are ligated. A $V_H$ gene segment then recombines with the rearranged $D_HJ_H$ segment. Light (L)-chain rearrangement is mediated by the same mechanism.

known as V(D)J recombination, occurs in the fetal liver or adult bone marrow, in the absence of antigen stimulation, and must be successful to continue with B cell maturation. Here, we present the molecular process, and later the functional and developmental consequences are discussed (see the B Cell Development section).

V(D)J recombination happens sequentially, beginning with the joining of one $D_H$ segment to one $J_H$ segment; then a $V_H$ segment will be targeted to the rearranged $D_HJ_H$ fragment. The absence of an in-frame recombination leads to recombination of the second allele. L-chain recombination also occurs in a stepwise manner. First, the κ locus is rearranged; in the absence of a productive κ-chain rearrangement, the λ locus undergoes recombination.[25]

The recombination machinery is composed of specific enzymes, including recombination-activating gene 1 and 2 (RAG-1 and RAG-2). The complex recognizes recombination signal sequences (RSS) that flank the V, D, and J gene segments. These highly conserved sequences are composed of a palindromic heptamer (7 base pairs) followed by DNA spacers that are 12 or 23 base pairs in length and an adenosine/thymidine (AT)-rich nonamer.[26] Once the complex recognizes its target, it generates double-stranded DNA (dsDNA) breaks at the RSS sites. Next, the cellular DNA repair complex recognizes and joins the cleaved segments.

The random recombination that occurs among V, D, and J gene segments can generate a diverse Ig repertoire without the need for a large number of germline H- and L-chain genes. During H-chain recombination, nucleotides may be added at $V_HD_H$ and $D_HJ_H$ junctions by the enzyme terminal deoxynucleotidyl transferase (TdT). These non–germline-encoded sequences are known as *N additions*. As long as these nucleotide changes do not disrupt the reading frame or lead to the incorporation of premature stop codons, the random addition of N sequences increases the diversity of the amino acid sequence. Imprecise ligation at the coding junctions may result in the loss of nucleotides, thereby also enhancing diversity. Finally, random pairing of the H chains and L chains further diversifies the Ig repertoire.

## B CELL DEVELOPMENT

The final outcome of the maturation process is the generation of a pool of mature B cells with a diverse repertoire of Ig specificities that can recognize foreign and pathogenic antigens without compromising the integrity of the self. Therefore the process of generation of Ig diversity is coupled with the censoring of autospecificities.

B cells, as with all cells in the hematopoietic lineage, begin with the differentiation of noncommitted $CD34^+$ hematopoietic stem cells to lymphopoietic precursors with restricted lineage potential. These cells, known as *common lymphoid progenitors* (CLPs), have the potential to give rise to NK, T, and B cells. Early B cell progenitors begin to express genes for DNA rearrangement, as well as B cell program transcription factors. Multiple transcription factors act in concert, but Ikaros, E2A, EBF, and Pax5 appear to be the most important in B cell development.[27] Pax5 is considered the master transcriptional control for B cells because it is induced in early stages of B cell commitment and plays a dual role by repressing genes required for differentiation to the myelomonocytic lineage and activating B cell–specific genes such as Ig genes, CD19, and signaling molecules.[28]

### Niches for Human B Lymphopoiesis

The development of hematopoietic stem cells ($CD34^+$) into mature B cells begins in the first weeks of uterine life. By the eighth gestational week, early B cell precursors can be identified in the fetal liver and omentum. From gestational week 34 and through adulthood, the bone marrow is the primary site of B lymphopoiesis.[29] It has been unequivocally established that there are differences between the B cells that originate during fetal and adult lymphopoiesis in mice, and it is becoming clear that these differences extrapolate to human lymphopoiesis as well. B cell precursors are susceptible to estrogen, and the maturation of maternal B cells is arrested at the pro–B cell stage during pregnancy; in contrast, fetal B cell precursors lack estrogen receptors and consequently are unaffected by exposure to hormones.[30] B cells originating during prenatal life have a bias in the usage of $D_H$ and $J_H$ gene segments, and this, along with low expression of the enzyme TdT, leads to a more restricted Ig repertoire with shorter CDR3.[31]

Whether during fetal or adult lymphopoiesis, the maturation of B cells from multipotential progenitors is contingent on the presence of stromal cells that provide both contact-dependent and soluble signals. Although the nature of the interactions provided by the stromal cells to create a lymphopoiesis-permissive environment is still largely unknown, they include both survival and proliferative signals. Stromal-derived factor 1 (SDF-1) is required for homing of the early B cell precursors to sites of lymphopoiesis.[32] Interactions between vascular cell adhesion molecule-1 (VCAM-1) on the membrane of the stromal cells and its ligand, very late activation antigen-4 (VLA-4), on early B cell progenitors are required for B cell differentiation. The molecules interleukin (IL)-7, IL-3, and the Fms-like tyrosine kinase-3 (Flt-3) ligand promote B cell lymphopoiesis, although IL-7 appears to be dispensable for human B cell development. Matrix molecules in the microenvironment such as heparan sulfate proteoglycan are assumed to "trap" critical soluble factors.[33]

### B Cell Ontogeny

The stages of B cell development are defined by the state of Ig gene rearrangement and the expression of intra-cellular and surface proteins. The nomenclature and classification of particular stages vary slightly among different laboratories working in this field. For simplicity, we have divided the stages of B cell lymphopoiesis into early B cell progenitors, pro-B, pre-B, immature, transitional, and mature naïve B cell (Table 13-2). When a CLP begins to transcribe mRNA coding for the proteins required for B cell maturation, E2A and EBF, the cell becomes an early B cell progenitor. Once these two transcription factors are expressed, they enable transcription of the proteins involved in the recombination machinery (RAG-1/RAG-2). The beginning of D to J recombination on the IgH locus marks the progress to a pro-B stage.

**TABLE 13-2** Human B Cell Maturation Markers during B Cell Development

| Marker | HSC | Pro-B | Pre-B | Immature | Transitional 1 | Transitional 2 | Plasma |
|---|---|---|---|---|---|---|---|
| CD34 | + | + | – | – | – | – | – |
| CD19 | – | + | + | + | + | + | + |
| CD10 | – | + | + | + | + | + | – |
| CD20 | – | + | + | + | + | + | – |
| CD21 | – | – | – | – | – | + | – |
| CD22 | – | – | + | + | + | + | – |
| CD23 | – | – | – | – | – | + | – |
| CD38 | – | + | + | + | + | + | + |
| CD40 | – | + | + | + | + | + | – |
| CD45 | – | + | + | + | + | + | + |
| CD138 | – | – | – | – | – | – | + |
| RAG-1 | – | + | + | +/– | +/– | +/– | – |
| RAG-2 | – | + | + | +/– | +/– | +/– | – |
| TdT | – | + | + | – | – | – | – |
| Igα | – | + | + | + | + | + | + |
| Igβ | – | + | + | + | + | + | + |
| Heavy chain | – | – ($D_H$–$J_H$) | + ($V_H$–$D_H$-$J_H$) | + | + | + | + |
| Pre-BCR | – | – | + | – | – | – | – |
| Surface IgM | – | – | – | + | + | + | – |
| Surface IgD | – | – | – | – | – | + | – |
| Light chain | – | – | + ($V_κ$-$J_κ$ $V_λ$-$J_λ$) | + | + | + | + |

BCR, B cell receptor; CD, cluster of differentiation; HSC, hematopoietic stem cell; Ig, immunoglobulin; RAG, recombination-activating gene; Tdt, terminal deoxynucleotidyl transferase.

## Pro-B Cells

The pro-B cell stage is defined by the rearrangement of the IgH chain gene segments and synthesis of a μ-polypeptide. Pro-B cells are dependent on interactions with endothelial cells present in the stroma. The VLA-4 integrin and CD44 both mediate adhesion to stromal cells, are highly expressed at this stage, and are believed to be important for continued development.[34] Pro-B cells also express high levels of B cell lymphoma 2 (Bcl-2), a molecule that protects cells from apoptosis. At the onset of the pro-B cell stage, the variable gene segments of both H- and L-chain loci are in the unrearranged germline configuration but accessible to the recombination machinery. A $D_H$ gene segment on one H-chain chromosome rearranges with a $J_H$ gene segment residing on the same chromosome, often with the inclusion of nontemplate nucleotides at the junction of these two segments. Next, a $V_H$ gene rearranges to the $D_HJ_H$ gene fragment. Completion of $V_HD_HJ_H$ gene rearrangement leads to the generation of an H-chain transcript that also contains the IgM constant region ($C_μ$), which is the constant region gene most proximal to the variable region genes on the chromosome (see Figure 13-2).

The generation of a μ-polypeptide and its subsequent expression on the surface of the cell, together with a surrogate L chain formed by the λ5 and Vpre-B polypeptides, as well as the Igα/Igβ dimer—a complex known as the pre-B cell receptor (pre-BCR)—marks the end of this phase of gene recombination. This constitutes a critical developmental checkpoint and the entrance to the next developmental stage, known as the *pre-B cell*.[35] The requirement for a pre-BCR complex ensures that B cells without a productive H chain will not undergo further differentiation.

The pre-BCR stimulates pro-B cells with productive IgH rearrangement to proliferate. It also transduces a signal that the V(D)J rearrangement was successful and halts recombination of the second H-chain allele. This process, known as *allelic exclusion*, ensures that all Ig molecules generated within a single B cell are identical and have the same antigenic specificity. If no μ chain is generated, rearrangement is initiated on the other chromosome. If the second rearrangement also results in a nonproductive H-chain molecule, the absence of a pre-BCR–mediated signal induces apoptosis. The odds of generating a productive rearrangement are one in three, and consequently, approximately 50% of the cells that begin recombination will be unable to proceed along a developmental pathway.

## Pre-B Cells

The pre-B cell stage is characterized by L chain recombination. Initiation of this stage requires the presence of the pre-BCR and functional signal transduction machinery.

At the transitional stage from pro-B to pre-B cells, the expression of the pre-BCR induces a proliferative burst that generates daughter cells with the same H chain and

potential for multiple specificities within daughter cells, each of which may produce a different L chain. Targeted disruption of genes encoding the pre-BCR complex such as the IgM transmembrane constant region domain, λ5, or the Igα and Igβ accessory molecules results in a profound decrease in developing B cells. In addition, defects in the adapter molecule BLNK or the tyrosine kinase Btk lead to a serious impairment in pre-B cell maturation. It appears that the charged residues on pre-BCR induce self-aggregation, which activates constitutive internalization and signaling of the pre-BCR complex that leads to clonal expansion of B cell precursors with a fit pre-BCR.[36]

The expression of the pre-BCR is transient. After the proliferative burst, the μ H chain is present only in the cytoplasm as the pre-B cell rearranges an L chain. The general rearrangement process is similar to V(D)J rearrangement and is dependent on RAG-1/RAG-2 expression. Because TdT is not expressed in this stage, L chains do not usually contain N sequences at the $V_LJ_L$ junction. At the end of this process, pairing of the newly minted L chain with the μ H chain leads to the surface expression of an IgM molecule, complexed with Igα and Igβ, to form the BCR complex. It is believed that surface expression of the BCR on immature B cells transduces signals that enforce allelic exclusion at the L-chain locus and downregulate expression of the RAG genes. This immature B cell has completed the gene rearrangement process and is now subject to repertoire selection.[37]

### Immature B Cells

Once B cells express surface IgM in the bone marrow, they are subject to *repertoire censoring*. During this stage, cross-linking of the BCR by antigen leads to the activation of one of several tolerance mechanisms. These mechanisms include deletion, receptor editing, and anergy and diminish the fraction of autoreactive cells present in the mature repertoire (see later discussion on negative selection).

During the maturation process in the bone marrow, the cells become less dependent on interactions with the stroma and move toward the sinusoidal lumen. Once they express IgM, they begin to move into blood, where they are called *transitional cells*.

## Peripheral Naïve B Cell Subsets

As B cells mature and their dependence on stromal cells decreases, they leave the bone marrow and finish their maturation in the spleen before homing to other lymphoid tissue such as the lymph nodes, tonsils, and Peyer's patches of the intestine. It is in these secondary lymphoid organs where mature B cells interact with foreign antigen and specific humoral immune responses are activated.

### Transitional B Cells

Once immature B cells egress from the bone marrow, they are called *transitional cells*. These cells are the earliest B cells found in the periphery in healthy subjects and move to the spleen to finish their maturation.

Transitional cells are the last B cell subpopulation that expresses the developmental marker CD24. At this stage B cells begin to express surface IgD, which harbors the same specificity as the IgM because the IgD H chain is encoded by the same VDJ fragments as IgM but expresses the $C_\delta$ instead of the $C_\mu$ domain. It is the expression of IgD that separates transitional cells into two different maturation stages. Transitional 1 (T1) B cells that do not express IgD are the recent bone marrow emigrants, and transitional 2 (T2) B cells that begin to express IgD represent the subsequent maturational stage.[38] The existence and functional characteristics of a third transitional stage (T3) are still debated.

Transitional cells constitute a stage subject to multiple regulatory processes. First, transitional cells must compete with naïve B cells already present in the periphery for a developmental niche. Transitional B cells are extremely dependent on a B cell survival factor known as *B cell–activating factor of the tumor necrosis factor family* (BAFF or BLyS). In its absence, B cell development does not progress beyond the T1 stage.[39] Second, T1 transitional cells are still highly susceptible to tolerance induction after BCR cross-linking. In T1 cells, cross-linking of the BCR ex vivo has been shown to lead to cell death, whereas T2 cells respond to BCR cross-linking by proliferation and differentiation to the mature naïve B cell stage.

### BAFF Family of Cytokines

The cytokine milieu surrounding the B cell is diverse and spatially and temporarily regulated. Although B cells are modulated by multiple cytokines, in recent years two members of the tumor necrosis factor (TNF) family, BAFF and APRIL, have emerged as key survival factors, particularly at two regulatory points in development and differentiation: the transition from an immature to a naïve B cell in the periphery and the survival of the newly produced plasma cells. BAFF (B cell–activating factor, BLyS) and APRIL (a proliferation-inducing ligand) are proteins produced by cells that take part in the innate response such as macrophages and DCs, as well as stromal cells, and are present as membrane-bound proteins or soluble trimers. They have three known receptors (BAFF-R, TACI, and BCMA) that are present on the membrane of B cells from the T2 stage to their final differentiation to plasma cells. BAFF binds the three receptors, whereas APRIL binds only TACI and BCMA. BAFF induces survival and activation of B cells when bound to BAFF-R, whereas BAFF signaling through TACI decreases the size of the B cell pool. APRIL does not participate in B cell homeostasis but seems to be critical to the survival of plasmablasts in the bone marrow.[40]

Enhanced survival and activation of autoreactive B cells have been demonstrated in mice that overexpress BAFF.[41] An increase in serum levels of BAFF has been observed in some patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, and Sjögren's syndrome and is thought to contribute to pathogenesis because autoreactive B cells that would normally be censored can survive in the presence of excess BAFF[42] (Figure 13-3).

### Mature B Cells

The final stages of maturation that occur in the spleen and give rise to the naïve B cell subset have not been fully

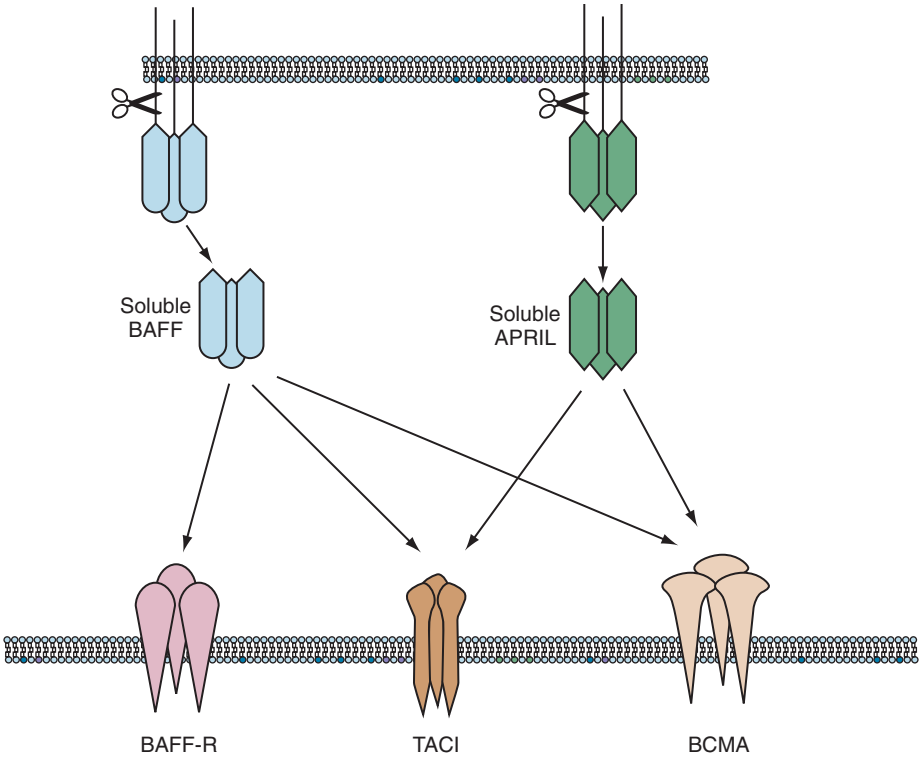



**Figure 13-3** The BAFF family of cytokines and their receptors. BAFF (also known as BLyS) and APRIL are expressed as membrane-bound proteins that can be cleaved by proteases to produce the soluble proteins. BAFF might bind the three known receptors: BAFF-R, TACI, and BCMA. APRIL only binds to BCMA and TACI.

elucidated, but the prevailing theory is that T2 B cells give rise to the circulating mature naïve cell population. In the mouse, two populations of phenotypically and functionally different naïve B cells are recognized: follicular and marginal zone (MZ) B cells. Human naïve B cells constitute 60% to 70% of the circulating B cell repertoire and populate the spleen and lymph nodes. They include the equivalent of mouse follicular B cells and represent the circulating, nonantigen-exposed B cell subpopulation characterized by surface IgM and IgD expression, lack of CD27, and the presence of the membrane transporter ABC.[43] There is also a population in blood of $IgM^+$, $CD27^+$ B cells that have been likened to MZ B cells, which do not recirculate in the mouse.[44]

### Marginal Zone B Cells

MZ B cells are a population of noncirculating mature B cells located in the MZ of the rodent spleen. In rodents, MZ B cells present clear phenotypic and functional differences to the cells present in the follicles, responding to blood-borne pathogens and to repetitive antigenic structures such as the ones present on polysaccharide antigens.

In the human spleen, the structural definition of the MZ does not correspond exactly to the area surrounding B cell follicles. However, there is a population with the functional characteristics of mouse MZ B cells: low activation thresholds, highly responsive to polysaccharide antigens and with a clear surface phenotype, can be differentiated. These cells, which are sometimes named *MZ-like* or *unswitched memory*, are not restricted to the human spleen but are found circulating in the peripheral blood, as well as in the lymph nodes, tonsils, and Peyer's patches.[44,45] They are defined as $IgM^{high}$, $IgD^{low}$, $CD27^+$, $CD21^+$, and $CD1c^+$.[45,46]

Given that these cells possess the "memory" marker CD27, it has been suggested that they have experienced antigenic exposure; however, the presence of MZ-like B cells in subjects with X-linked agammaglobulinemia (a CD40L deficiency) suggests that even if antigen exposure has occurred, T cell help has not.

Interestingly, MZ-like B cells, although already present at birth, do not seem to be fully functional at that time; infants up to age 2 years are particularly susceptible to infections by capsulated bacteria. This phenomenon might be due to the functional immaturity of the cells or to the lack of development of the antigen-trapping microstructure.

### B1 Cells

In mice, B1 cells represent a minor population of B cells that reside predominantly in the pleural and peritoneal cavities. They are named B1 because it is assumed that is the first population of B cells to develop during intrauterine life.

Functionally, B1 cells have been characterized as self-renewing cells that possess a limited BCR repertoire and respond with low affinity to a broad array of antigens, mainly phospholipids and carbohydrate structures in the bacterial cell wall. Despite their low numbers, these cells secrete most of the natural antibodies of the organism (antibodies that appear without evidence of previous immunization) and are assumed to be the precursors of most of the plasma cells that

home to the intestinal *lamina propria*. Phenotypically, these cells are defined as $IgM^{high}$ and $IgD^{low}$. About 70% of them express the marker CD5.

In humans, the definition of B1 cells is still unresolved. The CD5 marker has been used extensively as a surrogate marker for B1 cells with mixed success, given that activated human B cells also upregulate the expression of CD5.[47]

## SITES OF B CELL HOMING AND ACTIVATION

After the immature B cell stage, B cells home to secondary lymphoid organs, which contain the microenvironment and architecture necessary for the retention and activation of B cells. These organs include the spleen and lymph nodes, as well as lymphoid structures in mucosal tissue (e.g., Peyer's patches, appendix, and tonsils). The secondary lymphoid tissue is adapted to trap circulating antigen and expose the B cells to it and to provide interactions with T cells and other co-stimulatory cells. Peripheral lymphoid tissue contains specialized antigen-presenting cells known as *dendritic cells*. In the Peyer's patches of the intestines, foreign antigen is taken up in specialized epithelial cells known as *M cells*. Even though peripheral lymphoid tissues vary in structure and cellular organization, they all possess antigen-presenting cells and B cell–containing follicles surrounded by T cell–rich zones.[48] As explained in the following section, antigen, T cells, and DCs are required for B cell activation and differentiation into Ig-secreting plasma cells or memory B cells.

### Circulation and Homing

B1 cells typically home to the peritoneal and pleural cavities and, to a lesser extent, the spleen. Naïve B cells enter the peripheral circulation by passing through the endothelial lining of the sinusoids of secondary lymphoid tissue and recirculate throughout the follicles of secondary lymphoid tissues. The entry, retention, and recirculation of B cells through secondary lymphoid organs depend on both adhesion molecules and chemokine receptors.[49,50] First, expression of LFA-1 and VLA-4 is required for entry into the lymphoid tissue, and then the chemokine receptors CXCR5 and CCR7 direct localization within the tissue. The CXCR5 molecule is expressed on all mature B cells and mediates B cell migration to follicles in response to the chemokine CXCL13, which is produced by follicular stromal cells. These cells, in turn, are regulated by lymphotoxin made by B cells. In the follicle, the B cells scan for antigen, making contact with potential antigen-bearing cells such as FDCs, subcapsular macrophages, and DCs. If the B cell does not encounter a cognate antigen, it will exit the lymphoid organ through the efferent lymphatics in response to the molecule sphingosine 1 phosphate (S1P). Neutralization of S1P leads to sequestration of B cells in lymphoid organs.[51]

Upon antigenic encounter, the B cells are retained in the lymphoid organ because of the upregulation of CCR7. The ligands for CCR7 (CCL19 and CCL21) mediate the organization of the T cell zone and attract antigen-activated B cells to this area, where cognate T cell–B cell interactions occur.

In contrast, it is assumed that MZ-like B cells respond to antigen without the help of cognate T cells. In mice, MZ B cells are present exclusively in the spleen and do not recirculate; in humans they are found in the spleen and tonsils, as well as in blood. MZ B cells localize to the outer layers of the follicles, making them among the first cells to encounter blood-borne antigens. The adhesion molecules ICAM-1 and VCAM-1, as well as S1P and cannabinoid receptor 2, are responsible for sequestering MZ B cells within the marginal sinuses.

The interplay of chemokine expression and the induction of chemokine receptors play an important role in the germinal center (GC) response. The chemokine CXCL12 retains centroblasts in the dark zone during the process of somatic hypermutation and isotype class switching. CXCL13 regulates migration to the light zone, where survival and selection events are mediated by interactions with CXCR5-expressing T follicular helper (Tfh) cells and FDCs.[52] Later, CXCL12 promotes the migration of plasmablasts to the bone marrow, where they undergo further development into long-lived plasma cells.

### Mucosa-Associated Compartments

Within the mucosal tissue, the sites of induction of immune responses are distinct from the site where the effector cells reside. There are two main sites for the induction of an immune response. The first is the mucosa-associated lymphoid tissue (MALT) that includes Peyer's patches, nasopharynx-associated tissue, and isolated lymphoid follicles, where exogenous antigen is displayed by specialized M cells that transport antigen to the follicle. The second site of induction includes mucosa-draining lymphoid nodes such as the mesenteric and cervical lymph nodes.[52]

B cells reach these sites through the systemic circulation. Once they are stimulated by antigen and induced to differentiate, they home to the effector sites in the intestinal and respiratory lamina propria, where they differentiate into plasma cells and produce antibody mainly of the IgA isotype. An interesting characteristic of the plasma cells induced in the mucosal compartments is their selective homing to mucosal effector sites. Nasal activation leads to IgA-secreting cells with high levels of CCR10 and $\alpha4\beta1$ integrins that home to the respiratory and genitourinary tracts in response to their ligands, CCL28 and VCAM-1. Migration to the intestinal lamina propria, in contrast, seems to be dependent on orally induced activation and subsequent expression on B cells of the chemokine receptor CCR9 and integrins that bind to CCL25 and MADCAM1/VCAM-1, respectively.[53]

## B CELL ACTIVATION AND DIFFERENTIATION

Engagement of surface Ig by antigen triggers a series of cellular events that regulate B cell proliferation and differentiation. Receptor cross-linking leads to relocation of the BCR to microdomains known as *lipid rafts*, which leads to the rapid activation of proximal mediators of the BCR signal transduction pathway.[54] This results in the activation of second messengers such as phospholipase C, phosphatidylinositol 3-kinase (PI3K), and Ras pathways. Induction of these pathways ultimately transmits signals to the nucleus,

initiating new gene expression. Depending on the type of signal delivered and the stage of maturation of the B cell, B cells can undergo either differentiation into memory B cells and plasma cells, or apoptosis. Along with surface Ig, several other membrane receptors modulate antigen-induced signal transduction.

## B Cell Receptor Signaling

B cell activation is triggered by the binding of specific antigen to the BCR. The end result of this process will depend on the characteristics of the antigen, the B cell subpopulation activated, and the co-stimulatory signals provided by the antigen itself, T cells, and the microenvironment.

The BCR complex is composed of surface Ig, noncovalently bound to a dimer formed by the molecules Igα and Igβ. The surface Ig on the naïve B cell includes both IgM and IgD. The role of surface Ig is to recognize foreign antigen; the Igα and Igβ molecules transduce the signal through their cytoplasmic tails that contain a particular amino acid sequence known as an *immunoreceptor tyrosine-based activation motif* (ITAM). This sequence contains two tyrosine residues that can be phosphorylated upon activation. After phosphorylation, the ITAM acts as a docking site for the Src homology-2 (SH2) domain to recruit tyrosine kinases and other signaling molecules.

BCRs on resting B cells are highly mobile within the plasma membrane, and they generate a ligand-independent tonic signal that is essential for B cell survival.[55,56] After cross-linking by antigen, BCRs aggregate and translocate to cholesterol- and sphingolipid-enriched membrane microdomains named *lipid rafts*.[54] The signal transduction events that occur after BCR cross-linking are mediated by the subsequent recruitment and activation of intra-cellular kinases including Lyn, Fyn, Btk, and Syk. The most proximal event after BCR cross-linking is the activation of Lyn, which results in the activation of the phosphatase CD45. CD45 removes the inhibitory phosphates on the ITAMs of Igα and Igβ, and the activation of Lyn leads to the activation of Syk and Btk.[57] There is evidence that ligation of CD19, an activating co-receptor of the BCR, leads to recruitment and activation of Vav, PI3K, Fyn, Lyn, and Lck.[58] Subsequently, the tyrosine kinases Syk and Btk are activated by tyrosine phosphorylation. The phosphorylation of Syk triggers the activation of phospholipase C (PLC), PI3K, and Ras pathways. The activation of Syk appears to be absolutely critical for BCR-mediated signal transduction because Syk-deficient cell lines exhibit a loss of BCR-induced signaling. Btk also appears to be required for the activation of second messenger pathways. In some patients with X-linked agammaglobulinemia, a mutation in the *BTK* gene results in impaired BCR signaling at the pre-B cell stage.[57] As a consequence, these patients have a greatly reduced number of mature B cells and generate poor antibody responses. In mice, however, a mutation in *Btk* leads to a disease known as *X-linked immunodeficiency*. B cell development is impaired at the transitional T2 stage, and B cells that do go on to maturity are unable to respond to certain T cell–independent antigens.

After recruitment and activation of the intra-cellular kinases, downstream pathways are initiated. Btk, Syk, and the adapter molecule BLNK are required for the activation of phospholipase C gamma. This leads to breakdown of phosphatidylinositol 4-phosphate to DAG and inositol 1,4,5-triphosphate ($IP_3$) to trigger calcium release from intra-cellular stores and the subsequent translocation of nuclear factor of activated T cells (NFAT) to the nucleus. In addition, Btk activates Ras, which leads to nuclear translocation of the transcription factor activator protein-1 (AP-1). BCR cross-linking also activates mitogen-activated protein (MAP) kinases (Figure 13-4).

Induction of these pathways ultimately transmits signals to the nucleus, where signals are integrated to regulate gene expression. One of the main transcriptional activators related to B cell activation is nuclear factor (NF)-κB, a family of transcriptional factors consisting of homodimers or heterodimers of different subunits. NF-κB regulates cellular processes leading to activation, differentiation into memory B cells and plasma cells, and apoptosis.

## Surface Co-Receptors

Along with surface Ig, many molecules can modulate BCR signal transduction by either enhancing or diminishing the signal activated by antigen. These molecules include, but are not limited to, the B cell co-receptor complex (CD19/CD21/CD81/Leu-13), CD45, SHP1, SHP2, SH2 containing inositol 5-phosphatase (SHIP), CD22, FcγRIIB1, CD5, CD72, PIR-B, and PD-1 (see Figure 13-4). The integration of all these signals sets the threshold for activation of the BCR signal.

The main positive regulator of B cell activation is the B cell co-receptor complex, composed of CD19, CD21, CD81, and an interferon-inducible molecule called *Leu-13*.[59] After antigen cross-linking of surface Ig, specific tyrosine residues contained within the CD19 cytoplasmic domain rapidly become phosphorylated. Although the natural ligand for CD19 is not known, in vitro studies have demonstrated that ligation of CD19 with anti-CD19 antibody lowers the threshold required for BCR-mediated B cell activation and enhances the proliferative effect of anti-IgM treatment on B cells.[60] A role for CD19 in B cell activation has been clearly defined in mice that either lack or overexpress CD19.[61] The CD19 molecule is required for T cell–independent and T cell–dependent B cell responses and for GC formation. The CD21 molecule serves as a receptor for cleavage fragments of the C3 component of complement. Cross-linking of the BCR and CD21 with complement-coated antigen has been proposed to trigger the activation of the cytoplasmic tail of CD19. Mice deficient in CD21 also have impaired responses to T cell–dependent and T cell–independent antigens and a defect in GC formation.[62] The functions of the remaining two components of the B cell co-receptor complex, CD81 and Leu-13, have not been characterized, although it has been suggested that these molecules may mediate homotypic cell adhesion.

In contrast, CD22 that also associates with the BCR is primarily a negative regulator of BCR activation. Although CD22 contains an ITAM motif and is able to recruit Src tyrosine kinases to its cytoplasmic domain,[63] it also contains a specific motif known as the *immunoreceptor tyrosine-based inhibition motif* (ITIM). Similar to the ITAM, the ITIM contains a critical tyrosine residue. After activation of

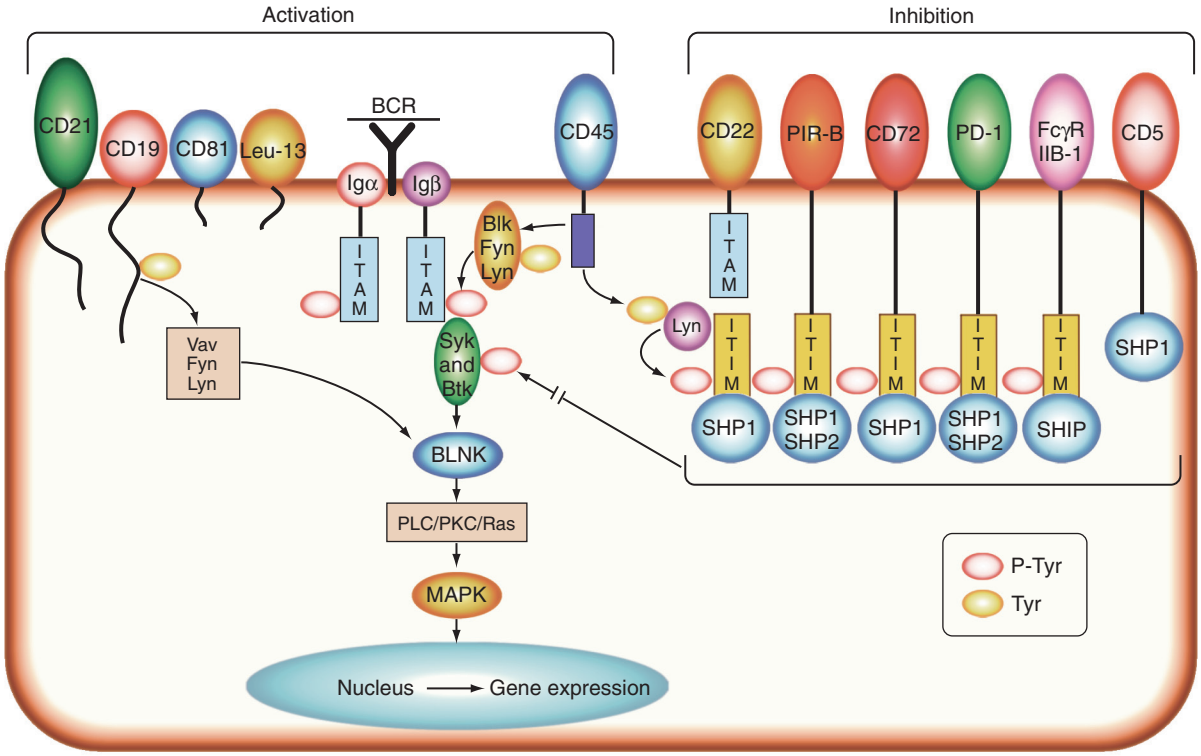



**Figure 13-4** Molecules that regulate the activation state of B cells. Co-ligation of surface immunoglobulin results in tyrosine phosphorylation at specific tyrosine residues present in the immunoreceptor tyrosine activation motif (ITAM) of Igα and Igβ cytoplasmic domains. This phosphorylation occurs after the removal of the inhibitory tyrosine residues of the B cell receptor (BCR)–associated cytoplasmic kinases such as Blk, Fyn, and Lyn, which is mediated by CD45. The phosphorylated ITAMs recruit and activate the Syk and Btk kinases, which in turn activate a series of second messenger pathways (PLC, PKC, and Ras) that result in the upregulation of genes required for B cell activation and survival. Co-ligation of the pre-BCR complex (CD19, CD21, CD81, and Leu-13) results in phosphorylation of tyrosine residues residing in the cytoplasmic domain of CD19. Cytoplasmic kinases including Vav, Fyn, and Lyn become activated and enhance the signaling mediated by the BCR. After the activation of distal mediators of BCR signaling such as PLC, PKC, and Ras, molecules of the mitogen-activated protein kinase (MAPK) pathway become activated and translocate to the nucleus to regulate gene expression. Signals mediated by CD22, PIR-B, CD72, PD-1, FcγRIIB1, and CD5 deliver negative signals that block the activation of distal molecules. After phosphorylation of the immunoreceptor tyrosine inhibition motif (ITIM), present in the cytoplasmic tail of these molecules, the phosphatases SHP1, SHP2, and SH2-containing inositol 5-phosphatase (SHIP) are recruited and activated.

Lyn, it is believed that the ITIM of CD22 is phosphorylated by Lyn, leading to the recruitment and activation of phosphatases such as SHP1 that downregulate the activation cascade.[64]

### FcγRIIB

Simultaneous ligation of both the BCR and FcγRIIB1 sends an inhibitory signal to diminish antigen activation of naïve and memory B cells. This inhibitory signal is activated by the presence of immune complexes and provides a negative feedback mechanism to attenuate an antigen-induced antibody response. After co-ligation of FcγRIIB1 and the BCR, Lyn phosphorylates FcγRIIB1.[65] The SHIP then associates with the FcγRIIB1 and mediates the dephosphorylation of phosphatidylinositol (3,4,5)-triphosphate ($PIP_3$), the major product of PI3K, thus inhibiting the recruitment and activation of Btk and Akt and attenuating BCR signaling.

### CD5

The role of CD5 in B1a cell function is not well understood. After BCR cross-linking, CD5 is thought to mediate signals that induce apoptosis and block proliferation.[66] Cross-linking of CD5 with an anti-CD5 monoclonal antibody results in apoptosis. Some evidence indicates that CD5 recruits the inhibitory protein tyrosine phosphatase SHP1 to its cytoplasmic domain. However, unlike CD22 and FcγRIIB1, CD5 does not contain a consensus ITIM sequence and may recruit SHP1 indirectly.[67] The ligand-binding region of CD5 remains to be elucidated, but recent evidence suggests that CD5 may be a ligand for another negative regulator of BCR signaling, CD72.

### CD72

CD72 is a transmembrane receptor that is expressed as a homodimer. The cytoplasmic tail of CD72 contains ITIMs. Mice with a targeted disruption of the CD72 gene reveal that CD72 plays a negative role in B cell activation, presumably through recruitment of SHP1. The B cell of CD72-deficient mice is similar to that of viable moth-eaten mice with expansion of B1 cells and B cells that are hyperresponsive to BCR cross-linking and are more resistant to BCR-mediated apoptosis.[68] There are several putative ligands for CD72, including CD5 and CD100.

### PIR

Paired Ig-like receptor (PIR)-A and PIR-B are expressed in a pairwise fashion, as the names imply. These receptors are believed to have opposing functions, with PIR-A inducing an activation signal and PIR-B inducing an inhibition signal. The ligands for PIR-A and PIR-B remain to be elucidated. Although little is known about the role of PIR-A in B cell activation, recent data demonstrate that PIR-B plays a role in downregulating B cell responses. The cytoplasmic tail of PIR-B possesses several ITIMs that recruit SHP1. Mice that harbor a targeted disruption of the PIR-B gene exhibit a phenotype similar to mice that are deficient in the other ITIM-bearing inhibitory receptors such as an expansion of B1 cells and B cell hyperresponsiveness.[69]

### PD-1

PD-1 is an inhibitory molecule expressed predominantly on activated B and T cells. The ligand-binding domain binds PD-1L, and the cytoplasmic tail of PD-1 contains ITIMs that recruit SHP2 to attenuate BCR signals. The B cells of PD-1–deficient mice are hyper-responsive to BCR signaling, and these mice display an augmented response to T cell–independent type II antigens. On certain genetic backgrounds, PD-1 deficiency leads to an autoimmune phenotype.[70]

## Phosphatases

In general, intra-cellular signaling is regulated by a balance of phosphorylation and dephosphorylation. Protein tyrosine phosphatases (PTPs) play a major role in restraining the magnitude and duration of BCR signaling; among these, SHP1 is a potent negative regulator. SHP1 is found in association with transmembrane proteins such as CD22, FcγRIIB1, CD5, CD72, and PIR-B. SHP1 antagonizes BCR signaling by countering the action of protein tyrosine kinases.[67] The function of SHP1 has been extensively studied in mice that bear a naturally occurring mutation in the SHP1 gene. Mice with this genetic defect are known as moth-eaten mice because of the appearance of their fur. These mice have a decreased number of conventional B cells and an expansion of B1 cells, and they develop a fatal autoimmune and inflammatory disease.[71] The severe defect in B cell regulation underscores the importance of SHP1 in limiting the extent of BCR signaling. In contrast, the intra-cellular tyrosine phosphatase SHP2, although structurally similar to SHP1, seems to be a positive regulator of BCR signaling because it augments ERK responses.[72] The important role played by PTP in disease is also demonstrated by the discovery that a single mutation in the PTPN22, a protein that regulates Src family kinases, augments the risk of multiple autoimmune diseases.[73]

Another important regulator, SHIP, is an inositol phosphatase that inhibits B cell activation by removing the 5 phosphate from $PIP_3$, a critical component that recruits membrane association of pleckstrin homology (PH) domain containing signaling molecules, including PLCγ2, Btk, and Akt. Similar to SHP1 and SHP2, SHIP is recruited to an ITIM on FcγRIIB after BCR cross-linking. Mice deficient in SHIP display splenomegaly and elevated levels of serum antibody.[74]

## Signal Transduction in Immature versus Mature B Cells

An important event in BCR signaling is the recruitment of signaling components to lipid rafts, which are lipid-rich microdomains of the membrane that serve to bring together requisite signal molecules to facilitate integrated functions in three dimension. After antigen engagement, the BCR translocates into lipid rafts and the BCR signaling cascade ensues because of the clustering of signaling components. In addition to activating signals, however, inhibitory components also localize to lipid rafts. As discussed later, BCR ligation mediates negative selection during the immature and transitional B cell stages and B cell activation during the mature B cell stage. The reason for these two distinct outcomes is not clear because the same signal components are present; however, differences in membrane cholesterol limit recruitment of the BCR to lipid rafts in immature B cells.[75] Evidence also indicates that the signal strength and duration and stage-specific expression patterns of signaling elements differ between immature and mature B cells.[76]

## B Cell Activation

B cells develop without bias for a particular antigenic specificity, ensuring that a diverse repertoire of different Ig molecules is produced. After being stimulated by antigen and accessory cells such as DCs and T cells, antigen-activated B cells undergo clonal expansion; B cells that do not interact with antigen are destined to undergo programmed cell death in a matter of days or weeks. The B1 and B2 cell subsets are regulated by different activation mechanisms and are involved in different immune responses (Table 13-3).

### B1 Cell Activation

B1 cells present in the pleural and peritoneal cavities respond to T cell–independent antigens. There are two classes of T cell–independent antigens: type I, which includes lipopolysaccharide, and type II, which includes large multivalent antigens with repetitive epitopes, often found on the surface of bacteria. T cell–independent antigens can directly activate B cells, resulting in the secretion of antibody. Soluble factors such as IL-5 and IL-10 also appear to be involved in the maintenance and activation of B1 cells.

B1 cells do not require interaction with antigen-specific T cells. However, activated T cells and macrophages may augment B1 cell activation, enhance Ig production, and influence isotype class switching such that B1 cells may also produce IgA and IgG.

### Marginal Zone B Cell Activation

MZ B cells are situated in the marginal sinuses, where Ds and specialized macrophages trap and remove antigen from the circulation. Like B1 cells, many MZ B cells express polyreactive BCRs that are specific for microbial antigens,

**TABLE 13-3** Markers of Human Mature B Cell Subsets

| Characteristic | Naïve | Unswitched Memory | Switched Memory | B1 |
|---|---|---|---|---|
| Surface IgM | High | High | Low | High |
| Surface IgD | Low | Low | High | Low |
| CD5 | + | – | – | –/+ |
| CD21 | – | – | + | ++ |
| CD23 | – | – | + | – |
| CD11b/CD18 | + | + | – | – |
| Bone marrow progenitors | – | + | + | + |
| Self-renewal capacity | + | + | – | – |
| Response to T cell–independent antigens | + | + | +/– | + |
| Response to T cell–dependent antigens | +/– | +/– | + | +/– |
| Predominant isotype | IgM | IgM | IgG | IgM |
| Anatomic locations | Peritoneum, pleura, spleen | Peritoneum, pleura, spleen | Spleen, lymph nodes, Peyer's patches, tonsils, peripheral blood | Spleen, tonsils |

CD, Cluster of differentiation; Ig, immunoglobulin.

including both protein and carbohydrate molecules. If micro-organisms reach the lymphoid organ from the blood, MZ B cells recognizing microbial carbohydrates elicit an innate-like T cell–independent response. MZ B cells can also mount a T cell–dependent response against microbial protein antigens. Soluble factors such as BAFF and T cell–derived cytokines are important for MZ B cell activation. MZ B cell activation can also be enhanced by CD40 ligand expressed on T cells, even though an antigen-specific (cognate) interaction with T cells is not necessary. Although the mechanism is not fully understood, MZ B cells are constitutively partially activated and differentiate rapidly into short-lived antibody-secreting plasma cells after activation by antigen. MZ B cells secrete predominantly IgM and, to a lesser extent, IgG antibodies without an affinity maturation process. Thus these antibodies display low to intermediate affinity.

## Follicular B Cell Activation

Engagement of the BCR by antigen signals the B cells to engulf the antigen, process it intra-cellularly, and express peptide fragments bound to class II MHC molecules on its surface. After the expression of peptide fragments bound to class II MHC molecules, antigen-activated B cells present peptide to primed T helper (Th) cells. B cells and T cells interact through T cell receptor recognition of a peptide-MHC complex on the B cell and through engagement of co-stimulatory molecules B7 and CD40 on the B cell by CD28 and CD40L, respectively, on T cells (Figure 13-5). Expression of the B7 molecule is upregulated on B cells after antigen stimulation. B cell activation during a T cell–dependent immune response also depends on engagement of the CD40 receptor expressed on B cells with the CD40 ligand (CD40L) expressed on T cells.[77] The importance of signal transduction events mediated by CD40-CD40L engagement is evident from studies of patients with X-linked hyper-IgM syndrome, an immunodeficiency disease resulting from a defect in CD40L. These individuals do not mount strong immune responses to T cell–dependent

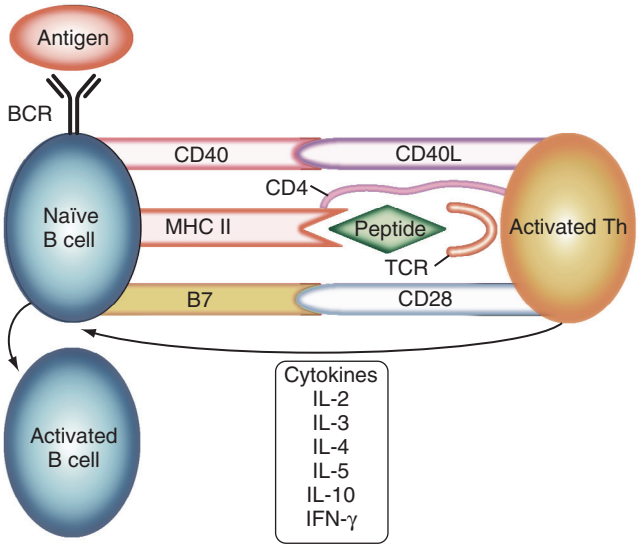



**Figure 13-5** B cells as antigen-presenting cells. Antigen bound to surface immunoglobulin on naïve B cells triggers endocytosis and intracellular processing of the antigen. B cells engage antigen-specific T helper (Th) cells through the recognition of foreign peptide by the T cell receptor (TCR) and through the binding of a conserved region of the class II major histocompatibility complex (MHC) molecule by CD4. Along with antigen binding, co-ligation of CD40 and B7 (expressed on B cells) with CD40L and CD28 (expressed on T cells) provides critical co-stimulatory signals and the secretion of cytokines required for B cell activation. BRC, B cell receptor; IFN, interferon; IL, interleukin.

antigens; they have high concentrations of circulating IgM but only trace amounts of IgG and no affinity maturation of the antibody response. Th cells secrete cytokines such as IL-2, IL-3, IL-4, IL-5, IL-10, IL-17, IL-21 and IFN-γ and provide co-stimulatory signals that are important for B cell maturation and differentiation.[78]

Upon encountering antigen, a naïve B cell with high affinity for the antigen will activate and differentiate to a plasma cell that will secrete IgM or IgG antibodies without somatic mutations. If the activated B cell, however, harbors a receptor with low to intermediate affinity for antigen, the

cell will migrate first to the T cell area of the follicle and will begin the process of creating a GC.[79]

## Germinal Centers

As B cells progress to form GCs, they interact with cognate T cells that are a specialized subpopulation of helper cells known as *T follicular helper* (Tfh) cells. These Tfh cells express CXCR5 and S1P receptor 2 and therefore can migrate to and be retained in the B cell follicle, where, along with the activated B cells, they form discrete structures known as *germinal centers* (GCs). It is in the GC that antigen-activated B cells undergo the process of class switch recombination (CSR) and affinity maturation through somatic hypermutation (SHM), and finally differentiate into memory B cells or long-lived plasma cells.

GCs can be subdivided into separate regions where the different stages of B cell differentiation take place (Figure 13-6). The dark zone is the initial site of rapid proliferation, and B cells within the dark zone are called *centroblasts*. They are derived from a relatively small number of antigen-activated B cells (Table 13-4). Expression of the anti-apoptotic Bcl-2 protein is low in these cells, whereas expression of the proapoptotic Fas protein is upregulated. Low levels of Bcl-2 expression render developing B cells sensitive to apoptosis, but these cells can be rescued by antigen and CD40-CD40L interactions provided by antigen-specific Th cells. When these cells migrate to the light zone, they are termed *centrocytes* and encounter a dense network of FDCs and Tfh (Figure 13-7). B cells that express low affinity BCRs are outcompeted by B cells bearing higher affinity BCRs, whose enhanced antigen acquisition enables them to better interact with cognate Tfh, and thus they are selected to undergo more cell division.[80]

The process of SHM is activated during the centroblast stage. During this process, a nucleotide base-pair change is introduced in the DNA sequence of the variable region of Ig H- and L-chain genes, at approximately $10^{-3}$ per base pair per cell division. The mechanism for SHM is complex and

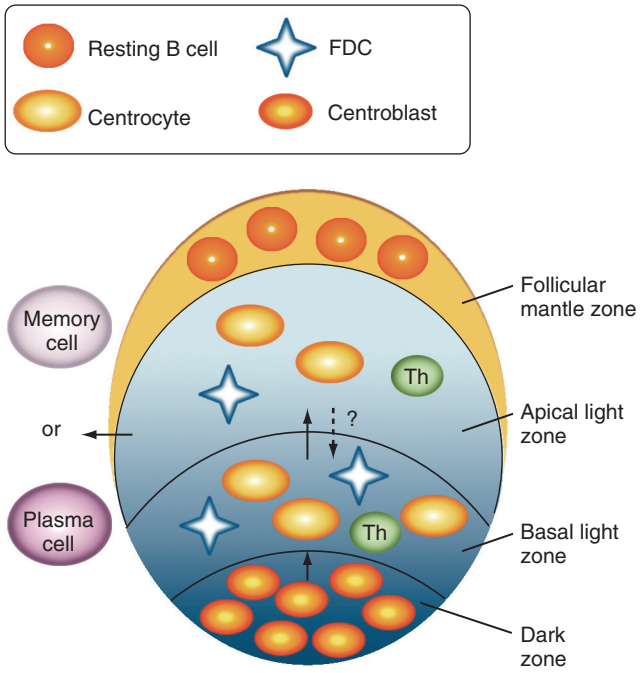



**Figure 13-6** B cell maturation in germinal centers. After exposure to antigen, B cells in the primary follicles form germinal centers or migrate to previously formed germinal centers. Centroblasts located in the dark zone undergo proliferation and acquire somatic mutations. A small number of proliferating centroblasts can give rise to a larger number of centrocytes present in the basal light zone. As these cells pass through a dense network of follicular dendritic cells (FDCs) and T helper (Th) cells, centrocytes bearing surface immunoglobulin receptors with high affinity for antigen undergo positive selection. Centrocytes in the apical light zone are nondividing cells that undergo differentiation into memory B cells or plasma cells. It has been suggested that centrocytes may return to the dark zone, where additional somatic mutations may be acquired. Resting B cells that are not activated by antigen are pushed aside to form the follicular mantle zone.

**TABLE 13-4** Markers of Antigen-Activated B Cells in Secondary Lymphoid Tissue

| Marker | Naïve | Centroblast | Centrocyte | Memory | Plasma |
|---|---|---|---|---|---|
| Surface IgD | + | – | – | – | – |
| Surface IgM, IgG, IgA, or IgE | + | – | + | + | – |
| CD10 | – | + | + | – | – |
| CD20 | + | + | + | + | – |
| CD38 | – | + | + | – | + |
| CD77 | – | + | – | – | – |
| Presence of somatic mutation | – | + | + | + | + |
| Isotype class switch | – | – | + | + | + |
| Bcl-2 | + | – | +/–* | + | + |
| Fas | + | + | + | + | – |
| AICDA | – | + | – | – | – |
| Blimp-1 | – | – | – | ? | + |

AICDA, Activation-induced cytidine deaminase; Bcl-2, B cell lymphoma 2; Blimp-1, B lymphocyte–induced maturation protein 1; CD, cluster of differentiation; Ig, immunoglobulin.

*Bcl-2 is expressed in centrocytes only after interaction with follicular dendritic cells.

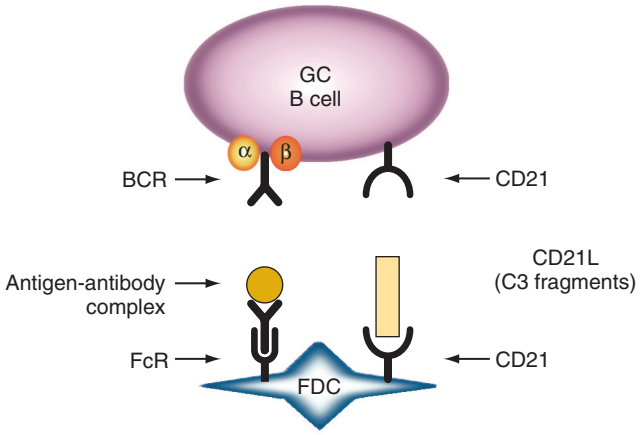



**Figure 13-7** Engagement of B cells with follicular dendritic cells (FDCs). Interaction between FDCs and B cells results in signals that mediate the positive selection of B cells in germinal centers (GCs). Antigen-antibody complexes trapped on the FDC surface deliver a signal to the B cell receptor (BCR). A second signal is delivered by the binding of CD21 on B cells to C3 complement components on the surface of FDCs. FcR, Fc receptor.

requires the enzyme activation-induced cytidine deaminase (AICDA) to target to specific hotspot sequences.[81] The process of SHM is responsible for the affinity maturation of the antibody response. B cell clones that express surface Ig with an increased affinity for antigen are selectively expanded during the affinity maturation process, whereas B cells that express somatically mutated Igs with low affinity for antigen or novel binding to self-antigens are targeted for apoptosis or inactivation. The importance of somatic mutation and affinity maturation during an immune response is underscored by the fact that patients with mutations in the *AICDA* gene are immunocompromised.

AICDA is critical for not only somatic hypermutation but also for CSR. Although expression of IgM and IgD in B cells occurs through alternative splicing of a long transcript that contains coding regions for both the μ and δ chain, expression of any other Ig isotype requires the excision of all the H-chain genes between the recombined V(D)J region and the isotype to be expressed. Isotype switching requires preactivation of the particular H-chain locus to be involved in the recombination event, which is controlled by the cytokine milieu in which the cell is activated. AICDA induces the deamination of cytidine, creating dU:dG pairs (instead of dC:dG pairs) that activate the cellular DNA repair machinery and ultimately create circumstances that favor both CSR and SHM. For CSR, CD40-CD40L interactions and cytokines are also required.

As centroblasts become centrocytes, they require survival signals from FDCs to overcome their low level of Bcl-2 expression and high level of Fas expression. FDCs are stromal-derived cells that trap antigen-antibody complexes by FcγR and complement receptors in bodies known as *iccosomes* (immune complex-coated bodies) on the cell surface. Iccosomes deliver an antigen-specific signal to B cells through the BCR (Figure 13-8). Centrocytes with specificity for antigen on FDCs are saved from apoptosis by upregulation of the Bcl-2 molecule. Engagement of the complement receptors CR1 and CR2 (CD21 and CD35, respectively) on B cells by components of the C3 complement protein (iC3b, C3dg, and C3d) bound to FDCs may mediate a secondary co-stimulatory signal.[82] If centrocytes do not receive these positive selection signals, they rapidly die through a Fas-dependent pathway. Should they receive survival signals, they continue to differentiate into memory B cells or plasma cells.

## Ectopic Lymphoid Structures

Despite being the prototypical structure for the induction of humoral immune responses, GCs are not found exclusively in lymphoid tissue. Ectopic lymphoid structures with GC-like characteristics are present in sites of chronic inflammation such as the synovium in persons with rheumatoid arthritis, pancreatic islands in persons with type 1 diabetes, and salivary glands of persons with Sjögren's syndrome.[83]

These GC-like structures seem to develop as a consequence of chronic inflammation, which leads to the release of soluble mediators such as the chemokines CCL21 and CXCL12 that recruit lymphocytes.[84] These cells, once activated, secrete cytokines such as lymphotoxin that act in a paracrine manner and contribute to the organization of a GC-like structure that includes a dark and light zone with local induction of AICDA. In contrast to the germinal centers of the secondary lymphoid organs, these structures are not encapsulated. The B cells in these structures, therefore, are continuously exposed both to the local antigens that might be absent from the lymphoid organs[85] and to the inflammatory microenvironment that may facilitate bypass of the regulatory points in B cell differentiation, hence contributing to a potential autoimmune bias in these sites. Although no evidence exists that these structures are the underlying cause of any disease, in some diseases they may contribute to tissue pathology and augment the pool of autoreactive plasma and memory cells. Not all ectopic lymphoid foci share characteristics with the GC. It is not known which structures are more damaging to tissue.

## B Cell Differentiation

After antigen activation, B1 and MZ B cells rapidly differentiate into antibody-secreting plasma cells independent of T cell help. This innate-like humoral response generates mostly short-lived plasma cells that produce antibodies with low affinity, but it provides an early and fast protection against blood-borne microbes. It is antigen-activated follicular B cells that generate adaptive antibody responses through a GC reaction that give rise to long-lived memory B cells and plasma cells.

### Memory B Cells

Postgerminal center memory B cells express Ig genes that have undergone CSR and SHM, and in humans they are distinguished by the presence of the marker CD27. The CD40-CD40L interaction contributes to directing GC B cells to mature into long-lived memory B cells. The exact life span of memory B cells is unknown. It has been postulated that these B cells either persist throughout the lifetime of the host[81] or are renewed constantly through either nonspecific stimulation[86] or antigen-specific stimulation.[87]

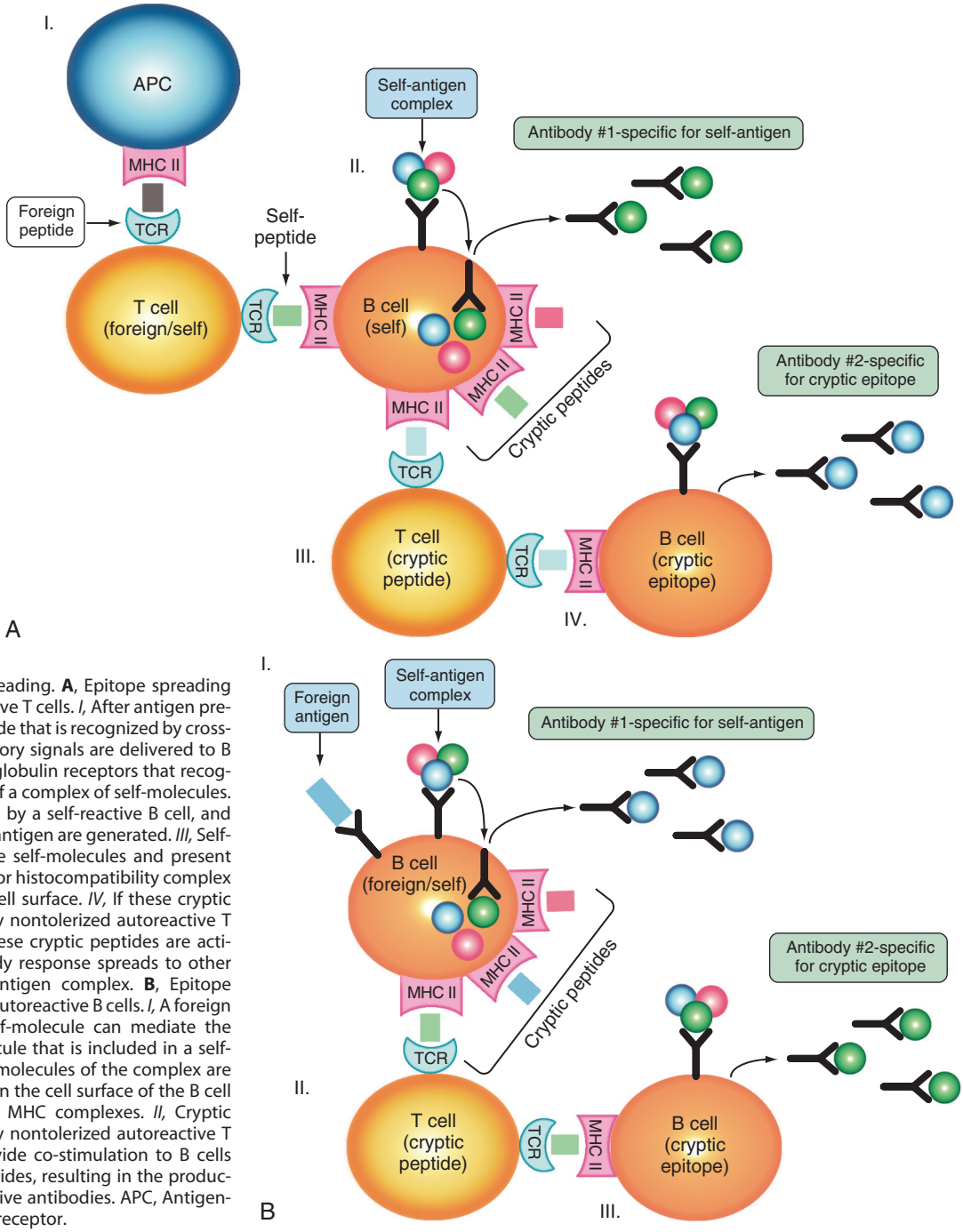



**Figure 13-8** Epitope spreading. **A**, Epitope spreading by activation of cross-reactive T cells. *I,* After antigen presentation of a foreign peptide that is recognized by cross-reactive T cells, co-stimulatory signals are delivered to B cells with surface immunoglobulin receptors that recognize a self-antigen as part of a complex of self-molecules. *II,* The complex is engulfed by a self-reactive B cell, and antibodies specific for self-antigen are generated. *III,* Self-reactive B cells process the self-molecules and present cryptic peptide–class II major histocompatibility complex (MHC) complexes on the cell surface. *IV,* If these cryptic peptides are recognized by nontolerized autoreactive T cells, B cells specific for these cryptic peptides are activated and the autoantibody response spreads to other components of the self-antigen complex. **B**, Epitope spreading by activation of autoreactive B cells. *I,* A foreign antigen that mimics a self-molecule can mediate the endocytosis of a self-molecule that is included in a self-antigen complex. The self-molecules of the complex are processed and expressed on the cell surface of the B cell as cryptic peptide–class II MHC complexes. *II,* Cryptic peptides are recognized by nontolerized autoreactive T cells. *III,* These T cells provide co-stimulation to B cells that recognize cryptic peptides, resulting in the production of additional self-reactive antibodies. APC, Antigen-presenting cell; TCR, T cell receptor.

Memory B cells circulate throughout the body in a quiescent state until specific antigen is re-encountered and triggers a potent secondary immune response. Memory B cells respond to antigen much faster, require lower amounts of antigen, and can even be induced in its absence by soluble mediators such as IL-2 or IL-15, in part because the BCR is already localized to lipid rafts. Subsequently, just like naïve B cells, memory B cells ingest antigen and express peptide–class II MHC fragments. After antigen presentation of peptide to Th cells, memory B cells undergo expansion and may differentiate to plasma cells.

### Plasma Cells

The B cell differentiation cascade ends with the generation of a plasma cell. At the molecular level, the differentiation program is directed by the transcriptional repressor known as *B lymphocyte–induced maturation protein 1* (Blimp-1).[88] Blimp-1 induces a program in which plasma cells lose expression of several markers because they no longer express surface Ig, MHC molecules, or CD20. They initiate increased protein synthesis and secretion and consequently exhibit a large cytoplasm with a well-developed endoplasmic reticulum devoted to antibody production.

Blimp-1 also silences the expression of CXCR5, and thus B cells differentiating into plasma cells exit the lymphoid follicles and migrate to extrafollicular regions of secondary lymphoid tissue or to the bone marrow, where the final stage of plasma cell maturation occurs. B cells in the GC are induced to become plasma cells by IL-5, IL-6, and IL-21. Plasma cells have a variable life span that may be days for short-lived plasma cells that originate extrafollicular responses to years for long-lived plasma cells that arise from the GC response and home to the bone marrow.[89]

The longevity of plasma cells in the bone marrow depends on the presence of a niche that provides the survival factors CXCL12, IL-6, APRIL, and TNF. Cross-linking the FcγRIIb expressed by plasma cells induces apoptosis, a phenomenon that has been credited with pruning the plasma cell repertoire.[6]

### Trafficking of Postimmune Cells

Plasma cells downregulate CXCR5 expression and elevate expression of EBI2, a chemotactic receptor that guides cells to outer follicle areas and extrafollicular regions. Both memory and plasma cells express CXCR4 that directs homing to the bone marrow but also allows localization in lymphoid tissues. Therefore CXCR4-expressing plasma cells can be found surrounding the GC or B cell follicles.[90] A subset of memory cells expresses CXCR3 and therefore migrates toward inflammatory chemokines such as CXCL9 and CXCL10.[91]

### Nonconventional B Cell Activation

Although it is a central paradigm that B cells require cognate antigen for activation, it has also been known for years that infections induce an antibody response in which only a fraction of the responding B cells are specific for microbial antigen. This phenomenon, known as *polyclonal activation*, is induced by several mediators such as superantigen, cytokines, or noncognate T cell co-stimulation and leads to the production of self-reactive antibodies.

B cell superantigens include the *Staphylococcus aureus* protein A (SpA), the protein gp120 of HIV-1, and the erythrocyte membrane protein 1 of *Plasmodium falciparum*. These proteins share the ability to bypass the need to be recognized in the CDRs and bind instead to framework regions common to Ig gene families. They can therefore activate multiple clones; for example, SpA recognizes Igs of the $V_H3$ family that constitutes up to 50% of the IgM repertoire.[92] Although the activation of multiple B cell clones by an organism might be seen as an advantage for the host, polyclonal activation leads to an extrafollicular response that, after a transient hyperglobulinemia, exhausts the B cell pool, leaving a vulnerable organism.

Noncognate activation of B cells clearly occurs in memory B cells that are easily activated by the presence of soluble mediators such as IL-2, IL-15, or CpG (see the Memory B Cells section).[86] In chronic infections, polyclonal activation seems to be a consequence of two nonmutually exclusive mechanisms. The simpler one is bystander activation of noncognate B cells by inflammatory cytokines. The second is $CD4^+$ T cell–mediated co-stimulation. Noncognate B cell activation carries the intrinsic risk of activating self-reactive B cells, as is observed in both experimental and natural infections.[93]

### Mucosal T–Independent Class Switch Recombination

In the mucosa, IgA specific to commensal bacteria is induced through machinery that is T cell independent and occurs outside of organized lymphoid tissue.[94] Because this phenomenon is independent of T cells, other interactions must provide the required signals for CSR. One of the candidate molecules is BAFF (BLyS), which is expressed by DCs in mucosal tissue. BAFF has been shown to induce CSR in B cells in the presence of IL-10 or TGF-β, both of which are present in the mucosal microenvironment.[95]

## REPERTOIRE SELECTION

The ability to discriminate between foreign and self-antigens is as important as the capacity to mount a protective immune response. Any molecule derived from the intracellular or extra-cellular components of the host can be considered self-antigen; the censoring of the responses against these antigens occurs throughout B cell maturation and includes multiple mechanisms in the B cells themselves and in cells that cooperate in their activation and differentiation.

### Tolerance

The random process of V(D)J recombination generates a diverse repertoire of BCRs that recognize virtually all antigens and thus, inevitably, also self-antigens. SHM during GC reactions also give rise to B cells with autoreactive BCRs. These autoreactive B cells have to be purged by a process known as *tolerance*.

Tolerance is achieved through signals provided by the BCR, co-receptors, inflammatory mediators, and metabolic by-products that are integrated by the cell according to its developmental stage. BCR cross-linking during development or in the absence of co-stimulatory signals activates tolerance mechanisms in immature and transitional B cells. The same mechanisms that induce tolerance to self-antigen can induce tolerance to pathogens, and because some autoantigens are sequestered in sites of immune privilege, cells specific to them are not subject to tolerance mechanisms.

The tolerance mechanism activated depends on the stage of the B cell and the strength of the BCR signal delivered by antigen. The strength of the signal depends on the degree of cross-linking of the BCR, which in turns depends on the concentration of self-antigen and the affinity of the antibody for self-antigen. With little receptor cross-linking, because of low antigen concentration or low affinity for antigen, there is no BCR signaling, and the autoreactive B cell is not tolerized. Three mechanisms mediate tolerance: receptor editing, anergy, and deletion. When mechanisms that regulate autoreactive B cells fail, the breakdown of self-tolerance can lead to the development of autoimmune disease.

During B cell development, autoreactive B cells are generated in the bone marrow after V(D)J rearrangement and expression of surface Ig on immature B cells. Because of the vast number of different antibody molecules that can be

formed through the random recombination of H- and L-chain variable region genes and the random association of H and L chains, all individuals generate autoreactive B cells. The tolerance process that prevents the maturation of autoreactive B cells occurs with such efficiency that despite the fact that more than 75% of immature B cells bear receptors with some degree of autoreactivity, about 30% of naïve B cells do so.[96]

### Receptor Editing

A high degree of cross-linking of the BCR during development results in a process known as *receptor editing*. This process involves a secondary gene rearrangement of the H-chain or L-chain genes. Receptor editing requires reactivation of the recombination machinery and re-expression of RAG-1/RAG-2. When successful, receptor editing produces a BCR receptor with low or no affinity to the antigens present in its environment, and the cell is allowed to continue its development. RAG-1/RAG-2 can be seen in some cells in the GC or extrafollicular region as well, and some authors have interpreted this as evidence that receptor editing also occurs later in B cell development.

### Deletion

Extensive BCR cross-linking also leads to apoptosis, thus deleting the autoreactive B cell. This was the first mechanism of tolerance described for B cells[97] and was long believed to be the main mechanism of central tolerance. However, deletion occurs only when cells are not able to decrease their autoreactivity by editing the BCR. In the periphery, deletion occurs if a B cell is extensively activated in the absence of Th cell co-stimulation. Tolerance through deletion is mediated primarily through the activation of a series of endogenous proteases. In B cells the Fas pathway and the Bcl-2 pathway play important roles in regulating apoptosis.

Fas (also known as *CD95* or *Apo-1*), a member of the TNF receptor gene family, and Fas ligand are transmembrane proteins expressed on a variety of cell types. Because Fas ligand is a homotrimeric molecule, it can bind three Fas molecules. Clustering of Fas on the cell surface, which occurs when Fas molecules bind Fas ligand, activates apoptosis.[98] It appears that when B cells engage CD40L expressed on Th cells in the absence of BCR ligation, Fas signaling induces apoptosis.[99,100] Mutations in Fas *(lpr)* or Fas ligand *(gld)* in mice result in a SLE-like syndrome characterized by the production of pathogenic autoantibodies and lymphadenopathy. In humans, similar mutations lead to lymphadenopathy and anti-erythrocyte antibodies, but anti-DNA antibodies and glomerulonephritis are not found in these individuals.[101]

The Bcl-2 gene family is composed of molecules that either protect against or induce apoptosis in many cell types. Relative levels of these molecules dictate life and death of a cell. For example, excess Bcl-2 or Bcl-xL promotes cell survival, whereas excess Bax or Bim induces cell death.[102] Bcl-2 and Bcl-xL are upregulated at critical points during B cell development but can be easily counterbalanced on BCR cross-linking. The fact that certain mouse strains that overexpress Bcl-2 in B cells produce autoantibodies highlights the importance of apoptosis in tolerance.[103]

### Anergy

Anergy is a hyporesponsive state considered to be induced in immature B cells when they undergo a modest degree of BCR cross-linking. Anergic B cells downregulate surface Ig receptors and display a desensitization of the BCR, blocking activation of downstream signaling. Anergic B cells are short-lived. Goodnow and colleagues[104] performed classic studies on B cell tolerance induction in mice engineered to express an anti–hen egg lysozyme (HEL) antibody, along with soluble HEL, to act as a self-antigen. In the anti-HEL transgenic mouse model, B cells that encounter soluble, monovalent HEL are anergized. These B cells populate secondary lymphoid tissue but do not secrete anti-HEL antibody and are not recruited into B cell follicles. This phenomenon is known as *follicular exclusion*.[105]

Although anergy implies that the cells are not activated through BCR engagement, they *can* be activated by non–antigen-specific T cell co-stimulation, lipopolysaccharide, or IL-4. Exposure of anergic B cells in vivo to multivalent antigen in the presence of activated Th cells may also lead to their activation.[106] Consequently, it has been suggested that anergic B cells may serve as a potential source of autoantibody and may be activated in inflammatory conditions. Recently, it has been shown that B cells can be blocked from activation if they are chronically exposed to antigen and IL-6. If IL-6 is removed from the microenvironment, those chronically activated B cells will secrete antibody.

## B Cells as Immune Regulators

B cells produce cytokines in response to their environment. Several subsets of B cells have been reported to be able to suppress autoimmunity, including $CD1d^{hi}CD5^{+}$ B cells and transitional B cells. All these B regulatory cells (Bregs) produce IL-10 to suppress immune responses.[107] The function of Bregs depends on stimulation through BCR and CD40. In healthy persons, Bregs secrete IL-10 in response to CD40 engagement, whereas the equivalent population in patients with SLE fails to do so.[108] Bregs are also able to mediate immunosuppression through an IL-10–independent mechanism. Recent studies have identified IL-35 as an additional effector molecule for Breg function.[109,110] Some IL-35–producing Bregs express CD138 and Blimp-1. Thus, activated B cells and plasma cells play an important role in regulating immune responses. These Bregs not only harness autoimmunity but also restrain immune responses against microbial infection.

## Regulation by Small Molecules

Beyond the classic activators and regulators, the molecules described in the following sections play a particularly important role in the biology of B cells and are highlighted given their potential as biomarkers and therapeutic agents.

### Vitamin D

Vitamin D is acquired from the diet or synthesized in the skin, followed by a conversion into a biologic product in the liver and kidney. The active metabolite,

1,25-dihydroxyvitamin $D_3$, has been shown to decrease activation and proliferation of B cells, as well as differentiation to plasma cells. Circulating levels of vitamin D tend to be decreased in patients with autoimmune disease; whether this phenomenon contributes to the disease process is not known.

### Estrogens

The role of estrogens in B cell–mediated autoimmune diseases has long been suggested by the female gender predominance within autoimmune diseases and may reflect a variety of effector mechanisms. However, estrogens have been shown to modify the B cell repertoire, allowing survival of autoreactive B cells, and to alter the peripheral compartments in mice.[111]

### Leptin

Although its first described role was as an endocrine hormone with a primary role in the control of metabolism, leptin was later shown to exhibit immune regulatory effects. For example, a murine model of experimentally induced arthritis is attenuated in leptin receptor–deficient mice.[112] More recently, it has been shown that leptin promotes B cell survival and proliferation through induction of Bcl-2 and cyclin D1.[113]

## B CELL–MEDIATED AUTOIMMUNITY

B cell–mediated autoimmunity is the consequence of the production of self-reactive antibodies. We have detailed multiple mechanisms operating throughout B cell maturation and differentiation that are designed to avoid autoreactivity. The failure of only one tolerance checkpoint rarely leads to autoimmune disease[114]; it may, however, increase the level of circulating autoantibodies, without clinical disease.

The generation of a B cell–mediated autoimmune disease must involve (1) the generation of B cells bearing autoreactive BCRs; (2) failure of mechanisms that in the normal event will abrogate their maturation to short- or long-lived plasma cells; and (3) tissue malfunction induced by the autoantibody that leads to clinical symptoms.

### Origin of Autoreactive B Cells

Theoretically, autoreactive cells can arise from any B cell subpopulation. In mice, B1 cells that bear BCRs with low affinities but high polyreactivity produce autoantibodies, but these autoantibodies help remove cellular debris and can protect the body from pathogenic autoreactivity. In addition, evidence indicates that MZ B cells secrete autoantibodies, which may also provide a physiologic rather than pathologic function.

### Autoreactivity in the Preimmune B Cell Repertoire

Studies of the reactivity of human B cells have shown that in the healthy peripheral B cell compartment, about 30% of the naïve B cells bear some degree of autoreactivity; however, few of those can be considered potentially pathogenic given their low affinity for autoantigen.[96] In persons with SLE, the frequency of autoreactive cells is as high as 50% in the naïve B cell population[115]; the frequency is greatest when disease is active and diminishes during periods of disease quiescence,[116] demonstrating that an inflammatory milieu may alter B cell selection.

### Autoreactivity in the Postimmune B Cell Repertoire

Most of the autoantibodies in patients with autoimmune disease are generated through GC reaction because they have undergone CSR and display extensive somatic mutation. This observation suggests that the GC does not possess a fail-proof mechanism to effectively purge mutated autoreactive cells, although post-GC receptor editing and deletion of autoreactive B cells has been shown to occur. The understanding of the tolerance mechanisms that prevent the generation of autoreactive memory B cells and plasma cells is still incomplete.

### Molecular Triggers of Autoimmunity

Several prevailing theories attempt to explain the activation and expansion of autoreactive B cells that should normally be silenced. Autoimmunity is thought to arise by a combination of environmental factors such as infectious agents that initiate an autoimmune response and genetic defects that alter B cell regulation. Proposed models for autoimmunity include (1) cross-reactivity of foreign antigen with self-antigen, (2) inappropriate co-stimulation, and (3) altered thresholds for BCR signaling.

Much of our understanding of the breakdown of self-tolerance and the progression of autoimmunity comes from the examination of mouse models. Autoimmune mouse models can be divided into two broad categories: induced autoimmunity and spontaneously occurring autoimmunity. Even though the progression of autoimmunity in humans is thought to be a highly complex process that involves multiple genetic and environmental factors, these animal models have provided much information about the molecular events that lead to a loss of self-tolerance.

#### Molecular Mimicry

One proposed model for the initiation of autoreactivity is that cross-reactive anti-self, anti-foreign B cells escape central tolerance because self-antigen is present at too low a concentration to trigger tolerance induction or because the affinity of the antibody for autoantigen is below the signaling threshold. These B cells become activated in the periphery by foreign pathogens resembling self-antigen and produce antibodies that bind both foreign and self-antigen. This cross-reactivity is known as *molecular mimicry*, and it is a popular model to explain the induction of many autoimmune disorders.[117] Once the pathogen is cleared, the autoantibody response is usually diminished because antigen-specific T cell help is no longer present, unless a high number of long-lived plasma cells have been generated. In the case of autoimmune-prone individuals, it is proposed that intrinsic B cell defects prevent the

**TABLE 13-5** Evidence for Antibody Cross-Reactivity between Foreign and Self-Antigens

| Foreign Antigen | Self-Antigen |
|---|---|
| Yersinia, Klebsiella, Streptococcus[a] | DNA |
| Epstein-Barr virus nuclear antigen 1[a] | Ribonucleoprotein SmD |
| Streptococcus M protein[b] | Cardiac myosin |
| Coxsackie B3 capsid protein[c] | Cardiac myosin |
| Klebsiella nitrogenase[d] | Human leukocyte antigen B27 |
| Yersinia lipoprotein[e] | Thyrotropin receptor |
| Mycobacteria heat shock protein[f] | Mitochondrial components |
| Escherichia, Klebsiella, Proteus[g] | Acetylcholine receptor |
| gpD derived from herpes simplex virus[g] | Acetylcholine receptor |

Autoimmune disorders exhibiting cross-reactive antibodies: *a*, systemic lupus erythematosus; *b*, rheumatic fever; *c*, myocarditis; *d*, ankylosing spondylitis; *e*, Graves' disease; *f*, primary biliary cirrhosis; *g*, myasthenia gravis.

downregulation of autoantibody production, even after foreign antigen clearance. Several data support molecular mimicry as a trigger for B cell–mediated autoimmunity in some instances; for example, antibodies to infectious agents have been identified that cross-react with self-molecules associated with specific autoimmune diseases[118] (Table 13-5). Intriguing examples include the cross-reactivity observed between the M protein of group A *Streptococcus* and cardiac myosin in rheumatic heart disease and the cross-reactivity between *Campylobacter* and aquaporin.

Because both nonautoimmune and autoimmune-prone persons have the capacity to generate autoantibodies, it is unlikely that cross-reactivity between foreign and self-antigens is solely responsible for breakdown of tolerance. A plausible explanation is that foreign antigen acts as a molecular trigger to initiate an immune response to self-molecules, and a defect in the mechanism that regulates B cell activation leads to the propagation of an autoimmune response.

In general, an initial immune response is generated against a dominant set of epitopes, followed by a later response to secondary or "cryptic" epitopes, a process known as *epitope spreading*.[119] Epitope spreading is an important aspect of a protective immune response because the ability to recognize multiple antigenic determinants increases the efficiency of the neutralization and removal of pathogens. When an autoimmune response has been triggered, epitope spreading can lead to the production of additional autoantibodies with specificity for multiple self-antigens. There are several proposed mechanisms by which epitope spreading triggers a cascade of T and B cell activation. For instance, antigen-presenting cells may present a foreign peptide that mimics a self-peptide to T cells (see Figure 13-8A). Such cross-reactive T cells become activated and provide co-stimulation to autoreactive B cells that recognize self-antigen, which results in the production of autoantibodies specific for the antigen recognized by the T cell. After internalization of the self-antigen by the autoreactive B cells, the autoantigen is processed and new cryptic epitopes of the self-antigen are presented to T cells. A B cell binding to the self-antigen internalizes not only that self-antigen but also any complex of molecules that includes the self-antigen. The B cell may, therefore, present cryptic epitopes of many self-antigens and activate autoreactive T cells representing multiple autospecificities. In the periphery, T cells are present that have not been tolerized to these (cryptic) epitopes and thus are activated by self-peptide. These activated T cells in turn help provide co-stimulation and activate other autoreactive B cells.

Alternatively, cross-reactive B cells may be activated first after exposure to foreign antigen and T cell help (see Figure 13-8B). These B cells internalize self-antigen and present cryptic peptides to T cells that have not been tolerized, leading to activation of autoreactive T cells and initiation of the cascade. Thus molecular mimicry and epitope spreading could lead to the activation of T cells and B cells specific for multiple autoantigens as long as the autoantigens form a complex in vivo.

### Supraoptimal B Cell Co-Stimulation

It is evident that co-stimulatory signals provided by T cells play a critical role in B cell activation. Therefore inappropriate co-stimulation may lead to the propagation of an immune response directed against a self-antigen. The interaction between B7 on B cells and CD28 on T cells is crucial for the activation of antigen-specific T cells and B cells. When a genetically engineered protein that inhibits B7-CD28 interactions is administered to autoimmune mice, progression of disease is blocked.[120] Reciprocally, autoreactive B cells present in mice that constitutively overexpress B7 are not sensitive to Fas killing, and the mice display high serum autoantibody titers.[118] Overexpression of CD40 or CD40L may also activate autoreactivity. In vitro studies have demonstrated that CD40-CD40L ligation in the presence of IL-4 activates anergic cells. It has been suggested that CD40L may be overexpressed in lymphoid cells of patients with SLE.[5,121]

Roquin-1/RC3H1, a putative ubiquitin E3 ligase, regulates differentiation of Tfh. Roquin-1 is an RNA binding protein that mediates mRNA degradation.[122] It represses the expression of multiple inflammatory cytokines, including ICOS, a co-stimulatory molecule that plays an important role in Tfh development and function. Tfh cells provide strong co-stimulation in the GC. Mice harboring a mutation in the gene encoding Roquin display high-affinity dsDNA antibodies as a result of increased numbers of Tfh and GC B cells.[123]

Interferon regulatory factor-4 binding protein (IBP) has also been shown to regulate T cell co-stimulatory signals.[124] IBP is an activator of Rho guanosine triphosphatases (GTPases) and is recruited to the immunologic synapse after T cell receptor cross-linking to mediate the reorganization of the cytoskeleton. Mice deficient in IBP exhibit an autoimmune phenotype characterized by the production of dsDNA antibodies and glomerulonephritis. IBP plays an important role in the survival and effector function of memory T cells. In addition, IBP sequesters the transcription factor IRF4, thus preventing it from turning on expression of IL-17 and IL-21.[125]

TLRs belong to a family of pathogen recognition receptors that initiate innate immune responses to various

components of pathogens. TLRs are expressed on the cell surface or are localized in the endosomes. Thus TLR4 on the plasma membrane binds bacterial lipopolysaccharides and endosomal TLR7 and TLR9 recognize RNA and hypomethylated CpG-containing nucleic acid sequences, respectively, within B cells. Beside their role in DC maturation and T cell activation and differentiation, TLRs have been shown to be directly involved in multiple steps of antibody responses. Some T cell–independent antigens, such as LPS, engage both BCRs and TLR signaling pathways that induce strong B cell activation. During a T cell–dependent response, co-stimulation of TLR enhances BCR-mediated uptake of antigen and boosts AICDA induction for CSR and GC reactions. TLR activation has also been linked with chronic inflammation and autoimmune diseases. Numerous studies suggest that engagement of the BCR and TLR with immune complexes containing nuclear antigens triggers the activation of anti-nuclear B cells, implying that TLR7 and TLR9 can function to enhance the activation of autoreactive B cells under some circumstances.[126-128]

### B Cell Signaling Thresholds

The effects of altering the threshold for BCR signaling have been demonstrated in several mouse models. In transgenic mice that overexpress the BCR co-receptor complex component CD19, anergic B cells are activated and secrete autoantibody.[129] This result suggests that a decrease in the minimal requirement for antigen engagement of the BCR can lead to inappropriate activation of autoreactive B cells. Viable moth-eaten mice also develop an autoimmune syndrome due to a naturally occurring deficiency in the SHP1 phosphatase, a potent negative regulator of BCR signaling.[71] In these mice, B1 cells are responsible for the production of IgM anti-DNA antibodies. Transgenic mice deficient in other signaling molecules that alter threshold activation such as CD22[62] and Lyn[65] also produce autoantibodies. Thus, lowering thresholds for antigen-induced B cell activation can lead to the activation of autoreactive B cells.

## CONCLUSION

The generation of a diverse repertoire of antibody molecules provides an important line of defense against microbial infections. The immune system is exquisitely controlled at multiple levels to allow the maturation of B cells that produce protective antibodies while attempting to avoid the production of autoantibodies (Figure 13-9). Only a small percentage of B cell precursors generated completes the maturation pathway. During the pro-B and pre-B cell stages of development, B cells with aberrantly rearranged H- or L-chain genes are eliminated. As the remaining precursor cells transit into the immature B cell stage, they are subject to negative selection; that is, immature B cells with

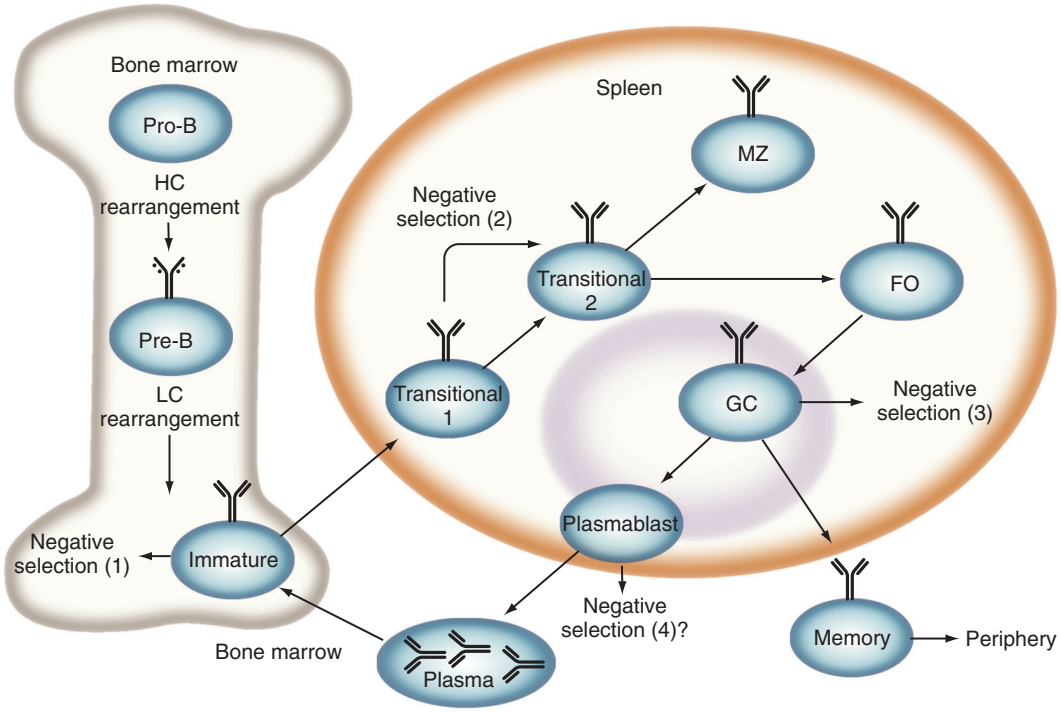



**Figure 13-9** Selection checkpoints during B cell maturation. Autoreactive B cells can be censored at multiple developmental checkpoints: *(1)* After surface expression of surface immunoglobulin, immature B cells that encounter autoantigen in the bone marrow are subject to negative selection. *(2)* B cells that are not eliminated in the bone marrow may undergo negative selection during the transitional B cell stage. Transitional B cells that emerge from this development stage give rise to follicular (FO) or marginal zone (MZ) B cells. Follicular B cells activated by antigen and the help of cognate T cells progress to the germinal center (GC) B cell stage. *(3)* Germinal center B cells that acquire high affinity for autoantigen by the process of somatic hypermutation may be eliminated in the germinal center to block their further maturation into long-lived plasma cells or memory cells. *(4)* There is evidence that autoreactive plasmablasts may also be subject to negative selection. Long-lived plasma cells that emerge from the selection process home primarily to the bone marrow, and memory B cells circulate throughout the periphery. HC, Heavy chain; LC, light chain.

autospecificity are either deleted or inactivated, whereas nonautoreactive B cells are released into the periphery. B cells that are stimulated by foreign antigen are selectively expanded and undergo further Ig gene diversification in peripheral lymphoid tissue. During this stage of development, B cells that express high-affinity BCRs undergo positive selection, whereas B cells with a diminished affinity or those that have acquired autoreactivity are eliminated. B cells that pass through these critical developmental checkpoints differentiate into long-lived memory B cells or plasma cells. The underlying causes of B cell–associated autoimmunity are not fully understood, but just as there are multiple checkpoints for the survival or activation of autoreactive B cells, it seems likely that multiple defects in the regulatory mechanisms that control B cell maturation and differentiation contribute to autoimmune disease.

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CHAPTER 14

# Fibroblasts and Fibroblast-like Synoviocytes



Christopher D. Buckley • Andrew Filer

**KEY POINTS**

- Fibroblasts are programmed epigenetically to determine the unique structure and function of different organs and tissues. However, these unique features might contribute to organ-specific disease.
- Tissue fibroblasts may be recruited from a number of sources and cell types including the bone marrow, blood, and local stromal cells and act as organ-specific innate immune sentinel cells.
- Under inflammatory conditions, fibroblasts become key immune system players by recruiting and modulating the behavior and survival of infiltrating immune cells.
- Fibroblasts can be programmed epigenetically through exposure to inflammatory and environmental stress such that they inappropriately prolong inflammation, which becomes persistent.
- Within the synovium, persistent abnormal behavior of fibroblasts results in continued damage to vital joint structures such as cartilage and bone, which, if untreated, will result in deformity and functional impairment.

## WHAT IS A FIBROBLAST?

The architecture of organs and tissues is closely adapted to their function in order to provide microenvironments in which specialized functions may be carried out efficiently. The nature and character of such microenvironments are primarily defined by the stromal cells that reside within the tissues. The most abundant cell types of the stroma are fibroblasts, which are responsible for the synthesis and remodeling of extra-cellular matrix (ECM) components. In addition, their ability to produce and respond to growth factors and cytokines allows reciprocal interactions with adjacent epithelial and endothelial structures and with infiltrating leukocytes. Fibroblasts also integrate microenvironmental stimuli such as oxygen tension and pH. As a consequence, fibroblasts play a critical role during tissue development and homeostasis and are often described as having a "landscaping" function.

### Fibroblast Identity and Microenvironments

Tissue-resident macrophages in the liver (Kupffer cells) and lung (alveolar macrophages) perform very different functions compared with macrophages in the brain (glial cells) or skin (Langerhans cells), yet they are all members of the monocyte/macrophage family. Until recently fibroblasts had been thought of as ubiquitous, generic cells with a common phenotype even within different tissues. However, we now know that fibroblasts from different organs are more like their macrophage counterparts, with unique morphologic features and repertoires of ECM proteins, cytokines, co-stimulatory molecules, and chemokines specialized to the different microenvironments in which they are found. This characterization also extends to their function as "immune sentinel" cells, expressing innate immune system pattern recognition receptors such as Toll-like receptors (TLRs), which trigger a pro-inflammatory response when ligated by bacterial or viral determinants.

Examination of fibroblast transcriptional profiles with use of microarray techniques reveals that fibroblasts retain a strong memory of their anatomic position and function in the body. Early studies demonstrated that fibroblast transcriptomes (i.e., the global picture of transcribed genes measured using microarrays) could be clustered into peripheral (synovial joint or skin fibroblasts) versus lymphoid (tonsil or lymph node) groups according to their organ of origin, with the potential to shift their transcriptional profiles upon exposure to inflammatory mediators such as tumor necrosis factor (TNF), interleukin (IL)-4, or interferon (IFN)-γ. More extensive analysis of expression profiles from primary human fibroblasts by Rinn and colleagues[1] has shown large-scale differences related to three broad anatomic divisions: anterior-posterior, proximal-distal, and dermal-nondermal. Genes involved in pattern forming, cell signaling, and matrix remodeling were found to predominantly account for these divisions.[1] The gene expression profile of adult fibroblasts may therefore play a significant role in assigning positional identity within an organism.

More recently, it has become clear that these stable changes in gene transcription are brought about through epigenetic activation and silencing of the HOX family of landscaping genes.[2] Such epigenetic patterning, whereby covalent modifications are made to regulatory regions of DNA or to the histones around which the DNA is wrapped in order to control access of transcriptional complexes, is a prototype for the stable changes that are also seen in fibroblasts. Epigenetic modifications result in stable changes in gene expression that persist over cellular generations in the absence of mutation of the primary DNA sequence and therefore drive the persistence of disease, as is described in more detail in Chapter 22.

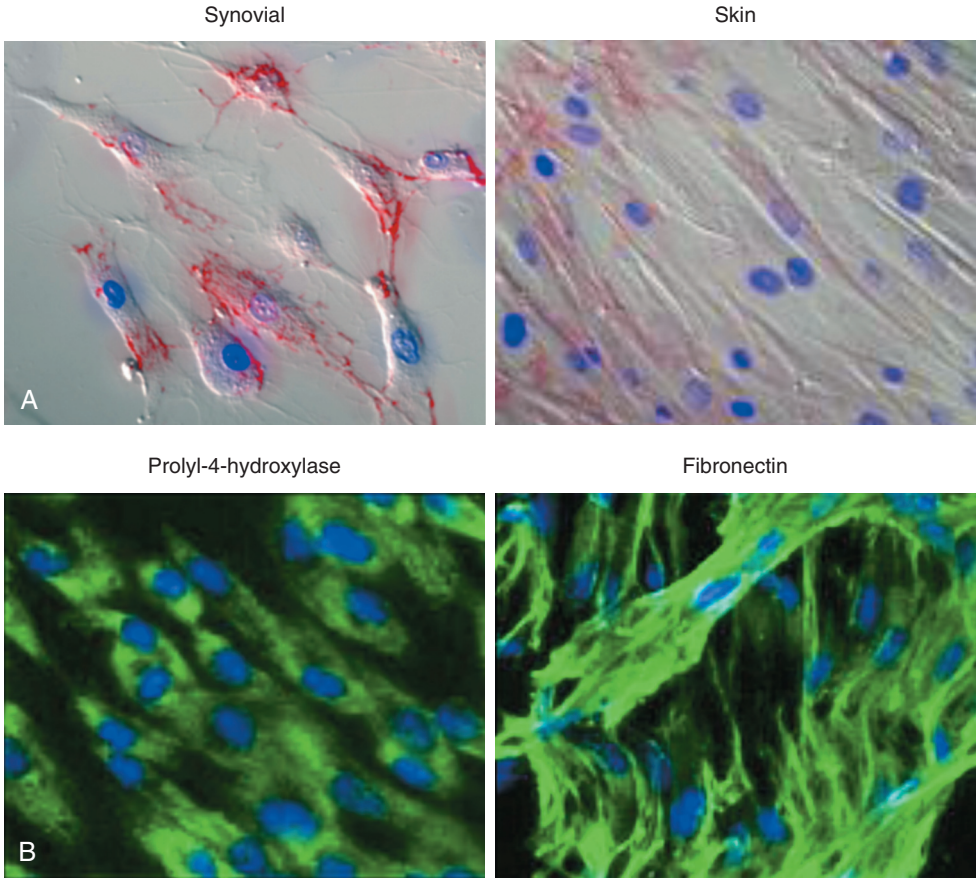



**Figure 14-1** Fibroblast phenotype. **A,** Staining and differential interference contrast microscopy of live fibroblast cells in culture illustrating typical morphologic features and marked differences between synovial fibroblasts of the rheumatoid arthritis joint and skin fibroblasts. Red stain (fibronectin) demonstrates matrix production. Blue stain indicates nuclei. **B,** Stromal cell status is confirmed by fluorescence microscopy of cells showing collagen synthetic enzymes (prolyl-4-hydroxylase) within and matrix production (fibronectin) on the surface of skin fibroblasts.

## Embryologic Origins

The problem of distinguishing fibroblasts of differing origin or maturity has historically been difficult because of a lack of specific cell surface markers. Whereas cluster of differentiation (CD) markers have revolutionized the isolation and study of leukocyte subsets, relatively few, poor-quality discriminatory markers have been identified that allow the identification of fibroblast subpopulations. Therefore fibroblasts traditionally have been identified by their spindle-shaped structure (Figure 14-1), elaboration of ECM, and lack of positive markers for endothelium, epithelial, and hemopoietic cells.

However, growing evidence indicates that fibroblasts are not a homogeneous population but exist as subsets of cells, much like tissue macrophages and dendritic cells (DCs). It is likely that connective tissue contains a mixture of distinct fibroblast lineages with mature fibroblasts existing side by side with more immature fibroblasts that are capable of differentiating into other connective tissue cells. Recent studies have begun to identify novel markers that demarcate distinct subpopulations of stromal cells during development and have the potential to act as markers for different subpopulations of fibroblasts, each with different roles. Such markers include smooth muscle actin, which marks out a population of secretory, activated cells termed *myofibroblasts*, and more recently discovered markers such as CD248 and gp38 (podoplanin) (Table 14-1[3-14]; also see later discussion). Fibroblasts have been defined in terms of their embryologic origins and lineage relationships and are generally considered to be mesenchymal in origin. However, cell populations that appear to blur the distinction between hemopoietic and nonhemopoietic populations have now been identified. In addition, other unexpected shifts in lineage have been reported, including differentiation from neural stem cells into myeloid and lymphoid hemopoietic lineage. Classification by such lineages is therefore becoming increasingly untenable.

## Origins of Fibroblasts in Tissue

Both inflammation and wound healing are characterized by the formation of new tissue. However, recent findings suggest that the new cells that form the remodeled tissues might not be derived from the proliferation of resident cells in the adjacent noninjured tissue, as was previously assumed. This finding is important because in both rheumatoid arthritis (RA) and fibrotic pathologic conditions, fibroblasts accumulate in excessive numbers despite apparently low proliferative rates. The principle origin for fibroblasts is from primary mesenchymal cells, and upon appropriate stimulation, fibroblasts can proliferate locally to generate

**TABLE 14-1** Synovial Stromal Markers and Their Geographic and Functional Significance

| Marker | Associated Cell Type | Synovial Location | Functional Significance |
|---|---|---|---|
| CD55 | Fibroblast-like synoviocyte | Lining layer | Receptor/ligand for synovial macrophage CD97[3] |
| VCAM-1 | Fibroblast-like synoviocyte | Lining layer | Activated lining layer fibroblasts; adhesion molecule[4] |
| α-SMA | Myofibroblast | Variable, minority subpopulation | Secretory, profibrotic fibroblast[5] |
| CD248/endosialin | Pericyte | Sublining fibroblasts, pericytes | Acute inflammation,[6] cancer and vasculogenesis[7] |
| gp38/podoplanin | Pericyte and lymphoid endothelium | Lining layer fibroblasts, pericytes, lymphoid endothelium | Structural, proangiogenic lymph node role[8]; promotes motility in cancer[9] |
| 5B5/prolyl-4-hydroxylase | Broad fibroblast marker in vivo | Lining and sublining cells | Marks collagen synthetic machinery[10] |
| S-100A4/FSP-1/Mts-1 | — | Lining and sublining cells, invasive regions | Cancer, invasiveness roles via motility and impaired apoptosis[11] |
| FAP | Associated with α-SMA$^+$ fibroblasts[12] | Lining layer | Role in cancer fibroblasts,[13] protective if ectoenzyme blocked in rheumatoid arthritis[14] |

α-SMA, α-Smooth muscle actin; FAP, fibroblast activation protein; VCAM-1, vascular cell adhesion molecule-1.

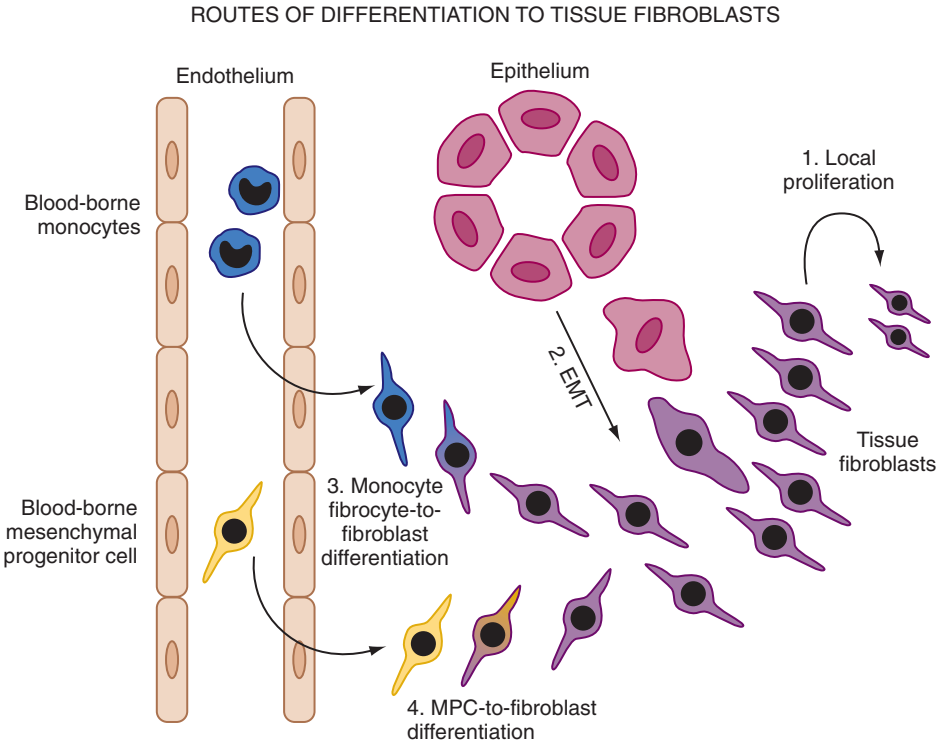



**Figure 14-2** Routes of differentiation to tissue fibroblasts. In response to wounding or inflammation, increased numbers of fibroblasts are produced within tissue. *1,* Fibroblasts can proliferate locally to generate new fibroblasts. *2,* The transition of epithelial to stromal cell populations occurs in cancer and diseases of the lung, kidney, and possibly the synovium. *3,* Fibrocytes arise from the monocyte population in blood and then differentiate toward fibroblasts in tissue. *4,* Blood-borne mesenchymal progenitor cells (MPC) may be recruited to tissues and undergo local differentiation to tissue fibroblasts. EMT, Epithelial to mesenchymal transition.

new fibroblasts; however, although an increase in fibroblast numbers caused by local proliferation does occur, fibroblasts also may arise from other sources (Figure 14-2). The first of these sources is local epithelial to mesenchymal transition (EMT). EMT is an essential, physiologically important developmental mechanism for diversifying cells in the formation of complex tissues. However, fibroblasts also appear to be derived by this process in adult tissue after epithelial stress such as inflammation or tissue injury. EMT both disaggregates epithelial cells and reshapes them for movement. The epithelium loses polarity as defined by the loss of adherens junctions, tight junctions, desmosomes, and cytokeratin intermediate filaments. Epithelial cells also rearrange their F-actin stress fibers and express filopodia and lamellipodia. A combination of cytokines and matrix metalloproteinases (MMPs) associated with digestion of the basement membrane is believed to be secreted and is important in the process. The transition of epithelial to mesenchymal cell populations has been shown to occur in cancer and in diseases of the lung and kidney, in which the

process has been implicated in fibrotic disease.[15] Early evidence suggests that a similar process may occur within the RA synovium.[16]

An alternative explanation for the accumulation of stromal cells in persons with chronic inflammatory conditions such as RA lies in the possibility of blood-borne precursors. In the mid 1990s it was shown that vascular precursors (angioblasts) circulate in the blood of healthy people and that they could be recruited to sites of vasculogenesis in a rabbit ischemic hind limb model.[17] This finding demonstrated that circulating mesenchymal precursors exist outside the hemopoietic system. Subsequent work has confirmed the presence of circulating cells of a mesenchymal phenotype in human subjects. These cells bear a remarkable resemblance to the synovial fibroblasts found in the joints of persons with RA, which accumulate in large quantities in the joint lining despite little evidence of proliferation. Interestingly, Marinova-Mutafchieva and colleagues[18] showed that an influx of such cells preceded inflammation in a mouse collagen-induced arthritis model, suggesting that a role may exist for blood-borne stromal cell precursors in the initiation of inflammatory diseases. Furthermore, synovial fibroblasts themselves may migrate in the bloodstream, at least between distant sections of human cartilage in severe combined immunodeficiency (SCID) mice,[19] raising an intriguing parallel to cancer and the radical concept of RA as a metastatic disease of the stroma.

Another circulating precursor cell that could account for the accumulation of fibroblasts in disease is the fibrocyte. Fibrocytes constitute 0.1% to 0.5% of nonerythrocytic cells in peripheral blood and rapidly enter sites of tissue injury and contribute to tissue remodeling in models of inflammatory lung disease.[20] Fibrocytes are adherent cells with a spindle-shaped structure. They express class II major histocompatibility complex (MHC) and type I collagen and arise from within the $CD14^+$ (monocyte) fraction of peripheral blood.[21] They are capable of matrix elaboration and differentiate along fibroblast lineages under the influence of cytokines, particularly transforming growth factor (TGF)-β. The mere fact that a cell type apparently arising from within the monocyte lineage may become a "mesenchymal" stromal cell such as a fibroblast implies a further degree of plasticity and blurring of the apparently clear dividing line that was previously thought to exist between hemopoietic and nonhemopoietic lineages.

### Fibroblasts versus Mesenchymal Progenitor Cells

The potential role of circulating mesenchymal cell precursors (variously termed *mesenchymal stem cells* [MSCs], *mesenchymal stromal cells*, or *mesenchymal progenitor cells* [MPCs]) as sources of tissue fibroblasts is highlighted by the remarkable capacity of these cells to differentiate into other members of the connective tissue family, including cartilage, bone, adipocyte, and smooth muscle cells. This ability was initially demonstrated in bone marrow stromal cells, RA synovial fibroblasts, and circulating mesenchymal cells. Therefore, a characteristic mesenchymal phenotype could be defined on the basis of the hypothesis that the rheumatoid synovium becomes populated by a large proportion of circulating mesenchymal progenitor cells exported from the bone marrow. However, the property of trilineage differentiation ("pluripotentiality") has now been shown to be a property of many adult tissue fibroblasts, although varying somewhat between fibroblasts from different tissues, implying a hitherto unsuspected degree of plasticity in the body's stromal cell populations.[22] The two previously separate fields of mesenchymal precursor cell biology and largely disease-centered fibroblast biology have therefore rapidly converged. However, the concept of bone marrow stromal precursors remains interesting; in chimeric murine models with bone marrow Green fluorescent protein (GFP) expression, arthritic joints contained significantly more $GFP^+$ cells than did nonarthritic joints, supporting a bone marrow origin for expanded fibroblast populations.[23]

## PHYSIOLOGIC CHARACTERISTICS AND FUNCTIONS OF FIBROBLASTS

### Production of ECM Components

Ensuring ECM homeostasis is one of the primary functions of fibroblasts. To perform this function, fibroblasts must be able to produce and degrade ECM, as well as adhere to and interact with existing matrix components. Fibroblasts produce a number of ECM molecules—both fibrous proteins and polysaccharide gel components such as collagens, fibronectins, vitronectin, and proteoglycans—which are then assembled into a three-dimensional network. This mechanism provides a framework through which other cell types, which use varying strategies to navigate through the ECM, can move.[24] It also provides a substrate for the deposition of haptotactic (tissue rather than fluid-based) gradients of chemokines and stores of growth factors to direct cellular movement and behavior in a regional fashion.[25] The types of ECM molecules produced by individual populations of fibroblasts differ from tissue to tissue, reflecting the diversity of fibroblasts in different organs. For example, dermal fibroblasts produce significant amounts of type VII collagen, which adheres to the epidermal and dermal layers in the skin. Fibroblasts in other organs such as the lung and kidney produce mainly interstitial, fibrillar collagens (particularly types I and III).

In the synovial membrane, fibroblasts also have a barrier function, in that they provide the joint cavity and the adjacent cartilage with lubricating molecules such as hyaluronic acid, along with plasma-derived nutrients. Anatomically, the intimal synovial membrane is an unusual structure in that barrier function is maintained in the absence of a laminin-rich basement membrane, as occurs in epithelial structures. In addition to lacking a basement membrane, cellular contacts between the fibroblast-like synoviocytes also lack tight junctions and desmosomes. However, strong homophilic adhesion between synoviocytes is mediated by the adhesion molecule cadherin-11 (see later discussion), which is largely responsible for fibroblast organization into synovial tissue. In the presence of disease, fibroblasts must migrate to sites of tissue injury or remodeling and interact with ECM molecules through specific surface receptors. Through such receptors, fibroblasts must sense changes in both the structure and the cellular composition of connective tissues. They respond dynamically by adjusting the production of ECM components and cross-linking them into the appropriate matrix.

**TABLE 14-2** Fibroblast Cell Adhesion Molecules and Their Receptor/Ligand Molecules

| Family | CAM | Alternative Names | Ligands |
|---|---|---|---|
| Integrins | $\alpha_1\ \beta_1$ | VLA-1 | Laminin, collagen |
| | $\alpha_2\ \beta_1$ | VLA-2 | Laminin, collagen |
| | $\alpha_3\ \beta_1$ | VLA-3 | Laminin, collagen, fibronectin |
| | $\alpha_4\ \beta_1$ | VLA-4, CD49d/CD29 | VCAM-1, CS1 fibronectin |
| | $\alpha_5\ \beta_1$ | VLA-5 | Fibronectin |
| | $\alpha_6\ \beta_1$ | VLA-6 | Laminin |
| | $\alpha_L\ \beta_2$ | LFA-1, CD11a/CD18 | ICAM-1, ICAM-2, ICAM-3, JAM-A |
| | $\alpha_M\ \beta_2$ | Mac-1, CR3, CD11b/CD18 | ICAM-2, iC3b, fibrinogen, factor X |
| | $\alpha_X\ \beta_2$ | P150, 95, CD11c/CD18 | iC3b, fibrinogen |
| | $\alpha_E\ \beta_2$ | | E-cadherin |
| | $\alpha_4\ \beta_7$ | CD49d | Fibronectin, VCAM-1, MadCAM-1 |
| | $\alpha_v\ \beta_3$ | CD52/CD61, vitronectin receptor | Vitronectin, fibronectin, osteopontin, thrombospondin-1, tenascin |
| Ig superfamily | ICAM-1 | CD54 | LFA-1, Mac-1 |
| | ICAM-2 | | LFA-1 |
| | ICAM-3 | | LFA-1 |
| | VCAM-1 | | $\alpha_4\ \beta_1$, $\alpha_4\ \beta_7$ |
| | MadCAM-1 | | $\alpha_4\ \beta_7$, L-selectin |
| Cadherins | E-cadherin | Cadherin-1 | E-cadherin |
| | N-cadherin | Cadherin-2 | N-cadherin |
| | Cadherin-11 | OB-cadherin | Cadherin-11 |

ICAM, Intercellular adhesion molecule; Ig, immunoglobulin; JAM, junctional adhesion molecule; LFA, lymphocyte function–associated antigen; Mac-1, macrophage 1 antigen; MAdCAM, mucosal addressin cell adhesion molecule; OB-cadherin, osteoblast cadherin; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

## Attachment to and Interaction with Extra-cellular Matrix

### Integrins

Integrins are key mediators of both cell-to-matrix and cell-to-cell adhesive interactions. They are expressed as transmembrane heterodimers containing one α- and one β-subunit, of which at least 25 αβ combinations are known (Table 14-2). The main adhesion molecules responsible for the attachment of fibroblasts to collagen are $\alpha_1\beta_1$ and $\alpha_2\beta$ integrins, whereas other $\beta_1$ integrins such as $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins mediate attachment of fibroblasts to fibronectin and its spliced variants. In addition, $\alpha_v$ integrins are responsible for attachment to vitronectin.

### Syndecans

In addition to conventional integrin-to-ligand binding, additional accessory molecules allow for the integration of adhesive contacts and local growth factor signaling. Syndecans are a family of four single transmembrane domain proteins that carry three to five heparan sulfate and chondroitin sulfate chains, allowing for interaction with a large variety of ligands including fibroblast growth factors, vascular endothelial growth factor (VEGF), TGF-β, and ECM molecules such as fibronectin.[26] Syndecans are expressed on fibroblasts in a tissue-specific and development-dependent manner. Data from syndecan knockout mice indicate that syndecan-4 is involved in wound healing and that the response of syndecan-4–deficient fibroblasts to fibronectin attachment is significantly altered.[27]

### Immunoglobulin Superfamily Receptors

The immunoglobulin (Ig) superfamily is a diverse group of transmembrane glycoproteins defined by the presence of one or more Ig-like repeats of 60 to 100 amino acids with a single disulfide bond.[28] Although it includes numerous adaptive immune system genes (e.g., Igs, T cell receptor, and MHC), adhesion proteins such as intercellular adhesion molecules (ICAMs) 1 to 3, vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule (MAdCAM) mediate both cell-to-cell interactions and adhesive interactions with integrins (see Table 14-2).

### Cadherins

Cadherins mediate homotypic, calcium-dependent adhesive interactions with the same cadherin species expressed by neighboring cells.[29] Classical cadherins possess five extracellular domains, a single-pass transmembrane domain, and a highly conserved cytoplasmic tail. The cytoplasmic tail interacts with β-catenin, which in turn binds α-catenin, forming a linkage between the cadherin-catenin complex and the actin cytoskeleton. Tightly regulated expression of cadherins is essential to embryogenesis but is also critical for tissue morphogenesis and tissue-specific cell differentiation. Cadherins also modulate cell proliferation and invasion through activation of intra-cellular signal transduction pathways, modulation of MMP production, and association with growth factor receptors.[30-32]

### Adhesion Molecule-Mediated Signaling

Importantly, interaction with adhesion molecules not only regulates adhesion and motility but also directly influences activation status, apoptosis, and pro-inflammatory and anti-inflammatory responses in fibroblasts and other cells. The engagement of cell adhesion molecules such as integrin receptors on the surface of fibroblasts results in the formation of focal adhesion complexes, which activate intracellular signaling cascades that regulate cell proliferation

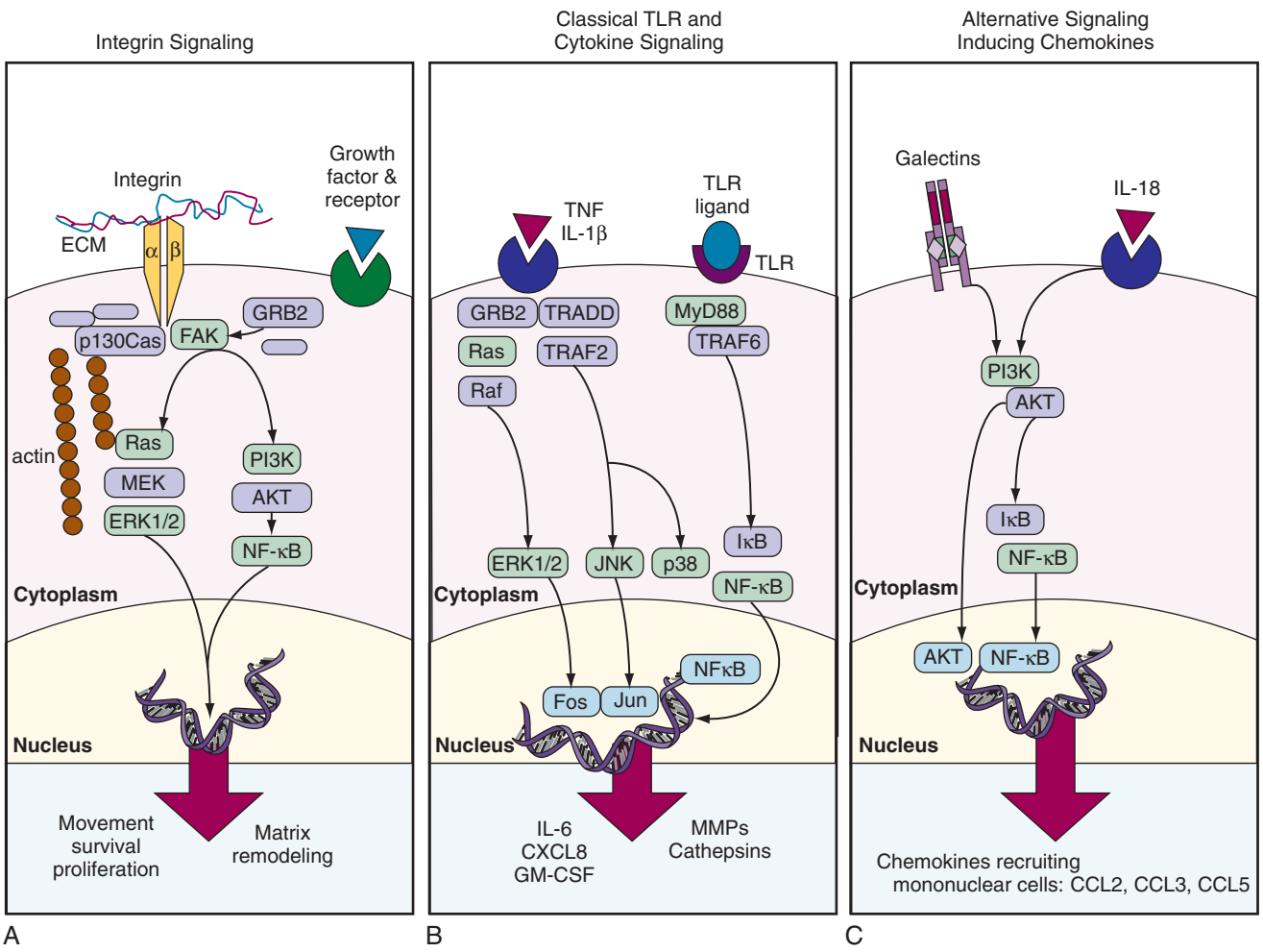



**Figure 14-3** Important signaling pathways in synovial fibroblasts. **A,** Integrin signaling in fibroblasts. The engagement of integrins and extra-cellular matrix (ECM)–bound growth factors on the cell surface of fibroblasts results in the initiation of signaling cascades that result in changes in (1) cell motility through reorganization of the cytoskeleton, (2) cell survival (e.g., through activation of the Akt-NF-κB pathway), and (3) the production of matrix molecules, matrix-degrading enzymes, and soluble mediators through the activation of mitogen-activated protein kinases (MAPK). **B,** The three MAPK pathways are also pivotal in pro-inflammatory cytokine activation of synovial fibroblasts, with tumor necrosis factor (TNF), interleukin (IL)-1β, and IL-6 all capable of activating the three main pathways. In particular, c-Jun N-terminal kinase (JNK) and p38 MAPK pathways are crucial to the production of matrix metalloproteinases (MMPs) such as the collagenases. Fos family members and jun dimerize to form the activator protein-1 (AP-1) transcription factor for which binding sites are present on multiple pro-inflammatory genes, including the MMPs. **C,** There is evidence for a discrete pro-inflammatory pathway for some ligands, which might bypass the classical MAPK and nuclear factor-κB (NF-κB)/AP-1 pathways, signaling via phosphatidylinositol 3-kinase (PI3K) to elicit secretion of chemokines. The chemokines specifically recruit the mononuclear cell population, which predominates in persistent inflammatory disease. GM-CSF, Granulocyte-macrophage colony-stimulating factor; TLR, Toll-like receptor.

and survival, the secretion of certain cytokines and chemokines, and matrix deposition and resorption. In particular, integrin-to-fibronectin engagement induces MMP expression, linking adhesion-to-matrix remodeling[33] (Figure 14-3). Among the signaling molecules that transmit signals from the integrins to the cell interior, focal adhesion kinase (FAK) plays a central role.[34] FAK, a tyrosine kinase, is recruited into newly established focal contacts and, in turn, recruits other adapter proteins such as p130Cas and Grb2. This process leads to phosphatidylinositide 3-kinase (PI3K) and Src-kinase activation and promotes the initiation of a variety of signaling cascades, culminating in phosphorylation of the extra-cellular regulating kinase (ERK) mitogen-activated protein kinases (MAPKs) and activation of transcription factors. Such pathways can also be activated through FAK-independent signaling events, such as through growth factor receptor ligation. The exact mechanisms by which different signals cooperate to mediate a specific response of fibroblasts and how this translates into distinct diseases are not yet fully defined.

## Degradation of Extra-cellular Matrix by Fibroblasts

Remodeling of the ECM requires fibroblasts to express an extensive repertoire of matrix-degrading enzymes

with varying specificity. Although these matrix-degrading enzymes are crucial to tissue maintenance and repair, inappropriate overexpression of such enzymes is a key factor in the joint damage, particularly to cartilage, that occurs in inflammatory disease. Such enzymes fall into a number of families, including MMPs, tissue inhibitors of metalloproteinases (TIMPs), cathepsins, and aggrecanases, which are covered in detail in Chapter 8.

With the exception of MMP-2 and the membrane-type (MT)-MMPs, which are constitutively expressed by fibroblasts, MMP expression is regulated by extra-cellular signals via transcriptional activation in fibroblasts. Three major groups of inducers can be differentiated: pro-inflammatory cytokines, growth factors, and matrix molecules. Among the cytokines, IL-1 is perhaps the most potent inducer of a variety of MMPs, including MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14. Fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) are also known inducers of MMPs in fibroblasts because they potentiate the effect of IL-1 on MMP expression. All MMP promoter regions except MMP-2 contain activator protein-1 (AP-1) binding sites; however, there is good evidence that all MAPK families (ERK, c-Jun N-terminal kinase [JNK], and p38 pathways; see Figure 14-3), in addition to activators of nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT), and ETS transcription factors participate in MMP regulation.[35-39] Matrix proteins (i.e., collagen and fibronectin), and especially their degradation products, also activate MMP expression in fibroblasts, providing the possibility for site-specific MMP activation in regions of matrix breakdown.[40]

## Fibroblasts as Innate Immune Sentinels

Classically, macrophages have been studied as sources of inflammatory cytokines and chemokines in response to innate immune stimuli and portrayed as immune sentinel cells accordingly. However, when activated by substances released during tissue injury or the products of invading microorganisms, fibroblasts are capable of elaborating a broad repertoire of inflammatory mediators, which fully justifies their classification as immune sentinel cells. Through expression of TLRs 2, 3, and 4, fibroblasts respond to bacterial products such as lipopolysaccharide (LPS) by activating the classical NF-κB and AP-1 inflammatory pathways, generating chemokines capable of recruiting inflammatory cells, and generating metalloproteinases capable of degrading matrix.[41-43] However, TLR expression may be increased by pro-inflammatory cytokines TNF and IL-1β within the local microenvironment[44] and may also be activated by endogenous cellular debris such as necrotic cells in synovial fluid, leading to widespread fibroblast activation in disease.[45] As immune sentinels, fibroblasts are able to bridge the innate and adaptive immune responses through expression of the molecule CD40. This molecule was initially assumed to be restricted in its expression to antigen-presenting cells such as macrophages and DCs. However, it is widely expressed by fibroblasts within discrete tissues. CD40 engagement by its ligand CD40L expressed on a restricted population of immune cells including activated T lymphocytes, is critical for the further induction of pro-inflammatory cytokines and chemokines during an immune response, as well as for antibody production by CD40-expressing B lymphocytes.

Fibroblasts also need to be able to respond to more generic danger signals. The intra-cellular apparatus for response to danger signals such as high levels of urate has recently been identified as the nucleotide-binding oligomerization domain (NOD)-like receptor family, which is made up of NOD and NALP (i.e., NACHT domain, leucine-rich repeat [LRR] domain, and pyrin domain [PYD]-containing protein) receptors. A high local level of urate released by dying cells triggers formation of the active NALP3 inflammasome complex, which results in release of IL-1.[46]

Expression of high levels of NOD-1, NOD-2, and NALP3 (cryopyrin) are seen in the RA synovium and can be induced in fibroblasts by TLR ligands and/or TNF. Furthermore, synergy between TLR and NOD stimulation, with increased IL-6 production in response to NOD-1, TLR2, and TLR4 ligands, has recently been demonstrated. The cytokine IL-17 also regulates multiple TLRs in RA synovial fibroblasts.

## Role of Specialized Fibroblast Subsets within Tissue Microenvironments

Combining surface markers with consistent function has been the key to decades of development in the field of leukocyte biology. By comparison, stromal cell biologists have had remarkably few such stable markers. However, this situation is now gradually changing, and certain areas of developmental biology have spearheaded identification of putative markers (such as CD248) through approaches such as immunization of animals with human fibroblasts and digesting and identifying stromal cell subpopulations in tractable organ systems. One example is the murine thymic stroma, in which subsets with both geographic and functional consistency have been identified. For instance, Link and colleagues identified $CD45^{-}$, $gp38^{+}$ stromal cells in the thymus as T-zone fibroblastic reticular cells.[47] This population of cells, which is geographically restricted to the T zone, provides a limited pool of essential homeostatic survival factors, IL-7 and CCL19, for T lymphocytes, serving a key niche function for which adaptive immune cells must compete.[47] Also, gp38 marks populations of fibroblastic reticular cells within the lymph node that modulate trafficking of DCs.[48]

A further subpopulation of specialized fibroblast-like cells of mesenchymal origin is the pericyte. These cells ensheath small blood vessels (i.e., arterioles, capillaries, and venules) and are involved in vasculogenesis, matrix stabilization, and immunologic defense. Pericytes have been hypothesized to represent the extralymphoid source of mesenchymal progenitor cells and express markers consistent with mesenchymal stem cells. Their further definition with newer stromal cell markers such as CD248 and CD146 will be able to establish a mesenchymal progenitor cell niche.[49]

## Fibroblast-like Synoviocytes in the Normal Synovium

The normal synovium provides an excellent prototypic model of fibroblast subsets defined by known markers, some of which are responsive to disease. In healthy people the

synovium is a delicate, thin, two-layer structure attaching bone and the joint capsule. One layer, a two- to three-cell-thick lining layer, is formed in roughly equal proportions of $CD68^+$, phagocytic type A macrophage-like synoviocytes, and type B mesenchymal, fibroblast-like synoviocytes (FLS). This layer serves a barrier function, and FLS secretes lubricative substances, including hyaluronic acid and lubricin, along with secreting the lining layer matrix. The second layer is the sublining layer, which is composed of less densely packed fibroblasts and macrophages in a loose tissue matrix along with blood vessel networks. FLS in the lining layer are associated with a number of cellular markers (see Table 14-1), including CD55 (decay accelerating factor [DAF]), VCAM-1 (which, outside of T cell–to-integrin interactions, is generally only expressed by bone marrow fibroblasts providing support for the B cell developmental niche[50]), uridine diphosphoglucose dehydrogenase (UDPGD), reflecting the ability to synthesize hyaluronan, and the novel marker gp38.[51] Sublining FLS are instead marked by the nonspecific cellular marker CD90 (Thy-1), which also recognizes endothelium, and by the recently discovered marker CD248, which marks both pericytes and stromal fibroblasts. Gp38 marks cells in the sublining region, including lymphatic endothelium and pericytes (Figure 14-4).

As mentioned earlier, the unique lining layer barrier function is not supported by a basement membrane and conventional tight junctions but instead by homotypic interactions between cadherin-11 molecules.[52] Randomly assorted cells expressing classical cadherins such as cadherin-11 will sort themselves in a cadherin-specific manner, emphasizing their importance in the generation and maintenance of organ integrity. Cadherin-11 mediates selective association of mesenchymal rather than epithelial tissues, a function that is carried forward after embryogenesis in structures such as the joint, lung, and testis.[53] Cadherin-11 knockout mice exhibit a hypoplastic synovial lining that lacks the normal numbers of synovial lining cells and is deficient in ECM quantity.[54] Adhesion between type A and type B synoviocytes is maintained by ICAM-1:$\beta_2$ integrin and VCAM-1:$\alpha_4\beta_1$ integrin interactions.

By virtue of their role in defining the geography of specialized tissues, fibroblasts and other stromal cells exist in living organisms within three-dimensional environments, whereas the vast majority of experiments performed using fibroblasts in the laboratory are still conducted within two-dimensional environments. Furthermore, fibroblasts are frequently grown in nonphysiologic stimuli such as serum, to which fibroblasts would not normally be exposed unless tissue damage were to occur. Behavior is significantly different when cells are cultured in artificial three-dimensional environments.[55] It is therefore all the more remarkable that fibroblasts cultured using conventional two-dimensional

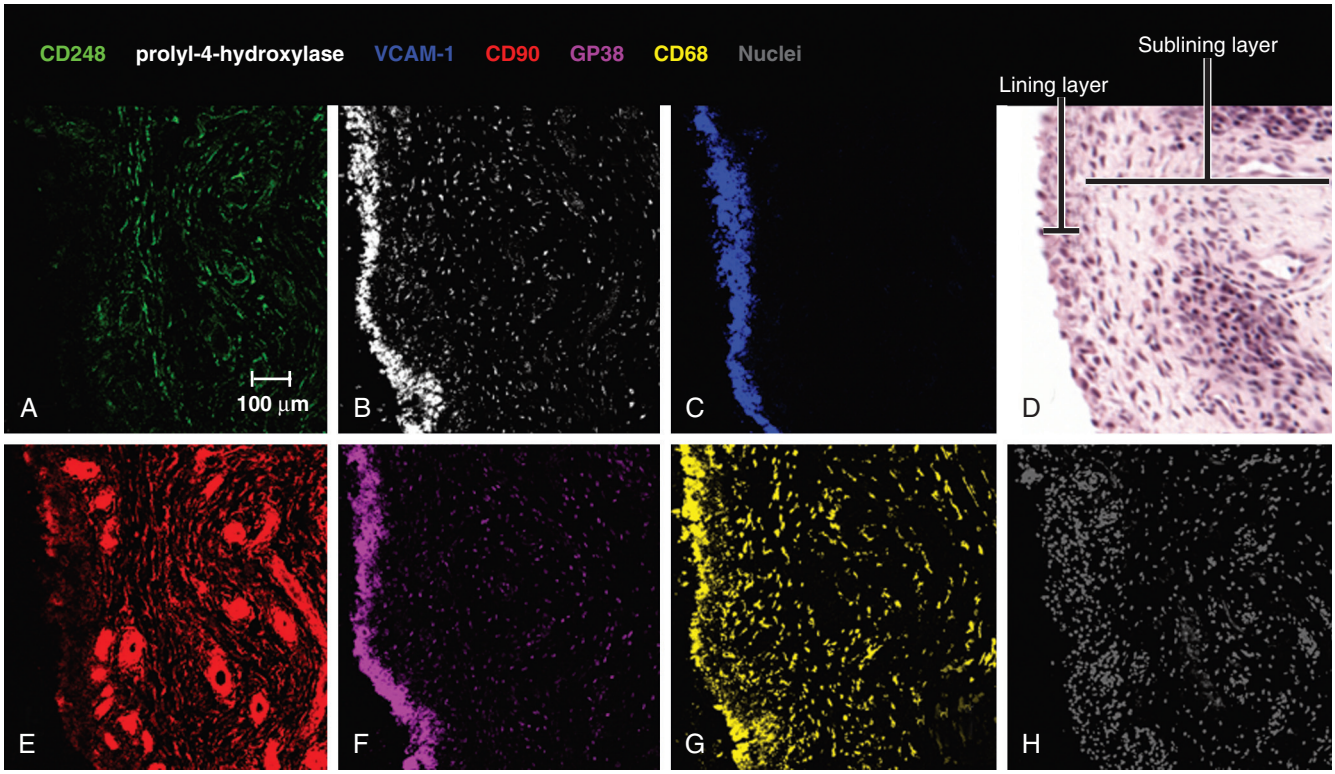



**Figure 14-4** Microscopic appearance of the synovium and stromal cell markers. The microscopic structure of hematoxylin and eosin–stained synovium is illustrated in **D,** indicating lining and sublining layers. This geographic structure is reflected in serial frozen sections of rheumatoid arthritis synovium stained for stromal markers **(A-C, E-G).** For reference, a nuclear stain is shown in **H. A,** CD248 stains only sublining fibroblasts. **B,** Prolyl-4-hydroxylase stains most populations of synovial fibroblasts. **C,** Vascular cell adhesion molecule-1 (VCAM-1) (CD106) characteristically stains only the lining layer. **E,** CD90 (Thy-1) stains predominantly sublining layers but also strongly stains endothelial cells, outlining synovial vasculature. **F,** gp38 marks lining layer cells and a proportion of sublining cells. **G,** CD68 highlights macrophage-like synovial cells in the lining layer and resident tissue macrophages in the sublining layer.

techniques retain characteristics such as positional memory and unique cytokine profiles.

Recent work has addressed the issue of three-dimensional synovial models. In so-called "micromass cultures," FLS, but not dermal fibroblasts, within laminin-containing spheres reproduced a lining layer structure with production of lubricin, support for co-cultured monocytic cells, and expansion of the membrane upon stimulation with pro-inflammatory stimuli such as TNF. Some cells also remained in a "sublining" zone of low density.[56] FLS therefore have the ability to self-organize in a tissue organoid, which recapitulates some of the key features of the synovium. This finding is further evidence of the robustness of epigenetic programming, which determines site and organ specialization.

## FIBROBLASTS IN RHEUMATIC DISEASES

### Role of Fibroblasts in Persistent Inflammation

Inflammatory reactions proceed against the backdrop of specialized stromal microenvironments. The response to tissue damage involves a carefully choreographed series of interactions among diverse cellular, humoral, and connective tissue elements. In order for an inflammatory lesion to resolve, dead or redundant cells that were recruited and expanded during the active phases of the response must be removed. In addition, resident fibroblasts attempt to repair damaged tissue.

It is becoming increasingly clear that fibroblasts are not only passive players in immune responses but also actively determine the switches that govern progression from acute to chronic inflammation, as well as those governing resolution or the progression to chronic, persistent inflammation. The "switch to resolution" is an important signal that permits tissue repair to take place and enables immune cells to return to draining lymphoid tissues (lymph nodes) in order for immunologic memory to become established. However, in chronic immune-mediated inflammatory diseases such as RA, fibroblasts contribute to the inappropriate recruitment and retention of leukocytes in a site- or organ-dependent manner, leading to tissue- and site-specific initiation and subsequent relapse of chronic persistent inflammatory disease, effectively a "switch to persistence."[57]

It is now recognized that fibroblasts themselves may undergo fundamental changes while responding to such environmental stimuli. It is known that during wound healing and under profibrotic conditions, some fibroblast-like cells are transformed into myofibroblasts, which are distinct from tissue fibroblasts in terms of both their phenotype and their behavior.[58] The mechanisms underlying such persistent phenotypic change, which is maintained through cellular generations, are highly likely to involve epigenetic modifications of gene promoters and their closely related histones (see Chapter 22). This has been shown recently in both human and murine renal fibrotic disease, where hypermethylation of the promoter region of a ras oncogene inhibitor led to gene silencing, ras pathway activation, and hence persistent fibrogenesis.[59] Such fibrotic transformation of fibroblasts is also characteristic of systemic sclerosis, a generalized fibrotic disorder that affects the skin and various internal organs such as the lungs, heart, and gastrointestinal tract (see Chapter 84). The overproduction of ECM components, particularly type I, III, VI, and VII collagen, by skin fibroblasts is a hallmark of this disease and is closely linked to the disease-specific activation of these fibroblasts. This pattern of activation includes not only a distinct profile of ECM overproduction but also altered responses to both inflammatory mediators and immune cells.[60] Although the phenotype of fibroblasts in RA is not fundamentally profibrotic in this sense, the hallmark of these cells, both in vitro and in vivo, is also a persistently imprinted phenotype that is maintained even in the absence of continuous stimulation by inflammatory triggers or leukocytes.

## FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

In inflammatory arthritis such as RA, the two compartments of the synovium undergo radical change. The lining layer undergoes dramatic hyperplasia, sometimes reaching 10 to 20 cells in depth with both type A and type B cell populations expanded and becoming merged with the sublining. At the articular borders of the synovium, the thickened synovial lining layer may become a mass of "pannus" tissue rich in FLS and osteoclasts, which aggressively invade the adjacent articular cartilage and subchondral bone, respectively. The sublining layer also undergoes expansion, with sometimes huge infiltrates of inflammatory cells including macrophages, mast cells, T cells, B cells, and plasma cells in addition to DCs. T and B lineage cells may remain in diffuse infiltrates or may coalesce into aggregates of cells varying from simple perivascular "cuffs" a few cells in diameter to structures resembling B cell follicles in up to 20% of samples.[61] This increased activity is supported by further ECM production and neoangiogenesis, although the inflamed synovium remains in a state of relative hypoxia.[62]

As mentioned previously, cadherin-11 serves a vital role in preserving the integrity of the synovial lining layer, and cadherin-11 knockout mice display a hypoplastic lining layer. However, when cadherin-11 knockout mice are evaluated in the K/BxN serum transfer model, invasiveness is reduced with a 50% reduction in inflammation. Similarly, cultured fibroblasts with mutant cadherin-11 constructs also demonstrate impaired invasiveness into cartilage.[54,63] Cadherin-11 expression is also much higher in RA than in osteoarthritis (OA) or normal synovium. This unique structural molecule may therefore emerge as a therapeutic target[54]; because of shared roles in invasive disease, targeting of this molecule in breast cancer is currently in development.[64]

### Persistent Activated Fibroblast Phenotype in the Rheumatoid Arthritis Synovium

Increased expression of cadherin-11 is but one facet of the persistent, activated phenotype of rheumatoid FLS, which remains stable even after culturing in vitro for many months. These cells play a direct role in tissue damage through secretion of multiple MMPs and cathepsins, which degrade cartilage and bone tissues in the joint. In vitro functional assays such as the laminin invasion assay produce intriguing results, in which the degree of invasion with a given in vitro cultured fibroblast sample correlates with the degree of radiographic progression seen in the joints of the patient

from whose samples the fibroblasts were initially cultured.[65] The most compelling evidence for a persistent phenotype is the attachment to and invasion of fibronectin-rich matrix such as human cartilage in the absence of functioning leukocyte immune cells in the SCID mouse model of arthritis.[66] Here, fibroblasts in a tissue construct with human cartilage are implanted under the kidney capsule or skin of immune-incompetent SCID or $Rag^{-/-}$ mice. Multiple-passage cultured rheumatoid FLS, but not OA or normal FLS, invade and destroy the co-implanted human cartilage. This model has been used to explore the in vivo mechanisms governing invasiveness. For example, targeting MMP-1 and cathepsin L using ribozymes inhibits cartilage destruction.[67,68] The effectiveness of glucocorticoids and the relative efficacy of different formulations of methotrexate in preventing erosions have also been examined.[69,70]

Unbiased approaches to determining the key regulators of fibroblast invasiveness have made rapid recent progress. Transcriptomic approaches linking gene expression in RA fibroblasts and macrophages have revealed invasiveness pathways within fibroblasts regulated by complementary macrophage inflammatory pathways that are strongly driven by IL-1β stimulation. Key genes include periostin osteoblast-specific factor (POSTN) and twist basic helix–loop–helix transcription factor 1 (TWIST1).[71,72] The impact of TNF and IL-17 stimulation on the transcriptome of RA fibroblasts has been examined, revealing critical hypoxia regulated genes linked to invasiveness, including MMP-2 and the chemokine receptor CXCR4, which is already implicated in disease persistence.[73]

An alternative unbiased approach to dissecting function involves the parallel study of cellular enzymes mediating tyrosine phosphorylation of key signaling molecules (protein tyrosine phosphatases [PTPs]). Investigation of the PTPome of synovial fibroblasts in RA compared with OA revealed a dual role for SH2 domain–containing phosphatase 2 (SHP2); knockdown of this enzyme reduced both invasiveness and survival of RA FLS, suggesting a pivotal signaling molecule.[72]

Fibroblasts implanted with cartilage migrate to a contralateral cell-free implant and that subcutaneous, intraperitoneal, and intravenously injected fibroblasts will also migrate to sections of human cartilage, suggesting a tropism to damaged cartilage tissue. This important finding raises the question of which cell populations are grown from the synovium when tissue is digested and adherent cells are cultured in vitro: lining layer cells, sublining cells, or a mixture of both? From a methodologic perspective, answering this question is a challenge. However, we do know from transcriptomic approaches that the phenotype remains more stable in tissue culture than might be expected, with little transcriptional divergence over the first two to four passages and the level of differentially expressed genes between parallel cultures rising to greater than 10% only after passage 7.[74]

These models demonstrate the remarkably stable and disease-specific phenotype of cultured RA synovial fibroblasts, which includes high basal and stimulated expression of signature cytokines such as IL-6 and chemokines (discussed later).[75] RA synovial fibroblasts also express characteristic adhesion and immune-modulating molecules such as VCAM-1, galectin-3, and a specific repertoire of TLRs, which initiate innate immune cellular responses. A satisfactory molecular explanation for the stable phenotype of RA synovial fibroblasts has until recently evaded the field. However, epigenetic changes including DNA methylation; histone modifications such as acetylation, methylation, and citrullination; and altered micro RNA (miRNA) expression have now been suggested to underlie the observed persistent changes in fibroblast gene transcription and post-transcriptional repression (see Chapter 22). Further characteristic aspects of the RA FLS phenotype and their biology are discussed extensively in Chapter 69.

## Interactions of Fibroblasts with Leukocytes

### Recruitment of Inflammatory Infiltrates into the Joint

Stromal elements such as synovial fibroblasts are subject to a pro-inflammatory cytokine network within the inflamed synovium. Direct-contact interactions with other infiltrating cells such as T lymphocytes lead to high levels of expression of many inflammatory chemokines (see Figure 14-3). Neutrophil-attracting chemokines are expressed at high levels by stimulated fibroblasts and include CXCL8 (IL-8); CXCL5 (ENA-78); and CXCL1 (GRO-α).[76-78] Monocytes and T cells are recruited by a range of chemokines found at high levels in the synovium; CXCL10 (IP-10) and CXCL9 (Mig) are highly expressed in synovial tissue and fluid.[79] CXCL16 is also highly expressed in the RA synovium and acts as a potent chemoattractant for T cells.[80] CCL2 (MCP-1) is found in synovial fluid and is known to be produced by synovial fibroblasts; it is considered to be a pivotal chemokine for the recruitment of monocytes.[81] CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES) are chemotactic for monocytes and lymphocytes and are known products of synovial fibroblasts.[79,82] CCL20 (MIP-3α) is also overexpressed in the synovium and has a similar chemoattractant profile via its specific receptor, CCR6.[83] CX3CL1 (fractalkine) is also widely expressed in the rheumatoid synovium. A number of chemokine receptors have been shown to differ between peripheral blood and synovial leukocytes, suggesting that they are enriched in the synovium either though their selective recruitment by endothelial-expressed chemokines or after upregulation by the microenvironment after their recruitment.

### Fibroblast Support for Leukocyte Survival

Stromal cell support for the survival of leukocyte populations fulfills a physiologic role in certain organs within the body. The selective recruitment and support of hemopoietic subsets is an essential physiologic function of stromal cells in specific microenvironments. For instance, immature B lymphocytes are completely dependent on factors such as IL-6 produced by bone marrow stromal cells. Although the bone marrow niche plays a critical role in the early development of all hemopoietic leukocyte populations, it also acts as an active reservoir for terminally differentiated leukocyte subpopulations, including CD4 and CD8 T cells and neutrophils. The bone marrow stromal microenvironment therefore maintains not only the selective survival, differentiation, and proliferation of all lineages of immature hemopoietic cells but also, in some

cases, the survival of their mature counterparts. The stromal microenvironment plays a crucial role in the maintenance of such survival niches, which are not generic but are highly specific to certain organs and tissues, resulting in site-specific differences in the ability of different stromal cells to support the differential accumulation of leukocyte subsets.

In the case of an inflammatory response, successful resolution requires the removal of the vast majority of immune cells that were recruited and expanded during the active phase of the inflammation. A number of studies have shown that during the resolution phase of viral infections, the initial increase in T cell numbers in peripheral blood that is seen within the first few days is followed by a wave of apoptosis occurring in the activated T cells. This situation is mirrored within tissues, where apoptosis induced by the molecule Fas occurs at the peak of the inflammatory response and may be responsible for limiting the extent of the immune response. In contrast, the resolution phase appears to be principally triggered by cytokine-deprivation–induced apoptosis, during which leukocytes compete for a shrinking pool of survival factors provided by the microenvironment, leading to programmed death of those cells, which are surplus to requirements.

In RA the resolution phase of inflammation becomes disordered. Recent studies have shown that a failure of synovial T cells to undergo apoptosis contributes to the persistence of the inflammatory infiltrate. The T cell survival pathway shares all the essential hallmarks of a stromal cell, cytokine-mediated mechanism (high B cell lymphoma [Bcl]-$X_L$, low Bcl-2, and lack of cell proliferation). Type I IFNs (IFN-α and -β), which are produced by synovial fibroblasts and macrophages, have been identified as one of the principal factors responsible for prolonged T cell survival in the rheumatoid joint (Figure 14-5).[57] Interestingly, although type I IFN is beneficial in multiple sclerosis (a disease in which tissue scarring and low levels of T cell infiltrates are observed), these results suggest that type I IFN is not likely to be a successful therapy for persons with RA, a prediction that has been borne out in clinical trials.[84] It is likely that this mechanism of stromal cell–induced leukocyte survival occurs in many chronic inflammatory conditions in which T cells accumulate.

Not surprisingly, other leukocyte subpopulations have been shown to derive support from stromal cells. Although fibroblast support for T cell and B cell survival exhibits site-specific properties, neutrophil survival is dependent on prior cytokine activation of fibroblasts and shows no differences between fibroblasts taken from different anatomic sites.[75] Plasma cells are, of course, rescued from apoptosis within the bone marrow stem cell niche,[85] but mast cells of the gut are rescued by intestinal fibroblasts,[86] whereas dermal fibroblasts maintain Langerhans-like cells in the skin.[87] Fibroblast modulation of inflammatory and differentiation pathways in monocyte lineage cells has become a recent focus as the role of differentially polarized macrophage populations becomes more prominent. Recent transcriptomic analyses of co-cultured RA FLS and macrophages demonstrated not only that shared inflammatory pathways are present in both cell types but that co-cultured RA FLS modulate up to a third of TNF-regulated pathways in macrophages.[88]

### Fibroblast-Mediated Retention of Leukocytes in Tissue

Although inhibition of T cell death by stromal cells at sites of chronic inflammation contributes to T cell accumulation, it is unlikely to be the only mechanism because lymphocytes should be able to leave the inflamed tissue during the resolution of inflammation, even if their death is inhibited. A number of studies have recently reported that the synovial microenvironment contributes directly to the inappropriate retention of T cells within the joint by an active chemokine-dependent process. The presence of high levels of inflammatory chemokines, produced by stromal cells, is a characteristic of environments such as the rheumatoid synovium. However, recent data suggest that, paradoxically, constitutive chemokines, which are involved in the recruitment of lymphocytes to secondary lymphoid tissues, are ectopically expressed in immune-mediated inflammatory diseases. The constitutive chemokine CXCL12 (SDF-1) and its receptor CXCR4 emerged as unexpected but crucial players in the accumulation of T lymphocytes within the rheumatoid synovial microenvironment. This chemokine-receptor pair plays an important role, both in the constitutive traffic of lymphocytes and in the recruitment and retention of hemopoietic cells within the bone marrow. Unexpectedly, $CD45RO^+$ T lymphocytes in the rheumatoid synovium were found to express CXCR4 receptors at high levels in the rheumatoid synovium. The CXCR4 ligand CXCL12 was highly expressed on endothelial cells at the sites of T cell accumulation.[89,90] In addition, stromal cell–derived TGF-β is responsible for upregulation of CXCR4 receptors on T cells in the synovium.[89] Evidence also suggests that the stability of lymphocyte infiltrates is reinforced by a positive feedback loop whereby tissue CXCL12 promotes CD40 ligand expression on T cells, which in turn stimulates further CXCL12 production by CD40-expressing synovial fibroblasts. Furthermore, levels of CXCL12 secreted by synovial fibroblasts are controlled in part by T cell–derived IL-17.[91]

Therefore, clear evidence supports the hypothesis that aberrant ectopic constitutive expression of chemokines such as CXCL12, CCL19, and CCL21 by synovial stromal cells contributes to the retention of T cells within the RA synovium.

Other cell constituents of the rheumatoid inflammatory infiltrate may be affected by the CXCL12/CXCR4 axis. Blades and colleagues[92] have shown increased expression of CXCL12/CXCR4 by monocyte/macrophage cells in RA compared with OA. In addition, using implanted human synovial tissue in SCID mice, they demonstrated that monocytes are recruited into transplanted synovial tissue by CXCL12.[92] Contact-mediated B cell survival induced by synovial fibroblasts has also been shown to depend on CXCL12, B cell activating factor (BAFF)/BLyS, and CD106 (VCAM-1)–dependent mechanisms that are independent of TNF.[50,93] Overexpression of CXCL12 has also been identified as a distinct feature of RA, as opposed to OA synovia, using complementary DNA arrays. Data validating these findings in vivo have come from a collagen-induced arthritis model of RA in DBA/1 IFN-γ receptor deficient-mice, where administration of the specific CXCR4 antagonist AMD3100 significantly ameliorated disease severity.[94] In another murine collagen-induced arthritis model, the small

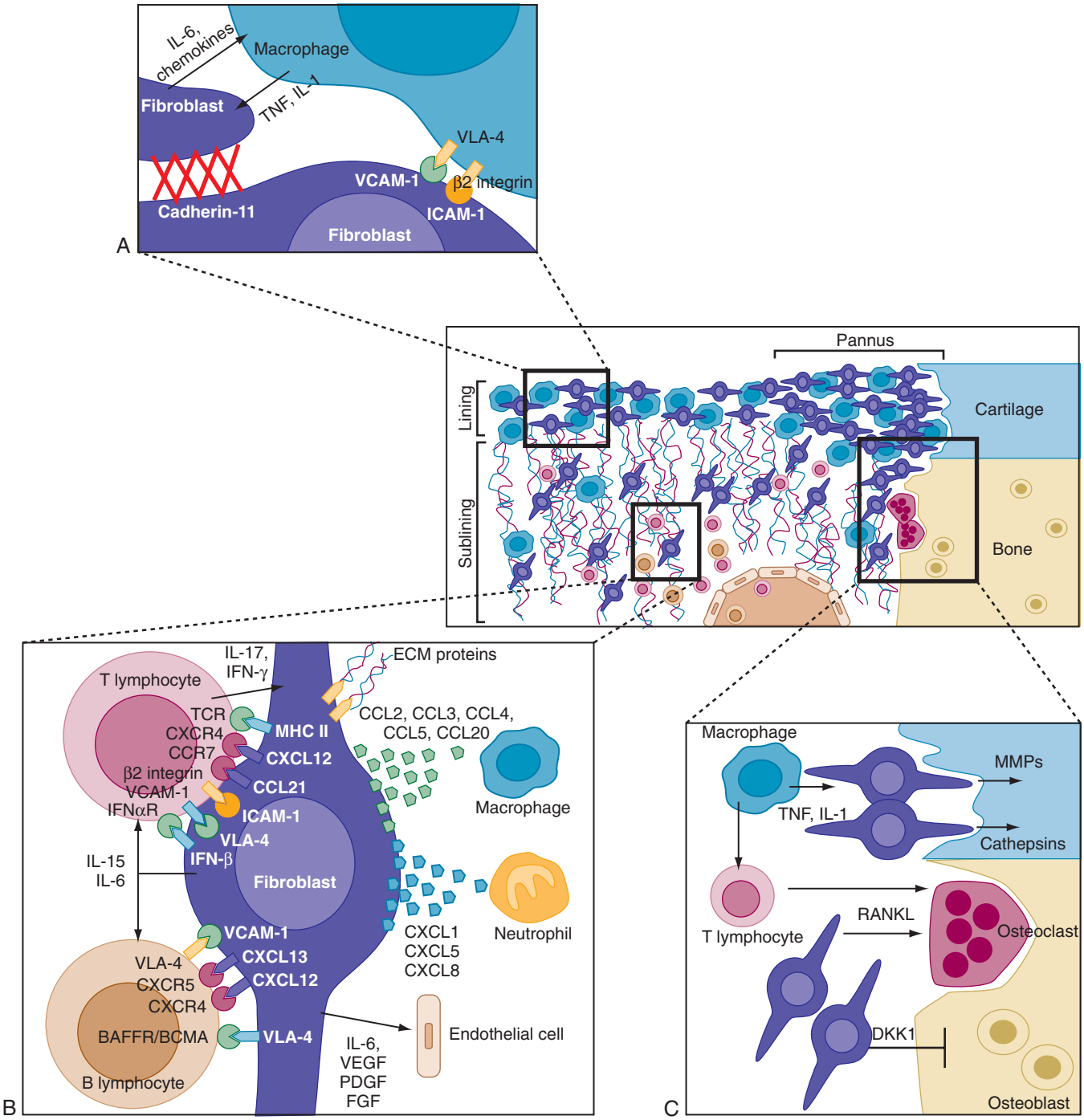



**Figure 14-5** Cell-cell interactions in the synovium. Synovial fibroblasts interact with multiple cell types in the rheumatoid arthritis (RA) synovium to maintain persistence of inflammation and continued joint destruction. **A,** Fibroblast-like synoviocytes in the RA synovial lining interact with macrophage-like synoviocytes through both secretion of soluble factors and cell surface receptor interactions to maintain lining layer structure and promote the activation of both cell types. Key soluble interactions include production of interleukin (IL)-1 and tumor necrosis factor (TNF) by macrophages and IL-6 by fibroblasts. Adhesive interactions consist of integrin-receptor interactions as described in the text and the critical presence of homotypic interaction through cadherin-11. **B,** Sublining synovial fibroblasts interact with numerous cell types including mast cells and plasma cells (not shown), T cells, B cells, interstitial macrophages, and endothelial cells, leading to their recruitment, retention, activation, and differentiation. Both cell surface receptor interactions and secreted mediators are important in this process. T cell–fibroblast interactions include T cell recruitment and retention by fibroblast-secreted chemokines such as CXCL12, CCL5, and CX3CL1 (fractalkine). In addition, fibroblasts may activate T cells through antigen presentation, co-stimulatory receptors (e.g., CD40 and intercellular adhesion molecule-1 [ICAM-1]), and cytokine secretion. Fibroblast cytokines such as IL-6 and IL-15 may be particularly important for differentiation of the Th17 T cell subset, and secretion of interferon (IFN)-β supports T cell survival. Fibroblasts, in turn, are activated by these cell surface interactions and by T cell cytokines, including IL-17 and IFN-γ. B cells are similarly recruited and retained through fibroblast secretion of chemokines such as CXCL12 and CXCL13 and through cell surface adhesion interactions (e.g., very late activation antigen-4 [VLA-4] and vascular cell adhesion molecule-1 [VCAM-1]). Critical survival and differentiation signals are maintained through fibroblast secretion of BAFF (BLyS) and APRIL. Neutrophils and monocyte/macrophage lineage cells are also recruited by fibroblast chemokine production. Macrophages, in turn, help activate synovial sublining fibroblasts through the production of cytokines such as IL-1 and TNF. Finally, synovial sublining fibroblasts promote angiogenesis through the production of proangiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) and may help direct endothelial recruitment of inflammatory cells through secretion of cytokines such as IL-6. **C,** Pannus tissue, an extension of the hyperplastic synovial lining consisting of both activated macrophage-like synoviocyte (MLS) and fibroblast-like synoviocyte (FLS), actively degrades both cartilage and bone through production of matrix degrading enzymes such as matrix metalloproteinases (MMPs) and cathepsins. In addition, fibroblasts and T cells secrete receptor activator of nuclear factor-κB ligand (RANKL), which promotes osteoclast differentiation and activation, leading to bone erosions. Furthermore, production of dickkopf-1 (DKK1) inhibits wnt signaling pathways, which normally promote anabolic osteoblast activity, preventing repair of bone erosions. CD40L, CD40 ligand; ECM, extra-cellular matrix; FGF, fibroblast growth factor; IFNαR, interferon-α/β receptor; MHC II, type II major histocompatibility complex; TCR, T cell receptor.

molecule CXCR4 antagonist 4F-benzoyl-TN14003 ameliorated clinical severity and suppressed delayed-type hypersensitivity (DTH) responses.[95] The CXCL12/CXCR4 constitutive chemokine pair therefore seems to play an important role in lymphocyte retention in RA.

These experiments demonstrate that understanding the behavior of fibroblasts and leukocytes within microenvironments necessarily requires that we model the interactions of all the cellular populations concerned. An elegant example of this approach in vitro is the work of Lally and colleagues, who developed a flow-based model of cellular recruitment to the rheumatoid synovium.[96] Co-culturing fibroblasts from skin and RA synovial membrane with endothelial cells showed that IL-6 released from synovial (but not skin) fibroblasts was able to induce production of chemokines and adhesion molecules, resulting in greater neutrophil recruitment by synovial fibroblasts. Subsequent work interrogating the system using low-density gene arrays demonstrated that the effect of neutrophil-attracting chemokines such as CXCL5 released from synovial fibroblasts was dependent on the function of the chemokine transporter molecule DARC (Duffy antigen receptor for chemokines), which was also induced by fibroblast-to-endothelial cell co-culture.[96]

### Constitutive Chemokines and Lymphoid Neogenesis

RA is one of a number of inflammatory diseases in which the organization of the inflammatory infiltrate shares characteristics of lymphoid tissue. Follicular hyperplasia with germinal center formation can occur in autoimmune thyroid disease, myasthenia gravis, Sjögren's syndrome, and RA and may occur during infection with *Helicobacter pylori* and *Borrelia burgdorferi*. The lymphoid infiltrates in the rheumatoid synovium can be divided into at least three distinct histologic groupings, varying from diffuse lymphocyte infiltrates through organized lymphoid aggregates to clear germinal center reactions. Moreover, there is conflicting evidence that such distinct histologic types correlate with other serum indicators of disease activity. This form of inflammatory lymphoid neogenesis relies on inappropriate but highly organized temporal and spatial expression by fibroblasts of the constitutive chemokines, particularly CXCL13 and CCL21, which are required for physiologic lymphoid organogenesis.

The elegant choreography of lymphocyte-stromal interactions within lymph nodes is organized by expression of adhesive and chemotactic cues in overlapping and combinatorial fashions. Once they have encountered new antigen, DCs specialized in the presentation of antigen to lymphocytes undergo a process of maturation under the local influence of inflammatory cytokines and bacterial and viral products. As a result, inflammatory chemokine receptors are downregulated and upregulation of the constitutive receptors CCR4, CCR7, and CXCR4 occurs, causing DCs to migrate into local draining lymphatics and thereby into peripheral lymph nodes. Trafficking of B and T cells is regulated by CXCL13 (BCA-1), its receptor CXCR5, and CCL21 and CCL19 (ELC), which are both CCR7 agonists. Within the lymph node, CXCR5-bearing B cells are attracted to follicular areas, whereas T cells and DCs are maintained within parafollicular zones by local expression of CCL21 and CCL19. Some T cells that have been successfully presented with their cognate antigen by DCs then upregulate CXCR5, allowing them to migrate toward and interact with B cells.[97-99]

The genesis of lymphoid follicular structures in diseases such as diabetes and RA appears to rely on expression of such constitutive chemokines, in association with the lymphotoxins α and β (LT-α and LT-β) and TNF.[100] In this context it is important to note that transgenic animals overexpressing the TNF gene display increased formation of focal lymphoid aggregates and develop a chronic arthritis similar to RA.[101] Clearly, one of the many mechanisms of action of TNF inhibitor therapy may be the dissolution of such aggregates. In transgenic mouse models, expression of CXCL13 in the pancreatic islets was sufficient for the development of T and B cell clusters, but because they lacked follicular dendritic cells, it was not sufficient for true germinal center formation.[102] CCL21 does appear to be sufficient in some cases for lymph node formation; murine pancreatic islet models have demonstrated formation of lymph node–like structures in the presence of CCL21 and lymphoid infiltrates in response to CCL19 expression. The degree of lymphoid organization seen in the rheumatoid synovium correlates with expression of the chemokines CCL21 and CXCL13, although these chemokines are also associated with less organized lymphoid aggregates.[103] Expression of CCL21 is restricted to a population of perivascular fibroblastic reticular cells with common phenotype and function in secondary lymphoid and inflammatory aggregate tissues.[104] CXCR5 is overexpressed in the rheumatoid synovium, consistent with a role in recruitment and positioning of B and T lymphocytes within lymphoid aggregates of the RA synovium. It therefore seems likely that expression of lymphoid-constitutive chemokines contributes significantly to the entry, local organization, and exit of lymphocytes in the RA synovium. It also seems that the ectopic expression of chemokines is a general characteristic of a number of chronic rheumatic conditions because another B cell–attracting chemokine CXCL13 (BCA-1) is inappropriately expressed by fibroblasts in the salivary glands of patients with Sjögren's syndrome.[105]

Interestingly, the ectopic lymphoid structures seen in RA are capable of appropriate secondary lymphoid tissue structures, including the production of class-switched high-affinity antibody production, as evidenced by the expression of activation-induced cytidine deaminase (AID), the enzyme required for somatic hypermutation and class switch recombination (CSR) of Ig genes.[106] CXCR3-expressing plasma cells are also present in the rheumatoid synovium, and their recruitment is once more supported by ectopic production of the CXCR3 ligand CXCL9 by fibroblasts, particularly in the sublining region where aggregates are located.[107]

## Role of Fibroblast Subsets in Disease

It is possible that the various potential sources of expanded populations of synovial fibroblasts discussed earlier may correspond with functionally diverse fibroblast lineages and subpopulations. Through use of microarray analysis, Kasperkovitz and collaborators showed that the transcriptional profile of RA synovial fibroblasts clustered into two

broad groups representing "high" (myofibroblastic) and "low" (growth factor producing) populations.[5] These clusters were shown to be representative of the heterogeneity and of the degree of inflammation in the tissue of origin, suggesting that transcriptionally and functionally distinct populations of fibroblasts exist in joints. For example, some $CD248^+$ cells may correspond with a pluripotential, stem cell–like population of pericytic cells lying in close apposition to endothelial cells, which provide a supply of new stromal cells during inflammation.[108] Interestingly, deletion or removal of the intra-cellular portion of CD248 can reduce stromal cell accumulation and ameliorate models of arthritis such as murine collagen antibody-induced arthritis (CAIA).[109] Furthermore, the prevailing conditions of hypoxia within the rheumatoid synovium may enhance expression of CD248, which is regulated by hypoxia-inducible factor-2 (HIF-2) binding to a hypoxia response element, and which in turn participates in angiogenesis.[110]

Whether such markers remain associated with functionally distinct subpopulations or simply contribute to a larger local pool of multipotential mesenchymal precursors is as yet unknown, but the discovery of markers apparently linked to function has provided the tools with which such questions can be answered. The surface marker gp38 (podoplanin) is a potential therapeutic target that marks populations of fibroblasts (fibroblastic reticular cells) that modulate trafficking of immune cells such as dendritic cells within the lymph node.[48] The surface marker gp38 is highly expressed in lining layer fibroblasts in RA, and its synovial expression has been shown to be reduced during TNF blockade. In vitro data support a relationship with inflammation, because gp38 expression is induced by pro-inflammatory cytokines, whereas silencing reduces IL-6 and IL-8 production.[111,112] The results of studies demonstrating the association of stromal subpopulations with disease outcomes and response to therapy are eagerly awaited, as are the first attempts to target stromal markers therapeutically. The other group of markers that has been associated with synovial fibroblasts has come from the field of oncology.

## Epigenetic Regulation of Fibroblast Gene Expression in Rheumatic Disease

Epigenetic regulation implies heritable alterations in regulation of gene transcription in the absence of genetic mutation. The epigenetic code, itself regulated by dedicated enzyme complexes, consists of two main groups of covalent modifications: first, methylation and hydroxymethylation of CpG dinucleotides in DNA gene promotor regions, and second, multiple modifications of the histone proteins around which DNA is packaged in chromatin, including acetylation, methylation, and citrullination[113]; these modifications control the access of transcription complexes to chromatin. Global changes in DNA methylation have been recorded in RA FLS, paralleling those seen in human tumors; using demethylating agents to induce global DNA demethylation in "normal" synovial fibroblasts induces genes suggestive of change toward an RA FLS-like phenotype.[114] Similarly, global changes in histone acetylation and levels of the histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes that are responsible for their control have been demonstrated in RA FLS.[115,116] HDAC inhibitors have also been suggested to show beneficial effects in vitro and in ex vivo models using synovial tissue.[117,118]

Transcriptomics has been used to identify genes overexpressed in RA FLS, which has identified promoter hypomethylation in the critical T-box transcription factor 5 *(TBX5)* gene and specific local histone modifications such as histone 4 lysine 4 trimethylation that correspond with open chromatin structure and active transcription. TBX5 emerges as a target of cytokine (IL-1β) and TLR stimulation and a driver of multiple pathways, including chemokine expression, thus identifying a key element in the epigenetic regulation of established RA FLS.[119] Other genes recently identified as subject to epigenetic regulation in RA FLS include the cytokine IL-6[120] and the chemokine CXCL12.[121] Cytokine stimulation can regulate promoter DNA methylation, demonstrating a reversible, short-term hypomethylation of candidate genes in response to IL-1β in OA and RA fibroblasts mediated via changes in DNA methyltransferase activity. However, although this suggests that pro-inflammatory cytokines can drive epigenetic regulation, the repertoire of promoters modified by IL-1β exposure was limited, suggesting that additional factors influence the persistent changes seen in long-standing disease.[122] Whole methylome comparative analysis of RA FLS and OA FLS reveals epigenetic change in key genes, including the key signaling component STAT3, and MAP3K5 (ASK1), a key component of TLR-induced inflammatory pathways and apoptosis in response to cellular stress.[123,124]

Rapid advances helped define the altered, profibrotic phenotype of fibroblasts in systemic sclerosis. Once again initial studies have focused on genome-wide regulation; blockade in dermal fibroblasts of histone 3 lysine 27 trimethylation, a well-characterized histone modification associated with gene silencing, led to dramatic increases in collagen release in vitro and in animal models of fibrosis.[125] Focused work on proteins interacting with Wnt pathways that regulate both bone turnover and fibrotic processes revealed hypermethylation of the promoters of DKK1 and SFRP1. Use of the whole genome demethylating agent 5-aza-cytidine reversed this modification and ameliorated experimental fibrosis.[126] Although this represents a non-specific approach to modifying regulation of individual genes, 5-aza-cytidine is already in use in the treatment of multiple myeloma, and such drugs are the precursors of newer agents that will target regulation of specific groups of genes via blockade of regulatory subunits of the chromatin modifying complexes that generate and remove epigenetic modifications.[127]

Proof of the concept that epigenetic regulation determines disease phenotype comes from a recent genome-wide DNA methylation study comparing fibroblasts from scleroderma patients with diffuse disease, patients with limited disease, and control subjects. Not only were differentially methylated DNA sites found between the groups, but these sites were also frequently related to key genes in fibrotic pathways, including the metalloprotease ADAM12, collagen genes, and transcription factors of the RUNX family that are known to drive collagen synthesis. Multiple additional candidates identified by this study will further elucidate the roles and interactions of fibroblasts in scleroderma.[128]

Targeting global epigenetic change, although a potential therapeutic in the context of a hematologic malignancy such as myeloma, is unlikely to be acceptable in the treatment of chronic inflammatory disease, as reflected in the high levels of adverse events seen in early phase trials in persons with juvenile idiopathic arthritis (JIA).[129] Our ability to target specific epigenetic modification complexes and thus influence individual genes or groups of genes is currently limited[130] but will rapidly expand over coming years.

### MicroRNAs and Fibroblast-like Synoviocytes

MiRNAs are small, approximately 22-nucleotide-long RNAs that regulate the expression of multiple genes through interactions with both the 3′ untranslated region (UTR) of gene transcripts and RNA-induced silencing complexes.[131] This in turn interferes with translation of messenger RNA (mRNA) into protein or induces mRNA degradation. Currently around 1900 miRNAs have been identified in humans, although many of the target genes of miRNAs are yet to be described.

MiRNAs are frequently grouped alongside epigenetic modifications as key controllers of altered gene expression in disease. Underlying this association is the high degree of epigenetic regulation of miRNA genes. This phenomenon has been demonstrated at the level of individual genes, as in the case of miR-203, which is overexpressed in RA FLS under the control of DNA methylation, leading to enhanced expression of MMPs and IL-6.[132] A similar regulatory relationship has also been demonstrated at a whole genome level: Comparing RA FLS and OA FLS, a range of differentially expressed miRNAs were identified and shown to be in close proximity to differentially methylated CpG rich regions of DNA, suggesting that differences in miRNA profiles are closely related to DNA methylation. These differential CpG region methylation and miRNA expression data sets have been further combined in silico to predict networks of miRNA targets that may be modified in the aggressive RA FLS phenotype.[133]

Two miRNAs, miR-146a and miR-155, are vital for immune function, are overexpressed in RA FLS compared with OA FLS, and are subject to induction by cytokines and TLR ligands. The miRNA miR-155 drives changes in MMP repertoire,[134] although in vitro studies suggest a regulatory role leading to overall repression of MMP production.[135] The importance of miR-146a in anti-inflammatory regulatory networks has been highlighted through work with the arthritogenic Chikungunya virus. Chikungunya virus–infected synovial fibroblasts upregulate miR-146a, which in turn downregulates pro-inflammatory signaling factors such as TRAF6, IRAK1, and IRAK2, resulting in a corresponding decrease in NF-κB phosphorylation. This finding highlights the critical involvement of this miRNA in the regulation of inflammation.[136]

Increased RA FLS survival is linked to miRNAs; the miR-34 promoter becomes hypermethylated in RA FLS, leading to reduced responsiveness to FasL and TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.[137] More recently, mutations in p53 in RA have been linked to miRNA-regulated proliferation of RA FLS; mutated forms of p53 are not capable of driving the expression of miR-22, which represses the pro-proliferation protein Cry61. This finding fits with observations of decreased miR-22 expression in RA compared with OA synovial tissue, providing a potential explanation for increased RA FLS numbers in the synovium.[138] MiR-20 has also been implicated in regulation of cell survival and inflammation; overexpression of MiR-20 in RA FLS reduced ASK1 mRNA stability and inhibited LPS-induced IL-6 and CXCL10 expression.[139] This finding adds to the increasing data demonstrating a role for miRNAs in regulation of TLR-induced responses; for example, previous work has shown that miR-19a/b regulate TLR2 expression in RA FLS.[140]

Modulation of miRNA expression has significant therapeutic potential as a means of regulating networks of related genes rather than current approaches targeting single cytokines or cell types. Multiple approaches are being taken to develop miRNA therapeutics, including administering exogenous miRNAs and treatment with compounds that either stimulate or inhibit the expression of endogenous miRNAs. For instance, the compound denbinobin upregulates the expression of miR-146a, which in turn ablates the responsiveness of OA FLS to IL-1β.[141]

## Lessons Learned from Cancer

Alongside the field of inflammation, oncology has also been experiencing a surge in interest in the biology of fibroblasts and stromal cells, as well as the mechanisms by which they interact with primary transformed tumor cells.[142] A number of important cytokines that contribute to cancerous transformation of healthy cells by so-called cancer-associated fibroblasts have been described, including hepatocyte growth factor (HGF) and TGF-β. Crucially, tumor-associated fibroblasts appear able to transform normal cells, in addition to premalignant cells.[142] The importance of tumor-associated fibroblasts, termed cancer-associated fibroblasts (CAFs), has been demonstrated in breast cancer: human breast cancer implants were unable to grow successfully when implanted into mice without their co-administration with human tumor–derived fibroblasts.[143] Intriguingly, similar molecular signals have been implicated in the predilection for cancer cells to metastasize to certain sites. In particular, the ectopic expression and function of the CXCL12/CXCR4 ligand-receptor pair, in a manner reminiscent of RA, has been implicated in the persistence and tissue tropism of metastatic cells in breast cancer. Tumor-associated fibroblasts secrete CXCL12, resulting in increased promotion of carcinoma cell proliferation, migration, and invasion compared with control fibroblasts but also leading to recruitment of endothelial cell precursors.[144,145]

Furthermore, molecules that mark fibroblast subpopulations in the joint are associated with active, invasive cancer. These molecules include the expression of tumor-associated stromal markers such as fibroblast activation protein (FAP),[12, 13] galectin-3,[146,147] and S-100A4.[148] Interestingly, galectin-3 has been shown to be subject to epigenetic regulation.[147,149] Both gp38 and CD248 are also heavily implicated in tumor progression.[9,150]

These similarities between RA synovial fibroblasts and CAFs overlap with observations that the persistent phenotype of RA synovial fibroblasts itself includes elements

normally associated with "transformed" cells. These elements include loss of density and anchorage limitation for growth, which usually curtails in vitro fibroblast culture, firm adherence to ECM components of cartilage, and the invasiveness that is most aptly demonstrated in the chimeric SCID mouse model. Another defining characteristic of RA synovial fibroblasts that helps to explain their phenotype is dysregulation of proto-oncogenes and tumor suppressor genes. Once again, epigenetic regulation is likely to underlie this phenotype. However, the precise mechanisms maintaining the persistent phenotype at a whole genome level are yet to be elucidated.

Furthermore, RA is a systemic disease involving multiple joints; therefore, whether the fibroblast phenotype results from a global change in fibroblast gene expression or whether the phenotype is locally imprinted by exposure to a characteristic cytokine, matrix, and cellular milieu is yet to be established. Recent data now appear to confirm that human RA synovial fibroblasts within the SCID mouse model may travel systemically through lymphatics and the bloodstream to unpopulated samples of cartilage and then invade.[19] Therefore, at least one possibility is that locally imprinted, "activated" fibroblasts may export destructive arthritis to joints where mild injury or immune response has occurred over time. In the cancer field, the concept of tumor stroma "normalization" has now become an accepted aspect of new oncology therapies. Clinical studies of angiogenesis inhibitors and antibodies against ECM components such as tenascin have been favorable, whereas inhibitors of MMPs, overexpression of TIMPs, and blockade of integrin signaling have all shown promise in pre-clinical trials.[151] Results of studies examining the interactions between endothelial cells and their associated pericytes underlie the importance of targeting the stroma as a whole. Bergers and colleagues[152] have shown that endothelial cells release PDGF, which induces VEGF production from pericytes, leading to bidirectional conversations between the two cell types. Interrupting these conversations by using PDGF inhibitors proved to be more effective therapy than using VEGF inhibitors alone. Interestingly, although VEGF inhibitors lost their inhibitory effect in later stage tumors, targeting of the pericytes helped even late-stage tumors to regress.[152] The authors have subsequently shown that pericyte precursors are partly recruited from the bone marrow to tumor perivascular sites.[153]

The cyclin-dependent kinases (CDKs) are a family of enzymes that maintain cellular proliferation and survival under tight control, balanced by specific inhibitors (CDKis). CDK dysregulation has been demonstrated in many tumors, and their manipulation has consequently been exploited to develop anticancer drugs including the CDKi roscovitine.[154] RA fibroblasts express low levels of the CDKi p21,[155] and adenovirus-mediated p21 gene transfer into RA fibroblasts both induces cell cycle arrest and downregulates expression of cytokines, MMPs, and cathepsins,[156] suggesting a novel therapy targeting a disease-specific phenotype in RA FLS. Inhibition of TNF pathways and CDK inhibition have recently been shown to synergize in ameliorating murine collagen-induced arthritis without increasing immune suppression. This approach, currently in early phase trials in RA, may therefore have significant benefits.[157]

## CONCLUSION

Fibroblasts are structural mesenchymal cells that form the cellular infrastructure for most internal organs, as well as for bordering membranes such as the synovial membrane. They are prominently involved in the deposition and resorption of the ECM and thus are responsible for maintaining tissue homeostasis. However, fibroblasts are far more than structural, passively responding cells that build the "backbone" for organ-specific function. Rather, they are sensitive to environmental changes. They react in a specific manner to a variety of stimuli and are capable of actively influencing not only the composition of the ECM but also the cellular composition of tissues and barrier membranes. Under inflammatory disease conditions, fibroblasts act as organ-specific, innate immune system sentinel cells and are involved in the progression of organ damage, as well as in the switch from acute resolving to chronic persisting inflammation. We now know that functionally distinct fibroblast subsets exist and can be identified with new markers to understand better mechanisms of developmental patterning, wound healing, and persistent inflammatory responses, which appear to depend in large part on epigenetic modifications. This notion is particularly true for fibroblast-like synoviocytes, which play a critical role in the pathogenesis of RA and possess a characteristic, invasive, and activated phenotype. In addition to contributing to the recruitment of inflammatory cells to the joint, they modulate the survival and behavior of these cells and are, in turn, regulated by the newly recruited cells. More importantly, fibroblast-like synoviocytes are crucial components in the hyperplastic lining layer and in cartilage destruction. New data raise the possibility of epigenetically programmed aggressive cells exporting arthritis from inflamed to uninflamed joints in the early stages of arthritis, but at the same time offering the possibility of specifically targeting stromal subpopulations of choice.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 15



# Mast Cells

Peter A. Nigrovic

**KEY POINTS**

- Mast cells arise in the bone marrow, circulate as immature precursors, and develop into functional mast cells after entering peripheral tissues.
- The phenotype of mast cells is diverse, plastic, and governed by signals from lymphocytes, fibroblasts, and other elements of the microenvironment.
- In healthy tissues, mast cells serve as immunologic sentinels and participate in innate and adaptive immune responses to bacteria and parasites.
- Mast cells accumulate in injured and inflamed tissue, where they may amplify or suppress inflammation.
- Mast cells have been implicated in multiple immune-mediated diseases, including inflammatory arthritis, although their role as a therapeutic target remains uncertain.

Although mast cells are best known for their role in allergy and anaphylaxis, the immune function of this bone marrow–derived lineage far outstrips its participation in immunoglobulin (Ig)E-driven disease. Mast cells are resident broadly in vascularized tissues but cluster near interfaces with the external world, in the linings of vulnerable body cavities, and near blood vessels and nerves. In these locations, mast cells serve as immune sentinels and are equipped with an array of pathogen receptors, along with an armamentarium of mediators capable of rapidly recruiting immune effector cells. Mast cells also accumulate in sites of tissue injury and chronic inflammation, although their role in such locations remains uncertain. Other functions for this lineage, conserved by evolution for more than 500 million years, continue to be defined.

Circumstantial and experimental evidence implicates mast cells in the pathogenesis of rheumatic diseases. Mast cells reside constitutively in the normal synovium and are found in large numbers in inflamed synovial tissue, whereas mast cell mediators are identified in inflammatory joint fluid. Moreover, models have indicated that mast cells can make an important contribution to the pathogenesis of experimental arthritis. Mast cells have also been implicated in other autoimmune conditions, including multiple sclerosis, bullous pemphigoid, and systemic sclerosis.

## BASIC BIOLOGY OF MAST CELLS

### Development and Tissue Distribution

Mast cells are distinctive in appearance. Ranging in size from 10 to 60 μm and with a centrally located round or oval nucleus, their abundant cytoplasm is filled with multiple small granules. They were named *Mastzellen* in 1878 by the German pathologist Paul Ehrlich, who believed that they were overfed connective tissue cells (*mästen*, in German, means to feed or fatten an animal).[1] Electron microscopy reveals that the plasma membrane of the mast cells exhibits multiple thin cytoplasmic extensions, providing a broad interface with the surrounding tissue (Figure 15-1A). The tissue distribution of mast cells is extensive; within tissue, mast cells tend to cluster around blood vessels and nerves and near epithelial and mucosal surfaces. They are also found in the lining of vulnerable body cavities such as the peritoneum and the diarthrodial joint. Given this localization, mast cells are among the first immune cells to encounter pathogens invading into tissue from the external world or via the bloodstream, consistent with their role as immune sentinel cells.

Mast cells are of hematopoietic origin, arising in the bone marrow and depositing in tissues after migrating through the bloodstream[2,3] (Figure 15-2). Unlike other myeloid cells, such as monocytes and neutrophils, mast cells do not terminally differentiate in the bone marrow but rather circulate as progenitors, bearing the surface signature $CD34^+/c\text{-}Kit^+/CD13^+$.[4] Further developmental details have been worked out most extensively in the mouse. A burst of circulating progenitors late in gestation suggests the possibility that mast cells may populate the tissues primarily during early development, with later recruitment restricted to the inflammatory context, as appears to be the case for certain populations of murine tissue macrophages.[3] After entering the tissues, murine mast cells may mature into classic granulated cells or remain as ungranulated progenitors, awaiting local signals to differentiate fully. Comparison of murine lung and intestine has demonstrated that these tissues use distinct pathways to regulate the constitutive and inducible recruitment of mast cell progenitors, illustrating that mast cell homing is a precisely controlled process.[5,6]

Once resident in tissues, mast cells may live for many months. Mature mast cells remain capable of mitotic division, although recruitment of circulating progenitors appears

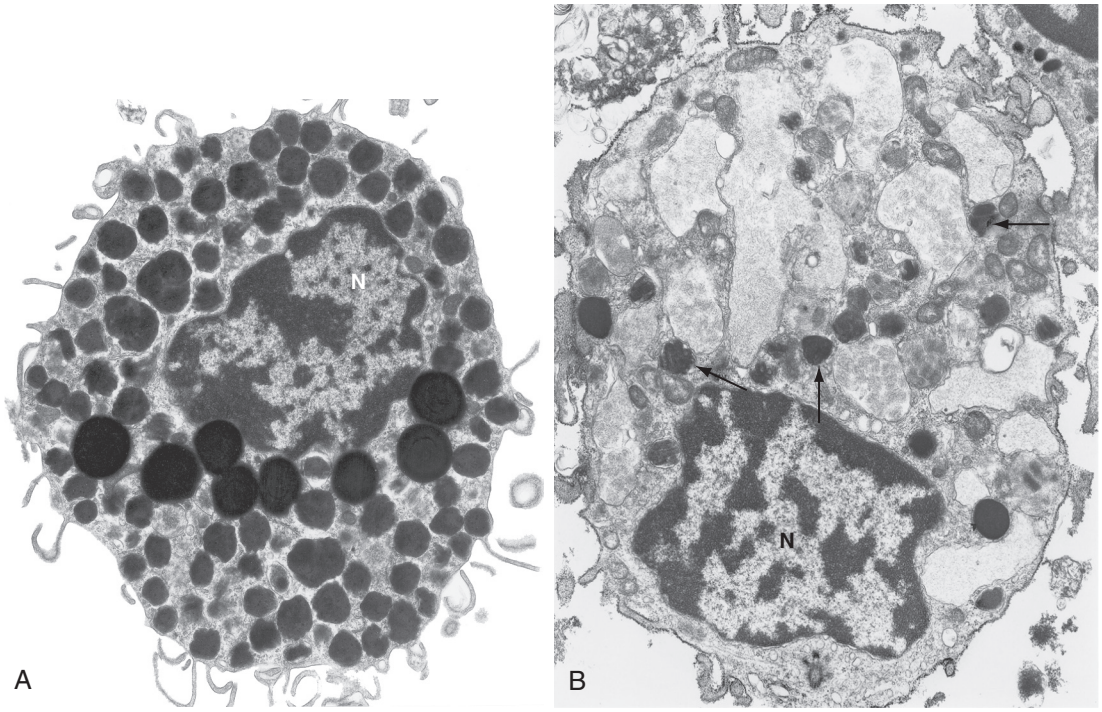



**Figure 15-1** Mast cell morphology. **A,** An intact mast cell. **B,** A mast cell that has undergone anaphylactic degranulation. Note how fusion of intracellular granules has resulted in the formation of a labyrinth of interconnected channels by which granule contents may be expelled from the cell. Arrows indicate remaining granules. N, Nucleus. *(Images courtesy Dr. A. Dvorak, Beth Israel Deaconess Medical Center, Boston, Mass. From Dvorak AM, Schleimer RP, Lichtenstein LM: Morphologic mast cell cycles,* Cell Immunol *105:199–204, 1987; and Galli SJ, Dvorak AM, Dvorak HF: Basophils and mast cells: morphologic insights into their biology, secretory patterns, and function. In Ishizaka K, editor:* Progress in allergy: mast cell activation and mediator release, *Basel, 1984, S Karger, pp 1–141.)*

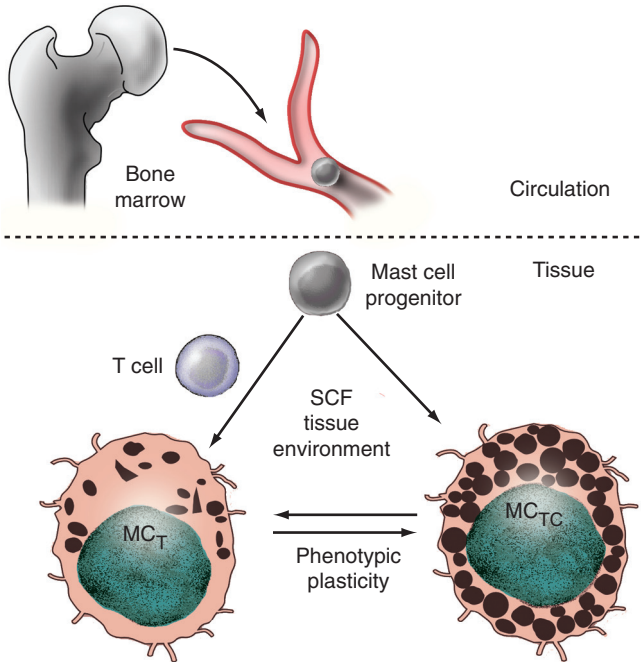



**Figure 15-2** Mast cell origin and differentiation. Mast cells arise in the bone marrow, circulate as committed progenitors, and differentiate into mature mast cells upon entering tissue. Human mast cells may be classified on the basis of granule proteases into tryptase$^+$ mast cells ($MC_T$) and tryptase$^+$/chymase$^+$ mast cells ($MC_{TC}$), with characteristic tissue localization and mediator production. SCF, Stem cell factor.

to greatly exceed local replication as a pathway to expand the number of tissue mast cells. Mechanisms of reducing mast cell numbers include apoptosis, as demonstrated in tissue mast cells deprived of the cytokine stem cell factor (SCF), a critical survival signal for mast cells.[7] Under certain conditions, mast cells can also emigrate via the lymphatics, appearing in draining lymph nodes much in the manner of dendritic cells.[8] The pathways that license mast cell accumulation in inflamed tissues are incompletely defined but include tissue production of factors such as interleukin (IL)-4 and IL-33 that attenuate mast cell apoptosis.[9,10]

## Mast Cell Heterogeneity: Common Progenitor, Multiple Subsets, and Phenotypic Plasticity

Whereas all types of mast cells derive from a common progenitor lineage, the phenotype of fully differentiated tissue mast cells is heterogeneous. Human mast cells are conventionally divided into two broad classes on the basis of the protease contents of their granules (Figure 15-2). $MC_{TC}$ display rounded granules that contain the enzymes tryptase and chymase, whereas the smaller, and more irregularly shaped granules of $MC_T$ contain tryptase but not chymase. $MC_{TC}$ also express other proteases, including carboxypeptidase and cathepsin G. These subtypes differ in tissue distribution. $MC_{TC}$ tend to be found in connective tissue, such as in normal skin, muscle, the intestinal submucosa, and the normal synovium, whereas $MC_T$ predominate in

mucosal sites, including the lining of the gut and respiratory tract, although in fact both are present in many locations. Beyond the protease signature, other differences between these subsets include their profile of cytokine elaboration, as well as cell surface receptor expression; however, tissue-specific phenotypic differences are noted within each type as well (i.e., neither $MC_{TC}$ nor $MC_T$ are homogenous cellular subsets).

The relationship between $MC_{TC}$ and $MC_T$ mast cells is controversial. Are they committed subsets, akin to CD4 and CD8 lymphocytes, or are they functional states assumed by mast cells under the influence of the microenvironment? In mice, in which an analogous distinction exists between connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs), evidence for phenotypic plasticity is strong. Both in culture and in vivo, single CTMCs may differentiate into (or give rise to) MMCs and vice versa.[11,12] Mast cells with intermediate protease expression are found, and serial observations suggest that exposure to an inflammatory stimulus can induce progressive change from one class to another, although whether this change occurs at a single-cell level has not been definitively established.[13] Similarly, in murine and human mastocytosis, clonally expanded mast cells display divergent phenotypes depending on the tissue of residence.[14,15] In aggregate, these data favor the hypothesis that mast cells assume a particular phenotype under the control of the local signals but can alter this phenotype if conditions change.

## Stem Cell Factor

One of the most important signals from tissue to local mast cells is SCF. The receptor for SCF, c-Kit, is expressed widely on hematopoietic lineages early in differentiation, but among mature lineages, only mast cells express c-Kit at a high level. Stimulation of mast cells by SCF promotes maturation and phenotypic differentiation, blocks apoptosis, and induces chemotaxis. It may also activate mast cells directly to release mediators. In both mice and humans, SCF remains an irreplaceable survival signal for tissue mast cells. Accordingly, mice with defects in SCF or c-Kit are strikingly deficient in mature tissue mast cells (examples include $W/W^v$, $Sl/Sl^d$, and $W^{sh}$ mouse strains). Similarly, clonal mast cells obtained from patients with systemic mastocytosis commonly exhibit activating mutations in *c-kit*.

SCF occurs in two alternate forms resulting from differential mRNA splicing: soluble and membrane bound. The importance of this latter form is clear from $Sl/Sl^d$ mice, which lack only the membrane-bound isoform yet exhibit very few tissue mast cells.[16] SCF is synthesized by multiple lineages, including mast cells themselves. Expression by fibroblasts is especially important, given the intimate physical contacts observed between fibroblasts and mast cells in situ. The SCF/c-Kit axis mediates cell-cell adhesion between mast cells and fibroblasts independent of the kinase activity of the receptor.[17] Rodent mast cells co-cultured with fibroblasts demonstrate enhanced survival, connective-tissue phenotypic differentiation, and heightened capacity to elaborate pro-inflammatory eicosanoids, effects mediated at least in part by direct contact, including interactions between SCF and c-Kit.[18,19] The extent of similar regulation in human mast cells is uncertain.[20] Expression of SCF has also been documented on other lineages including macrophages, vascular endothelium, and airway epithelium and is likely a critical pathway by which tissues modulate the local mast cell population.

## T Lymphocytes and Other Cells

T lymphocytes exert a profound effect on mast cell phenotype. Severe combined immunodeficiency (SCID) mice that lack T cells fail to develop mucosal mast cells, a defect that can be corrected by T cell engraftment.[21] An analogous observation has been made in humans deficient in T cells due to congenital immunodeficiency or acquired immunodeficiency syndrome (AIDS). Intestinal biopsy in these patients shows that MMCs ($MC_T$) are strikingly reduced, whereas CTMCs ($MC_{TC}$) are present in normal numbers.[22] The pathways by which T cells exert this striking effect are not defined, although it is clear that T cell cytokines such as interleukin (IL)-3, IL-4, IL-6, IL-9, and transforming growth factor (TGF)-β may have profound effects on the phenotype of mast cells matured in culture. By contrast, interferon (IFN)-γ inhibits mast cell proliferation and may induce apoptosis. These observations imply that cells recruited to an inflamed tissue may have a profound impact on the phenotype of local mast cells. The rheumatoid synovium may well exemplify this phenomenon. The synovium is normally populated by $MC_{TC}$ mast cells, but large numbers of $MC_T$ mast cells are identified in the inflamed synovium, typically in regions rich in infiltrating leukocytes, whereas $MC_{TC}$ mast cells reside in deeper, more fibrotic areas of the joint.[23] Interestingly, regulatory T cells (Treg cells) can also have a direct impact on mast cell function, including recruitment, receptor expression, degranulation, and cytokine production.[24-27]

Other cells aside from T cells can potentially interact with mast cells in the tissues. In particular, fibroblasts and mast cells commonly demonstrate close physical interactions. Beyond SCF, fibroblasts elaborate cytokines such as the IL-1 family member IL-33, which can exert effects on mast cell protease expression, effector phenotype, and survival.[10,28,29] Dendritic cells have also been implicated in the recruitment of mast cells into inflamed tissues.[30]

## Different Functions for $MC_T$ and $MC_{TC}$ Mast Cells

The preservation of distinct types of mast cells in multiple species implies distinct and nonoverlapping roles for these subtypes. However, the understanding of functional differences between $MC_T$ and $MC_{TC}$ remains limited. One hypothesis is that $MC_T$ play a pro-inflammatory role and $MC_{TC}$ specialize in matrix remodeling.[31] This hypothesis makes sense of (1) the promotion of $MC_T$ development by T cells patrolling the tissues; (2) the partition of $MC_T$ and $MC_{TC}$ mast cells to inflamed and fibrotic areas, respectively; and (3) the preferential expression of the pro-inflammatory mediators IL-5 and IL-6 by $MC_T$ and the profibrotic IL-4 by $MC_{TC}$.[32] Not all observations fit comfortably into this dichotomy, however. For example, the potently pro-inflammatory anaphylatoxin receptor C5aR (CD88) is expressed on $MC_{TC}$ but not on $MC_T$ [33]. Ultimately, too little is known about the actual functional importance of these subsets to permit firm conclusions.

## Mast Cell Activation

### Immunoglobulin E

The canonical pathway to mast cell activation is via IgE and its receptor FcεRI. With a $K_a$ of $10^{10}$/M, this receptor is essentially constantly saturated with IgE at typical serum concentrations. Such binding not only sensitizes mast cells to the target antigen but also helps promote mast cell survival and, in some cases, cytokine production. Cross-linking FcεRI-bound IgE by multivalent antigen induces a brisk and vigorous response. Within minutes, granules within the mast cell fuse together and with the surface membrane, creating a set of labyrinthine channels that allow rapid release of granule contents (see Figure 15-1B).[34] This compound exocytosis event, termed *anaphylactic degranulation*, is followed within minutes by the elaboration of eicosanoids newly synthesized from arachidonic acid cleaved from internal membrane lipids. Finally, signals transduced via FcεRI induce the transcription of new genes and elaboration of a wide range of chemokines and cytokines (Figure 15-3). Upon termination of the stimulation event, the surface membrane closes over the granule-formed channels, which subsequently bud off within the cytoplasm, re-creating discrete granules using the original membranes.[34] These granules are then recharged with mediators in a process that occurs gradually over days to weeks.[35]

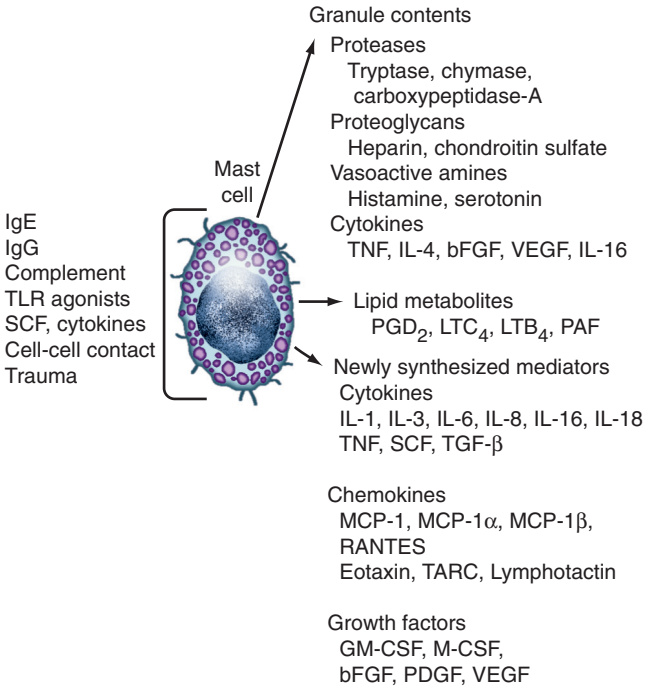



**Figure 15-3** Mediator production by human mast cells (partial list). The set of mediators liberated upon activation will vary depending on the state of differentiation of the mast cell and the nature of the stimulus. See Reference 91 for a complete mediator list and references. bFGF, Basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; Ig, immunoglobulin; $LTB_4$, leukotriene $B_4$; $LTC_4$, leukotriene $C_4$; MCP, monocyte chemoattractant protein; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; $PGD_2$, prostaglandin $D_2$; RANTES, released upon activation, normal T-cell expressed and secreted; SCF, stem cell factor; TARC, thymus and activation-regulated chemokine; TGF-β, transforming growth factor-β; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

### Immunoglobulin G and Immune Complexes

IgE is only one among many pathways of mast cell activation. One key trigger for mast cell activation in both human and mouse is IgG, acting via receptors for the Fc portion of IgG (FcγR). The importance of this pathway was demonstrated first in mice rendered genetically deficient in IgE. Contrary to expectations, these animals remain susceptible to anaphylaxis mediated through IgG and the low-affinity IgG receptor FcγRIII.[36,37] The human counterpart of this receptor, FcγRIIa, is equally capable of inducing activation of human mast cells.[38,39] Human mast cells can also be induced to express the high-affinity IgG receptor FcγRI, rendering them susceptible to IgG-mediated activation.[40,41]

These IgG receptors contribute to involvement of mast cells in IgG-driven diseases. Thus, in the mouse, mast cells participate in IgG-mediated immune complex peritonitis, the cutaneous Arthus reaction, and experimental murine bullous pemphigoid.[42-44] Activation via Fc receptors also mediates mast cell participation in antibody-mediated murine arthritis.[45]

### Soluble Mediators and Cell-Cell Contact

Mast cells coordinate with immune and nonimmune cell lineages via mechanisms beyond antibody response, including soluble mediators and surface receptors. Examples of such signals include the cytokine tumor necrosis factor (TNF) and the neurogenic peptide substance P, which can induce mast cell degranulation.[46,47] Physical contact with other cells can also induce mast cell activation. For example, CD30 on lymphocytes can interact with CD30L on mast cells to induce the production of a range of chemokines.[48] Interestingly, ligation of CD30L does not induce release of granule contents or lipid mediators, illustrating the selectivity of response of which mast cells are capable.

### Danger and Injury

Mast cells are equipped to recognize danger in the absence of guidance from other lineages via a range of pathogen receptors, including multiple toll-like receptors (TLRs) and CD48, a surface protein recognizing the fimbrial antigen FimH. These receptors are implicated in the response of mast cells to pathogens and could potentially contribute to mast cell engagement in diseases such as atopic dermatitis, in which skin becomes abnormally colonized with bacteria.[49,50] Mast cells may also be activated through complement, including the anaphylatoxins C3a and C5a.[39,47] Mast cells can respond directly to physical stimuli including trauma, temperature, and osmotic stress.[51] Finally, mast cells can be triggered by danger signals released by injured bystander cells, such as IL-33 and monosodium urate crystals.[52-54] Together, these receptors enable mast cell involvement in a broad range of immune and nonimmune processes.

### Inhibitory Signals for Mast Cells

As with other immune lineages, mast cells are subject to both negative and positive regulation. Cytokines that inhibit aspects of mast cell function include TGF-β and

IL-10.[55,56] Examples of inhibitory receptors on the surface of mast cells include the IgG receptor FcγRIIb and the phosphatidyl serine receptor CD300A. The importance of these receptors is demonstrated in genetically deficient animals. Mice lacking FcγRIIb demonstrate a striking propensity to activation via both IgG and IgE (which binds with low affinity to both FcγRIIb and FcεRI).[57,58] CD300A mediates suppression of mast cell activation by apoptotic debris.[59] Modulating the surface expression of inhibitory receptors is a potentially important mechanism for regulation of the activation threshold of mast cells in tissues.

## Mast Cell Mediators

### Granule Contents: Proteases, Amines, Proteoglycans, and Cytokines

Mature mast cells package a range of mediators in their granules that are ready for immediate release through fusion with the surface membrane. The most abundant of these mediators are the neutral proteases, named for their enzymatic activity at neutral pH, but vasoactive amines, proteoglycans such as heparin, and certain pre-formed cytokines play distinct roles in the biologic consequences of mast cell degranulation. The release of these mediators is not all or none. In addition to anaphylactic degranulation, mast cells may release only a few granules at a time in a process termed *piecemeal degranulation*.[60] Further, mast cells can release one type of granule but not another.[61] Alternately, mast cells may be induced to elaborate cytokines and chemokines without any release of granule contents, as illustrated by activation via CD30L.[48] Thus, although the mast cell is well equipped to release large volumes of pre-formed mediators, it is equally capable of responses tailored to the activating stimulus.

**Tryptase.** Named for its enzymatic similarity to pancreatic trypsin, tryptase is the most abundant granule protein in human mast cells. It is also an essentially specific marker for mast cells. Tryptase is synthesized in scant amounts by basophils but by no other lineage. The enzyme found in granules is the β isomer, which is the product of two distinct genes. It is enzymatically active upon formation of a homotetramer that relies on the scaffolding function of the proteoglycan heparin.[62] Mast cells also synthesize α tryptase, a protein incapable of forming homotetramers and thus enzymatically inactive. Unlike β tryptase, the α isomer is not stored in granules but is constitutively released into circulation, where its function is unknown. The distinction between tryptase isomers is important for diagnostic reasons: as a marker of degranulation, systemic levels of β tryptase constitute a marker of recent anaphylaxis.[63] By contrast, α trypsin levels reflect total body mast cell load and serve as a useful biomarker in systemic mastocytosis.[64]

Tryptase cleaves structural proteins such as fibronectin and type IV collagen and enzymatically activates stromelysin, an enzyme responsible for activating collagenase.[65] Fibrinogen is another substrate, potentially implicating mast cells in prevention of fibrin deposition and blood coagulation in the tissues.[66] Tryptase also promotes hyperplasia and activation of fibroblasts, airway smooth muscle cells, and epithelium. Cleavage of protease-activated receptors such as PAR2 may contribute to some of these activities, although other studies document PAR2-independent tryptase activation of mesenchymal cells.[67] In aggregate, these effects suggest an important role for tryptase in matrix remodeling. A further contribution to the inflammatory milieu is suggested by the capacity of tryptase to promote neutrophil and eosinophil recruitment and to cleave C3, C4, and C5 to generate anaphylatoxins.[68-70] Interestingly, tryptase can potentially suppress inflammation by cleaving IgE and IL-6.[71,72]

**Chymase.** Chymase, a chymotrypsin-like neutral protease, is found in the $MC_{TC}$ subset of human mast cells, packaged within the same granules as tryptase. Like tryptase, chymase can cleave matrix components and activate stromelysin, although it can also activate collagenase directly, suggesting a role in matrix remodeling.[73] Chymase can activate angiotensin I, leading to angiotensin-converting enzyme (ACE)–independent activation of the potent vasoconstrictor angiotensin II.[74] Chymase can also affect cytokine function, with the capacity to cleave pro-IL-1β to generate active cytokine and to inactivate pro-inflammatory cytokines such as IL-6 and TNF, as well as "alarmins," including heat shock protein 70 and IL-33.[75]

**β-Hexosaminidase.** β-Hexosaminidase is an enzyme found in lysosomes from many cell types. In mast cells it is abundant in secretory granules, where it is released into the surrounding environment during degranulation. Recent studies have found that this enzyme is capable of degrading bacterial cell peptidoglycan, and release by mast cells plays an important role in the defense against experimental staphylococcal infection in mice.[76]

**Vasoactive Amines.** Human mast cells are able to synthesize and store the biogenic amines histamine and serotonin, which are implicated in instances of vascular leak.[77] Histamine, which is by far more abundant than serotinin, is a vasoactive amine found in both $MC_T$ and $MC_{TC}$ mast cells, although it is not unique to this lineage. Histamine is involved in the wheal-and-flare response to cutaneous allergen challenge via augmented vascular permeability, transendothelial vesicular transport, and neurogenic vasodilation. These effects are mediated principally via the H1 receptor. Three other histamine surface receptors, H2, H3, and H4, are distributed widely on immune and nonimmune lineages, with effects as diverse as gastric acid secretion, Langerhans cell migration, and B cell proliferation.[78] Another important mechanism underlying mast cell–induced vascular leak is heparin-mediated bradykinin production (see the next section).

**Heparin and Chondroitin Sulfate E.** Heparin and chondroitin sulfate E are large proteoglycans that enable the ordered packing of mediators within human mast cell granules.[79,80] Negatively charged carbohydrate side chains complex tightly with positively charged proteins, allowing very high concentrations of β tryptase and other proteases. Heparin, which is produced exclusively by mast cells, facilitates the activity of tryptase by making possible proteolytic self-activation within the granule and stabilizing the active tetrameric form of this enzyme. Heparin also has a wide range of effects beyond the mast cell. It is potently angiogenic. Heparin binding activates antithrombin III, providing the basis for use as an anticoagulant, while inhibiting chemokines and both classical and alternative pathways of complement activation, as well as the function of Treg

cells.[81,82] The physiologic role of these extra-cellular activities is uncertain. Of more evident in vivo relevance is that the negatively charged surface of heparin enables activation of factor XII. This zymogen then activates kallikrein, an enzyme that in turn cleaves high-molecular-weight kininogen to generate bradykinin, a potent mediator of vascular leak. This mechanism contributes to the antihistamine-resistant edema observed in patients with hereditary angioedema from lack of C1 esterase inhibitor, an inhibitor of activated factor XII and kallikrein.[83]

**Pre-Formed Cytokines and Chemokines.** Mast cells are able to store certain mediators in their granules for rapid release. The first of these mediators to be documented was TNF.[84] In the mouse, this pool of TNF is implicated in the rapid recruitment of neutrophils to the peritoneum during peritonitis.[42,85] Exocytosed granules appear to release TNF gradually into the environment, enhancing the immunostimulatory effect of the cytokine as TNF-containing granules reach draining lymph nodes, helping to promote development of the mature immune response.[86-88] Other cytokines that may be stored in granules include IL-4, IL-16, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF). Stored chemokines include the neutrophil chemoattractants CXCL1 and CXCL2.[89]

### Newly Synthesized Mediators: Lipid Mediators, Cytokines, Chemokines, and Growth Factors

Beyond pre-formed mediators stored within granules, activated mast cells elaborate a range of mediators that are generated de novo. These mediators are released minutes to hours after stimulation, broadening and extending the impact of activated mast cells on surrounding tissues.

**Lipid Mediators.** Within minutes of activation, mast cells begin to release metabolites of membrane phospholipids. This process is rapid because the relevant enzymes, beginning with phospholipase A2, which is responsible for harvesting phospholipids from the outer leaflet of the nuclear membrane, are already present in the cytoplasm and need only to be activated through signals mediated by calcium flux and the phosphorylation of intra-cellular messengers. The hallmark prostaglandin of human mast cells is prostaglandin $D_2$ ($PGD_2$), which induces bronchoconstriction, vascular leak, and neutrophil recruitment. Smaller amounts of other prostaglandins, as well as thromboxane, are also made. Mast cell–derived leukotrienes (LTs) have similar but generally more potent activity. $LTC_4$ (the major LT species generated by human mast cells), together with its metabolites $LTD_4$ and $LTE_4$, serve as potent inducers of vascular leak. Smaller amounts of the chemotaxins $LTB_4$ and platelet-activating factor (PAF) are also generated. The particular profile of lipid mediators produced by mast cells can change with local environmental signals and the resulting state of differentiation. Thus mast cells from skin generate $PGD_2$ in excess of $LTC_4$, whereas both species are elaborated in roughly equal proportion by mast cells isolated from lung and osteoarthritic synovium.[90]

**Cytokines, Chemokines, and Growth Factors.** Within hours of activation, mast cells begin to elaborate newly synthesized mediators as the end result of induced gene transcription and translation. The range of such mediators is broad (see Figure 15-3). They include the canonical pro-inflammatory mediators TNF, IL-1, and IL-6; the Th2 cytokines IL-4, IL-5, IL-10, and IL-13; the "alarmin" IL-33; chemotactic factors including IL-8, macrophage inflammatory protein (MIP)-1α, CXCR1, CXCR2, and RANTES (regulation upon activation normal T cell expressed and presumably excreted); and growth factors for fibroblasts, blood vessels, and other cells such as bFGF, VEGF, and platelet-derived growth factor (PDGF).[91] IL-17 is detected in tissue mast cells and can be generated by mast cells stimulated in culture.[92-94] As noted earlier, some of these cytokines may also be stored pre-formed in granules for rapid release. The panel of mediators generated depends on the state of differentiation, as well as the activating signal, and may occur in the absence of degranulation.

## ROLE OF MAST CELLS IN HEALTH AND DISEASE

The understanding of the role of mast cells in health and disease has been aided greatly by the availability of mice that lack mast cells entirely or are deficient in mast cell–specific products such as heparin or granule proteases. These mice are viable, excluding an obligate role for mast cells in the structure and function of most tissues. Yet when they are under physiologic stress, such as that imposed by experimental models of disease, multiple differences from wild-type mice become evident. In many cases, these abnormalities may be corrected by engraftment with cultured mast cells,[95] directly implicating mast cells in a remarkably broad range of disease processes (Table 15-1). The extrapolation of such experiments to human disease is limited by multiple factors. Most evidently, mice are not humans, and the experimental systems used typically model at best only certain aspects of the corresponding human condition. Further, many of these mice exhibit "off-target" phenotypes outside of the mast cell lineage, complicating interpretation of the data, especially when results in one mast cell–deficient mouse strain diverge from those observed in another. These discrepancies have occasioned considerable controversy in the mast cell–related literature.[96,97] However, together with in vitro experiments using human mast cells and careful study of individuals in different states of health and disease, animal experiments have contributed greatly to the understanding of mast cell physiology and pathophysiology.

### Mast Cells in Allergic Disease: Anaphylaxis, Allergic Disease, and Asthma

Mast cells are the primary mediator of IgE-mediated systemic anaphylaxis. This phenomenon is demonstrated in mast cell–deficient mice, in which resistance to anaphylaxis may be restored by engraftment with mast cells.[98] In humans, participation of mast cells in anaphylaxis has been documented through the detection of elevated serum levels of β tryptase, a specific marker of mast cell degranulation.[63] Of note, anaphylaxis may be mediated by IgG, a context in which mast cells play only a partial role, while neutrophils assume primacy.[99] Mast cells accumulate in atopic mucosal tissues where they degranulate upon exposure to antigen and contribute prominently to tissue edema and the overproduction of mucus.[100] Mast cells also accumulate in the asthmatic airway, including within the smooth muscle

**TABLE 15-1** Participation of Mast Cells in Murine Models of Disease (Partial List)

| Beneficial to Host | Harmful to Host |
|---|---|
| Angiogenesis | Anaphylaxis* |
| Anxiety control | Arthritis* |
| Bacterial cystitis | Aortic aneurysm* |
| Bacterial peritonitis* | Asthma* |
| Bone remodeling | Atherosclerosis* |
| Brain trauma | Atopic dermatitis |
| Dengue fever | Atrial fibrillation |
| Dermatitis* | Autoinflammatory disease |
| Envenomation* | Bacterial cystitis |
| Glomerulonephritis* | Burn |
| Graft tolerance* | Bullous pemphigoid* |
| Intestinal epithelial barrier* | Cardiac fibrosis |
| Lung infection, bacterial* | Cardiomyopathy |
| Lung infection, viral* | Chronic obstructive pulmonary disease |
| Parasites, intestine* | Colitis |
| Parasites, muscle | Colon polyps |
| Parasites, skin | Dermatitis, irritant* |
| Peptic ulcer disease | Dermatitis, sunburn |
| Thromboembolism | Gastritis |
| Tumor suppression* | Glomerulonephritis* |
| Wound healing* | Gout |
| | Immune complex peritonitis* |
| | Ischemia-reperfusion injury |
| | Kidney injury* |
| | Lung fibrosis |
| | Malaria |
| | Multiple sclerosis* |
| | Myositis* |
| | Neurogenic inflammation* |
| | Obesity* |
| | Peritonitis, irritant* |
| | Peritoneal adhesions |
| | Pneumonitis |
| | Renal fibrosis* |
| | Scleroderma |
| | Sepsis* |
| | Tumor angiogenesis |

Mast cells are implicated in these processes by virtue of phenotypic abnormalities in mast cell–deficient mice or in mice lacking mast cell-specific mediators. The asterisk (*) indicates that the phenotype has been shown to be reversible by engraftment with cultured mast cells, providing more direct evidence for a role for this lineage.

lining the airways, and have been implicated by human and animal data in airway hyper-reactivity and mucosal changes.

## Mast Cells in Nonallergic Inflammation

### Pathogen Defense: Mast Cells as Sentinels of Innate Immunity

The involvement of mast cells in atopic disease is clear but does not explain their remarkable evolutionary conservation.[101] Rather, mast cells must somehow contribute to the survival of the organism. The most probable mechanism by which mast cells convey a survival advantage is in the defense against infection. This role is suggested by the localization of mast cells near epithelial surfaces, around blood vessels, and in other locations of potential invasion by pathogens.

Mast cells are competent defensive cells against bacteria. They express TLRs and other receptors against bacterial antigens, and upon activation are able to phagocytose bacteria and generate anti-microbial molecules such as cathelicidin.[102,103] However, given their relatively small numbers, the most important function of mast cells in immune defense is likely as sentinels, monitoring for early traces of infection and rapidly mobilizing neutrophils and other inflammatory cells when needed. Such a role has been clearly demonstrated in mouse models of bacterial peritonitis, in which mast cell–deficient animals exhibit a high mortality. This susceptibility correlates with delayed recruitment of neutrophils via TNF and leukotrienes; both neutrophil influx and survival may be restored by correction of the mast cell deficit, although in the case of severe infection, mast cell TNF may actually contribute to mortality.[85,104-106] Clearance of bacteria from the lungs is delayed in mast cell–deficient mice and can be similarly restored by exogenous mast cells.[85] Analogous observations have been made in other models of bacterial infection. Thus mast cells likely play an important role in the defense of the host against bacterial infection.

Mast cells are also implicated in the defense against parasites. Mast cell–deficient animals exhibit abnormal clearance of multiple parasites from the gut and skin, in a manner promoted by IgE.[107,108] The mechanism of this defense remains uncertain but may include direct attack upon pathogens, recruitment of inflammatory lineages such as neutrophils and eosinophils, and lysis of tight junctions in the mucosal lining to facilitate the expulsion of helminths.[107,109,110] More limited data implicate mast cells in the defense against viruses, including dengue, vaccinia, and cytomegalovirus. The role of mast cells in this setting includes recruitment of CD8 T cells and other cytotoxic lineages, as well as production of type I IFNs to help neighboring cells resist infection.

### Mast Cells and the Adaptive Immune Response

In addition to recruiting innate effector cells, mast cells mobilize T and B lymphocytes, the adaptive arm of the immune system.[91] Mast cells may express major histocompatibility complex (MHC) II, as well as co-stimulatory molecules such as CD80 and CD86, rendering them effective antigen-presenting cells for CD4 T cells. Mast cells can also

mobilize and potentiate CD8 T cell responses.[111] They can migrate from peripheral tissues to lymph nodes carrying antigen and contribute to the recruitment of T cells to lymph nodes via mediators including MIP-1β and TNF.[8,86,112] Indeed, infection-induced lymph node hyperplasia is abrogated in the absence of mast cells. Further, mast cells can recruit CD4 and CD8 effector T cells to peripheral tissues via $LTB_4$, among other mediators.[113-115] Finally, mast cells contribute to the migration of cutaneous Langerhans cells and other dendritic cells to lymph nodes via mediators including histamine.[116-118] By means of the inducible expression of CD40L and cytokines, mast cells stimulate B cells and induce class switching to IgA or IgE.[119,120] The physiologic importance of these effects will vary with circumstances. For example, under some conditions, delayed-type hypersensitivity responses in skin are dependent on mast cells, whereas under other conditions, mast cells play no role.[91] The potential importance of the mast cell in adaptive immunity is highlighted by the recent demonstration that mast cell activators are effective vaccine adjuvants.[87]

### Neurogenic Inflammation

In addition to their perivascular localization, mast cells cluster near and sometimes within peripheral nerves. A discrete function for them in these locations has not yet been defined, although the potential for bidirectional neuroimmune interaction is clear. Mast cell mediators such as histamine can directly activate neurons, whereas mast cells residing near stimulated neurons can be induced to degranulate.[121] Indeed, vascular leak and neutrophil infiltration arising from infiltration of skin with the neurogenic mediator substance P are mediated by mast cells.[122,123] Thus neurons might recruit mast cells as local effectors of neurogenic inflammation.

### Autoimmune Disease

Reconstitution experiments in mast cell–deficient mice have implicated mast cells in a variety of pathologic conditions (see Table 15-1), including murine models of autoimmune diseases such as bullous pemphigoid, multiple sclerosis, scleroderma, and inflammatory arthritis. In mice with pemphigoid, mast cells that are triggered via IgG antibodies against a hemidesmosomal antigen recruit neutrophils, which are responsible for blister formation.[44] The role of mast cells in murine experimental autoimmune encephalomyelitis (EAE) is more complex. Although the resistance of mast cell–deficient $W/W^v$ mice to EAE corrects with mast cell engraftment, these cells fail to repopulate the brain and spinal cord, indicating that mast cells are not obligate local effector cells in this model.[124,125] One mechanism for this activity appears to be the promotion of the adaptive immune response, because mast cell engraftment into $W/W^v$ animals improves T cell responses to immunization with the inciting myelin antigen.[126,127] The contribution of mast cells to human scleroderma remains unknown, although they are recognized to express TGF-β in sclerodermatous skin and to interact closely with local lymphocytes and fibroblasts, even to the point of gap junction formation and therefore cytoplasmic continuity with the latter cells.[128,129] Humans with polymyositis, but not dermatomyositis, exhibit increased density of mast cells in inflamed muscle; a similar phenotype is noted in dermatomyositis skin.[130,131] Murine studies suggest that these cells may play a role in disease pathogenesis, because experimental myositis is attenuated in mast cell–deficient animals, a defect that corrects partially with mast cell engraftment.[131] The participation of mast cells in arthritis is discussed in detail later.

## Mast Cells as Anti-Inflammatory Cells

Within the past decade, it has become evident that mast cells may also help moderate the immune response. One mechanism for this effect is degradation of pro-inflammatory mediators. Mast cell proteases may cleave and inactivate the cytokines IL-5, IL-6, IL-13, IL-33, and TNF, as well as endothelin-1 and the anaphylatoxin C3a.[69,75,132-134] Proteases also degrade tissue-derived danger signals, including heat shock protein 70.[75] The importance of this anti-inflammatory activity has been demonstrated in a murine sepsis model, where mast cells reduced mortality by restraining excess inflammation in a protease-dependent manner.[134]

More broadly, mast cells can produce mediators such as IL-10 that have immunosuppressive activity, and even otherwise pro-inflammatory mediators such as TNF and granulocyte-macrophage colony-stimulating factor (GM-CSF) can be immunosuppressive under appropriate circumstances.[135,136] Thus mast cells promote immunologic tolerance to skin grafts and limit tissue inflammation related to ultraviolet light injury.[72,136-138] Data suggest a role for mast cell–produced IL-2 in the promotion of Treg responses that limit experimental dermatitis, as well as in the development of IL-10–producing regulatory B cells.[139,140] Mast cells may play a permissive role in the immunosuppressive activity of murine myeloid-derived suppressor cells, an activity that may be beneficial or harmful to the host.[141,142]

## Mast Cells and Connective Tissue

### Wound Healing and Tissue Fibrosis

Mast cells have long been noted to accumulate at the borders of healing wounds. In healthy human subjects undergoing experimental wounding and recurrent biopsies, mast cell numbers increase sixfold by day 10 after the initial incision. These mast cells localize preferentially to fibrotic areas of the wound and strongly express IL-4, a cytokine capable of inducing fibroblast proliferation and collagen synthesis.[143] In vitro studies confirm the stimulatory effects of mast cells on fibroblast growth[144]; candidate fibroblast mitogens in addition to IL-4 include tryptase, histamine, $LTC_4$, and bFGF. Indeed, $W/W^v$ animals exhibit delayed contracture and healing of skin wounds in a manner reparable by local engraftment with cultured mast cells, although other mast cell–deficient mice exhibit no defect in wound healing.[145,146]

Mast cells also accumulate in sites of pathologic fibrosis, including the skin and lungs of patients with scleroderma. Because experimental skin fibrosis proceeds in mast cell–deficient mice with few if any differences in intensity or kinetics, it is unlikely that mast cells are an obligate effector

lineage in human scleroderma, although they may contribute to disease progression.

### Bone

Mast cells are also implicated in the remodeling of bone. Mast cells accumulate in sites of healing fracture, and under normal circumstances, they may contribute to normal bone turnover.[147,148] However, mast cells accumulate in osteoporotic bone, and systemic osteoporosis is a known complication of systemic mastocytosis.[149,150] Heparin is one potentially important mediator of bone loss because it directly promotes differentiation and activation of osteoclasts.[151] Mast cell products such as IL-1, TNF, and MIP-1α have similar activity.

### Angiogenesis

Another potentially important activity of mast cells on the stroma is the promotion of angiogenesis. Mast cells are not required for the development of the normal vasculature, as is evident in the viability of mast cell–deficient mice. However, mast cells cluster at sites of early blood vessel growth in tumors and contribute appreciably to physiologic angiogenesis under certain experimental conditions.[152,153] Heparin was the first proangiogenic mast cell mediator identified; bFGF and VEGF are other potent stimulators of endothelial migration and proliferation.

## Mast Cells in Arthritis

The normal synovium is populated by a limited number of mast cells. These cells are not found in the immediate lining layer but rather reside in the synovial sublining near blood vessels and nerves, constituting almost 3% of cells within 70 μM of the synovial lumen.[154] In both mice and humans, their phenotype is principally $MC_{TC}$, similar to mast cells found in most other connective tissue sites.[23,155] The severalfold increased density of mast cells in the immediate vicinity of the synovial lining, compared with more distant connective tissue, supports the hypothesis that mast cells contribute to immune surveillance of the articular cavity.[23] Extrapolating from the activity of mast cells near other vulnerable body cavities, such as the peritoneum, it is likely that synovial mast cells help monitor the joint for early evidence of infection.

In the presence of inflammatory arthritis, the population of synovial mast cells expands remarkably (Figure 15-4). More than two-thirds of synovial specimens from patients with rheumatoid arthritis (RA) exhibit abnormal numbers of mast cells, averaging in excess of tenfold above normal.[156] Consistent with these histologic findings, synovial fluid from rheumatoid joints contains appreciable quantities of histamine and tryptase.[157,158] Unlike the normal joint, in the RA joint both subtypes of mast cells are present in roughly equal numbers, with $MC_T$ cells located nearer to the pannus and infiltrating leukocytes, whereas $MC_{TC}$ cells cluster in deeper, more fibrotic areas of the synovium.[23] Mast cells have been noted near the junction of pannus and cartilage.[159] Rare mast cells are also identified in synovial fluid.[160] The absence of mitotic figures and of staining for the proliferation antigen Ki-67 suggests that they arise not from local replication but rather by recruitment of circulating progenitors.[161] Although the signals driving this recruitment are unknown, inflammatory cytokines such as TNF enhance expression of the mast cell chemotactic and survival factor SCF on synovial fibroblasts, suggesting one mechanism.[162] Indeed, degree of inflammation is the best predictor of the number of mast cells within the joint.[156,163,164] Incompletely identified factors in RA synovial fluid can potently promote mast cell differentiation and growth.[165]

Hyperplasia of the mast cell population is not specific for RA but is observed in a wide range of inflammatory joint disorders (Table 15-2). Expansion is also noted in osteoarthritis (OA), often to densities observed in RA.[166] The levels of histamine and tryptase in OA synovial fluid are also comparable. Interestingly, unlike in RA, the expansion in OA results from an increase in numbers of $MC_T$ mast cells, the subtype generally associated with T cells and inflammation.[23,167]

Studies of human synovial mast cells confirm that they can be activated via pathways relevant to arthritis biology, including complement, Fc receptors, and TLR ligands.[41,168,169] Mast cells are also a potential source of citrullinated

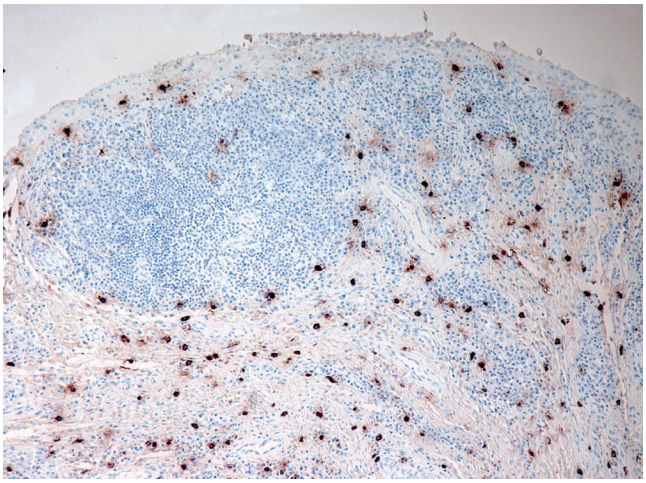


**Figure 15-4** Mast cells in the rheumatoid synovium. Stained red by an antibody against tryptase, mast cells are abundant in this synovial biopsy from a patient with chronic rheumatoid arthritis. Note the proliferation of mast cells in the synovial sublining. *(From Nigrovic PA, Lee DM: Synovial mast cells: role in acute and chronic arthritis.* Immunol Rev *217:19–37, 2007.)*

**TABLE 15-2** Joint Diseases with Documented Synovial Mastocytosis

| |
|---|
| Chronic infection |
| Gout |
| Juvenile idiopathic arthritis |
| Osteoarthritis |
| Psoriatic arthritis |
| Rheumatoid arthritis |
| Rheumatic fever |
| Traumatic arthritis |
| Tuberculosis |

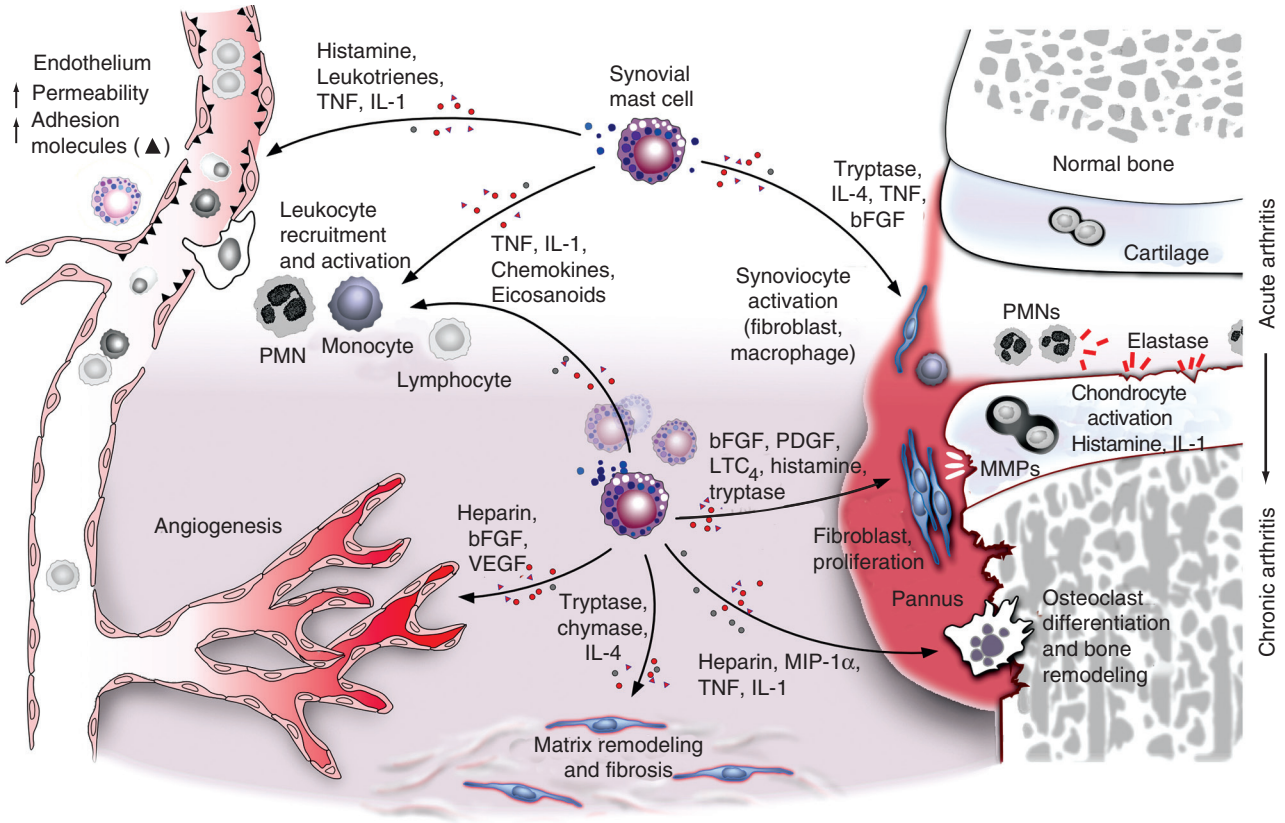



**Figure 15-5** Potential roles of mast cells in acute and chronic arthritis. In the acute phase of joint inflammation, mast cells may contribute to initiation of arthritis by inducing vascular permeability, recruiting and activating circulating leukocytes, and stimulating local fibroblasts and macrophages. In established arthritis, these activities may be joined by effects on the stroma, including the promotion of pannus formation, angiogenesis, fibrosis, and injury to cartilage and bone. Potential anti-inflammatory effects of mast cells are not depicted. The mediators listed are representative and do not represent a complete list. bFGF, Basic fibroblast growth factor; IL, interleukin; $LTC_4$, leukotriene $C_4$; MIP-1α, macrophage inflammatory protein-1α; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PMN, polymorphonuclear neutrophil; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. *(From Nigrovic PA, Lee DM: Synovial mast cells: role in acute and chronic arthritis,* Immunol Rev *217:19–37, 2007.)*

autoantigens implicated in RA, although their relative importance in this context is unknown.[170]

## Mast Cells in Acute Arthritis: Insights from Animal Models

Experimental work in mice has shed light on the role of mast cells in inflammatory arthritis. Several mast cell–deficient strains demonstrate striking resistance to arthritis induced by IgG autoantibodies, a defect that may be repaired by engraftment with cultured mast cells expressing receptors for IgG and C5a.[39,45,171-173] A number of mechanisms contribute to this arthritogenic activity. First, mast cells induce vascular permeability, facilitating entry of autoantibodies into the joint.[174,175] Second, mast cells release proinflammatory mediators including IL-1 that help establish inflammation, presumably via effects on endothelium and other local populations such as macrophages and fibroblasts.[45,173] These actions appear to be most critical at the initiation of disease, constituting a "jump start" for acute inflammation within the joint. This function is in line with the activity of mast cells in other models of IgG-mediated disease, such as IgG-mediated immune complex peritonitis, murine bullous pemphigoid, and anaphylaxis. In each of these models, mast cells resident in tissue for the purpose of immune defense become co-opted by autoantibodies to initiate inflammatory disease (Figure 15-5, *top*). Mast cells have also been implicated in monosodium urate crystal sensing and cytokine production in experimental gout.[54]

Of note, not all mice lacking mast cells are resistant to experimental arthritis.[45,176,177] The basis for this discordance is not fully understood but likely reflects factors that include the intensity of the arthritogenic stimulus and the susceptibility of the background strain. This suggestion is reinforced by studies in mice lacking specific mast cell proteases, where partial protection from arthritis is evident, but only in the context of a submaximal arthritis stimulus.[67,178] These data suggest that mast cells represent one potential contributor to joint inflammation but that their importance will vary with context.

## Mast Cells in Chronic Arthritis

In contrast to the acute phase of joint inflammation, the role of mast cells in established arthritis is poorly understood. The sheer numbers of these cells in arthritic synovium implies a substantial role. Taking into account the spectrum of mast cell activity elsewhere, it is likely that mast cells

participate both in the inflammatory process and in the mesenchymal response (Figure 15-5, *bottom*).[166]

An ongoing contribution of mast cells to inflammatory arthritis is suggested by several observations. First, as noted, prominent among infiltrating synovial mast cells are $MC_T$ cells, typically associated elsewhere with the elaboration of cytokines such as IL-6 with documented pathogenic activity in RA. Immunofluorescence staining has identified TNF and IL-17 in mast cells from inflamed synovium,[7,93,179] and the elaboration of other pro-inflammatory mediators is probable. Second, mast cells from RA but not OA synovium express the receptor for the anaphylatoxin C5a, a mediator readily documented in synovial fluid.[168] Immune complexes within RA joints represent a likely pathway to activation of synovial mast cells.[180] Indeed, ultrastructural data support ongoing degranulation of mast cells in the RA synovium.[167] Finally, studies of c-Kit inhibitors in murine and human arthritis suggest efficacy, although it remains unclear whether these agents functionally antagonize tissue mast cells and whether such antagonism explains their efficacy, because these agents have an impact on multiple kinases and affect a broad range of lineages.[181,182]

The effect of mast cells on the established inflammatory synovial infiltrate is difficult to predict. As in acute arthritis, activated mast cells may promote the recruitment and activation of leukocytes. Alternately, protease cleavage of inflammatory mediators and the elaboration of cytokines such as IL-10 and TGF-β could suppress inflammation, potentially by promoting Treg function, as has been observed in tolerance of skin grafts in mice.[137]

Mast cells likely modulate the stromal response to inflammation. Expansion and activation of synovial fibroblasts is a key pathogenic process within RA, and the capacity of mast cells to promote such changes is well established. Through interaction with osteoclasts, mast cells could promote both focal erosions and periarticular osteopenia. Mast cell tryptase not only promotes inflammation but can contribute directly to joint injury by activating synovial fibroblasts to produce chemokines and by cleaving substrates such as cartilage aggregan, both directly and through proteolytic activation of matrix metalloproteinases.[67,178,183-185] Finally, by producing proangiogenic mediators, mast cells may enable growth of the vascular supply required for the profound expansion of the thin synovial layer into thick pannus. Confirmation of these roles awaits further experimental data.

## Therapeutic Potential of Mast Cell Antagonism in Rheumatic Disease

The broad involvement of mast cells in health and disease suggests that targeting this lineage could have potential therapeutic value. Multiple strategies for blocking mast cell activity are under active consideration.[186] In the context of atopic disease, targeting IgE and its receptor FcεRI offers the appealing prospect of antagonizing a pathway of direct disease relevance while retaining most protective mast cell functions. In rheumatic diseases, however, such specificity will be more difficult to achieve because the pathways by which mast cells are engaged, and their downstream effector responses, are typically relevant for immune defense and are shared by multiple hematopoietic lineages.

### Mast Cell Protease Inhibition

Mast cell proteases make up the bulk of the protein content of these cells and are in many cases largely or entirely specific to this lineage, as previously described. Targeting these proteins therefore offers a pathway to selective mast cell antagonism, in particular because mast cell proteases are implicated directly in arthritis pathogenesis, both as pro-inflammatory factors and as mediators of tissue injury.[67,178,185] Protease inhibitors have been generated but are not currently in active clinical development for arthritis.[186]

### SCF/c-Kit Antagonism

Given the critical importance of the SCF/c-Kit axis for the development and survival of mast cells, attention has focused on the potential of kinase inhibitors as mast cell antagonists. Although no inhibitors are fully specific to c-Kit, the tyrosine kinase inhibitors imatinib and mastinib and the downstream protein kinase C inhibitor midostaurine exhibit relative selectivity for c-Kit and have clinical utility in some cases of systemic mastocytosis.[187] These treatments may be complicated by neutropenia and thrombocytopenia, reflecting the importance of c-Kit in other hematopoietic lineages. Imatinib is effective in murine arthritis, in doses that affect in vitro mast cell activation; similarly, imatinib treatment of human synovial cultures induces apoptosis of mast cells and limits cytokine production.[7,93,181] However, the action of this agent on multiple synovial lineages obscures the role of mast cell targeting in these results, even in vitro.[181] Interestingly, imatinib is less effective at antagonizing c-Kit stimulation from immobilized SCF, as between tissue-resident mast cells and their adjacent fibroblasts.[17] Further, both in vitro and in vivo data suggest that c-Kit signaling can be at least partially replaced by other survival signals from the inflamed environment, such as IL-33.[10,188] Together, these results suggest that c-Kit blockade is unlikely to represent a mast cell–specific therapeutic approach, although effects on mast cells may certainly contribute to the clinical improvement suggested by series of patients with rheumatic diseases, including RA, treated with kinase inhibitors.[182,189-191]

### Signaling Pathways

Mast cells share pathways of intra-cellular signaling with other cells, but disproportionate use of individual pathways could offer the potential for pharmacologic specificity. Examples of such pathways include the delta isoform of phosphoinositide 3 kinase (PI3Kδ) and Ras guanine nucleotide-releasing protein-4 (RasGRP4). PI3Kδ is involved in intra-cellular signaling in mast cells, including downstream of c-Kit, and mice lacking this receptor exhibit a partial deficiency of mast cells in some tissues and a striking reduction of IgE-mediated signaling and anaphylaxis.[192,193] Mice lacking RasGRP4, a guanine nuclear exchange factor expressed principally in mast cells, exhibit impaired mediator production by this lineage and impaired inflammation in models of colitis and arthritis.[194,195] Antagonism of targets such as these could hold the promise of limiting the contribution of mast cells to human disease,

although the clinical efficacy and side effect profile of such blockade will depend greatly on the availability of redundant pathways within human mast cells and the expression of these targets in other lineages.

## CONCLUSION

Mast cells are potent immune cells characterized by phenotypic diversity and an extremely broad range of functions in health and disease. In addition to mediating atopic disease, mast cells represent important sentinels against pathogen invasion. Under certain conditions it is likely that they also participate in the control of the immune response and the remodeling of tissue matrix. Aberrant activation of mast cells by autoantibodies and potentially other signals has been identified in a range of inflammatory diseases, including arthritis. Such activation may represent a key pathologic step in the development of tissue inflammation and injury and could present an interesting target for the development of anti-inflammatory therapies.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 16



# Platelets

Eric Boilard • Peter A. Nigrovic

**KEY POINTS**

- Platelets are small subcellular fragments that circulate in blood, where they promote hemostasis.
- Platelets express receptors and a multitude of mediators recognized as being active in inflammation.
- Inhibition of platelet functions generally involves blockade of G protein–coupled receptor soluble agonists (e.g., thromboxane, thrombin, and adenosine diphosphate). However, platelet immunoreceptor tyrosine-based activation motif signaling at the site of inflammation is also implicated.
- Platelets likely contribute to inflammation in rheumatic diseases, including rheumatoid arthritis and systemic lupus erythematosus.
- Specific blockade of platelet pro-inflammatory functions could represent a new approach to treatment of the rheumatic diseases and their associated cardiovascular risks.

Platelets patrol the vasculature to promote hemostasis. In blood, platelets represent the second most abundant cellular lineage after the red blood cell, outnumbering leukocytes by several orders of magnitude. When damage to the vasculature occurs, platelets quickly respond to prevent blood loss. However, in addition to their recognized role in thrombosis, platelets are emerging as important participants in the separation of blood and lymphatic systems, in maintenance of vasculature integrity in inflammation, and in immune responses. Accumulating evidence suggests that platelets and their bioactive mediators are important contributors to the pathogenesis of rheumatic diseases.

## PLATELET STRUCTURE

Platelets are small subcellular fragments that circulate in blood. In humans, normal platelet counts range between $150 \times 10^6$/mL and $450 \times 10^6$/mL, making them the second most abundant cell lineage present in blood after erythrocytes. Structurally, resting platelets resemble irregular discs that are 2 to 5 μm in diameter and 0.5 μm thick, with a volume of 6 to 10 femtoliters. In comparison, lymphocytes and neutrophils have a volume of 218 and 330 femtoliters, respectively.[1,2] Because of their small size and discoid shape, platelets are continuously pushed by the blood flow toward the edge of blood vessels, positioning them optimally to recognize endothelial injury. Thus because of their structure and abundance in the blood, platelets are positioned to fulfill their hemostatic function: the maintenance of vascular integrity.[3]

The cytoskeleton is necessary for maintaining the platelet's discoid shape.[4] A spectrin-based cytoskeleton is associated with the cytoplasmic side of the plasma membrane, microtubule coils form along the circumference of the cell, and 2000 to 5000 linear polymers of actin, the most abundant protein expressed by platelets, fill the cytoplasm.[5-7] These cytoskeletal components maintain the platelet's structure in the setting of high fluid shear forces and also enable conformational changes with activation to form finger-like filopodia and pseudopods.[8] Under these conditions, the platelet structure is remodeled dramatically, changing from a disk to spiny sphere.

The resting platelet plasma membrane is composed of phospholipids and, on electron microscopy, it appears smooth with a fine corrugated surface, resembling the surface of the brain.[9,10] The surface membrane contains channels and sinuous invaginations called the open canicular system (OCS).[11] The OCS increases the platelet surface area in direct contact with the extra-cellular milieu. Plasma components such as serotonin and fibrinogen are imported into platelets through this system, and the OCS serves as a conduit for the release of mediators stored within the platelet.[12,13] The channels that constitute the OCS are also a source of membrane necessary for the profound morphologic changes, such as the formation of filopodia and spreading that occur rapidly when platelets are activated, increasing their exposed surface by 420%.[14] Adherent platelets have been observed to trail long (250 μm) tendrils into the blood vessel lumen, further increasing their contact surface and helping them to interact with circulating leukocytes.[15]

The platelet cytoplasm includes numerous small organelles that include three main types of secretory granules: the α-granules, dense bodies (δ granules), and lysosomes (Table 16-1). The α-granules are the most abundant organelle in platelets (40 to 80 per platelet).[16,17] They are round or oval, have a diameter of 200 to 500 nm, and contain proteins in their lumens or membrane, including von Willebrand factor (vWF), P-selectin, coagulation factor V, thrombospondin, fibrinogen, platelet factor-4 (also known as CXCL4), and growth factors such as platelet-derived growth factor (PDGF) and tumor growth factor-β (TGF-β).[18] Dense bodies are smaller than α-granules, are fewer in number (4 to 8 per platelet), and are rich in calcium, magnesium, adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and histamine.[19,20] Calcium and

**TABLE 16-1** Platelet Components Implicated in the Inflammatory Response

| | Platelet Component | Actions |
|---|---|---|
| Surface molecules | P-selectin (CD62P), PECAM (CD31), GPIbα | Adhesive targets for leukocytes |
| | PAF, ROS | Neutrophil activation |
| | CD154 (CD40 ligand) | Agonist for endothelial cells |
| Soluble factors | Serotonin, histamine | Regulators of vascular permeability |
| | β-Thromboglobulin, PF4 | Chemotaxis |
| | Acid hydrolases, ROS | Tissue destruction |
| | PDGF, TGF-β | Cellular mitogens, chemoattractant |
| End products of platelet procoagulant activity | Thrombin, fibrin | Promote leukocyte accumulation |

GPI, Glycosyl phosphatidylinositol; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PECAM, platelet–endothelial cell adhesion molecule; PF4, platelet factor-4; ROS, reactive oxygen species; TGF, transforming growth factor.

serotonin are responsible for the opacity of dense bodies on electron microscopy. Platelets also usually bear approximately one lysosome (sometimes none and never more than three). These lysosomes may serve as an endosomal digestion compartment, but the importance of this function in hemostasis is unknown. Proteolysis can also take place in platelet proteasomes.[21] The proteasome is necessary for platelet function, because its pharmacological blockade by the proteasome inhibitor bortezomib (given to patients with multiple myeloma) inhibits platelet thrombotic activities and platelet production.[22,23] Platelets express residual fragments of Golgi complexes and functional mitochondria (4 to 7 per platelet), which are implicated in the production of energy and platelet activation.[21,24]

## PLATELET PRODUCTION

The great majority of the platelets will never be involved in any hemostatic process and will be eliminated at senescence through the reticuloendothelial system in the liver and spleen. Because of their relatively short life span in circulation (10 days), approximately 100 billion platelets must be produced every day in the human body. Platelets are not cells per se; they are fragments derived from megakaryocytes and, consequently, they are deprived of a nucleus.[25,26] The megakaryocyte arises from a master stem cell in the bone marrow under the control of thrombopoietin, a hormone produced in the liver and kidneys that promotes megakaryocyte maturation, number, and size and forestalls megakaryocyte apoptosis.[27,28] Thrombopoietin is a soluble, 80 to 90 kDa protein that acts through binding to its receptor, c-Mpl, which is expressed by megakaryocytes and platelets. The binding of thrombopoietin to platelets induces its catabolism. More thrombopoietin is thus available in persons with lower platelet counts, thereby driving megakaryocyte activity. Further, aging platelets directly induce thrombopoietin expression in hepatocytes because they are cleared through an interaction between desialyated platelet surface glycans and the hepatic asialyloglycoprotein receptor.[29]

Cytokines such as interleukin (IL)-1, IL-3, IL-6, IL-11, stem cell factor (also called Kit ligand) and granulocyte-macrophage colony-stimulating factor amplify megakaryocyte production.[30-32] Enhanced platelet production can be assessed by measurement of the mean platelet volume (MPV), a measure that is often part of a complete blood cell count (CBC). A larger average platelet size is seen when platelet production by megakaryocytes is increased, generally reflecting compensation for accelerated platelet destruction as seen in immune thrombocytopenic purpura (ITP), myeloproliferative diseases, and Bernard-Soulier syndrome.[33]

The mature megakaryocyte is a giant cell (50 to 100 μm in diameter) and is capable of endomitosis, meaning that it undergoes repeated cycles of DNA replication without cell division, leading to polyploidy, typically up to 16N (128N can be observed).[34] This amplification of DNA results in gene amplification and increases protein production, which is necessary for the generation of up to 5000 platelets per megakaryocyte. During platelet formation, long (millimeters in length) cytoplasmic extensions emanate from the megakaryocyte and protrude out of the bone marrow in the interior of vascular sinusoids. These extensions, called proplatelet processes, elongate through the action of microtubules, which also serve as a track for the transportation of membranes, organelles, and granules from the mother megakaryocyte distally into nascent proplatelets. Actin filaments participate in proplatelet branching. Cytoplasmic continuity between the mother megakaryocyte and nascent proplatelets also enables platelets to bear functional microRNA and messenger RNA, as well as the molecular machinery required to translate the latter efficiently into protein.[35,36]

Fragments from proplatelet processes are next released into the blood as either globular pre-platelets or as barbell-shaped proplatelets (Figure 16-1). These interconvertible forms generate platelets upon fission. Furthermore, individual human platelets can divide.[37] These observations suggest that platelet maturation continues in the circulation. Interestingly, megakaryocytes can migrate into the bloodstream, and evidence suggests that an appreciable proportion of platelets is generated by the megakaryocytes that have taken up residence in the lungs.[25,38]

## PLATELETS AND HEMOSTASIS

The sum of the processes by which the loss of blood is prevented is called hemostasis. Intravital microscopic analyses, performed as early as in 1882 by the Italian scientist Bizzozero, demonstrated that platelets could recognize a damaged blood vessel and form a plug.[1] Since then, the molecular participants in the promotion of hemostasis have been described. The intact endothelium produces molecules, such as prostacyclin ($PGI_2$), nitric oxide, and thrombomodulin, which, conjointly with cell-associated ecto-adenophosphatase (ADPase; CD39),[39] inhibit platelet activation. Endothelial injury triggers vasoconstriction, which reduces blood flow and exposes the subendothelial matrix. At this stage, the main platelet glycoprotein (GP) receptors implicated are the members of the GP Ib-IX-V

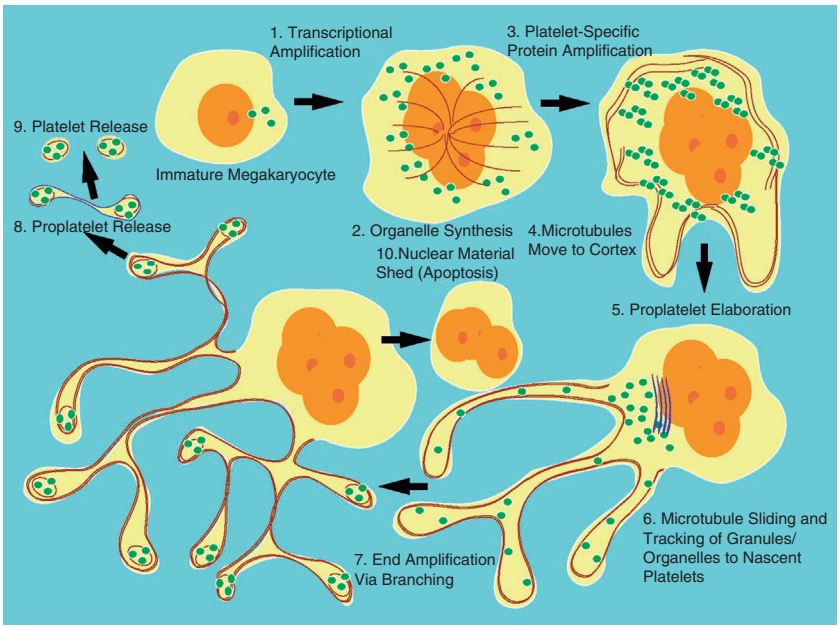



**Figure 16-1** Platelet biogenesis according to the proplatelet model. *(From Italiano JE Jr, Hartwig J: Megakaryocyte development and platelet formation. In Michelson AD, editor:* Platelets*, ed 3, Amsterdam, 2013, Elsevier, pp 27–50.)*

complex (25,000 copies/platelet), and the integrin αIIbβ3 (GPIIb-IIIa complex; 80,000 copies/platelet).[40,41]

Under high shear, the GPIb-IX-V complex attaches to vWF, which is itself bound to the wound rich in collagen fibers, mediating transient adhesion (also referred to as tethering).[42] Glycoprotein VI (GPVI), which is expressed as a complex with the homodimeric Fc receptor γ chain,[43,44] and the integrin α2β1 stabilize the association by binding collagen directly. Although GPIIb-IIIa is a receptor for several ligands (fibronectin, fibrinogen, vWF, thrombospondin, and vitronectin), only its activated form binds fibrinogen. Platelet activation through GPIb-IX and GPVI thus promotes inside-out signaling, leading to GPIIb-IIIa activation and the binding of the latter to fibrinogen, thereby bridging platelets together and recruiting additional platelets onto adhered spread platelets.[40-42,45,46] Deficiency of components of the GPIb-IX-V complex and the GPIIb-IIIa complex leads to the congenital bleeding disorders Bernard-Soulier disease and Glanzmann's thrombasthenia, respectively.[47,48] vWF catabolism is mediated by the metalloprotease ADAMTS-13, which is expressed in plasma and acts as a natural anti-thrombotic agent. Mutations of ADAMTS-13 and depletion by autoantibodies cause familial and acquired thrombocytopenic purpura, respectively.

Platelet activation engages intra-cellular signaling. The actin cytoskeleton pushes the granules toward the platelet membrane. Granule fusion with the plasma membrane is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) complexes, leading to release of granule content through the OCS route.[49] The release of ADP further activates platelets through P2Y12 receptors. Because phospholipids serve as a reservoir of fatty acid, such as arachidonic acid (AA), the cleavage of phospholipids by phospholipase A2 generates lysophospholipids, which can be metabolized into platelet activating factor (PAF) and AA. AA is the substrate of cyclo-oxygenase 1 (COX-1) present in platelets, leading to generation of thromboxane $A_2$.[50] Thromboxane $A_2$ is highly potent at inducing platelet aggregation and vasoconstriction, acting via the thromboxane $A_2$ receptor. As a result, growth of the platelet plug occurs and the blood coagulation cascade is initiated, leading to thrombin generation and fibrin clot formation. In line with the significance of these activation pathways, the inhibition of ADP activity using antagonists of P2Y12 receptors (such as clopidogrel) and of the biosynthesis of thromboxane $A_2$ using COX-1 inhibitors (such as acetylsalicylic acid) is frequently applied in persons at risk of myocardial infarction and thrombotic stroke.

Platelets also respond to activation and shear stress through the release of small extra-cellular vesicles, called microparticles. Microparticles, originally termed "platelet dust" by pioneer investigators,[51] are produced by cytoplasmic blebbing and fission. They measure approximately 100 to 1000 nm in diameter and are distinct from the exosomes that are smaller (50 to 100 nm in diameter) and originate from multivesicular bodies and α-granules through exocytosis.[52] Megakaryocytes can also elaborate microparticles directly, independent of platelet synthesis.[53] Platelets and megakaryocytes are the most abundant source of microparticles in circulating blood, as identified by expression of GPIIb (CD41).[54] During microparticle release, loss of membrane asymmetry generally occurs, leading to exposure of phosphatidylserine (PS) on the microparticle surface.[55] Exposed PS can support blood coagulation.[56] Platelet microparticles are implicated in the pathogenesis of several inflammatory pathologies, including rheumatic diseases (discussed further later).

Beyond their role in the defense of injured blood vessels, platelets appear to play an incompletely understood role in the maintenance of endothelial integrity. Patients with severe thrombocytopenia have been noted to develop

abnormalities of the endothelium such as thinning, fenestrations, and enhanced vascular permeability.[57,58] Accordingly, animals with profound experimental thrombocytopenia experience bleeding at the site of inflammation in skin, lung, or brain.[57-60]

## SIGNALING PATHWAYS IN PLATELET ACTIVATION

ADP, thromboxane $A_2$, and thrombin, the latter through its binding to protease-activating receptors (PARs), share signaling via G protein–coupled receptors (GPCRs).[60,61] However, GPCR signaling is dispensable for the prohemostatic role of platelets in inflammation, pointing to the existence of other significant activation signaling pathways in platelets.

Immunoreceptor tyrosine-based activation motif (ITAM) signaling can play such a role. Platelets express three receptors that belong to the family of ITAM receptors: Fc receptor γIIA (a low affinity receptor for IgG immune complexes),[62] Fc receptor γ chain, which is noncovalently associated with GPVI and is necessary for GPVI function,[43,44] and the C-type lectin 2 (CLEC2), a receptor for podoplanin.[63]

Fc receptor γIIA (FcγRIIA) is expressed on human platelets (but is absent in mice),[62,64] and its activation is critical in the pathogenesis of immune-mediated thrombocytopenia, bacterial sepsis–associated thrombocytopenia, disseminated intravascular coagulation, and various thrombotic manifestations of anti-phospholipid antibody syndrome.[60] Importantly, FcγRIIA also amplifies platelet aggregation and thrombus formation by contributing to integrin outside-in signaling.[60,61]

To determine how platelets prevent hemorrhage during inflammation (previously discussed), transgenic mice expressing a chimeric human IL-4 receptor α/GPIbα (hIL-4Rα/GPIbα) protein instead of GPIbα on the platelet surface were used to induce thrombocytopenia. By infusion of antibodies against hIL-4Rα, profound thrombocytopenia was generated and hemorrhages were detected in the skin after inducing an Arthus reaction. Because wild-type (WT) platelets are insensitive to platelet depletion by anti-IL4Rα (they don't express the chimeric α/GPIbα [hIL-4Rα/GPIbα] protein), WT platelets were transfused in thrombocytopenic mice and could rescue mice from bleeding at the site of inflammation.[59] Importantly, transfused platelets lacking the expression of GPVI and CLEC2 failed to prevent bleeding in skin during an Arthus reaction, demonstrating the key role of GPVI and CLEC2 signaling in the maintenance of endothelial integrity by platelets.[59]

Another prohemostatic role of platelet ITAMs is the separation of blood and lymphatic vessels.[65] Mouse embryos deficient for CLEC2 display blood-filled lymph sacs and die shortly after birth, reminiscent of lethality seen in mice lacking spleen tyrosine kinase (Syk) and SLP-76, two kinases downstream of CLEC2 signaling.[66-69] Blood-filled lymphatic vessels also develop in lethally irradiated mature mice transplanted with bone marrow lacking CLEC2, Syk, or SLP-76 and they die of lymphatic dysfunction, demonstrating that CLEC2 and its signaling are implicated in the separation of blood and lymph in the adult.[60,70] It is not known whether drugs intended to target Syk in the treatment of chronic inflammatory conditions, such as rheumatoid arthritis (RA), might have an impact on blood-lymphatic vessel separation.

Together, these observations illustrate that not all functions of platelets can be blocked simply through "classical" anti-platelet drugs such as antagonists of ADP, acetylsalicylic acid, and thrombin (Figure 16-2).

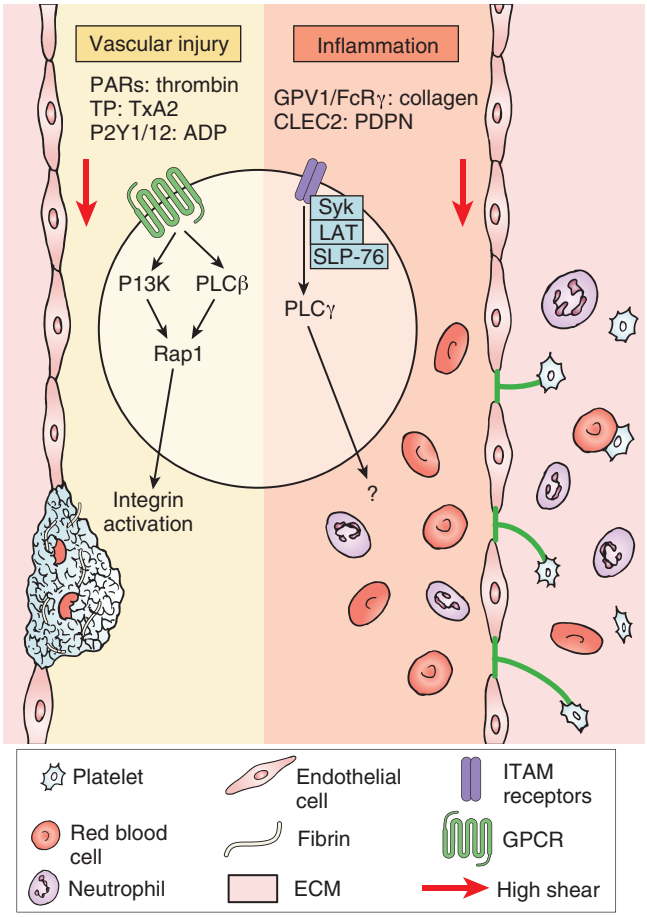



**Figure 16-2** Platelet-dependent hemostasis after vascular injury and at sites of inflammation. Schematic representation of important molecular mechanisms regulating platelet-dependent hemostasis. At sites of vascular injury, platelet activation and adhesion is strongly dependent on soluble agonists and their respective G protein–coupled receptors (GPCRs) expressed on the platelet surface. Engagement of GPCRs leads to the rapid activation of phospholipase Cβ2 (PLCβ2) and phosphatidylinositol 3-kinase (PI3K), events that are critical for the activation of the small guanosine triphosphatase Rap1, affinity regulation in platelet integrins, and platelet aggregate formation. The contribution of immunoreceptor tyrosine-based activation motif (ITAM)–coupled receptors to platelet activation at sites of vascular injury is weak when compared with GPCRs. In contrast, hemostasis at sites of inflammation depends primarily on platelet ITAM signaling and is independent of major platelet adhesion receptors. In these inflammatory conditions, platelets can also contribute to neutrophil extravasation and formation of neutrophil extra-cellular traps. These findings suggest a model in which platelets get activated under low-flow/no-flow conditions in the extravascular space, leading to the release of soluble factors that secure vascular integrity. Both the signaling response downstream of PLCγ2 and the platelet-derived mediator(s) critical for vascular integrity in inflammation are currently unknown. CLEC2, C-type lectin 2; ECM, extra-cellular matrix; FcRγ, Fc receptor γ chain; GPVI, glycoprotein VI; LAT, linker for activation of T cells; PAR, protease activated receptor; PDPN, podoplanin; SLP-76, SH2 containing leukocyte protein of 76 kDa; TP, TxA2 receptor; and TxA2, thromboxane $A_2$. *(Modified from Boulaftali Y, Hess PR, Kahn ML, Bergmeier W: Platelet immunoreceptor tyrosine-based activation motif [ITAM] signaling and vascular integrity.* Circ Res *114:1174–1184, 2014. Reprinted with permission from the American Heart Association.)*

## THE PLATELET AS AN INFLAMMATORY CELL

Nucleated thrombocytes, called hemocytes, participate both in hemostasis and in immune defense in lower vertebrates (amphibians, birds, fish, and reptiles).[71] It is generally accepted that these more primitive multifunctional cells slowly evolved to the more specialized role of the mammalian platelets. The platelet nevertheless maintains a broad repertoire of inflammatory mediators, and growing evidence points to an active role of the platelet in innate and adaptive immunity.

Inflammation occurs during innate and adaptive immune responses and in tissue repair and can sometime contribute critically to human disease. A central event in inflammation is the recruitment of leukocytes. When a blood vessel is damaged, not only do platelets rapidly seal the vascular leak, but they also recruit leukocytes that serve to combat potential infection at the injured site. Because of their abundance in blood and their adhesive receptors, platelets are perfectly positioned to have an impact on immune cell extravasation. Platelet P-selectin interacts with leukocyte P-selectin glycoprotein ligand-1 (PSGL-1), allowing leukocytes to roll on adherent activated platelets. Firm adhesion of leukocytes follows through the interaction between leukocyte integrin αMβ2 (CD11b/CD18, Mac-1) and platelet GPIb.[72,73]

CD40L (CD154) is a transmembrane protein part of the tumor necrosis factor (TNF) receptor family that is expressed by both T cells and platelets. Its counterligand CD40 is expressed by macrophages, dendritic cells, B cells, platelets themselves, and other lineages, including endothelial cells. Platelet-derived CD40L can support B cell differentiation and immunoglobulin class switching and induces the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and release of chemokine (CC motif) ligand 2 (CCL2) by endothelial cells, promoting leukocyte adhesion and extravasation.[71] Because platelets are the largest reservoir of soluble CD40L in human body, plasma levels of CD40L correlate with platelet activation.

Platelets also express Toll-like receptors (TLRs), which are members of a family of pattern recognition receptors that recognize well-conserved molecular motifs common among pathogens. Platelets express TLRs 1 through 9, and platelet TLR stimulation provokes thrombocytopenia and engages TNF production in vivo.[74-77] When platelet TLR4 is activated by bacterial lipopolysaccharide (LPS), it promotes the interaction of platelets with neutrophils, helping to trigger neutrophil degranulation and release of neutrophil extra-cellular traps (NETs) that stretch downstream of the platelet-neutrophil aggregate in blood.[78] NETs are readily observed in RA, systemic lupus erythematosus (SLE), and gout. Whether platelet-induced NETosis is beneficial or detrimental is unclear, but recent observations in gout suggest that NETs may form aggregates that trap cytokines and thereby limit inflammation.[79-82]

Apart from their surface receptors, platelet-derived soluble molecules also contribute to inflammation via mediators including growth factors, cytokines, chemokines, and lipid mediators (Table 16-1). Activated platelets release PDGF and TGF-β, which are responsible for the fibroproliferative response in chronic inflammation. PDGF is chemotactic for smooth muscle cells, fibroblasts, and macrophages and is central in wound healing.[83] Platelets contain a major pool of TGF-β in humans (milligrams of TGF-β/kilogram) and regulate its blood levels.[71] TGF-β is also chemoattractant for leukocytes, and its main function appears to be the inhibition of inflammation, because its systemic administration can reduce the development of inflammatory arthritis in rats,[84] and TGF-β is also necessary for the development of $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells.[71] However, fine control of its expression is important because TGF-β overexpression is potentially profibrotic.[85]

Other members of the platelet secretome implicated in the intertwined prohemostatic and pro-inflammatory reactions are the CXC chemokine PF4 and the CC chemokine RANTES, stored in α-granules. Because patients who have gray platelet syndrome (and thus are deficient in platelet α-granules) do not experience recurrent infections, it has been suggested that platelet pro-inflammatory activity may reside in other storage compartments or may reflect newly synthesized mediators. Indeed, activated platelets can rapidly synthesize and release IL-1β.[35,86] In sum, platelets are versatile players in inflammation. The fact that platelets express inflammatory mediators, which appear to be mostly dispensable for their hemostatic functions, supports the idea that they might contribute to inflammatory conditions, such as rheumatic diseases.

## PLATELETS AND RHEUMATIC DISEASES

### Rheumatoid Arthritis

Variations in platelet number and signs of platelet activation are seen in persons with RA. Thrombocytosis (increased platelet number) is frequent in persons with active RA[87,88] and correlates with disease severity and relapses, suggesting that their production might be induced by inflammatory cytokines such as IL-1, IL-6, or TNF.[31,89] By contrast, thrombocytopenia in RA may result from treatments such as gold, cyclophosphamide, methotrexate, and azathioprine that can suppress megakaryocyte development. More rare is drug-induced immune thrombocytopenia, in which immunoglobulin G (IgG)-coated platelets are eliminated in the spleen, a feature seen in 1% to 3% of patients injected intramuscularly with gold salts.[90] In Felty's syndrome, a rare but severe complication in persons with seropositive RA, splenomegaly results in enhanced platelet clearance, but hemorrhage is rare because platelets generally remain above 50,000 platelets per microliter.[91]

In RA, the hallmarks of platelet activation are apparent in both blood and synovial fluid. Platelet-leukocyte aggregates, platelet microparticles, soluble P-selectin, and soluble CD40L are increased in the blood of persons with RA compared with healthy persons. Platelets isolated from the blood of persons with RA are hyperresponsive to stimuli in vitro, suggesting that they might be primed in vivo.[92-100] Platelets and platelet-derived proteins have been identified in the inflamed synovial cavity,[88,101-103] and platelet CLEC2 is detected in synovium in conjunction with platelet thrombi and fibrin deposition in synovial vessels.[104-106] Platelet microparticles, identified through expression of GPIIb (CD41), have been detected in the synovial fluid of

persons with RA.[107-110] Platelet microparticles are much more abundant in RA synovial fluid than in osteoarthritis fluid, but they are also detected at significant levels in joint fluid from active psoriatic arthritis, juvenile idiopathic arthritis, and gout. In RA, platelet microparticles express autoantigens such as citrullinated fibrinogen and vimentin and are coated with autoantibodies and complement in synovial fluid, but not in blood of persons with RA or in psoriatic arthritis synovial fluid.[108,111] Platelet microparticles therefore could represent an important source of autoantigens in persons with RA. Further, platelet microparticles likely represent an important nucleating factor for the immune complexes that are abundant in seropositive, but not seronegative, persons with RA.[112,113]

Whereas adherence of platelets to migrating leukocytes represents one potential route for the accumulation of platelet-derived components in the synovial fluid, a permeable vasculature will also permit direct entry of platelet microparticles. Under inflammatory conditions, platelets can mediate the vascular permeability, leading to extravasation of fluid and tissue edema.[114] Injection of platelet extracts into the skin of human volunteers induces swelling, tenderness, and redness, and platelets injected in the skin of animals triggers neutrophil accumulation and edema.[115]

Because the synovial vasculature is more permeable during RA,[104,105,116,117] the role of platelets in vascular permeability was evaluated in arthritic mice using intravital imaging.[104] Gaps in the blood vessels of the inflamed joint allowed fluorescent microbeads that had been intravenously injected in the tail vein to accumulate inside the synovial tissue, outside the vasculature. Notably, these gaps were absent in healthy mice, and no microbeads could reach nondiseased joints. The gaps were created by platelet-derived serotonin. Serotonin is stored in dense granules after its import through the serotonin transporter, the same serotonin reuptake receptor that is a target of anti-depressant agents, and fluoxetine could reduce the vascular permeability in the arthritic joint.[104] Thus, at least in mice, platelets play a key role in the ongoing maintenance of enhanced vascular permeability in the inflamed synovium.

Platelet depletion in mice reduces joint inflammation in the K/BxN mouse model of arthritis (Figure 16-3).[104,107,118] Using this model, activation of platelets triggered microparticle production through GPVI and occurred independently of P2Y12, GPIb, and thromboxane $A_2$. GPVI-induced microparticles contain both IL-1α and IL-1β and thereby activate fibroblast-like synoviocytes to produce inflammatory cytokines.[107] These results point to GPVI and its ITAM signaling cascade as possible targets for the treatment of arthritis (Figure 16-4).

## Systemic Lupus Erythematosus

Elevated levels of thromboxane,[119] soluble and surface P-selectin,[120-122] platelet-monocyte aggregates,[123] platelet microparticles,[52,97,124] and platelet ultrastructural changes such as blebbing[125] are evident in the blood of patients with SLE. Complement C4d and IgG antibodies against GPIIbIIIa and GPVI[126-128] are reported on the platelet surface in persons with SLE, and it has been suggested that anti-DNA antibodies abundant in SLE may cross-react with GPIIbIIIa.[126]

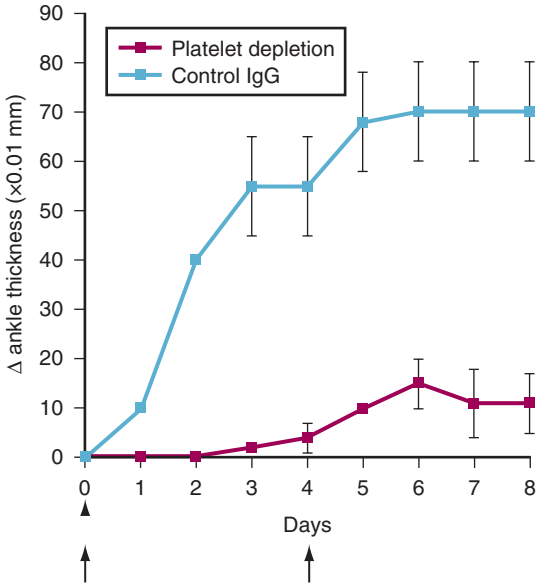



**Figure 16-3** Platelets can be involved in the development of arthritis. The passive K/BxN model of arthritis is induced by administration of arthritogenic serum containing antibodies to glucose-6-phosphate isomerase (GPI). The graph shows arthritis severity after K/BxN serum transfer in mice administered a platelet-depleting antibody *(red squares)* or isotype control *(blue squares)*. Data show the mean ± standard error of the mean (SEM).[42] *Arrows* indicate parenteral administration of platelet-depleting antibody; *arrowhead,* K/BxN serum administration. These findings suggest that platelets are required for arthritis development in vivo in this model. *(From Boilard E, Nigrovic PA, Larabee K, et al: Platelets amplify inflammation in arthritis via co llagen-dependent microparticle production.* Science *327:580–583, 2010.)*

Platelets may also bind immune complexes through FcγRIIA, a receptor implicated genetically in the pathogenesis of SLE.[129,130] Indeed, the sera of persons with SLE, but not of healthy volunteers, trigger expression of the platelet activation marker P-selectin via FcγRIIA.[131,132] Upon activation through FcγRIIA, platelets trigger production of IFN-α by myeloid and plasmacytoid dendritic cells, promoting generation of autoantibodies by B cells.[131] Using SLE-prone mouse models, platelets were shown to actively promote the disease, because platelet depletion and inhibition of platelet function (using P2Y12 blockers) both significantly ameliorate proliferative nephritis[131,133,134] (Figure 16-5).

Patients with SLE are at higher risk of thrombosis, potentially correlating with elevated levels of platelet-derived microparticles.[135] The elevated levels of circulating soluble CD40L in SLE are mainly due to the release of CD40L from activated platelets, correlating with disease activity.[131] The impact of the blockade of CD40L in SLE has been evaluated in two distinct clinical trials. Although inhibition of CD40L in patients with active proliferative lupus nephritis efficiently reduced proteinuria, the trial was terminated prematurely because of thromboembolic events seen in patients treated with the drug.[136] Correspondingly, thrombosis was induced in FcγRIIA transgenic mice intravenously injected with complexes composed of anti-CD40 and soluble CD40L, activating platelets through its unique Fc receptor.[137] In a second study, the inhibition of CD40L failed to have an impact on SLE features, and no adverse events were observed.[136,138]

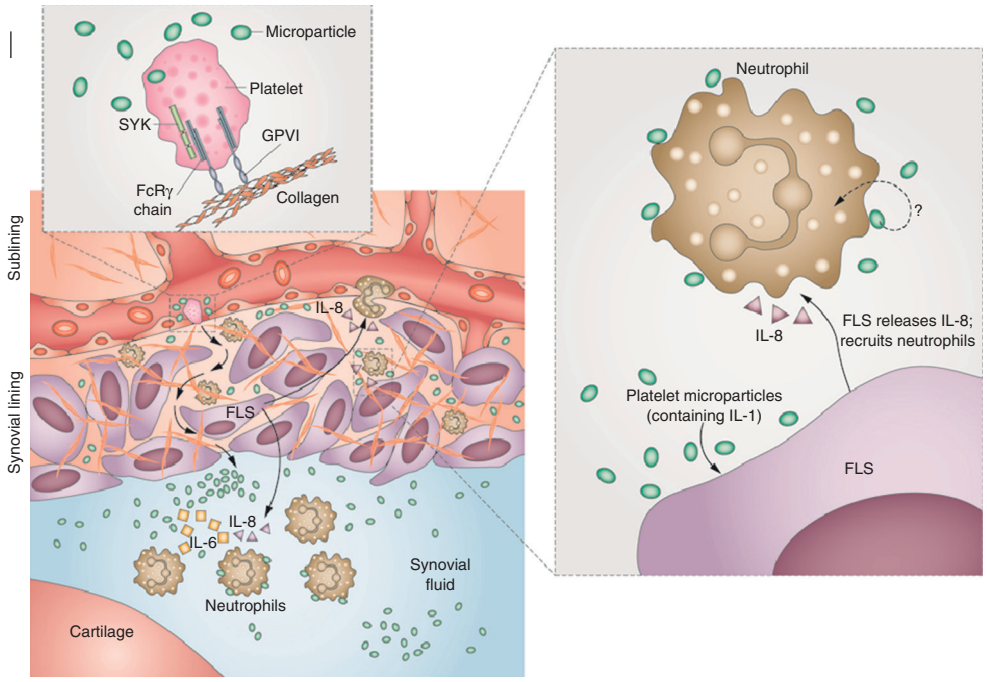



**Figure 16-4** Mechanism by which platelets can enhance inflammation in rheumatoid arthritis. Glycoprotein (GP)VI-expressing platelets activated by GPVI ligands (collagen or laminin) produce interleukin (IL)-1-rich microparticles *(main panel and insets)*. The precise anatomic location of platelet activation and the route by which microparticles enter the joint remain speculative but may implicate transportation by leukocytes and the presence of gaps between the endothelial cells in the joint vasculature and created by platelet-derived serotonin. GPVI stimulation mediates platelet activation via Src kinases and is dependent on SYK activity *(top inset)*. Platelet microparticles (~100 to 1000 nm in diameter), detectable at high levels in inflammatory synovial fluid, interact with tissue cells including fibroblast-like synoviocytes (FLS) and synovial fluid leukocytes *(right inset),* eliciting further inflammatory effector functions from target cells and thereby amplifying synovitis. In the case of FLS, platelet microparticles promote production of IL-6, IL-8 and other mediators capable of leukocyte chemoattraction to the joint *(right inset)*. Platelet microparticles attached to neutrophils, as found in diseased synovial fluid, might also stimulate neutrophil effector functions. *(From Boilard E, Blanco P, Nigrovic PA: Platelets: active players in the pathogenesis of arthritis and SLE.* Nat Rev Rheumatol *8:534–542, 2012. Reprinted with permission from Nature Publishing Group.)*

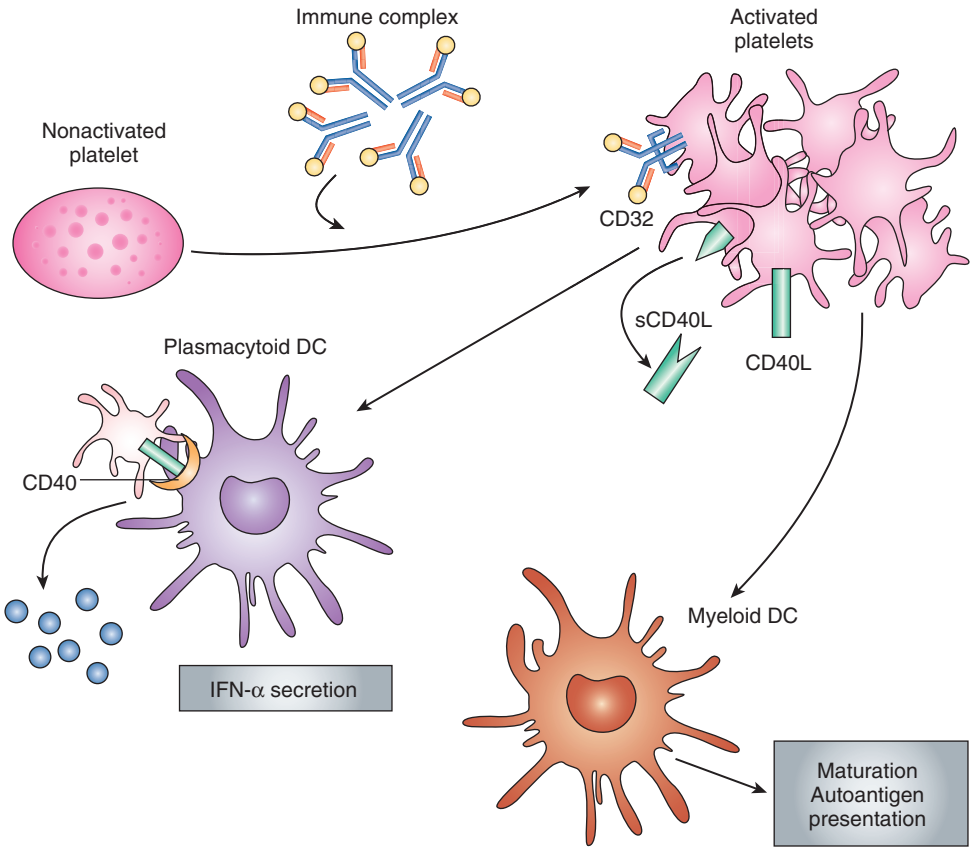



**Figure 16-5** Platelet activation processes involved in systemic lupus erythematosus. Platelest activated by circulating immune complexes express surface CD40 ligand (CD40L), which promote platelet aggregation, binding to myeloid dendritic cells (DCs) to induce their maturation, and binding to plasmacytoid DCs to promote interferon (IFN)-α secretion. sCD40L, Soluble CD40 ligand; SLE, systemic lupus erythematosus. *(From Boilard E, Blanco P, Nigrovic PA: Platelets: active players in the pathogenesis of arthritis and SLE.* Nat Rev Rheumatol *8:534–542, 2012. Reprinted with permission from Nature Publishing Group.)*

## Other Rheumatic Diseases

Platelets most likely participate in other rheumatic disorders. Activated platelets, platelet aggregates, soluble P-selectin, and platelet-derived microparticles have been observed in persons with anti-phospholipid syndrome, systemic sclerosis, ankylosing spondylitis, Arthus reaction, and Raynaud's phenomenon.[123,139-148] Although the treatment of Raynaud's phenomenon by anti-depressants targeting serotonin uptake appears to be effective,[149] pointing to a potential role for platelet-derived serotonin, the actual role of platelets in Raynaud's remains to be established.

# CONCLUSION

A key role of platelets in the prevention of hemorrhage is well known. Platelets also have less generally appreciated roles in the maintenance of endothelial integrity, in the regulation of vascular permeability, in separating blood vessels from lymphatics, and in modulating the severity and course of inflammation. Growing recognition of these non-hemostatic functions has led to a new appreciation of the potential role of platelets in the rheumatic diseases, including RA and lupus. Whereas multiple pathways can contribute to platelet activation, traditional anti-thrombotic agents are unlikely to be sufficient to intervene therapeutically in the immune biology of the platelet. Further research is required to advance our comprehension of the diverse roles of the platelet in host defense and in inflammatory disease, with the ultimate goal of developing ways to interfere selectively with the role of platelets in disease while maintaining their contribution to vascular integrity.

Full references for this chapter can be found on ExpertConsult.com.

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PART 3 EFFECTOR MECHANISMS IN AUTOIMMUNITY AND INFLAMMATION

CHAPTER 17



# Innate Immunity

Steven A. Porcelli

| KEY POINTS |
| --- |
| Innate immunity depends on recognition of conserved molecular patterns found in many microorganisms. |
| Several families of pattern recognition receptors are responsible for triggering innate immune responses. |
| Toll-like receptors and other pattern recognition receptors with leucine-rich repeat domains play a key role in innate immune recognition. |
| Anti-microbial peptides are important effectors of innate immunity. |
| Phagocytic cells and several types of innate-like lymphocytes are key cell types in mediating innate immunity. |
| Innate immune responses have a strong impact on the development of adaptive immunity. |
| Some defects in the innate immune system are associated with a predisposition to infection or to autoimmune disease. |

It has become common practice in immunology to divide the mechanisms involved in host defense into adaptive and innate components. This approach provides a useful framework for classifying the numerous cells, receptors, and effector molecules that combine to make up the vertebrate immune system (Table 17-1). A specific immune response, such as the production of antibodies or T cells against a particular pathogen, is referred to as *adaptive immunity* because it represents an adaptation that occurs during the lifetime of an individual as a result of exposure to that pathogen. Adaptive immune responses involve the clonal expansion of T and B lymphocytes bearing a large repertoire of somatically generated receptors that can be selected to recognize virtually any pathogen. The adaptive immune system of any given person is profoundly molded by the immunologic challenges encountered by that person during the course of a lifetime. A hallmark of adaptive immune responses is that they are highly specific for the triggering agent, and they provide the basis for immunologic memory. This property of memory endows the adaptive immune response with its "anticipatory" property, which provides increased resistance against future infection with the same pathogen and also allows vaccination against future infectious threats.

Adaptive immunity is essential for the survival of all mammals and most other vertebrates, but a wide variety of other mechanisms that do not involve antigen-specific lymphocyte responses are also involved in successful immune protection. These diverse mechanisms are collectively known as *innate immunity* because they are not dependent on prior exposure to specific pathogens for their amplification. Such responses are controlled by the products of germline genes that are inherited and similarly expressed by all healthy persons. Innate immune mechanisms involve both constitutive and inducible components and use a wide variety of recognition and effector mechanisms. It has become clear in recent years that innate immune responses have a profound influence on the generation and outcome of adaptive immune responses. This ability of the innate immune system to instruct the responses of the adaptive immune system suggests many ways in which innate immunity can influence the development of both long-term specific immunity and autoimmune disease.

## EVOLUTIONARY ORIGINS OF INNATE IMMUNITY

In spite of its obvious importance for most vertebrate organisms, the adaptive immune system is a relatively recent evolutionary development (Figure 17-1). In the great majority of present-day vertebrate species, the adaptive immune system is based on the ability to generate large families of variable lymphocyte receptors with immunoglobulin-like structures. This ability has been conserved as a result of the acquisition of a specialized recombination system that mediates the assembly of gene segments in the T cell and B cell receptor families, which most likely occurred through invasion of the genome of a primitive vertebrate by a transposable element or virus carrying this machinery.[1] This critical step in the evolution of the immune system can be traced back to the emergence of the ancestors of present-day jawed fish, which represent the most primitive extant species known to have adaptive immune systems based on the generation of large families of specific immunoglobulin-type receptors.[2] Recently, other systems of variable lymphocyte receptors that are unrelated to immunoglobulins but also provide the basis for an adaptive immune response have been discovered in primitive jawless fish such as lampreys

**TABLE 17-1** Contrasting Features of Innate and Adaptive Immune Systems

| Property | Innate Immune System | Adaptive Immune System |
|---|---|---|
| Receptors | Relatively few (several hundred?)<br>Fixed in genome<br>Gene rearrangement not required | Many (potentially $10^{14}$ or more)<br>Encoded in gene segments<br>Gene rearrangement required |
| Distribution | Nonclonal<br>All cells of a class identical | Clonal<br>All cells of a class distinct |
| Targets | Conserved molecular patterns<br>Lipopolysaccharides<br>Lipoteichoic acids<br>Glycans and peptidoglycans<br>Others | Details of molecular structure<br>Proteins<br>Peptides<br>Carbohydrates |
| Self–non–self-discrimination | Perfect: selected over evolutionary time | Imperfect: selected in individual somatic cells |
| Action time | Immediate or rapid (seconds to hours) | Delayed (days to weeks) |
| Response | Microbicidal effector molecules<br>Anti-microbial peptides<br>Superoxide<br>Nitric oxide<br>Cytokines (IL-1, IL-6, others)<br>Chemokines (IL-8, others) | Clonal expansion or anergy of specific T and B lymphocytes<br>Cytokines (IL-2, IL-4, IFN-γ, others)<br>Specific antibody production<br>Specific cytolytic T cell generation |

IFN, Interferon; IL, interleukin.

Modified from Medzhitov R, Janeway CA Jr: Innate immune recognition. *Annu Rev Immunol* 20:197, 2002.

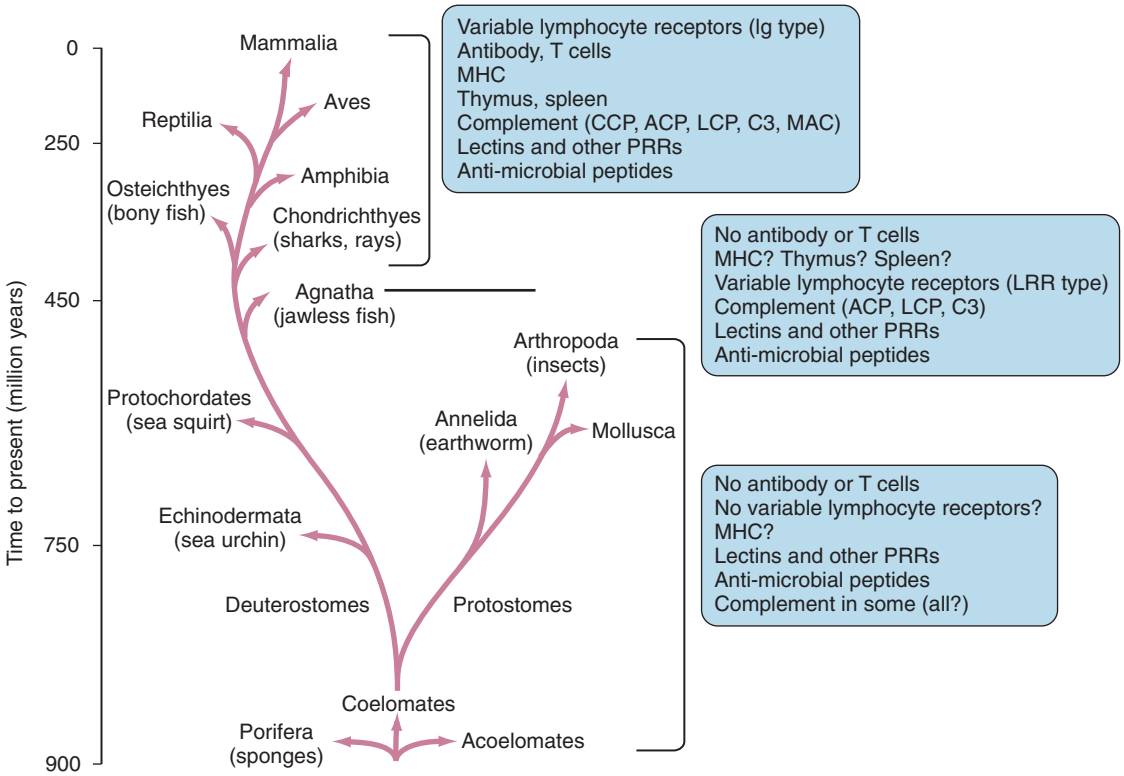



**Figure 17-1** Ancient evolutionary origin of the innate immune system. Studies of the immune systems of a wide range of vertebrates and invertebrates have revealed that even the most primitive invertebrates possess many components of innate immunity (e.g., pattern recognition receptors of the lectin and Toll-like families, anti-microbial peptides, and complement proteins). The innate immune system is thus extremely ancient, having arisen early in the evolution of multicellular life. In contrast, the adaptive immune system is a much more recent development that did not appear until emergence of the ancestors of present-day sharks and rays, approximately 400 million years ago. The first species to acquire an adaptive immune system based on immunoglobulin (Ig)-type receptors must have arisen after the appearance of the direct ancestors of present-day jawless fish (lampreys and hagfish), which are the most highly evolved living species that lack the ability to generate large families of variable Ig-type lymphocyte receptors *(arrow)*. ACP, Alternative complement pathway; CCP, classic complement pathway; LCP, lectin-activated complement pathway; LRR, leucine-rich repeat domain; MAC, membrane attack complex; MHC, major histocompatibility complex; PRR, pattern recognition receptor. *(Modified from Sunyer JO, Zarkadis IK, Lambris JD: Complement diversity: a mechanism for generating immune diversity?* Immunol Today *19:519, 1998.)*

and hagfish.[3] This finding shows that at least two different strategies for the creation of an adaptive immune system emerged at the dawn of vertebrate evolution about 500 million years ago, and it emphasizes the importance of adaptive immunity for survival and further evolution of the vertebrate lineages.

Given this key role of adaptive immunity in the evolution and survival of vertebrates, it is surprising that all invertebrate animals appear to lack the ability to generate lymphocyte populations bearing large families of clonally diverse antigen receptors.[4,5] In these animals, protection against pathogen invasion depends entirely on innate immunity, elements of which appear to exist in all animals and plants and must have evolved with the earliest multicellular forms of life. In many cases, components of the innate immune system are significantly conserved in structure and function in animals from the lowliest invertebrates to the most complex vertebrates. This preservation of innate immune mechanisms, with their functions largely intact, over such vast evolutionary distances is a clear indication of their importance, even in animals that have developed sophisticated adaptive immune responses.

## PATHOGEN RECOGNITION BY THE INNATE IMMUNE SYSTEM

Some mechanisms of innate immunity are constitutive, meaning that they are continuously expressed and are not significantly modulated by the presence or absence of infection. Examples include the barrier functions provided by epithelial surfaces continuously exposed to microbial flora, such as those of the skin and intestinal and genital tracts. In contrast, the inducible mechanisms of innate immunity involve increased production of mediators and upregulation of effector functions that eliminate microorganisms. Induction occurs as a result of exposure to a wide variety of microbes and represents a less specific form of immune recognition than that associated with the specific antibodies and T cells that mediate adaptive immunity. The basic principle underlying this form of response is a process known as *pattern recognition*. This recognition strategy is based on the detection of commonly occurring and conserved molecular patterns that are essential products or structural components of microbes.

## PAMPS AND DAMPS: PATTERNS FOR INNATE IMMUNE RECOGNITION

### Pathogen-Associated Molecular Patterns

The general name given to the targets of innate immune recognition in microbes is *pathogen-associated molecular patterns* (PAMPs). These structural features or components distinctive for microorganisms are not normally found in the animal host. The best-known example of a PAMP is bacterial lipopolysaccharide (LPS), a ubiquitous glycolipid constituent of the outer membranes of gram-negative bacteria. Another important example is the peptidoglycan structure present as the basic cell wall component in nearly all bacteria. These structures may vary partially from one bacterium to another, but the basic elements are conserved, thus providing the possibility of recognizing a broad array of pathogens by sensing a single or a relatively small number of PAMPs. Many PAMPs that serve as targets of recognition for the innate immune response are now known to be associated with bacteria, fungi, and viruses.

In addition to allowing direct recognition of molecules produced by various microorganisms, the innate immune system is able to respond to the patterns of host-derived molecules released by cells undergoing necrotic death. The molecules recognized are generally referred to as *damage-associated molecular patterns* (DAMPs) and include multiple different families of proteins, as well as nonproteinaceous substances such as uric acid microcrystals.[6,7] Thus the response to DAMPs can be an indirect response to microbial invasion, or it can be triggered by other types of tissue damage such as ischemia to result in sterile inflammation.

### Pattern Recognition Receptors

Recognition of PAMPs and DAMPs is mediated by a collection of germline-encoded molecules known collectively as *pattern recognition receptors* (PRRs) (Table 17-2). These receptors are host proteins that have evolved, through many millions of years of natural selection, to possess precisely defined specificities for particular PAMPs or DAMPs expressed by microorganisms. The total number of PRRs present in complex vertebrates such as humans is estimated to be several hundred—a number limited by the size of the genome of any animal and the number of genes it can dedicate to immune protection. The human genome, for example, is estimated to contain approximately 20,000 to 35,000 genes, most of which are not related directly to the immune system. This example demonstrates one of the strong points of contrast between innate and adaptive immune systems, because the latter can possess in the range of $10^{14}$ different somatically generated receptors for foreign antigens in the form of antibodies and T cell receptors. With its much more limited array of receptors, the innate immune system uses the strategy of targeting highly conserved PAMPs that are shared broadly by large classes of microorganisms. Because most pathogens contain PAMPs, this strategy allows the generation of at least partial immunity against most infections.

PRRs are expressed by many cell types; some are specialized effector cells of the immune system (e.g., neutrophils, macrophages, dendritic cells, and lymphocytes), and others are not generally regarded as part of the immune system (e.g., epithelial and endothelial cells). Unlike the T and B cell receptors used for adaptive immune recognition, expression of PRRs is not clonal, which means that all receptors displayed by a given cell type (e.g., macrophages) have identical structure and specificity. When PRRs are engaged by recognition of their associated PAMPs or DAMPs, effector cells bearing the PRRs are triggered to perform their immune effector functions immediately, rather than after undergoing proliferation and expansion, as in the case of adaptive immune responses. This mechanism accounts for the much more rapid onset of innate immune responses.

Considerable progress has been made toward identifying many of the important PRRs involved in the induction of innate immunity. These receptors can be classified into three functional classes: secreted, endocytic, and signaling PRRs (see Table 17-2). In addition, many of the known

**TABLE 17-2** Pattern Recognition Receptors

| Receptor Class | Examples | Prominent Sites of Expression | Major Ligands | Function |
|---|---|---|---|---|
| Secreted PRRs | Collectins<br>Mannan-binding lectin<br>Ficolins<br>Surfactant proteins (SP-A, SP-B)<br>Pentraxins<br>Short pentraxins (CRP, SAP)<br>Long pentraxins | Plasma | Carbohydrate arrays typical of bacterial capsules, fungi, and other microbes<br>Apoptotic cells and cellular debris, including chromatin | Complement activation<br>Opsonization |
| Endocytic PRRs | Lectin-family receptors<br>Macrophage mannose receptor<br>DEC-205<br>Dectin-1<br>Scavenger receptor A<br>MARCO<br>Complement receptors<br>CD11b/CD18 (CR3)<br>CD21/35 (CR2/1) | Macrophages, dendritic cells, some endothelia, epithelia, and smooth muscle cells | Cell wall polysaccharides (mannans and glucans), LPS, LTA, and opsonized cells and particles | Pathogen uptake by phagocytes<br>Delivery of ligands to antigen-processing compartments<br>Clearance of cellular and extra-cellular debris |
| Signaling PRRs | Toll-like receptors<br>CARD/NOD proteins<br>Pyrin domain proteins<br>PYHIN proteins<br>RIG-I-like receptors | Macrophages, dendritic cells, epithelia | Multiple conserved pathogen-associated molecular patterns (LPS, LTA, dsRNA, lipoproteins, flagellin, viral or bacterial DNA, others) | Activation of inducible innate immunity (anti-microbial peptides, cytokines, reactive oxygen or nitrogen intermediates)<br>Instruction of adaptive immune response |

CARD, Caspase activation and recruitment domain; CR, complement receptor; CRP, C-reactive protein; DEC-205, dendritic and epithelial cells, 205 kDa; dsRNA, double-stranded RNA; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MARCO, macrophage receptor with collagenous structure; NOD, nucleotide-binding oligomerization domain; PRR, pattern recognition receptor; PYHIN, pyrin and HIN-200 domain containing proteins; RIG-I, retinoic acid inducible gene-I; SAP, serum amyloid P protein; SP, surfactant protein.

PRRs can be classified into structurally defined families on the basis of a few characteristic protein domains. Among these, the best known include proteins with calcium-dependent lectin domains, scavenger receptor domains, and leucine-rich repeat domains (LRRs).

### Pattern Recognition Receptors of the Lectin Family

Calcium-dependent lectin domains are common modules of secreted and membrane-bound proteins involved in the binding of carbohydrate structures. A well-characterized PRR belonging to this class is the *mannan-binding lectin* (MBL), also known as *soluble mannose-binding protein*, which represents a secreted PRR that functions in initiation of the complement cascade (Figure 17-2).[8,9] This protein is synthesized primarily in the liver on a constitutive basis, although its production can be increased as an acute phase reactant after many types of infection. MBL binds to carbohydrates on the outer membranes and capsules of many bacteria, as well as fungi, some viruses, and parasites. Although mannose and fructose sugars bound by MBL can also be found on the surfaces of normal mammalian cells, they are present at too low a density or in the wrong orientation to efficiently engage the lectin domains of MBL. In contrast, the coats of many microorganisms contain an array of these sugars, which allows strong binding of MBL. Thus, in this case, the spacing and orientation of specific carbohydrate residues constitute the PAMP that triggers the activation of innate immunity by MBL. MBL functions as one of a small number of secreted PRRs that can initiate the lectin pathway of complement activation. At least two other soluble proteins with lectin activity in human plasma, known as *ficolins* (ficolin/P35 and H-ficolin), can also activate this pathway after their interaction with bacterial polysaccharides.[10]

Several of the soluble lectin-type PRRs also play an important role in the opsonization of microbes by binding to their surfaces and directing them to receptors on phagocytic cells. Among these are two pulmonary surfactant proteins, SP-A and SP-D, which similarly recognize and bind to the surface sugar codes of microbes in the respiratory tract.[11] These molecules are similar in structure to MBL, having both collagen-like and lectin domains, and together they constitute a family of soluble PRRs known as *collectins*. Another family of soluble PRRs that performs a similar function in plasma is the pentraxins, so called because they are formed by the association of five identical protein subunits.[12] This family includes the acute-phase reactants C-reactive protein (CRP) and serum amyloid P protein (SAP), along with a number of so-called long pentraxins, which have an extended polypeptide structure with homology to the classic short pentraxins (i.e., CRP and SAP) only at their carboxy-terminal domains. Long pentraxins are expressed in a variety of different tissues and cells, and their specific functions are mostly unknown. However, the long pentraxin PTX3 has been shown to play an important, nonredundant role in resistance to fungal infection in mice, and recent studies indicate that PTX3 is essentially a functional ancestor of antibodies that recognizes microbes and promotes their clearance through complement activation and phagocytosis.[13]

In addition to these soluble proteins, a large number of membrane-bound glycoproteins with lectin domains are known to exist; some of them participate in innate immunity by serving as endocytic PRRs for the uptake of microbes or microbial products[14,15] (Figure 17-3). One of the

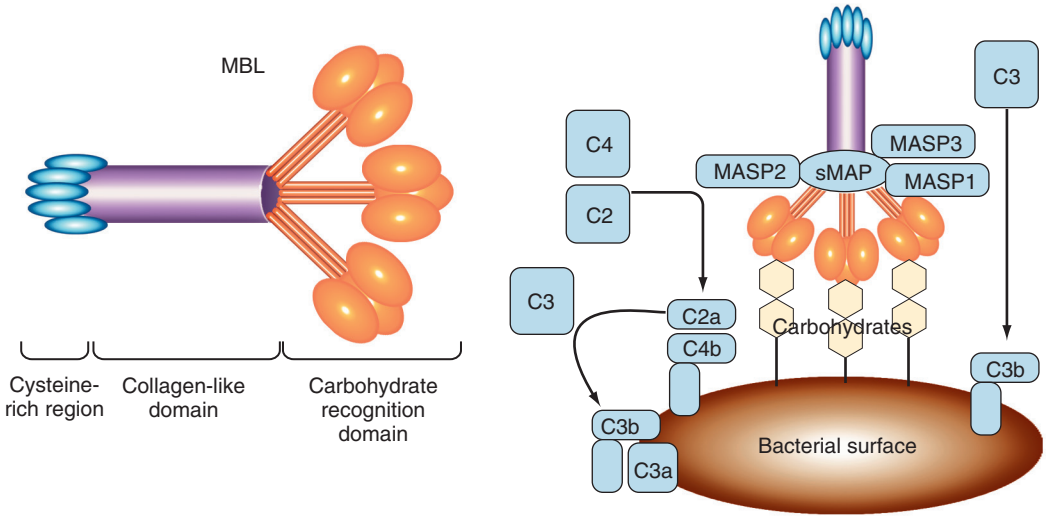



**Figure 17-2** Structure and function of mannan-binding lectin (MBL), a soluble pattern recognition receptor. *Left,* MBL is a multimer protein structure with multiple carbohydrate-binding lectin domains. Three identical 32 kDa polypeptides associate to form a subunit, which then oligomerizes to form functional complexes (the trimeric form consisting of three subunits is illustrated, which is one of several different oligomer sizes that has been observed for MBL). Each polypeptide in the subunit contains an N-terminal cysteine-rich domain, a collagen-like domain, a neck region, and a C-terminal carbohydrate recognition domain. *Right,* Initiation of the lectin pathway for complement activation by MBL. The carbohydrate recognition domains of MBL bind to carbohydrates that are characteristic of bacterial surfaces, which leads to the recruitment of several other serum proteins, including small MBL-associated protein (sMAP) and the three MBL-associated serine proteases (MASP1, MASP2, MASP3). The protease activity of MASP2 cleaves complement C4 and C2 subunits, generating the C3 convertase (C4bC2a). MASP1 is able to cleave C3 directly. The deposition of C3 cleavage products on the bacterial surface results in opsonization and phagocytosis of the bacterial cell.

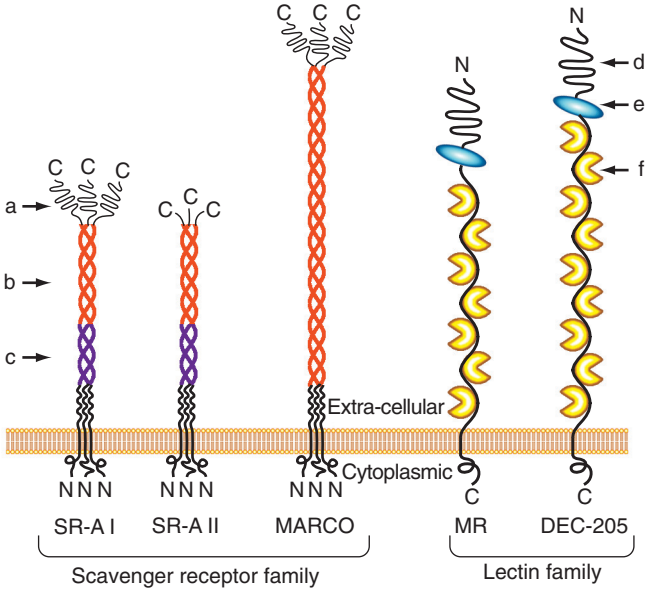



**Figure 17-3** Endocytic pattern recognition receptors of the scavenger receptor and lectin families. *Left,* Illustrations of three members of the scavenger receptor family. These trimeric complexes of type II transmembrane polypeptides have their N-terminals positioned in the cytoplasm and their C-terminals in the extra-cellular space. Three distinct extra-cellular structural domains are indicated: (a) the scavenger receptor cysteine-rich (SRCR) domain (absent in SR-A II), which has no currently known function; (b) the collagen-like domain, which is implicated in the binding of polyanionic ligands; and (c) the α-helical coiled-coil domain (absent in macrophage receptor with collagenous structure [MARCO]), which is believed to assist in receptor trimerization. *Right,* Two examples of multilectin domain endocytic pattern recognition receptors: macrophage mannose receptor (MMR) and DEC-205. Distinct extra-cellular domains in these receptors include (d) a cysteine-rich N-terminal domain, (e) a fibronectin-like domain, and (f) multiple calcium-dependent (C-type) lectin domains that bind various carbohydrate ligands. *(Modified from Peiser L, Mukhopadhyay S, Gordon S: Scavenger receptors in innate immunity.* Curr Opin Immunol *14:123, 2002.)*

most extensively studied of these is the macrophage mannose receptor (MMR).[16] Although originally identified on alveolar macrophages and known to be expressed on macrophage subsets throughout the body, this receptor is also expressed on a variety of other cell types, including certain endothelia, epithelia, and smooth muscle cells. The MMR is a membrane-anchored, multilectin domain–containing protein that mediates the binding of a broad range of pathogens, leading to their internalization via endocytosis and phagocytosis. Although the major function of the MMR appears to be directing the uptake of its ligands, evidence suggests that this receptor may be capable of signaling to modify macrophage functions after receptor engagement.[17] Another member of this receptor family, the β-glucan binding cell-surface lectin known as *dectin-1*, has a role in the modulation of inflammation in a mouse model of infection-induced arthritis.[18]

## Pattern Recognition Receptors of the Scavenger Receptor Family

The scavenger receptor family contains a broad range of structurally diverse cell surface proteins that are expressed most prominently on macrophages, dendritic cells, and endothelial cells[19] (see Figure 17-3). Although they were originally defined by their ability to bind and take up modified serum lipoproteins, they also bind a wide range of other ligands, including bacteria and some of their associated products. Multiple members of this family have been implicated as PRRs for innate immunity, including the scavenger receptor A (SR-A) and a related molecule called the *macrophage receptor with collagenous structure* (MARCO).[20] Both of these molecules contain a scavenger receptor cysteine-rich domain in the distal ends of their membranes and a collagen-like stalk with a triple-helical structure. Both

are known to bind bacteria, and SR-A also binds well-known PAMPs such as lipoteichoic acids and LPS.[21,22] Mice that have been made deficient in SR-A by targeted gene disruption show increased susceptibility to infections caused by a variety of bacteria, thus providing strong evidence of the role of scavenger receptors in protective immunity, most likely through the activation of innate immune mechanisms.[23,24] Members of the class B scavenger receptor family, including CD36 and SR-BI/CLA-1, have also been found to recognize a variety of pathogen-derived molecules.[19] Although these members of the scavenger receptor family clearly function as endocytic PRRs in the uptake of microbes, their potential to serve as signaling receptors has not yet been established. However, several scavenger receptors play a co-receptor role in the signal transduction process mediated by members of the Toll-like receptor (TLR) family (discussed later), most likely by capturing specific ligands and transferring them to adjacent TLRs.[19]

## Pattern Recognition Receptors with Leucine-Rich Repeat Domains

LRRs are structural modules found in many proteins, including PRRs involved in signaling the activation of innate immunity. Molecules in this class include, most notably, the family of mammalian TLRs, which are membrane-bound signal-transducing molecules that play a central role in the recognition of extra-cellular and vacuolar pathogens.[25] Two families of cytoplasmic LRR-containing receptors have also been identified; they play a prominent role in the innate immune recognition of PAMPs expressed by intra-cellular pathogens. These include the families of caspase activation and recruitment domain (CARD) proteins and of pyrin domain proteins.[26] Molecules are closely related in structure and function to proteins found in invertebrates and plants involved in pathogen resistance, highlighting the ancient origin of these pathways for host defense; they appear to have been recognizably conserved throughout approximately 1 billion years of evolution.

**Toll-Like Receptors.** The first member of the Toll family to be discovered was the *Drosophila* Toll protein, which was identified as a component of a signaling pathway that controls dorsoventral polarity during development of the fly embryo.[27] The sequence of Toll showed it to be a transmembrane protein with a large extra-cellular domain containing multiple tandemly repeated LRRs at the N-terminal end, followed by a cysteine-rich domain and an intra-cellular signaling domain (Figure 17-4). A role for Toll in immune responses was suggested by the observation that its intra-cellular domain shows homology to the mammalian interleukin (IL)-1R cytoplasmic domain.[28] This association was later confirmed in studies showing that Toll was critical for the antifungal response in the fly, linking this pathway for the first time to innate immunity.[29] Identification of *Drosophila* Toll eventually led to a search for similar proteins in mammals; this effort has been richly rewarded, yielding

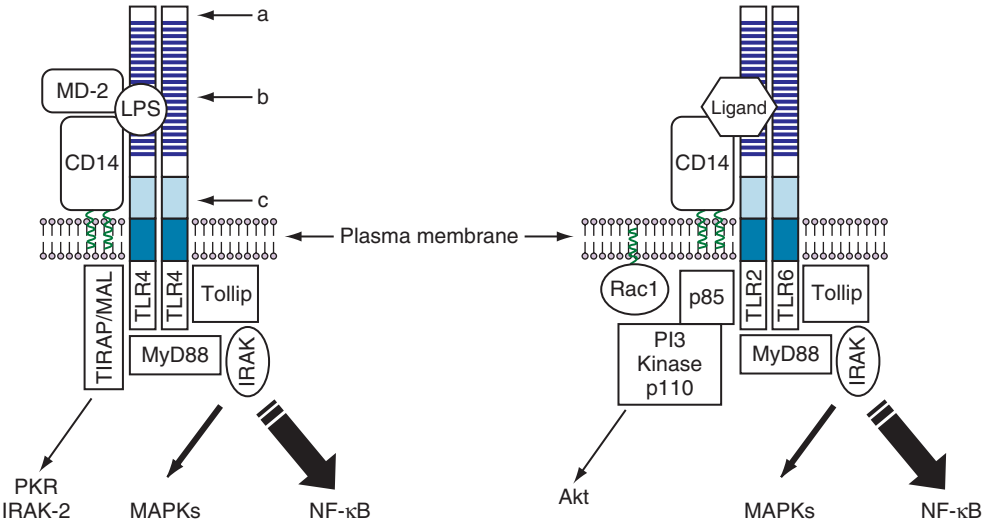



**Figure 17-4** Toll-like receptors (TLRs) and associated proteins. *Left,* TLR4 is a transmembrane polypeptide present in the plasma membrane as a homodimer. The TLR4 polypeptide has three distinct extra-cellular regions: (a) an N-terminal flanking domain; (b) the leucine-rich repeat (LRR) region, which contains 21 leucine-rich motifs and is thought to be directly involved in binding to lipopolysaccharide (LPS) and other ligands; and (c) a C-terminal flanking cysteine-rich domain. The cytoplasmic domains of TLR4 and all other TLRs have homology to the human interleukin (IL)-1 receptor and are designated Toll-IL-1 receptor (TIR) domains. The extra-cellular portion of TLR4 associates with at least two other proteins, CD14 and MD-2, which are involved in ligand recognition. The intra-cellular TIR domains associate with multiple adapter proteins (MyD88, TIRAP/MAL, Tollip), which link the receptor complex to kinases that activate signaling cascades. For TLR4, and probably for most other TLRs as well, activation of the IL-1 receptor–associated kinase (IRAK) is an important step leading to release of the active form of transcription factor nuclear factor-κB (NF-κB). In addition, signaling through TLR4 leads to signal transduction through the activation of mitogen-activated protein kinases (MAPKs), double-stranded RNA-binding protein kinase (PKR), and other members of the IRAK family such as IRAK-2. *Right,* A different set of ligands is recognized by TLR2, which functions as part of a heterodimeric complex with other TLRs such as TLR6. The TLR2-TLR6 complex shares many features with the TLR4 complex in terms of its associated proteins. However, the TIR domain of TLR2 also appears to recruit phosphatidylinositol-3-OH kinase (PI3 kinase, p85, and p110 subunits) and the membrane-associated guanosine triphosphatase Rac1, which allows the activation of other signaling molecules such as the serine-threonine kinase Akt. Thus, although the major signaling pathways activated by different TLRs are similar or identical (i.e., activation of NF-κB and MAPKs), it is likely that each TLR complex has subtle differences in its secondary pathways of signal transduction. These differences may lead to partially overlapping but distinct outcomes in response to ligands recognized by different TLR complexes. *(Modified from Underhill DM, Ozinsky A: Toll-like receptors: key mediators of microbe detection.* Curr Opin Immunol *14:103, 2002.)*

a family of 10 TLRs in humans and 12 in mice.[30] Among these, TLR1 through TLR9 are conserved between mice and humans, TLR10 is present only in humans, and TLR11 through TLR13 are expressed only in mice.[30] All these molecules contain large extra-cellular domains with multiple LRRs, as well as intra-cellular signaling domains known as Toll/IL-1R, or TIR, domains.[31] Many of these TLRs have been linked to innate immune responses against various PAMPs of different microorganisms.[32]

**Toll-Like Receptor 4 and the Response to Lipopolysaccharide.** The first human TLR to be identified was the molecule now designated TLR4, which is a major component in the response to one of the most common of all PAMPs: bacterial LPS.[33] Earlier studies on the response to LPS had identified two proteins—CD14 and LPS-binding protein—as molecules involved in the binding of LPS to the surface of LPS-responsive cells. However, these molecules did not possess any potential for transducing signals into the cell, so it was unclear how LPS binding would lead to the activation of cellular responses associated with gram-negative bacterial infection. The answer was provided by positional cloning studies of the gene responsible for the phenotype in the LPS-hyporesponsive C3H/HeJ mouse.[34] This study revealed a single amino acid substitution in the signaling domain of TLR4. Specific deletion of the *TLR4* gene by targeted gene disruption in mice subsequently confirmed the essential role of this molecule in the response to LPS, because TLR4 knockout mice have almost no response to LPS and are highly resistant to endotoxic shock.[35,36] Biochemical studies provide further support for TLR4 as a component of the LPS receptor; they show that LPS bound to the surface of cells is in close contact with both CD14 and TLR4, as well as another protein called *MD-2*, which appears to perform an accessory function in the binding of LPS to the receptor complex.[37] Additional studies have elucidated many of the downstream elements in the signaling pathways that connect TLR4 to the activation of genes associated with inducible innate immunity[38] (see Figure 17-4). Studies of Toll signaling pathways in *Drosophila* have identified the transcription factor nuclear factor-κB (NF-κB) as one of the key effectors of gene activation after the engagement of Toll. This basic pathway in the fly appears to be largely conserved in TLR signaling in higher animals, including mammals.[39]

**Other Pathogen-Associated Molecular Patterns Recognized by Toll-Like Receptors.** The search for ligands that lead to signaling through various TLRs has demonstrated that this family of PRRs is collectively responsible for innate immune responses to an extraordinary array of PAMPs. In addition to its central role in the signaling of responses to LPS, TLR4 is involved in responses to multiple different self-ligands and non–self-ligands.[32] The antimitotic agent and cancer chemotherapy drug paclitaxel (Taxol) has been shown to mimic LPS-induced signaling in mouse cells through a pathway that requires both TLR4 and MD-2.[40] Other foreign ligands of TLR4 include the fusion protein (F protein) of respiratory syncytial virus[41] and the heat shock protein 60 (HSP60) of chlamydia.[42] TLR4 can signal in response to mammalian HSP60, a protein expressed at increased levels and most likely released by stressed or damaged cells.[43] This represents a variation of the pattern recognition principle in which the pattern is an endogenous molecule released by damaged host cells that serves as a DAMP, rather than a PAMP produced directly by a pathogen. Other examples of recognition of DAMPs by TLR4 include responses to oligosaccharide breakdown products of tissue hyaluronans and responses to the extra-domain A region of fibronectin produced by alternative RNA splicing in response to tissue injury or inflammation.[32,44]

The range of PAMPs recognized through TLR2 is probably even greater than for TLR4. TLR2 is known to be involved in signaling in response to multiple PAMPs of gram-negative and gram-positive bacteria, including such structures as bacterial glycolipids, bacterial lipoproteins, parasite-derived glycolipids, and fungal cell wall polysaccharides.[32] TLR2 does not function independently in responding to these PAMPs; rather, it forms heterodimers with TLR1 or TLR6. This ability to pair with other TLRs appears to be unique to TLR2, because other TLRs that have been studied carefully (e.g., TLR4 and TLR5) most likely function only as monomers or homodimers. Other TLRs with currently defined ligands are TLR5 (involved in the response to bacterial flagellin), TLR3 (double-stranded RNA), TLR7 (single-stranded RNA), and TLR9 (unmethylated bacterial DNA).[32] It is apparent that most, if not all, microbes contain multiple PAMPs that are recognized by different TLRs. For example, a typical bacterium expressing LPS also contains unmethylated DNA and thus generates signals not only through TLR4 but potentially through TLR9 as well. Because different TLRs are capable of activating distinct signaling cascades (see Figure 17-4), the ability of a single cell to detect several different features of a pathogen simultaneously with multiple TLRs may help the innate immune response to be more finely tuned to respond to a particular challenge.[45]

**CARD and Pyrin Domain Proteins.** A large number of cytosolic proteins that have structural similarities to membrane-bound TLRs and that function as sensors for PAMPs of intra-cellular pathogens and as regulators of innate immune responses have been identified. Many of these proteins contain LRR domains and have been classified on the basis of their incorporation of a CARD or pyrin domain. The nomenclature and classification schemes for this growing family of innate immune sensors and regulators are still evolving, and it has been proposed that they should be grouped and classified as members of a single family designated the CATERPILLER (CARD, R [purine]-binding, pyrin, lots of leucine repeats) family.[46] However, the most recent literature shows a trend toward referring to this group of proteins as the *NLR family*, an acronym that stands for either "nucleotide-binding domain, leucine-rich repeat proteins"[47] or "Nod-like receptors."[30] The first intra-cellular microbial sensors in this family to be described were the Nod1 and Nod2 proteins, which contain LRR domains linked to a central nucleotide binding and oligomerization (NOD or NACHT) domain and an N-terminal CARD domain.[48] As in the case of TLRs, the LRR domains of these proteins appear to be involved in the recognition of pathogen-derived molecules and a variety of host components that function as DAMPs, and their CARD domains are linked to downstream signaling for the activation of innate immunity. Although originally implicated in the response to bacterial LPS, it is now well accepted that both

Nod1 and Nod2 are primarily involved in the recognition of muropeptide monomers released from bacterial cell wall peptidoglycans.[26] Signals resulting from the recognition of peptidoglycan components by Nod1 and Nod2 lead to activation of the NF-κB pathway, as in the case of TLR signaling. However, other signaling pathways also appear to be engaged, such as activation of procaspase-1 and caspase-9 by CARD domain interactions, leading to increased production of IL-1β and cell death through a process referred to as *pyroptosis*.[47]

The pyrin domain–containing proteins represent a major subgroup of NLRs that are believed to signal in response to microbial invasion or cellular stress. The prototype member of this family is pyrin, which is the product of the gene that is mutated in persons with familial Mediterranean fever.[46] Although pyrin itself lacks an LRR domain, numerous other members of this family contain an LRR linked to a central NOD domain and an N-terminal pyrin domain. These family members include cryopyrin (also known as NLRP3 or NALP3), which is mutated in patients with a range of hereditary inflammatory diseases referred to collectively as *cryopyrin-associated periodic syndromes*.[47] Cryopyrin, together with these multiple related proteins, constitutes a large set of related proteins known as the NLRP (NLR-PYRIN domain) or NALP (NACHT-LRR-PYRIN domain–containing proteins) family.[26] The human genome contains 14 genes encoding NLRP proteins, the precise functions of which are still largely unknown.[30] However, several NLRP proteins, particularly NLRP3 and NLRP1, have been identified as key components in the formation of intra-cellular complexes known as *inflammasomes*. These cytosolic protein complexes serve as activating platforms that are involved in the activation of caspases—that is, intra-cellular proteases required for the processing of inflammatory cytokines such as IL-1β and IL-18.[47,49] Direct recognition of specific PAMPs by NLRP proteins remains to be established, although initial studies implicate these proteins as direct or indirect sensors of various stimuli, including constituents of bacteria (peptidoglycan, bacterial RNA, and exotoxins), viruses (double-stranded RNA), and uric acid crystals.[30,47,50–53]

In addition to the likely role of NLRP proteins in sensing foreign nucleic acids as PAMPs, recent studies have characterized a complex array of other proteins that serve as sensors for intra-cellular DNA or RNA and are likely to play major roles in anti-viral innate immunity. One prominent example is the family of PYHIN proteins, which are cytosolic or nuclear proteins containing PYRIN domains linked to a DNA-binding domain known as HIN-200.[54] Some of these proteins, such as the absent in melanoma-2 (AIM2) protein, have been clearly established to act as sensors for intra-cellular viral DNA that can trigger a range of innate immune responses. A range of other candidate intra-cellular proteins have been identified that may make important contributions to the innate response to DNA, particularly those that act through signaling pathways mediated by the adaptor protein called stimulator of interferon genes (STING).[55] Among the growing list of cytosolic RNA sensing proteins, the most well studied at present are the RIG-I-like receptors (RLRs). The RLRs include the retinoic acid–inducible gene-I (RIG-I), melanoma differentiation factor 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) proteins.[56] These proteins are expressed in the cytosol of most cells and respond to structural motifs present in many viral RNA molecules to induce type I interferon (IFN) and other anti-viral effectors.

## EFFECTOR MECHANISMS OF INNATE IMMUNE RESPONSES

The ability to recognize pathogens through PRRs allows activation of numerous anti-microbial effector mechanisms by the innate immune response. These responses lead to the killing of pathogens through production of effector molecules with direct microbicidal activities, including the membrane attack complex of complement, a variety of anti-microbial peptides, and the caustic reactive oxygen and reactive nitrogen intermediates generated within phagocytic cells. In invertebrates, these mechanisms represent virtually the entire protective response against microbial invaders. However, in most vertebrates, including mammals, innate immune recognition also has profound effects on triggering and programming the adaptive immune response that follows somewhat later. This ability of the innate immune system to instruct the adaptive response has major implications for the development of long-term protective immunity to infection and may play a critical role in mechanisms leading to autoimmunity.

## CELL TYPES MEDIATING INNATE IMMUNITY

Many types of cells have the ability to mount at least a limited response to PAMPs, but the most effective cell types in this regard are the specialized phagocytes, such as macrophages, neutrophils, and dendritic cells. Upon recognition of microbial stimuli, these cells have the ability to upregulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by assembling the components of this enzyme complex on phagosomal membranes, leading to an oxidative burst that produces microbicidal superoxide ions.[57] Many phagocytic cells also increase their expression of inducible nitric oxide synthase (iNOS, or NOS2) upon contact with various PAMPs.[58] This increased expression leads to the production of reactive nitrogen intermediates, including nitric oxide and peroxynitrite, which have potent direct antimicrobicidal activities. These responses are synergistic because the anti-microbial activity of the phagocyte oxidase system is frequently enhanced by the expression of reactive nitrogen intermediates.

### Innate-like Lymphocytes

A number of distinct lymphocyte subsets also play important roles in innate immune responses. Among these subsets are a complex mixture of lymphocytes that lack markers of the T and B cell lineages and are collectively referred to as innate lymphoid cells (ILCs). The complexity of ILC populations is currently still being unraveled. Recent classifications recognize three distinct lineages designated ILC1, ILC2, and ILC3 cells that are distinguished by phenotypic markers and by secretion of different cytokines.[59] The ILC1 subset is composed of IFN-γ–secreting populations, which include natural killer (NK) cells. These lymphocytes do not express receptors generated by somatic recombination and thus depend on germline-encoded receptors for signaling

their responses against pathogen-infected cells.[59] NK cells participate in the early innate response against virally, and probably bacterially, infected cells through expression of cytotoxic activity and secretion of cytokines.[60]

Several other subsets of lymphocytes belonging to the T and B cell lineages have been identified as participants in the rapid response against pathogens to which the host has not previously been exposed. Although these cells express clonally variable, somatically rearranged antigen receptors (T cell antigen receptors or membrane immunoglobulins) and thus could be classified as components of the adaptive immune system, their manner of functioning is much more characteristic of innate than adaptive immunity. These innate-like lymphocytes (ILLs) may represent remnants of the earliest primitive adaptive immune system, and they appear to have been conserved to varying degrees because they continue to make specialized contributions to host immunity.[61]

Among the currently recognized ILLs are two B cell populations, known as the B1 and marginal zone B cell subsets.[62,63] These B cell populations are involved in the spontaneous production of natural antibodies, which are largely germline-encoded immunoglobulins that are reactive to commonly expressed microbial determinants. In addition, both of these B cell populations generate rapid T cell–independent responses after bacterial challenges and thus contribute to the first line of immune defense that precedes the onset of adaptive immunity.

Among the T cells, three populations of ILLs have been identified and characterized in detail: γδ T cells, NK T cells, and mucosal-associated invariant T (MAIT) cells. The γδ T cells express somatically rearranging receptors that use a limited number of variable region genes and are thought to recognize a narrow spectrum of foreign or self-ligands.[64] In humans, the specificities of two subsets of γδ T cells have been at least partially defined. One of these subsets, the major circulating population expressing the Vδ2 gene product, responds rapidly and without prior immunization to a variety of small alkyl phosphate and alkyl amine compounds that are produced by many bacteria. Another subset, characterized by its expression of the Vδ1 gene product, responds to class I major histocompatibility complex (MHC)–related self-molecules of the class I MHC chain–related A and B (MICA/B) and CD1 families.[64] These molecules may serve as markers of cellular stress and are upregulated on cells in the context of infection or inflammation, leading to the activation of Vδ1-bearing γδ T cells.

A similar principle appears to be involved in the functioning of NK T cells, which are so named because of their co-expression of an αβ T cell antigen receptor and a variety of receptor molecules that are typically associated with NK cells.[65] Similar to γδ T cells, NK T cells have somatically rearranged antigen receptors that use a limited array of V genes and most likely recognize a narrow range of foreign or self-antigens. A major population of NK T cells is reactive with the class I MHC–like CD1d molecule, and these ILLs appear to be activated by recognition of a variety of lipid or glycolipid ligands that can be presented by CD1d. Recently, several bacterial glycolipids have been identified as specific antigens that stimulate NK T cells, suggesting that these cells may be rapid responders that contribute to innate antibacterial immunity.[65] A wide variety of mouse disease models have shown that NK T cells also make significant contributions to the development of adaptive immune responses and may play a particularly important role in immunoregulation to prevent autoimmunity.[65] Similarly, MAIT cells are a separate population of innate-like T cells that have recently been shown to use their αβ T cell antigen receptors to recognize common vitamin B metabolites that are produced and secreted by a wide range of bacteria.[66]

### Anti-microbial Peptides

Anti-microbial peptides are the key effector molecules of inducible innate immunity in many invertebrates and are being increasingly recognized as important elements of innate immunity in higher animal species, including mammals.[67] They are evolutionarily ancient components of host defense that are widely distributed throughout all multicellular organisms in the animal and plant kingdoms. More than 1000 such peptides have been identified, and their diversity is so great that it is difficult to categorize them. However, at a structural and mechanistic level, most of these peptides share several basic features. They generally are composed of amino acids arranged to create an amphipathic structure with hydrophobic and cationic regions. The cationic regions target a fundamental difference in membrane design between microbes and multicellular animals, which is the abundance of negatively charged phospholipid head groups on the outer leaflet of the lipid bilayer. The preferential association of anti-microbial peptides with microbial membranes leads to membrane-disrupting activity, most likely involving the interaction of the hydrophobic regions of the peptide with membrane lipids.[68]

Anti-microbial peptides produced in response to engagement of various PRRs account for most of the inducible immunity against microbes noted in many invertebrate animals and plants. Although these peptides are probably less central to host immunity in most vertebrates, evidence indicates that they make important contributions to immunity in more highly evolved animals, including mammals.[69] In humans, active anti-microbial peptides, such as the α- and β-defensins, are constitutively or inducibly produced in skin and epithelia of the gastrointestinal and respiratory tracts.[70] These molecules most likely act as natural preservatives of epithelia that are colonized or frequently exposed to microbial flora. Because the acquisition of resistance against these agents by sensitive microbial strains is extremely unusual, anti-microbial peptides are of great interest as templates for the development of new anti-microbial pharmaceuticals.[70,71]

## INFLUENCE OF INNATE MECHANISMS ON ADAPTIVE IMMUNITY

In addition to functioning as a first line of defense against invading pathogens, a critical feature of the innate immune system in higher animals such as mammals is its effect on activating the adaptive immune system. In fact, it is now clear that in most situations, the adaptive immune system mounts a response to a pathogen only after the pathogen has generated signals via PRRs of the innate immune system. This principle serves as the basis for the adjuvant

effect, which is the observation that antibody and T cell responses are efficiently generated against protein antigens only if these are introduced together with a nonspecific activator of the immune system, which is generically known as an *adjuvant*. Most adjuvants are in fact extracts or products of bacteria, and it is clear that in most or all cases, adjuvant effects result from activation of the innate immune response.[72]

Innate immune responses can prime or potentiate the adaptive immune response in many ways (Figure 17-5). In the case of T cell responses, one extremely important and well-recognized mechanism involves the upregulation of co-stimulatory molecules. T cells require at least two signals to become activated from a naïve resting state. One signal is provided through the T cell antigen receptor by its binding to a specific peptide ligand presented by an class I or II MHC molecule. The second signal is provided by one of several co-stimulatory ligands that are expressed by specialized antigen-presenting cells such as dendritic cells. The best studied of these are the molecules of the B7 family—B7-1 (CD80) and B7-2 (CD86)—which engage the activating receptor CD28 on the surface of the T cell. Expression of B7 family co-stimulatory molecules on the surface of antigen-presenting cells is controlled by the innate immune system, such that these molecules are induced to appear at functional levels only after PRRs, such as members of the TLR family, have been activated by recognition of their cognate PAMPs or DAMPs.[33]

Recent studies have shown that innate immune signaling through TLRs has a major impact on the responses of phagocytic antigen-presenting cells; it also provides an important second signal for immunoglobulin production by B cells. In the case of phagocytic cells, uptake of microbes by phagocytosis and subsequent maturation of the phagosome are stimulated by concurrent TLR signaling.[73] In dendritic cells, which are the predominant antigen-presenting cells for the priming of T cell responses, TLR signaling has a major impact on whether antigens from phagocytosed microbes are effectively presented on class II MHC molecules.[74] For B cells responding to foreign antigens, it has been demonstrated that concurrent signaling through TLRs is necessary for the efficient stimulation of T cell–dependent differentiation into plasma cells and subsequent antibody secretion.[75] This concept is also relevant for T cell responses

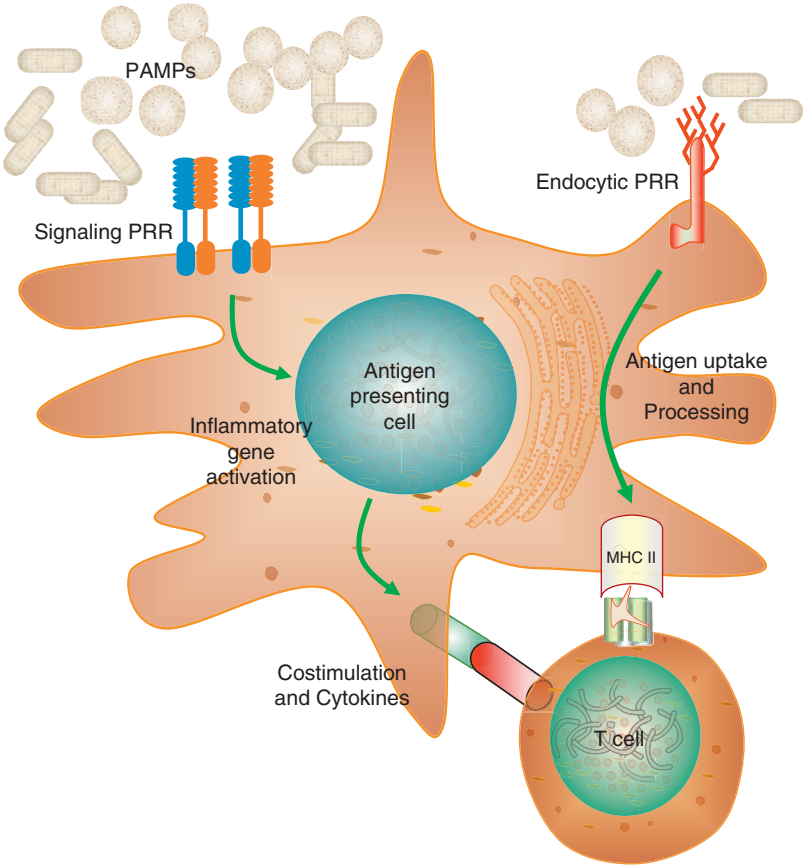



**Figure 17-5** Instruction on the adaptive immune response by the innate immune system. When an antigen-presenting cell (APC) comes into contact with pathogen-bearing pathogen-associated molecular patterns (PAMPs), responses are triggered via innate immune mechanisms that dramatically alter the ability of the APC to stimulate an adaptive (T cell–mediated) immune response. For example, signals generated by contact with PAMPs such as lipopolysaccharide (LPS) with Toll-like receptor 4 (TLR4) lead to the activation of transcription factor nuclear factor-κB (NF-κB), which enters the nucleus of the APC and assists in switching on genes for cytokines (e.g., interleukin [IL]-1, -6, and -12 and a variety of chemokines) and co-stimulatory molecules (e.g., the B7 family members CD80 and CD86). In addition, binding of the pathogen to endocytic pattern recognition receptors (PRRs) such as the mannose receptor (MR) leads to delivery of the pathogen to endosomes (Endo) and lysosomes (Lys). There, the protein antigens of the pathogen are partially degraded to generate antigenic peptides that can be presented by class II major histocompatibility complex (MHC) molecules for recognition by the T cell antigen receptors (TCRs) of specific T cells. These effects of pattern recognition by the innate immune system lead to expression of the signals required for activation of quiescent antigen-specific T cells and the subsequent generation of specific antibodies.

to autoantigens, including several prominent nuclear antigens that are targets of autoantibodies in rheumatic disease.[76,77]

Innate immune responses also trigger the production of many cytokines and chemokines, which enhance the development of adaptive immune responses and change the nature of the adaptive response generated. For example, contact between dendritic cells and PAMPs such as LPS or bacterial lipoproteins leads to the production of IL-12 as a result of signaling through TLRs.[72,78] This cytokine acts on antigen-specific T cells to promote their differentiation into T helper type 1 cells, which are associated with the production of IFN-γ and other effector mechanisms that favor the clearance of bacterial pathogens.[79] In the case of myeloid lineage dendritic cells, signaling through TLRs (and potentially other PRRs) induces a process known as *maturation*, which is associated with increased expression of antigen-presenting and co-stimulatory molecules that enables the efficient priming of naïve antigen-specific T cells.[80]

This requirement for the innate immune response to "switch on" the expression of molecules required for the priming and differentiation of T cell responses helps ensure that pro-inflammatory adaptive immune responses occur mainly in the setting of a relevant infectious challenge. After activation, helper T cells control other components of adaptive immunity, such as the activation of cytotoxic T cells, B cells, and macrophages. Innate immune recognition, therefore, appears to control all major aspects of the adaptive immune response through the initial recognition of infectious microbes by PRRs. The discovery of self-molecules that act as DAMPs further extends this paradigm to include immune responses that are triggered by tissue damage. This more extended view, sometimes referred to as the "danger model," helps explain why certain self-ligands produced or released in the setting of infection or tissue damage can function in essentially the same manner as the PAMPs associated with microorganisms.[81,82]

## DISEASE ASSOCIATIONS INVOLVING INNATE IMMUNITY

Given the obvious role of the innate immune response in virtually all types of infectious disease, one might expect that gross defects in the mechanisms of innate immunity occur relatively rarely and in association with clinical immunodeficiency. In fact, increasing evidence indicates that mutations that inactivate various innate immune pathways can lead to increased pathogen sensitivity in both laboratory mice and humans.[8,23,69,83,84] Because many of the pathways leading to innate immunity are amplified during recurrent or prolonged activation of the immune system, they must also participate in mediating tissue damage in chronic inflammatory disease. In addition, certain self-molecules that are produced or released at increased levels as a result of inflammation, including heat shock proteins, nucleic acids, and microcrystals of monosodium urate or calcium pyrophosphate, may act as DAMPs.[42,76,77,85,86] These self-molecules may signal through TLRs or other PRRs to stimulate adjuvant-like effects that increase the potential for autoreactive lymphocytes to be activated.

Perhaps a more surprising finding has been that some defects in innate immunity are associated with a markedly increased predisposition to autoimmune disease. Several different mechanisms have been proposed to explain this paradoxical association. Mechanisms of the innate immune response play an important role in the clearance of self-antigens released from necrotic or apoptotic cells, resulting in a non-inflammatory clearance of self-antigens that tends to favor tolerance rather than the stimulation of immune responses.[87] Failure of such clearance may lead to excessive exposure to self-antigens, triggering normally silent autoreactive lymphocyte clones to expand and differentiate into effector cells. This mechanism may account for the development of lupus-like autoimmunity in mice with targeted deletion of the gene for the short pentraxin SAP, which, along with other components of the innate immune system, appears to play a significant role in the clearance of DNA-chromatin complexes.[88] Reduced levels of serum mannose-binding lectin in humans also appear to be a risk factor for the development of systemic lupus erythematosus, possibly because of the role of this soluble PRR in facilitating the clearance of apoptotic cells.[89]

Deficiencies of early components of the classic pathway of complement activation have been strongly associated with lupus-like autoimmunity in both humans and mouse models.[90-94] This association may be the result of alterations in the clearance of apoptotic cells or other sources of self-antigens, resulting in increased stimulation of normally silent autoreactive lymphocytes.[95,96] An alternative, but nonexclusive, mechanism relates to involvement of the complement system, particularly the early components C1 and C4, in facilitating the induction of self-tolerance by the adaptive immune system by increasing the localization of autoantigens such as double-stranded DNA and nucleoproteins within the primary lymphoid compartment.[90,97,98] Thus, a deficiency of C1 or C4 appears to result in the failure to delete or functionally inactivate autoreactive B cell clones as they arise during lymphopoiesis in the bone marrow.[97,99] Studies carried out in mouse models suggest that this tolerance-inducing mechanism is partially disrupted in animals that are deficient in a variety of other components of innate immunity, including SAP and the complement receptors CD21/CD35.[88,97]

Multiple examples of links between defects in signaling receptors of the innate immune system and chronic inflammatory diseases have emerged from studies of the CARD and pyrin families of cytosolic PRRs.[30,47] The first association of this type was provided by genetic mapping studies that identified the Nod2 protein as the product of the inflammatory bowel disease 1 (IBD1) locus, which contributes to disease susceptibility in a subset of patients with Crohn's disease.[100-103] This soluble PRR of the CARD family normally functions by inducing cytokine production in response to bacterial peptidoglycan, but mutant alleles associated with increased risk of Crohn's disease are defective in this function.[100] In this case, it may be failure of innate immunity to adequately control bacterial colonization or infection in the intestine that leads to the final expression of disease. Consistent with this view, a recent study has demonstrated diminished expression of a class of anti-microbial peptides (β-defensins known as *cryptdins*) in Paneth cells from the ileum of patients with Crohn's disease and *Nod2* mutations.[104] Other findings suggest that defective signaling by mutant variants of *Nod2* can result in reduced production

of immunoregulatory cytokines such as IL-10, perhaps resulting in uncontrolled inflammation in the intestine.[105] Other studies have established links between various members of the pyrin family and specific chronic inflammatory disorders. These links include the causative association of mutations in pyrin with familial Mediterranean fever and of cryopyrin with cryopyrin-associated periodic syndromes.[46,47] These diseases and other chronic inflammatory or autoimmune disorders associated with specific deficiencies in innate immune mechanisms are frequently considered together as autoinflammatory diseases.[106] Recognition that these diseases are frequently associated with dysregulation of inflammatory cytokine production, in particular IL-1β, has led to some striking therapeutic advances in the treatment of selected patients using systemic administration of the IL-1 receptor antagonist anakinra.[107-109]

Deficiencies in at least two populations of ILLs—NK cells and NK T cells—have been associated with multiple autoimmune syndromes in both humans and mice.[65,110-112] This finding is believed to reflect a significant role for these ILLs in regulating adaptive immune responses, although the precise mechanisms by which they act still are not fully understood. Given the complex interplay between innate immunity and adaptive immunity, it is extremely likely that associations between alterations in innate immunity and autoimmune diseases will continue to emerge. As for some of the examples cited here, a fuller understanding of these associations is likely to lead to new and successful therapies for autoimmune and autoinflammatory diseases.

## FUTURE DIRECTIONS

In the past two decades of research in immunology, we have seen a great emphasis on the fundamental role played by innate immune mechanisms in all immune responses. The innate immune system in humans represents the accumulation of many stages of evolution and natural selection that began with the most primitive organisms. Because of the ancient origins of the innate immune system, some of the most important discoveries in the field of innate immunity have come from studies of relatively simple animals such as flies and worms. Now that many of the pieces of this elaborate system have been discovered and categorized, continued research efforts are turning increasingly toward exploring the roles of various components of innate immunity in the human immune system. These efforts are very likely to yield insights into many currently unexplained diseases and may provide targets for new therapeutics.

## CONNECTION TO THE CLINIC

- Innate immune responses serve as the foundation of all immunity; they participate at some level in all infectious, inflammatory, and autoimmune diseases.
- Drugs targeting specific innate immune effectors are beginning to emerge as useful therapeutic agents.
- Innate immune receptors are responsible for triggering the clinical syndromes associated with uric acid crystals and potentially other types of crystal-induced arthritis.
- Primary defects in specific innate immune molecules have been identified as the cause of a range of uncommon disorders known as *autoinflammatory diseases*.
- Many systemic autoimmune diseases are associated with genetic polymorphisms of molecules involved in innate immune responses.
- Research into innate immunity has provided important new insight into potential mechanisms for common autoimmune diseases such as Crohn's disease and systemic lupus erythematosus.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 18



# Adaptive Immunity and Organization of Lymphoid Tissues

Michael L. Dustin

**KEY POINTS**

- Lymphocytes are born, and many self-reactive cells are deleted, in primary lymphoid tissues.
- Immune responses are usually initiated in secondary lymphoid tissues.
- Tertiary lymphoid tissue can be generated at sites of inflammation and may promote tissue-specific autoimmunity.
- Self-tolerance is established by antigen recognition in primary lymphoid tissues or elsewhere in the absence of inflammation.
- Generation of an adaptive immune response is dependent on innate immune system activation.
- Some innate immune cells use antigen receptors.
- Dendritic cells sense both tissue-specific factors and innate immune stimuli in shaping T cell responses to antigens.
- Gut microbes that alter T cell differentiation increase susceptibility to joint inflammation and specific gut microbiota signatures are associated with new-onset rheumatoid arthritis.

The adaptive immune system is so named because it can adapt to virtually any pathogen or toxin that enters the body. Although invertebrates defend themselves through innate immunity alone,[1] all vertebrates have developed some form of adaptive immunity—that is, the ability to generate novel receptors by genetic recombination mechanisms that can then be selected to recognize diverse macromolecules associated with rapidly evolving pathogens.[2] A molecule that can be recognized by the adaptive immune system is known as an "antigen" (Table 18-1).

The ability to produce molecules and cells that can attack any biologic structure is a double-edged sword.[2] Although pathologic autoimmunity is not described in invertebrates, which evolved with a hard-wired immune system, it is a common problem in vertebrates.[3] Self-recognition must be offset by redundant mechanisms to produce self-tolerance. We discuss some mechanisms involved in this process and the anatomy behind these mechanisms in this chapter. In addition to self-antigens, animals with adaptive immunity are also exposed to many harmless environmental antigens that have the potential to induce allergic reactions.[4] Mechanisms to distinguish self-macromolecules from foreign macromolecules, as well as benign foreign macromolecules from harmful foreign macromolecules, are critical processes in successful adaptive immunity. At the core of these mechanisms is the essential partnership of adaptive and innate immunity, which was first formulated by Charles Janeway, Jr.[5] Although the adaptive immune system can recognize any foe and focus powerful effector mechanisms to destroy this foe, its ability to calculate the relative risks of mounting an immune response or becoming tolerant to a given recognized structure is guided by innate recognition of pathogen-associated molecule patterns (PAMPs) or inflammation triggered by tissue damage–associated molecular patterns (DAMPs).

The function of the adaptive immune system is tightly linked to its anatomy.[6,7] In some respects, T lymphocytes are cells without boundaries that can be found in almost any tissue at any time. T and B lymphocytes are readily monitored in patients because they are reliably found in the blood of healthy persons. However, the vast majority of the T and B lymphocytes are located in secondary lymphoid tissues, where they search for antigens. Three types of tissues concentrate lymphocytes: primary lymphoid tissues, where these cells are born; secondary lymphoid tissues, where they search for antigens and initiate immune responses; and tertiary lymphoid tissues that form at sites of chronic inflammation. Secondary and tertiary lymphoid tissues have a characteristic and functionally important organization into B cell and T cell zones. In fact, all of the previously mentioned histologic findings are directly related to functional goals of the adaptive immune system, as will be clarified subsequently.

This chapter addresses the basic concepts of adaptive immunity and its partnership with innate immunity in the context of the anatomic sites in which these responses take place.

## LYMPHOCYTE MIGRATION PARADIGMS FOR HOMING, INTERSTITIAL NAVIGATION, AND EGRESS

Because we talk about adaptive immunity in the context of secondary lymphoid tissues, it is worthwhile to first review what is known about lymphocyte migration. Since the early 1960s it has been appreciated that lymphocytes "recirculate" between secondary lymphoid tissues and the blood, and more recently we have learned that, upon activation, they take on different tissue-specific homing properties that are important for function.[8] If one arbitrarily begins this circuit with a naïve T cell in the blood, then three distinct transitions can be considered: (1) interaction with the vessel wall and extravasation; (2) interstitial locomotion in the tissue parenchyma; and (3) egress from the tissue parenchyma back into the blood, sometimes via lymph. General

**TABLE 18-1** Terms and Definitions

| Term | Definition |
|---|---|
| Antigen | Any molecular structure recognized by the adaptive immune system; the ligand for B cell receptor (BCR)/antibody or T cell receptor (TCR) |
| Chemokine | Family of small secreted or shed proteins (typically 8 kDa) that bind to G protein–coupled receptors and activate or attract cells; a chemical *(chemo-)* that induces movement *(-kine)* |
| Integrin | Family of adhesion molecules that are specialized for adhesion in the context of migration and generation of contractile force/mechanical stabilization; noncovalent heterodimers that are regulated rapidly by signaling from chemokine receptors and antigen receptors |
| Selectin | Family of adhesion molecules that are specialized for mediating initial attachment of leukocytes to the vessel wall from flowing blood; they have amino-terminal calcium-dependent lectin domains and interact with carbohydrate ligands that can incorporate protein determinants |

features of these three steps are addressed here, and relevant details will be commented on in different functional contexts in relation to adaptive immunity.

## Multistep Paradigm for Extravasation

The movement of lymphocytes from blood to tissues is complicated by the high-flow rates in blood vessels. Springer, Butcher, and colleagues[9-15] established the current paradigm called the *multistep model* for T cell extravasation.

The first step is the initial tethering of the free-flowing leukocyte to the vessel wall. A special class of adhesion molecules known as selectins and their carbohydrate ligands mediate this step (see Table 18-1).[10] The selectin family comprises three members: L-, E-, and P-selectin. L-selectin is expressed on naïve T and B lymphocytes and is essential for the entry of these cells into lymph nodes, but not into the spleen.[11] The ligand for L-selectin is a complex of sulfated sialic acid bearing complex carbohydrates linked to different protein backbones expressed on high endothelial cells in postcapillary venules in primary, secondary, and tertiary lymphoid tissues.[10] The distinctive morphology of these endothelial cells was first noted by Gowans and colleagues[8] and is due to the large number of transmigrating lymphocytes that distend the otherwise flat cells. E- and P-selectin are expressed on activated endothelial cells at sites of inflammation in diverse tissues.[12] They bind to glycoprotein ligands expressed on leukocytes that have terminal sialyl Lewis-X blood group antigens and can also incorporate other structural modifications.[13] The high-affinity ligand for P-selectin is the protein backbone P-selectin glycoprotein-1 (PSGL-1) with a stretch of sulfated tyrosines that make up part of the ligand. PSGL-1 can be expressed by lymphocytes without the necessary secondary modification to make it a ligand for P-selectin. Therefore a fusion of P-selectin to immunoglobulin Fc that can be purified and fluorescently tagged is the best probe to determine if a leukocyte is competent to bind to P-selectin–expressing endothelial cells. The genetic basis of forming selectin ligands is complex with requirements for expression of core proteins, specific sialotransferases, fucosyltransferases, and sulfotransferases.[13] Defects in fucose metabolism are the basis for a rare genetic immunodeficiency/mental retardation syndrome called *leukocyte adhesion deficiency type 2* (LAD2).[14] Selectins will only mediate leukocyte tethering and rolling, not arrest and extravasation.

Selectin-mediated tethering allows the leukocyte to bring G protein–coupled receptors close enough to the vascular wall to bind ligands attached to glycoproteins.[15] Chemokine receptor signaling is critical to activate closely linked integrin family members to bind their ligands.[16] This chemokine signal is pertussis toxin–sensitive, indicating that it involves $G_{i\alpha}$-coupled receptors. Three chemokine receptors are important with respect to lymphocyte recirculation: CCR7, CXCR4, and CXCR5. CCR7, which binds to ligands CCL19 and CCL21, is the most important chemokine system for entry of T cells into secondary lymphoid tissues.[17] The binding of CXCR4 to its ligand CXCL12 also contributes to entry of T and B cells.[18] The binding of CXCR5 to its ligand CXCL13 is the major system that controls entry of B cells into secondary lymphoid tissues.[19] After activation, CCR7 is downregulated on many effector T cells and other chemokine receptors are upregulated, allowing these cells to home to peripheral sites of inflammation.[20] Activated endothelial cells in these tissues express ligands that selectively recruit subsets of activated T cells. In contrast, activated B cells either become memory cells that retain CXCR5 and CCR7 expression[21] or differentiate into plasma cells that downregulate CXCR5 and CCR7 and upregulate CXCR4, targeting these cells to medullary cords via a novel migratory mechanism and bone marrow.[22]

Integrin family members are the immediate recipients of chemokine signals to produce rapid arrest of rolling leukocytes and initiate the extravasation process.[16] The major integrin that mediates homing to secondary lymphoid tissues is leukocyte function–associated antigen 1 (LFA-1), which is composed of the αL and β2 subunits. LFA-1 is expressed only on leukocytes and binds to intercellular adhesion molecules (ICAMs), of which the best characterized are ICAM-1 and ICAM-2.[23] ICAM-1 and ICAM-2 are the major ICAMs expressed on endothelial cells, with ICAM-1 displaying regulated expression in response to inflammatory mediators like tumor necrosis factor (TNF) and interferon (IFN)-γ.

The deficiency of the integrin β2 subunit is the basis of a rare genetic syndrome known as *leukocyte adhesion deficiency type 1* (LAD1). In this disease, leukocyte extravasation at sites of inflammation is defective and patients are highly susceptible to bacterial infections of the skin and mucous membranes. Patients with LAD1 are developmentally normal and can be treated by bone marrow transplantation with a high success rate.[24] A leukocyte adhesion deficiency type 3 in which multiple leukocyte integrins show defects in regulation of Rap1, a small G protein important in LFA-1 regulation, has also been described.[25]

The structure of integrins reveals remarkable machinery for regulated adhesion.[26] The inactive form is folded into a compact globular structure, in which the ligand-binding domain points toward the leukocyte surface. After activation by chemokines, the integrin extends to two times its original height and projects the ligand binding site to greater than 20 nm from the leukocyte membrane, with orientation toward the endothelial cell surface.[27] This

dramatic change is closely coupled to cytoskeletal association, providing the anchorage needed for arrest and cell spreading after ligand binding.[28]

A second integrin expressed on naïve and activated lymphocytes, very late activation antigen-4 (VLA-4), is composed of the α4 and β1 subunits; it can play a small role in entry into lymph nodes but a major role in entry into inflamed sites. Its ligand is vascular cell adhesion molecule (VCAM), which is also a member of the immunoglobulin superfamily regulated by inflammatory cytokines.[29] Inhibition of VLA-4 by a monoclonal antibody (natalizumab) is the basis of an approved treatment for multiple sclerosis that decreases leukocyte entry into the central nervous system.[30] Natalizumab therapy has been associated with rare cases of progressive multifocal leukoencephalopathy, a severe infection caused by reactivation of latent John Cunningham (JC) virus.[31] Thus risks and benefits need to be weighed carefully with immunosuppressive therapies even when only a single specific pathway is targeted. When T lymphocytes are activated to home to mucosal effector sites, they upregulate expression of the integrin β7 (which also associates with α4 to form the gut-homing integrin α4β7) and bind a different immunoglobulin superfamily ligand called MAdCAM (i.e., *m*ucosal *ad*dressin cell *a*dhesion *m*olecule), which is expressed on endothelial cells in the gut.[32]

The extravasation process involves the movement of lymphocytes between or through endothelial cells.[33] Endothelial junctional complexes include special adhesion molecules that need to be transiently disengaged to allow lymphocyte passage between endothelial cells. The transcellular pathway may be dominant in situations in which the endothelial junctions are particularly sturdy, as in the brain, thymus, or lymph nodes. Both junctional and transcellular routes involved active processes in the leukocyte and endothelial cells, but this step is not thought to be regulated to control homing decisions.

To summarize, three types of receptor ligand pairs define the key regulated steps in lymphocyte homing. The compatibility of all three is required to gain entry into the tissue. If a compatible selectin-ligand pair is not available, the leukocyte will not be able to initiate adhesion to the endothelial wall and will flow past. If a chemokine receptor-chemokine pair is not available, it will be impossible to activate integrins, even if the selectin-ligand pair mediates rolling or tethering, and the cell will eventually release and remain in the blood. If the integrin-ligand pair is not compatible, the cells will not be able to arrest and extravasate, even if the selectin-ligand pair and chemokine-ligand systems are engaged. Thus these molecular pairs can be thought of as a hierarchic area code for lymphocyte homing, with the digits defined by the compatible interactions, each of which must be correctly engaged to allow entry.[34]

## Tissue Organization and Interstitial Migration

Classical histology and mouse genetics have been used to establish the molecular mechanisms that account for the segregation of T cells and B cells within secondary lymphoid tissues. The T cell zone is defined by the production of CCL19 and CCL21 by stromal cells and the expression of CCR7 on T cells.[17] Interestingly, these signals are the same ones that trigger the arrest of T cells on endothelial cells for tissue entry. T cell zones are also amply populated by conventional dendritic cells (DCs), which express CCR7 as they mature. DCs appear to form a network on a scaffold of reticular fibers.[35] The parenchyma of lymph nodes and splenic white pulp nodules are crisscrossed by thick collagen bundles sheathed in fibroblastic reticular cells.[36] The inner compartment of these fibers forms a network of conduits in the lymph node and spleen that allows DCs in the parenchyma access to the afferent lymph or blood, respectively. The B cell follicles depend on CXCL13 expressed by follicular stromal cells and CXCR5 expressed on B cells. In fact, the balance of CXCR5 and CCR7 expression by B cells controls their proximity to the boundary between T cell zone and B cell zone, where B cells can encounter T helper (Th) cells.[21] B cell zones are also populated by variable numbers of follicular DCs, which are differentiated stromal cells rather than cells of hematopoietic origin.

Two-photon laser scanning fluorescence microscopy has revealed the dynamics of immune cells in secondary lymphoid tissues. Fluorescently labeled T cells moved with an average speed of 12 μm/min in T cell zones, and B cells moved 30% slower in the follicles of acute organ-cultured lymph nodes of live mice.[37] The paths taken by T and B cells appeared random, but subsequent analysis of fluorescent lymphocyte movement in the presence of differentially labeled stromal cells revealed that the lymphocytes were guided by a ramified stromal network and changed direction frequently as they encountered branches in the stromal scaffolding.[38] The DC network is supported by the stromal network, ensuring that T cells contact DCs as they follow a random pathway through the stromal network.[35] Thus the process by which a DC that has brought antigen from the periphery can show this antigen to many T cells is based on forming random contacts with thousands of T cells per hour. Because of its random nature, this model has been referred to as "stochastic repertoire scanning."

The major signal that stimulates the rapid and random migration of T and B cells in lymph nodes is thought to be chemokines presented on the surface of stromal cells. The speed of B lymphocyte migration is significantly reduced in mice lacking $G_{i\alpha 2}$ subunit of G protein–coupled receptors, further supporting the model that chemokines may have a role in this process.[39] Chemokines can act as chemoattractants but can also stimulate migration in a random fashion—called *chemokinesis*—under some conditions. Rapid T cell motility in vitro can be recapitulated by surfaces coated with only CCR7 ligands.[40] Somewhat surprisingly, integrins such as LFA-1 contribute little to the migration speed of DCs or T cells.[40,41]

Although the initial scanning process is random, once antigen-bearing DCs begin to interact with antigen-specific T cells, a number of nonrandom elements of migration are established. B cells that recognize antigen upregulate CCR7 and downregulate CXCR5, such that they are attracted to the boundary between the T and B cell zones.[21] DCs that have been in contact with $CD4^+$ T cells produce CCL3 and CCL4, which attract $CD8^+$ T cells under inflammatory conditions.[42] This directed migration of $CD8^+$ T cells biases the scanning of the $CD8^+$ T cell repertoire toward DCs that have received T cell help and thus have come into contact with interesting antigens. This process likely increases the efficiency of repertoire scanning.

### Immunologic Synapses Maintain Antigen-Specific Interactions with Dendritic Cells

The most extreme change in lymphocyte migration during an immune response is the near full arrest referred to as an *immunologic synapse*.[43] In vitro analysis has revealed that underlying the arrest of T cell migration are elaborately organized structures that are in contact with DCs bearing appropriate MHC-peptide complexes.[44] In vivo imaging analysis has repeatedly revealed stable T cell–DC interactions as a common feature of both tolerance and immunity induced by high-affinity ligands.[45] Although LFA-1–ICAM-1 interactions are not necessary for migration in lymph nodes, these adhesion molecules are required for stable synapses.[46] Immunologic synapses have been proposed to lead to asymmetric cell divisions that generate effector T cell and memory T cell precursors.[47] Another possibility is that stable immunological synapses during priming are related to delivery of premitotic effector functions involving regulatory cytokines such as interleukin (IL)-2.[48] Dynamic T cell–DC interactions referred to as *kinapses* may set up symmetric divisions that help maintain the differentiated state of these early precursors.[49]

### Egress from Lymph Nodes and the Thymus: Sphingosine 1 Phosphate

The recirculation of lymphocytes requires that they periodically cease their local scanning activity or activation process in secondary lymphoid tissues and exit to the lymph or blood, which is referred to as *egress*. Egress is also important for recently matured lymphocytes as they leave the bone marrow or thymus to move to secondary lymphoid tissues. Insight into the molecular processes controlling egress from lymph nodes and the thymus were provided through investigation of the fungal metabolite derivative FTY720, which has been approved as a drug (fingolimod) for treatment of relapsing-remitting multiple sclerosis.[50] FTY720 administration rapidly decreases T and B cell numbers in blood. FTY720 is phosphorylated by sphingosine kinase and acts as an agonist for a number of sphingosine 1 phosphate receptors that are expressed on lymphocytes and endothelial cells. Further insight was provided by the study of bone marrow chimeric mice in which fetal liver cells from embryonic lethal sphingosine 1 phosphate receptor 1 (S1PR1) knockout mice were used to reconstitute lethally irradiated syngeneic wild type mice.[51] The S1PR1-deficient, recently matured lymphocytes are unable to exit the thymus. Furthermore, when the mature S1PR1-deficient lymphocytes trapped in the thymus were transferred into wild-type recipients, they were able to enter lymph nodes normally but could not egress from lymph nodes because of failure to counterbalance CCR7-dependent retention signals. These results suggested a T cell autonomous defect in egress from both the thymus and lymph node. Although FTY-720-P is an agonist of S1PR1, it has the effect of downregulating expression of the receptor such that the compound recapitulates the knockout phenotype. A parallel study with reversible S1PR1 agonists and antagonists suggests that S1PR1 also has a role in controlling lymphatic endothelium permeability, and this effect would contribute to arrest of egress in a similar manner to the T cell autonomous effects.[52]

## PRIMARY LYMPHOID TISSUES: SITES WHERE T AND B CELLS ARE GENERATED AND SELF-TOLERANCE MECHANISMS ARE INITIATED

The critical event in the birth of T and B cells is the rearrangement of the antigen receptor genes to generate T cell antigen receptors (TCRs) and B cell antigen receptors (BCRs) that are expressed on the cell surface. Each T cell and B cell has a single antigen receptor as birth certificate and identification card. The life, death, or expansion of each of these cells controls the availability of this antigen-binding structure to the adaptive immune system. This is the heart of the clonal selection theory first proposed by Burnett in the 1950s. Self-reactive cells need to be deleted or retasked shortly after their birth. This process takes place in the bone marrow for B cells and in the thymus for T cells. The anatomy of these two sites is different, in keeping with the dramatically different recognition mode employed by B and T cells. First I will discuss the bone marrow space, followed by the functional anatomy of the thymus.

### B Cell Development in the Bone Marrow

Immature B cells express surface BCRs composed of surface immunoglobulin (Ig) noncovalently complexed to the signal transducing subunits Ig$\alpha$ and Ig$\beta$[53] (see also Chapter 13). The majority of immature B cells are reactive to self-antigens and even display "polyreactivity," meaning that they produce antibodies that bind not just one but many self-antigens.[54] These autoreactive cells are mostly lost from the repertoire in two checkpoints—the encounter of immature B cells with antigens in the bone marrow or by newly matured B cells upon entry into secondary lymphoid tissues.[54] Checkpoint 1 depends on antigen display in the bone marrow. If BCRs on immature B cells encounter surface immobilized antigens on bone marrow cells or extracellular matrix, they will undergo apoptosis. The second source of self antigens is the blood. The bone marrow parenchyma is bathed in blood plasma antigens due to fenestrated endothelial cells lining bone marrow sinusoids. When soluble antigens bind to their BCRs, they may undergo apoptosis if the antigen is multivalent and cross-links the BCR, inducing strong signaling. If the antigen is monovalent, the B cell may become anergic—a form of nonresponsiveness that is induced by weaker signaling through the BCR. In the second checkpoint, newly matured B cells that have egressed from the bone marrow will encounter antigens on a variety of cells. For example, DCs present intact antigens to B cells,[55] and because DCs sample many tissue antigens, this may provide a wider array of tissue-specific self-antigens to check against the BCR. These selection checkpoints decrease the frequency of self and polyreactive B cells by greater than 10-fold, but some polyreactive B cells persist in the repertoire. Polyreactivity may contribute to pathogen recognition, as was recently shown for neutralizing antibodies to low-density envelope glycoprotein of HIV.[56] Thus the adaptive immune system may always need to tolerate some self-reactivity to defend us against the universe of pathogens.

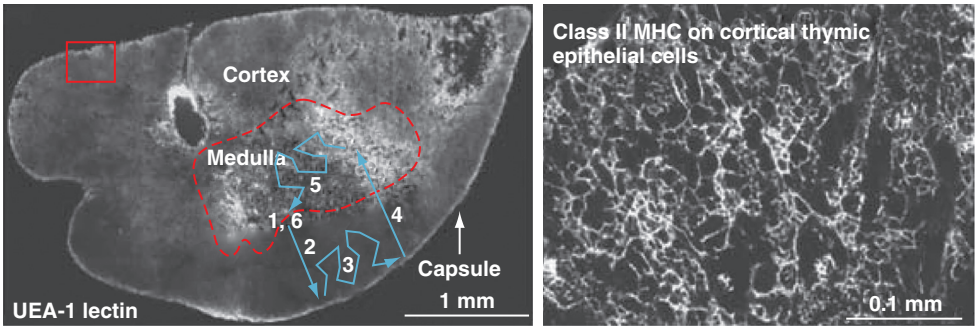



**Figure 18-1** Section of mouse thymus stained with *Ulex europeus* antigen-1 (UEA-1) lectin *(left)* or anti–class II major histocompatibility complex (MHC) *(right)* visualized with immunofluorescence microscopy. The UEA-I staining is strongest in the medulla but also highlights the capsule and stroma of the cortex. The cortical thymic epithelial cells are strongly positive for MHC molecules, which allow negative and positive selection of thymocytes. The *blue arrows* indicate a hypothetical path of a developing T cell (thymocyte) through the thymus: (1) entry via the corticomedullary junction, (2) migration into the cortex, and (3) scaffolded random walk in the cortex during which positive or negative selection can take place; (4) if positive selection takes place, the thymocyte undergoes directed migration to the medulla where negative selection or conversion into a thymic regulatory T cell (see below) remains possible; (5) the mature single positive thymocyte migrates back to the corticomedullary junction and (6) egress under control of sphingosine-1 phosphate mediate chemotaxis. *(Images courtesy Richard Lewis, Stanford University.[57])*

## T Cell Development in the Thymus

T cell precursors arrive in the thymus and initiate TCR gene rearrangement in this microenvironment (see also Chapter 12). The thymus is an epithelial organ formed during development from the third pharyngeal arch. Histologically, the tissue has a clearly distinguishable cortex, medulla, and vascular corticomedullary junction.[7] Figure 18-1 is adapted from a recent article examining the dynamics of T cell selection in the thymus.[57] It shows fluorescence staining with *Ulex europeus* antigen-1 (UEA-1) lectin, a marker for the thymic medulla, on the left side and class II major histocompatibility complex (MHC) antigen in the thymic cortex on the right side. The stepwise path of thymocyte migration is outlined in the left panel. Early $CD4^-$ and $CD8^-$ progenitors enter via postcapillary venules at the corticomedullary junction (step 1) and migrate to the subcapsular region of the cortex, where TCR gene rearrangement takes place and CD4 and CD8 become expressed on the surface of immature T cells, also called *thymocytes* (step 2). These cells then migrate randomly among thymic epithelial cells in the cortex, sampling self-MHC-peptide complexes (step 3). If they express a TCR that recognizes self-MHC-peptide complexes with low affinity, they will undergo "positive selection." Positively selected thymocytes then mature into $CD4^+$ or $CD8^+$ cells that migrate to the medulla under control of CCR7 ligands[58] (step 4). Medullary thymic epithelial cells express a transcription factor called *AIRE* that mediates expression of a number of tissue-specific genes by amplifying expression from poorly expressed chromatin regions.[59] A small number of persons lacking expression of AIRE have a complex autoimmune syndrome characterized by polyendocrinopathy and other tissue-specific autoimmune diseases. The hypothesis is that AIRE-mediated transcription of otherwise tissue-specific genes in thymic epithelial cells promotes negative selection of some autoreactive T cells and may also promote generation of tissue antigen-specific regulatory T cells (step 5). Conventional positive selection requires Ras activation on intra-cellular membranes, whereas stronger Ras activation at the plasma membrane mediates negative selection.[60] Regulatory T cell generation requires signaling through protein kinase C-θ and c-Rel transcription factors.[61] The small percentage of thymocytes that express a TCR and pass both positive and negative selection downregulate CD69 and S1PR1 and exit the lymph nodes as naïve "conventional" T cells (step 6).

## SECONDARY LYMPHOID TISSUES: SITES WHERE ANTIGEN FINDS RARE SPECIFIC T AND B CELLS

The frequency of naïve T cells that recognize any particular antigen is so low that it has been challenging to estimate. Recently, enrichment methods using antibody-coated magnetic beads have been developed to assist direct measurement of precursors by flow cytometry with high-avidity MHC-peptide–based probes known as *tetramers*.[62] The number of cells specific for commonly used model antigens is approximately 500 in most mouse strains when pooling cells from all secondary lymphoid tissues (spleen and lymph nodes). Approximately 500 million total $CD4^+$ T cells are present in these tissues, and thus antigen-presenting DCs need to make contact with a million irrelevant T cells to find one specific T cell. Thus a small number of antigen-positive DCs early in an infection must have access to highly concentrated swarms of T cells in secondary lymphoid tissues to sample enough T cells to find a few specific precursors to activate and expand. As described earlier, this search process is initially random but may become more directed and efficient as a response progresses. Although naïve T cells recirculate in an unbiased manner through secondary lymphoid tissues, the antigen-carrying cells, primarily DCs, have a high degree of regional bias in their movement, such that they are thought to relay information about both innate immune stimulation and tissue of origin of antigens to T cells.

Once antigen-specific T cells come into contact with antigen-bearing DCs, they initially form synapses with multiple DCs to (1) integrate signals through the T cell receptors and co-stimulatory ligands (which represents part of the innate immune system's contribution to T cell activation) and (2) form more stable synaptic interactions to deliver early effector cytokines to DC and other T cells; they then

divide more than 20 times with short cell cycle times of approximately 6 hours.[63] Expansion of $CD8^+$ T cells, which give rise to cytotoxic effector cells, is greater in general than for $CD4^+$ T cells, which give rise to various types of Th cells. After expansion, which peaks around day 7 to 10, the infectious agent is often eradicated and most of the effector T cells undergo apoptosis. The flulike symptoms associated with viral infections are due to cytokines produced by the dividing and differentiating T cells.

Because primary adaptive responses take a week or longer to develop, the host is dependent on innate immune mechanisms like natural antibodies, neutrophils, interferons, and natural killer (NK) cells to control the infection until sufficient numbers of effector T cells are generated. Thousands of the expanded T cells that survive after the pathogen is destroyed become memory cells.[64] Although the process of asymmetric division may contribute to the establishment of memory and effector cells, models in which events cannot be attributed to a single asymmetric first division but must incorporate additional stochastic events that have an impact on the lineages of multiple precursors are required to account for results in in vivo fate mapping experiments.[65]

There are two subsets of memory T cells: (1) central memory cells that express L-selectin, CCR7, and high LFA-1 and recirculate via secondary lymphoid tissues, and (2) effector memory cells that lack L-selectin and CCR7 but may express P- and E-selectin ligands and other chemokine receptors such as CXCR3, CCR5, CCR4, and/or CCR9.[66] Macrophages in the subcapsular sinus of lymph nodes produce CXCL9, which recruit $CXCR3^+$ memory T cells into the interfollicular area, where they are positioned to intercept incoming DCs as they move through the subcapsular sinus into the T cells zones.[67] Effector memory cells largely migrate to peripheral sites of inflammation and are equipped for rapid effector function if they encounter antigen. Memory cells respond rapidly to recurrence of the same infection, and together with antibodies, they rapidly eradicate that same agent if it is encountered a second time. These memory cells may also cross-react with other pathogens, and depending on the degree of cross-reaction, this type of response can result in rapid clearance of a new pathogen or, sometimes, an impaired response.

## Antigens from Blood Are Detected Most Efficiently in the Spleen and Liver (Portal System)

The spleen is a large visceral organ that filters approximately 5% of the cardiac output. The red pulp is an important location for removal of aged red blood cells from the circulation. The red pulp also contains many macrophages that specialize in this clearance process,[68] as well as DCs that come into direct contact with naïve T cells in the blood. The function of these red pulp DCs is not known. Most attention has been focused on the white pulp nodules and the marginal zone as sites of T cell–DC interaction and antigen capture, respectively (Figure 18-2, *left*). Blood flows into the spleen via an artery that splits into arterioles that empty into venous sinuses in the marginal zone and red pulp (Figure 18-2, *right*). The blood is then re-collected into a venous system that drains to the liver, joining with the portal tract. The arterioles are surrounded by sheaths of T cells (the periarterial lymphoid sheath [PALS]) that make up the T cell zones of the white pulp. Bridging channels connect the red pulp, where lymphocytes leave the blood, to the PALS, where they migrate in a pertussis toxin–sensitive manner.[69] B cell follicles and the marginal zone

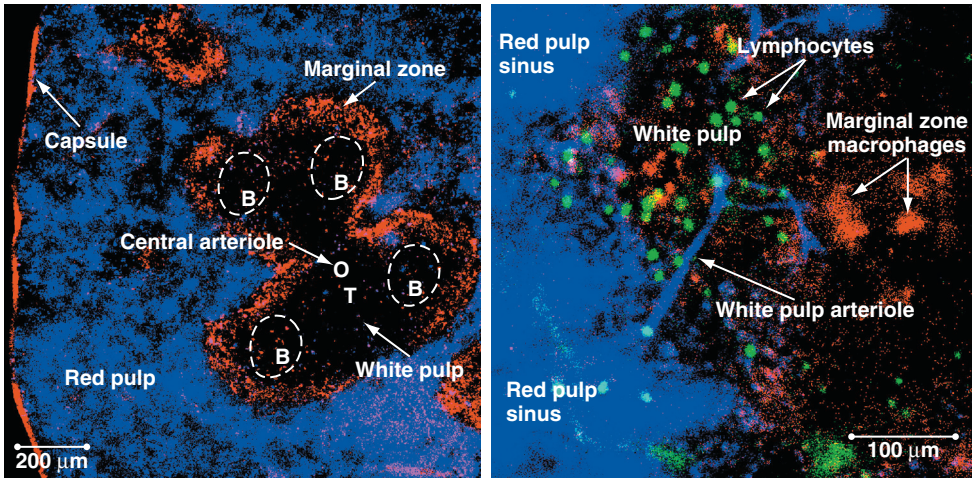



**Figure 18-2** Organization of the spleen. *Left,* Tissue section from a mouse injected with low-molecular-weight *(blue)* and high-molecular-weight *(red)* fluorescent dextrans. The low-molecular-weight dextran labels the red pulp *(blue)* and the high-molecular-weight dextran labels macrophages in the marginal zone *(red)* such that the white pulp nodule, which contains approximately $10^9$ lymphocytes per $cm^3$, is dark. The white pulp nodule forms around a central arteriole (O) from which smaller arterioles branch to the red pulp sinuses. B and T cells are segregated into follicles (B) and the periarteriolar lymphoid sheath (T). (Scale bar 0.2 mm.) *Right,* Image of a live mouse spleen during injection of low-molecular-weight fluorescent dextran. The blood carrying the dextran passes through the white pulp in small blood vessels *(arrows)* emanating from the central arteriole (not shown) and connected to red pulp sinuses that rapidly fill with blood, which fills the marginal sinus but not the white pulp itself. In this image approximately 0.3% of the T cells in the PALS are labeled with a fluorescent dye. These areas are packed with T and B cells. Marginal zone macrophages and B cells have direct access to blood to capture pathogens. White pulp lymphocytes are not in direct contact with blood and need antigen presentation by cells that migrate from the marginal zone or antigens that are delivered to dendritic cells via reticular fibers.[108] (Scale bar 0.1 mm.) *(Courtesy Janelle Waite, New York University.)*

then surround the PALS. Macrophages, DCs, and marginal zone B cells line the marginal zone, where they have direct access to blood antigens. DCs that pick up antigens in the marginal zone migrate to the PALS within 9 hours.[70] A reticular fiber network connects the marginal zone to the PALS and allows soluble antigens from the blood to reach resident DCs in the PALS. Thus the spleen provides multiple opportunities to mount primary and recall responses to particulate or soluble antigens in the blood.

As mentioned earlier, venous blood from the spleen drains into the liver. Immune responses in the liver are poorly understood, but this site is important because many pathogens colonize the liver. Two blood circulations supply the liver: the portal vein with deoxygenated blood from the gut and spleen, and the hepatic artery with oxygenated blood. These circulations mix in the liver sinusoids, a low-pressure network of blood spaces between sheets of hepatocytes connecting the portal tract and the central collecting vein. Thus most of the liver parenchyma appears as plates of hepatocytes alternating with blood-carrying sinusoids (Figure 18-3, *left*). The liver is rich in a type of sessile macrophage called a *Kupffer cell* and patrolling lymphocytes, particularly NK T cells in the mouse and mucosal-associated invariant T (MAIT) cells in humans (Figure 18-3, *right*).[71,72] Both of these cell types participate in antibacterial immunity. The major antigen-presenting cells in the liver are not Kupffer cells but the perivascular Ito cells.[73] The liver also plays an important role in the clearance of B cells mediated by therapeutic anti-CD20 antibodies that are used to treat patients with rheumatoid arthritis.[74]

## Antigens from Mucosal Surfaces Are Detected Most Efficiently in Peyer's Patches and Mesenteric Lymph Nodes

The mucosal-associated lymphoid tissues include the tonsils, Peyer's patches, lamina propria, cryptopatches, and appendix. Peyer's patch and lamina propria DCs have different mechanisms for sampling the contents of the gut lumen. Peyer's patches are composed of large B cell follicles with smaller T cell zones (Figure 18-4, *top* and *bottom right*). They have high endothelial venules that allow efficient entry of naïve T and B cells, as well as memory cells with gut homing phenotypes. The large size of the follicles in the Peyer's patches causes a domelike effect with the epithelium protruding into the lumen. Smooth-surfaced M cells replace some of the microvilli-laden absorptive epithelial cells in this dome region of Peyer's patches. These cells act as relay points for entry of enteric pathogens into the dome region of Peyer's patches, where a population of $CCR6^+$ DCs efficiently presents pathogen-related antigens to induce rapid local T cell responses[75] (Figure 18-4, *bottom left*). In contrast to the Peyer's patches, activated T cells, plasma cells, DCs, and macrophages populate the core of the villi in the terminal ileum. The DCs migrate to mesenteric lymph nodes, where they present antigens to stimulate production of regulatory T cells and effector T cells to balance gut tolerance and immunity.[76] $CX3CR1^+$ macrophages actively sample the gut contents and then may present antigens to effector and regulatory T cells that patrol the lamina propria[77] (Figure 18-4, *bottom left*). The gut presents a barrier between the host and billions of commensal bacteria and food antigens. There are also many pathogens, such as bacteria, viruses, protozoans, and worms, that exploit this niche. Recently it has been found that new-onset rheumatoid arthritis is associated with expansion of particular microbial taxa in the gut.[78] This area of research is rapidly developing and is touched on again in the context of T cell differentiation.

## Antigens from Other Tissues and Solid Organs Are Detected in Peripheral Lymph Nodes

An extensive network of peripheral lymph nodes filters lymph from the skin, visceral organs, and nervous system. Lymph is composed of fluid that leaves blood vessels and

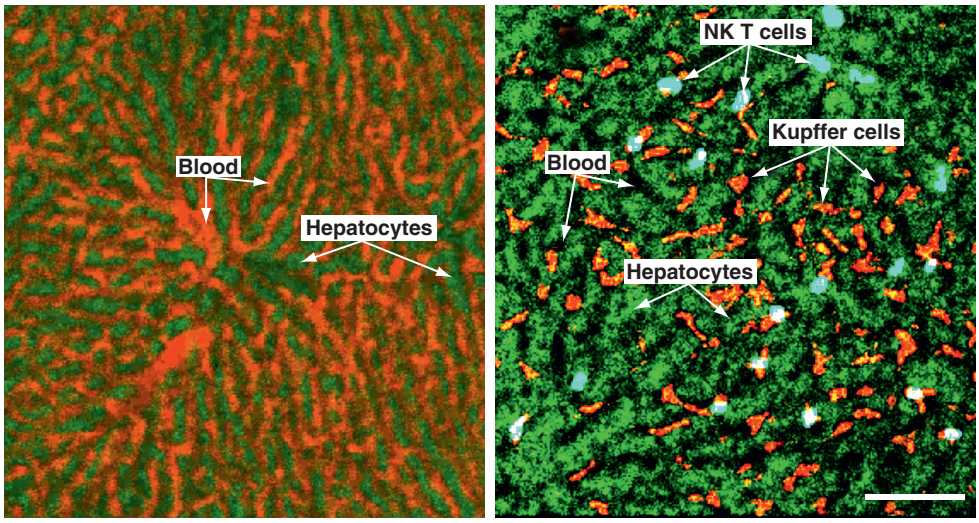



**Figure 18-3** Microcirculation and immune cells in liver. *Left,* Intravital imaging of blood space in mouse liver. Confocal imaging of hepatocytes (green autofluorescence) and blood space (red fluorescent dextran) is shown. The flow pattern is toward the center of the image panel. *Right,* Intravital imaging of natural killer T (NK T) cells (blue-green) and SIGN $R1^+$ Kupffer cells in liver sinusoids (dark spaces) between hepatocytes (green autofluorescence). The Ito cells would also be lining the sinusoids, but on the other side of the endothelial cells in the space of Disse. *(Courtesy Tom Cameron, New York University.)*

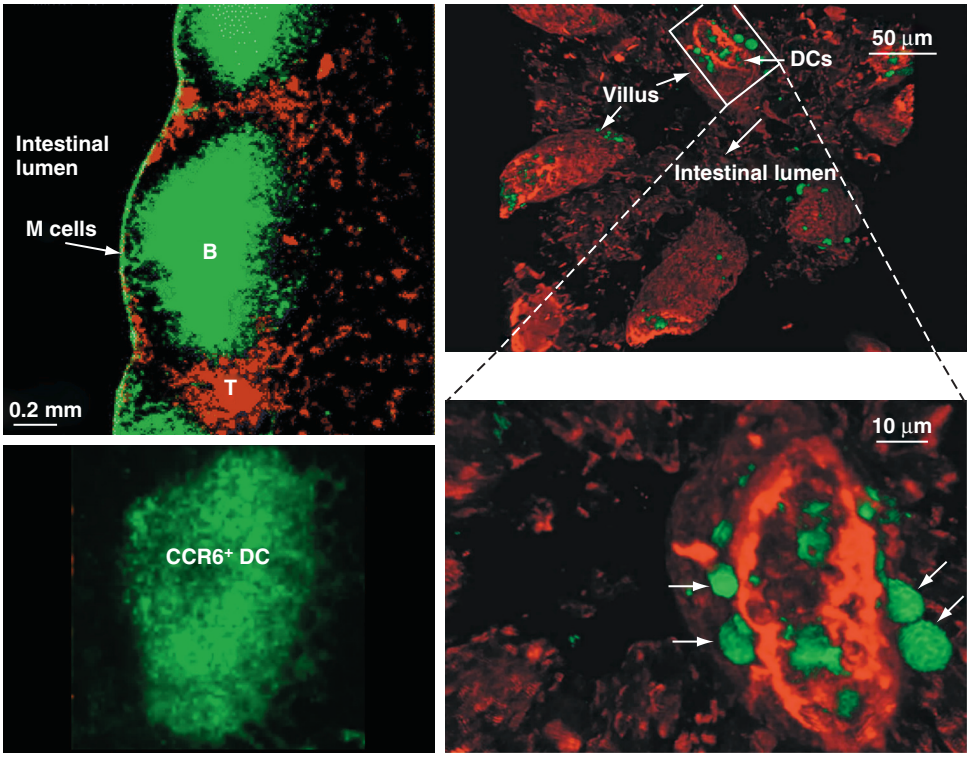



**Figure 18-4** Gut-associated lymphoid tissues. *Top left,* Immunofluorescence image of Peyer's patch with B cells (B) in green and T cells (T) in red.[109] *Bottom left,* A similarly oriented view of Peyer's patches from mouse in which CCR6$^+$ dendritic cells (DCs) also express green fluorescent protein due to targeting of *gfp* to CCR6 locus.[72] *Right,* Images of CD11c$^+$ DCs in the tips of villi in the terminal ileum. The DCs extend processes through the epithelial layer to assist uptake of luminal antigens (*arrows* in bottom right point to the DC projections in the gut lumen).[110]

then must be collected from the tissues in afferent lymphatic vessels, passes through at least one lymph node, exits the lymph node as efferent lymph, and is returned to the blood via the thoracic duct. The afferent lymphatics of a lymph node connect to the capsule of the node and drain into the subcapsular sinus, which contains many macrophages (Figure 18-5). The floor of the subcapsular sinus covers the lymph node parenchyma and is the point of origin of the reticular fiber network that connects to the high endothelial venules and medullary cords, where cells move from the parenchyma into the efferent lymph. Lymph does not come into direct contact with cells in the parenchyma, but cells lining the reticular fibers and a space between the high endothelial venules and the reticular fibroblast sheath are exposed to lymph fluid.[36] The parenchyma is divided into T and B cell zones defined by distinct populations of stromal cells producing CCL19/21 and CXCL13, respectively (see Figure 18-5). DCs migrate from peripheral tissues in a CCR7-dependent manner and join networks of DCs in the T cell zones with scattered cells in the follicles.[35] Emigrant DCs have been reported to array around high endothelial venules, where they are efficiently encountered by newly extravasated T and B cells. In experimental models, antigen-positive DCs have been shown to stop both B and T cell migration during antigen encounters.[45,55] These immunologic synapses, discussed earlier, play an important role in antigen transfer for B cells and for priming of the proliferative response in T cells. It has been noted that different populations of DCs such as dermal DCs and Langerhans cells actually populate different subregions of the T cell zones, but the significance of this phenomenon is not clear.

## Peripheral Tolerance Induction under Steady-State Conditions

Lymph nodes are associated with peripheral tolerance induction. The presentation of antigens in the steady state by DCs induces proliferation of T cells that are then deleted or anergic by 7 days.[79] This process may be particularly important to tissue-specific antigens that are not present in the thymus in the steady state.[80] It is known that low-affinity self-antigens can escape peripheral tolerance induction by this mechanism.[80] Low-affinity self-antigen–specific T cells that are then activated in the context of infection by a pathogen with strong innate stimulation may then induce tissue-specific autoimmunity.[80]

## Regulatory T Cells Reduce Autoreactivity by Inhibiting Immunologic Synapse Formation

Regulatory T cells are CD25$^+$ IL-2 dependent T cells that express the transcription factor FoxP3 and have the ability to suppress immune responses to tissue-specific self-antigens.[81] These cells represent another layer in redundant mechanisms to prevent autoimmune attack by adaptive immunity. One way in which regulatory T cells function is to block immunologic synapse formation between T cells and autoantigen-presenting DCs.[82] This mechanism appears to operate on low-affinity self-antigens that are the most

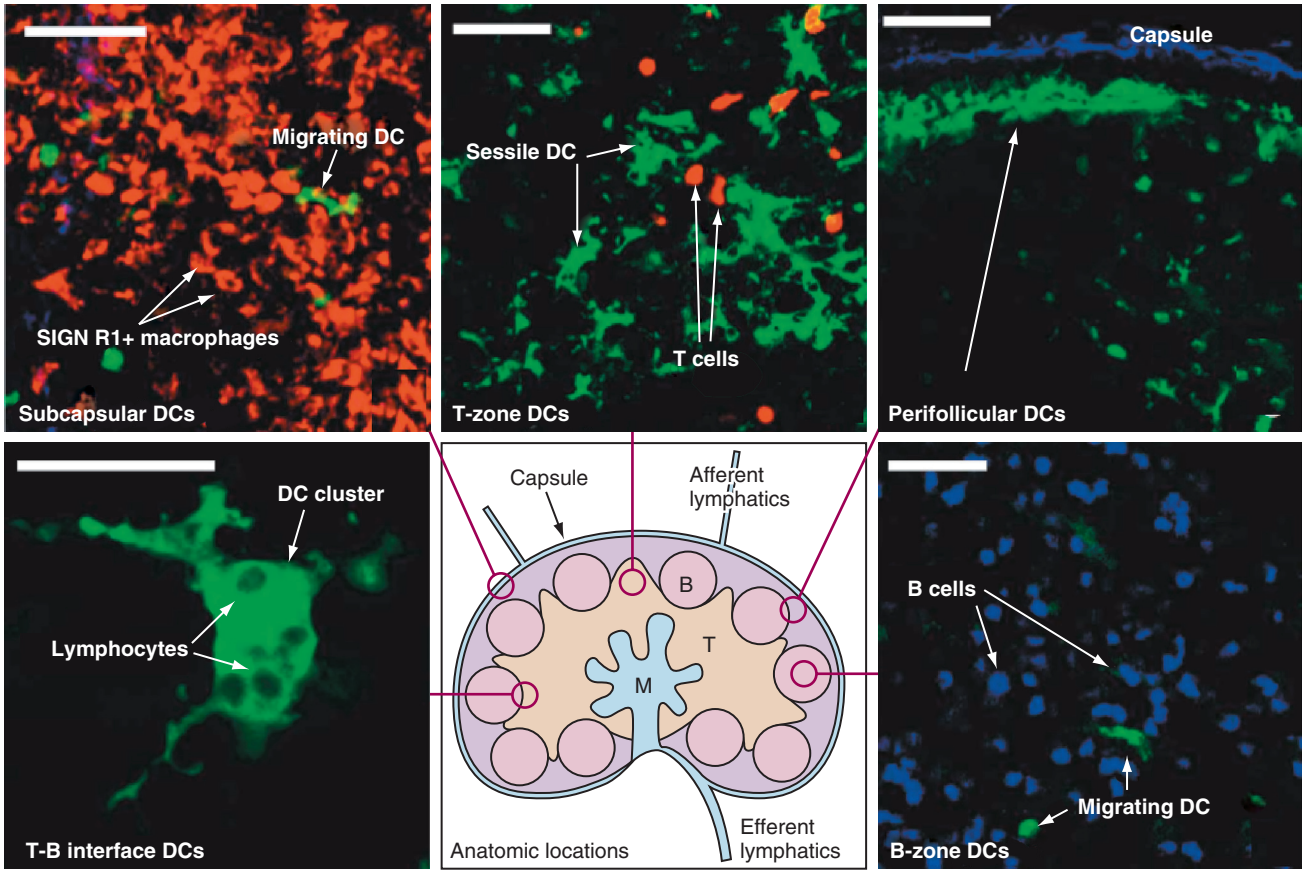



**Figure 18-5** Lymph node schematic and dendritic cell (DC) structures. Intravital microscopy–based images of DCs in lymph nodes of CD11c-YFP marker mice are linked to a schematic of the lymph node. (All scale bars 50 μm.) *Schematic:* Lymph enters via the afferent lymphatics and exits via the efferent lymphatics. T cells enter the lymph node via the high endothelial venules and exit via medulla to the efferent lymphatic. B, B cell follicles; M, medullary cords; T, T cell zone. *(From Lindquist RL, Shakhar G, Dudziak D, et al: Visualizing dendritic cell networks in vivo.* Nat Immunol *5:1243–1250, 2004.)*

likely type of self-reactive T cell to escape other peripheral tolerance mechanisms. Prevention of long-lived T cell–DC interactions may reduce proliferation and cytokine production by autoreactive T cells.

## Changes in the Lymph Node during Infection/Vaccination

Infection or vaccination in tissues induces a strong reaction in draining lymph nodes. Innate signals trigger production of inflammatory cytokines that lead to increased blood flow, increased adhesion molecule and chemokine expression to increase T and B cell entry, and suppression of T and B cell exit by downregulation of S1PR1.[83] DCs in reactive lymph nodes express higher levels of co-stimulatory ligands such as CD80 and CD86 and promote robust proliferation, survival, and differentiation of antigen-specific T cells. T cells that are activated in the lymph nodes regain expression of S1P1 after 3 to 4 days, along with a new repertoire of homing molecules, and migrate to effector sites.

## Tissue Environment of Immature Dendritic Cells Determines T Cell Imprinting

When T cells are activated in lymph nodes, the repertoire of homing molecules expressed by the activated effector T cells is determined in part by the origin of the DC.[84] DCs arise from a common monocyte-like precursor that migrates into tissues via the blood. DCs that drain from the gut produce retinoic acid from vitamin A. After maturation and migration to draining lymph nodes, these DCs secrete retinoic acid, which induce activated T cells to express gut-homing chemokine receptors like CCR9 and gut-specific integrins like α4β7. Because gut-associated postcapillary venules express MAdCAM and present CCL25 (a CCR9 ligand), these effector T cells tend to home to the gut. In the absence of retinoic acid produced by gut-derived DCs, the signals induced by skin-derived DCs favor expression of ligands for E- and P-selectin and CCR4 on T cells.[84] Because endothelial cells of skin-associated postcapillary venules express P-selectin and present CCL17, these T cells tend to home to inflamed skin. Recently, it has been shown that DCs in the skin metabolize vitamin D to generate a signal for T cell expression of CCR10, which allows these cells to migrate to the epidermis in response to CCL27. Although DCs will strongly skew T cells to home back to the sites from which the DCs migrated, the expression of homing receptors and chemokines on lymphocytes also has a stochastic component, which means that these effector cells and memory cells will also show up in diverse peripheral sites scattered throughout the animal—thus giving effector T cells their ability to appear in any tissue at any time.

Overall, these results show that DCs sense their tissue environment and innate immune activation signals in the process of shaping T cell responses.

## Germinal Center Reactions: Sites of Antibody Affinity Maturation and Class Switch Recombination

Low-molecular-weight antigens that enter the subcapsular sinus are directly accessible to B cells.[85] Particulate antigens are captured by subcapsular macrophages and transferred to B cells, which then mediate their transport to follicular dendritic cells in a nonantigen-specific process requiring complement receptors.[86] B cell activation by antigens in conjunction with co-stimulation via complement receptor or other forms of innate immune stimulation can lead to immediate proliferation of the B cells and the formation of plasma cells producing IgM antibodies from daughter cells.[87] A specific subset of follicular helper T (Tfh) cells promotes the formation of germinal centers within the B cell zone of any secondary lymphoid tissues in a manner critically dependent on the inducible T cell co-stimulator (ICOS).[88] Germinal centers are roughly spherical collections of hundreds of antigen-specific B cells that undergo interaction with Tfh cells in the light zone and proliferation in the dark zone[89] (Figure 18-6). The light zone is populated by stromal follicular DCs (which, unlike conventional DCs, are of nonhematopoietic origin) and Tfh cells. Follicular DCs use complement receptors to hold immune complexes on their surface for sampling by antigen-specific B cells. The Tfh cells provide help to B cells that maintain or increase their affinity for antigens and can also provide cytokine signals to promote class switching. Intravital microscopy studies demonstrate that germinal centers are dynamic open structures in which antigen-specific B cells are continuously in motion and follicular DCs are accessible to interaction with B cells that have diverse receptor specificity.[90] Thus antigen-specific B cells can be recruited into germinal center reactions at any time in the process and can compete openly with B cells that were present earlier.[91] Interactions between Tfh cells and centrocytes in the light zone are also highly dynamic and depend on the SLAM associated protein (SAP) adapter protein.[92] T cells help control interzonal migration and affinity maturation.[84] Because most somatic mutations destroy the BCR or lower its affinity, a large amount of apoptosis is present in the germinal center in addition to proliferation to provide substrates for mutations.

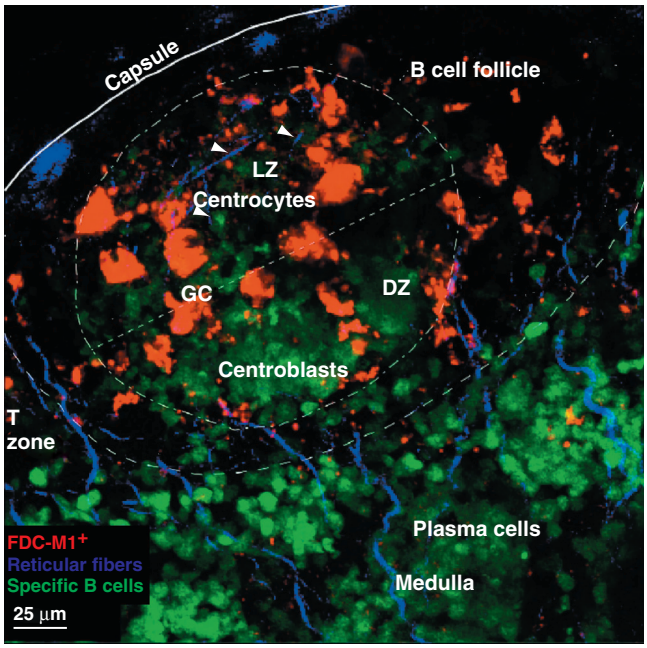



**Figure 18-6** Germinal center (GC) in living mouse lymph node. A GC was induced by vaccination after introduction of fluorescent antigen-specific B cells and antigen. Follicular dendritic cells were labeled with FDC-M1 antibody conjugated to a red fluorescent dye. Reticular fibers, which serve as antigen conduits into the follicle, are labeled *blue*. The GC is outlined and divided into a dark zone (DZ) containing aggregated centroblasts *(green)* and a light zone (LZ) containing loosely aggregated centrocytes, follicular dendritic cells (FDC-M1$^+$), and follicular T cells (not shown). The surrounding follicle is densely packed with bystander B cells (not shown), which often enter and traverse the LZ. *Green cells* in the medullary region are plasma cells produced in the germinal center reaction. *(From Schwickert TA, Lindquist RL, Shakhar G, et al: In vivo imaging of germinal centres reveals a dynamic open structure.* Nature *446:83–87, 2007.)*

## TERTIARY LYMPHOID TISSUES ARE GENERATED AT SITES OF CHRONIC INFLAMMATION

Tertiary lymphoid tissues resemble secondary lymphoid tissues in many respects but are formed in the adult in response to chronic inflammation in locations where such tissues do not exist in steady-state conditions. The induction of tertiary lymphoid tissues can be compared with the formation of lymph nodes during normal development. Normal lymph node development involves the colonization of connective tissues in characteristic vascular nexuses by RORγt-dependent, CD4$^+$, lymphotoxin-positive lymphoid tissue inducer cells.[93] These cells use surface lymphotoxin and TNF to induce local stromal cells to express ICAM-1 and VCAM-1 and produce CCL19/21 or CXCL13, leading to development of lymph node stroma with a reticular fiber conduit system that is integrated into the developing lymphatic vessel system. Thus steady-state presentation of inflammatory cytokines plays a key role in this process. This normal process can also be recapitulated in the adult because chronic inflammation induces production of TNF and lymphotoxin in normal tissues, leading to the induction of stromal cells to form organized follicles and T cell zones within the inflamed tissues. Induction of stromal cells to produce CCL19/21 and/or CXCL13 is probably important in this process because transgenic expression of CXCL13 in ectopic locations in mice leads to the formation of fully developed B cell follicles in these tissues.[94] Thus B cells may have a particular capacity to induce tertiary lymphoid tissues. The efficacy of anti-TNF therapies in autoimmune disease may relate to the suppression of such tissues.[95]

Tertiary lymphoid tissues are often associated with autoimmune diseases (including rheumatoid arthritis) and the local infiltration of naïve T cells in an area with high concentrations of tissue-specific self-antigens, and innate stimulation may promote progression of disease by recruiting new T and B cell specificities into the autoimmune process.[96] Breaking this cycle may be a key target of anti-cytokine and

anti–B cell therapies that have been remarkably effective and are discussed in later chapters.

## FIVE MAJOR TYPES OF EFFECTOR T CELLS

$CD8^+$ effector T cells are generally cytotoxic and involved in killing of infected cells. $CD8^+$ T cell responses can be initiated without the help of $CD4^+$ T cells, but CD4 T cell help is required for effective $CD8^+$ T cell memory.[97] Important transcription factors for effector CD8 T cell differentiation are Tbet and eomesodermin.[98] Three major types of effector $CD4^+$ T cells are recognized (see also Chapter 12). The first defined axis for CD4 T cell differentiation was between IFN-γ–producing T cells expressing the master transcription factor Tbet and IL-4–producing T cells using the master transcription factor GATA3.[99] The IFN-γ–producing cells are referred to as *Th1 cells*, which help inflammatory cytotoxic T cell and macrophage responses, and the IL-4–producing cells, called *Th2*, help anti-parasitic B cell responses, leading to IgE production and eosinophilic infiltration. The major cytokines that initiate Th1 responses are IFN-γ and IL-12 in the absence of IL-4, whereas IL-4 is the major cytokine that initiates the Th2 program. Recently it has been appreciated that a third type of effector T cell produces IL-17 and is important in many inflammatory diseases. Differentiation of these Th17 cells requires the nuclear hormone receptor RORγt.[100] The cytokine conditions that lead to development of Th17 cells are transforming growth factor (TGF)-β plus IL-6, or IL-1β with IL-23 for maintenance.[101] The contribution of TGF-β places Th17 cells on the same axis with induced regulatory T cells (Tregs), with the deciding factor being the presence of IL-6 or IL-1β to favor Th17 over Treg induction.[102] Th17 cells are highly pro-inflammatory T cells that trigger recruitment of neutrophils to combat extra-cellular bacterial infections. The generation of gut-associated Th17 cells in mice has been linked to a single species of segmented filamentous bacteria.[103] The presence of the commensal bacteria improves the clearance of intestinal pathogenic bacteria that require a Th17 response, but it also leaves the host more susceptible to autoimmune arthritis.[104] The most recently recognized Th lineage is the Tfh cells, which share the expression of the Bcl-6 transcription factor with germinal center B cells. The signature cytokine of Tfh cells is IL-21, which is critical for promoting B cell differentiation.[105]

## CONCLUSION

Adaptive immune responses have a degree of flexibility that is unparalleled in molecular recognitions systems. This flexibility has a dangerous side that is constrained by anatomy and strong coupling to innate immunity. Autoimmune disease may result when weak points in tolerance mechanisms intersect with infection or tissue damage, leading the adaptive system to attack its own organs in a self-amplifying process of tissue destruction, inflammation, and inappropriate anatomic adaptation such as tertiary lymphoid tissue genesis. These processes are highly relevant to rheumatoid diseases. For example, a mouse model for autoimmune rheumatic disease called the *KRN transgenic mouse* develops a T cell– and B cell–dependent joint disease.[104] Similarly, mutations in the T cell signaling protein ZAP-70 lead to failure of tolerance mechanisms in the SKG mouse, which recognizes autoantigens that are shared with human rheumatoid arthritis.[106] Strategies to break these cycles by attacking innate or adaptive immune system components nonspecifically have met with success, but more specific strategies may reduce negative consequences of general immunosuppression.[107]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 19

# Autoimmunity



Dwight H. Kono • Argyrios N. Theofilopoulos

**KEY POINTS**

- Autoimmunity ranges from physiologic levels of autoreactivity to overt autoimmune disease, in part reflecting the complexity of the immune system, the presence of multiple layers of tolerance mechanisms, and genetic heterogeneity.
- Autoimmune diseases can be classified by the extent of organ involvement (organ-specific to systemic), innate immune system requirements, and effector mechanisms. However, each type of autoimmune disease has unique pathophysiologic characteristics.
- Advances in defining the mechanisms underlying autoimmune diseases have been greatly facilitated by delineation of the innate and adaptive immune systems.
- Autoimmune disease susceptibility is multifactorial, involving genetic, environmental, sex, and other factors, with genetic predisposition usually playing a central role. The contributions of these factors are typically heterogeneous, partial, and additive.
- Animal models have been critical for understanding the pathophysiology of autoimmune diseases and provide fundamental insights into genetic, mechanistic, and pathologic processes. Spontaneous animal models do not exist for most autoimmune diseases.
- Progress in delineating pathways has revealed many key genes and molecules in autoimmune diseases that are of potential therapeutic relevance.

Autoimmune diseases represent a significant health burden for 3% to 9% of the general population, and rheumatology, perhaps more than any medical subspecialty, encompasses a broad array of such diseases that affect a wide range of organ systems (Table 19-1).[1-3] Consequently, rheumatologists have a great interest in defining the causes and pathophysiology of autoimmunity and in applying this information in the clinic.

The immune system must effectively defend against a diverse universe of pathogens while simultaneously maintaining tolerance to self-antigens. Recent advances have begun to clarify how this equilibrium is established and sustained and, importantly, have identified many critical factors and processes involved in the pathogenesis of autoimmune diseases. This chapter provides a general overview of autoimmunity, covering the definition of the term, general tolerance mechanisms for T and B lymphocytes, theories of how tolerance can be breached, and ways in which genetic and environmental factors have been implicated in breaking tolerance and producing disease. Emphasis is placed on manifestations and mechanisms related to rheumatologic pathoses.

## DEFINITION AND CLASSIFICATION OF PATHOGENIC AUTOIMMUNITY

Autoimmunity—that is, the immune response against self—evokes the specter of "horror autotoxicus," a term coined by Paul Ehrlich at the turn of the 20th century to depict the perceived disastrous consequences of this condition.[4] In fact, autoreactivity is more nuanced, ranging from a low "physiologic" level of self-reactivity essential for lymphocyte selection and maintenance of normal immune system homeostasis, to an intermediate level of autoimmunity that manifests as circulating autoantibodies and tissue infiltrates that are not associated with clinical consequences, to pathogenic autoimmunity associated with immune-mediated dysfunction or injury. From the clinical perspective, it is the transformation to pathogenic autoimmunity that demarcates significant from insignificant self-reactivity.

The diagnosis of an autoimmune disease is generally based on the presence of adaptive immune system–mediated disease caused by self-reactive antibodies, T cells, or both. For many common autoimmune diseases, more definitive evidence for an autoimmune cause has come from studies showing transfer of disease by autoantibodies or self-reactive T cells and from animal models exhibiting congruent characteristics. However, no universally accepted criteria exist, and some less well-characterized diseases that are currently considered to be autoimmune may turn out to have other causes.

An example of disorders that exhibit some characteristics of autoimmunity but are distinct in their pathogenesis are the autoinflammatory diseases.[5-8] These diseases are predominantly rare monogenic disorders typified by intermittent bouts of fever, rash, serositis, and arthritis caused by a widening spectrum of defects in the control of basic inflammatory pathways. Included in this category are familial Mediterranean fever, the cryopyrinopathies, hyperimmunoglobulinemia D with recurrent fever, familial cold urticaria, Blau syndrome, and others. These syndromes could be considered part of a broader definition of autoreactivity in which autoinflammatory diseases mediated entirely through the innate arm of the immune system comprise one end of the spectrum, while at the other end are autoimmune diseases that require both the innate and adaptive responses. Indeed, the possibility of a less stringent demarcation has been suggested by assessment of disorders such as Behçet's disease, systemic juvenile rheumatoid arthritis, and Crohn's

**TABLE 19-1** Rheumatologic Autoimmune Diseases

| |
|---|
| Rheumatoid arthritis |
| Juvenile inflammatory arthritis |
| Systemic lupus erythematosus |
| Neonatal lupus |
| Systemic sclerosis<br>CREST syndrome |
| Mixed connective tissue disease |
| Anti-phospholipid syndrome |
| Vasculitis<br>Giant cell arteritis/polymyalgia rheumatica<br>Takayasu arteritis<br>Granulomatosis with polyangiitis<br>Churg-Strauss syndrome<br>Polyarteritis nodosum<br>Microscopic polyangiitis |
| Polymyositis/dermatomyositis |
| Relapsing polychondritis |
| Sjögren's syndrome |
| Behçet's disease |
| Kawasaki's disease |
| Sarcoidosis |

CREST, Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia.

disease, which appear to manifest various degrees of both autoinflammatory and autoimmune features.

Autoimmune diseases can be classified as systemic or organ-specific depending on the extent of their clinicopathology (Table 19-2). The systemic category includes systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, primary Sjögren's syndrome, dermatomyositis, and systemic vasculitides. In systemic disease, autoimmunity targets ubiquitously expressed self-antigens, and end-organ injury is typically mediated by autoantibodies and, less commonly, T cells. In contrast, in organ-specific diseases, the self-antigens are typically cell- or tissue-specific in location or accessibility, and end-organ damage can be mediated by antibodies and/or T cells. Some of the more notable examples in this group, which span virtually all organ systems, include Hashimoto's thyroiditis, Graves' disease, multiple sclerosis (MS), type 1 diabetes mellitus (T1DM), anti-phospholipid (aPL) syndrome, pemphigus vulgaris, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, and myasthenia gravis. It should be emphasized, however, that although the distinction of systemic and organ-specific disorders provides a conceptual framework, the individual pathophysiologies are more diverse than is implied by this simple classification. Autoimmune diseases are also classified by hypersensitivity reaction type based on the mechanism of adaptive immune system–mediated injury,[9] a topic that will be discussed later in the chapter.

## ANIMAL MODELS OF AUTOIMMUNITY

Much of what is known about the immune system and autoimmunity has been derived from studies in animals, particularly the mouse, which has an immune system and genome composition similar to that of humans. Many well-characterized autoimmune models exist in which investigators can manipulate genomes and immune systems, test interventions, and modify environments. Animal models of autoimmune disease can be divided into three main types based on their derivation: (1) spontaneous, (2) genetically modified, and (3) induced. Some of the more common or notable models are listed in Table 19-3.

Within the spontaneous group are models of SLE, RA, and T1DM. The lupus-prone strains commonly develop anti-DNA and immune complex–mediated kidney damage, but they also possess unique phenotypic characteristics and differ in the genes underpinning susceptibility.[10] The SKG arthritis model is a spontaneous, inflammatory, and erosive arthritis caused by a *ZAP70* mutation that, similar to RA, is associated with rheumatoid factor (RF) and antibodies to citrullinated proteins.[11] T1DM develops in nonobese diabetic (NOD) mice and biobreeding (BB) rats as a result of T cell–mediated destruction of β-islet cells.[12]

The genetically modified group, encompassing transgenic, site-directed genetic replacement (gene knockout or knockin) and *N*-ethyl *N*-nitrosourea (ENU)-mutagenized mice, is by far the largest, with more than 95 different models of lupus and many models of organ-specific diseases, particularly T1DM and MS.[10,12] The lupus models, primarily single gene knockout or transgenic mice, have provided a wealth of information related to immune tolerance and disease pathogenesis. Some examples are (1) confirmation of human SLE gene associations and elucidation of mechanisms, such as C1q and fragment, crystallizable γ receptor IIb (FcγRIIb) knockout mice; (2) discovery of novel mechanisms such as altered messenger RNA (mRNA) regulation in sanroque and miR-17-92 transgenic mice; and (3) identification of new pathways relevant to therapy, such as B cell–activating factor of the tumor necrosis factor family (BAFF) transgenic.

An example of an arthritis model created by genetic modification is the K/BxN mouse, a B6 × NOD hybrid expressing a transgenic T cell receptor, named KRN, that recognizes a bovine ribonuclease peptide on H-2K.[13] An acute severe inflammatory arthritis develops in these mice that is caused by antibodies to glucose-6-phosphate isomerase (GPI), an antigen that, although intra-cellularly and ubiquitously expressed, is mainly accessible to antibodies in joints.[14] Although GPI is not a major target in RA or other arthritides, the model has nevertheless been useful for investigating inflammatory mechanisms in arthritis.[15]

Other genetically modified models of spontaneous arthritis include the human T-lymphotropic virus (HTLV)-1 tax transgenic, the tumor necrosis factor (TNF) transgenic, the interleukin-1 receptor antagonist (IL-1ra) transgenic, the IL-1 transgenic, and a gain-of-function knockin mutant of CD130, the signaling component of receptors for several cytokines including IL-6, IL-11, IL-27, and leukemia inhibitory factor (LIF).[16] Several genetically modified models of T cell–mediated organ-specific disease have been developed that consist of T cell receptor (TCR) transgenic T cells that recognize tissue-specific antigens in organs such as the brain and pancreatic islets or employ a slightly modified version in which mice are double transgenic for both an antigen expressed in a tissue of interest and the corresponding TCR.[17-20] By allowing analysis of single autoreactive T cell clones, these models have yielded considerable insights into

**TABLE 19-2** Classification, Mechanisms, and Models of Autoimmune Diseases

| Syndrome | Autoantigen | Consequence | Hypersensitivity Type | Animal Model (Example) |
|---|---|---|---|---|
| **Systemic** | | | | |
| aPL syndrome | β2-GP1 (apoH) | Vascular thrombosis, recurrent miscarriages | II | (NZW × BXSB)F1 |
| Microscopic angiitis | p-ANCA (MPO) | Glomerulonephritis, leukocytoclastic vasculitis, mononeuritis multiplex, lung inflammation | III | anti-MPO |
| Granulomatosis with polyangiits | c-ANCA (PR3) | Vasculitis of kidney, upper airway, and lungs | III | None |
| Cryoglobulinemia | Unknown | Cutaneous vasculitis, glomerulonephritis | III | MRL-*Fas*$^{lpr}$ |
| SLE | Nucleic acids | Glomerulonephritis, skin lesions, arthritis, CNS lupus, and others | III | MRL-*Fas*$^{lpr}$, BWF1, BXSB, NZM2410 |
| Systemic sclerosis | Unknown | Fibrosis of multiple organs | III | Tsk/$^{+}$ mice, bleomycin-induced |
| RA [†] | RF IgG immune complexes, citrullinated proteins, other joint antigens | Arthritis and associated manifestations: rheumatoid nodules, rheumatoid lung, Felty's syndrome | III | CIA, PGIA, AA, SKG, K/BxN, BxD2, citrullinated-protein-immunized DR4-IE transgenic |
| **Organ-Specific** | | | | |
| Graves' disease | TSH receptor | Stimulation of receptor; hyperthyroidism | II | EAT |
| Myasthenia gravis | ACh receptor | Receptor blockade/modulation; neuromuscular paralysis | II | EAMG |
| Autoimmune hemolytic anemia | RBC surface antigen | C′ and FcγR-mediated cell destruction; anemia | II | NZB |
| Idiopathic thrombocytopenic purpura | Platelet integrin GpIIb:IIIa | Thrombocytopenia; purpura, bleeding | II | (NZW × BXSB)F1 |
| Goodpasture's syndrome | Type IV collagen* and other basement membrane antigens | Pulmonary hemorrhage, glomerulonephritis | II | Anti-CIV, anti-laminin |
| Pemphigus vulgaris | Epidermal cadherin (Dsg3) | Bullous skin lesions | II | anti-Dsg3 |
| Neonatal lupus | Ro/La | Cutaneous LE, heart block | II | Anti-Ro52[254] |
| T1DM | Pancreatic β-cell antigens | Pancreatic islet inflammation, diabetes | IV | NOD, BB |
| Multiple sclerosis | CNS antigens; MBP, PLP, MOG in animal models | Progressive CNS inflammation and paralysis | IV | EAE, Theiler's virus infection |

*Most likely hypersensitivity type.
[†]Both systemic and organ-specific.
AA, Adjuvant arthritis; ACh, acetylcholine; ANCA, anti-neutrophil cytoplasmic antibody; anti-CIV, anti–type IV collagen; anti-Dsg3, anti-desmoglein 3; aPL, anti-phospholipid antibody; apoH, apolipoprotein H; β2-GP1, β2-glycoprotein 1; c-ANCA, cytoplasmic anti-neutrophil cytoplasmic antibody; CIA, collagen-induced arthritis; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; EAMG, experimental autoimmune myasthenia gravis; EAT, experimental autoimmune thyroiditis; Ig, immunoglobulin; LE, lupus erythematosus; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MPO, myeloperoxidase; NOD, nonobese diabetic; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; PGIA, proteoglycan-induced arthritis; PLP, proteolipid protein; PR3, proteinase 3; RA, rheumatoid arthritis; RBC, red blood cell; RF, rheumatoid factor; SLE, systemic lupus erythematosus; T1DM, type 1 diabetes mellitus; TSH, thyroid-stimulating hormone. Other abbreviations are mouse strains.

tolerance mechanisms and disease pathophysiology. Similarly, autoreactive B cell receptor transgenics or knockin models have been developed that have been crucial for defining B cell tolerance mechanisms.[21-27]

The induced models encompass a wide variety of both systemic and organ-specific diseases. More commonly studied models of systemic disease include tetramethylpentadecane (TMPD; also called pristane)-induced autoimmunity, mercury-induced autoimmunity, and chronic graft-versus-host disease.[28-30] All models bear similarities to human SLE in producing anti-nuclear antibodies and immune complex deposits but differ in pathophysiology and strain susceptibility. For the induced models of organ-specific diseases, a common approach is to immunize rodents with a self-antigen or closely related peptide or foreign antigen, plus a strong adjuvant, usually complete Freund's. This approach makes it possible to induce autoimmunity in virtually all organ systems and to produce diseases mediated by cellular as well as humoral mechanisms. Some of the more commonly studied organ-specific models developed by this approach include collagen-induced arthritis (CIA), proteoglycan-induced arthritis (PGIA), and experimental autoimmune encephalomyelitis (EAE), but many others exist that are directed to the thyroid, eye, gonad, nerves,

**TABLE 19-3** Partial List of Autoimmune Disease Models

| Model | Autoimmune Disease | Species | Notable Characteristics |
|---|---|---|---|
| **Spontaneous Variant** | | | |
| MRL-Fas$^{lpr}$ | SLE | Mouse | Fas mutation, defective apoptosis, lymphoproliferation, human counterpart ALPS |
| (NZB × NZW)F1 | SLE | Mouse | Female predominance |
| NZB | SLE, AIHA | Mouse | Anti-RBC |
| BXSB | SLE | Mouse | Yaa mutation (duplication of X chromosome containing *TLR7* gene on Y chromosome) |
| (NZW × BXSB)F1 | SLE, APS, ITP | Mouse | Anti-cardiolipin, anti-platelet |
| BXD2 | SLE, RA | Mouse | Lupus and inflammatory arthritis |
| SKG | RA | Mouse | *ZAP70* mutation |
| NOD | T1DM | Mouse | MHC (H-2$^{g7}$) similar to T1DM-predisposing HLA |
| BB | T1DM | Rat | Lymphopenia |
| **Genetically Modified** | | | |
| C1q ko | SLE | Mouse | Defective clearance of apoptotic cells |
| FcγRIIb ko | SLE | Mouse | Impair regulation of B cell and APCs |
| BAFF Tg | SLE | Mouse | Enhanced survival of B cells |
| TLR7 Tg | SLE | Mouse | Enhanced survival and activation of B cells and DCs |
| *Sanroque* mice (*roquin* gene) | SLE | Mouse | *Rc3h1* M199R mutation increases ICOS expression and Tfh cell expansion |
| miR-17-92 Tg | SLE | Mouse | miRNA-induced autoimmunity |
| K/BxN, TCR Tg | RA | Mouse | Arthritis mediated by anti-GPI |
| MBP-specific TCR Tg mice | MS | Mouse | Spontaneous autoimmune encephalomyelitis |
| Double Tg: anti-GP TCR and insulin promoter-GP (GP = LCMV glycoprotein) | T1DM | Mouse | Ignorant Tg T cells are activated by infection with LCMV, causing insulitis and diabetes |
| **Induced** | | | |
| TMPD (pristane)-induced autoimmunity | SLE | Mouse | IFN-α- and TLR7-dependent |
| Hg-induced autoimmunity | SLE | Mouse, rat | IFN-γ dependent |
| Chronic graft-versus-host disease | SLE | Mouse | Parent into F1 |
| Collagen-induced arthritis | RA | Mouse, rat | Autoimmunity to type II collagen |
| PG-induced arthritis | RA | Mouse | Autoimmunity to proteoglycan |
| Adjuvant arthritis | RA | Rat | Inflammatory arthritis induced by complete Freund's adjuvant or mineral oil |
| Citrullinated-protein-immunized DR4-IE transgenic | RA | Mouse | Autoimmunity to citrullinated-fibrinogen or enolase (human and bacterial) |
| EAE | MS | Mouse, rat | Autoimmunity to MBP, MOG, or PLP depending on MHC haplotype |
| EAT | Thyroiditis | Mouse, rat | Autoimmunity to thyroglobulin |
| EAMG | MG | Rat | Autoimmunity to acetylcholine receptor |

AIHA, Autoimmune hemolytic anemia; ALPS, angiolymphoproliferative syndrome; APC, antigen-presenting cell; APS, anti-phospholipid syndrome; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; EAMG, experimental autoimmune myasthenia gravis; EAT, experimental autoimmune thyroiditis; GPI, glucose-6-phosphate isomerase; HLA, human leukocyte antigen; ICOS, inducible co-stimulator; IFN, interferon; ITP, idiopathic thrombocytopenic purpura; ko, gene knockout; LCMVB, lymphocytic choreomeningitis virus; MBP, myelin basic protein; MHC, major histocompatibility complex; miRNA, micro RNA; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PG, prostaglandin; PLP, proteolipid protein; RA, rheumatoid arthritis; RBC, red blood cell; SLE, systemic lupus erythematosus; T1DM, type 1 diabetes mellitus; TCR, T cell receptor; Tfh, follicular helper T cell; Tg, transgenic; TMPD, 2,6,10,14-tetramethylpentadecane.

neuromuscular junction (acetylcholine receptor), muscle, heart, adrenal gland, bladder, stomach, liver, inner ear, kidney, and prostate tissues. In certain susceptible strains of rats, a progressive inflammatory erosive arthritis called adjuvant arthritis can also be induced by intradermal injection of complete Freund's adjuvant or by mineral oil components of Freund's adjuvant such as TMPD. Other induced models of arthritis include streptococcal cell wall and antigen-induced arthritis.[16] Arthritis characterized by synovial hyperplasia and ankylosis was reported in human leukocyte antigen (HLA)-DR4-IE transgenic mice immunized with human citrullinated fibrinogen or enolase.[31,32]

**TABLE 19-4** Multiple Tiers of Tolerance

| Type | Cell Type | Site | Mechanism |
|---|---|---|---|
| **Central Compartment** | | | |
| Central tolerance | T cells<br>B cells | Thymus<br>Bone marrow | Primarily deletion, anergy, possibly editing<br>Editing, anergy, deletion |
| **Peripheral Compartment** | | | |
| Immature B cell tolerance | Transitional 1 (T1) B cells | Periphery | Deletion, anergy upon activation |
| Peripheral anergy | T and B cells | Secondary lymphoid organs and peripheral tissue | Inadequate signal induces cell inactivation |
| Ignorance | T cells; maybe B cells | Peripheral and secondary lymphoid organs | Insufficient self-antigen or co-stimulation |
| Inaccessible self-antigen | T and B cells | Peripheral organs | Sequestration, crypticity |
| Regulation | T and B cells | Secondary lymphoid organs and site of inflammation | Suppression by regulatory cells via intercellular signals and cytokines |
| Clonal deletion after activation | T and B cells | Site of inflammation and secondary lymphoid organs | Apoptosis caused by a decline in survival factors |
| Cytokine deviation | T cells | Site of inflammation and secondary lymphoid organs | Differentiation toward less pathogenic Th subsets |
| Postsomatic hypermutation | B cells | Germinal center | Insufficient CD4 T cell help, deletion (via *Fas*) |
| Tissue resistance | B and T cells | Peripheral tissues | Inhibitory intercellular signals and cytokines |
| **Innate Mechanisms** | | | |
| PRR engagement required for activation | Innate cells | Site of inflammation | Simple mechanism for self-nonself discrimination |
| Suppression of adaptive immune responses | Immature and mature DC | Site of inflammation and secondary lymphoid organs | Delivery of inhibitory signals and activation of Treg |
| Clearance of apoptotic cells | Complement, phagocytes | Peripheral tissues | Removal of potential pro-inflammatory material and self-antigens |
| Complement mediated effects on adaptive responses | Lymphocytes, innate cells | Secondary lymphoid organs and peripheral tissue | Modulation of activation |

PRR, Pattern recognition receptor; Treg, regulatory T cell.

## TOLERANCE MECHANISMS

Increasing insights into mechanisms of self-nonself discrimination have emerged during the past six decades in parallel with growing knowledge of the immune system. Just over 50 years ago, Burnet and Medawar advanced the critical concept that tolerance was imposed by clonal deletion of self-reactive lymphocytes during early development—that is, central tolerance.[33,34] The subsequent discovery that mature B cells undergo somatic hypermutation in the periphery led to the hypothesis by Bretscher and Cohn that the production of autoantibodies might be impeded by the need for both B and T cell compartments to breach tolerance.[35] In 1975, while studying allogeneic responses, Lafferty and Cunningham posited that activation of T cells involved the passing of a second signal that need not be antigen-related, thereby implicating co-stimulation from antigen-presenting cells (APCs) as a critical factor in lymphocyte activation.[36] In 1987, the nature of the co-stimulation, or two-signal, requirement was further defined when Jenkins and Schwartz showed that engagement of antigen receptor alone without a second signal resulted in functional inactivation of T cells.[37] An elegant mechanism for self-nonself discrimination was then advanced by Janeway in 1989 when he hypothesized that APCs remain quiescent unless activated by the engagement of pattern recognition receptors (PRRs) by microbial products.[38] This concept was further extended by Matzinger in 1994 to a "danger model" that includes activation of the immune system by both foreign and endogenous factors associated with tissue stress and damage.[39] These models laid the foundation for the current, more complex view of self-nonself discrimination in which tolerance is imposed by both innate and adaptive immune systems through layers of mechanisms occurring at various stages throughout lymphocyte development and activation (Table 19-4).

### Clone-Specific Self/Nonself Recognition

In contrast to innate immune cells, which are activated primarily by hardwired microbial PRRs, lymphocytes have broad specificity and therefore self-nonself discrimination must be implemented at the clonal level. To achieve this objective, T and B cells utilize several mechanisms that can be grouped into three general strategies. First, the type of response is controlled by developmental stage. For example, immature lymphocytes respond to strong antigen receptor stimulation by cell death, whereas a similar signal in mature cells leads to activation. Through this mechanism, self-reactive clones are eliminated from the nascent lymphocyte repertoire before they can cause injury. Second, activation of mature lymphocytes requires, in addition to antigen receptor engagement, a second co-stimulatory signal, the absence of which results in anergy or cell death. For the most part, this requirement limits reactivity to self because co-stimulatory signals are largely provided by cells of the

innate immune system. Third, lymphocytes are fine-tuned in various ways by a fairly extensive list of modulating factors necessary for controlling self-reactive clones.[40-42] In B cells, but not T cells, this includes an inverse correlation of antigen receptor (membrane immunoglobulin [Ig]M) expression and ability to flux calcium with the degree of self-reactivity.[43]

Examples of defects affecting a broad range of other surface receptors on lymphocytes include those with prosurvival (IL-7R, BAFF receptor [BAFFR], and IL-2R), proapoptotic (TNF receptors [TNFRs], Fas ligand [FasL], and TNF-related apoptosis-inducing ligand [TRAIL]), co-stimulatory (CD28, CD40, and Toll-like receptors [TLRs]), differentiating (IL-12R, IL-4R, interferon γ receptor [IFNγR], IL-23R, retinoic acid receptor, transforming growth factor-β receptors [TGF-βRs], signaling lymphocyte activation molecule [SLAM]/SLAM-associated protein [SAP] family members, OX40, and inducible co-stimulator [ICOS]/ICOS ligand [ICOSL]), inhibitory (FcγRIIb, CD22, cytotoxic T lymphocyte–associated antigen-4 [CTLA4], and programmed cell death protein 1 [PD-1]), antigen receptor signal modulating (CD19 and CD45), and activating (SAP/SLAM family members) functions, and they have been shown to influence the development of autoimmunity.[10] Collectively, these self-nonself recognition mechanisms provide the basic cellular means by which the innate and adaptive immune systems render T and B cell clonotypes tolerant to self-antigens and resistant to autoimmune disease.

## The Innate System and Tolerance

Given its vital role in initiating and modulating adaptive immune responses, it is not surprising that the innate immune system strongly influences both tolerance and autoimmunity. Indeed, although its contributions have yet to be fully delineated, several ways in which self-tolerance is influenced by the innate arm have been defined.

First, activation of the innate immune system under normal circumstances typically requires engagement of microbial PRRs, which endow the immune system with a direct and simple way to distinguish foreign antigens from self-antigens.[44] PPRs recognize common structures on pathogens and also a few self-molecules, such as certain damage-associated molecular patterns (DAMPs)—for example, heat shock proteins[45]—which are released during cellular stress, injury, or death. PPRs can be classified by their location into the following types: secreted (e.g., collectins, pentraxins, and ficolins), transmembrane (e.g., TLRs, certain C-type lectin receptors, and N-formyl methionine receptors), and endosomal (e.g., TLRs) or cytosolic (e.g., retinoic acid inducible gene I [RIG-I]-like receptors, NOD-like receptors, and DNA receptors: IFI16, AIM2, and the cyclic guanosine monophosphate [GMP]–adenosine monophosphate [AMP] synthase–stimulator of interferon genes [STING] pathway).[46-48] The importance of this mechanism is illustrated by the finding that overexpression of TLR7 by spontaneous gene duplication or transgenic approaches promotes systemic autoimmunity.[49] This outcome occurs because certain PRRs, such as TLR7 and TLR9, which equally sense both foreign and self-nucleic acids, avoid significant exposure to endogenous nucleic acids by virtue of their location in subcellular compartments.[50] However, in the case of TLR7, overexpression makes it possible for normally substimulatory amounts of endogenous RNA to activate immune cells, thereby bypassing the usual requirement for microbial exposure.

Second, some cells of the innate immune system actively suppress adaptive immune system activation under certain conditions. For example, both immature and mature dendritic cells (DCs) have been shown to promote tolerance by inducing CD4 T regulatory (Treg) cells and other mechanisms.[51,52]

Third, failure of the rapid non-inflammatory clearance of apoptotic cells, another critical function of the innate immune system,[53,54] can result in an increased supply of self-antigenic material including nucleic acids, secondary necrosis, and release of pro-inflammatory factors, leading to systemic autoimmunity.[55] Accordingly, deficiencies in several key apoptotic cell clearance molecules are associated with autoimmunity, including (1) the Tyro3-Axl-Mer receptors on phagocytes that bind, through Gas6 or protein S, the exposed phosphotidylserine (PS) on apoptotic cells[56], (2) the milk fat globule–epidermal growth factor (EGF) 8 (MFG-E8) protein that bridges the αvβ3 integrin on phagocytes and PS on apoptotic cells,[57,58] (3) Tim4 expressed on the surface of resident macrophages that binds PS,[59] (4) the 12/15 lipoxygenase that controls which macrophage subpopulations phagocytize apoptotic cells,[60] and (5) natural IgM, C1q, or C1q receptor (SCARF1) that bind to and enhance clearance of apoptotic cells.[61-64]

Fourth, several complement components have been directly implicated in autoimmunity. For example, SLE is associated with deficiencies of proximal components of the classical pathway, including C1q, C4, and C2. Although the mechanism for this association is uncertain, both defective clearance of apoptotic material/immune complexes and a shift in the activation threshold of lymphocytes have been suggested.[65] Another example is that deficiency of CD55 (or decay-accelerating factor), a cell surface protein that restricts complement activation, is associated with enhanced T cell responses and exacerbation of neuroinflammation and lupus in animal models.[66,67]

Fifth, the innate system could be a major source of self-antigenic material. For example, neutrophil extra-cellular traps (NETs), the nuclear material–containing weblike structure extruded by neutrophils to combat bacterial infection, contain a compelling collection of modified autoantigens, but thus far their role in patients remains to be firmly established.[68] Thus, at many levels, the innate immune system plays a critical role in maintaining tolerance and controlling autoimmunity.

## T Cell Tolerance

T cells are critical players in not only achieving but also fine-tuning tolerance to a high degree of specificity. Several mechanisms have been identified that can be divided into three main areas: central tolerance wherein T cells first acquire their antigen receptor, peripheral tolerance where T cells encounter self-antigens not present in the thymus, and postactivation regulation wherein activated and expanded T cell clones are returned to their resting state. Central tolerance, as alluded to earlier, is

imposed on T cells with self-reactive specificities during thymocyte development by mechanisms utilizing primarily deletion, to a lesser extent anergy, and possibly receptor editing of the TCR α chain.[69,70] This process, although highly effective, is not completely efficient, and T cells with autoreactivity—primarily those with intermediate to low affinity to self or that recognize self-antigens poorly expressed in the thymus—emigrate in significant numbers. This leakiness is probably necessary to generate a broad repertoire, but it then creates vulnerability to autoimmunity and necessitates peripheral tolerance mechanisms.

In the periphery, multiple mechanisms for avoiding autoreactivity have been identified. Among these, a common explanation is that most self-antigens are not accessible to trigger a response, a situation that can be caused by low abundance, specific characteristics of the self-antigen, or location. This mechanism is supported experimentally by the finding that T cells expressing a transgenic TCR to certain tissue-specific antigens are not deleted or activated, nor do they cause autoimmune disease. Yet, these so-called "ignorant" T cells are fully functional and respond to self-antigen when presented in a conventional context.[20,71] For a few self-antigens, such as those expressed in the anterior chamber of the eye, central nervous system (CNS), or other so-called immunologic privileged sites—as originally defined by their ability to accept allograft transplants—resistance to self-reactivity is also partly from anatomic sequestration caused by limited access of blood-borne cells and the absence of conventional lymphatic drainage.[72] The latter is important because T cells are typically first activated in secondary lymphoid organs and subsequently migrate to target organs where reactivation by local APCs and the production of pro-inflammatory factors leads to tissue damage.[73] Anatomic sequestration alone, however, is not sufficient for tissues to support immunologic privilege and, as will be discussed later, such sites employ a host of additional local mechanisms.[74,75]

Another peripheral mechanism is the aforementioned two-signal paradigm in which T cell activation requires both TCR engagement and a co-stimulatory signal usually provided by CD28. Because the two ligands for CD28, CD80, and CD86 are primarily expressed at high levels on activated professional APCs, presentation of self-antigen by quiescent APCs would lead to tolerance. Indeed, immature DCs promote tolerance in this manner by constitutively presenting low doses of self-antigen on major histocompatibility complex (MHC), resulting in cell death or anergy of the corresponding T cells.[76]

Peripheral tolerance is also maintained by active suppression via immunoregulatory cells of the immune system, among which $CD4^+$ Tregs are the best characterized.[77-80] They constitute a distinct αβ–T cell subset generated in the thymus (natural Treg [nTreg]) or are differentiated in the periphery from naïve or mature $CD4^+$ T cells exposed to TGF-β (induced Treg [iTreg]) and developmentally induced by the forkhead box P3 (FOXP3) transcription factor. $CD4^+$ Tregs typically express high levels of the IL-2 receptor component, IL-2Rα (CD25), and require IL-2 for survival. Tregs participate in every adaptive immune response, are critical for maintaining the proper level of immune response, and are activated at the same time as conventional T cells. They are thought to suppress the magnitude of the response by initially downregulating DC function and then inhibiting T cell activation by competing for IL-2, producing immunosuppressive cytokines such as TGF-β, IL-10, and IL-35, and by cell-cell inhibitory interactions that lead to cell killing or the induction of negative signals.[81] These inhibitory actions suppress T cells that are in proximity to Treg cells regardless of their antigen specificity.[82] Other T cells with regulatory activity in autoimmunity, including Tr1, CD8 Treg, Qa-1/HLA-E–restricted CD8 T cells, and γδ T cells, have been described but are less well characterized.[83-87]

Tissues themselves also employ mechanisms that suppress self-reactivity and contribute to the establishment of immune privilege.[74,88-90] These tissues can be considered to comprise three general categories: First, certain tissues are decorated with cell surface inhibitory molecules, such as the proapoptotic FasL and TRAIL, lymphocyte inhibitory and Treg promoting PD-L2, and complement regulatory proteins, CD55 and CD46, that can potentially eliminate or impede the activation of autoreactive T cells. Second, soluble inhibitors of inflammation and immune activation are secreted by particular tissues. Notably, in the aqueous humor of the eye, there is a broad spectrum of such factors that include TGF-β, α–melanocyte-stimulating hormone, vasointestinal peptide, calcitonin gene–related protein, somatostatin, macrophage inhibitory factor, and complement inhibitors. Third, lymph node resident stromal cells have been shown to induce tolerance of $CD8^+$ T cells that recognize peripheral tissue-restricted self-antigens.[91,92] Thus it has been proposed that stromal cells in lymph node and tissues may serve to provide a means to eliminate T cells that bind to tissue-restricted antigens not expressed in the thymus. Fourth, the anterior chamber of the eye elicits a unique type of altered immune response through a complex multistep process termed "anterior chamber–associated immune deviation" (ACAID) that leads to a dampened, less tissue-destructive response.[74,90,93] Although ACAID was long thought to be important for tolerance, it has recently been argued that its primary function may be to modulate the immune response so the eye can respond to infection without damaging its integrity.[94]

Another possible peripheral mechanism is immune deviation wherein polarization away from a predisposing cytokine pattern—such as from a Th1 to a Th2 profile—by administering or blocking specific cytokines suppresses the development of autoimmune disease.[95] Similarly, activation of natural killer (NK) T cells with α-GalCer, which induces IFN-γ production, is associated with dampening of the adaptive Th1 and Th17 effector responses and protection from experimental autoimmune uveitis.[96] In these models, autoreactive T cells are activated but do not produce the pro-inflammatory factors necessary for tissue damage.

In addition to central and peripheral tolerance, the immune system must also avert autoimmunity by suppressing or eliminating T cells after their activation or expansion. This regulation is mediated by several processes, including upregulation of inhibitory receptors such as CLTA4 and PD-1,[97] expression of proapoptotic receptors such as Fas, synthesis of metabolic enzymes such as indoleamine-2,3-dioxygenase (IDO),[98] and release of intracellular proapoptotic factors such as Bim. Deficiencies of such mediators that control the magnitude of T cell

responses are associated with severe expansion of lymphocyte populations and with varying degrees of autoimmunity.

## B Cell Tolerance

B cells are not only required for antibody production but serve as potent APCs for T cells and follicular DCs and can also act in regulation.[99] Moreover, depletion of B cells with rituximab has shown promise even in autoimmune diseases considered to be mediated by T cells such as T1DM[100] and MS.[101] Therefore, substantial interest exists in defining both mechanisms of B cell tolerance and the specific role of B cell tolerance in autoimmune disease.

Before discussing specific tolerance mechanisms, however, it should be emphasized that the fate of B cell antigen receptor (BCR) after cross-linking is highly dependent on developmental stage, context and strength of signal, and the nature of the antigen, probably more so than for T cells because B cells are subjected to less stringent selection during central tolerance. Several checkpoints considered important for controlling autoreactive B cells and maintaining self-tolerance have been identified and include many central and peripheral mechanisms similar to those described for T cells, as well as a few additional ones.

Central tolerance of B cells takes place in the bone marrow during pre-B to immature B cell transition as they express rearranged Ig genes on their surface.[102] It appears that the dominant mechanism for B cells with high affinity to membrane-bound self-antigen is receptor editing (replacement of light [L] chain) and, to a lesser extent, deletion, whereas soluble self-antigens induce both receptor editing and anergy.[103,104] Anergic B cells are detectable in the periphery as an $IgD^{+}IgM^{-}$ population[105] or in mice as splenic transitional 3 (T3) B cells.[106] They are short-lived at least in part because they downregulate the BAFFR, which is required for their survival, putting them at a competitive disadvantage with other immature B cells, and they are less able to enter B cell follicles.

In the periphery, the earliest tolerance checkpoint occurs at the transitional 1 (T1) B stage over a 2-day interval before maturation to T2 and later naïve B cell subsets.[102,107-109] T1 B cells are the immediate bone marrow emigrant population, retain an immature phenotype, and are dependent on BAFF for survival. Importantly, they undergo apoptosis and not activation when stimulated, which results in deletion of B cells that recognize peripheral self-antigens not expressed in the bone marrow compartment. Thus, this mechanism, which is unique to B cells, essentially represents an extension of central tolerance to the periphery. Self-reactive B cells can also downmodulate their surface IgM,[43] which allows them to escape deletion and affords the host a broader repertoire despite the risk of autoimmunity.

Other peripheral tolerance mechanisms are achieved in much the same way as those previously described for T cells but differ qualitatively as a result of distinct differentiation pathways and differences in antigen recognition by B cells and T cells—that is, BCRs can bind to virtually all tertiary structures, whereas TCRs are restricted to recognizing self-MHC/peptide complexes on host cell surfaces. Accordingly, B cells can be ignorant of their corresponding self-antigen because of insufficient quantity or access[21] or can undergo anergy and ultimately cell death if engagement of the BCR occurs without co-stimulation, that is, two signals.[110]

Another notable checkpoint occurs during T-dependent immune responses as B cells undergo affinity maturation in germinal centers (GCs) and acquire new specificities that may include self-reactivity. Evidence suggests that tolerance at this juncture is often defective in autoimmune diseases, because most autoantibodies have acquired autoreactivity through somatic hypermutation and are class-switched,[111-113] both of which are indicative of GC maturation. Although recent studies have provided significant insights into GC processes involved in the selection of B cell clones with high affinity to foreign antigens, the manner in which tolerance of class-switched autoreactive B cells is achieved remains less well defined. Nevertheless, the strongest evidence suggests that (1) autoreactive B cells compete poorly for the cognate T cell help essential for GC B cell survival because the autoreactive BCR would bind less well to the original antigen, resulting in less internalization of antigen for processing and presentation to T cells,[114-116] and (2) B cells that acquire BCRs with reactivity to high-density membrane self-antigens are deleted by a Fas-dependent mechanism.[117,118]

# THEORIES OF AUTOIMMUNITY

Development of autoimmune diseases is influenced by genetic and, to varying degrees, environmental, gender, and other factors, with current evidence supporting a model in which genetic predisposition is required (Figure 19-1). Therefore, theories of autoimmunity and loss of tolerance are closely intertwined with genetic influences. In addition, such theories must also explain how tolerance is breached when autoimmunity is induced in otherwise healthy animals. Upon taking both these factors into account and applying a reductionist perspective, theories of autoimmune disease can be consolidated into two main mechanisms representing separate ends of a continuum, with most diseases having some elements of both. On one end, loss of tolerance and consequent autoimmune disease is

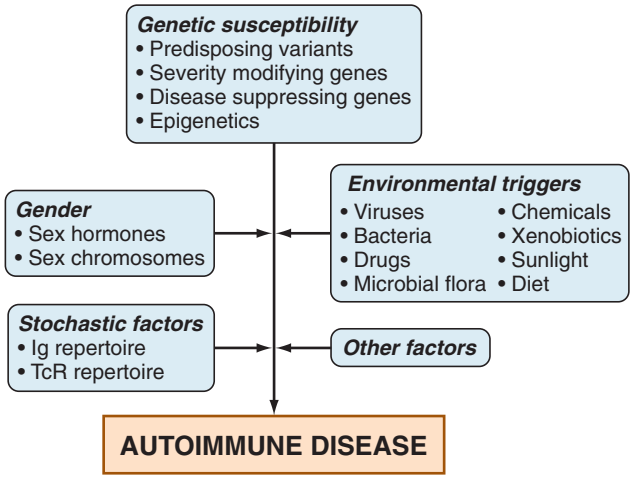



**Figure 19-1** Etiology of autoimmune diseases. Autoimmune diseases are usually caused by the additive effects of autoimmunity-promoting environmental, gender, and other factors superimposed on significant underlying genetic predisposition. IG, Immunoglobulin; TcR, T cell receptor.

**TABLE 19-5** Mechanisms of Autoimmunity

| Examples | Disease | Mechanism |
|---|---|---|
| **Defective Tolerance** | | |
| **Central Defects** | | |
| *AIRE* deficiency | APECED syndrome | Failure to delete autoreactive T cells because of reduced expression of peripheral antigens in thymus |
| *ZAP70* deficiency | Inflammatory erosive arthritis (mice) | Defective T cell activation and thymic selection |
| **Peripheral Defects** | | |
| FAS/FASLG deficiency | ALPS | Defective apoptosis |
| Rc3h1 (M199R) mutation | Lupus (mice) | Increased ICOS on Tfh cells promotes their expansion |
| TREX1 (DNase II) deficiency | Aicardi-Goutières syndrome, chilblain lupus | Accumulation of intra-cellular DNA induces IFN-α production |
| FOXP3 deficiency | IPEX syndrome | Absence of Treg cells |
| PD-1 deficiency | Lupus, myocarditis (mice) | Defective peripheral tolerance of T cells |
| **Activation of Nontolerant Lymphocytes** | | |
| Penetrating injury | Sympathetic ophthalmia | Release of self-antigen in an inflammatory milieu |
| Coxsackie B virus infection | T1DM (mice) | Infection mediated release of self-antigen in an inflammatory milieu |
| Immunization with self-antigen and strong adjuvant | EAE (mice) | Activation of ignorant T cells |
| Citrullination of proteins | RA | Generation of neo self-antigens |
| Altered structure of collagen IV caused by sulfilimine bonds | Goodpasture's syndrome | Formation of conformational neo self-antigens |
| Cross-reactivity of group A streptococcal and cardiac antigens | Rheumatic fever | Molecular mimicry |
| Lymphopenia caused by disease-associated IL-21 production | T1DM (NOD mice) | Lymphopenia-induced homeostatic proliferation |

AIRE, Autoimmune regulator; ALPS, autoimmune lymphoproliferative syndrome; APECED, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy; DNase, deoxyribonuclease; EAE, experimental autoimmune encephalomyelitis; ICOS, inducible co-stimulator; IFN, interferon; IL, interleukin; IPEX, immune dysregulation, polyendocrinopathy, enteropathy X-linked; NOD, nonobese diabetic; PD-1, programmed cell death protein 1; RA, rheumatoid arthritis; T1DM, type 1 diabetes mellitus; Tfh, follicular helper T cell; Treg, regulatory T cell.

caused by genetically imposed defects in central and/or peripheral tolerance mechanisms, whereas on the other end, autoimmunity arises from the conventional immune response to self-antigens for which tolerance is normally incompletely established (Table 19-5). In general, most systemic autoimmune diseases are caused by tolerance defects, whereas organ-specific diseases can be mediated by either mechanism.

## Defective Tolerance

Although it can be inferred that loss of tolerance underlies autoimmunity, the specific tolerance defects causing common autoimmune diseases and polygenic animal models have been difficult to delineate, presumably because of modest defects at multiple checkpoints. Nevertheless, studies of monogenic human autoimmune diseases and animal models have identified a wide range of defects in the various layers of central and peripheral tolerance. Such defects are caused by diverse genetic abnormalities and are mediated by a variety of lymphoid and nonlymphoid cell types. A few representative examples will be discussed.

Defects in central tolerance have long been suspected to cause autoimmunity because of the well-documented role of central tolerance in eliminating nascent self-reactive lymphocytes, but until relatively recently, solid evidence for this mechanism was lacking. A breakthrough came from the discovery that mutations in a transcription factor, autoimmune regulator (AIRE), was the cause of autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), a rare inherited disease associated with T cell–mediated autoimmune destruction of multiple endocrine organs.[119-121] *Aire*-deficient mice developed a similar syndrome caused by the reduced expression of thousands of mostly peripheral tissue genes in the thymic medullary epithelium and consequent failure to eliminate T cells recognizing those gene products. Evidence suggests that AIRE achieves this selectivity by binding to certain repressive transcription complexes such as MBD1-ATF7ip that target specific methylated cytosine-phosphatidyl-guanine (CpG) dinucleotides in genetic regions encoding tissue-specific antigens. Thus, it appears that the main function of AIRE is to prevent autoimmunity by deleting T cells that recognize peripheral tissue self-antigens.

Another example of altered thymic selection leading to autoimmunity, in this instance caused by an intrinsic defect in the T cells, is the aforementioned ZAP70 arthritis model.[122] Here, a function-impairing mutation in the C-terminal SH2 domain of ZAP70, a Syk family tyrosine kinase activated by the TCR complex ζ-chain, reduces TCR signaling, causes defective conventional T cell and nTreg development in the thymus, and seemingly enhances positive selection of autoreactive T cells.[123] Interestingly, using several ZAP70 mutants, it was shown that susceptibility to arthritis was altered by only slight differences in ZAP70-mediated signaling strength.[123,124]

For B cells, major defects in central tolerance leading to autoimmunity have been more difficult to prove, and no AIRE-like equivalent has been discovered, presumably because immature B cells continue the selection process for a couple of days after egress from the bone marrow, allowing direct exposure to peripheral antigens. Nevertheless, defective central tolerance of B cells in autoimmunity has been suggested by the finding of a higher frequency of naïve mature B cells with self-reactivity in patients with SLE and RA.[125-127]

In the periphery, FAS deficiency provides an example of defective peripheral tolerance promoting autoimmunity.[128] FAS is a proapoptotic surface receptor that plays a critical role in maintaining immunologic homeostasis by eliminating undesired cells. Defects in FAS cause autoimmune lymphoproliferative syndrome (ALPS, also called Canale-Smith syndrome) and, in mice, lymphoproliferative (lpr) disease, both of which exhibit massive enlargement of secondary lymphoid organs caused mainly by the accumulation of a normally rare, so-called "double-negative" subset of T cells lacking both CD4 and CD8 co-receptors, along with a variety of autoimmune manifestations. Lpr mice are defective in eliminating B cells that acquire self-reactivity in the periphery, a process that typically occurs in the GC during somatic hypermutation.[117] Similar abnormalities are found in humans and mice with defects in the ligand for FAS (FASLG).[129]

Peripheral tolerance is also breached by overexpression of the co-stimulatory molecule ICOS on T follicular helper (Tfh) cells in sanroque mice. These mice have a point mutation in the *Rc3h1* gene, a RING-type ubiquitin ligase that impairs its ability to degrade ICOS mRNA.[130] Increased expression of ICOS promotes the expansion of Tfh cells and GCs, production of IL-21, and autoimmunity.[131]

An example of a lymphocyte-extrinsic cause for loss of tolerance and autoimmunity is deficiency in the 3′ repair exonuclease 1 (TREX1, deoxyribonuclease [DNase] III). Loss-of-function mutations in TREX1 have been implicated in Aicardi-Goutières syndrome, a rare progressive encephalopathy associated with elevated IFN-α levels in the cerebrospinal fluid, and chilblain lupus, a rare form of SLE characterized by painful bluish-red inflammatory skin lesions typically affecting areas exposed to cold temperatures.[132,133] Autoimmunity is thought to be caused by the intra-cellular accumulation of single-stranded DNA (ssDNA) derived from endogenous retro-elements normally degraded by TREX1, resulting in the activation of the intra-cellular DNA sensor STING and consequent overproduction of IFN-α.[134-136] Intriguingly, oxidized DNA, which is abundant in NETs and in ultraviolet skin lesions, is resistant to TREX1 degradation and can promote STING activation and type I IFN production,[137] suggesting that the TREX1 pathway may play a more general role in SLE pathogenesis. The importance of DNA disposal in lupus has been further illustrated by the association of DNase I deficiency with development of lupus in humans and mice.[138,139]

Defective regulation can also result in loss of tolerance and autoimmune disease, as illustrated by the absence of Tregs.[140,141] In humans, monogenic deficiency of the *FOXP3* gene, which is required for Treg development, is associated with the immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome, a severe systemic autoimmune disease associated with diarrhea, eczematous dermatitis, and endocrinopathy that is usually fatal within the first year. T1DM, autoimmune cytopenias, and nephritis are among other less common autoimmune manifestations of this syndrome. Similar findings are observed in scurfy mice, which have a spontaneous function-impairing mutation of *Foxp3*.

## Autoimmunity Caused by Activation of Intolerant or Partially Tolerant T Cells

The other main theory is that autoimmunity develops through the conventional activation of self-reactive T cells that have not been deleted in the thymus and remain oblivious to the corresponding self-antigen after emigration. Such ignorant T cells, commonly found in the periphery of both healthy humans and animals, can be activated by antigen presented by professional APCs in the context of an innate inflammatory milieu. Once activated, T cells are able to gain access to virtually all tissues and, when activated again locally, can elaborate pro-inflammatory factors, causing tissue damage.[73] Breach of tolerance by this mechanism is most often associated with organ-specific diseases, presumably because tissue-specific antigens are less likely to be expressed in the thymus. This theory is supported by the finding that type 1 diabetes is prevented in BB rats and NOD mice by intrathymic transplantation of pancreatic islets[142,143] or inhibited in NOD mice by intrathymic administration of glutamic acid decarboxylase (GAD), a major β-islet autoantigen in this model.[144,145] Similarly, EAE can be prevented by intrathymic administration of either the immunizing antigen, myelin basic protein, or its major encephalitogenic epitope,[146] and autoantibody production can be delayed in lupus-prone mice after intrathymic injections of polynucleosomes.[147] Taken together, these data support the concept that autoimmunity can be caused by the activation of lymphocytes to self-antigens for which there was incomplete central tolerance. This mechanism is similar to the mechanism underlying the APECED syndrome caused by AIRE deficiency, but in this instance central tolerance is not known to be defective.

Breach of tolerance by ignorant lymphocytes has been shown to depend on many factors, including (1) the nature of the self-antigen, (2) the extent of exposure to antigen, (3) antigen receptor affinity, (4) frequency of autoreactive lymphocytes, (5) types and levels of co-stimulatory molecule expression, (6) cytokine and chemokine profiles, and (7) presence of inflammation.[20,148-154] It should be also emphasized that despite the presence of lymphocytes that recognize self-antigen, under normal conditions, peripheral tolerance mechanisms are difficult to overcome. Consequently, although the experimental autoimmune models are highly reproducible, they require supra-physiologic amounts of self-antigen, strong adjuvant, specific MHC haplotypes, and a susceptible background to break tolerance. This finding is consistent with the fact that autoimmunity does not develop in most individuals and speaks to the robustness of the tolerance process that must be overcome. Although it is difficult to prove which of these mechanisms are applicable in patients, they provide frameworks for understanding how autoimmunity can develop, along with guidance in devising interventions.

Similar to immune responses in general, key factors for the initiation of autoimmunity are innate inflammatory and co-stimulatory factors that promote the initial activation and expansion of naïve autoreactive lymphocytes. This mechanism is thought to contribute to the autoimmune response by the release of self-antigens after cell damage or death, increased MHC/peptide expression, upregulation of co-stimulatory factors, and activation of professional APCs.[149,150,155] Indeed, moderate to severe tissue necrosis is often associated with some evidence of autoreactivity, although this condition rarely progresses to autoimmune disease.[156,157] In autoimmune diseases, chronic cell and tissue injury and the continual release of self-antigens under conditions favorable for antigen presentation and co-stimulation promotes epitope spreading and the activation of an expanding repertoire of lymphocytes that recognize autoantigens beyond the initiating autoantigen.[158,159] This process is thought to at least partially account for the often progressive course of autoimmunity. Overall, these and other findings support the theory that, under certain conditions such as infection or trauma, self-antigens are released and, in the presence of inflammatory factors and an activated innate immune system, there is triggering and expansion of previously ignorant self-antigen–recognizing lymphocytes that can lead to autoimmune disease.

In addition to the release of sequestered self-antigens, the initial activation of non-tolerant lymphocytes has been postulated to occur in several other ways. Some investigators have suggested that the initial response may be directed toward certain determinants[160,161] based on the concept of "crypticity," essentially the presence of a hierarchy of dominant and cryptic epitopes on a protein because of differences in binding affinity to the MHC, protein processing, preference for presentation by different APCs, and the repertoire of epitope-specific T cells.[159] It is therefore posited that during thymic selection, T cells that engage the few dominant epitopes are eliminated, whereas those recognizing the less antigenic but more abundant cryptic epitopes are spared. The latter T cells then emigrate to the periphery, where they can be activated by the corresponding cryptic epitope under certain inflammatory conditions.

Another mechanism by which self-antigens can activate nontolerant lymphocytes is through the production of neo self-antigens after post-translational or chemical modifications. A prominent example of this mechanism is the formation of citrullinated proteins caused by the deimination of arginine residues by peptidylarginine deiminase (PADI) enzymes. Several citrullinated proteins are not only major targets of autoantibodies in a subset of RA but are thought to play a significant role in disease pathogenesis.[162-164] Deficiency of protein L-isoaspartate O-methyltransferase (PIMT), which catalyzes the repair of isoaspartyl (isoAsp) proteins formed by the spontaneous conversion of aspartic acid to its isoAsp derivative, has also been shown to result in accumulation of isoAsp proteins and development of lupus in a mouse model.[165] Furthermore, in the PL/J model of EAE, acetylation of the encephalitogenic Ac1-11 peptide of myelin basic protein (MBP) is required for T cell activation even though unmodified peptide binds to MHC.[166] These and other studies suggest that protein modifications can generate new epitopes either directly by creating new structures such as citrulline or indirectly by altering MHC binding or modifying sites of peptide processing to produce new and/or cryptic self-peptides.[167]

Neo self-antigens can also arise from changes in overall structure—for example by the formation of immunogenic IgG-containing immune complexes from nonantigenic soluble monomeric IgG, which can induce rheumatoid factors, antibodies to the Fc portion of IgG.[168,169] Likewise, in Goodpasture's disease, a configuration change in type IV collagen due to sulfilimine bonds produces a neotarget for pathogenic autoantibodies, a mechanism coined *conformeropathy*.[170]

Another potential mechanism for triggering autoimmunity in susceptible persons is lymphopenia-induced homeostatic expansion of T cells.[171,172] Expansion occurs because of greater availability of survival-promoting cytokines (IL-7 and IL-15) that, when combined with low-affinity engagement of the TCR to self-peptide/MHC, induces low-grade proliferation without full activation. It was therefore hypothesized that the requirement for self-reactivity could, after repeated rounds of lymphopenia, result in the preferential expansion of autoreactive T cells and, consequently, autoimmunity.[171] This hypothesis is supported by the presence of lymphopenia in persons with certain autoimmune diseases, such as SLE and RA, autoimmune manifestations in some primary immunodeficiencies with lymphopenia, and several experimental autoimmune models.

In addition to self-antigens, foreign antigens with sufficient sequence or structural similarity can cross-activate nontolerant self-recognizing T (and B) lymphocytes, a mechanism termed *molecular mimicry*.[173] For T cells, several findings support this possibility: (1) cross-reactivity requires only short peptide lengths of 8 to 15 amino acids; (2) T cell recognition is highly degenerate, depending on only a few key amino acid residues, and it is possible to have mimotopes with no identical amino acids at any position[174,175]; (3) estimation that a single T cell, despite requiring anchor residues to bind the HLA, can react with $10^4$ to more than $10^8$ different peptides[176]; (4) MBP-specific T cells cloned from patients with MS can be stimulated by diverse microbial peptides[177,178]; and (5) infection with a modified Theiler's virus expressing a foreign cross-reactive peptide (*Haemophilus influenzae* protease IV protein that shares only 6/13 amino acids) induced T cells against a myelin protein, proteolipid protein (PLP), resulting in autoimmune CNS disease.[179] To date, however, no compelling evidence exists to connect a specific microbial T cell mimotope to any autoimmune disease.[180,181]

In contrast, fairly good evidence indicates that molecular mimicry affects self-reactive B cells in a few autoimmune diseases. The best examples include cross-reactivity of (1) bacterial adhesin FimH with lysosome-associated membrane protein 2 (LAMP-2) in anti-neutrophil cytoplasmic antibody (ANCA)-positive pauci-immune focal necrotizing glomerulonephritis[182]; (2) group A streptococcal carbohydrate epitope, N-acetyl glucosamine, and M protein with cardiac myosin in rheumatic fever[183]; and (3) *Campylobacter jejuni* lipo-oligosaccharide with ganglioside GM1 on peripheral nerves in the acute motor axonal neuropathy subtype of Guillain-Barré syndrome because of an identical determinant, Gal β1–3 GalNAc β1–4 (NeuAc α2-3) Galβ.[184,185] Overall, however, although molecular mimicry is an attractive hypothesis, supportive evidence for most autoimmune diseases is lacking. Whether this situation is because of

technical issues that impede detection, such as multiple mimotopes from diverse sources, diverse HLA haplotypes, and the considerable plasticity of the T cell receptor, or whether mimicry is an uncommon cause of autoimmune disease remains an open question.

## IMMUNOLOGIC MECHANISMS OF TISSUE INFLAMMATION AND DYSFUNCTION

The same effector mechanisms used by the immune system to neutralize pathogens are exploited in autoimmunity to inflict a wide range of deleterious effects on self-molecules, cells, and tissues. These mechanisms have been broadly grouped into hypersensitivity types II to IV, which encompass antibody-, immune complex–, and T cell–mediated processes, respectively (see Table 19-2).

In type II reactions, pathologic autoantibodies bind to self-antigens primarily located on cell surfaces or in tissues and mediate autoimmune disease by three general mechanisms: (1) altering the function of the target antigen, (2) promoting cell injury or death, and (3) inducing inflammation. Blocking or enhancing self-molecule function represents a special kind of type II hypersensitivity response in which autoantibodies alone are sufficient to effect autoimmune manifestations. Examples include the agonist anti–thyroid-stimulating hormone (TSH) receptor antibodies in Graves' disease that stimulate thyrocyte growth and production of thyroid hormone, the anti-acetylcholine receptor antibodies that block neuromuscular transmission in myasthenia gravis, and anti-β2-glycoprotein I antibodies in anti-phospholipid syndrome that alter the regulation of anticoagulant activity.[186] Direct cell injury or death is mediated by the binding of IgM or IgG to surface antigens, leading to cell lysis directly by complement activation or to phagocytosis via interaction of deposited C3 fragments with CR1 and CR3 receptors. Bound IgG also promotes phagocytosis through its interaction with Fc receptors. Examples include autoimmune hemolytic anemia, idiopathic thrombocytopenia, and autoimmune neutropenia. Finally, antibodies bound to tissue antigens promote inflammation by activating complement, which generates the chemoattractant C5a and leukocyte-activating C3 fragments, and by FcγR binding, which activates immigrating leukocytes such as neutrophils and macrophages, as well as tissue resident mast cells and basophils. These cells produce proinflammatory factors that further expand the inflammatory response by recruiting and then activating additional circulating leukocytes.

Type III responses are caused by abnormal deposition of immune complexes of IgG antibody and soluble antigen in tissues. Such complexes, which also contain bound C3 complement fragments, are normally cleared from the circulation by complement receptors on red blood cells (RBCs) and by complement receptors and FcγRs on mononuclear phagocytes and platelets. However, this clearance can be overwhelmed under certain conditions such as overabundant production or immune complexes composed of excess antigen wherein less antibody coverage reduces complement deposition and aggregation of Fc regions, leading to less efficient clearance. Once deposited in tissues, immune complexes initiate, through complement activation and FcγR binding, the same inflammatory cascades as type II responses. SLE and RA are autoimmune diseases mediated by this mechanism.

Type IV hypersensitivity encompasses cell and tissue injury mediated by activated T cells through their cytolytic activity in the case of CD8 T cells or the production of pro-inflammatory factors primarily by the CD4 subset. Apart from direct evidence of this mechanism in animal models using approaches that are not feasible in human studies, indirect evidence supporting this mechanism includes a higher frequency of autoreactive T cells with effector function in patients with autoimmune diseases, immunopathologic findings similar to T cell–mediated autoimmune models, and inhibition with T cell–blocking agents such as cyclosporin A.[187] T1DM and MS are examples of type IV reactions.

It should be mentioned that these classifications are general, and for some diseases, the underlying effector mechanism may be more complex. In RA, for example, the mechanism responsible for injury or dysfunction probably involves more than one hypersensitivity type,[188,189] whereas in others, such as SLE, different clinical manifestations are mediated by different mechanisms; for example, anti-neuronal antibody-mediated CNS disease is a type II process, whereas glomerulonephritis is a type III process.[190-193] Finally, for diseases such as dermatomyositis and systemic sclerosis, the type of mechanism mediating tissue injury remains to be defined.

## PATHOPHYSIOLOGY OF AUTOIMMUNE RHEUMATIC DISEASES

Although the general principles underlying loss of tolerance and autoimmunity provide a useful conceptual foundation, the actual pathophysiologies suggested by the few better-defined autoimmune diseases are likely to also employ specific and unique mechanisms. Two notable examples of this phenomenon are the pathophysiology of anti-nuclear antibody production in SLE and arthritis in RA. These examples will be briefly discussed in the context of their broader significance to autoimmune diseases.

Recent findings suggest a model of SLE pathophysiology that provides a mechanism explaining why anti-nuclear antibodies are virtually always present in lupus despite substantial genetic and clinical heterogeneity.[49,194] First, autoreactive B cells are activated when self-reactive BCRs bind to nucleosomes or ribonucleoprotein (RNP) and internalize them into the endolysosome compartment, where the released nucleic acids engage TLR7 and TLR9 and provide a second signal. Such activated B cells act as potent APCs for T lymphocytes and after class-switch recombination produce IgG autoantibodies. Next, immune complexes form by the binding of these autoantibodies to material containing nucleic acid activate plasmacytoid DCs (pDCs) and DCs after their internalization via FcγRIIA (FcγRIII in mice) and then engagement of TLRs by nucleic acids. Elaboration of lupus-promoting cytokines such as type I IFN and BAFF by pDCs and DCs, as well as enhanced antigen presentation, are thought to further drive loss of tolerance, activation of autoreactive B cells, and autoantibody production, thereby resulting in an amplification loop. Thus, in persons susceptible to lupus, confinement of nucleic acid–binding TLRs to endolysomes is not enough of a barrier to

block their activation by normally innocuous amounts of self-nucleic acids. This mechanism provides an explanation for the high prevalence of autoantibodies in SLE that either bind to nucleic acids or antigenic cargoes containing nucleic acids, such as DNA, nucleosomes, RNP, myeloperoxidase (ANCA), and even RBCs, or that exhibit cross-reactivity to such antibodies. Importantly, this explanation suggests the possibility that other autoimmune diseases might also be mediated by specific PRRs.

The pathophysiology of RA also involves common and specific pathways that together promote inflammatory synovitis in roughly three phases,[195-199] which is best described in the HLA-DR1*401 (DR4) anti-citrullinated protein antibody $(ACPA)^+$ subset of patients with RA, who have greater disease severity. The initial pre-clinical phase that can occur years before the onset of arthritis is postulated to involve the activation of T cells and B cells at extra-articular inflammatory sites where CCPs are produced in part by the release of peptidylarginine deiminase from apoptosing granulocytes and monocytes. The HLA-DRB1 shared epitope, which has been shown to specifically bind and present citrullinated self-peptides, is thought to predispose to this type of response. Targets for ACPA include fibrinogen, vimentin, α-enolase, and others, and these antibodies are thought to play a major role in disease pathogenesis, although the mechanism remains uncertain. The specific triggers of the ACPA response are also not firmly established, but evidence strongly suggests that inflammation at mucosal sites caused by environmental factors such as smoking, silicosis, periodontal disease, and the gut microbiome are responsible for initiating a limited ACPA response that expands in scope and consistency with clinical progression.

The next early arthritis phase is characterized by localization of the immune response and inflammation to joints. The resulting immune complexes and activated T cells lead to further stimulation of macrophages, synovial fibroblasts, endothelial cells, mast cells, and osteoclasts, and the eventual production of pro-inflammatory factors such as TNF, IL-1, IFN-γ, IL-17A, IL-23, chemokines, matrix metalloproteinases, osteopontin, and many others that contribute to synovitis, pannus formation, bone erosion, and cartilage destruction. This activity leads to a predominantly inflammatory and tissue-damaging phase largely mediated by activated fibroblast-like synoviocytes, which produce a wide array of pro-inflammatory mediators that promote recruitment and activation of circulating and resident immune cells.[200] It has also been suggested that the spread of arthritis to unaffected joints could, in fact, be mediated by transmigration of these activated fibroblast-like synovial cells.[201] The pathophysiology of the ACPA subset of RA provides an additional example of the collaboration of the innate and adaptive arms of the immune system in autoimmune disease, but it also has two unique features; first, the major autoantigen, citrullinated protein, is a neoantigen specifically targeted in this disease, and second, tissue damage is to a large extent mediated by fibroblast-like synoviocytes.

## GENETICS OF AUTOIMMUNE DISEASES

During the past two decades, substantial insight into the genetic landscape responsible for autoimmune disease susceptibility has emerged from both human and animal studies. This progress was greatly facilitated by the availability of genomic sequences, improved definition of genetic variations and haplotypes among human populations, consortia with collections of patients and control subjects in the thousands, and numerous major technical and analytical advances.[202,203] In particular, genetic studies have progressed from testing a few specific candidate polymorphisms to genome-wide family analyses of hundreds of cases, and even larger scale genome-wide association studies (GWAS) involving thousands,[204,205] making it possible to not only verify known genetic associations but also to capture common disease-predisposing variants with modest effects. Combined, these approaches have identified more than 100 candidate genes or loci in SLE and RA and in other rheumatologic diseases such as systemic sclerosis, Kawasaki's disease, Behçet's disease, spondylitis, and ANCA-associated vasculitis (reviewed in references 206-219; http://www.genome.gov/gwastudies).[206-219] These candidates span the gamut of innate and adaptive immune systems but also include some loci with genes of unknown immunologic function. For example, in SLE—one of the best defined at the genetic level of any rheumatic disease—candidate genes are involved in antigen presentation (HLA-DR3), B and T cell receptor signaling (*PTPN22*, *BANK1*, and *BLK*), CD4 T helper cell regulation (OX40L or *TNFSF4*), T cell–mediated regulation (*PDCD1*), cytokine signaling (*STAT4*), interferon and TLR7/9 signaling (*IRF5*, *TNFAIP3*, *IRAK1*, *IRF7*, and *TYK2*), Fc receptor function, one of which has been implicated in the transport of immune complexes containing nucleic acid to TLR7/9-containing endosomes (*FCGR2A*), neutrophil function (*ITGAM*), clearance of self-antigens (*C1Q*, *C2*, and *C4*), clearance of intracytoplasmic DNA (*TREX1*), and also several loci containing genes with no known connection to the immune system or lupus. Together, these candidates provide clues to specific pathways involved in SLE, which indeed has dovetailed well with genetic studies in mice.[10]

From GWAS and other studies, including those in animal models, several general conclusions can be made about genetic susceptibility in the more common autoimmune diseases. First, autoimmune diseases are associated with a large number of susceptibility genes that have been shown to have an impact on a wide range of immunologic, cellular, and end-organ functions in ways that enhance, modify, or even suppress relevant pathophysiologic processes.

Second, considerable genetic heterogeneity exists at both the individual and population levels regardless of whether the phenotype is relatively uniform, as in RA, or diverse, as in SLE. Thus, although a large number of predisposing genes exist, having only a subset of these genes is sufficient for disease development. Whether this heterogeneity is caused by variants affecting a few common pathways or numerous unique pathways remains unclear.

Third, the vast majority of candidate genes or loci have only modest effect sizes with most odds ratios less than 1.5, although in some diseases, notably SLE, a few rare variants such as *C1Q* deficiency associated with a greater than 90% incidence of SLE and *TREX1* mutations leading to chilblain lupus have been identified that are highly penetrant. Another salient finding derived from GWAS analysis is

that, for most autoimmune diseases, HLA alleles consistently have the highest or among the highest effect sizes. This finding accords well with the central role of antigen presentation and T cells in directing the adaptive immune response to specific antigens. Overall, however, for most candidate variations, defining the mechanism and proving a role in autoimmune disease will be hampered by their low effect sizes.

Fourth, some of the variant genes and loci are shared among autoimmune diseases, suggesting common underlying mechanisms.[220] Noteworthy examples are the association of *PTPN22* with a wide range of autoimmune diseases, including T1DM, RA, SLE, juvenile idiopathic arthritis (JIA), Graves' disease, systemic sclerosis, myasthenia gravis, generalized vitiligo, and granulomatosis with polyangiitis, but not MS,[221] *STAT4* with RA, SLE, systemic sclerosis, and Sjögren's syndrome.[222,223] This finding supports a role for such broadly predisposing genetic factors in the known occurrence of multiple different autoimmune diseases in some families.

Fifth, common single nucleotide polymorphism-defined variants account for only a portion of overall heritability in autoimmune diseases, that is, approximately 20% to 60%,[202,203,224] of which the HLA region typically accounts for a substantial part. Several reasons for the missing heritability have been suggested: (1) failed detection because of inadequate single-nucleotide polymorphism (SNP) coverage or the presence of disease-promoting non-SNP genomic variations such as copy number variants; (2) a large number of common variants with modest to marginal effects (odds ratio, <1.1 to 1.2) that are undetectable despite large study sizes given that the statistical power is reduced by both smaller effect size and lower variant frequency in the population; and (3) uncommon variants (1% to 5%) or rare disease-associated risk alleles (<1% frequency). Illustrating the hurdles that must be overcome to identify these rare variants on a genomewide scale, it was estimated that 25,000 cases plus a replication set would be required for a well-powered study.[225] Any of these possibilities present significant challenges to further defining genetic susceptibility. It should also be noted that most common SNP variants associated with diseases do not affect coding regions, making it difficult to determine if the SNP variant is the actual change associated with disease and to decipher its effects on gene function and autoimmunity, although new genomic approaches are being developed to tackle this challenge.

Finally, information gleaned from genetic studies to date has not been useful for identifying persons at risk, and it is thought that this situation will remain so because of the small effect sizes and relatively high frequency of known risk alleles combined with incomplete coverage of heritability.[203] Nevertheless, these data have helped define disease-relevant pathways and within that context have provided clues and support for potential therapeutic targets.

## GENDER AND AUTOIMMUNITY

A significant female gender bias in autoimmunity was identified early on and was considered an important clue to disease pathogenesis.[226] The strength of this predilection, however, varies among autoimmune disorders, with female prevalences in the 80% to 95% range for thyroiditis, SLE, Sjögren's syndrome, and anti-phospholipid syndrome; in the 60% to 75% range for RA, scleroderma, myasthenia gravis, and MS; and close to 50% for T1DM and autoimmune myocarditis.[227] Why some autoimmune diseases are affected more by gender than others is not known, but the lack of common or distinguishing characteristics in diseases with higher female predominance suggests the possibility of multiple mechanisms.

Indeed, to differing extents depending on the disease, both sex hormones and sex chromosomes have been implicated in this dichotomy.[228,229] In addition, fetal-maternal chimerism has been suggested as another factor, but this possibility remains controversial.[230] In terms of sex hormones, substantial evidence from in vitro, animal, and clinical studies supports a role for both female and male sex steroids in modifying the incidence and severity of autoimmunity. For example, in persons with SLE, both estrogens and prolactin have been shown to exacerbate disease. In animal studies, they promote loss of tolerance and expansion of B cell populations, which, for estrogens, was also shown to be associated with enhanced survival and greater expression of B cell lymphoma 2 (Bcl-2).[231] Importantly, studies of lupus-prone mice involving combinations of castration and hormone replacement convincingly demonstrated a disease-promoting effect of female hormones on spontaneous systemic autoimmunity.[232]

Less extensive evidence also supports a role for sex chromosomes in autoimmune disease susceptibility. Persons with Klinefelter's syndrome (XXY) have a higher than expected incidence of SLE, whereas there is contrastingly suggestive under-representation in Turner's syndrome (XO).[233,234] Furthermore, using genetic and gonad manipulation in mice that allowed direct comparison of the effects of one or two X chromosomes on autoimmunity, susceptibility to both EAE in SJL mice and TMPD-induced lupus was shown to be more severe in mice with XX.[235,236] Thus it appears that both hormonal and chromosomal influences play roles in female predilection to autoimmunity.

## MICROBIAL AND OTHER ENVIRONMENTAL TRIGGERS

Considerable evidence indicates that environmental factors can exert varying degrees of influence on the development of autoimmune diseases. A clear causal effect has been documented for a few disorders, whereas the low concordance rate (20% to 50%) among monozygotic twins in common autoimmune diseases supports the presence of a significant environmental component. Nevertheless, the specific environmental factors and the extent to which they contribute to disease induction and exacerbation remain largely unknown for most autoimmune disorders. The reasons for this lack of knowledge are likely manifold and, depending on the disease, may include unsuspected or unknown environmental culprits, multiple independent factors, additive factors contributing to cumulatively small portions of the overall environmental effect, a highly variable or prolonged interval between exposure and disease onset, and a low incidence of autoimmune disease after exposure. Moreover, another general impediment is the difficulty of proving causation with epidemiologic data. Because of these difficulties, animal studies have been invaluable in allowing controlled

experimentation, and much of what we understand mechanistically has come through this approach.

Despite the aforementioned limitations, a broad range of environmental factors have been implicated, albeit with varying levels of confidence.[237] Notably, the most prominent are infection and exposure to microbial products, which epidemiologic and animal models suggest can either enhance or inhibit autoimmunity depending on the type of exposure and disease.[238,239] Rheumatologic examples include Reiter's syndrome after certain enteric infections, chronic Lyme arthritis resulting from *Borrelia burgdorferi*, and the association of oral *Porphyromonas gingivalis* that expresses a peptidylarginine deiminase that can citrullinate proteins with RA. Several mechanisms by which microbes are postulated to induce or exacerbate autoimmunity include (1) molecular mimicry,[183] (2) bystander activation of autoreactive T cells by pathogen-activated antigen-presenting cells,[240] (3) inflammation of the target tissue and the release of immunologically hidden self-antigens,[241] and (4) production of disease-promoting cytokines, such as IFN-α.[49] Inhibition of autoimmune diseases by pathogens was initially suggested by epidemiologic evidence in persons with T1DM and MS consistent with the "hygiene hypothesis"—that is, a lower incidence of infection is responsible for an increasing incidence of allergic disease in Western countries.[242] This concept has since been supported by additional epidemiologic and experimental data, although the microbial pathogens, type of exposure, and mechanisms remain uncertain. Studies of T1DM-prone NOD mice, which worsen in clean conditions and improve with exposure to microbial products, showed that alterations in gut microbiota, which can interact with and modulate the host immune and inflammatory apparatus, could account for a significant portion of the environmental effects and even influenced the female gender bias.[243-246] Many of these mechanisms do not require a specific pathogen but can be mediated by a wide spectrum of organisms. Finally, in some animal models, autoimmune disease develops under germ-free conditions, thereby excluding an absolute requirement for microbial environment or infection. In this case, one would infer that the trigger is provided by self, most likely cell damage–derived products. Examples include T1DM in NOD mice and BB rats, APECED in *Aire*-deficient mice, IPEX in the presence of Foxp3 deficiency, and lupus in MRL-lpr mice.[247-251] Interestingly, however, in MRL-*lpr* mice, the addition of a filtered diet containing fewer pathogen-associated molecular patterns (PAMPs) reduces disease severity. This finding suggests that microbial products at least partially affect disease susceptibility in this model and points to the difficulty of completely excluding a role for microbial organisms.

In terms of the other nonmicrobial environmental factors, some of the general types include (1) drugs such as procainamide, gold salts, and interferons that typically cause mild autoimmunity that resolves after discontinuation; (2) trauma, exemplified by sympathetic ophthalmia caused by penetrating injury to the globe; and (3) diverse environmental agents such as adulterated oil (which causes toxic oil syndrome), ultraviolet radiation (which exacerbates systemic autoimmunity), iodine (which causes autoimmune thyroiditis), silica (which causes RA, SLE, and systemic sclerosis), and tobacco smoke (which causes $ACPA^+$ $HLA\text{-}DR4^+$ RA).[164,237]

## CONCLUSION

Since 1904, when Donath and Landsteiner reported the first evidence of self-reactivity in paroxysmal hemoglobinuria,[252] the field of autoimmunity has progressed tremendously at both the clinical and basic science levels. Notably, recent progress since the last edition of this book has made it possible to define with greater detail and confidence the interrelationships between genetic susceptibility, environment, and pathophysiology, and indeed, novel pathways have been identified that link cause with immunopathology. Advances have continued to broaden appreciation for the central role of the innate response in initiating autoimmunity and in determining the type of adaptive autoimmune response and type of autoimmune disease. Large genome-wide association projects have progressed to identifying and characterizing causative variants. Advances in the understanding of disease pathogenesis primarily in more common rheumatologic diseases have further solidified and extended existing frameworks from which researchers can build to identify critical pathways and molecules. Of importance to patient care has been the successful bench-to-bedside translation approach that has resulted in the continued advancement of therapeutic targeting of immune-related molecules to the clinic. Despite this progress, many important facets of autoimmunity are unresolved, and patient care remains suboptimal. Importantly, the highly sought-after objective of specifically re-establishing tolerance in patients has remained elusive despite considerable effort,[253] and current, more broadly immunosuppressing therapies are only partially effective and are associated with infection and cancer risks. Accordingly, there continues to be a critical need to investigate the basic processes involved in autoimmune diseases and to develop new and more effective clinical management.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 20



# Metabolic Regulation of Immunity

Ruoning Wang • Tingting Wang • Stephen Tait

**KEY POINTS**

- Immune signaling drives metabolic reprogramming in both innate and adaptive immune cells.
- The metabolic shift renders immune cells highly dependent on certain metabolic pathways.
- Mitochondria serve as signaling hubs for directing innate and adaptive immune responses.
- The availability of extra-cellular metabolites mediates the intercellular metabolic cross-talk that affects the immune response.

## OVERVIEW

The evolution of vertebrate immunity has culminated in an effective and complex chain reaction, which is composed of a rapid activation of specialized innate immune cells—largely neutrophils, macrophages, and dendritic cells (DCs)—followed by the proliferative burst and functional polarization of adaptive immune cells, that is, B and T cells. Because the invading pathogens of vertebrates often rapidly reproduce and spread in their host, an effective host-mediated immune response must be fast and energy intensive. As we discuss in this chapter, an immune signaling–driven metabolic reprogramming in both innate and adaptive immune cells may be essential for their activation, proliferation, and polarization and for the subsequent functional events elicited by these cells (Figure 20-1).

## METABOLIC REWIRING IN INNATE IMMUNITY

### Macrophage Metabolism

Macrophages, together with DCs, are considered first-line effectors of innate immunity. Based on their specific functional activities after pathogen or cytokine stimulation, macrophages can be largely defined as two different subtypes: the classically activated macrophage subset (M1) and the alternatively activated macrophage phenotype (M2). The classic activation of macrophages is often induced by a combination of bacterial product lipopolysaccharide (LPS) and cytokines such as interferon-γ (IFN-γ), whereas the polarization of macrophages via the alternative activation program is triggered by exposure to the cytokine interleukin (IL)-4 or IL-13.[1] M1 macrophages produce nitric oxide (NO), a product of inducible nitric oxide synthase (iNOS)–mediated breakdown of arginine, reactive oxygen species (ROS), and pro-inflammatory cytokines, including tumor necrosis factor (TNF), IL-1β, IL-6, and IL-12, thus mounting a rapid, effective response against highly proliferative intra-cellular pathogens. In contrast, M2 phenotype macrophages produce high levels of IL-10 and IL-1 receptor antagonist (IL-1ra), meanwhile shifting arginine catabolism from iNOS-mediated production of NO to an arginase I (Arg I)–mediated breakdown of arginine into urea and ornithine, thus functioning in anti-parasitic responses, promoting tissue healing, and, in general, dampening inflammation.[2-4] Therefore, M1 macrophages promote inflammation, whereas M2 macrophages suppress inflammation and promote tissue repair. In addition to the differential engagement of an iNOS-dependent or Arg I–dependent arginine catabolic program, the differences of M1 and M2 macrophages are also reflected in their other metabolic profiles. Whereas the inflammatory M1 macrophages predominantly engage glycolysis and the pentose phosphate shunt (PPP), the anti-inflammatory M2 macrophages actively engage lipid oxidation.[1,5]

Heightened glycolysis is required for adenosine triphosphate (ATP) generation in M1 macrophages and also provides many glycolytic intermediate metabolites, which are direct precursors for lipid and amino acid biosynthesis. Whereas newly synthesized lipids are involved in the dramatic intra-cellular membrane reorganization after pathogen invasion, both lipids and amino acids are required for the production and secretion of pro-inflammatory cytokines.[6-9] Meanwhile, the PPP pathway provides nicotinamide adenine dinucleotide phosphate (NADPH), which functions in maintaining reduced glutathione and limiting oxidative stress in M1 macrophages.[10-12] Enhanced glycolysis and PPP in M1 macrophages also results in glucose depletion and an acidified microenvironment, resulting in a hostile environment that may suppress pathogen proliferation.[13] It has been demonstrated that the metabolic reprogramming in M1 macrophages is tightly coordinated by transcriptional and post-translational regulation of metabolic enzymes. LPS stimulation in M1 macrophages leads to a transcriptional induction of glycolytic enzymes such as phosphoglycerate kinase (PGK), the glucose transporter-1 (GLUT-1), and ubiquitous 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase (uPFK2) to promote glycolysis, meanwhile suppressing the expression of mitochondrial enzymes.[14,15] In addition, LPS stimulation results in a robust NO production, which not only plays an indispensable role in destroying invading microorganisms but also results in a suppression of mitochondrial oxidative phosphorylation, likely via S-nitrosylation of mitochondrial metabolic

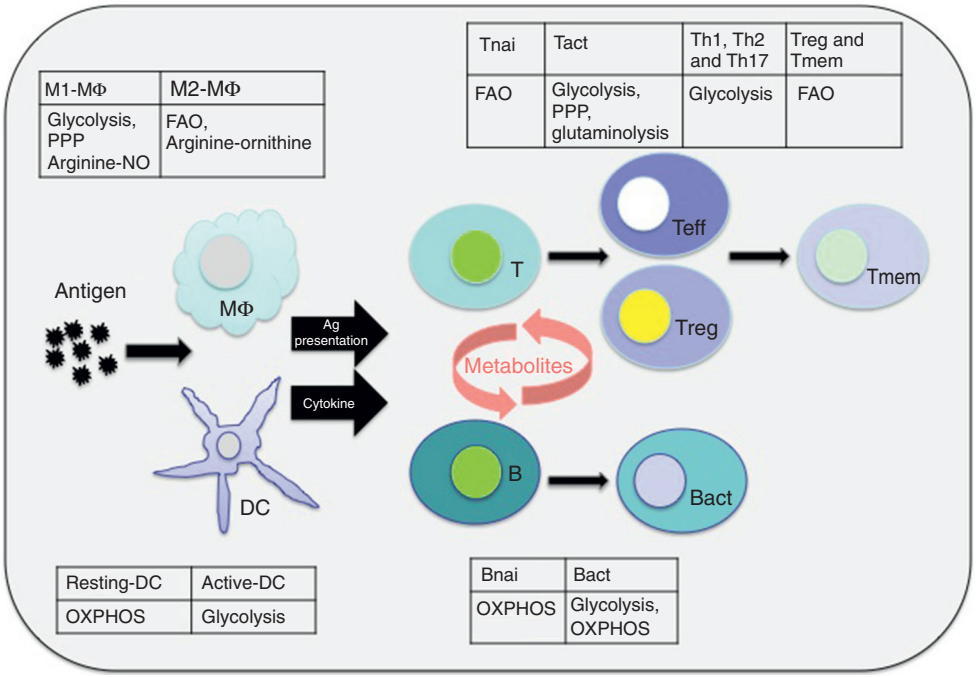



**Figure 20-1** Metabolic reprogramming and interplay in immunity. Resting dendritic cells (DCs) mainly rely on the mitochondrial oxidative phosphorylation (OXPHOS) metabolic pathway, whereas active DCs rapidly switch from OXPHOS to glycolysis after activation. M1 macrophages (MΦ) engage glycolysis, pentose phosphate shunt (PPP), and inducible nitric oxide synthase (iNOS)–mediated arginine catabolism that generates nitric oxide (NO), whereas M2 MΦ predominantly rely on fatty acid oxidation (FAO) and convert arginine to ornithine and urea. Upon antigen and cytokine stimulation, naïve T (Tnai) cells become active and undergo a metabolic reprogramming from FAO to glycolysis, PPP, and glutaminolysis. Active T (Tact) cells then differentiate into effector T (Teff) cells with heightened glycolysis, as well as regulatory T (Treg) and memory T (Tmem) cells that depend on FAO. Unlike T cells, naïve B (Bnai) cells rely on OXPHOS, whereas active B (Bact) cells display a balanced increase in glycolysis and OXPHOS. Beyond this, the different immune populations may interplay with each other through competing nutrients or formation of metabolic symbiosis.

enzymes.[16,17] The transcriptional factor hypoxia-inducible factor-1α (HIF-1α) has also been implicated in the regulation of glycolysis, in addition to its role in promoting the transcription of proangiogenic factors and pro-inflammatory cytokines in macrophages.[15,18] On the other hand, a recent study found that a rapid downregulation of carbohydrate kinase–like protein (CARKL) after LPS stimulation is required for shunting glucose catabolism into the oxidative arm of the PPP pathway in M1 macrophages. It was suggested that CARKL possesses the same catalytic activity as the sedoheptulose kinase, which promotes metabolic flux through a nonoxidative arm and consequentially reduces metabolic flux through the oxidative arm of the PPP. The nonoxidative arm of the PPP is designed to generate ribose-5-phosphate (R5P), whereas the oxidative arm of the PPP produces NADPH, thus modulating redox balance. Intriguingly, M2 macrophages display higher CARKL expression than do M1 macrophages, implicating CARKL as a rheostat for coordinating macrophage metabolic routes and functional polarizations.[19] Beyond this, IL-4, an M2 macrophage stimulator, dramatically induces the signal transducer and activator of transcription 6 (STAT6), which promotes the transcription of Arg1, thus shifting arginine catabolism from iNOS-mediated production of NO to the production of urea and ornithine.[20] STAT6 also cooperates with peroxisome proliferator-activated receptor γ (PPARγ)-coactivator-1β (PGC-1β) to induce the expression of genes involved in fatty acid oxidation (FAO) and mitochondrial biogenesis in M2 macrophages.[21]

## Dendritic Cell Metabolism

DCs, which are key players in immunity and tolerance, play a crucial role in driving T cell activation and differentiation via antigen presentation and production of cytokines. DCs can be divided into several subsets, including conventional DCs (cDCs), inflammatory DCs (infDCs), and plasmacytoid DCs (pDCs).[22-24] Resting DCs, which are largely immature and poorly immunogenic, mainly rely on the mitochondrial oxidative phosphorylation (OXPHOS) metabolic pathway. Upon the stimulation of pathogen-derived toll-like receptor (TLR) ligands, DCs become active and immunogenic and undergo a rapid metabolic switch from OXPHOS to glycolysis to fulfill their bioenergetic needs.[25-27] In addition, many intermediate metabolites of the glycolytic pathway provide a carbon source for amino acid biosynthesis and de novo fatty acid synthesis, the latter of which is required for the synthesis of endoplasmic reticulum (ER) and Golgi membranes to promote the synthesis, transportation, and secretion of proteins associated with DC activation and maturation.[25,28] Mechanistically, the engagement of TLR ligands leads to the activation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, which may directly promote GLUT membrane translocation and enhance glucose uptake in DCs.[25,29,30] As an essential downstream signaling node of the PI3K/Akt signaling pathway, the mammalian target of rapamycin (mTOR) has also been implicated in modulating metabolic programs in both cDCs and infDCs.[25,29,31] The activation of

mTOR results in the expression and stabilization of transcription factor HIF-1α, which regulates the expression of genes involved in glycolysis. The transcription factor sterol-regulatory element binding protein (SREBP) is another downstream target of mTOR and is responsible for controlling genes relevant in lipid synthesis.[32-34] Similar to M1 macrophages, an iNOS-derived NO may play a critical role in suppressing mitochondrial OXPHOS, thus promoting the metabolic shift from mitochondrial-dependent metabolism to glycolysis in DCs. Other reports also suggested that the autocrine production of type I IFN is required for the metabolic switch in cDCs.[26,27,35] Whereas the engagement of glycolysis is required for TLR-induced DC activation and immunogenicity, mitochondrial OXPHOS is likely involved in DC-mediated immune tolerance. Consistent with findings in other immune cells, recent studies indicated that adenosine monophosphate (AMP)–activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α form a signaling axis to coordinately regulate mitochondrial biogenesis, OXPHOS, and other catabolic metabolisms in DCs, thus favoring the acquisition of tolerogenic DCs.[25,36-39]

## METABOLIC REWIRING IN ADAPTIVE IMMUNITY

### T Cell Metabolism

As an essential component of adaptive immunity, T cells can recognize foreign antigen and rapidly transit from a quiescent to an active state that is concomitant with cell growth (increase of cell size) and proliferation. Subsequently, activated and proliferating T cells can differentiate into various functional subsets, which are determined by the nature of antigen stimulation and the surrounding cytokine milieu. Subsequent to the peak of T cell expansion and antigen clearance, the vast majority of T cells will die by programmed cell death (apoptosis) during a phase of contraction. The remaining population returns to a quiescent state and gives rise to the memory subset, which responds more quickly and effectively upon encountering with the same pathogen. To fulfill their bioenergetic and biosynthetic demands coupled with various functional stages, T cells actively engage distinct signaling pathways and transcriptional modulators to alter their metabolic programs accordingly.

#### T Cell Activation

Upon the engagement of antigen and co-stimulatory molecules, resting T cells undergo a rapid growth and proliferation process. Concomitant with this process, and as a result of activation signaling, T cells reprogram their metabolic profile, shifting from FAO to robust aerobic glycolysis, PPP, and glutaminolysis. Naïve T cells rely on OXPHOS, generating energy to meet the basic needs for cell function and survival. Heightened aerobic glycolysis and glutaminolysis in activated T cells not only support ATP generation but also provide biosynthetic intermediates as building blocks of amino acids, nucleotides, and lipids to fulfill the biosynthetic demands of rapid cell growth and proliferation. In addition, glutaminolysis and glycolysis in active T cells provide carbon and nitrogen for other growth and proliferation-associated biosynthetic pathways, such as hexosamine and polyamine biosynthesis. Shunting of glucose into the PPP pathway results in the production of R5P and NADPH. Whereas R5P is a precursor for ribonucleotide biosynthesis, NADPH determines cellular redox balance and coordinates to FFA and cholesterol biosynthesis through the provision of reducing equivalents.[40-43]

The rewiring of metabolic pathways upon T cell activation is coordinately regulated by several signaling pathways, including mitogen-activated protein kinase (MAPK)/extra-cellular signal-regulated kinase (ERK) and PI3K/Akt/mTOR cascades.[40,44] The activation of Akt signaling promotes the expression and cell surface trafficking of GLUT-1 to the cell surface, facilitating glucose uptake.[45,46] On the other hand, ERK signaling promotes glutamine uptake via modulation of sodium-dependent neutral amino acid transporter-2 (SNAT2) expression and cell membrane trafficking.[47] Beyond the regulation of glucose and glutamine uptake, T cell activation signaling drives a global metabolo-transcriptome, including most of the key metabolic enzymes involved in the aforementioned catabolic and biosynthetic pathways. Interrogation of the promoters of these genes and subsequent genetic modulation of candidate transcriptional factors in T cells revealed that the proto-oncogene Myc is required in T cell activation–driven glucose and glutamine catabolism.[40,44] Meanwhile, metabolic genes involved in lipid metabolism and de novo cholesterol biosynthesis and transport are under the dynamic regulation of transcriptional factors, nuclear receptor liver X receptor (LXR), and the orphan steroid receptor estrogen receptor-related α (ERRα).[48-50]

#### T Cell Differentiation

After a rapid initial growth phase, T cells enter a proliferation phase and subsequently differentiate into various phenotypic and functional subtypes. In response to the distinct antigen challenge and extra-cellular cytokine signal, activated $CD4^+$ T cells differentiate into immune suppressive regulatory T cells (Tregs) or inflammatory T effector cells, such as T helper (Th)1, Th2, and Th17 (see Chapter 12). Th1 cells mediate responses to intra-cellular pathogens. Th2 cells control responses to extra-cellular bacteria and helminthes. Th17 cells are important in anti-fungal defense and inflammation.[51-53] Despite their distinct functions in immunity, Th1, Th2, and Th17 cells all sustain heightened glycolysis, whereas Treg cells show decreased glycolysis.[49,54] Although the regulatory mechanism of elevated glycolysis in Th1 and Th2 cells remains unclear, HIF-1α is indispensable for driving Th17 differentiation and sustaining elevated glycolysis during Th17 differentiation.[49,54] Consistent with the crucial role of mTOR in regulating T effector development,[55,56] the expression of HIF-1α is dependent on the function of mTOR during Th17 differentiation. Whereas heightened glycolysis is necessary for Th17 differentiation and function,[54] HIF-1α appears to also directly regulate Th17 differentiation, at least in part through direct transcriptional activation of the Th17 master transcription factor, RAR-related orphan receptor γ (RORγt), thereby enhancing Th17 differentiation.[57] On the other hand, HIF-1α suppresses Treg differentiation, partially by antagonizing

forkhead box protein 3 (Foxp3), a master transcriptional factor for Treg differentiation.[57] Treg cells act as immunologic suppressors by dampening T cell activation and inflammatory response. In contrast to other Th cells that actively engage glycolytic programs, Treg cells exhibit a reliance on mitochondrial-dependent oxidation of lipids for energy production.[49] Consistent with this mechanism, exogenous fatty acid supplementation inhibits Th1, Th2, and Th17 differentiation while modestly enhancing Treg differentiation.[49] In addition, butyrate, a commensal microbe-derived short-chain fatty acid, preferentially induces Treg differentiation. However, this effect may be due to the inhibition of histone deacetylase activity by butyrate.[58,59] Similar to CD4 T cells, activated CD8 T cells also switch from FAO to aerobic glycolysis and sustain elevated glycolysis and anabolic metabolism for CD8 T cell growth and differentiation into cytotoxic T cells.[60] After the peak of cell proliferation and differentiation, the metabolic profile of T cells shifts from glycolysis back to FAO, partially as a result of decreased mTOR signaling.[61-63] Therefore, the metabolic switch is postulated to be required for the generation of memory CD8 T cells. Taken together, T cell activation and differentiation are tightly coupled with metabolic reprogramming.

### B Cell Metabolism

B cells, which produce antibodies against pathogens, represent another critical component in adaptive immunity. Whereas T cells rapidly engage robust glycolytic programs upon antigen stimulation, B cells display a balanced increase in aerobic glycolysis and mitochondrial glucose oxidation after the engagement of the B cell antigen receptor (BCR) or LPS-mediated activation of TLR signaling. This balanced increase is likely because of a proportionally upregulated GLUT and mitochondrial mass.[64,65] Accumulating evidence has indicated that the engagement of glycolysis is tightly regulated by intra-cellular signal transduction pathways after B cell activation and is required for B cell proliferation and antibody production. It has been reported that the PI3K/Akt pathway is indispensable for glucose uptake and utilization, because the activation of Akt is sufficient to increase glucose metabolism in B cells.[66-68] Differentiation of B cells into immunoglobulin-secreting plasma cells is accompanied by the expansion of the intra-cellular membrane network, where immunoglobulin is produced and secreted. Such endomembrane network expansion requires the engagement of de novo lipogenesis. The PI3K/Akt signaling pathway is required to activate ATP-citrate lyase (ACLY), a key enzyme that channels the carbon of glucose to lipids through conversion of citrate to cytosolic acetyl-coenzyme A (CoA).[69,70] In addition, IL-4 has been shown to promote B cell survival by regulating glucose metabolism via the Janus kinase 1/3 (JAK1/3)-STAT6 signaling pathway.[65] Poly (adenosine diphosphate [ADP]-ribose) polymerase 14 (PARP14), an ADP ribosyltransferase, may represent another important downstream effector of IL-4 signaling, interplaying with STAT6 and modulating glycolysis in B cells.[71] Although HIF-1α is required for regulating glycolysis in B cell development in bone marrow,[72] it is dispensable for driving glycolysis in active B cells. Rather, an Myc-dependent upregulation of GLUT1 and likely other glycolytic genes are required for engaging glycolysis after B cell activation.[64]

## MITOCHONDRIA AND IMMUNITY

It has recently become apparent that mitochondria play multiple, key roles in immunity. In addition to their critical biosynthetic function, mitochondria are intimately involved in immunity, where they serve as both initiators and transducers of various signaling cascades. Immunity can be divided into innate, pre-existing, or acquired, such that it develops after pathogenic challenge. Direct signaling roles for mitochondria have been best described in the context of innate immunity. Innate immune cells including macrophages or DCs detect infectious pathogens or damaged cells through pathogen recognition receptors (PRRs).[73] PRRs recognize conserved molecular patterns shared by microorganisms (pathogen-associated molecular patterns [PAMPs]) and damaged cells (damage-associated molecular patterns [DAMPs]). PRR pathway activation leads to the production of various pro-inflammatory and anti-microbial cytokines, including type I IFNs and IL-1. Through their action, these cytokines simultaneously create an anti-microbial environment and stimulate the development of adaptive immunity against the invading pathogen. As we will now discuss, mitochondria regulate multiple aspects of innate immune signaling, where they serve as both initiators and effectors of PRR signaling. We will review the roles of mitochondria in transducing signals elicited by three separate PRR families: retinoic acid inducible gene (RIG-I)–like receptors (RLRs), TLRs, and nuclear oligomerization domain (NOD)–like receptors (NLRs).

### Mitochondria and NOD-Like Receptor Signaling

Many PAMPs and DAMPs activate cytoplasmic complexes called *inflammasomes*.[74] After activation, inflammasomes activate the protease caspase-1, which, in turn, cleaves various pro-inflammatory cytokines, leading to their maturation and cellular release. The best-described cytoplasmic NLR is NLRP3. NLRP3 inflammasomes recruit and activate caspase-1 via the adaptor protein ASC. Various roles for mitochondria in promoting NLRP3-activation have been proposed; for example, recent data implicate the mitochondrial protein MAVS in NLRP3 inflammasome activation. MAVS is a key player in anti-viral immunity engaged by RLR, however, it has also recently been shown to interact with NLRP3, leading to NLRP3 mitochondrial recruitment, thus promoting NLRP3-inflammasome assembly and activation.[75,76] Nevertheless, although it facilitates NLRP3 activation, MAVS is not essential because MAVS-deficient cells retain NRLP3 activity.[77] In a separate study, microtubule-dependent transport of mitochondria to the endoplasmic reticulum (ER) promotes NLRP3-inflammasome activation by bringing together the key inflammasome-adaptor molecule, ASC (present on mitochondria) with NLRP3 (present on the ER).[78] Supporting this model, previous work has shown that active NLRP3 inflammasomes reside at mitochondrial-ER contacts.[79] Interestingly, mitochondrial transport depends upon acetylated tubulin, suggesting that mitochondrial metabolism (producing the acetyl-CoA

required for acetylation) may also regulate inflammasome activity. Why would recruitment of NLRP3 to the mitochondria, either via MAVS or ASC, facilitate inflammasome activation? Potentially, mitochondria simply act as a physical scaffold that promotes inflammasome assembly. Alternatively, mitochondria may actively participate in inflammasome activation. Along these lines, different studies have shown that mitochondrial ROS promote NLRP3 activity.[79,80] Various DAMPs and PAMPs trigger mitochondrial ROS, and blocking ROS production (using ROS scavengers) can effectively block NLRP3-inflammasome activation.[79] It is not clear how ROS promotes NLRP3-inflammasome activation; similarly, it is not known how such diverse stimuli give rise to ROS. Besides mitochondrial ROS, other mitochondrial molecules such as cardiolipin and mitochondrial DNA (mtDNA) have been proposed to facilitate NLRP3 activation.[80,81] As we have discussed, there are numerous means by which mitochondria may promote NLRP3 activity; nevertheless, these means remain controversial. Whether mitochondria are required for NLRP3-activation is a question that may be readily addressed using a recently described method to generate mitochondria-deficient cells.[82]

## Mitochondria and RIG-I-Like Signaling

RLRs are the primary means by which cytoplasmic viral double-stranded RNA (dsRNA) is detected. RLR signaling ultimately leads to the production of type-1 IFN and pro-inflammatory cytokines, thereby inhibiting viral replication and promoting acquired immunity. In simplistic terms, binding of viral dsRNA to RIG-I or melanoma differentiation–associated protein 5 (MDA5) triggers its binding to MAVS, which is located on the mitochondrial outer membrane. MAVS then undergoes oligomerization, permitting it to bind adaptor molecules TRAF3 and TRAF6; these molecules subsequently activate interferon regulatory factor (IRF) and nuclear factor-κB (NF-κB) transcription factors, leading to anti-viral interferon and pro-inflammatory cytokine production.[83] MAVS is a resident mitochondrial outer membrane protein, and various reports highlight an active role for mitochondrial dynamics in regulating RLR, MAVS-dependent signaling. Mitochondria are constantly undergoing rounds of fission and fusion with one another, thereby promoting mitochondrial homeostasis. Interestingly, RLR signaling via MAVS requires mitochondrial fusion because cells deficient in mitofusins (MFN) 1 and 2 or optic atrophy type 1 (OPA-1)—all proteins that are required for mitochondrial fusion—are defective in MAVS signaling.[84,85] Similarly, Ψ disruption of mitochondrial membrane potential ($\Psi_m$), leading to mitochondrial fragmentation, also inhibits MAVS-dependent signaling.[86] It remains unclear why an intact mitochondrial network supports MAVS signaling, although in research that potentially addresses this question, MAVS activation has been shown to occur in a prion-like manner, whereby one activated MAVS molecule activates another.[87,88] Potentially, a continuous, fused mitochondrial network facilitates sufficient MAVS oligomerization to support downstream signaling. It is important to note that because of its pleiotropic effects, disruption of mitochondrial membrane potential may affect MAVS signaling by additional means beyond disrupting mitochondrial fusion. Similarly, MFN2 has additional fusion-independent functions, for example, in mitochondrial-ER tethering.[89]

## Mitochondria and Toll-Like Receptor Signaling

TLRs are localized at both the plasma membrane and various intra-cellular organelles that include lysosomes and endosomes. This nine-membered family responds to various PAMPs derived from bacteria, viruses, fungi, and parasites, transducing signals that ultimately lead to the production of pro-inflammatory cytokines.

A key facet of innate immunity is the destruction of intra-cellular bacteria. After phagocytosis, ROS are induced, leading to the killing of intra-cellular bacteria. Most ROS produced during this process originate from NADPH oxidases; however, recent evidence also demonstrates an important role for TLR-driven mitochondrial ROS.[90] Triggering of specific TLRs triggers translocation of the TLR-adaptor molecule, TRAF6, to the mitochondria. At the mitochondria, TRAF6 ubiquitylates evolutionarily conserved signaling intermediate in toll pathways (ECSIT), a protein implicated in complex I assembly.[91] Through means that are unclear, ubiquitinated ECSIT triggers mitochondrial ROS production and pathogen clearance. Beyond these effects, TLR engagement has been shown to effect mitochondrial metabolism in other ways. For example, LPS increases the mitochondrial Krebs cycle intermediate metabolite, succinate, that induces pro-inflammatory IL-1β expression.[18]

## Mitochondria as a Source of Danger Signals

Thus far we have discussed mitochondria as key signaling platforms in a variety of innate signaling cascades. Likely stemming from their bacterial ancestry, mitochondria also represent a rich source of DAMPs. Chief among these is mtDNA, which contains hypomethylated cytosine-phosphatidyl-guanine (CpG) motifs similar to bacterial DNA. In line with a pro-inflammatory function, direct injection of mtDNA, but not nuclear DNA, leads to inflammation.[92] Moreover, systemic release of mtDNA after trauma has been proposed to underlie systemic inflammatory response syndrome, a form of septic shock.[93] Besides mtDNA, other mitochondrial molecules may also serve as DAMPs. Again, similar to bacteria, mitochondria also use *N*-formyl-methionine as the translation initiating residue. Both can stimulate cytokine production after binding of formyl peptide receptors (FPRs).[93]

# METABOLIC INTERPLAY IN THE IMMUNE MICROENVIRONMENT

Aerobic glycolysis, glutaminolysis, and other amino acid catabolism are dominant metabolic routes for many pathogen-encountered immune cells. Thus, those heightened catabolic pathways cause local depletion of nutrients (e.g., glucose and glutamine) and local accumulation of metabolic end- or by-products (e.g., lactate, proton, and NO) in infection and inflammatory sites. The similarity of metabolic programs among some immune cells may lead to a potential metabolic antagonism for limited nutrient

sources. On the contrary, some immune cells may preferentially utilize metabolic products of others to form a potential metabolic symbiosis (see Figure 20-1).

### Metabolic Antagonism in Immunity

The metabolic competition among active T cells, B cells, and DCs may lead to a rapid, albeit transient, nutrient depletion after immune activation. The restriction of glucose and glutamine results in metabolic stress and, consequentially, elicits signaling responses through AMPK and mTOR to modulate immune responses.[41,56,94] In addition, the depletion of extra-cellular tryptophan or arginine by DCs and macrophages that express amino acid catabolic enzymes such as indolamine 2,3-dioxygenase (IDO), tryptophan-2,3-dioxygenase (TDO), and arginase I (Arg I) often results in the depletion of local amino acids, and consequentially the activation of the protein kinase GCN2 (general control nonrepressed 2) in T cells.[95-98] Therefore Th17 differentiation is suppressed, whereas Treg development and T cell anergy are enhanced.[95,99] Furthermore, the secreted metabolic products may dramatically change the local metabolic environment and may form additional metabolic antagonism to shape immune cell functions. The lactate and carbon dioxide produced from glycolysis and glutaminolysis lead to microenvironment acidification, which suppresses T cell proliferation, impairs NK cell and T cell cytokine production, and has a profound impact on monocyte differentiation.[100-103] Beyond this, the cross-membrane transport of sodium ions is intimately coupled with proton and amino acids transport and has a profound impact on immune function.[104,105] Recent studies showed that high-sodium chloride conditions induce the development of pathogenic Th17 cells with elevated release of pro-inflammatory cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF], TNF, and IL-2) and thus promote tissue inflammation.[106,107] Also, iNOS-mediated breakdown of arginine into NO affects both the intra-cellular and extra-cellular redox balance and consequentially elicits immune modulatory effects.[108,109]

### Metabolic Symbiosis in Immunity

Lactate has been shown to mediate a form of metabolic symbiosis in muscle, brain, and certain tumors.[110-112] Although it has not been demonstrated, the preference of mitochondrial-dependent oxidative metabolism of Treg cells indicate the possibility that Treg cells may utilize lactate and form a metabolic symbiosis with other lactate-producing immune cells. The concentration of lactate in vertebrate plasma ranges from 1 to 30 mM under physiologic and pathologic conditions.[113] Early studies have shown that lactate enhances Treg differentiation through the stimulation of IL-2 production and, in another case, promotes the development of myeloid-derived suppressor cells (MDSCs).[100,114,115] In addition, lactate and acidic environments have a profound impact on tumor-associated macrophages (TAMs), promoting tumor angiogenesis.[103,116-118] Beyond this, the breakdown of tryptophan to kynurenine and potentially other intermediate metabolites in antigen-presenting cells results in the accumulation of nature ligands of aryl hydrocarbon receptor (AHR) in the local microenvironment, which plays a broad role in modulating immunity.[119,120] As such, extra-cellular accumulation of kynurenine elicits an AHR-mediated response to reciprocally enhance function of Tregs and suppress the function of effector T (Teff) cells and immunogenicity of DCs.[121-123] Thus kynurenine may mediate another form of metabolic symbiosis in immunity.

## CONCLUSION

In this chapter, we illustrate the relevant metabolic aspects of immune cells that have recently been revealed. In addition, we discuss the potential regulatory mechanism of metabolic reprogramming and the consequences of metabolic intervention on specific metabolic pathways in the immune response. The metabolic shift in immune cells during the transition between rest and activation is often associated with dramatically increased bioenergetic and biosynthetic demands. This may also lead active immune cells to become "addicted" to certain metabolic pathways in ways that resting cells are not. Thus, the modulation of such addiction, in terms of the biologic effects of enhancement or inhibition of specific pathways in immune cells, may offer novel therapeutic regimes to improve immunologic unresponsiveness or to suppress excessive immune responses, respectively. In addition to other known soluble protein factors, such as cytokines and chemokines, the availability of specific metabolites in the infection/inflammation microenvironment may be part of a pro-inflammatory or anti-inflammatory signaling circuit that affects the immune response. This is independent of their roles of bioenergetic fuels and may represent a general feature of the intercellular metabolic cross-talk mediated by metabolites. The revived interest in cell metabolism has revealed many fundamental biological insights and will likely generate new therapeutic strategies for immunologic diseases in the near future.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 21

# Genetics of Rheumatic Diseases



Stephen Eyre • Peter Gregersen • Anne Barton

**KEY POINTS**

- In recent years, major advances in our knowledge of the genetic basis for musculoskeletal diseases have been achieved.
- In many cases the causal gene and causal variants of disease have not yet been defined, although a locus of association has been identified.
- Exploiting the genetic data that are available is providing insights into the key risk pathways and primary cell types responsible for disease and may highlight targets for novel drug development.
- Translating genetic testing into the clinical setting is still premature; more work and possible integration with other data is required to identify signatures of drug response and prognosis.

Many of the musculoskeletal diseases seen by rheumatologists are thought to arise as a result of an environmental insult that triggers disease in a genetically susceptible person. As such, these diseases are known as complex diseases because both genes and the environment contribute to the risk of disease development. Genetic risk factors are easier to study than environmental risk factors because genetic variants are present from conception (and therefore must have been present before disease onset and could be causal), are stable throughout life, and are easily measured. In contrast, information about environmental risk factors is often collected after disease has developed, and the exposure could have occurred many years before disease onset, thereby introducing recall bias, or the exposure is measured after initial symptom onset, making it difficult to separate cause from effect. Furthermore, environmental risk factors often cannot be reliably or consistently measured. Thus, although research has enabled the identification of a few environmental factors that lead to a predisposition to disease, in comparison, an explosion of knowledge has occurred about the genetic contribution to many rheumatic diseases.

## EVIDENCE FOR A GENETIC COMPONENT TO RHEUMATIC DISEASES

To justify an investigation of the genetic basis of any disease, it is first necessary to have some evidence that genes play a role in the disease. This evidence most commonly comes from twin or family studies, although findings of adoption and migration studies can also support a genetic component. Classic twin studies compare the incidence of disease concordance in monozygotic (MZ) and dizygotic (DZ) twin pairs. Higher disease concordance in MZ twins provides support for a genetic cause and can be used to estimate the heritability of disease. The concordance of disease in MZ twins at one point in time will underestimate the genetic contribution to risk in diseases that generally feature a late age at onset, such as many musculoskeletal disorders, because as time progresses, the concordance rate in MZ twins is likely to increase as their age increases. Thus the concordance rate from one twin study in the United Kingdom was 15% in MZ twins versus 4% in DZ twins, equating to a 60% heritability.[1]

For less common rheumatic diseases, it may be impossible to collect enough data on twin pairs to interpret the data reliably. However, family studies can also indicate a genetic contribution to disease. The sibling recurrence risk ratio is defined as:

$$\lambda_s = \frac{\text{Risk of recurrence in a sibling of affected individuals}}{\text{General population risk}}$$

Diseases that show an increased prevalence in family members are likely to have a genetic component. Obtaining a reliable value of $\lambda_s$ depends on having accurate estimates of disease prevalence in the two comparison groups, which is not a trivial matter. A firm diagnosis of rheumatic disease is difficult to make in large population surveys, with errors possible in both directions. Underestimation may occur because of the lack of reporting of disease that is no longer active, and overestimation may result from inadequate distinction among different forms of rheumatic diseases. Table 21-1 shows the heritability estimates (where available) and sibling recurrence risk ratios for some rheumatic diseases.

If genome-wide genotype data are available, it is now also possible to estimate heritability from the data itself using statistical methods that assume that patients with a disease are more genetically similar overall than control subjects, a process called genome-wide complex trait analysis (GCTA).[2] However, in some cases the genetic data can suggest lower heritability than the original twin/family estimates; for example, an estimation of "callous-unemotional" behavior based on twin study methodology yielded a heritability estimate of 64%, compared with genomic sequencing that yielded a heritability estimate of 7%.[3]

## STUDY DESIGN

A number of important considerations exist when designing a study to identify genetic variants contributing to disease. These considerations include the use of linkage

(Table 21-2) versus association approaches, case-control versus family-based studies, the choice of marker to be tested, and candidate gene versus whole genome methods. The choice may be driven by cost considerations, power, and/or the availability of samples. The differing approaches are outlined in the following sections.

## Linkage Studies

Linkage methods depend on the ability to track polymorphic markers in families and to show that these genetic markers co-segregate with the disease phenotype in families in which multiple members are affected. Thus multiplex families are required for linkage analysis. The details of the statistical methods are complex but are in general based on examining the likelihood of a particular pattern of co-inheritance of marker and disease (linkage) compared with the likelihood that there is no linkage (the null hypothesis). A measure of this likelihood is referred to as the log of the odds (LOD) score, with a LOD score greater than 3 generally interpreted as indicating significant evidence of linkage when markers across the entire genome are examined. Ott and Bhat[4] described linkage methods in detail in 1999.

**TABLE 21-1** Sibling Recurrence Risks and Heritability Estimates for Some Common Rheumatic Diseases

| Rheumatic Disease | Sibling Recurrence Risk | Heritability (%) |
|---|---|---|
| Rheumatoid arthritis | 3-19 | 60 |
| Juvenile idiopathic arthritis | 15-20 | |
| Psoriatic arthritis | 40 | |
| Ankylosing spondylitis | 54 | >90 |
| Systemic lupus erythematosus | 20-40 | 66 |
| Osteoarthritis | | |
| Hip | 2-4 | 60 |
| Knee | 2-5 | 40 |
| Hand | 4 | 60 |

One advantage of linkage analysis is that the entire genome can be effectively interrogated by using less than 500 informative microsatellite markers, or approximately 5000 single-nucleotide polymorphism (SNP) markers. Linkage analysis has been applied with great success to the analysis of rheumatic diseases that exhibit a clear Mendelian pattern of inheritance (e.g., dominant or recessive). For example, in 1992, familial Mediterranean fever was mapped to chromosome 16,[5] and this mapping led to the identification of the gene for this disease in 1997.[6] In addition, an entirely new class of familial periodic fever syndromes has been localized to mutations in the tumor necrosis factor (TNF) receptor 1 gene on chromosome 12.[7] Thus, for highly penetrant Mendelian disorders, classic linkage analysis is a powerful means of identifying the underlying molecular basis of disease. However, one of the drawbacks to classic linkage analysis is that, to be most useful, it should be applied to disorders with a high penetrance and a known genetic model (e.g., dominant vs. recessive). An alternative approach based on linkage, broadly called *allele sharing*, is preferred for the study of musculoskeletal diseases that have a complex genetic basis.[8]

The most common approach to allele sharing is the affected sibling pair (ASP) method. This method is based on a simple question: When two siblings are both affected with a disease, do they share alleles at particular genetic markers more frequently than would be expected by chance? Figure 21-1 illustrates this basic approach. In this family, two siblings are affected and the first-born sibling (sibling 1) has inherited alleles 1 and 3 at a marker locus, X. By the laws of Mendelian inheritance, sibling 2 has a 25% chance of inheriting these same two alleles and a 25% chance of inheriting neither of these alleles (i.e., sibling 2 inherits 2,4 or shares nothing with sibling 1 at locus X). By a similar reasoning, there is a 50% chance that these two siblings will share one allele in common. This 25:50:25 distribution of sharing zero, one, or two haplotypes is expected if there is no linkage between the disease and the marker locus. However, if a gene that lies near the marker locus is involved in disease risk, a significant deviation toward increased sharing among affected siblings will be observed. The closer

**TABLE 21-2** Glossary of Terms

| | |
|---|---|
| Allele | Alternative form, or variant, of a gene at a particular locus |
| Alloantisera | Antisera that detect antigenic differences between individuals in the population; the term is most often used to refer to sera that detect antigenic (i.e., structural) differences among human leukocyte antigen molecules carried by different individuals |
| Haplotype | A group of alleles at adjacent or closely linked loci on the same chromosome that are usually inherited together as a unit |
| Heterozygote | An individual who inherits two different alleles at a given locus on two homologous chromosomes |
| Heterozygosity | A measure at a particular locus of the frequency with which heterozygotes occur in the population |
| Linkage | The tendency toward the co-inheritance within a family of two genes that lie near each other on the genome; complete linkage occurs when parents who are heterozygous at each locus are unable to produce recombinant gametes |
| Linkage disequilibrium | The preferential association in a population of two alleles or mutations that occurs more frequently than predicted by chance; linkage disequilibrium is detected statistically, and except in unusual circumstances, it implies that the two alleles lie near each other on the genome |
| Polymorphism | The degree of allelic variation at a locus within a population; specific criteria differ, but a locus is said to be polymorphic if the most frequent allele does not occur in >98% of the population; occasionally, polymorphism can be used in the same way as allele to refer to a particular genetic variant |
| Penetrance | The conditional probability of disease (or phenotype) given the presence of a risk genotype |

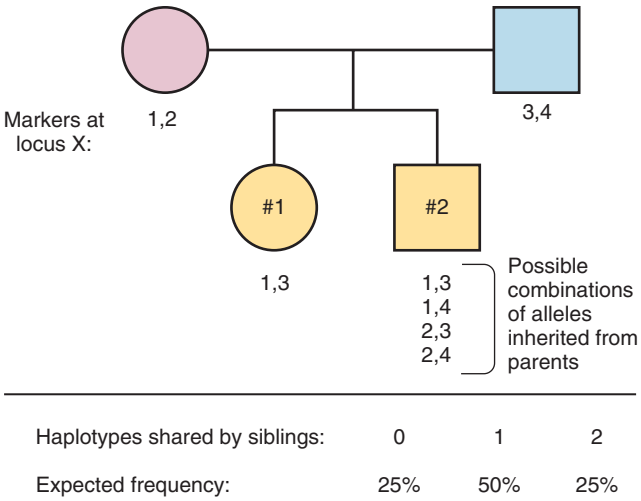



**Figure 21-1** A nuclear family with two affected children (affected sibling pair). The possible distribution of alleles at an autosomal locus, X, is shown for sibling 2, along with the predicted frequency of shared haplotypes among the siblings. In these families, researchers can detect linkage using affected sibling pair analysis (see text).

the marker is to the disease locus, the greater the deviation will be from a 25:50:25 distribution. By examining large numbers of affected sibling pairs in this manner, the investigator can develop statistical evidence that this is the case using a standard $\chi^2$ analysis, with the null hypothesis being that there is no increased sharing at the marker locus.

ASP analysis has a number of distinct advantages and disadvantages. Because only affected individuals are used, the problem of falsely assigning a family member as "unaffected" is eliminated, which is a major issue for many musculoskeletal diseases in which the disease may not express itself until later in life. ASP analysis also can be performed without assuming a specific model of inheritance (i.e., recessive or dominant). However, as with linkage in general, the ASP methods are limited in that they have relatively low power to detect genes that confer only modest risk. As a result, large numbers (hundreds or thousands) of families with affected siblings are required to obtain statistically significant results (see Figure 21-1).

Linkage analysis based on allele sharing began to be applied to genetically complex rheumatic and autoimmune diseases in the 1990s and has resulted in a few successes, most notably the identification of the *NOD2* gene as a major risk factor for Crohn's disease.[9,10] Linkage analysis has provided evidence for numerous risk genes involved in systemic lupus erythematosus (SLE).[11] The presence of a linkage peak (LOD score >3.5) on chromosome 2q in rheumatoid arthritis (RA) ASPs[12] led to the identification of the *STAT4* gene as a risk gene for RA and SLE.[13] Thus, although linkage is challenging to carry out, occasionally it can be applied successfully to complex diseases that do not have a clear Mendelian pattern of segregation.

## Population-Association Studies and the Calculation of the Odds Ratio, an Estimate of Relative Risk

The ideal way to establish whether a genetic variant (allele) confers risk for a disease is by performing a prospective cohort study. In this type of study, a group of individuals carrying (exposed to) the risk variant is compared with a matched control group that does not carry the risk variant. These two groups are followed up over time (preferably over a lifetime) to see if disease develops more frequently in the exposed group. The results can be displayed in a contingency table (Table 21-3). By examining the upper half of Table 21-3, it is apparent that the fraction of exposed individuals who get the disease is a/(a + b), whereas the fraction of unexposed individuals who get the disease is c/(c + d). The ratio of these two fractions is known as the relative risk (RR): a/(a + b) ÷ c/(c + d) = (ac + ad)/(ac + bc). If the disease is rare in the population, ac is small and the RR is approximated by (a × d)/(b × c), also referred to as the *cross-product*. In reality, such prospective cohort studies are usually impractical, and therefore a retrospective case-control design is used. In this type of study, subjects are initially identified according to whether they have the disease, and persons without the disease are the control subjects. The data can be tabulated as in the lower half of Table 21-3. In this case, the cross-product or (a × d)/(b × c) is known as the odds ratio (OR). In practice, this quantity is often reported as the estimated RR because the cross-product is close to the RR when the disease is rare. An OR of 1 indicates that the genetic factor confers no risk for the disease. An OR less than 1 suggests that the genetic factor under study is negatively associated with the disease (i.e., it is protective). Traditionally, ORs are reported with respect to the less common genetic variant (minor allele), and thus an OR of less than 1 indicates that the major allele confers risk. Sometimes, however, the OR with respect to the risk variant (whether the major or minor allele) is reported, and in that case, the OR is always greater than 1 if the result is statistically significant.

**TABLE 21-3** Contingency Table for Cohort and Case-Control Studies*

**Cohort Study**

| | Disease | No Disease |
|---|---|---|
| Exposed | a | b |
| Not exposed | c | d |

**Case-Control Study**

| | Exposed | Not Exposed |
|---|---|---|
| Disease | a | b |
| No disease | c | d |

*The letters a, b, c, and d represent the number of persons observed in each category.

One of the potential drawbacks of case-control association studies is that spurious association can occur if the case subjects and control subjects are not sampled from the same population (population stratification)—for example, if all subjects with disease were from a Scottish population and all control subjects were from a Spanish population. The gene variant conferring red hair color is more prevalent in the Scottish population with or without disease. Therefore, the red hair color gene would be associated with disease in such a study, but the association would be a false positive. In reality, if information is available on a large number of

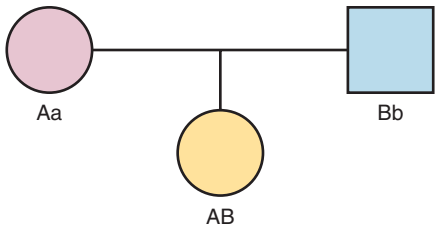



**Figure 21-2** Illustration of the trio family used for transmission disequilibrium test analysis.

genetic variants across the genome or for variants known to differ across populations of different ancestry, it is possible to correct for population stratification in the analysis, but the best solution is to consider this potential confounder in the study design.

## Family-Based Versus Case-Control Approaches

Family-based control subjects can also be used for genetic association studies. Consider the family shown in Figure 21-2. The affected child carries alleles A and B, each of which is inherited from one parent. The laws of Mendelian inheritance specify that one allele from each parent is not inherited by any given offspring—in this example, a in the father and b in the mother. These two noninherited haplotypes can be thought of as forming a genotype for a "control" individual. In this manner, issues of population stratification (see earlier) are eliminated because patients and control subjects are sampled from the identical (parental) gene pool. Falk and Rubinstein[14] originally proposed this approach to disease association and called it the *haplotype RR method*. Its validity depends on a number of assumptions, including that the genetic marker under study does not influence mating preference or the production of gametes. An extension of this approach is called the *transmission disequilibrium test* (TDT).[15] Using the family in Figure 21-2, for a given heterozygous parent (such as the father carrying a,c), there is a probability of 0.5 that any given allele, such as "a," will be transmitted to the child. If the "a" allele has no bearing on disease risk, the probability of transmission (T) to an affected child is equal to the probability of nontransmission (NT). This can be stated simply as P (T/D) = P (NT/D), in which D indicates the presence of disease in the offspring. However, if the allele being examined is associated with disease risk, then the following equation applies: P (T/D) > P (NT/D). If large numbers of heterozygous parents with affected offspring are examined, the TDT can establish an association between disease and the test allele compared with the (noninherited) control alleles. Although the TDT is an elegant solution to getting around the issue of population stratification, it is generally more expensive to identify and genotype trio families, and the TDT has less statistical power than a standard case-control approach to association (Figure 21-2) .

## Choice of Genetic Marker to Test

An explosion in new genetic knowledge has occurred during the past few years and is likely to continue for some time. A major foundation was laid by the completion of the Human Genome Project,[16] which provided a draft reference sequence for the entire 3.2 billion base pairs of the human genome. Subsequently, the human HapMap Project (http://hapmap.ncbi.nlm.nih.gov) has provided a catalog of the common sequence variation across the human genome. The most common form of variation across the genome is where a single base change occurs—for example, from adenine (A) to guanine (G) in the DNA sequence. Such changes are called SNPs, and more than 30 million SNPs are now referenced for *Homo sapiens* in dbSNP, an online SNP reference database (www.ncbi.nlm.nih.gov/SNP). In addition to SNPs, there are tens of thousands of variable numbers of tandem repeats (VNTRs) variants, a common form of which is termed a *microsatellite*. Microsatellites were used extensively for linkage analysis in the era before genome-wide association studies (GWAS) with SNPs. Large insertions and deletions are also quite common across the genome, some of which arise de novo,[17] providing yet another source of individual genetic variation that can be associated with human disease. For example, Huntington's disease is caused by a variable number of repeats of a CAG triplet within the Huntington gene. Smaller insertions and deletions are just beginning to be cataloged, and thus the number of these genetic variants will grow substantially as more human genomes are fully sequenced.

## Candidate Gene Versus Genome-wide Association Studies

The first wave of genetic studies of complex diseases focused on candidate gene studies. The candidate genes investigated were selected either on the basis of biologic plausibility, because they were associated with another disease, or on the basis of animal model studies. However, the previous probability of detecting an association to one gene among at least 30,000 known protein-coding genes was very low, especially when only one or a few markers were tested at the genomic locus, as was generally the case in such candidate gene studies. Furthermore, few studies tested large sample sizes of case and control subjects, and thus they were underpowered to generate robust findings. Nonetheless, several genes of large effect sizes were robustly and consistently detected, notably the *HLA* and *PTPN22* genetic associations observed with many complex diseases.

The acceleration in the knowledge of genetic variation across the genome due to initiatives such as The HapMap project, coupled with sweeping technologic advances, have meant that it is now possible to test SNP markers spanning the genome for evidence of association, so-called GWAS. The era of SNP-based GWAS began in earnest in 2005, when the complement regulatory protein factor H was identified as a significant risk factor for age-related macular degeneration.[18] Shortly thereafter, a second major GWAS led to the identification of the interleukin (IL)-23 receptor as a risk gene for Crohn's disease.[19] A major advance was made in 2007 with the realization that for most complex diseases, effect sizes of individual variants on disease would be modest, and hence large sample sizes would be required to robustly detect association. This advance was heralded by the pioneering Wellcome Trust Case Control Consortium (WTCCC) study.[20] The WTCCC study was the first

to use a respectably powered study design, including 2000 cases from seven diseases and 3000 common control subjects. Association was tested to 500,000 SNP markers spanning the genome, and robust statistical thresholds were applied to claim confirmed significance. Novel loci were identified and subsequent, better powered studies in larger independent sample sizes identified further loci. In the intervening 10 years, GWAS have proven to be particularly fruitful when applied to autoimmune disorders. Together, nearly 200 distinct chromosomal regions have now been identified as containing risk loci for major autoimmune diseases (https://www.genome.gov/26525384). As large datasets are assembled for some of the rarer or less studied autoimmune disorders, GWAS will continue to play a major role in dissecting the genetic contribution to musculoskeletal diseases.

## Genome-wide Association Studies

Compared with linkage analysis, association methods have much greater statistical power to detect genetic effects.[21] However, in contrast to the relatively modest numbers of markers used for linkage studies, hundreds of thousands or even millions of genetic markers are currently used to carry out GWAS using association methods. For scanning the entire genome, GWAS have generally replaced linkage methods except in special situations in which large multiplex families suggest the presence of a Mendelian pattern of inheritance.

The issue of testing so many markers raises the possibility of detecting association by chance alone. Hence, the genetics community has implemented thresholds for claims of confirmed association, based on the fact that there are estimated to be 1 million independent (noncorrelated) SNPs across the genome; applying a Bonferroni correction for 1 million markers equates to exceeding a statistical threshold of $P < 5 \times 10^{-8}$ to report confirmed association. In turn, this has implications for study power and is related to the effect size of the locus, the statistical threshold, and the frequency of the risk allele, which all have an impact on the sample size required to detect association. Thus the smaller the effect size expected, the lower the *P* value threshold used, and the lower the frequency of the minor allele, the larger the sample size required to have power to detect association. The effect size reflects the contribution of a particular locus to disease susceptibility and is measured by the OR. ORs are often overestimated in the first study in which a locus is reported to be associated with a disease because of the phenomenon of "winner's curse,"[22] because most studies are underpowered to detect all susceptibility loci. If association is detected, it is likely that, by chance, the risk allele is enriched in the population tested. In independent populations, the true frequency of the risk allele will be lower so a larger sample size is required to replicate the association.

The success of whole genome association studies is critically dependent on taking advantage of the underlying haplotype structure of the genome, which in turn reflects the ubiquitous presence of linkage disequilibrium (LD) across the genome. LD is discussed in greater detail in the next section and refers to the fact that genetic variants at adjacent loci often tend to be found together more frequently than expected by chance. LD over long distances is a particularly prominent feature of the human leukocyte antigen (HLA) region, especially for certain haplotypes.

### Linkage Disequilibrium

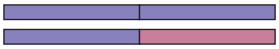
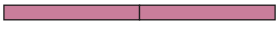
The concept of LD is central to understanding the significance of any genetic association with disease. LD exists when the frequency of two alleles occurring together on the same haplotype exceeds that predicted by chance. For example, a common major histocompatibility complex (MHC) haplotype that exhibits LD in the white population carries a certain combination of alleles, A*0101-B*0801-DRB1*03011, commonly referred to as the A1-B8-DR3 haplotype, and more recently designated the "8.1" haplotype.[23] This haplotype is present in approximately 9% of the Danish population, a typical white Northern European group. To understand why this reflects the presence of LD, consider the fact that the A1 allele is present in 17% of Danes and the B8 allele is present in 12.7% of Danes. They could be expected to be found together only 12.7% × 17% = 2.1% of the time, much less than what is observed (9%). This simple difference between the expected and observed association between alleles is a measure of LD, known as *D*; in this case D = (0.09 – 0.021) = 0.069. Table 21-4 provides the general calculation of D for a simple two-locus, two-allele situation. Because the magnitude of D is strongly influenced by the relative frequencies of the various alleles, normalized measures of LD are used in practice, including D′ and $r^2$, as described in Table 21-4.

It is useful to understand these measures of LD because detailed maps of LD are widely available online for the entire human genome, with visualization tools that are easy to use (see http://hapmap.ncbi.nlm.nih.gov). The lower portion of Figure 21-3 shows a visualization of LD using the D′ measure for a region around the *PTPN22* gene on chromosome 1. The D′ value between any two markers is reflected by the heat map (red D′ = 1; white D′ = 0). In this case, LD extends well beyond *PTPN22* itself. Indeed, as indicated in Figure 21-3, the SNP marker rs6679677 was used by the WTCCC[20] to detect the underlying association of RA with *PTPN22*, even though this marker is 100 kb distant from the causative SNP at rs2476601, which lies within the *PTPN22* gene.

To understand how LD occurs, it is useful to remember that, during meiosis, recombination occurs, resulting in a shuffling of the genome. An analogy is shuffling a pack of cards. Three likely explanations for LD exist. First, the population may have originated from a mixture of two populations, one of which had a high frequency of a particular haplotype. If this phenomenon happened recently, there would not have been time (i.e., a sufficient number of generations) to randomize alleles at closely linked loci by recombination at meiosis, which is known as population admixture. In the analogy of the playing cards, consider the situation of two suits of cards (diamonds and spades, for example) that have been shuffled together less than 10 times to mix them. When the cards are dealt, there will be large runs of sequential cards of the same suit. Inasmuch as human history is marked by large population migrations, it is probable that population admixture explains many examples of LD. A second explanation, related to the first, rests

**TABLE 21-4** Measuring Linkage Disequilibrium

Consider a region of a chromosome with two adjacent loci, A and B. At each locus, there are two possible alleles, 1 and 2. Four combinations of alleles, or haplotypes, are possible. These possible combinations are shown below, including a color bar representation and designations for the frequency of these haplotypes in the population.

$A_1$____$B_1$  frequency = $x_{11}$
$A_1$____$B_2$  frequency = $x_{12}$
$A_2$____$B_1$  frequency = $x_{21}$
$A_2$____$B_2$  frequency = $x_{22}$

Designate the allele frequencies at locus A as $p_1$ and $p_2$.
Designate the allele frequencies at locus B as $q_1$ and $q_2$.

Then

$p_1 = x_{11} + x_{12}$
$p_2 = x_{21} + x_{22}$
$q_1 = x_{11} + x_{21}$
$q_2 = x_{12} + x_{22}$

A simple measure of D is then calculated as $D = x_{11} - p_1 * q_1$. This value directly measures the difference between the observed haplotype frequency (in this case $x_{11}$) from that expected from random association of the alleles at each locus (in this case $p_1 * q_1$). For any allelic combination in a given dataset, the magnitude of D will be the same, although the sign (+ or –) may change according to the direction of the allelic association on the haplotypes.

A more standardized measure of D is more useful in practice and is designated D′. D′ is the ratio of the observed D to the maximal (or minimal) possible value of D given the observed allele frequencies.

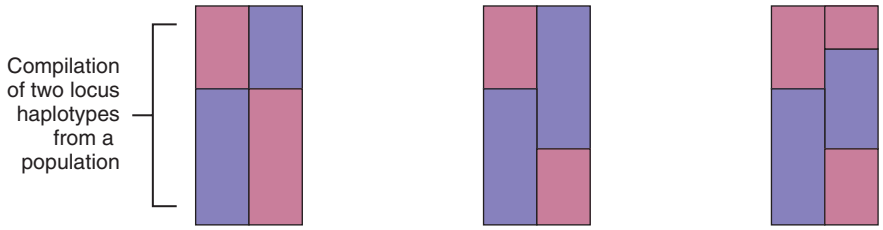
$$D' = \frac{D}{D_{max}} \text{ when } D \geq 0 \quad \text{and} \quad D' = \frac{D}{D_{min}} \text{ when } D < 0.$$

D′ can therefore take on values between 0 and 1, and it is a measure of linkage disequilibrium (LD) that is normalized for variations in allele frequencies at the two loci.

Another useful measure of LD is the correlation coefficient, $r^2$, between alleles at A and B. Like D′, the value of $r^2$ can also vary between 0 and 1. However, unlike D′, the value of $r^2$ is a more global measure of how alleles at the two loci are associated and is given by:

$$r = \frac{D}{\sqrt{p_1 p_2 q_1 q_2}}$$

When $r^2$ is = 1, there are only two possible haplotypes, and knowing the allele at locus A is completely predictive of the allele present at locus B. In this case, D′ also = 1. However, D′ can = 1 when $r^2 < 1$. In this case, there will only be three possible haplotypes in the population. If D′ is < 1, there will be four haplotypes in the population, which is illustrated in the colored two-locus phased haplotype displays shown below.




A presentation of phased haplotypes similar to these is shown for the region around the *PTPN22* gene in Figure 21-3.

on the observation that certain regions of the genome tend to exhibit relatively low levels of meiotic recombination (i.e., in the card analogy, for some reason, some cards are never separated by shuffling but are stuck together) for reasons related to the underlying genomic structure. Thus genetic variants within these regions tend to stay together on the same haplotype over many generations, even if haplotypes were introduced into a population in the distant past. The importance of this concept for understanding LD is still under active debate in the genetics community.

A third explanation for LD posits that the alleles in LD may be maintained together because of a selective advantage. For example, going back to the A1-B8-DR3 haplotype mentioned earlier, one could postulate an advantage for immune defense when alleles on this haplotype are maintained and regulated together in the same individual. Thus individuals with this haplotype may have had a survival advantage in times when infection in childhood was the greatest cause of death. The haplotype would become more common in the population, but now that people are surviving into in older age, the same haplotype may predispose to autoimmune disease as a result of a heightened immune response. Although plausible, this hypothesis is difficult to prove for any particular haplotype.

LD is useful for mapping associated variants because, as Figure 21-3 shows, for the region around the *PTPN22* gene

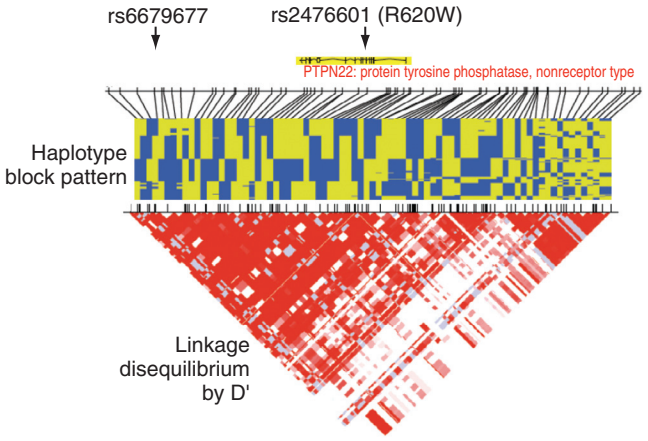



**Figure 21-3** Map of the region around the *PTPN22* locus on chromosome 1p13 covering approximately 200,000 base pairs. The blue and yellow haplotype pattern in the central part of the figure was generated by looking at combinations of single-nucleotide polymorphism (SNP) alleles in 90 white subjects from the HapMap Project. It is analogous to the presentation of linkage disequilibrium discussed in Table 21-7. Note that despite the large number of SNPs, a limited number of haplotype patterns are observed, generating a kind of bar code for each subject. The lower portion of the figure shows a heat map in which the intensity of the red color reflects the degree of correlation (linkage disequilibrium [LD] measured by D′) among SNPs across the region (indicated by *tick marks*). Note that widely separated SNPs are highly correlated. Two markers associated with type 1 diabetes (and other autoimmune diseases) are shown at the top. Marker rs2476601 is likely to be the causative variant in this region and results in an amino acid change at codon 620. Note that another marker (rs6679677) nearly a distance of 100 kb also strongly associates with diabetes, emphasizing that it is difficult to assign the causative locus on the basis of associations alone when extensive LD exists in a region.

on chromosome 1, common variations in large segments of most genetic regions can be interrogated using just a few markers that "tag" the common haplotypes. One can think of these patterns of haplotype variation as a kind of bar code of common haplotypes across a genomic region, as shown in the central region of Figure 21-3 and described in Table 21-4. As previously mentioned, the International HapMap Project has established online resources that allow users to easily explore the haplotype structure of any region of the genome. The HapMap Web site also contains informative tutorials on genetic diversity and the use of this resource.

## Common Versus Rare Variants

Debate continues about the overall genetic "architecture" of human disease.[24] Until recently, it was assumed that common allelic variants are likely to account for a large portion of the genetic risk for common diseases in the population. This assumption was based on the "common disease, common variant" hypothesis that assumes that common diseases will be caused by variants in the population that do not individually have a large effect on disease risk and thus are common in the population.[25] If a variant had a large effect on disease risk, it would be likely to reduce reproductive fitness and therefore would not survive over generations, and thus its frequency would be rare. The fact that common diseases are common suggests that the variants that cause them do not affect reproductive fitness and thus would be expected to persist over generations and be frequent in the population. By common variants, we generally mean variants that are present in the population at frequencies of 5% or more, and certainly not less than 1%.

Findings from many complex diseases provide partial support for this theory; for example, the HLA alleles and the *PTPN22* gene variant that are associated with many rheumatic diseases are examples in which the risk variants occur at a reasonable frequency in the population. However, the common variants identified to date do not explain all of the genetic contribution to disease, and there is no a priori reason to reject the hypothesis that many rare variants actually account for a significant fraction of the genetic burden of disease. The main reason that common variants have been a focus of research is because the current technologies are particularly well suited to investigating them. Therefore, the common disease, common variant hypothesis currently is a self-fulfilling prophecy because the technology platforms have focused on testing common variants. This situation is about to change with the advent of new technologies that permit resequencing on a massive scale. Routine sequencing of entire individual human genomes is probably not more than 5 years away, and examples of rare variants contributing to musculoskeletal diseases are already beginning to emerge.[26] This advancement in turn has stimulated the development of sophisticated statistical approaches to the analysis of large datasets, while at the same time highlighting the need to integrate the genetic knowledge with clinical data in ways that are manageable and meaningful to researchers and, ultimately, to practitioners. The imminent availability of massive genetic datasets now mandate new, more efficient ways to store and analyze the data. Thus we are still relatively early in the whole genome era of genetic mapping.

## Interpreting Statistical Association from Case-Control Studies

Almost all of the studies of complex diseases in recent years have reported statistical associations that are detected by means of retrospective case-control studies. It is essential to understand the strengths and weaknesses of this approach to genetic analysis to judge the significance of these associations.

In general, there are three possible reasons for detecting an association between a particular allele and a disease, once acceptable statistical criteria are met (see preceding text). First, the allele under investigation may be directly involved in the pathogenesis of the disease. A second reason that must be considered is the possibility that the result is an artefact of population stratification of patients and control subjects. The specific concern is that the control group may not be genetically matched to the disease group at loci that are unrelated to disease, which often results from a failure to study a control group that is ethnically matched to the disease group. This lack of genetic matching is generally a major issue in genetic case-control studies, and several approaches to address this issue have been proposed,[27] including the use of panels of genetic markers that specifically reflect ethnic background. These methodologies for correcting for underlying population genetic substructure are now widely accepted and are often required for publication in leading genetics journals. It is generally not adequate

to accept self-reported ethnicity as a basis for matching cases and control subjects.

Finally, a third (and common) reason for observing a genetic association is that the causative gene is actually in LD with the marker allele being tested, be it an SNP or a particular HLA variant. Therefore, once an association signal is detected, further fine mapping is required to locate the markers with the greatest evidence of statistical association and explore whether these variants could be functional and, therefore, causal.

## Rheumatoid Arthritis Susceptibility Genes

Before the GWAS era, researchers could with confidence assign only two loci—the *HLA-DRB1* and *PTPN22* genes—as having a role in the risk of susceptibility to RA, and they are still the largest genetic risk factors known to date for RA. As with the majority of autoimmune diseases, the HLA region confers, by far, the largest genetic risk for RA, accounting for approximately 60% of the genetic load. This large effect size enabled robust conclusions to be made regarding the involvement of the HLA region in RA some 30 years before the modern genetic era, using quite modest sample sizes.

## Class I and Class II Human Leukocyte Antigen Isotypes: Functional Correlates

Class II HLA molecules have a restricted tissue distribution that is generally limited to antigen-presenting cells of the immune system, such as B cells, macrophages, dendritic cells, and some subsets of T cells. This phenomenon reflects the fact that class II HLA molecules are primarily involved in presenting foreign antigens to $CD4^+$ T cells during the initiation and propagation of the immune response. However, the expression of class II HLA molecules can also be induced on a variety of other cell types by inflammatory cytokines such as interferon (IFN)-γ, enabling these cells to engage in antigen presentation to $CD4^+$ T cells. In contrast, class I HLA molecules are widely distributed on all somatic cells, with the exception of red blood cells. This distribution reflects their predominant role in presenting antigen to $CD8^+$ effector or cytotoxic T cells.

Another major functional difference between class I and class II molecules is related to the source of peptide antigens that are found in the antigen-binding cleft. In general, class I molecules present peptide antigens derived from proteins that are actively synthesized within the endoplasmic reticulum, whereas class II HLA molecules present antigens that are taken up from outside of the cell by endocytosis. These differences are reflected in the antigen-processing machinery and the different trafficking patterns of class I and class II molecules inside the cell. This complex process is discussed in detail in Chapters 10 and 18.

## Rheumatoid Arthritis: *HLA-DRB1* Associations and the "Shared Epitope"

Stastny[28] reported the first associations of RA with class II HLA alleles in the 1970s. This work was performed using cellular and antibody reagents that are no longer routinely used for HLA typing; however, the nomenclature for HLA alleles still derives from these early typing methods. The *DRB1*0401* allele (corresponding to the "Dw4" type in Stastny's original report) was the first HLA polymorphism to be associated with RA. Numerous studies have generally confirmed that this allele is the most strongly associated with RA, at least in white populations. However, several other *HLA-DRB1* alleles have also been associated with RA, although the strength of these associations varies. In some ethnic groups, RA is not associated with *HLA-DR4* alleles but rather with *HLA-DR1*[29] or *HLA-DR10*.[30] Experts now widely accept that the following alleles are the major contributors to RA risk at the DRB1 locus: *DRB1*0401*, **0404*, **0405*, **0101*, and **1001*. In addition, minor variants of these alleles and others (e.g., *DRB1*1402*) may also contribute to susceptibility, and *DRB1*0901* is a susceptibility allele in Asians, where this allele is common. Most of these risk alleles share a common sequence, as shown in Table 21-5. This consensus amino acid sequence—Q or K-R-R-A-A—has been termed the *shared epitope* (SE).[31] This structural feature is located in the third hypervariable region on the α-helical portion of the DR β chain in a position where it may influence both peptide binding and T cell receptor (TCR) interactions with the DRB1 molecule. In the case of the *DRB1*1001* risk allele, one amino acid varies from this consensus by a conservative change, with an R at position 70, as does *DRB1*0901*, which is commonly associated with RA in Asian populations (Table 21-6).

A number of different hypotheses have been advanced to explain the SE association with RA. Given that this region of the genome codes for amino acids that sit within

**TABLE 21-5** Genotype Relative Risks of DRB1 Genotypes for Rheumatoid Arthritis

| DRB1 Genotype | Relative Risk | *P* Value |
|---|---|---|
| 0101/DRX | 2.3 | $10^{-3}$ |
| 0401/DRX | 4.7 | $10^{-12}$ |
| 0404/DRX | 5 | $10^{-9}$ |
| 0101/0401 | 6.4 | $10^{-4}$ |
| 0401/0404 | 31.3 | $10^{-33}$ |

**TABLE 21-6** Amino Acid Substitutions that Compose the Shared Epitope at Positions 70 through 74 of DRB1 Alleles Associated with Rheumatoid Arthritis

| | AMINO ACID POSITION | | | | |
|---|---|---|---|---|---|
| DRB1 Alleles | 70 | 71 | 72 | 73 | 74 |
| 0101 | Gln | Arg | Arg | Ala | Ala |
| 0401 | — | Lys | — | — | — |
| 0404 | — | — | — | — | — |
| 0405 | — | — | — | — | — |
| 0408 | — | — | — | — | — |
| 1402 | — | — | — | — | — |
| 1001 | Arg | — | — | — | — |

and inform the shape of the groove of the DRB1 protein responsible for binding and presenting foreign bodies (antigens) to the immune system, an obvious possibility is that a specific RA antigen, probably an autoantigen, is being presented to the immune system in such a way as to illicit an erroneously controlled immune response, leading ultimately to the inflammatory destruction of joint tissue. Many years of investigations have failed to convincingly demonstrate the RA autoantigen presented by the shared epitope, although many antigens have been suggested as possibilities. In view of the strong association of the SE alleles with anti-citrullinated protein antibodies (ACPA), it is of interest that citrullinated peptides may have a particular affinity for *DRB1*0401* alleles.[32]

A second major hypothesis posits that these risk alleles regulate the formation of the peripheral T cell repertoire by acting to select for particular TCRs during thymic selection. Elegant experimental evidence in humans supports a role for DR4 alleles in shaping the peripheral T cell repertoire.[33] However, it is unclear whether this effect on the TCR repertoire is really related to disease susceptibility. Researchers have proposed a number of other interesting hypotheses, involving molecular mimicry,[34,35] allele-specific differences in intra-cellular trafficking,[36] and regulation of nitric oxide production,[37] but these hypotheses require further experimental confirmation.

The SE hypothesis has come under scrutiny in recent years because it is quite clear that it is not a complete explanation for the *HLA* associations with RA, as indicated by the fact that not all SE-positive alleles carry the same degree of genetic risk; in addition, the strength of the association varies in different populations. In general, *DRB1*0101* alleles carry lower levels of RR for RA than the *DRB1*0401* and **0404* alleles,[38] and yet *DRB1*0101* is the major risk allele in some ethnic groups. The SE itself does not appear to associate strongly with RA in African American and some Hispanic populations.[39,40] Furthermore, certain combinations of *DRB1* alleles carry especially high risk.[41] Thus the combination of *DRB1*0401* with **0404* carries a RR of higher than 30 in Caucasian populations.[38] In comparison, RR values are in the range of 4 or 5 for either allele alone. Table 21-4 summarizes some of these relationships.

The idea of the SE involvement in RA, which was first suggested in 1987, is still largely correct but has been modified slightly in recent years. Using massively high throughput genetic technologies, coupled with powerful bioinformatic and statistical analysis, the association to RA in the *HLA-DRB1* gene has been refined to three amino acids that are independently associated with susceptibility to RA, with two of these amino acids located within the SE region.[42] Current understanding of the RA associations within the HLA region suggests that an amino acid at position 11 of *HLA-DRB1*—still within the peptide binding groove but not within the classical SE motif—is most associated with disease susceptibility, followed by two further associations at amino acids 71 and 74 within the SE. In addition, amino acids in *HLA-B* (at position 9) and *HLA-DPB1* (at position 9) also show robust association with RA, even after correcting for the association with HLA-DRB1 amino acids. Together, these five amino acids explain almost all of the association observed at the MHC genomic region in ACPA-positive European subjects with RA.

The other gene robustly associated with RA susceptibility in the premodern genetics era is *PTPN22*. In 2004, an association between the intra-cellular phosphatase, *PTPN22*, was reported for a number of autoimmune diseases including type 1 diabetes,[43] RA,[44] SLE,[45] and autoimmune thyroid disease.[46] With ORs consistently in the range of 1.5 to 2, this was the first compelling demonstration that a specific common allelic variant outside of the *HLA* gene region could confer risk for multiple different autoimmune phenotypes, and the finding has been consistently replicated.[47] In this case, a nonsynonymous change in one of several SH3 binding sites in PTPN22 (a tryptophan substitution for arginine at codon 620) was shown to disrupt the normal association of PTPN22 with a Csk, an intra-cellular tyrosine kinase.[43,44] Knockout of *PTPN22* in rodents leads to dramatic overactivity of T cells, but the exact functional consequence of the human risk allele remains controversial. However, there is little doubt that PTPN22 is involved in setting thresholds for TCR signaling through Lck,[48,49] as well as B cell receptor signaling.[50] PTPN22 is also found in many other hematopoietic cells, and its function in these cells is largely unknown.

Interestingly, all of the autoimmune diseases associated with *PTPN22* have a prominent autoantibody component, and thus a specific role in regulating humoral immunity appears to be a unifying feature. Notably, the *PTPN22* risk allele is actually protective for Crohn's disease and has no role in risk for multiple sclerosis, emphasizing the likely presence of distinct mechanisms of pathogenesis in these disorders.[51] This scenario offers a fascinating insight into disease mechanism and obviously has implications in areas such as drug development, where an agonist may well be effective for RA and other autoimmune diseases but may exacerbate Crohn's disease. Thus *PTPN22* is a prime example of how the discovery of new disease associations with relatively modest effect can redirect hypothesis-driven research into new pathways. The theme of common variants in genes that share susceptibility alleles across different diseases with common underling mechanisms begs the question of how these genetic variants affect the normal immune response. Do these genetic variants confer survival advantages? Could one examine the "normal" range of immune response phenotypes to detect a state of risk, without actually defining the complex underlying genetics? The entire issue of what constitutes the normal range of immune function has received renewed interest[52] and has driven interesting studies of the function of autoimmune risk loci in subjects without disease.[53] Indeed, the study of the normal immune system may be the key to understanding how complex arrays of risk alleles actually lead to disease in an individual.[54] Thus, in addition to further expansion of gene discovery efforts, advances in the genetics of autoimmunity are going to require more sophisticated ways of phenotyping the normal immune system, as well as the diseases themselves.

## The Genome-Wide Association Studies Era of Rheumatoid Arthritis Genetics

The step change in the rate of discovery of RA susceptibility genes was achieved in 2007 with the WTCCC[20] and followed from the huge advances in the knowledge of the

genetic architecture of the human genome and the enormous advancements in genotyping technology, enabling the design of chip arrays capable of analyzing more than 500,000 variants in a single experiment at a cost-effective price. It also coincided with the realization by the genetics community that large sample sizes of both cases and control subjects would be required to achieve adequate power and that new, robust statistical methodologies would need to be developed to analyze the resulting data. GWAS approaches have been improved over time, with ever greater numbers of genetic variants being analyzed in ever-increasing sample sizes, such that the latest RA genetic study incorporated 29,880 RA cases and 73,758 control subjects and brought the total number of confirmed genetic loci involved in susceptibility to 101.[55] We can learn much from the 101 loci currently known for RA:

1. *There are probably more genetic loci associated with RA left to discover.* It is notoriously difficult to estimate the true size of the genetic component of a complex disease like RA. The twin studies used to estimate the heritability of RA have been relatively small and have led to quite disparate estimates, but even using the lower estimates, the current confirmed genetic findings only account for approximately 60% of the genetic component of RA susceptibility. Many theories have been suggested for this "missing" genetic heritability,[56] including the role of epistasis, a multiplicative interaction between genes or between genes and environmental factors that confers greater risks than simple additive carriage alone. Other theories include the involvement of genetic variations, which have not yet been well investigated, such as copy number variants and rare variants. These types of variants generally are not well captured using current genotyping technologies. Epigenetics—that is, non–sequence-related changes to the DNA—may also play a role. It appears likely, though, that a combination of overestimating the genetic component of RA and the existence of many more genetic loci that contribute to the susceptibility of RA with ever-decreasing effect sizes are likely to play the major role in "missing" inheritance. A large number of genetic variants are strongly associated with disease susceptibility but at a significance level where findings cannot be claimed as confirmed ($P < 10^{-5}$ but $> 10^{-8}$). Studies of other diseases and traits (e.g., height and inflammatory bowel disease) have illustrated that the more samples that are tested, the more confirmed loci are obtained—a story found, and likely to be continued, in RA.[2,57]
2. *Not all risk variants are required in every patient.* The genetic variants that are being discovered to be associated with disease are generally quite common—that is, they are present in more than 5% of the population. As such, many people without disease have a "genetic risk score" and carry risk variants. Similarly, all patients will have a genetic risk score that is generally higher than that of control subjects but is based on carriage of a subset of risk variants, never all of them. Determining which specific variants are important in subsets of patients has the potential to stratify patients into more homogeneous subgroups, potentially with benefits for treatment therapy and outcome predictions. Statistical modeling has indicated that patients who carry a subset of risk variants (*HLA*, *PTPN22*, and *STAT4*) can have an OR of developing RA of greater than 15 compared with persons who do not have these variants.[58] This work and other continuing efforts will provide insights into the highest risk groups and their eventual outcome and response to different therapies.
3. *HLA associations are different in the serotypes of RA and, although they are by far the strongest genetic association, they are neither necessary nor sufficient to cause disease.* The long-established association of RA with the HLA locus gives us a great wealth of information about the disease. The vastly increased frequency of a distinct set of amino acids of the DRB1 locus in HLA suggests that the presentation of a common antigen is important in most but not all patients with RA. This situation is clearly distinct from that of other autoimmune diseases, which may be strongly associated with the HLA locus but with different genes or alleles. Furthermore, HLA-risk–negative patients with RA may be exposed to different environmental antigens that trigger the onset of disease. Indeed, evidence clearly indicates that ACPA seropositive and seronegative patients show association to different risk alleles within the *HLA-DRB1* gene, reflecting evidence that the amino acids themselves are different in seropositive and seronegative disease.[59] Similar to the landscape in seropositive disease, seronegative disease has independent genetic associations with both HLA class II *(HLA-DRB1)* and class I *(HLA-B)* genes. Again, the independent associations are seen at position 11 in *HLA-DRB1* and at position 9 in *HLA-B*, both within the peptide-binding grooves. These amino acid positions therefore have an association to both forms of disease, but importantly, the risk of seropositive and seronegative disease is to distinct amino acid residues, suggesting that separate antigens may be important in the different forms of RA. For example, Serine at position 11 was protective for $ACPA^+$ disease but conferred risk of $ACPA^-$ disease.[59] Along with other evidence that the genetic loci associated with the disease serotypes are different in both strength and effect,[60] the HLA locus provides compelling evidence that the two forms of disease are indeed genetically distinct. Within the seropositive subgroup, there is a hierarchy of risk dependent on the amino acid present at each of the five most important sites within the HLA locus, and this hierarchy of risk also correlates with disease severity (see reference 60a).[42]
4. *Both overlaps and clear differences exist between the genetic risk factors for RA in different populations and ethnicities.* The latest large-scale meta-analysis, which brought the confirmed number of genes associated with RA to 101, was an international study using large cohorts of Asian and Caucasian samples.[55] The study showed that many RA loci are shared among these groups, including key immune genes such as *IL6R*, *STAT4*, *TNFAIP3*, and *IRF5*. Although these loci are shared among patients with RA from different ethnic backgrounds, it is still to be established whether the same genetic variant is causal in both populations. Association with different causal genetic variants mapping to the same gene is observed in different autoimmune diseases; for example, different genetic variants within and around the *TNFAIP3* gene

are associated with both RA and SLE, but it is not clear whether this is true of RA in different populations.[61,62] If different causal variants were identified in different populations of the same disease, it would indicate that the gene is fundamental to disease susceptibility but that the genetic mutation arose on different ancestral backgrounds. Some genetic loci are shared between the two ethnicities, but as yet with no obvious candidate gene as the likely causal target. For example, strong associations with RA can be found to the promoter region of AFF3, a transcription factor with an unknown function in immune disease, in both the Asian and Caucasian populations.[55] Associated loci also exist that are population-specific, offering a fascinating insight into the different pathways, different evolutionary constraints, and different gene/environment interactions that may play a role in disease onset. These genes, surprisingly, include the *REL* gene, a subunit of nuclear factor-κB (NF-κB), which is a key driver of the immune response and a pathway strongly implicated in the majority of autoimmune diseases. The association at *REL* is only found in European populations and is absent in Asian cohorts. Other European-only susceptibility loci include *IL2RA*, *PRKCQ*, *CD5*, *CD28*, and *IFNGR2*. Loci restricted to Asian populations include *PRKCH*, *CD83*, and *IL3*, perhaps indicating disparate immune pathways that ultimately lead to RA in the different populations.

5. *Overlap exists between autoimmune diseases*. One of the remarkable findings in the modern genetic GWAS era has been the unexpected and significant overlap in genetic risk factors between different diseases, particularly among autoimmune diseases. In fact, this finding led to the instigation of a successful study to design a custom Illumina genotyping array—"immunochip"—that densely mapped the shared genetic loci in a wide range of autoimmune diseases, including RA, type 1 diabetes, inflammatory bowel disease, and celiac disease.[63,64] That study is the starting point for in-depth investigations as to the extent of genetic sharing between diseases, whether that be the same variant associated with groups of diseases or shared loci, but with different causal variants. Some of the most interesting insights may be gained by understanding which loci are associated uniquely with a particular disease. Already the analysis has offered a fascinating insight into cross-disease genetic risk. For example, although the majority of autoimmune diseases, including RA, share a risk variant within the *PTPN22* gene, the same variant is protective for Crohn's disease. Similarly, at the *IL6R* gene, a genetic variant protective for RA and cardiovascular disease confers risk for asthma. Interestingly, this variant is highly correlated with soluble IL-6R levels and functions by increasing the cleaving of IL-6R from the membrane bound to the soluble form. This increase in soluble IL-6R mimics the action of the therapeutic agent, tocilizumab, a soluble IL-6R agent used in the treatment of RA. For RA, the greatest overlap with disease currently appears to be with type 1 diabetes, although this outcome may be due to the larger sample sizes tested in these diseases, yielding larger numbers of confirmed associations. Perhaps the more interesting aspect of the genetic overlap between diseases is the genes that are unique to a disease. For RA it appears that only 2 genes of the 101 identified to date are unique to disease susceptibility. The first is the *PADI4* gene, which encodes a protein responsible for the citrullination of proteins and may be expected to be RA specific, given the specificity of ACPA autoantibodies in disease. More surprisingly, the gene encoding a general cytokine, CCL21, also seems to be uniquely associated with RA. This chemokine, which is responsible for the formation of lymph nodes (including tertiary, ectopic nodes) and T cell migration across lymph nodes, and has also been implicated in angiogenesis, may well explain the pannus formation and nodule formation characteristic of RA.
6. *Genetics can provide clues as to how disease is initiated and maintained, including the cell type that is the most important in disease initiation*. Both T and B cells have been implicated as the key drivers of RA. By studying the epigenetic marks that show regions of the DNA genome that are active in each cell type, it has been reported that there is an enrichment of RA genetic associations in regions of active DNA $CD^+$ T cells, implicating this cell type in susceptibility.[65] However, these findings are preliminary because it is becoming increasingly apparent that different regions of the genome are active under different conditions of stimulation and chronicities[66]; whether the enrichment of RA loci in active DNA regions in $CD4^+$ T cells will continue to be observed under these different conditions requires confirmation. Ongoing work will illuminate if, for example, a genetic change is more important in the synovial tissue to maintain disease progress from early inflammatory arthralgia to full-blown RA.

## Juvenile Idiopathic Arthritis

Juvenile idiopathic arthritis (JIA) is perhaps the most heterogeneous of complex rheumatic disorders. JIA is classified into several subgroups, incorporating number and chronicity of joint involvement, as well as comorbidity and autoantibody status. Although the subgrouping of JIA into classes may be contentious, clear delineation exists between the major classes. Subgroups exist that also have psoriasis, spine involvement, a systemic disease, and both antibody-positive and antibody-negative disease. It may be speculated that these subgroups are early forms of psoriatic arthritis (PsA), ankylosing spondylitis (AS), SLE, RA, and "true" JIA, and genetics has a clear role in gaining insight into the overlap between these forms of disease. In turn, this may inform a more rational or targeted approach to disease management.

The major genetic locus in the susceptibility to JIA is the *HLA* region, conferring up to 13% of the total genetic risk of disease.[67] Because of the genetic complexity of the *HLA* region and the difficulties in collecting sufficient sample sizes for each of the JIA subgroups to ensure adequate power to determine differences between subgroups, analysis of the *HLA* region in JIA lags behind RA but is ongoing. Current evidence suggests that, similar to RA, both class I and class II genes play a role in susceptibility to disease, although to different alleles. For example, the class I alleles *HLA-A2* and *HLA-B27* and the class II genes *HLA-DRB1* and *HLA-DP* have all been associated with various

subtypes of JIA. In the future it will be fascinating to discover the HLA types and amino acids that drive the risk of disease in more homogeneous subtypes of JIA and whether they mirror the adult diseases (e.g., AS and psoriasis). There is a large subgroup that are clinically distinct from adult forms of inflammatory arthritis, including the early onset, anti-nuclear antigen $(ANA)^+$ subgroup, and again, it will be interesting to explore the overlap with SLE, as well as to understand the key differences between subgroups.

Outside the *HLA* region, GWAS and immunochip studies have led to identification of 17 genes robustly confirmed to be associated with JIA susceptibility.[67] Knowledge of these genes has already provided insights into the pathogenesis of disease. For example, JIA shares many loci with the adult form of RA, including association at the *PTPN22*, *IL2*, and *IL2RA* genes. However, the *IL2* pathway appears to be more prevalent in the juvenile form of disease, with *IL2* and *IL2RA* conferring larger effect sizes than in RA, and other genes in the pathway also being associated, including *PTPN2* and *RUNX1*. The *IL2* pathway is enriched in persons with type 1 diabetes, a disease that appears to share more genetic loci with JIA than it does with RA.

## Psoriatic Arthritis

Genealogical and family studies suggest that the genetic contribution to PsA is greater than that to psoriasis alone. Family studies have estimated the sibling recurrence risk to be in the region of 40, yet many of the susceptibility loci identified are shared with psoriasis. This finding is hardly surprising because most patients with PsA will have psoriasis, and thus far, GWAS studies have been performed using only modest sample sizes of patients with PsA. Early GWAS studies identified the *TRAF3IP2* gene as being associated with PsA, with an effect size larger than psoriasis.[68] However, association at this locus was also observed in samples from patients with psoriasis, some of whom may also have had PsA, illustrating the difficulty of proving the existence of PsA-specific loci. In a follow-up study investigating 17 loci that did not reach genome-wide levels of significance in the original GWAS, association with the *RUNX3* gene was confirmed in both PsA and psoriasis.[69] The largest genetic study to date used the immunochip array to test nearly 2000 patients with PsA and 9000 control subjects.[69a] Eight loci showed confirmed evidence for association at genome-wide significance levels, seven of which have previously been reported to be associated with psoriasis (*HLA-C*, *TRAF3IP2*, *IL12B*, *IL23R*, *IL23A-STAT2*, *TNIP1*, and *TYK2*). However, the SNPs predisposing to disease appear to be different between psoriasis and PsA for at least one locus, *IL23R*. Furthermore, two PsA-specific loci were identified: one at chromosome 5q31 and the other in the MHC (*HLA-B27*). The association with the MHC is complex, with three major associations detected with PsA: the classic psoriasis-associated *HLA-C*0602; HLA-B27*, and *HLA-A*02*. An independent study investigating just the MHC locus also found that the HLA-B association differentiated subjects with PsA and subjects with psoriasis alone. In that study, the amino acid at position 45, found on the *HLA-B27* allele, was associated with PsA exclusively.[70] It is likely that as sample sizes improve for PsA, further loci will be identified, and it will be possible to dissect the shared and distinct loci for these overlapping diseases. This dissection is important because it would potentially pave the way to screen patients with psoriasis for the risk of the development of PsA.

## Ankylosing Spondylitis

Family studies indicate that AS has a substantial genetic component, with a sibling recurrence risk of 9.2% compared with 0.1% to 0.4% in the general population.[71] Based on these figures, the heritability has been estimated to exceed 95%.

The strongest genetic susceptibility factor for AS is carriage of the *HLA-B27* allele. In white populations, more than 90% of patients with AS carry *HLA-B27*, in contrast to approximately 8% of healthy persons, giving estimated RR values of 50 to 100 or higher.[72] However, AS will develop in only 2% of people who are *HLA-B27* positive, indicating the presence of other genetic, environmental, and stochastic risks. The consistency of the association with AS across most ethnic groups lends support to the contention that the *HLA-B27* alleles are directly involved in disease pathogenesis.[73,74] *HLA-B27* is also associated with reactive arthritis and with the arthritis seen in the context of inflammatory bowel disease. The serologic specificity of HLA-B27 actually encompasses many distinct class I HLA alleles. These alleles differ from one another at a number of amino acid positions, most of which involve amino acid substitutions in and around the peptide-binding pocket. This fact leads naturally to the question of whether differences exist among these B27 alleles in terms of disease association. Most data indicate that this is not the case, although some exceptions may exist in some populations.[73] These exceptions may provide clues to the role of the HLA-B27 molecule in pathogenesis. Overall, however, it appears that most of the structural differences among the *B27* alleles do not affect disease risk.

Outside the *HLA* gene region, recent genetic studies have identified 25 additional risk loci to date.[75] These findings have highlighted several important insights into underlying disease pathways and mechanisms. First, the association of several aminopeptidase genes (*ERAP1*, *ERAP2*, *LNPEP*, and *NPEPPS*) has reinforced the importance of antigen presentation in AS pathogenesis because these genes encode proteins that trim peptides to be presented to HLA molecules. Interestingly, the *ERAP1* association is only found in *HLA-B27*$^+$ persons and was one of the first examples of genetic epistasis; that is, the presence of both *HLA-B27* and *ERAP1* risk variants increased the disease risk multiplicatively.[76] In contrast, *ERAP2* is also associated with *HLA-B27*$^-$ AS. Studies in mice specifically implicate *ERAP1* in viral peptide generation and presentation. Second, there is enrichment of genes involved in the *IL23* pathway, including *IL23R*, *IL-12B*, and *IL27*. The pathway drives the differentiation of $CD4^+$ Th17 cells, which produce IL-17. Encouragingly, early phase clinical trials of biologic drugs targeting the IL-23 and IL-17 signaling pathways have shown promising results in patients with AS. Third, pathways involving T cell differentiation (*EOMES*, *IL7R*, *RUNX3*, *ZMIZ1*, *BACH2*, and *SH2B3*) and G-protein coupled receptors (*GPR35*, *GPR37*, *GPR65*, and *GPR25*) have also been identified. Although the *IL1* gene cluster has

been implicated in some studies, findings have been inconsistent, and trials of an anti–IL-1 biologic drug, anakinra, were not successful for the treatment of AS.[77,78] Finally, there appears to be considerable overlap in the genes predisposing to AS and Crohn's disease. Given that up to 60% of patients with AS have been reported to show histopathologic evidence of gut inflammation and that a pathway comprising genes involved in the defense against pathogens has been identified in Crohn's disease, there is now considerable interest in the role of the gut microbiome in the predisposition to AS.[79]

## Systemic Lupus Erythematosus: Identification of the Interferon Pathway

In 2005, researchers showed that IFN regulatory factor 5 *(IRF5)* was associated with susceptibility to systemic lupus,[80] and they replicated this finding shortly thereafter.[81] This observation was satisfying because activation of IFN pathways is clearly central to the pathogenesis of lupus and related disorders.[82] Since the time of these original observations, it has become apparent that multiple genes in IFN pathways are involved in lupus susceptibility.[83] IFN has emerged as a potential drug target, and there is renewed appreciation and interest in the role of IFN regulation in the immune response generally.[84] The involvement of multiple genes in IFN pathways as risk factors for autoimmune diseases provides important support for continuing biologic studies in this field, as well as the potential for new insights into the details of how this pathway is regulated.

Researchers into the genetic contribution to SLE have pioneered the way in determining critical biologic pathways that lead to disease. These breakthroughs have arisen from the usual GWAS and immunochip routes but have also incorporated robust findings from family and single-gene studies and transethnic studies. Monogenetic disorders, such as Aicardi-Goutières syndrome, share many of the phenotypes of the complex genetic form of SLE.[85] Using family linkage approaches, key genes and biologic pathways have been uncovered in these studies, including *TREX1* (which is important in IFN-α production), complement deficiency, and Fas ligand (which is implicated in apoptosis).

Putting these discoveries together with the data generated from case-control association studies has led to the provisional identification of four key pathways involved in SLE susceptibility. These pathways are the type 1 interferon pathway, as exemplified by the association of *IRF5*, *IFIH1*, and *TYK2*; the NF-κB pathway (*TNFAIP3* and *IRAK1*); B and T cell signaling pathways (*PTPN22* and *BLK*); and apoptosis (*ITGAM* and *FCGR2A*).[86] These groundbreaking findings are the first steps toward a stratified medicine approach whereby patients with SLE with a B cell signal–driven disease, for example, could be grouped together and may well respond better to a specific therapeutic approach.

## Osteoarthritis

Osteoarthritis (OA) is the most common musculoskeletal disorder, but because of its high prevalence in so-called "healthy control populations," identifying the genes underlying this disease has been very challenging because large numbers of "controls" will go onto experience OA in the future. Furthermore, different patterns of clinical joint involvement are apparent and were described before the era of genetics. Genetic studies have confirmed that different genes seem to underlie these phenotypic subgroups. GWAS have now confirmed association to 11 genomic intervals at genome-wide thresholds[87] (Table 21-7).

**TABLE 21-7** Summary of Genes/Loci Associated with Osteoarthritis at Genome-wide Levels of Significance as of August 2014

| Gene/Chromosome Locus | Joint | Population |
|---|---|---|
| *GDF5* | Knee | European and Asian |
| *7q22* | Knee | European |
| *COL6A4P1* | Knee | Asian |
| *HLA-BTNL2* | Knee | Asian |
| *MCF2L* | Knee and hip | European |
| *GLN3* | Knee and hip | European |
| *ASTN2* | Hip total joint replacement | European (women) |
| *CHST11* | Hip total joint replacement | European |
| *FILIP1/SENP6* | Hip | European |
| *KLHL42* | Hip | European |
| *DOT1L* | Hip | European (men) |

It should be noted that, as in other GWAS, rather than being able to pinpoint a single gene, it is often a chromosomal region that is found to be associated with the disease. The locus is often named according to the gene closest to the strongest association signal or the most likely candidate based on existing knowledge. However, in most cases, the gene that is conferring risk has not yet been conclusively identified. The most thoroughly investigated locus to date is the *GDF5* locus mapping to chromosome 20. Interestingly, this is one of two regions (*GDF5* and *DOT1L*) that are also inversely associated with height (i.e., the risk allele for OA is associated with being smaller[88]). The risk alleles for *GDF5* are absent in the African population, are common in European populations, and show positive selection in East Asians, suggesting that they may confer a survival advantage, but what that survival advantage is remains unclear. It has been suggested that the WNT signaling pathway plays a key role in OA, and genetic studies provide some evidence to support this suggestion. For example, the *GDF5* gene has been found to reduce matrix metalloproteinase 13 expression via the WNT pathway[89]; the 7q22 region contains the *HBP1* gene, which has been reported to suppress WNT signaling,[90] whereas the *DOT1L* gene encodes a methyltransferase that acts on the same pathway.[91] However, further functional studies will be required to confirm the importance of the pathway in OA etiopathogenesis. It is evident that many more genes have yet to be identified for OA, and this endeavor will require further GWAS in larger sample sizes.

## Clinical Translation

GWAS is only a starting point in understanding the genetic basis of musculoskeletal diseases. The technique often identifies a region of interest for which further fine-mapping or resequencing studies are required to refine the peak of association and identify a group of likely causal variants. Bioinformatics analysis can be used to prioritize the likely candidate gene in a region and the likely causal variant, but ultimately, experimental verification and functional studies are required to prove causation. Only once the causal genes have been defined can reliable pathway analysis be undertaken. At the time of the writing of this chapter, the causal gene within an associated locus has been unequivocally identified in only a few cases.[63] However, genetic studies could potentially be used to inform clinical practice in other ways, as outlined.

### Identification of Drug Targets: Lessons from Rheumatoid Arthritis Genetics

Three of the genes identified in RA GWAS are the targets for drugs that are highly effective in the management of disease activity: abatacept is an analogue of the molecule encoded by the *CTLA4* gene; tocilizumab is a biologic drug that perfectly mimics the action of the RA-associated variant within the *IL6R* gene; and tofacitinib acts on the JAK/STAT pathway in which the RA-associated gene, *TYK2*, is crucially involved. Indeed, drug targets have been reported to be enriched in the largest analysis of RA genetic studies, which identified more than 100 RA susceptibility genes.[55] Therefore, genetic studies can highlight novel targets or pathways for drug development. Supported by genetic data, several drugs targeting the IL-17 and IL-23 pathways in PsA and AS are in early phase clinical trials, along with drugs targeting the IFN pathway in SLE.

### Prognosis

It is recognized that huge variability exists in the course of musculoskeletal diseases, but few reliable predictors of outcome have yet been identified. In RA, the presence of ACPA as measured by ACPA antibody status is more predictive of the development of erosions than the presence of rheumatoid factor at baseline but does not completely explain the variance in outcome.[92] However, it is expected that disease severity will be genetically determined, and a family study in early RA has provided support for this expectation.[93]

A number of other non-HLA genes or genetic loci have been investigated in terms of whether they can predict severity. The most consistent evidence for association with severity is for *HLA-DRB1* SE alleles. The *TRAF1/C5* locus has also been associated with joint erosions in multiple populations[94-97] but was not replicated in a subsequent study.[98] Two studies have reported association of the *IL4R* gene with outcome.[99,100] An additional two studies have reported association of the *IL2RA* gene with erosions.[101,102] However, findings for many have not been replicated in all studies, and none of the variants provides sufficient discrimination to be of clinical utility alone.

### Treatment Response

Several biologic therapies are available, particularly for RA, that target specific pathways. However, for each drug or drug class, fewer than half of the patients treated achieve remission. Currently, drugs are used on a trial-and-error basis, often in the order they came to market rather than on any scientific rationale. It would be tempting to speculate that remission rates would be improved if the initial biologic used was selected to target the major pathway mediating inflammation in individual patients, which is the concept of precision medicine. The prerequisite is to define the pathways involved in individuals. Currently this approach is limited because, at most associated loci, the gene responsible is not known. As previously explained, gene names are often assigned to a locus based on biologic plausibility or because they are the closest gene to the most associated variant in a region. However, numerous examples exist of this assumption having been proved wrong. For example, rs12740374, an SNP strongly associated with cholesterol levels, is found within the gene *CELSR2*, but it has been convincingly shown to confer its functional effect on cholesterol levels by changing the expression of *SORT1* in the liver, a gene separated from *CELSR2* on the chromosome by 2 other genes (*PSRC1* and *MYBPHL*).[103] If the wrong gene is assigned to a locus, then downstream pathway analysis to define subgroups of patients by the predominant pathway involved may be catastrophically compromised, and treatment response rates based on such groupings are unlikely to be improved. Therefore, precision medicine with use of genetic biomarkers is only likely to become a reality once the genes responsible for the association signal in a region are identified.

In the meantime, efforts are ongoing to assign patients to strata according to their known treatment response to drugs, which is known as stratified medicine. In several countries, longitudinal cohort studies are under way in which genetic data are being generated from patients who have received a drug, usually a biologic drug, and whose response to therapy has been recorded using standardized definitions of response. In Europe and, increasingly, in the United States, the outcome measure used is usually the change in disease activity score across 28 joints (DAS28) or European League against Rheumatism (EULAR) response criteria, which are based on the DAS28.[104,105] Studies to identify genetic predictors of response lag behind those of studies of susceptibility for several reasons. First, the difference between responders to a drug and nonresponders is more subtle than between patients with a disease and control subjects without a disease. Issues about how moderate responders are classified can complicate the phenotype definition, for example. Furthermore, the outcome measure itself is a composite score comprising both subjective measures (e.g., tender joint count and patient global health score) and objective measures (e.g., swollen joint count, erythrocyte sedimentation rate, or C-reactive protein). Second, the genetic studies performed thus far have been in modest sample sizes. For example, one of the first GWAS of anti-TNF response was performed in 566 patient samples, encompassing good, bad, and moderate responders, and thus study power has been a major limitation.[106] Third, biologics acting on the same pathway have been grouped, as in the

study described, but evidence shows that etanercept has different properties than monoclonal anti-TNF drugs. Thus progress in stratified medicine has been slow, but international collaboration recently led to the first moderately sized GWAS, which identified the *CD84* gene as approaching genome-wide significance levels for association with response to etanercept.[107] Independent studies from Denmark and Spain have replicated association to the *PDE3A-SLCO1C1* and, in a combined analysis, the association statistic exceeded genomewide thresholds.[108] The *PTPRC* gene has been associated with anti-TNF response by two independent groups, but a combined analysis still does not meet thresholds to claim confirmed association.[109,110] Efforts are continuing, but we can already conclude that there is no major genetic determinant of anti-TNF response of an effect size similar to that of the *HLA* region with RA. Instead, response is likely to be mediated by a large number of genes, each with a small individual effect, and signatures of response might be more realistic.

## IDENTIFICATION OF HIGH-RISK GROUPS

Because of the low prevalence of many rheumatic diseases (with the exception of OA) and the low effect sizes for most of the risk loci identified, it is unlikely that population screening to identify groups at risk of disease will ever be feasible. Even for age-related macular degeneration, for which large effect sizes at a modest number of genes have been found, the sensitivity and specificity of the testing means that population screening will probably not be cost-effective. However, genetic testing may be of more use in an already high-risk group, for example, in RA, those with a family history of disease, or those who are ACPA$^+$. It may be more feasible for PsA, because patients with psoriasis are already at higher risk for developing PsA than the general population. This could pave the way for preventative therapies for high-risk individuals in the future, and several cohort studies are under way to identify other factors that may increase risk further.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 22



# Epigenetics of Rheumatic Diseases

Amr H. Sawalha

**KEY POINTS**

- Epigenetic mechanisms include DNA methylation, histone modifications, and noncoding ribonucleic acid regulation. Collectively, epigenetic mechanisms determine chromatin architecture, accessibility of genetic loci to transcriptional machinery, and gene expression levels.
- Epigenetic changes are cell-type specific, and epigenetic regulation plays an important role in regulating the normal immune response, such as T cell differentiation.
- Epigenetic alterations are increasingly recognized as playing an important role in the pathogenesis of a growing number of rheumatic diseases.
- Epigenome-wide studies in rheumatology have focused primarily on DNA methylation changes and have resulted in the identification of novel target genes and pathogenic mechanisms in immune-mediated diseases.
- The epigenome is dynamic, allowing for the development of novel biomarkers for disease activity, specific disease manifestations, and response to treatment.
- Some epigenetic changes reflect the effect of environmental triggers that cause disease in a genetically susceptible host. An integrated "omics" approach would help to better understand disease pathogenesis in rheumatology.

Epigenetics refers to changes in gene regulation without changes in the DNA sequence. These changes are often described as "heritable," indicating a relative stability of epigenetic changes during cell division. Three main epigenetic mechanisms are thought to operate in concert to regulate chromatin architecture, thereby controlling the accessibility of regulatory regions within the genome to transcriptional machinery. Therefore, epigenetic changes are often described as "silencing" or "activating," indicating the net effect of a given epigenetic change on gene expression (transcription). DNA methylation, histone modifications, and regulatory RNAs, such as microRNAs, have evolved in mammalian systems to provide a complex array of epigenetic changes that determine specific chromatin architecture in any given genetic locus to regulate gene expression. These epigenetic mechanisms are essential during development and tissue differentiation, as well as in dictating the repertoire of genes expressed by the various cell types and tissues. Every nucleated cell contains the same repertoire of the human genome, yet each cell type maintains a specific assortment of expressed genes that is necessary for its function, while the rest of the genome is silenced. This silencing is achieved by epigenetic changes and the relative presence or absence of transcription factors. Indeed, two elements must co-exist for any gene to be expressed in any cell type: chromatin accessibility and appropriate transcription factors. If either is absent in a particular gene locus, that gene cannot be expressed.

## EPIGENETIC REGULATION

The genetic material (DNA) that carries the genetic code within nucleotide bases is tightly packed within the nucleus with histone proteins to form the chromatin. The chromatin basic units are known as nucleosomes, which consist of ~147 base pairs (bp) of DNA wrapped twice around a core protein octamer consisting of two of each histone protein, H2A, H2B, H3, and H4.[1] This architectural arrangement serves two main purposes; first, it allows for the genomic material to be compacted in such a way that it fits within the tight space of the nucleus, and second, it provides an avenue for regulating gene expression through epigenetic modifications, as will be described later in more detail.

DNA methylation is the most studied epigenetic mechanism and is considered the cornerstone of epigenetic regulation. DNA methylation refers to the addition of a methyl group (–CH3) to the fifth carbon of cytosine rings.[2] This reaction is mediated by a group of enzymes called DNA methyltransferases (DNMTs). DNMT3A and 3B are considered de novo DNMTs because they determine and establish DNA methylation patterns in utero.[3] DNMT1 is the "maintenance" DNMT because it maintains DNA methylation patterns throughout cell division ex utero.[4-6] Although this is a general rule, naturally some exceptions exist, and some redundancies within the DNMTs as a group in specific situations and cell types have been described. The methyl group in DNA methylation reactions is derived from S-adenosyl-methionine, which gets converted to S-adenosyl-homocysteine after donating a methyl group (Figure 22-1). Therefore, dietary changes that affect micronutrient levels in S-adenosyl-methionine metabolism can potentially alter DNA methylation and provide a link between metabolism and gene expression patterns.[7] DNA methylation most often occurs in cytosine residues within cytosine-guanosine (CG) dinucleotides. However, non-CG methylation, which is very frequent in plants, has been recently described in mammalian cells. Non-CG methylation typically occurs in cytosines within CA or CT dinucleotides and is generally restricted to embryonic stem cells and developing brain tissue.[8,9]

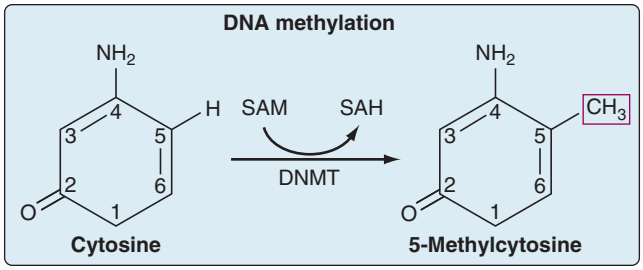



**Figure 22-1** DNA methylation refers to the addition of a methyl group to the fifth carbon in cytosine rings. Most DNA methylation occurs in cytosine residues within cytosine-guanosine (CG) dinucleotides, although non-CG cytosine methylation has also been recently described. DNA methylation is mediated by DNA methyltransferase enzymes (DNMTs) that use S-adenosyl-methionine as the methyl group donor. SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine.

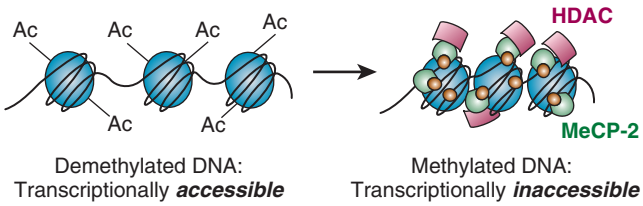



**Figure 22-2** DNA methylation represses gene expression. Transcriptionally accessible regions in the genome are generally characterized by DNA demethylated and histone tails acetylation. When DNA gets methylated *(red circles)*, it recruits a methyl-binding domain including proteins such as MeCP-2 *(green)*, which in turn recruits histone deacetylase (HDAC) 1 and 2. HDACs cleave off the acetyl group from histone tails, increasing the charge attraction between DNA and histone cores and thereby increasing chromatin compaction and reducing accessibility to transcription factors. The presence of methyl-binding proteins and HDACs also physically hinders transcription factor binding.

DNA methylation in promoter or regulatory sequences within the genome generally silences gene expression. Demethylation or hypomethylation, by contrast, is associated with active transcription. DNA methylation inhibits gene expression by several mechanisms. One important mechanism is inducing histone deacetylation (Figure 22-2). Methylated DNA recruits methyl-binding, domain-containing proteins, such as methyl-cytosine-phosphatidyl-guanine (CpG)-binding protein 2 (MeCP-2). MeCP-2 recruits and binds histone deacetylases (HDAC1 and 2), and the latter cleave off the acetyl groups from acetylated histone tails, thereby increasing the charge attraction between the histone core and DNA strands and inducing a compact chromatin architecture that becomes inaccessible to the transcriptional machinery, resulting in gene silencing.[10,11]

A number of histone tail modifications play a role in epigenetic regulation. As previously mentioned, histone acetylation (primarily of histones H3 and H4) is associated with transcriptional accessibility and active gene expression. Deacetylation of H3 and H4 silences gene expression. Other histone tail modifications have variable effects on gene expression, depending on the specific modification and the specific location of these modifications within histone proteins. The details of these histone changes are beyond the scope of this chapter, but generally they include acetylation, methylation, phosphorylation, ubiquitination, and others.[12] Increasingly, the complexity of the effects of histone changes on chromatin architecture has been recognized. Mapping chromatin modifications across the genome and in multiple cell types and tissues has resulted in a better understanding of chromatin regulation and architecture, as has been recently demonstrated by the ENCODE project.[13]

The importance of gene regulation by noncoding (nc) RNA (such as microRNAs) has been recognized by a recent Nobel Prize in Medicine and Physiology awarded in 2006 to Andrew Fire and Craig Mello. MicroRNAs are small RNA molecules of generally between 19 to 25 nucleotides that regulate the expression of target genes by binding to regulatory elements within their specific target genes, such as the 3′ untranslated regions (3′-UTR).[14] Our knowledge of the different types of regulatory RNAs, their biology and regulatory potential and function, and the specific target regions and genes they regulate is constantly evolving. In this chapter, we will focus on microRNAs as the most studied regulatory RNA in the context of autoimmune and rheumatic conditions.

The timeline for some of the major epigenetic discoveries and progress is outlined in Figure 22-3.

## EPIGENETICS AND THE IMMUNE RESPONSE

Epigenetic mechanisms are critically involved in the regulation of multiple aspects of the normal immune response.[15] We will discuss only a few examples in this chapter to demonstrate two important points: the role of epigenetic changes in the normal functions of immune cells that are thought to be key in the development of autoimmune and inflammatory diseases, and the utmost importance of examining specific cell types (not a mixture of cells) in epigenetic studies of immune-mediated diseases.

T cell activation is associated with a rapid production of interleukin (IL)-2, resulting in a feedback loop of further expression of the high-affinity IL-2 receptor α (IL-2Rα or CD25) and more IL-2 production (autocrine mechanism). This rapid and efficient IL-2 production is associated with a robust and rapid demethylation of the *IL2* promoter sequence. Indeed, this demethylation starts within minutes after T cell stimulation and therefore is clearly independent of cell cycle or cell division.[16,17] This characterization was in opposition to the dogma that DNA demethylation is passive and cell cycle dependent. In subsequent years we witnessed the characterization of DNA demethylation as an active process mediated by the ten-eleven translocation (TET) system and through the generation of 5-hydroxymethylcytosine and 5-caboxylmethylcytocine, both of which are intermediates in a demethylation reaction during the conversion of methylated to unmethylated cytosine residues.[18,19]

The differentiation of naïve $CD4^+$ T cells to T helper (Th)1 and Th2 effector cells is accompanied by essential and locus-specific epigenetic changes.[15] In naïve $CD4^+$ T cells, the Th1 locus *(IFNG)* and the Th2 locus (*IL4*, *5*, and *13* common locus control region) are inaccessible for transcription because the DNA is methylated and histone tails in these two loci are deacetylated, resulting in compact chromatin that is transcriptionally repressive. Upon Th1 differentiation, the Th1 locus *(IFNG)* gets demethylated and histone tails become acetylated, leading

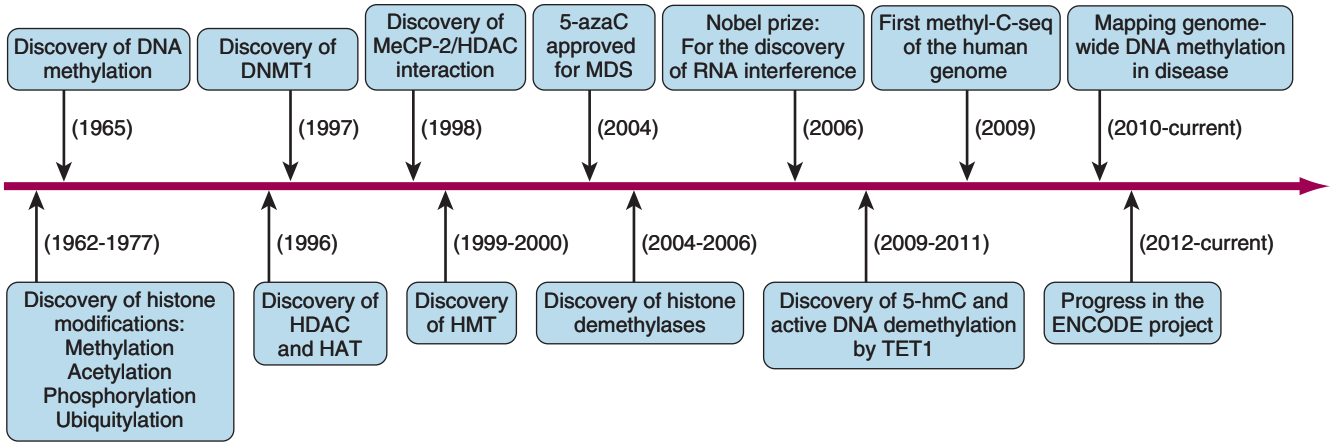



**Figure 22-3** The timeline for key discoveries and progress in epigenetics. DNMT1, DNA methyltransferase 1; HDAC, histone deacetylase; HAT, histone acetyltransferase; HMT, histone methyltransferase; MDS, myelodysplastic syndrome; TET1, ten-eleven translocation methylcytosine dioxygenase 1; ENCODE, Encyclopedia of DNA Elements.

to a locus-specific chromatin accessibility and the production of the key Th1 cytokine interferon (IFN)-γ. At the same time, the Th2 locus control region becomes even more methylated compared with naïve $CD4^+$ T cells to ensure no production of Th2 cytokines.[20,21] The exact opposite happens in the Th2 locus control region and the Th1 *IFNG* locus upon naïve $CD4^+$ T cell differentiation into Th2 effector cells.[22-24] Importantly, these locus-specific methylation changes are maintained upon memory Th1 and Th2 cell generation to ensure a rapid clonal T cell response upon re-exposure to the same pathogen. Indeed, the 2 to 3 days it generally takes for an initial Th1 or Th2 immune response is largely the time needed for the aforementioned locus-specific methylation changes to happen, and this explains why a second-exposure response from memory T cells is much faster.[25] The development of IL-17–producing Th17 cells is similarly accompanied by chromatin remodeling in the *IL17A* and *17F* loci.[26] Regulatory T cells (Tregs) demethylate the *FOXP3* genetic locus, resulting in the ability to produce FOXP3, a key transcription factor in the immune-regulatory function of these cells.[27] Therefore, locus-specific epigenetic changes play a critical role in the differentiation of T cells into specific subsets with a specific cellular function and cytokine-producing capacity.

## EPIGENETICS IN RHEUMATIC DISEASES

### Systemic Lupus Erythematosus

The etiology of systemic lupus erythematosus (SLE) is not completely understood. A strong and growing body of literature supports a role for epigenetic changes in the pathogenesis of SLE.[28-30] Most of these studies are focused on T cells, because demethylated T cells are sufficient to induce SLE in animal models. Initial studies in the epigenetics of SLE, and in rheumatology in general, originated from Dr. Bruce Richardson's work at the University of Michigan. As a result of this work, and subsequent work by other investigators, epigenetics is now in the center of the current paradigm of SLE pathogenesis. Indeed, epigenetics provides a link between environmental triggers and genetic susceptibility in autoimmune diseases.

#### DNA Methylation Regulation and Candidate Gene Studies in Systemic Lupus Erythematosus T Cells

Initial studies demonstrated that procainamide and hydralazine cause drug-induced SLE by demethylating T cells, thereby resulting in T cell autoreactivity. Both procainamide and hydralazine inhibit the activity of the maintenance DNA methyltransferase DNMT1. Procainamide directly inhibits DNMT1 in T cells, whereas hydralazine inhibits MEK/ERK signaling, which regulates and mediates DNMT1 expression.[31,32] $CD4^+$ T cells treated with procainamide or hydralazine, the DNA methylation inhibitor 5-azacytidine, or MEK/ERK pathway inhibitors overexpress messenger (m)RNA and proteins encoded by methylation sensitive genes (such as CD11a, CD70, perforin, CD40L, and killer cell immunoglobulin-like receptor [KIR] family) and become autoreactive, capable of killing autologous macrophages and stimulating B cell immunoglobulin production in vitro.[28] In vivo experiments, using adoptive transfer of similarly treated demethylated T cells into syngeneic mice, cause autoantibody production and an SLE-like disease.[28] T cells isolated from patients with SLE demonstrate reduced MEK/ERK signaling, reduced DNMT1 expression and activity, and demethylation of the promoter sequences, resulting in overexpression of CD11a, CD70, perforin, CD40L, and KIRs, similar to 5-azaC–treated T cells.[28] Therefore, T cells from patients with SLE resemble normal T cells treated with MEK/ERK pathway inhibitors or DNA methylation inhibitors. The extent of the MEK/ERK signaling defect and reduction in DNMT1 expression in SLE T cells is proportional to disease activity, with T cells isolated from patients with active SLE showing more extensive demethylation compared with patients with inactive disease.[33]

To further provide a proof of principle that reduced MEK/ERK signaling in T cells can result in T cell demethylation and SLE, rather than the alternative possibility that

the observed MEK/ERK signaling defect in SLE is the result of and not causal to the disease, a transgenic mouse model with an induced T cell MEK/ERK signaling defect was generated.[34] These mice demonstrated that inducing a defect in the MEK/ERK signaling pathway resulted in reduced DNMT1 expression, demethylation of methylation sensitive genes, and overexpression of these same genes, similar to T cells isolated from patients with SLE.[34,35] Further, this mouse model developed anti–double-stranded (ds)DNA antibodies and a T cell interferon expression signature, reminiscent of the interferon signature in patients with SLE.[34] When they have a genetic background permissive to autoimmunity, these mice also developed clinical SLE-like disease such as glomerulonephritis.[35] Taken together, inducing a DNA methylation defect in T cells is sufficient to cause autoimmunity in subjects with an autoimmune-resistant genetic background and a clinical SLE-like phenotype in the presence of a genetic background that is permissive to autoimmunity. This finding is similar to the finding that, in humans taking hydralazine (which is an MEK/ERK inhibitor), autoantibodies develop in the majority of patients and a clinical SLE-like disease develops in only a small fraction of patients, presumably those with a genetic background for SLE. This observation provides a basis for genetic-epigenetic interaction in the pathogenesis of SLE; other evidence for this interaction is discussed in later sections.

In subsequent studies, investigators mapped the origin of the MEK/ERK signaling defect in SLE T cells that causes the observed DNA methylation defect to an upstream signaling defect in protein kinase C (PKC)-δ.[36] Interestingly, oxidative stress such as oxygen free radical species can induce a defect in PKC-δ signaling similar to that observed in SLE T cells, resulting in T cell DNA demethylation.[37,38] These data provide a link between environmental triggers of oxidative stress, such as infections, and autoimmunity in the susceptible host. Infections are known to induce flares in patients with SLE, and this reaction might be mediated at least partly by increased oxidative stress, resulting in T cell demethylation. In fact, mitochondrial dysfunction resulting in increased production of reactive oxygen species and reduced levels of the antioxidant glutathione have been reported in SLE T cells.[39] Increased oxidative stress in SLE T cells also results in activation of the mammalian target of rapamycin (mTOR) signaling pathway, which also inhibits DNMT1.[39] Treatment of patients who have SLE with the antioxidant and glutathione precursor N-acetylcysteine blocked mTOR in T cells and improved disease activity.[40]

Recent data have also provided evidence for additional mechanisms that play a role in inducing the DNA methylation defect in SLE T cells. For example, protein phosphatase 2A (PP2A), which is involved in multiple cellular processes, including proliferation and activation, is overexpressed in SLE T cells. Increased PP2A expression and activity has been linked to reduced IL-2 production, which is characteristic of SLE T cells.[41] Recent evidence suggests that increased PP2A might be inducing a DNA methylation defect in SLE T cells by causing an MEK/ERK signaling defect, thereby reducing DNMT1 expression. Indeed, suppressing PP2A in SLE T cells enhanced MEK/ERK signaling, increased *DNMT1* mRNA expression, and reduced the expression of methylation-sensitive genes such as *CD70*.[42] Further, increased PP2A induces epigenetic changes in the *IL17* locus, enhancing IL-17 production from SLE T cells. PP2A catalytic subunit (PP2Ac) transgenic mice develop an IL-17–dependent glomerulonephritis.[43]

The growth arrest and DNA damage–induced 45α (GADD45α), which is involved in DNA repair and DNA demethylation, is overexpressed at the mRNA and protein levels in SLE $CD4^+$ T cells.[44] Inhibiting GADD45α in SLE $CD4^+$ T cells using siRNA resulted in increased methylation of *ITGAL* (encoding CD11a) and *CD70 (TNFSF7)* promoter regions and reduced *ITGAL* and *CD70* mRNA expression, reduced T cell proliferation, and reduced T cell autoreactivity as measured by B cell co-stimulation.[44]

Other contributing factors to the DNA methylation defect and overexpression of methylation-sensitive genes in SLE $CD4^+$ T cells include downregulation of regulatory factor X1 (RFX1). RFX1 recruits DNMT1 and HDAC1 and suppresses CD11a and CD70 expression, and underexpression of RFX1 is associated with demethylation and increased expression in CD11a and CD70 in SLE $CD4^+$ T cells.[45]

### Genome-wide DNA Methylation Studies in Systemic Lupus Erythematosus T Cells

Genome-wide DNA methylation studies have been performed recently in SLE. These studies provide an unbiased view of the DNA methylome and have identified a number of novel differentially methylated genetic loci that might be important in the pathogenesis of this disease. Using a group of twins discordant for SLE, Javierre and colleagues[46] investigated SLE-associated DNA methylation changes in ~1500 CpG sites in peripheral blood leukocytes. This first genome-wide study of DNA methylation in SLE was followed shortly by another study examining ~27,000 CpG sites in $CD4^+$ T cells from patients with SLE compared with healthy age-, sex-, and ethnicity-matched control subjects.[47] Several differentially methylated regions were identified in $CD4^+$ T cells, including hypomethylation of *CD9* (T cell co-stimulatory molecule), *MMP9* (matrix metalloproteinase involved in autoimmunity), *PDGFRA* (platelet-derived growth factor receptor-α precursor), *CASP1* (caspase 1), and IFN-regulated genes such as *IFI44L* and *BST2*.[47]

Subsequent work by Coit and colleagues[48] identified genome-wide DNA methylation changes across more than 485,000 methylation sites in naïve $CD4^+$ T cells in SLE. This study included a discovery and a replication cohort to robustly identify and replicate DNA methylation changes, coupled with gene expression profiling in a subset of the same samples. This study demonstrated for the first time a robust and consistent hypomethylation in IFN signature genes in SLE, which precedes gene expression.[48] Therefore, it appears that naïve $CD4^+$ T cells in patients with SLE are poised at the epigenetic level to produce type I IFN-regulated genes even before T cell activation (Figure 22-4). These data provided a mechanistic explanation for the previously reported increased type I IFN sensitivity in SLE peripheral blood mononuclear cells (PBMCs) and adds yet another pathogenic role for abnormal DNA methylation in the pathogenesis of SLE.[48] Hypomethylation of IFN-regulated genes was subsequently replicated and extended to B cells and monocytes in SLE.[49] More recently,

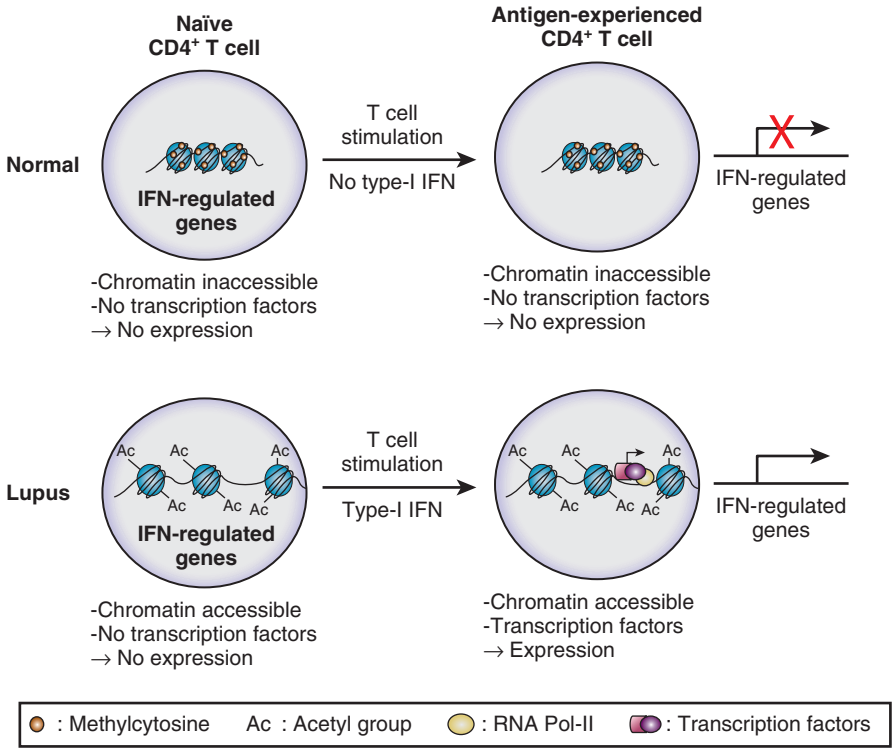



**Figure 22-4** Interferon (IFN)-regulated genes are epigenetically "poised" in naïve CD4+ T cells in systemic lupus erythematosus (SLE), because abnormal DNA demethylation exists in SLE T cells before activation. A model whereby interferon-regulated genes are epigenetically poised to respond to type I IFN upon T cell activation in SLE is proposed. These data provide evidence for an epigenetic architecture favoring—and providing an explanation for—type I interferon hyper-responsiveness in SLE T cells.

a genome-wide DNA methylation study coupled with transcriptome and microRNome analysis in SLE CD4+ T cells identified additional DNA methylation changes in SLE, such as the differential methylation and overexpression of novel target genes, including *NLRP2* (inflammasome component gene), *CD300LB* (a pro-inflammatory nonclassical activating receptor of the immunoglobulin superfamily), and *S1PR3* (a pro-inflammatory G protein–coupled receptor).[50] Further, this study localized some methylation changes that are specific to clinical disease subsets in SLE, such as kidney and skin disease.[50]

## Genetic–Epigenetic Interaction in Systemic Lupus Erythematosus

MeCP-2, encoded by *MECP2*, is a key transcriptional regulator that is intimately involved in DNA methylation–mediated transcriptional repression. Numerous studies have identified and replicated the genetic association between *MECP2* and SLE, suggesting that genetic variants within *MECP2* are associated with increased risk for SLE.[51-53] This was the first link suggesting a potential role for genetic-epigenetic interaction in the pathogenesis of SLE. Subsequent studies indicated that the SLE-risk variant in *MECP2* is associated with increased mRNA expression of one *MECP2* transcript variant in stimulated but not unstimulated T cells and that mice overexpressing human MeCP-2 develop anti-nuclear antibodies and a gene expression profile characterized by an IFN signature in activated CD4+ T cells.[54] Importantly, the DNA methylome of stimulated T cells from healthy persons carrying the SLE-risk variant in *MECP2* reveal hypomethylation of several IFN-regulated genes.[54]

Genetic-epigenetic interaction plays a role in the severity of flares in patients with SLE. A positive correlation exists between genetic risk to the T cell DNA methylation ratio and SLE disease activity in patients with SLE.[55] Moreover, male patients with SLE require a higher genetic risk and/or lower T cell DNA methylation to achieve an SLE flare of equal severity to that of women.[55] Genetic risk alone or T cell DNA methylation alone could not explain the difference between men and women in SLE flare severity. These data provide further evidence for genetic–epigenetic interaction in SLE and suggest that although genetic risk is relatively stable over time, dynamic changes in T cell DNA methylation might play an important role in SLE flares, in interaction with the genetic susceptibility for SLE.

## Histone Modifications in Systemic Lupus Erythematosus

Histone acetylation status is a product of the balance between histone acetyltransferases and histone deacetylases. As the name implies, the former increases histone acetylation, whereas the latter leads to its reduction. As previously discussed, histone acetylation/deacetylation determines chromatin accessibility and is influenced by the DNA methylation status (Figure 22-2). One of the earliest reports related to histone changes in SLE suggested that histone deacetylase inhibitors can abrogate SLE-like disease

in murine models. Histone deacetylase inhibitors downregulated the production of several cytokines including IFN-γ, IL-12, IL-10, and IL-6 in MRL/lpr mice. Further, mice treated with histone deacetylase inhibitors showed a global increase in histone H3 and H4 acetylation as expected, and more importantly, demonstrated significant improvement in renal diseases.[56] Similarly, NZB/W SLE-prone mice treated with a histone deacetylase inhibitor also showed improvement of renal disease, reduction in Th17 cells, and increased Tregs and acetylation of the Treg key transcription factor gene *FoxP3*. Therefore, histone deacetylase inhibitors might provide therapeutic effectiveness in SLE by altering T cell subsets and reducing pathogenic Th17 cells while increasing Tregs.[57]

Gene-specific studies in human disease demonstrated the importance of histone changes in suppressing IL-2 and inducing IL-17 production in SLE T cells.[58] In SLE monocytes, the *TNF* (encoding TNF) locus is more acetylated compared with normal control monocytes, and this hyperacetylation is associated with increased *TNF* mRNA expression.[59]

At a genome-wide level, although histone changes in SLE have not been as extensively studied as DNA methylation, $CD4^+$ T cells from patients with SLE demonstrate reduced global H3 and H4 acetylation.[60] This finding is consistent with a beneficial effect of HDAC inhibitors in SLE mice as previously discussed. Mapping histone H4 acetylation in SLE monocytes suggested that the majority of hyperacetylated loci are potentially regulated by type I IFNs.[61]

### MicroRNAs and the Pathogenesis of Systemic Lupus Erythematosus

Differential expression of microRNAs has been reported in multiple cell types in patients with SLE.[14,62] Because microRNA expression is involved in the regulation of multiple aspects of the normal immune response, it is not surprising that changes in the microRNA milieu can be associated with autoimmunity. Some of the dysregulated microRNAs in SLE tie in specific and credible pathogenic aspects of the disease and therefore can be targeted as novel therapeutic targets in the future. Overexpression of several microRNAs in SLE $CD4^+$ T cells contributes to the DNA methylation defect described in previous sections. The expression of microRNAs miR-126 and miR-148a is increased in SLE $CD4^+$ T cells, and they both directly target and inhibit *DNMT1*.[63,64] MiR-21, which is also upregulated in SLE $CD4^+$ T cells, inhibits DNA methylation by targeting *RASGRP1*, an upstream component in the MEK/ERK signaling pathway that regulates *DNMT1* expression.[64] These data demonstrate the interaction between two distinct epigenetic mechanisms in SLE, DNA methylation, and microRNA regulation.

An example of downregulated microRNAs in SLE is miR-31. Downregulation of miR-31, which directly targets and represses RhoA, contributes to impaired IL-2 production in SLE T cells.[65] Another downregulated microRNA in SLE is miR-146a, which targets several IFN-related genes, such as *IRAK1*, *TRAF6*, *IRF5*, and *STAT1*.[66,67] Downregulation of miR-146a results in increased activation of type I IFN, a characteristic pathogenic feature of SLE.[67]

Of interest, a genetic association between the *miR146a* locus and SLE has been described, and the risk variant in this locus is associated with downregulation of miR-146a.[68] This is another example of genetic-epigenetic interaction in SLE, whereby a genetic variant might contribute to SLE pathogenesis by affecting the expression of an epigenetic affecter molecule. Another such example is the genetic polymorphism in the 3′UTR region in *TLR7* in patients with SLE, which is located in a binding site for miR-3148, suggesting that this genetic variant in *TLR7* might induce pathogenicity by altering the epigenetic regulation of *TLR7*. Indeed, the SLE-associated variant in this locus is associated with increased TLR7 mRNA and protein expression, and the mRNA expression levels of *TLR7* in SLE and normal PBMCs was inversely correlated with miR-3148 expression.[69]

## Rheumatoid Arthritis

Fibroblast-like synoviocytes (FLS) are thought to play an important role in rheumatoid arthritis (RA). FLS are activated and produce a number of pro-inflammatory cytokines in joints of patients with RA. Further, this pro-inflammatory phenotype is associated with increased production of specific matrix metalloproteinases into the synovial fluid, leading to joint destruction. Earlier studies showed that FLS in patients with RA are characterized by global DNA hypomethylation, and as a result, express L1 retrotransposable elements; this global hypomethylation is presumably due to decreased expression of DNMT1.[70] Treating normal FLS with DNA methylation inhibitors results in phenotypic changes resembling activated FLS from patients with RA, as well as in overexpression of several gene products, including miR-203, which is increased in RA FLS and correlates with levels of IL-6 production.[71] Multiple other microRNAs are also overexpressed in RA FLS, such as miR-155, which is similarly upregulated in RA PBMC and synovial macrophages.[72,73] MiR-155 is a pro-inflammatory microRNA because miR-155–deficient mice are resistant to collagen-induced arthritis.[73] MiR-223 is overexpressed in the synovium, in peripheral blood naïve $CD4^+$ T cells, and in the serum in patients with RA, and its expression levels in the serum correlate with disease activity in treatment-naïve early RA.[74-76]

The overall role of miR-223 as a therapeutic target in RA remains controversial. Overexpression of miR-223 suppresses osteoclastogenesis in vitro, suggesting a potential beneficial therapeutic effect of miR-223 overexpression[75]; however, suppressing miR-223 in vivo ameliorates arthritis, bone erosions, and osteoclastogenesis in mice with collagen-induced arthritis,[77] suggesting a pathogenic effect of this same microRNA molecule. Of interest, miR-146a, which is downregulated in SLE peripheral blood leukocytes, is overexpressed in PBMCs and FLS from patients with RA,[72,78] although the exact role of miR-146a in RA has not been clearly elucidated.

Global reduction in T cell DNA methylation levels and DNA methyltransferase activity has been described in patients with RA, but to a lesser extent than has been observed in active SLE.[79] Further, a genome-wide DNA methylation study in peripheral blood leukocytes from twin pairs discordant for RA failed to show DNA methylation

differences, contrary to SLE in the same study.[46] A demethylated "senescent" $CD4^+$ T cell subset ($CD4^+CD28^-$ T cells) is expanded in RA and overexpresses a number of proteins encoded by methylation-sensitive genes such as CD70, perforin, and KIRs.[80] How much and what causal role this T cell subset plays in RA is currently not clear, but it is unlikely to be specific for RA because this T cell subset is expanded in multiple chronic inflammatory diseases. It is interesting, however, that this $CD4^+CD28^-$ T cell subset is found in atherosclerotic lesions and is implicated in plaque development and rupture,[81,82] because RA is an independent risk factor for atherosclerotic cardiovascular disease.

Recent genome-wide DNA methylation studies in persons with RA have focused on elucidating DNA methylation differences between FLS from patients compared with control subjects who have osteoarthritis (OA). In one study examining genomic DNA isolated from FLS of six patients with RA and five control subjects with OA, a number of differentially methylated genes that could play a role in RA were revealed, including hypomethylation of *CASP1*, *STAT3*, *MMP20*, *TRAF2*, and *MEFV*, among others.[83] Hypomethylation was enriched in pathways related to cell migration, adhesion, and extra-cellular matrix interactions. Interestingly, several CG sites in the TNF encoding gene *(TNF)* were hypermethylated in RA FLS.[83] Another study examined and integrated changes in the DNA methylome with microRNA expression and gene expression analysis in FLS from six patients with RA and six patients with OA.[84] This study identified differential methylation in novel genes in RA, including hypomethylation in *IL6R*, *CD74*, *TNFAIP8*, and *CAPN8* and hypermethylation in *DPP4*, *CCR6*, and *HOXC4*, among others. These data also demonstrated inverse correlation between DNA methylation levels and gene expression in more than 200 genes in RA. Importantly, several novel dysregulated microRNAs were revealed in this study, such as miR-503, -551b, -550, and -625.[84]

## Primary Sjögren's Syndrome

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of exocrine glands, primarily salivary and lacrimal glands, resulting in dryness of the eyes and mouth. Global DNA methylation levels in labial salivary gland epithelial cells from patients with pSS is decreased, and this reduction correlates with lower DNMT1 levels and increased expression of the demethylating co-factor GADD45α.[85] The presence and extent of the DNA methylation defect in salivary gland epithelial cells in pSS correlates with the presence of B cell infiltrates, which was confirmed in vitro using salivary gland epithelial cell/B cell co-culture.[85] However, no global reduction in peripheral blood B cell or T cell methylation levels were detected.[85] Gene-specific DNA methylation studies in peripheral blood $CD4^+$ T cells in pSS revealed demethylation and overexpression of the co-stimulatory molecule CD70, similar to what had been previously observed in patients with SLE.[86] In contrast, *FOXP3* is hypermethylation and transcriptionally repressed in $CD4^+$ T cells from pSS, which is consistent with the Treg functional defect reported in pSS.[87]

The only genome-wide DNA methylation study published to date in pSS examined DNA methylation differences in more than 485,000 methylation sites in naïve $CD4^+$ T cells compared with age-, sex-, and ethnicity-matched control subjects.[88] This study identified 753 differentially methylated sites, with the majority being hypomethylated in pSS compared with control subjects. Several hypomethylated genes of interest in pSS include *CD247*, *TNFRSF25*, *PTPRC*, *GSTM1*, and *PDCD1*. Several IFN-regulated genes were also hypomethylated, consistent with an IFN signature in pSS.[88] *LTA*, which encodes lymphotoxin-α (LTα, or TNF-β) and promotes IFN production, was also hypomethylated in naïve $CD4^+$ T cells in pSS. LTα is overexpressed in the serum and salivary glands from patients with pSS, and a clinical trial to block the lymphotoxin pathway as a therapeutic option in persons with pSS is under way. An interesting group of solute carrier protein genes was also differentially methylated in naïve $CD4^+$ T cells from pSS.[88]

## Systemic Sclerosis

Systemic sclerosis (scleroderma) is an autoimmune disease characterized by immune activation, vasculopathy including microvascular endothelial cell dysfunction, and excessive collagen production and fibrosis of the skin and internal organs. Epigenetic studies in scleroderma have therefore focused on fibroblasts, microvascular endothelial cells, and $CD4^+$ T cells.[89,90]

Fibroblasts from patients with scleroderma underexpress FLi-1, a negative regulator of collagen synthesis,[91] which is hypermethylated in fibroblasts from patients with scleroderma compared with control subjects.[92] Further, H3 and H4 acetylation levels are reduced in the *FLI1* promoter region, probably contributing to its transcriptional repression. Treating scleroderma fibroblasts with DNA methylation inhibitors combined with histone deacetylase inhibitors increases *FLI1* expression and restores collagen production to levels comparable with that of normal fibroblasts.[92] These data suggest that hypermethylation and hypoacetylation of *FLI1* in scleroderma fibroblasts might be playing a role in the pathogenesis of this profibrotic disease. Indeed, DNMT1 protein levels and the protein levels of the histone deacetylases HDAC1 and HDAC6 were shown to be elevated in scleroderma fibroblasts,[92] which could explain the hypermethylated and hypoacetylated repressive status of *FLI1*. Recent data suggest that the Wnt pathway signaling antagonists genes *DKK1* and *SFRP1* are underexpressed and silenced by hypermethylation in scleroderma fibroblasts.[93] Importantly, inhibiting DNMT1 reduced canonical Wnt pathway signaling in scleroderma fibroblasts and reduced bleomycin-induced fibrosis in mice.[93] Multiple microRNAs have been shown to be dysregulated in scleroderma fibroblasts compared with control subjects, with several targeting components in transforming growth factor (TGF)-β signaling. For example, miR-21 and -146 are overexpressed, putatively targeting SMAD4 and SMAD7, respectively.[90]

A recent genome-wide DNA methylation study in skin fibroblasts from patients with diffuse and limited scleroderma identified a large number of differentially methylated genes between patients and age-, sex-, and

ethnicity-matched healthy control subjects.[94] It is interesting that despite previous reports showing increased DNMT1 expression in scleroderma fibroblasts, most DNA methylation changes observed using this genome-wide unbiased approach were hypomethylation changes in patients compared with healthy control subjects. This report revealed methylation changes that are common between diffuse and limited disease, and also subset-specific DNA methylation changes. Hypomethylated genes in both diffuse and limited scleroderma included collagen genes such as *COL4A2* and *COL23A1*, *PAX9*, *TNXB*, *ITGA9*, *ADAM12*, and the RUNX transcription family members *RUNX1*, *RUNX2*, and *RUNX3*. Increased mRNA expression levels in several of these hypomethylated genes was also demonstrated in the same study.[94]

Studies in microvascular endothelial cells in scleroderma demonstrated increased methylation in *BMPR2* encoding bone morphogenic protein receptor II, which plays a role in endothelial cell resistance to apoptosis. BMPR2 is underexpressed in microvascular endothelial cells from patients with scleroderma, and in vitro treatment with DNA methylation/histone deacetylase inhibitors restores expression levels of BMPR2 to levels similar to those of healthy control subjects.[95]

$CD4^+$ T cells in patients with scleroderma express less DNMT1 compared with healthy control subjects[96] and overexpress proteins encoded by methylation-sensitive genes such as *CD70* and *CD40L*, and the promoter sequences of these genes are hypomethylated.[97,98] This observation is very similar to a previous observation in SLE $CD4^+$ T cells.

## Behçet's Disease

Behçet's disease is an immune-mediated inflammatory disease characterized by recurrent oral-genital ulcers, inflammatory eye disease, skin involvement, central nervous system involvement, and recurrent thrombosis. The cause of Behçet's disease is not completely understood, but unsurprisingly, genetic and environmental factors play a role in the pathogenesis of the disease. A recent genome-wide DNA methylation study of Behçet's disease shed light on the epigenetic architecture of $CD4^+$ T cells and monocytes in patients with Behçet's disease who have active untreated disease compared with healthy age-, sex-, and ethnicity-matched control subjects.[99] The study identified key differentially methylated loci and pathways across the genome in Behçet's disease that can be targeted for the development of novel therapeutic interventions in the future. Several regulatory and structural cytoskeletal components showed consistent DNA methylation change in both $CD4^+$ T cells and in monocytes in this disease. Differentially methylated genes in Behçet's disease include *RAC1*, *RGS14*, *FSCN2*, among others. Moreover, these epigenetic changes were dynamic, because the study showed clear evidence for reversibility of DNA methylation changes when the same patients were examined at a subsequent time after disease remission.[99] Therefore, this study of Behçet's disease provides evidence that DNA methylation does change throughout the course of the disease and that some of these epigenetically altered loci can be explored as novel disease biomarkers and therapeutic targets.

## Osteoarthritis

Several recent genome-wide DNA methylation studies in articular cartilage tissue have been reported and suggest that epigenetic profiling can identify novel candidate genes and disease aspects, therapeutic targets, disease subsets, and epigenetic biomarkers for disease severity in OA.[100-102] In a study examining DNA methylation in ~27,000 CG sites in cartilage tissue from patients with OA in the knee compared with healthy control subjects, 91 differentially methylated CG sites were identified between the two groups. Importantly, genome-wide methylation data and gene expression profiling identified a cluster of patients with OA who had a more "inflammatory" disease.[100] These data suggested that epigenetic and transcriptional profiling can identify disease subsets in OA, which could help target specific therapeutic options in a more individualized manner in the future.

Another study examined DNA methylation changes in hip joint OA across more than 485,000 methylation sites and used a unique approach comparing eroded with noneroded cartilage tissue from the same joint.[101] This approach eliminates any confounding effect from genetic variants across the groups. Remarkably, ~40% of genetic risk variants previously reported in OA showed evidence of differential methylation in this study, suggesting that genetic–epigenetic interaction might play a role in the pathogenesis of OA and that genetic or epigenetic alteration in these same genes are associated with OA risk. The study identified a total of 550 differentially methylated genes in OA, with more than two thirds of them hypomethylated. Bioinformatic analysis to identify common regulating factors among these differentially methylated genes identified TGF-β1 and several microRNAs such as miR-128, -27a, and -9. Importantly, this study showed correlation between DNA methylation in 20 CG sites and histologic severity scores in OA.[101]

# FUTURE DIRECTIONS

Epigenetic dysregulation has been increasingly recognized as a major factor involved in the pathogenesis of rheumatic diseases. In the past few years we have witnessed a growing interest in studying epigenetic differences between patients with various autoimmune, inflammatory, and non-inflammatory rheumatic conditions and healthy control subjects. The availability of unbiased genome-wide approaches has broadened our knowledge and allowed the identification of novel epigenetically altered genetic loci that can help us better understand disease pathogenesis and identify novel targets for therapy.

With this plethora of epigenetic and epigenomic studies in rheumatology, several issues need to be kept in mind. Epigenetic changes are cell specific, and therefore, moving forward, it is of utmost importance that very specific cell subsets should be examined to accurately capture epigenetic differences between patients and control subjects that represent genuine disease-associated differences, rather than the effect of differences in cellular constituents or cell activation status between patients and control subjects. Adequately powered studies to address the clinical heterogeneity of the disease of interest, careful phenotyping of patients,

and careful selection of matched healthy control subjects should be emphasized.

The dynamic nature of epigenetic changes would allow for the development of novel disease biomarkers. Indeed, in rheumatology there is a paucity of reliable biomarkers. Future studies would focus and expand on disease subset–specific epigenetic changes. We hope to answer questions such as what specific DNA methylation changes could predict nephritis in patients with SLE and what specific epigenetic changes could predict the development of lymphoma in patients with Sjögren's syndrome or severe lung involvement in patients with scleroderma. Some of the epigenetic changes that we need to discover and validate in future studies can also help us assess disease activity, predict disease flares, or determine what treatment option to use in an increasingly personalized medicine approach. One way to achieve this goal is to conduct longitudinal epigenetic studies in rheumatology—that is, to follow the same group of patients over time rather than using the more common cross-sectional approach. A longitudinal approach would also help address the question of "cause" versus "effect" in the epigenomic changes we associate with the various diseases. Ideally, a longitudinal approach that would entail following up and collecting biological samples from a group of persons before disease develops would be most informative in dissecting the issue of causality.

Future studies in epigenomics in rheumatology should also more comprehensively address genetic-epigenetic interaction, as well as the interaction between environmental triggers of disease, epigenomic changes, and the disease genetic background. Studies have already been initiated to examine allele-specific epigenetic changes to understand how some genetic variants that are associated with diseases induce risk. These efforts will need to be expanded to a genome-wide level. An integrated "omics" approach that includes the genome, epigenome, transcriptome, and exosome (environmental exposures) would be very informative to comprehensively understand and better treat rheumatic diseases in the very near future.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 23

# Complement System



Leendert A. Trouw

**KEY POINTS**

- The complement system is a delicately balanced protein activation cascade that includes several regulators that prevent unwanted and disproportional complement activation under physiologic conditions.
- In several forms of arthritis, complement is activated in the joints.
- Deposition of autoantibodies represents an important trigger for complement activation.
- Measuring complement levels and activation fragments is useful in the diagnosis and follow-up of patients who have systemic lupus erythematosus, but it is not equally validated in many other rheumatic conditions.
- Complement proteins may play additional roles outside the traditional complement activation cascades.

The time when complement was mainly considered an antibacterial defense mechanism via the pore-forming membrane attack complex is long gone. In addition to great progress in understanding the molecular functioning of complement proteins, substantial changes have occurred in the possibilities of modulating complement activation therapeutically in patients. Therefore the goal of this chapter is to provide rheumatologists with a fresh view on the physiologic and pathologic role of the complement system in the context of rheumatic diseases. Furthermore, a large array of tests is currently available to monitor complement levels and complement activation, but the interpretation of these results is not always straightforward. This chapter will provide guidance in the interpretation of laboratory test results in the context of the several rheumatic conditions.

## FUNCTIONS OF THE COMPLEMENT SYSTEM

The complement system was discovered more than a century ago and until relatively recently was considered to be a mechanism only involved in the defense against infections. In the experiments that led to the identification of complement, scientists learned that a substance with the capacity to kill bacteria was present in the cell-free fraction of blood. This substance had properties that were very different from the properties of antibodies. The substance was heat labile, unstable, and present in all individuals, whereas antibodies were heat stabile and required prior exposure to or immunization with, for example, certain bacteria. Sets of mixing experiments finally revealed that for the observed lytic effects, both antibodies and this substance were required. Because this substance was considered to "complement" the action of the antibodies to mediate lysis and host protection, the name *complement* was coined.[1]

Given that the identification of complement was based on lysis experiments and that for many years the laboratory readouts for complement activity were based on lysis assays, an important role for lysis as an effector mechanism of complement was implied. Today it is understood that although complement-mediated lysis does take place in vivo and may contribute to tissue damage, defense against infections is not dependent on this mechanism, indicating that complement has several other effector mechanisms that may overall have more impact than the simple insertion of lytic pores. Opsonization and generalized immune activation via anaphylatoxins represent more important mechanisms used by complement to mediate host protection in concert with the remainder of the innate and adaptive immune system.[1]

By now it is clear that complement plays a role in innate immune defense and instruction of adaptive immune responses. In addition, it is important in clearance of waste, such as apoptotic and necrotic cells, as well as immune complexes, prions, and amyloid-β aggregates. Finally, complement is involved in other cascades and processes such as coagulation, tissue repair, angiogenesis, placentation, and tumor cell survival. Unraveling the contribution of complement activation to a clinical condition is often difficult, because a similar deposition of C3b could either contribute to tissue damage or stimulate regeneration. The molecule C1q can contribute to immune complex–mediated damage in persons with systemic lupus erythematosus (SLE), whereas deficiency of C1q is the strongest genetic risk factor for the development of SLE. Collectively, these examples illustrate that the interpretation of laboratory results, including the activity levels of the pathways, the presence of circulating activation fragments, or tissue depositions, is not always straightforward. Although our understanding of the overall role of complement in rheumatic diseases is far from complete, this chapter provides an overview of current insights.

## TERMINOLOGY USED TO DESCRIBE COMPLEMENT AND ITS ACTIVATION FRAGMENTS

For scientists and clinicians who do not work with complement on an everyday basis, the nomenclature used to

describe the different complement factors, especially the convertases and the activation products, may appear confusing. However, some logic can be found in the nomenclature. Under the auspices of the International Complement Society (ICS) and the European Complement Network (ECN), a standing committee dedicated to enhancing the use of standardized terminology for both old and newly discovered proteins recently completed a much-needed update.[2] Complement proteins are mostly indicated by a capital letter followed by a number and a lowercase letter suffix (e.g., C5a and C5b). The molecular part designated with the letter "a" indicates the smaller molecular fragment, whereas the designation "b" indicates the larger fragment. The only exception to this rule is C2, for which the large cleavage fragment is indicated by the letter "a" and the small part by the letter "b." The Nomenclature Committee for Complement reported that no consensus has yet been reached to change this aberrant usage of "a" and "b" for C2, and therefore in this chapter it is referred to in the original manner.[2]

The three activation pathways of complement—that is, the classical pathway, the lectin pathway, and the alternative pathway—converge to cleave and activate the central component C3 (Figure 23-1). After cleavage of C3, all three pathways use the same terminal pathway that generates the membrane attack complex (MAC; Figure 23-1).

The nine proteins of the first pathway to be discovered, the classical pathway, are designated with an uppercase letter C, followed by a number (e.g., C2). Although most of these numbers are in a logical numeric order, one exception exists—C4—which is cleaved together with C2 and before C3. The components of the lectin pathway are largely similar to those of the classical pathway, but the proteins unique to the lectin pathway do not have a similar logical nomenclature. For the alternative pathway, however, most of the proteins involved are indicated by the word "factor" followed by an uppercase letter (e.g., factor B). Enzymatically active component or protein complexes are often indicated by a bar above the name (e.g., $\overline{C3Bb}$). After inactivation by complement inhibitors, several complement proteins can no longer participate in further complement activation and are indicated with a lowercase prefix "i," indicating "inactivated" (e.g., iC3b).

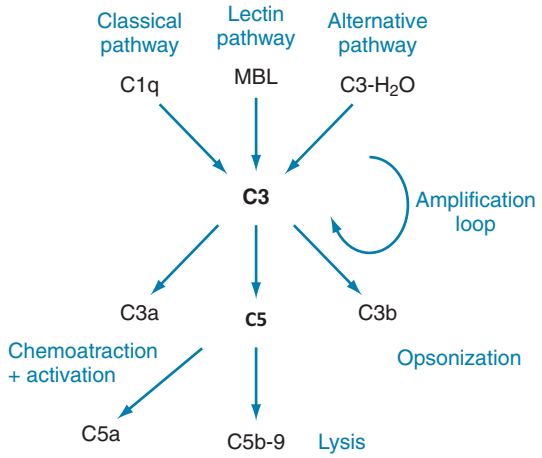



**Figure 23-1** Schematic overview of the different complement activation pathways. Key recognition molecules for each of the pathways are highlighted, as well as the key components and activation products. MBL, Mannan-binding lectin.

## ACTIVATION PATHWAYS

Traditionally, three different pathways of complement activation are distinguished: the classical pathway, the lectin pathway, and the alternative pathway (see Figure 23-1). In addition to these three pathways, several "shortcuts" collectively referred to as extrinsic complement activation also have been described and will be discussed only briefly.

The classical pathway becomes activated when its recognition molecule C1q binds to one of its ligands. Whereas initially mainly surface-bound immunoglobulin (Ig)M and complexed IgG were thought to be the main ligands for C1q, the list of relevant ligands has now been expanded and includes, among others, C-reactive protein (CRP), DNA, microbial components, and apoptotic and necrotic cells.[3] C1q is initially part of the C1 complex, which also comprises two C1r and two C1s molecules. After the binding of C1q to its ligands, a consecutive activation of C1r and C1s takes place.[4] The activated form of C1s now has the capacity to cleave C4 to C4a and C4b, exposing a hidden thioether residue that leads to the immediate covalent attachment of C4b on surfaces close to the place where C1q was binding to its ligands. This quick and covalent binding ensures that opsonization takes place at the right place.

Next, activated C1s also cleaves C4b-bound C2, generating C2a and C2b. The result of this cleavage is the formation of the classical pathway C3-convertase C4b2a. As the name implies, the C3 convertase is an enzymatic protein complex that has the capacity to cleave C3 into its active fragments C3a (an anaphylatoxin) and C3b (an opsonin), which, like C4b, bind instantly and covalently to nearby surfaces. C3 is considered to be the central component of complement activation because it is at this point that the three activation pathways converge to follow the same terminal pathway of complement activation, leading to the formation of the MAC (see Figure 23-1).

The lectin pathway is similar to the classical pathway, except for the fact that it uses different recognition molecules (mannan-binding lectin [MBL] and ficolins) and different serine proteases (the MBL-associated serine proteases [MASPs]; Figure 23-1). Most importantly, the ligands that are recognized by the lectin pathway are different and mainly comprise carbohydrate patterns. After binding of MBL, MASP-2 will cleave both C4 and C2, generating exactly the same C3 convertase as in the classical pathway.

The alternative pathway is truly different from the other two pathways and serves two purposes. It is an important activation pathway in its own right, using a tick-over mechanism, and it also serves as an amplification loop for the classical and alternative pathways. The tick-over mediated activation of the alternative pathway refers to the fact that a small fraction of the circulating C3 is hydrolyzed to $C3_{H2O}$. This hydrolyzed form of C3 exposes a binding site for factor B. After binding, factor B is cleaved by factor D, generating a fluid-phase C3-convertase $C3_{H2O}Bb$ that cleaves native C3 into C3a and C3b. Similar to C4b, this C3b also binds covalently to a surface and serves as the starting point for

the generation of a new C3 convertase, C3bBb. However, on host cells this initial binding of C3b is quickly neutralized by the action of several complement inhibitors, especially factor H, whereas insufficient inhibition of this alternative pathway initiation exists on the surface of foreign cells, resulting in strong deposition of C3b and C3 convertases, thereby making the difference between self and non-self.

Properdin is a molecule that stabilizes the alternative pathway C3 convertase by forming a complex, C3bBbP, which allows a longer half-life. However, properdin is now also thought to serve as a pattern recognition molecule that can localize complement activation to surfaces of pathogens or dead cells by attracting C3b, allowing the formation of the alternative pathway C3 convertase.[5] The amplification function of the alternative pathway is often neglected, but as much as 80% of the C3 deposited during complement activation via the classical or lectin pathway may be the result of the alternative pathway amplification loop.[6] This loop is activated simply by the deposition of C3b, which, independent of the pathway by which it was generated, serves as the starting point for more alternative pathway activation as the alternative pathway C3 convertase is built upon this bound C3b.

The terminal pathway of complement activation represents the common final pathway used by all three routes of complement activation. As more and more C3b fragments are generated by way of either the classical, lectin, or alternative pathway or its amplification loop, the C3 convertases also start to acquire additional C3b molecules, C4b2aC3b and C3bBbC3b. These complexes gain a unique property: they now can serve as C5 convertases that cleave C5 into C5a, a very potent anaphylatoxin, and C5b. C5b interacts with C6 and C7, and this complex becomes attached to the cell surface, followed by interaction with C8. However, it is not until several C9 molecules are inserted into the complex that a pore is actually formed in the cell membrane. This final complex is referred to as the MAC or as C5b9. Insertion of many copies of the MAC can lead to cellular activation, apoptosis, or lysis, depending on the dosage and the cell type involved.

As previously mentioned, other processes have been described that lead to complement activation via mechanisms that differ from the traditional complement pathways. Extrinsic complement activation refers to situations in which complement proteins can be cleaved, and hence activated, via noncomplement proteins such as plasmin, thrombin, elastase, and plasma kallikrein.[7,8] For example, cleavage of C5 by these proteases can yield bioactive C5a.[7,8]

## REGULATION OF COMPLEMENT ACTIVATION

Because the complement system is very aggressive and has the potential to be highly damaging, it is not surprising that the human body is equipped with a large array of complement regulators and inhibitors (Table 23-1). These proteins ensure that complement activation is limited in time and in place to ensure maximal effectiveness in fighting infections and clearing debris while minimizing the collateral damage to healthy host tissue.[9] One other important aspect of efficient regulation is the importance of maintaining sufficient levels of complement fragments so they are available to fight infections. In case of deficiencies of fluid-phase complement inhibitors factor I and factor H, the complement system becomes activated via the alternative pathway and does not stop until all complement is consumed, resulting in a secondary C3 deficiency. Both fluid-phase and membrane-bound complement inhibitors function primarily via a similar mechanism of putting a break on unwanted

**TABLE 23-1** Schematic Overview of the Main Complement Regulators

| Regulators | Alternative Names | Function |
|---|---|---|
| C1-INH | SERPIN1 | Inhibits C1r/s and MASPs |
| sMAP | MAP19 | Binds to MBL, competes with MASPs |
| MAP-1 | MAP44 | Binds to MBL/ficolins; inhibits C4 deposition |
| C4BP | C4b-binding protein | Accelerates decay of LP/CP convertases; cofactor for fI |
| Factor H | CFH | Recognizes self-surfaces; accelerates convertase decay; cofactor for fI |
| FHL-1 | Reconectin, CFHL1 | Accelerates convertase decay; cofactor for fI |
| MCP | CD46 | Membrane bound cofactor for fI |
| DAF | CD55 | Membrane bound accelerator of decay of convertases |
| CD59 | Protectin | Membrane bound protein; binds to C8 and C9; prevents assembly of TCC |
| CFHR-1 | FHR-1 | Recognizes self-surfaces and C5; inhibits C5 cleavage and TCC formation |
| Vitronectin | S-protein | Binds to C5b-9; prevents assembly of TCC |
| Clusterin | Apolipoprotein J; SP-40,40 | Binds to C7-C9; prevents assembly of TCC |
| Carboxypeptidase-N | | Degrades C3a and C5a to their des-Arg forms |

CFHR-1, Complement factor H–related protein 1; DAF, decay-accelerating factor; FHL-1, factor H-like protein 1; fI, factor I; MAP-1, MBL/ficolin associated protein-1; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; MCP, membrane cofactor protein; sMAP, small mannose-binding lectin–associated protein; TCC, terminal complement complex.

Modified from Ricklin D, Hajishengallis G, Yang K, Lambris JD: Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 11:785–797, 2010.

complement activation at several levels of the complement cascades: initiation, formation of C3 convertases, and MAC insertion.

Fluid-phase regulators circulate together with all the other complement proteins and prevent complement activation under physiologic conditions (Figure 23-2). C1 esterase (C1-INH) inhibits several enzymes of the classical and lectin pathways: C1r, C1s, and MASPs. One of the main fluid-phase inhibitors is factor H, which can serve both as a decay accelerator (decreasing the half-life of C3 convertases) or as a cofactor for the enzymatic degradation of C3b by factor I.[10] The degrading enzyme factor I is present in the circulation without a known inhibitor but only works to degrade C3b in the presence of a cofactor.[11] Once C3b is bound by, for example, factor H, then factor I comes into action and cleaves C3b to iC3b. The iC3b—that is, inactivated C3b—now can no longer serve as a starting point for the formation of a new C3 convertase, but it can still be recognized by complement receptors. Whereas factor H is the main fluid-phase regulator for the alternative pathway, C4b binding protein (C4BP) serves a similar role, mainly for the classical and lectin pathways.[12] Vitronectin and clusterin are fluid-phase regulators of the insertion of the MAC. Finally, carboxypeptidase-N quickly converts the potent anaphylatoxins C3a and C5a into less active, des-arginated forms.

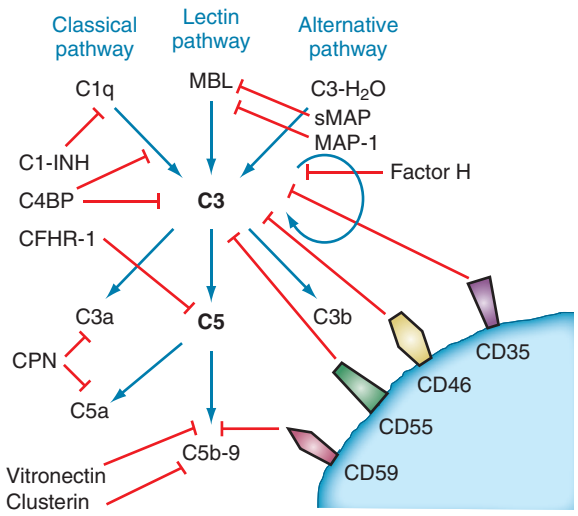



**Figure 23-2** Schematic overview of the different complement inhibitors acting on the complement cascade. Key complement inhibitors are highlighted, together with the parts of the complement cascade that they primarily inhibit. Note that factor I is not indicated; factor I is the enzyme that, together with some of the inhibitors highlighted in the scheme, actually cleaves and inactivates C3b and C4b. CPN, Carboxypeptidase N; MAP-1, MBL/ficolin associated protein-1; MBL, mannan-binding lectin; sMAP, small mannose-binding lectin–associated protein.

Membrane-bound regulators provide important protection against excessive complement attack on host cells (Figure 23-2). Most membrane-bound inhibitors work at the level of the C3 convertases, with the exception of CD59, which inhibits the insertion of the MAC into the cell membranes. Decay-accelerating factor (DAF; CD55) and complement receptor 1 (CR1; CD35) inhibit the action of C3 convertases by reducing their half-life. Membrane-cofactor protein (MCP; CD46) and CR1 serve as cofactors for the action of factor I.

## RECEPTORS FOR COMPLEMENT FRAGMENTS

Even though complement may be best known for its capacity to induce lysis via the MAC, its capacity to activate (immune) cells via complement receptors may actually be much more important in health and disease. Complement receptors (Table 23-2) are present on a large array of immune cells and stromal cells, and the integrated signals lead to a wide variety of biologic processes such as cellular activation, differentiation, and apoptosis. Three types of receptors can be distinguished depending on the type of ligands; C1q,

**TABLE 23-2** Schematic Overview of the Main Complement Receptors

| Receptors | Alternative Names | Function |
|---|---|---|
| CR1 | CD35; C3b/C4b receptor | Binds C3b/iC3b; induces phagocytosis: accelerates decay of convertases; cofactor for fI |
| CR2 | CD21; C3d receptor | Binds iC3b/C3dg/C3d; lowers threshold for B cell stimulation |
| CR3 | CD11b/CD18; Mac-1; integrin αMβ2 | Induces phagocytosis through interaction with iC3b; modulates IL-12 family in APCs |
| CR4 | CD11c/CD18; integrin αXβ2 | Induces phagocytosis through interaction with iC3b |
| C3aR | | Binds C3a; triggers pro/anti-inflammatory signaling |
| C5aR1 | CD88 | Binds C5a; triggers pro-inflammatory signaling |
| C5aR2 | C5L2, GPR77 | Binds C5a (strongly) and C5adesArg (weakly); might bind C3a/C3adesArg; function not fully defined |
| CRIg | Z93Ig, VSIG4 | Induces phagocytosis through interaction with iC3b/C3c; regulatory effect on C5 convertases |
| cC1qR | Calreticulin | Recognizes bound C1q; induces phagocytic signaling through CD91 |
| gC1qR | C1q-binding protein | Recognizes C1q; potential role in phagocytosis and signaling; modulates IL-12 on APCs |
| C1qRp | CD93 + unknown protein | Part of receptor complex that binds C1q and mediates phagocytosis |

APC, Antigen-presenting cell; fI, factor I; IL, interleukin.

Modified from Ricklin D, Hajishengallis G, Yang K, Lambris JD: Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* 11:785–797, 2010.

anaphylatoxins C3a and C5a, and C3 and C4 degradation products.

Several receptors for C1q have been proposed over the years, and most of these molecules actually do bind C1q, but whether they are essential C1q signaling molecules is sometimes debated. It is clear that C1q can have effects on cells, inducing migration and phagocytosis, but the nature or combinations of receptors and binding proteins remains the subject of further studies.

Detection of the anaphylatoxin C3a is mediated via the C3a receptor (C3aR), which is present on mast cells, smooth muscle cells, epithelial and endothelial cells, and cells of the myeloid lineage. Triggering of this receptor can lead to cellular activation, degranulation, and chemotaxis. Signaling via the C3aR may be either pro-inflammatory or anti-inflammatory depending on the context. Detection of anaphylatoxin C5a is mediated via the C5a receptor, C5aR1 (CD88). The C5a receptor is also present on a wide array of immune and nonimmune cells. Triggering results in strong chemotaxis, cellular activation, degranulation, and general immune activation. An alternative C5a receptor also exists, C5aR2 (also known as C5L2), which binds C5a strongly and $C5a_{desarg}$ weakly, and may also interact with $C3a/C3a_{desarg}$.

Cellular recognition of C3 and C4 fragments is mediated via the complement receptors CR1, CR2, CR3, and CR4. Despite their similar names, they have different structures, ligands, expression profiles, and function. CR1 (CD35), which is expressed on several immune cells and on erythrocytes, both enhances phagocytosis by binding to C3b and C4b and serves as a complement regulator that, via factor I, degrades its ligands.[13] CR1 is the only cofactor that allows a second cleavage of iC3b by factor I, generating C3dg. CR1 also serves an important role in transporting complement-opsonized immune complexes via erythrocytes. The C3b/C4b-opsonized immune complexes bind via CR1 to the erythrocytes (immune adherence), which deliver the immune complexes to the liver and spleen, where macrophage-like cells remove the immune complexes. These macrophages do not cleave the immune complexes but, rather, cleave CR1 to release the immune complexes from the erythrocytes.[14] The erythrocytes that re-enter the circulation therefore express less residual CR1 on their surface, a phenomenon sometimes observed in patients with lupus, in whom this immune complex transport and clearance is an important and frequent phenomenon. CR1 on granulocytes and monocytes will induce internalization and degradation of complement-opsonized immune complexes, whereas CR1 on follicular dendritic cells will result in the extra-cellular trapping and presentation of the immune complexes and antigens they contain.

CR2 (CD21) is expressed on B cells and follicular dendritic cells. On the B cells it serves as a co-receptor for signaling through the B cell receptor. The presence of C3d/C3dg on antigens reduces the threshold for B cell activation significantly, resulting in B cell activation and differentiation.[15]

CR3 (CD11b-CD18) and CR4 (CD11c-CD18) are integrin receptors expressed on myeloid cells that both bind iC3b. Both receptors strongly enhance phagocytosis, and CR3 is also involved in shaping the cytokine responses and immune activation in general.

# FUNCTIONS OF THE COMPLEMENT SYSTEM

## Innate Immune Responses

As was previously alluded to, complement plays an important role in the innate defense against invading micro-organisms. Whereas the MAC is responsible for complement-mediated lysis of myriad targets, it is surprising to see that C9 deficiency, which is relatively frequent in Japan, is only associated with infections with *Neisseria*. In contrast, deficiency for C3 is associated with a large array of recurrent infections. Together these findings indicate that the killing of microorganisms does not rely on the MAC, with the exception of *Neisseria*. Apparently, for all other types of infectious organisms, the innate immune defense uses mechanisms in addition to complement-mediated lysis to actually kill the microbes. This process is mediated largely via opsonization with C3b and cellular uptake via complement receptors and activation of immune cells via C3a and C5a. Complement also interacts with other systems of innate immune defense, such as the cellular Toll-like receptors (TLRs).[16] A clear two-directional interaction takes place between those systems; for example, triggering via the C3aR or C5aR/C5L2 may affect the cellular response to lipopolysaccharide (LPS) via TLR4.

Several proteins of the complement system also interact with the cascade of coagulation, collectively enhancing local clotting reactions to prevent the spread of possible pathogens. This bidirectional interaction involves, for example, C5a, which enhances the expression of tissue factor (TF)[17] and stimulates coagulation, whereas in the other direction thrombin has been shown to cleave C5 and generate C5a.[7]

## Clearing Immune Complexes and Apoptotic Material

The important contribution of complement toward clearance of immune complexes and dead cells became apparent from studies of complement-deficient persons. Deficiency of the early components of the classical pathway are associated with development of SLE, with greater risk associated with early components of the complement cascade (C1q–/– > C4–/– > C2–/–). When accumulation of apoptotic cells was also noted in C1q-deficient mice, the waste disposal hypothesis was put forward.[18] C1q can bind to apoptotic and necrotic cells and induce classical pathway activation.[19,20] In the absence of the early classical pathway components, apoptotic cells are not cleared efficiently.[21] Importantly, whereas early complement components bind to apoptotic cells to enhance opsonization and phagocytosis, the binding of fluid-phase inhibitors provides protection against excessive complement attack and lysis.[22]

Clearance of immune complexes is influenced by complement via two mechanisms. The first is the clearance of immune complexes to prevent their tissue precipitation,[23] a process mainly mediated via the classical pathway and transport via erythrocytes. The second mechanism involves solubilization of already existing immune complexes, which is mainly mediated via the alternative pathway. In addition to apoptotic material and immune complexes, many other forms of debris are cleared in ways that involve complement

activation, such as amyloid-β deposits, urate crystals, cholesterol crystals, oxidized lipids, and extra-cellular DNA.

### Regulating Adaptive Immune Responses

The complement system plays an important role in shaping the adaptive immune response. Whereas initially the focus was mainly on B cells and antibody responses, the focus has shifted to the T cell–dendritic cell interface, and now even toward the intra-cellular environment of T cells. C3dg, a degradation product of C3b, serves as a natural adjuvant by providing co-stimulatory signals to B cells via binding to CR2 in the B cell co-receptor complex.[24] In addition to activating B cells, CR2 is also involved in the capture and prolonged presentation of complement-opsonized antigens on follicular dendritic cells.[15] Complement also has an impact on the effector arm of antibodies, not just via the classical pathway, but also by influencing the expression levels of cellular Fcγ receptors. C5aR triggering alters the expression levels of activating and inhibiting Fcγ receptors in such a way that cells are more prone to respond to antibody triggering.[25] Interestingly, Fcγ receptor signaling can enhance synthesis of C5,[26] reinforcing the C5a-Fcγ receptor cross-talk.[25,26]

Direct effects of complement on T cell immunity were concluded from experiments with complement-deficient animals—for example, in the context of transplant rejection.[27] The local production of the anaphylatoxins C3a and C5a in the T cell–dendritic cell synapse highly determines the outcome of these cognate interactions.[28] Expression levels of complement inhibitory molecules such as DAF (CD55) have an impact on the degree of complement activation and hence the degree of dendritic cell and T cell activation. In addition, specific triggering of membrane-bound complement inhibitory molecules such as CD59 and DAF can limit their activation or even skew toward a regulatory T cell phenotype.[29,30]

### Measuring Complement Activation

Many different assays are available to monitor the activity and activation of complement and the antigenic levels of complement proteins. Depending on the clinical question, various assays or different combinations of assays are used. Deposition of activated complement fragments in target organs—for example, the renal glomeruli—provides important clinical information regarding the occurrence of lupus nephritis.

The most commonly used assays to determine the functional activity of complement are based on hemolytic assays for classical pathway and alternative pathway activity (CH50 and AP50). The assay for the classical pathway, CH50, records the ability of serum to lyse sheep erythrocytes that have been opsonized by rabbit IgG antibodies. The assay for the alternative pathway, AP50, records the ability to lyse 50% of rabbit erythrocytes in a buffer that does not allow the classical pathway to be active. The CH50 and AP50 determine the dilution of serum required to achieve this 50% lysis, and as such, these assays provide quantitation of overall activity from the initiation of the pathway to the insertion of the MAC. These assays can be used to screen for deficiencies but can also be used for the evaluation of disease activity and consumption of complement, as, for example, in the context of a flare in patients with SLE. More modern tests now provide platebound variants so that the classical, alternative, and lectin pathways can be screened for activity.[31] These assays are much easier to perform; however, they typically provide no quantitative insight but only a qualitative answer, and hence these assays are more suited to identify deficiencies than to monitor disease activity.

Current activation of complement can be concluded from the measurement of complement activation products—for example, C3a, C5a, C4d, C3d, and C5b-9. Monitoring of the levels of these markers over time allows evaluation of the underlying disease activity. However, in daily practice they are less often used because of the price, availability in routine laboratories, and difficulty of interpretation. As is the case with complement assays in general, the quality of the sample highly determines the reliability of the test. Complement is a heat-sensitive system in which some enzymes will relatively quickly lose their activity, and complement activation fragments may be generated during inappropriate handling of samples.

The antigenic screening for complement proteins C3 and C4 is regularly carried out in most laboratories around the world that perform routine tests, mostly based on nephelometric or turbidimetric assays. These robust assays are often used for the diagnosis and follow-up of patients with SLE in particular.

Several autoantibodies that react with complement proteins have been described.[32] In rheumatology, several autoantibodies such as anti-C1-INH, anti-CR1, and anti-Factor H autoantibodies have been described, but many laboratories that perform routine diagnostic tests offer only the detection of anti-C1q autoantibodies. These anti-C1q autoantibodies, which are relevant especially in the context of lupus nephritis,[33] will be further discussed in the section on SLE.

## COMPLEMENT DEFICIENCY

### Primary Complement Deficiency

Primary deficiencies have been described for nearly all complement proteins. The most common deficiencies are depicted in Table 23-3. Most deficiencies are inherited as autosomal recessive traits, except for the X-linked genes properdin and factor D, which display an autosomal dominant pattern. Almost all of the deficiencies are associated with increased risk for bacterial infections, although for some deficiencies this association only becomes apparent in otherwise immunocompromised hosts. At the time it came as a surprise that for the early classical pathway components—C1q, C4, and C2—their deficiency was highly associated with the development of clinical autoimmunity presenting as SLE-like syndromes.[34] Interestingly, this phenomenon is restricted to SLE, because these deficiencies have not manifested as Sjögren's syndrome, rheumatoid arthritis (RA), or vasculitis. Although SLE develops in a large portion of persons deficient for the early components of the classical pathway, only a very small fraction of all patients with SLE are genetically deficient for one of these early classical pathway components. During flares of their disease, many

**TABLE 23-3** Schematic Overview of the Main Complement Deficiencies

| Protein | Frequency | Disease Association |
|---|---|---|
| C1q | Rare; <100 cases | SLE; glomerulonephritis; infections |
| C1r or C1s | Rare; <50 cases | SLE; glomerulonephritis |
| C2 | 1:20,000 | SLE; infections |
| C4 | Rare; <50 cases | SLE; glomerulonephritis; infections |
| C3 | Rare; <50 cases | Recurrent infections; SLE; glomerulonephritis |
| MBL | Common; 1:10 | Susceptibility to infections |
| Factor D | Rare; <20 cases | Neisserial infections |
| Properdin | Rare; <100 cases | Meningococcal disease |
| C5/6/7/8 | Rare; <100 cases | Usually healthy; some *Neisseria* infections |
| C9 | 1:1000 in Japan | Usually healthy; some *Neisseria* infections |
| C1-INH | 1:50,000 | Hereditary angioedema |

*Estimated frequency in the Caucasian population (with the exception of protein C9).

MBL, Mannan-binding lectin; SLE, systemic lupus erythematosus.

Modified from Sturfelt G, Truedsson L: Complement in the immunopathogenesis of rheumatic disease. *Nat Rev Rheumatol* 8:458–468, 2012.

patients with SLE can display very low levels of these classical pathway components, but this phenomenon is related to immune complex–mediated activation and depletion and is hence a secondary deficiency, as outlined in the next section. C1q deficiency is most strongly associated with SLE (90%), followed by C4 (70%) and C2 (15%) deficiencies. C1q deficiency is rare, with approximately 70 cases currently known,[35] and C1r and C1s deficiency is also rare.[36] Next to increased risk for infections, these patients display a greater than 90% frequency of SLE.

The genes encoding C4 are polymorphic and have undergone ancestral duplication and mutation, giving rise to two genes designated *C4A* (encoding the acidic C4A) and *C4B* (encoding the basic C4B), which are not to be mistaken by the cleavage products C4a and C4b. It has been suggested that functional differences between *C4A* and *C4B* affect the risk for development of SLE, as well as the severity of disease. Homozygous deficiency of C4A, the form of C4 that can interact best with immune complexes, is a susceptibility factor for SLE.[37] In addition to complete genetic deficiency, low copy number variation also has been associated with increased risk for SLE,[38] but this association has not yet been well established.

C2 deficiency is relatively more common, with an estimated frequency of 1:20,000 in white populations.[39] The frequency of SLE in C2-deficient persons is approximately 15%, and the clinical presentation is suggested to be different from the SLE associated with C1q or C4 deficiency.[39]

Deficiencies of other complement factors such as C3, factor B, factor D, and properdin are particularly associated with severe infections.[40] Deficiencies of other factors such as MBL, with an estimated frequency of 1:10 in Caucasians, is associated with infections mainly in otherwise immunocompromised persons.[40] The question of whether MBL deficiency is associated with increased risk for SLE and RA remains controversial, but if any effect exists, it is most likely small. Interestingly, deficiencies of components of the MAC, such as C9, with an estimated frequency of 1:1000 in Japanese populations, do not lead to highly increased risk of infections in general but only to infections with *Neisseria*.[40]

## Secondary Complement Deficiency

Secondary, or acquired, complement deficiency is mainly the result of increased consumption via activation rather than decreased production. Secondary deficiency may occur as a consequence of lack of inhibition, such as in the absence of factor I or factor H, resulting in deregulated activation and depletion of the alternative pathway components, thus leading to a secondary C3 deficiency with an increased risk of infections.[41] However, by far most cases of (partial) secondary deficiency occur because complement activation is consuming more complement proteins than the liver and other tissues can synthesize. Especially in SLE, decreased levels of CH50 and AP50 are detected, which are proportional to decreased circulating levels of C1q, C3, and C4.[42] Hypocomplementemia is a bad sign in persons with SLE because it is related to complement consumption via activation on affected tissues such as the kidneys.[42] Decreased levels of classical pathway proteins are also observed in subsets of patients who have disorders such as mixed cryoglobulinemia and Sjögren's syndrome. In the latter condition, decreased levels of C3 and C4 have been repeatedly identified as markers of unfavorable outcomes, such as lymphoma, severe disease manifestations, and premature death.[43]

# TARGETED COMPLEMENT THERAPEUTICS

For rheumatic diseases, therapeutic intervention focused on complement is aimed either at (1) restoring normal complement function by replenishing deficient components or (2) inhibiting complement activation.

Reconstituting the primary deficiency is a method that has been used for many years. Either purified complement proteins such as MBL and C1-INH or fresh frozen plasma is infused to correct the missing factor. Many complement factors are produced by the liver, and it is worth noting that liver transplantation can reverse certain deficiency states, such as for MBL,[44] although obviously this is not a therapeutic option. However, for C1q, which is mainly produced by cells of hematopoietic origin such as macrophages and dendritic cells,[45] it is possible to reverse the deficiency phenotype completely through hematologic stem cell transplantation. This procedure has been successfully applied, resulting in restoration of circulating C1q levels and diminishment of lupus symptoms.[46]

Despite the important scientific insight that is provided by the association between complement deficiency and rheumatic diseases, it must be noted that this association represents a minute fraction of the patients who have these diseases. In line with what was called the *lupus paradox*, missing complement leads to SLE, but in most patients with SLE, complement contributes to tissue damage, and therefore complement inhibition might be the more appropriate

type of intervention. Several clinically approved interventions currently exist (reviewed in detail elsewhere).[47] One example is soluble CR1 (sCR1). CR1, which is mainly expressed on erythrocytes, mediates complement inhibition by serving as a cofactor for factor I–mediated inactivation of C3b and C4b and as an accelerator of the decay of C3 convertases.[13] Interestingly, the soluble version of CR1 also retains these activities, and modified forms of sCR1 have proven to be effective for the treatment of myocardial infarction.[48] Another main target of intervention is C5—either the C5 molecule itself or the interaction of C5a with the C5a receptor using blocking antibodies or peptides. A humanized antibody to C5 was developed and has been used successfully in the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), and it is currently being tested for several other conditions.[47] Peptides preventing the interaction between C5a and the C5a receptor have been tested in a small study in persons with RA, with no major effects.[49] Currently, blocking antibodies against the C5a receptor are being tested in clinical trials. Complement therapeutics in the context of RA will be discussed in more detail later.

## COMPLEMENT IN RHEUMATIC DISEASES

### Systemic Lupus Erythematosus

The involvement of complement activation in SLE is easily appreciated; not only are affected tissues decorated by activated complement fragments, but serum levels of several key complement components are decreased because of consumption.[42] As mentioned earlier, the lupus paradox makes it difficult to establish if complement plays an essential role in human SLE. On the basis of the tissue deposition of C1q and the heavily decreased levels of circulating C1q during a flare, one would assume that C1q would play an essential role by binding to deposited immune complexes and activating the classical pathway. However, C1q-deficient patients are not protected from lupus. On the contrary, they have a very high chance of developing SLE-like disease, which likely indicates that C1q is involved in two different processes. One process relates to the onset/development of SLE, and the other process relates to the final pathways of tissue destruction that are known to involve, but not totally depend on, complement activation.

SLE is mainly characterized by a breakdown of tolerance against nuclear components, such as double-stranded DNA. Immune complexes are formed between these anti-nuclear antibodies and their antigens, which are released from dead cells. These immune complexes deposit in tissues, where complement activation contributes to tissue damage by the formation of the MAC and the release of pro-inflammatory mediators such as C5a. In addition, the immune complexes stimulate plasmacytoid dendritic cells (pDCs) to produce type I interferons, further stimulating the disease process.[50] Lupus nephritis is a complicated process that is reviewed in detail elsewhere.[51] Substantial clinical information is available for lupus nephritis with regard to the relationship with tissue deposition of complement, decreased circulating levels, and clinical flares of the nephritis.

Circulating levels of C3 and C4 are included in the disease activity index SLEDAI.[52] Measuring cell-bound complement activation fragments, rather than decreased circulating levels of complement, is suggested as possibly being a more sensitive way to analyze complement activation in SLE,[42] but thus far this method has not replaced established assays in laboratories around the world that perform routine testing.

Anti-C1q autoantibodies can be detected in SLE, and their presence is associated with lupus nephritis.[53] In particular, the absence of anti-C1q autoantibodies is strongly associated with lack of renal involvement.[54] Because anti-C1q autoantibodies can also occur in healthy persons who do not have any renal problems, for many years it was unclear how anti-C1q autoantibodies could contribute to renal disease in persons with lupus.[33] However, mouse studies have revealed that anti-C1q autoantibodies only contribute to renal damage if sufficient immune complexes containing C1q are present in the glomeruli.[55]

Because complement seems to play such a prominent role in lupus nephritis, clinical trials have been conducted to study whether blocking complement would be beneficial.[56] However, the first small study on this topic did not reveal an effect during a relatively short follow-up period.[56]

### Rheumatoid Arthritis

A contribution of complement to the pathogenesis of RA can be concluded from the observed complement tissue deposition,[57] decreased levels of complement components in synovial fluid,[58] and the presence of complement activation fragments,[59,60] as previously reviewed.[61] However, the pronounced hypocomplementemia that is observed during flares in SLE is not observed in persons with RA. In addition, the complement deficiencies of the classical pathway that are associated with SLE may involve some arthritis but do not predispose to RA. Whereas over the years different views have existed regarding the contribution of autoantibodies, B cells, T cells, and other immune players, currently an emphasis has been placed on B cells and the autoantibodies they produce.[62] In addition to the well-known rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPAs)[63] and the recently identified antibodies against carbamylated proteins (anti-CarP autoantibodies)[64] are thought to be involved in the disease process.

Interestingly, these antibodies can already be detected in serum many years before the diagnosis of RA is made.[65,66] In addition, tissue-specific autoantibodies such as anti–type II collagen may be involved in a subset of patients.[67] A contribution of these autoantibodies is inferred from the clinical associations between the presence of the antibodies and more severe joint damage.[64,67,68] However, strong experimental evidence is currently lacking to confirm a directly pathogenic role for these autoantibodies.

In vitro, ACPAs have been shown to be able to trigger complement activation.[69] Interestingly, this action was mediated not just via the expected classical pathway but also via the alternative pathway of complement activation.[69] In mouse models for arthritis, it actually seems that only the alternative pathway is essential for antibody-mediated arthritis to develop, without any essential contribution of the classical pathway.[70]

Complement activation fragments can be detected in persons with RA, but the concentrations are much higher

in synovial fluid compared with serum.[59,60,71-73] These data indicate that complement is activated not systemically but mainly locally in the affected joints. The cause of this local complement activation is not known but likely involves several sources that are not mutually exclusive. Immune complexes formed by autoantibodies binding to joint specific antigens, dead cells that accumulate during chronic inflammatory reactions in the joint, or matrix components released from damaged cartilage may all contribute to the observed complement activation.[67,69,74,75] Release of C3a and C5a, as well as sublytic insertion of the MAC, may activate immune cells, along with synoviocytes. In addition, insertion of the MAC may be involved in the generation of citrullinated antigens in the joint,[76] possibly providing antigen for ACPAs to bind to and subsequently form immune complexes.

From animal models of arthritis it may be concluded that complement plays an essential role in the development of full-blown arthritis.[61] However, at present it is not known if complement activation plays an essential role in arthritis in humans. Several clinical intervention studies aimed at blocking complement activation or inhibiting the signaling via complement receptors have been performed or are currently under way. Peptide inhibition of C5a–C5a receptor signaling has thus far been unsuccessful.[49] Humanized monoclonal antibodies targeting C5 have been proven to be well tolerated in a phase I study and additionally showed beneficial effects in a phase II study.[56] However, thus far this biologic agent has not reached clinical use, most likely for economic reasons. Other studies using a blocking antibody to the C5a receptor are currently under way. Many experimental treatments that rely on different modes of action are being tested in pre-clinical models and include active vaccination against C5,[77] locally produced blocking minibodies,[78] or targeted inhibition using constructs consisting of CR2 to bind to activated complement fragments, as well as use of factor H domains to inhibit complement.[79,80]

For other forms of arthritis, the body of evidence supporting a role for complement in the disease processes is small.[81] For example, in psoriatic arthritis, increased levels of C3 were reported with no signs of activation, likely as a result of the acute-phase response.[82] Authors of several studies reported that complement activation in osteoarthritis (OA) and spondyloarthritis either did not exist or was limited, with only low levels of complement activation in reactive arthritis.[57,82,83] More recently one paper described a role for complement activation in OA.[84] However, overall it seems that determination of the relative contribution of complement activation to the disease processes underlying these conditions would benefit from additional studies, especially in patients.

### Other Systemic Rheumatic Conditions

In many and perhaps all systemic rheumatic conditions, a role for complement can be concluded on the basis of either changed serum levels or tissue deposition of activated complement components.[81] In several conditions hypocomplementemia—that is, low C3 and/or C4—have been included in criteria and disease activity scores.[34] However, the mechanism responsible for the observed effects on complement and how this contributes to the overall disease process in these systemic rheumatic conditions remains to be established.

## CONCLUSION

In this chapter an overview of the current understanding of the role played by the complement system in health and in rheumatic conditions is provided. For many years the role of complement in rheumatic diseases was largely limited to measuring complement consumption as biomarkers of disease activity. Now, as the first drugs that target complement activation are becoming available in the clinic, a new era has begun as we begin to understand the extent to which complement activation plays an essential role in disease processes. Because of all the positive effects of complement in the body, it will need to be established whether long-term complement inhibition in rheumatic diseases will ever become standard treatment or whether it will be limited to specific clinical conditions, such as a flare of lupus nephritis. In addition, the complement biomarker field is developing rapidly, and choosing the right biomarker for a specific clinical problem is an interesting future challenge.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 24



# Prostaglandins, Leukotrienes, and Related Compounds

Robert B. Zurier

**KEY POINTS**

- Eicosanoid biosynthesis is catalyzed by cyclooxygenases and lipoxygenases.
- Conversion of the endoperoxide intermediate prostaglandin $H_2$ requires activity of specific terminal synthases.
- Eicosanoids and their receptors regulate inflammatory and immune responses.
- Eicosanoid synthesis is modified by administration of precursor fatty acids.

The addition of oxygen to arachidonic acid and other polyunsaturated fatty acids that are not bound to membrane phospholipids in nearly all human cell types results in the formation of several classes of bioactive products termed *eicosanoids*. Eicosanoids include prostaglandins (PGs), prostacyclin (PGI), thromboxanes (TXs), leukotrienes (LTs), and lipoxins (LXs). All of these compounds are crucial to the regulation of immunity and inflammation, among other physiologic and pathologic processes. Although eicosanoids are derived from C20 polyunsaturated fatty acids (eicosa = 20), only a small percentage of these polyenoic acids form the eicosanoids: dihomo-γ-linolenic acid (DGLA), which is 8,11,14-eicosatrienoic acid; arachidonic acid (AA), which is 5,8,11,14-eicosatetraenoic acid; and eicosapentaenoic acid (EPA), which is 5,8,11,14,17-EPA (Figure 24-1).

Two groups of fatty acids are essential to the body: the omega-6 series derived from linoleic acid (18:2 n-6) and the omega-3 series derived from α-linolenic acid (18:3 n-3). The *n* refers to the number of carbon atoms from the methyl (omega) end of the fatty acid chain to the first double bond (i.e., omega-3 and omega-6 designations). Using this notation, *18* refers to the number of carbon atoms in the fatty acid. The degree of unsaturation (the number of double carbon-carbon bonds) follows the number of carbon atoms. Fatty acids are metabolized by an alternating sequence of desaturation (removal of two hydrogen atoms) and elongation (addition of two carbon atoms). Membrane phospholipids are the main storage site for polyunsaturated fatty acids and are particularly rich in eicosanoid precursors, which are located at the sn-2 position (Figure 24-2). Because mammalian cells cannot interconvert n-3 and n-6 fatty acids, the composition of membrane phospholipids is determined by exogenous sources of fatty acids.

## BIOSYNTHESIS OF EICOSANOIDS

### Phospholipases

The presence of phospholipase A2 ($PLA_2$) in lysosomes or bound to cell membranes catalyzes the breaking of the sn-2 bond, facilitating release of AA or other polyunsaturated fatty acids (see Figure 24-2). The enzyme is crucial to regulation of eicosanoid synthesis because it is in the nonesterified state that the polyunsaturated precursors enter into the cascades, leading to eicosanoid formation. Only a scant amount of oxidation at carbon 15 of AA occurs catalytically when the fatty acid is still covalently bound as part of a phospholipid.[1]

A large number of $PLA_2$ isoforms have been characterized and grouped based on primary structure, localization, and $Ca^{2+}$ requirements.[2] Cytosolic $PLA_2$ ($cPLA_2$) group IV is the major catalyst of arachidonate release that leads to PG and LT production. The structure of $cPLA_2$ reveals a monomeric cytosolic protein with a molecular size of 85 kD. Lysophospholipids "left over" after the action of $PLA_2$ are direct precursors of platelet-activating factor (PAF), a potent mediator of inflammation, which is generated by acylation (i.e., the addition of fatty acid) in the open sn-2 position of the lysophospholipid.

Four distinct types of $PLA_2$ activities hydrolyze fatty acids esterified at the sn-2 position. Secretory $PLA_2$ ($sPLA_2$) has small disulfide cross-linked proteins that require $Ca^{2+}$ in millimolar concentrations for optimal activity. $cPLA_2$ has larger proteins requiring $Ca^{2+}$ in micromolar concentrations, which are AA selective and can deacylate diacylphospholipids completely, preventing accumulation of potentially toxic lysophospholipids. $Ca^{2+}$-independent $PLA_2$ ($iPLA_2$) exhibits specificity for plasmalogen substrates. Finally, PAF acetylhydrolase (PAF-$PLA_2$) has a series of isozymes specific for short chains.[3,4]

Under basal conditions, AA is liberated by $iPLA_2$ and then reincorporated into cell membranes (reacylated). It is unavailable for appreciable eicosanoid biosynthesis. The acylase enzymes competitively inhibit the cyclooxygenase (COX) isoenzymes. After receptor activation and cell stimulation, intra-cellular $Ca^{2+}$ levels increase and the $Ca^{2+}$-dependent $cPLA_2$ liberates arachidonate at a rate that exceeds the rate of reacylation, leading to arachidonate metabolism by COX isoenzymes and by lipoxygenases (LOs). Initial formation of prostaglandin (PG)$E_2$ seems to be due to preferential coupling among $cPLA_2$, COX-1, and cytosolic PGH-PGE isomerase. More intense inflammation leads to participation of secreted $sPLA_2$ and amplification

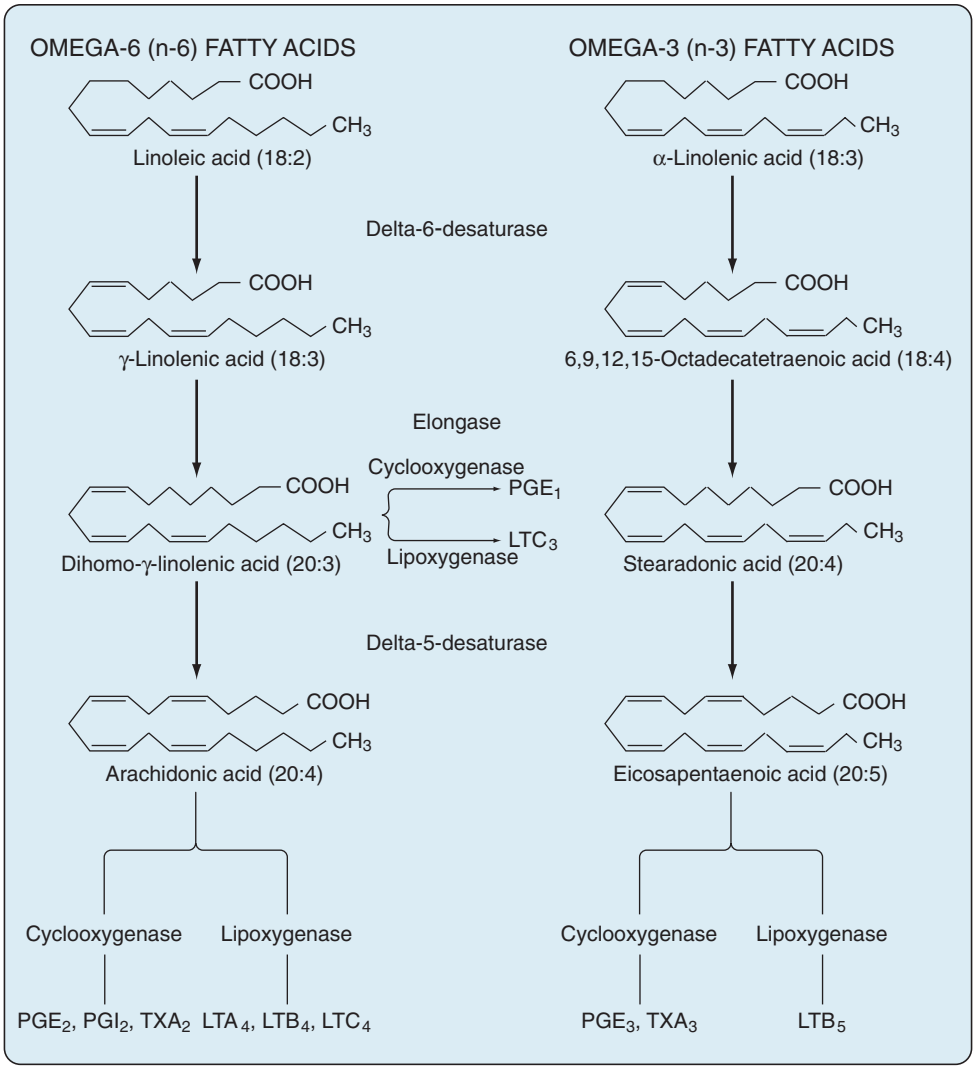



**Figure 24-1** Metabolic pathways of essential fatty acids. The pathways are ones of progressive desaturation alternating with elongation. Eicosanoid precursors include dihomo-γ-linolenic acid, arachidonic acid, and eicosapentaenoic acid. LT, Leukotriene; PG, prostaglandin; TX, thromboxane.

of $PGE_2$ biosynthesis by inducible COX-2. It is an oversimplification to regard the availability of AA as the sole rate-limiting step in cellular eicosanoid biosynthesis.

Phospholipase C (PLC) hydrolyzes the polar head group (e.g., inositol and choline) from phospholipids to yield diacylglycerol (DAG) and the polar head group. Direct protein isolation and molecular cloning studies have revealed multiple PLC isozymes in mammalian tissues. Phosphatidylinositol-PLC occurs in cytosolic (cPLC) and secreted (sPLC) forms and can be divided into three major classes (PLC-β, PLC-γ, and PLC-δ) based on substrate specificity. PLC with specificity for phosphatidylinositol and phosphorylated phosphatidylinositol is a key component of phosphatidylinositol-mediated signaling pathways. DAG is an activator of protein kinase C (PKC), and rapid production of this lipid by phosphatidylinositol-PLC hydrolysis of the phosphorylated phosphatidylinositol pool is a primary step in signaling. Further AA is made available by the sequential actions of diglyceride lipase and monoglyceride lipase.[5] PLC with activity on phosphatidylcholine has also been identified. Peripheral blood monocytes from patients with rheumatoid arthritis (RA) exhibit greater $PLA_2$ and PLC activity than do cells from healthy volunteers. $PLA_2$ concentrations did not correlate with disease activity, but the greatest increases in PLC enzyme activity were seen in cells from patients with the most severe, persistent, proliferative disease, not in cells from patients with the most active disease at the time the cells were studied.[6] In accord with the need for balance in regulation of the inflammatory response, $sPLA_2$, numbering nine members in humans, has both inflammatory and anti-inflammatory actions. Thus group V $sPLA_2$ counters the inflammatory activity of group IIA $sPLA_2$ by regulating cysteinyl LT synthesis and promoting immune complex clearance in a murine model of autoimmune erosive inflammatory arthritis. It is of interest that concentrations of IIA $sPLA_2$ are far greater than those of V $sPLA_2$ in synovial fluid from patients with RA.[7] In keeping with the fact that nothing in biology and the pathogenesis of disease is simple is the observation that treatment of patients who have RA with sPLA(2)-IIA inhibitors has only transient benefit and that sPLA(2)-IIA has activity (induction of COX-2) independent of its enzyme activity.[8]

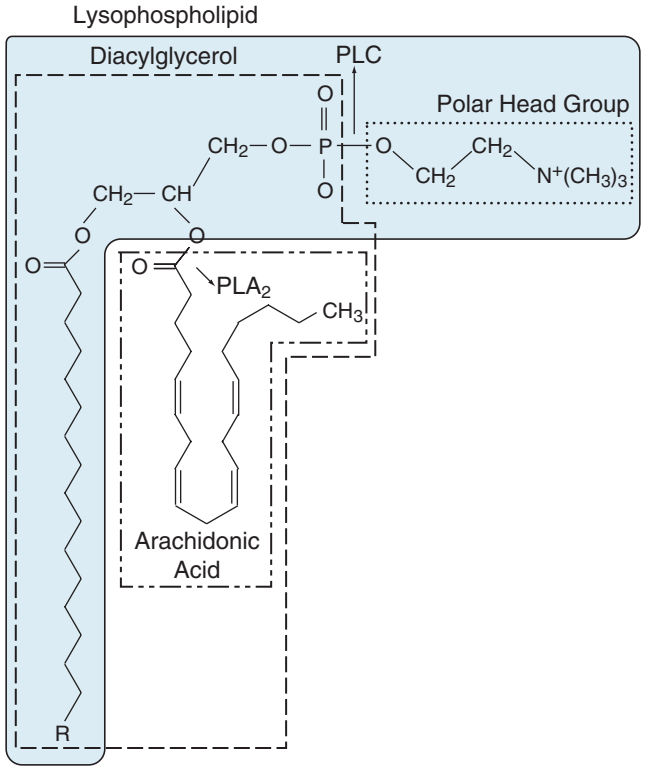



**Figure 24-2** Arachidonic acid release from phospholipid. Phosphatidylcholine, the major membrane storage site for polyunsaturated fatty acids, is depicted. $PLA_2$, Phospholipase $A_2$; PLC, phospholipase C.

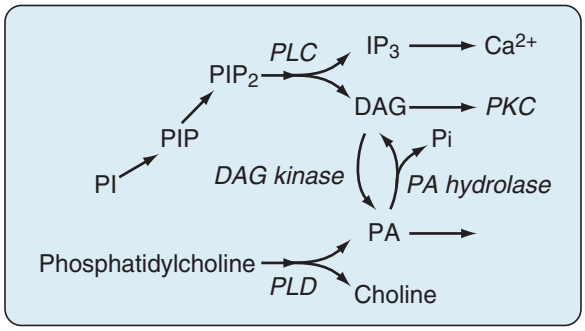



**Figure 24-3** Reactions catalyzed by phospholipases C and D, illustrating interconversion of diacylglycerol (DAG) and phosphatidic acid (PA). $IP_3$, Inositol-1,4,5-triphosphate; Pi, inorganic phosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; $PIP_2$, phosphatidylinositol-4,5-bisphosphonate; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D.

Hydrolysis of phospholipids by phospholipase D (PLD) produces phosphatidic acid (PA) and the respective polar head groups. The capacity of cells to interconvert PA and DAG through the action of specific cellular phosphatases and kinases (Figure 24-3) suggests that AA release from DAG and a variety of intra-cellular signaling and protein-trafficking events may be regulated by PLD activity. The targets of phosphorylation within the $PLD_2$ molecule that are key to its regulation have been mapped. PLD may be activated after or independent of PLC activation.[9]

The tetraenoic precursor (AA) is the most abundant of the three precursor fatty acids in cells of persons who eat usual Western-style diets. Metabolites of AA constitute the "2" series (dienoic) PGs (two double bonds in the molecule), and the metabolic pathway has acquired the familiar name "arachidonic acid (AA) cascade." However, diets enriched in eicosapentaenoic or γ-linolenic acid, the other eicosanoid precursors, lead to formation of different eicosanoids. Figure 24-4 illustrates the COX and 5-lipoxygenase (5-LO) pathways of the cascade.

Because phospholipases are involved in cell signaling and in acute inflammation, which sometimes escapes regulation and resolution, inhibitors of $sPLA_2$, $cPLA_2$, and PLD are being developed. It will be important to improve the selectability of these inhibitors.[10]

## Cyclooxygenase Pathway

The first step in the biosynthesis of the "prostanoids" (e.g., PGs, TXs, and PGI) is catalyzed by the bifunctional PG endoperoxide synthase 1 isozyme (also known as prostaglandin G/H synthase 1 [PGHS-1] and COX-1) and PGHS-2 (COX-2). COX is a homodimeric enzyme that integrates into only a single leaflet of the lipid bilayer of the cell membrane. The COX active site is a long hydrophobic channel. Aspirin and most other nonsteroidal anti-inflammatory drugs (NSAIDs) exclude arachidonate from the upper portion of the channel.[11] To form the characteristic five-carbon ring structure (TXs contain a six-member ring), the precursor fatty acids must have double bonds at carbons 8, 11, and 14 (numbering from the carboxyl group). When a molecule of oxygen is inserted across carbons 9 and 11, ring closure occurs enzymatically across C8 and C12, creating the unstable PG endoperoxide PGG. Subsequent peroxidation yields PGH with formation of the cyclopentane ring. PGH serves as the common precursor for PGs, PGI, and TXs that are formed under the influence of terminal synthases (see Figure 24-4). In addition to the activity of phospholipases, regulation of PG synthesis also occurs at the level of PGHS gene expression. PGHS levels are increased by interleukin (IL)-1, platelet-derived growth factor, and epidermal growth factor, which are agents that increase PG synthesis.

Cell membranes constitute the source of substrate AA and the site of action of eicosanoid-forming enzymes. PG synthesis can also form at lipid bodies, which are non–membrane-bound, lipid-rich cytoplasmic inclusions that develop in cells associated with inflammation. Lipid bodies isolated from human monocytes express PGHS activity, are reservoirs of arachidonyl phospholipids, and can function as domains of PG synthesis during an inflammatory reaction.[12]

PGHS, the well-known target of NSAIDs, exists in two isoforms. These isoforms are similar in terms of amino acid identities (about 60%), catalytic properties, and substrate specificity, but they differ in their genomic regulation.[13]

### Regulation of Cyclooxygenase-1 Expression

The gene for COX-1 *(PTGS1)* is preferentially expressed constitutively at high levels in selected cells, including endothelium, monocytes, platelets, renal collecting tubules, and seminal vesicles. Because the expression level of the enzyme does not vary greatly, it has been difficult to study

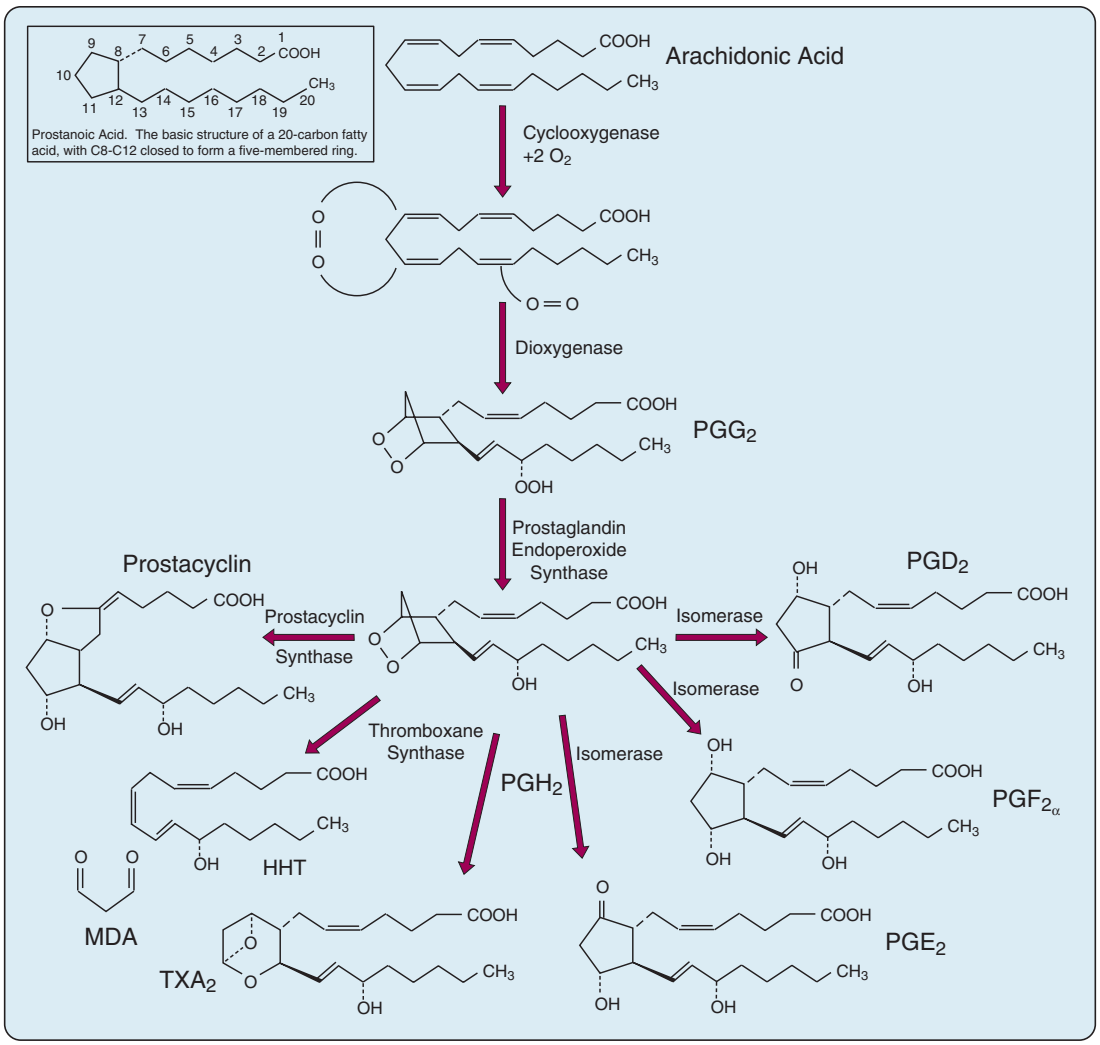



**Figure 24-4** Cyclooxygenase pathway of arachidonic acid metabolism. HHT, Hydroxyheptadeca-trienoic acid; MDA, malondialdehyde; PG, prostaglandin; TX, thromboxane.

its transcriptional regulation. The gene has a TATA-less promoter that contains multiple start sites for transcription. It is also known that Sp1/Cis regulatory elements in the promoter bond the Sp1 transcription factor to induce COX-1 gene expression. In addition, COX-1 and COX-2 splice variants may function in tissue-specific normal and pathologic processes and may represent new targets for therapy.[14] The localization of COX-1 in nearly all tissues under basal conditions suggests that its major function is to provide eicosanoids for physiologic regulation, which is seen clearly in platelets that do not have nuclei and cannot produce an inducible enzyme on activation. Rather, TXs are produced constitutively so that platelet aggregation can be completed.

### Regulation of Cyclooxygenase-2 Expression

The regulated formation of eicosanoids implies that cells have an appreciable ability to amplify the rate and amount of eicosanoid synthesis. Several processes contribute to that regulation, including silencing of $sPLA_2$ expression by COX-2 and autoinactivation ("suicide inactivation") of COX-2 and other oxygenases and synthases. In addition, the COX-2 transcript contains at least 12 copies of the AUUUA RNA motif, which makes it unstable and subject to rapid degradation. Factors that regulate COX-2 expression are specific for the physiologic processes involved. Expression of COX-2 in the macula densa in the kidney depends on luminal salt concentrations. Transcriptional activation of the COX-2 gene by mediators of inflammation such as IL-1β and tumor necrosis factor (TNF) is likely regulated by the transcription factors nuclear factor-κB (NF-κB) and CCAAT/enhancer binding protein (C/EBP). Perhaps the most crucial of the several demonstrated regulatory sequences in the 5′ flanking regions of the gene for COX-2 is the activating transcription factor/cyclic adenosine monophosphate (cAMP) response element (ATF/CRE), a site that is activated by the transcriptional activator protein-1 (AP-1) and the cAMP regulatory binding protein. Now that quantitative polymerase chain reaction analysis is routinely used to examine gene expression, it is clear that COX-2 is constitutively expressed under basal conditions at levels too low to be detected by Northern blot analysis.[15] Depending on cell and tissue specificity, several signaling

pathways (kinases, Rho, cyclic guanosine monophosphate, and Wnt) and transcription factors (NF-κB, AP-1, and NF of activated T cells [NFAT]) are involved in COX-2 expression.[16]

COX-1 and COX-2 affect a balance in several physiologic and pathologic situations. Of particular interest are their actions in the kidney and stomach. During times of low blood volume, the kidney releases angiotensin and other factors to maintain blood pressure by systemic vasoconstriction. Angiotensin also provokes PG synthesis in the kidney. COX-1, expressed in vessels, glomeruli, and collecting ducts, produces vasodilating PGs, which maintain renal plasma flow and glomerular filtration during conditions of systemic vasoconstriction. In the antrum of the stomach, COX-1 leads to production of PGs, which increase gastric blood flow and mucus secretion. Inhibition of COX-1 by NSAIDs prevents these protective mechanisms and results in renal ischemia and damage and gastric ulcers (mainly antral) in susceptible persons. These observations have led to the development of NSAIDs that selectively inhibit COX-2 and spare COX-1. AA gains access to the active site of the COX via a hydrophobic channel, and access is blocked by insertion of an acetyl residue on serine 530 in COX-1 and serine 516 in COX-2. The irreversibility of the interaction and the unique expression of COX-1 in the anucleate platelet is the reason for the clinical efficacy of low-dose aspirin. Nonacetylated NSAIDs compete with arachidonate for the active site and can interfere with the sustained effects of aspirin. Although the structures of COX isozymes are similar, COX-2 is characterized by a side-pocket extension to the hydrophobic channel, which is where the selective COX-2 inhibitors localize.[13]

The major adverse effects of NSAIDs, gastroduodenal injury, and impaired renal function, as well as the induction of myocardial infarction and stroke, are caused by inhibition of COX-1, whereas the analgesic and anti-inflammatory activities of NSAIDs rest in part on their ability to inhibit COX-2. However, COX-2 also seems to have a regulating role in renal, brain, gastrointestinal, ovarian, and bone function. COX-2 is also expressed in endothelial cells, and its inhibition suppresses prostacyclin synthesis by endothelial cells.[17] COX-2 acts in the initiation and resolution of inflammation. Its expression increases transiently early in the course of carrageenan-induced pleurisy in rats. Later in the response, COX-2 is expressed at even higher levels, leading to synthesis of $PGD_2$ and its dehydration product 15-deoxy-δ12,14-$PGJ_2$ (15δ$PGJ_2$). Early expression of COX-2 is associated with production of inflammatory PGs, whereas the later peak results in production of PGs that suppress inflammation.[18] That inflammation occurs in COX-2 knockout mice[19] reminds us, as Lewis Thomas stated,[20] "inflammation will take place at any cost." Nonetheless, the search for more selective and localized COX-2 inhibition continues. One strategy is achieved by using the mechanism of RNA interference (RNAi). Nonpathogenic *Escherichia coli* are engineered to invade tumor cells (and perhaps could be designed to invade synovial cells) and to generate anti–COX-2 small interfering (si)RNA molecules (siCOX-2), thereby silencing overexpressed COX-2. The involvement of micro (mi)RNAs in COX-2 post-transcriptional regulation suggests a possible endogenous silencing mechanism to reduce COX-2 expression.[21]

### Cyclooxygenase-3

Acetaminophen, similar to NSAIDs, suppresses pain and fever. However, acetaminophen is not an anti-inflammatory agent, and despite its extensive use, its mechanism of action has not been apparent. The finding that acetaminophen inhibits COX activity more in canine brain homogenates than in spleen homogenates gave rise to the concept that variants of the COX enzyme exist that are differentially sensitive to acetaminophen. COX-3 is a splice variant of COX-1 that retains the intron-1 gene sequence at the messenger (m)RNA level, which encodes a 30 amino acid sequence inserted into the N-terminal hydrophobic signal peptide of the enzyme protein. COX-3 protein and mRNA transcripts have been identified in human tissues, as well as in rats. They have been found most abundantly in the cerebral cortex and heart, although the mechanism for conversion of COX-3 mRNA to active enzyme is not clear.[11,22] These findings led to a search for other COX variants. A COX-2 variant induced by 0.5 mM diclofenac via stimulation of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (the receptor for the pro-resolving 15-deoxy-δ12,14 $PGJ_2$) has been discovered. Whereas induction of COX-2 by lipopolysaccharide (LPS) results in release of the inflammatory cytokines IL-6 and TNF, diclofenac-induced COX-2 leads to release of the anti-inflammatory cytokines transforming growth factor (TGF)-β and IL-10.

### Prostaglandin Synthases

Conversion of the endoperoxide intermediate $PGH_2$ to PGs requires the activity of specific terminal synthases. Hematopoietic PGD synthase (H-PGDS) catalyzes the isomerization of $PGH_2$ to $PGD_2$ in immune and inflammatory cells. Cytosolic PGE synthase (cPGES) is responsible for constitutive expression of $PGE_2$, and microsomal PGE synthase 1 (mPGES-1) induces PGE in response to inflammatory stimuli. At least 10 enzymes convert PG precursors into biologically active PGs, and reactive oxygen species generated during an inflammatory response lead to expression of mPGES-1.[23,24] Suppression of PG synthase activity might be considered as an alternative strategy that would fall between global blockade by inhibition of COX and blockade of a single eicosanoid receptor. Increased fibrosis may now be added to the well-known adverse effects of NSAIDs, including COX-2 selective agents.[25] Thus efforts have been directed at development of drugs that inhibit mPGES-1, rather than COX-2, thereby suppressing $PGE_2$ production while sparing production of prostacyclin.[26] Because $PGE_2$ is also important to the induction of matrix metalloproteinases (MMP)-3 and MMP-13, suppression of mPGES-1 might counteract articular cartilage degradation in patients with inflammatory arthritis.[27]

## PRODUCTS OF THE CYCLOOXYGENASE PATHWAY

### Prostaglandins

The basic structure of all PGs is a "prostanoic acid" skeleton, that is, a 20-carbon fatty acid with a five-membered

ring at C8 through C12 (see Figure 24-4, *inset*). The term *prostaglandins* is employed widely but should be used only to describe the oxygenation products that contain the five-membered carbon ring. A family of acidic lipids found first in human seminal fluid, PGs were misnamed because it was thought they were produced in the prostate gland rather than in the seminal vesicles.[28-30] The alphabetic PG nomenclature (e.g., PGE, PGF, and PGD) is related to the chemical architecture of the cyclopentane ring. PGE and PGF differ only in the presence of a ketone or hydroxyl function at C9 (see Figure 24-4). These compounds are made by different enzymes (e.g., $PGE_2$ and $PGD_2$ are made by isomerases, and $PGF_{2\alpha}$ is made by a reductase). In the nomenclature, a subscript numeral after the letters indicates the degree of unsaturation in the alkyl and carboxylic acid side chains. The numeral *1* indicates the presence of a double bond at C13-C14 ($PGE_1$), *2* marks the presence of an additional double bond at C5-C6 ($PGE_2$), and *3* denotes a third double bond at C17-C18 ($PGE_3$).

PGs are produced on demand and seem to exert their effects on the cell of origin or nearby structures. They are not stored in cells and are degraded rapidly in vivo by 15-hydroxyprostaglandin dehydrogenase (PGDH) during one passage through the lungs. Abundant experimental evidence supports the view that PGs participate in development of the inflammatory response. $PGE_2$ is a central component of the inflammasome-dependent induction of the eicosanoids that leads to loss of intravascular fluid.[31] However, PGs are probably better at potentiating the effects of other mediators of inflammation than they are at inducing inflammation directly. PGE compounds and intermediate hydroperoxides of AA increase pain sensitivity to bradykinin and histamine. The effects of PGE are cumulative, depending on concentration and time. Even small amounts of PGs, if allowed to persist at the site of injury, may in time cause pain.

$PGE_2$ stimulates bone resorption,[32] and its 13,14-dihydro derivative is nearly as potent, which is of interest because derivatives of the biologically active PGs are usually assumed to be of no functional significance. Addition of serum to the culture medium stimulates bone resorption, a process that is complement dependent and PG mediated. The mechanism may help explain bone erosion in joints of patients with RA, in which complement is activated and $PGE_2$ concentrations are high. The observation that $PGE_1$ can stimulate bone formation[33] suggests that PGs physiologically participate in coordination of bone formation and resorption. For example, primary idiopathic hypertrophic osteoarthropathy, a familial disorder, is associated with mutations in PGDH and subsequent impairment of PG degradation. These patients have chronically elevated levels of PGE and exhibit digital clubbing and evidence of both increased bone formation and resorption in their phalanges.[34] Many effects of IL-1 and TNF on cells are associated with stimulation of PG production and inflammation. Cartilage explants from patients with osteoarthritis express COX-2 (but not COX-1) and release 50 times more $PGE_2$ in culture than does cartilage from healthy subjects and 18 times more $PGE_2$ than does normal cartilage stimulated with cytokines plus LPS. Explants from patients with osteoarthritis release IL-1β, whereas normal cartilage does not express mRNA for pro–IL-1β or release IL-1β spontaneously. It seems that in osteoarthritis—and probably in RA—upregulation of cartilage IL-1β and subsequent production of $PGE_2$ leads to cartilage degradation.[35,36]

Mast cells, often overlooked as important in inflammatory responses, are seen in large numbers in synovium from patients early in the course of RA.[37] $PGD_2$, the major PG formed by mast cells, is also produced by eosinophils and type 2 (T helper [Th]2) lymphocytes. $PGD_2$ induces chemotaxis of Th2 cells via activation of the chemoattractant receptor-homologous molecule (CRTH2) expressed on Th2 cells, and CRTH2 antagonists block that action and reduce allergen-induced inflammation. $PGJ_2$, formed from the dehydration of $PGD_2$, functions as a brake on the inflammatory response. It reduces macrophage activation, reduces nitric oxide production from stimulated cells, and induces apoptosis in tumor cell lines. $PGJ_2$ is metabolized to 15-deoxy-δ12,14 $PGJ_2$ and δ12 $PGJ_2$, which are also biologically active.[38] The biologic activity of PGA, formed from loss of water from the cyclopentane ring, is not completely clear. However, in vitro, $PGA_2$ induces apoptosis in HL-60 cells and acts through a nuclear receptor to increase insulin sensitivity.[39]

## Prostacyclin

PGI, discovered in 1976,[40] has been purified, and the complementary (c)DNA for PGI synthase has been cloned. In addition to a cyclopentane ring, a second ring is formed by an oxygen bridge between carbons 6 and 9. PGI is generated from $PGH_2$ by a distinct prostacyclin synthase, a 56 kDa member of the cytochrome P-450 superfamily of enzymes found predominantly in endothelial and vascular smooth muscle cells.[15] Production of PGI can be stimulated by thrombin or generated by transfer of $PGH_2$ from platelets (the endoperoxide steal), or it can be stimulated by contact with activated leukocytes or by stretching of the arterial wall. PGI is a powerful vasodilator that inhibits platelet aggregation through activation of adenylate cyclase, which leads to an increase in intra-cellular cAMP. It is metabolized rapidly (the half-life in plasma is less than one circulation time) to the more stable, less biologically active 6-keto-$PGF_{1\alpha}$. The enzymatic products of its conversion—2,3-dinor-6-keto $PGF_{1\alpha}$ and 6,15-di-keto-2,3-dinor $PGF_{1\alpha}$—are also chemically stable and have little biologic activity. They are the major metabolites of prostacyclin excreted in urine, in which they can be assayed as indicators of PGI generation.

PGI generated in the vessel wall has anti-platelet and vasodilator actions, whereas $TXA_2$ generated by platelets from the same precursors induces platelet aggregation and vasoconstriction. These two eicosanoids represent biologically opposite poles of a mechanism for regulating the interaction between platelets and the vessel wall and of formation of hemostatic plugs and intra-arterial thrombi. Given the central role of platelets in inflammatory reactions, an appropriate PGI-TX balance is important to regulation of inflammation. The balance may be altered in patients with anti-phospholipid antibody syndrome, in patients treated with cyclosporine, and in patients treated with NSAIDs, especially patients treated with selective COX-2 inhibitors. Although COX-2 inhibitors reduce recurrence of colorectal

adenomas, they increase the risk for cardiovascular events, such as myocardial infarction and stroke.[41]

Intravascular infusion of PGI also reduces some of the clinical changes associated with pulmonary embolism. The instability of PGI makes it cumbersome to administer therapeutically. Nonetheless, it has been used with limited success to treat peripheral vascular disease, including Raynaud's phenomenon. New therapeutics for treatment of pulmonary hypertension, including patients with sarcoidosis and systemic sclerosis, are PGI analogues and PGI receptor antagonists.[42,43] Inhaled PGI analogues may help overcome some of the adverse effects due to intravenous administration of these drugs.[44]

## Thromboxanes

The endoperoxide $PGH_2$ can be converted into TXs after the action of the enzyme TX synthase, a microsomal 60 kDa member of the cytochrome P-450 family, which is quite active in the platelet. The gene that encodes the enzyme has been cloned. TXs contain a six-member oxane ring instead of the cyclopentane ring of the PGs. TX synthase converts $PGH_2$ into equal amounts of $TXA_2$ and 12L-hydroxy-5,8,10-heptadecatrienoic acid. $TXA_2$ stimulates platelet activation, contributes to intravascular aggregation of platelets, and contracts arteriolar and bronchiolar smooth muscles. It is hydrolyzed rapidly (the half-life is 30 seconds) to the inactive, stable, measurable product, $TXB_2$; its actions are limited to the microenvironment of its release.

The extraordinary rapidity with which platelets adhere to damaged tissue, aggregate, and release potent biologically active materials suggests that the platelet is well suited to be a cellular trigger for the inflammatory process. Efforts directed at suppression of TX synthesis and platelet aggregation may result in limitation of inflammatory responses, especially in coronary arteries. Inhibition of platelet aggregation may be important to the anti-inflammatory effects of aspirin and other NSAIDs. Long-term administration of low doses of aspirin (40 mg/day—the lowest dose predicted to cause total inhibition of TX formation in serum, according to mathematic modeling) has inhibitory effects on platelet function ex vivo that are indistinguishable from the effects caused by giving 325 mg/day of aspirin.[45] Aspirin acetylation of COX in platelets occurs in the portal vein, where the aspirin concentration is high before it is metabolized in the liver, which explains why the 81-mg dose of aspirin is so effective in prevention of heart attacks and strokes. Platelets lose their ability to aggregate until new platelets are formed in about a week.[11] Nonetheless, the response to aspirin can vary among individuals. Biomarker testing for TX production and assessment of aspirin efficacy—which is not done as routinely as necessary—can reduce the risks associated with aspirin therapy.[46] High platelet turnover in some patients with chronic inflammation may diminish the efficacy of aspirin, a phenomenon termed *aspirin resistance*.[47]

Selective inhibition of TX synthase represents an approach that may be used to suppress $TXA_2$ synthesis without depressing prostacyclin formation. The endoperoxide steal seems to function in vivo after administration of a TX synthase inhibitor. Antagonists of the receptors shared by endoperoxide and $TXA_2$ have been developed, and these agents inhibit platelet aggregation in patients who are recalcitrant to TX synthase inhibition. Novel agents targeting TX receptors and TX synthase have improved treatment of vasculitis and cardiovascular and renal diseases.[48] More specific inhibition of TX action may become possible now that TX receptors have been cloned and characterized.[49]

## Lipoxygenase Pathways

In contrast to the COX pathway, in which stable products have three atoms of oxygen covalently attached to AA from 2 moles of molecular oxygen, LOs insert a single oxygen atom into the molecular structure of AA. Separate LOs exist in certain cells and have strict structural requirements for their substrates. Three major mammalian LOs insert their oxygen atoms into the 5, 12, or 15 position of AA, with formation of a new double bond and hydroperoxy group. The hydroperoxy fatty acids (hydroxyperoxyeicosatetraenoic acid [HPETE]) can be reduced by peroxidases in the cell to yield the corresponding hydroxy fatty acids (hydroxyeicosatetraenoic acid [HETE]). The exclusive LO product of the human platelet is 12-HPETE, which, upon reduction of the hydroperoxy group, yields 12-HETE. In contrast, the human neutrophil makes predominantly 5-HPETE, but when high concentrations of AA are added, cells exhibit 15-LO. LOs that act on AA are found in the cytosol fraction of cells.

The human 5-LO gene has been isolated and characterized[50,51] and produces a 78 kDa enzyme. In myeloid cells the 5-LO pathway leads to formation of the biologically active LTs (Figure 24-5) that were originally found in leukocytes and that contain three conjugated double bonds (trienes). Cell activation leads to translocation of 5-LO from cytosol to the nuclear membrane, where it encounters the 18 kDa, 5-LO–activating protein (FLAP). AA is also translocated to FLAP for presentation to 5-LO. In addition, upon cell stimulation, $cPLA_2$ is activated and also associates with the nuclear membrane, close to FLAP. Thus FLAP inhibition represents an approach to suppression of inflammation.[52] The ability of macrophages and dendritic cells to respond appropriately during innate immune responses is likely regulated by 5-LO and 12-LO.[53] The unstable HPETE is the initial metabolite of each LO pathway. HPETE is reduced to the more stable HETE or is converted by 5-LO to $LTA_4$. $LTA_4$ can be converted to $LTB_4$ (in neutrophils and macrophages) or conjugated with reduced glutathione to form $LTC_4$ (in eosinophils, mast cells, endothelial cells, and macrophages). In contrast to LO, which is mainly distributed in myeloid cells, $LTA_4$ hydrolase (5,12-dihydroxyeicosatetraenoic acid), a zinc-requiring enzyme that converts $LTA_4$ to $LTB_4$, is widely distributed. From the cDNA sequence, it was suggested that mRNA for $LTA_4$ has a short half-life, which accounts for the properties of extremely rapid production and shutdown of $LTB_4$ and other eicosanoid biosynthesis.

$LTA_4$ can be exported from the cell of origin and converted in other cells by $LTA_4$ hydrolase to $LTB_4$. This variation on the endoperoxide steal—perhaps better called *transcellular metabolism*—also applies to conversion of $LTA_4$ to $LTC_4$ by $LTC_4$ synthase, a glutathione-*S*-transferase.[54] Although human endothelial cells do not produce terminal

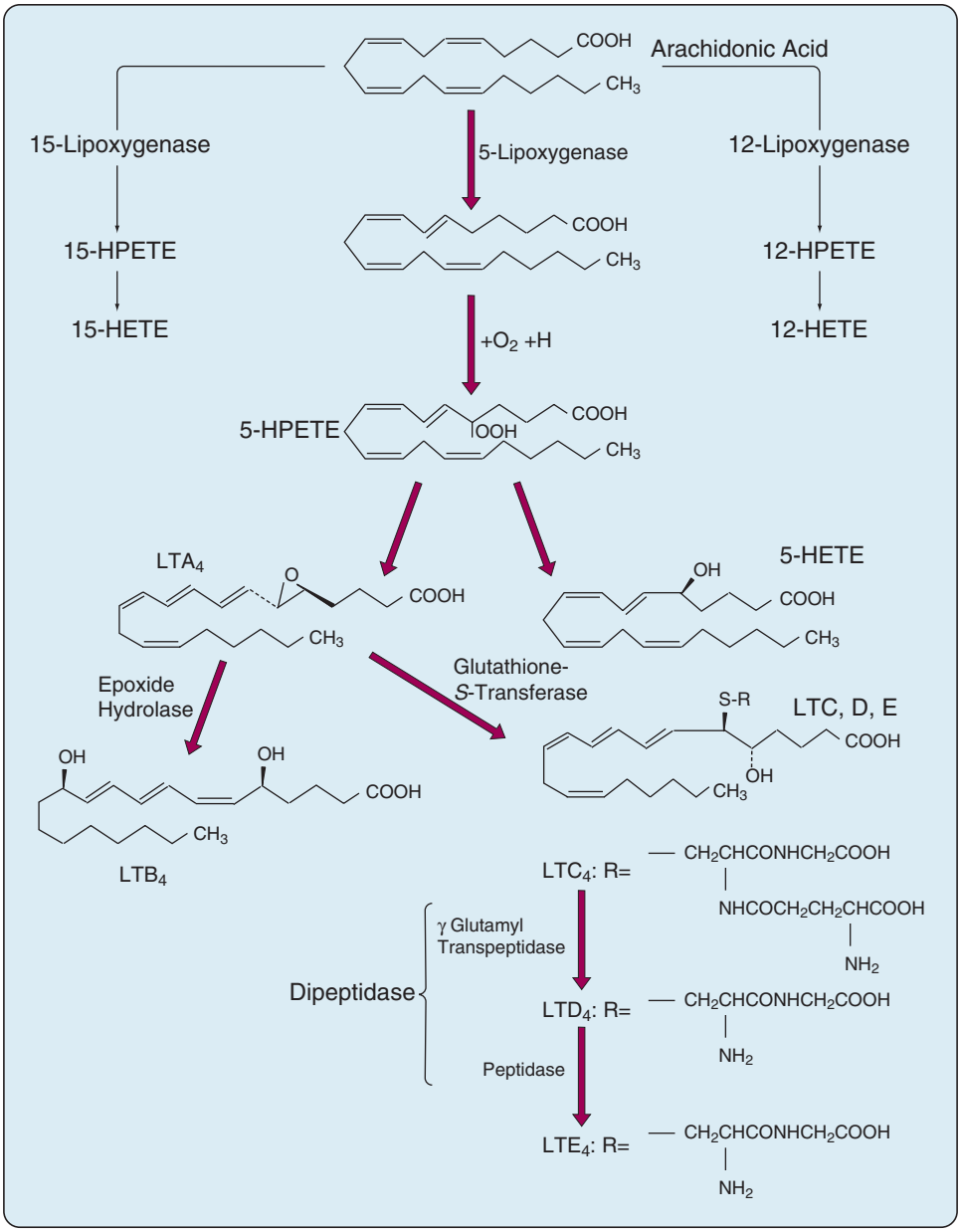



**Figure 24-5** 5-Lipoxygenase pathway of arachidonic acid metabolism. HETE, Hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene.

products of the 5-LO system, they do generate $LTC_4$ from $LTA_4$ provided by neutrophils. $LTC_4$ and its products, $LTD_4$ and $LTE_4$, constitute the biologic mixture previously known as slow-reacting substance of anaphylaxis. $LTD_4$ and $LTE_4$ arise from $LTC_4$ after sequential removal of γ-glutamic acid and glycine from $LTC_4$. The enzyme γ-glutamyl transpeptidase is present in many cells as part of a complex enzymatic system involved in glutathione biosynthesis and amino acid transport. In many systems the major sulfidopeptide LT has been reported to be $LTD_4$, rather than the precursor $LTC_4$. Removal of glycine from $LTD_4$ results in $LTE_4$, with concomitant loss of a significant amount of biologic activity. The principal route of inactivation of $LTB_4$ is by omega oxidation.

## Products of the Lipoxygenase Pathways

The biologic effects of compounds produced in the LO pathway indicate their importance in inflammatory diseases.[55] They are the major mediators of inflammation formed by the oxygenation of AA and are implicated as key mediators in several diseases, including inflammatory bowel disease, systemic sclerosis, psoriasis, and RA.

5-HETE and 5-HPETE stimulate the generation of superoxide in human neutrophils. These compounds also augment intra-cellular calcium levels, facilitating PKC-dependent activation of a superoxide-generating system of neutrophils. $LTB_4$ increases adherence of leukocytes to endothelial cells, a response that is augmented by exposure of the endothelial cells to TNF. $LTB_4$ does not seem to have

a direct vascular contractile action because it is inactive in the hamster cheek pouch preparation and several other microvasculature systems. In rabbit skin, administration of $LTB_4$ with a vasodilator PG induces plasma exudation, which suggests that $LTB_4$ may assist enhanced vascular permeability. Increased venule permeability does occur in response to $LTC_4$, $LTD_4$, and $LTE_4$. $LTB_4$ is a potent chemotactic factor for neutrophils and is weakly chemotactic for eosinophils. $LTB_4$ and, to a lesser extent, 5-HETE enhance migration of T lymphocytes in vitro. Synovial cells produce 5-HETE but do not produce significant amounts of $LTB_4$. However, macrophages that invade the synovium in patients with RA generate substantial quantities of 5-lipoxygenation and 15-lipoxygenation products, including $LTB_4$. In addition to local signs of inflammation induced by products of the LO pathway, these compounds may contribute to the pain, tenderness, and aching common in patients with RA. $LTB_4$ also seems to serve an immunoregulatory function. It stimulates differentiation of competent $CD8^+$ T lymphocytes from precursors lacking the CD8 marker. $LTB_4$ also stimulates interferon (IFN)-γ and IL-2 production by T cells and biosynthesis of IL-1 by monocytes.[56]

Synovial cell proliferation and endothelial cell proliferation are central to propagation of the rheumatoid joint lesion. $LTB_4$ and the cysteinyl LTs act as growth or differentiation factors for several cell types in vitro. These compounds also increase proliferation of fibroblasts when PG synthesis is inhibited.[57] These findings emphasize the importance of interactions between the COX and LO pathways and suggest limitations to NSAID therapy for patients with RA.

Strategies for inhibiting production or antagonizing the actions of LTs include development of selective LT receptor antagonists and inhibition of the production of LT by blocking the action of 5-LO. Inhibition of enzymes distal in the LT cascade, such as $LTA_4$ hydrolase,[58] is also a promising strategy for development of anti-inflammatory drugs. A compound that inhibits binding of 5-LO to FLAP exhibits anti-inflammatory effects in animal models. Although LO inhibitors have not been very useful for treatment of patients with inflammatory diseases, development of new compounds, based on a better understanding of the molecular aspects of inhibitory activities, seems more promising.[59] In addition, the existing agents may be aiming at the wrong target. Fibroblasts such as synovial cells do not make much $LTB_4$, but they do make 12-HETE, a growth factor, through a cytochrome P-450 pathway.[60] Cytochrome P-450 inhibitors may be more to the therapeutic point.[61]

LO activities do not lead solely to production of mediators of inflammation. DGLA is converted by 15-LO into 15-HETE, which is incorporated into DAG and exerts anti-inflammatory effects partly by interfering with PKC-β activity. An LO product of linoleic acid, 13-hydroxyoctadecadienoic acid, also suppresses inflammation and cell proliferation by a similar mechanism.[62] EPA is converted by LO into 15-hydroxyeicosapentaenoic acid, which also exhibits anti-inflammatory properties.[63]

## Lipoxins

Another large family of AA metabolites arises from the sequential action of 5-LOs and 15-LOs. Addition of 15-HPETE and 15-HETE to human leukocytes results in formation of a pair of oxygenated products containing a unique conjugated tetraene. One compound ($LXA_4$) was identified as 5,6,15L-trihydroxy-7,9,11,13-eicosatetraenoic acid, and the other proved to be its positional isomer ($LXB_4$), 5D-14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid (Figure 24-6). Because both of these compounds can arise through an interaction between LO pathways, the trivial name *lipoxins* (i.e., lipoxygenase interaction products) was introduced. Platelet 12-LO can transform neutrophil $LTA_4$ to LXs. The complete stereochemistry and multiple routes of biosynthesis for the biologically active $LXA_4$ and $LXB_4$ have been determined.[64]

The fact that macrophages of rainbow trout generate LXs rather than LTs or PGs as their major products of AA metabolism indicates that LXs have a long evolutionary

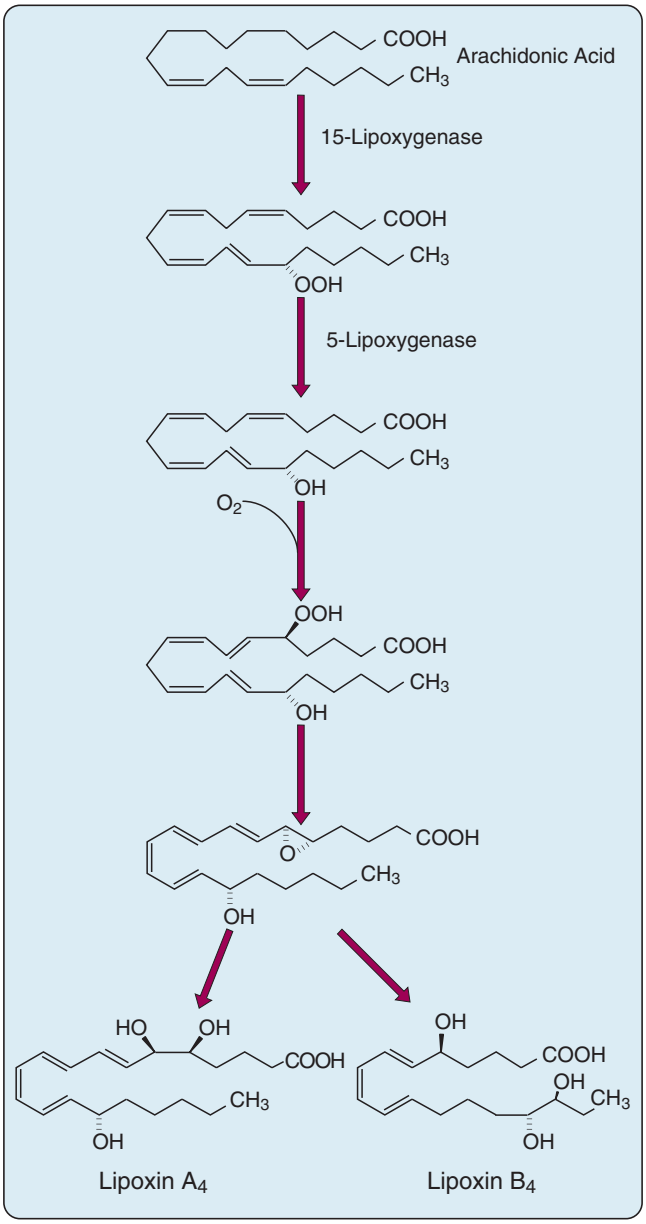



**Figure 24-6** Lipoxin biosynthesis. The lipoxins result from the sequential action of 15-lipoxygenase and 5-lipoxygenase on arachidonic acid.

history. LTs and LXs can be generated in parallel in fish. In humans, the process has diverged to a two-cell system. Biosynthesis of eicosanoids by transcellular and cell-cell interactions is recognized as an important way to generate and amplify lipid-derived mediators. LXs can be generated within the vascular lumen during platelet-leukocyte interactions and at mucosal surfaces via leukocyte–epithelial cell interactions. In humans, LXs are formed in vivo during multicellular responses such as inflammation, atherosclerosis, and in asthma. These tetraene-containing products serve as stop signals in that they prevent leukocyte-mediated tissue injury. Acute inflammation is a primitive protective response,[65] and chronic inflammation involves failure of mechanisms designed to stop the acute response. A major problem in joints of patients with RA, and of patients with other conditions associated with chronic inflammation and tissue injury, is that inflammation often does not resolve. LXs and aspirin-induced 15-epilipoxins are endogenous components of events governing resolution of inflammation. Aspirin acetylation of COX-2 in endothelial cells suppresses PG synthesis but leads to generation of 15R-HETE from AA, which is transformed to 15-epilipoxin by leukocytes in a transcellular biosynthetic route involving vascular endothelial cells or epithelial cells. These 15-epilipoxins exhibit anti-inflammatory and anti-proliferative actions in vitro and in vivo. Stable analogues of $LXA_4$ and of aspirin-triggered LX (ATL) also suppress inflammation in animal models. In addition, acting as antagonists at the receptor CysLT1, ATL analogues counteract the inflammatory actions of cysteinyl LTs.[66] These observations may lead to the development of new anti-inflammatory drugs. For example, induction by IFN of particular genes is important to the pathogenesis of systemic lupus erythematosus (SLE). A stable synthetic analogue of $LXA_4$ suppresses several IFN-induced genes and reduces kidney damage in a murine model of immune-mediated nephritis.[67]

LXs block human polymorphonuclear leukocyte chemotaxis but stimulate monocyte chemotaxis and adherence. Monocytes do not release mediators of inflammation in response to LXs, however, and LXs are converted rapidly by monocytes to inactive compounds. This selective effect on chemotaxis suggests that LXs play a role in wound healing. $LXA_4$ antagonizes $LTD_4$-induced vasoconstriction in vivo and blocks binding of $LTD_4$ to its receptors on mesangial cells. $LXA_4$ suppresses $LTB_4$-induced plasma leakage and leukocyte migration and blocks $LTB_4$-induced neutrophil inositol triphosphate generation and calcium mobilization, but not superoxide anion generation. Conversely, $LXA_4$ activates PKC and is more potent in this regard than DAG and AA. $LXA_4$ seems to be specific for the γ subspecies of PKC. These results indicate that LXs regulate the actions of vasoconstrictor LTs and suggest that $LXA_4$ may be an important modulator of intra-cellular signal transduction.

It was long thought that resolution of inflammation was a passive process. Contrary to this belief, the inflammatory response does not dissipate but is mediated actively by products of COX and LO and acetylation of these enzymes.[68] Discovery that omega-6 and omega-3 polyunsaturated fatty acids are also substrates for enzymatic oxygenation reactions that produce lipid mediators with potent anti-inflammatory and proresolution actions has advanced our understanding of the inflammatory response. In addition to LXs, entire spectra of specialized pro-resolving mediators (SPM) have been uncovered. These SPMs include resolvins, protectins, and maresins.[69] Like LXs, resolvins are formed via specific transcellular biosynthetic pathways from both n-6 and n-3 fatty acids. EPA-derived (E series) cellular interaction products formed in resolving exudates have been termed *resolvins* (RvE1, RvE2, and RvE3). The omega-3 docosahexaenoic acid (DHA) can be catalyzed via the 15-LO pathway to dihydroxy products (D series resolvins D1-D5) that also stimulate resolution of inflammation and enhance innate host defense mechanisms. LXs and resolvins act as endogenous receptor agonists at low concentrations (pM to nM) and at specific G protein–coupled membrane spanning receptors to actively downregulate inflammatory events and to stimulate resolution of an inflammatory exudate. A single cell type can also form oxygenated lipid mediators with counter-regulatory actions. DHA can also be catalyzed via the 15-LO pathway to a dihydroxy product termed *protectin D1* (PD1), which can protect against tissue damage and activate resolution of inflammation. In a second single oxygenation route, a lipid mediator termed *maresin* (macrophage mediators in resolving inflammation) is formed via the action of human 12-LO. The maresin biosynthetic pathway is activated in macrophages during phagocytosis. Maresin 1 (MaR1) reduces neutrophil migration and increases macrophage phagocytosis of apoptotic cells, thereby exhibiting the hallmarks of SPM. It now seems clear that failure of resolution programs contributes to progression of diseases characterized by chronic inflammation.[70]

## Isoeicosanoids

Isoeicosanoids, which are isomers of enzymatically derived eicosanoids, are derived from the free radical–mediated peroxidation auto-oxidation of both omega-6 and omega-3 fatty acids.[71] Isoeicosanoids include members of the F, D, and E isoprostanes, isothromboxanes, and isoleukotrienes. Analysis of the isoprostanes indicates that they reflect lipid peroxidation in vivo and are therefore used as markers of oxidative stress. One F-type isoprostane, isoprostane $F_{2\alpha}$ III (formerly 8-isoprostaglandin $F_{2\alpha}$), has been studied in detail because of its biologic activity in vitro. Isoprostane $F_{2\alpha}$ III (Figure 24-7) is a potent vasoconstrictor and may function as a mitogen, with actions that are blocked by TX receptor antagonists. Although isoprostanes may act as ligands at TX or PG receptors (e.g., 8,12-isoprostane $F_{2\alpha}$ III activates the $PGF_{2\alpha}$ receptor), they also activate specific isoprostane receptors.

### Isoprostanes

What all isoprostanes have in common, and what distinguishes them from the PGs, is the fact that the top (α) and the bottom (ω) side chains are always syn—that is, crowded together on the same face of the cyclopentane ring. The minimal requirement for generation of an isoprostane is a polyunsaturated fatty acid with three contiguous methylene-interrupted double bonds, a requirement met by dozens of naturally occurring polyunsaturated fatty acids. Brain tissue contains particularly high levels of fatty acids formed from DHA (C22:6 n-3). By the same peroxidation process undergone by AA, DHA in nerve cell membranes is converted

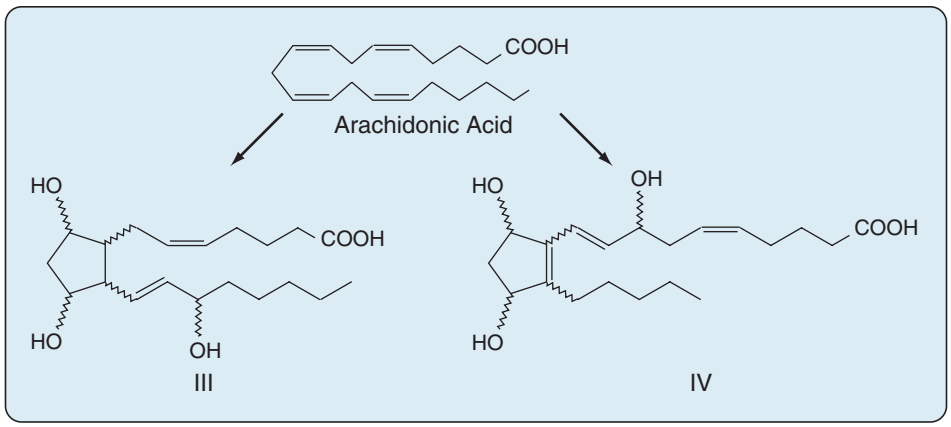



**Figure 24-7** Isoprostane $F_{2\alpha}$ structures. *Jagged lines* indicate that stereochemistry is uncertain.

to several neuroprostanes. Of these, F4-neuroprostane is a biomarker for several neurodegenerative diseases. A similar mechanism in plants yields phytoprostanes from α-linolenic acid. In contrast to conventional, enzymatically derived PGs that are formed intra-cellularly and released immediately, isoprostanes are formed in the cell membrane, cleaved by phospholipases, circulate in plasma, and are excreted in urine. As stable end products of lipid peroxidation, endogenously formed isoprostanes are useful markers of oxidative stress and independent risk markers for coronary artery disease. They are increased in several diseases, including acute respiratory distress syndrome, in which polymorphonuclear leukocytes generate reactive oxygen species that damage pulmonary epithelium. The immune cells in inflamed tissues are exposed to reactive oxygen intermediates produced by neutrophils and other phagocytic cells. Oxidants are also generated as mediators in intra-cellular signaling pathways by cytokines such as IL-1β and TNF. Isoprostanes are important to and are biomarkers for inflammatory conditions such as vasculitis and RA, and they are assayed in biologic fluids and tissues. Because isoprostanes are released pre-formed, their production is not blocked by NSAIDs, which suppress metabolism of free AA. It is possible that inflammation that is unresponsive to NSAIDs may yield to inhibition of isoprostanes. In keeping with the notion of eicosanoids as regulators, however, some isoprostanes suppress release of mediators of inflammation from macrophages, and a phytoprostane (E1-phytoprostane) signals through PPARγ and NF-κB to suppress IL-12 production by activated dendritic cells and to reduce cytokine production by Th1 and Th2 cells.[72,73]

## Endocannabinoids

Groups of naturally occurring members of the eicosanoid superfamily that can activate cannabinoid receptors and are derivatives of long-chain fatty acids have been referred to as *endocannabinoids*. They are not stored in cells; rather, they are synthesized rapidly in the manner of PGs and LTs from lipid precursors and are released from—among other cells—cells of the immune system during immune/inflammatory responses. They can then activate cannabinoid receptors on the same or adjacent cells and are metabolized rapidly by the specific serine hydrolase, fatty acid amide hydrolase (FAAH), or by monoglyceride lipase or *N*-acetylethanolamine. Thus inhibition of FAAH offers a strategy for treatment of chronic inflammatory pain.[74] One of the most important endocannabinoids is anandamide (from the Sanskrit word for "bliss"), the amide conjugate of AA and ethanolamine (arachidonoyl ethanolamide; Figure 24-8). Anandamides and other endocannabinoids, such as 2-arachidonylglycerol and virodamine, are involved in a wide range of regulatory functions, including pain perception and modulation of immune responses, actions mediated via cannabinoid 1 (CB1) and CB2 receptor subtypes, resulting in activation of G proteins of the G(i/o) family. Of particular relevance to joint tissue injury is the capacity of anandamide to suppress TNF-induced NF-κB activation by direct inhibition of I-κ-B kinase, an action that is independent of CB1 or CB2 activation.

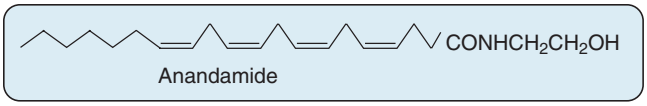



**Figure 24-8** Chemical structure of anandamide (arachidonoyl ethanolamide).

Acid congeners of anandamides are lipoamino acids (elmiric acids) that exist as endogenous substances, regulate tissue levels of anandamide, and exhibit anti-inflammatory effects and the capacity to assist resolution of inflammation. One such compound, *N*-arachidonylglycine (NAGly), is found in many tissues at higher concentrations than anandamide. It has analgesic actions similar to those reported for anandamide but does not exhibit psychotropic action. A library of elmiric acids (n-3 and n-6) has been synthesized. Several of these compounds exhibit anti-inflammatory activity, likely in part by increasing production of $PGJ_2$, and they also modulate immune responses.[75-77] Discovery of naturally occurring and synthetic analogues and metabolites of anandamide suggest that a family of such biologically active substances exists. The polyunsaturated amides dihomo-γ-linolenoyl (20:3 n-6) and adrenoyl (22:4 n-6) ethanolamides have been found in mammalian brain.[78] N-3 fatty acid ethanolamides also exist in mammalian tissues. That anandamide can enhance its own synthesis in macrophages suggests the presence of a rapid response to counter

excessive inflammatory or immune responses. Anandamide is converted by COX-2 (but not by COX-1) into $PGE_2$ or $PGF_{2\alpha}$ ethanolamide directly, without going through free AA.[78] These novel PGs ("prostamides") are pharmacologically active.[79] Because anandamide is a substrate for COX-2, inhibitors of COX-2 may reduce anandamide metabolism, with a subsequent increase in concentration of the anandamide. A combination of anandamide with NSAIDs reduces gastric damage and produces synergistic analgesia in experimental models.[80] NAgly, a naturally occurring lipoamino acid, is also a substrate for COX-2, giving rise to amino acid conjugates of the PGs.[81]

## EICOSANOID RECEPTORS

For many years, it was thought that the lipophilic eicosanoids—in contrast to the peptide molecules for which receptors were characterized routinely—simply "diffused" into cell membranes or were carried in by a binding protein. The isolation and cloning of eicosanoid receptors changed that thinking.[82]

### Prostaglandin Receptors

PGs exert most of their actions through G protein–coupled receptors (GPCRs). Receptors for the COX products are designated P receptors, depending on the prostanoid that has the most affinity for them. These receptors include the PGD receptors (DP); four subtypes of the PGE receptor (EP1 through EP4); the PGF receptor (FP); the $PGI_2$ receptor (IP); and the TX receptor (TP). The IP, DP, EP2, and EP4 receptors mediate increases in cellular cAMP, whereas TP, FP, and EP1 receptors induce calcium mobilization. EP2, EP4, and IP regulate macrophage cytokine production in a similar manner. As might be expected, signaling through these receptors is more complicated. Studies designed to understand $PGE_2$ signaling suggest involvement of phosphatidylinositol-3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and Wnt pathways in EP2R/EP4R regulation of cell growth, migration, and apoptosis.[34] $PGE_2$ is the most abundant of the major eicosanoids in the joint space of patients with RA. The molecular mechanisms whereby $PGE_2$ actions are mediated by each EP subtype have been explained using mice deficient in each subtype.[83] Identification of $PGD_2$ as the ligand for the DP2 receptor led to development of DP2 receptor antagonists for treatment of asthma and airway inflammation.[84]

Modification of immune cell and surrounding cell functions by prostanoids during immune or inflammatory responses is influenced by the different repertoire of PG receptors expressed on these cells. Mice deficient in each EP receptor subtype have been generated, and highly selective agonists for these receptors have been developed. As noted,[34] PGE can suppress or induce bone formation. EP2 and EP4 knockout mice exhibit impaired osteoclastogenesis and inflammation-induced bone resorption. The receptor mediating PGE-induced bone formation is unknown, although it has been proposed that EP2R and EP4R mediate the anabolic effects of $PGE_2$ on bone. In animal models, acute inflammation and pain are completely absent in IP-deficient mice. The profile of PG formation changes as the inflammatory response evolves to a more chronic state, so other receptors are probably involved. It is unlikely that blockade of one receptor will completely block an inflammatory response. More encouraging is the fact that PGs participate in allodynia, a pain response to a usually nonpainful stimulus. Knowing the contribution of P receptors to allodynia should lead to better treatment of neuropathic pain and myofascial pain syndromes such as fibromyalgia.

Stimulation of TX/endoperoxide receptors (TP) elicits platelet aggregation and contraction of vascular smooth muscle and promotes expression of adhesion molecules with subsequent movement of monocytes/macrophages from the circulation to tissue. Thus TP antagonists reduce vascular inflammation, are anti-thrombotic, and maintain vasodilation, actions that mark them as potential agents for treatment of conditions characterized by chronic inflammation.[85]

The availability of cloned P receptors should assist in the development of more effective receptor-active compounds. The $PGI_2$ analogue iloprost is useful for treatment of peripheral vascular disease and pulmonary hypertension. Although iloprost binds to IP with high affinity, it also binds to EP1 and EP3. It may be that targeting activation, blockade, or both of a single P receptor or a specific set of P receptors would provide advantages compared with compounds that work "upstream," such as the COX-2 inhibitors or traditional NSAIDs. For example, it is clear that E prostaglandins are the most important of the endogenous eicosanoids for modulation of the function and mucosal integrity of the gastrointestinal tract. Protection by $PGE_2$ against acid reflux esophagitis and against ethanol- and indomethacin-induced gastric mucosa injury is mimicked by EP1 agonists and attenuated by an EP1 antagonist, whereas EP4 antagonism does not affect the integrity of gastrointestinal mucosa. In addition, $PGE_2$ does not exhibit gastric cytoprotection in EP1 knockout mice. In the small intestine, the protective effect of $PGE_2$ on indomethacin-induced damage is mimicked by both EP3 and EP4 agonists.

Further evidence of the importance of eicosanoids in immune cell function and regulation of immune responses derives from experiments with mice deficient in PG receptors, which indicate that $PGE_2$ assists and amplifies IL-12–mediated Th1 cell differentiation and IL-23–mediated Th17 cell expansion. These $PGE_2$ actions and those of $PGD_2$ and $PGI_2$—also discovered using receptor-deficient mice—contribute to the development of immune diseases.[86] Mice lacking the $PGF_{2\alpha}$ receptor are protected against bleomycin-induced pulmonary fibrosis,[87] a finding with implications for treatment of systemic sclerosis.

The implications for therapy derived from this new knowledge are clear and exciting, but prostanoid analogues with selective binding properties need to be developed. Some progress has been made, and it seems that deletion of P receptors, with the exception of EP4, is not associated with serious problems of fetal development or physiologic function in animals.

### Leukotriene Receptors

Surface receptors for $LTB_4$, denoted BLT1 and BLT2 ($LTB_4$ R-1 and $LTB_4$ R-2), and for the cysteinyl (cys) LTs, produced by cells of the innate immune system, including Th2 lymphocytes, also exert their actions through

transmembrane-spanning GPCRs. High-affinity $LTB_4$ receptors transduce chemotaxis and adhesion responses, whereas low-affinity receptors are responsible for secretion of granule contents and superoxide generation. Recognition that cysLTs are important to the pathogenesis of diseases characterized by acute and chronic inflammation prompted development of selective cysLT receptor antagonists.[88,89] BLT2 knockout mice express normal levels of BLT1 but are protected from development of disease in the K/BxN model of inflammatory arthritis.[90] The COX-1–derived ligand 12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid (12-HHT), which is produced during thromboxane synthesis, is an endogenous high-affinity ligand for BLT2 (now called BLT2/HHTR), another example of a connection between the LO and COX pathways. $LTB_4$ in particular among the LTs is involved in development of atherosclerosis, which has emerged as a major concern for patients with inflammatory arthritis and lupus. The receptor for cysLTs includes two subtypes: $CysLT_1$ and $CysLT_2$. The molecular structure of $CysLT_2$ has been constructed. $CysLT_1$ contains 336 amino acid residues, and the gene encoding the receptor is located on the X chromosome. $CysLT_2$ contains 345 amino acids with 40% sequence identity to $CysLT_1$. $CysLT_1$ mediates calcium mobilization and inhibition of adenylate cyclase, whereas $CysLT_2$ mediates calcium mobilization and increased cAMP concentrations. The preferred ligands for $CysLT_1$ are $LTD_4 > LTC_4 > LTE_4$. $CysLT_2$ binds $LTC_4$ and $LTD_4$ equally, whereas $LTE_4$ exhibits low affinity for the receptor. Both receptors have wide tissue and cellular distribution, including a presence in cells that participate in immune responses.[3] Most actions of the cysLTs are mediated by $CysLT_1$. More than a dozen chemically distinct, specific, and selective antagonist drugs that block the binding of LT to $CysLT_1$ have been identified. Clinically, these compounds mainly have been used in the treatment of asthma. Development of selective $LTB_4$ receptor antagonists, which are useful in pre-clinical models, may lead to their use in inflammatory disease.[91] A challenge to the design of "anti-receptor therapy" is the genetic variation in GPCRs that can be associated with disease.[92] Adding to the complexity is the fact that variants may result in altered predisposition to disease, rather than manifestation of the disease. Each variant provides an opportunity to understand receptor function such as recycling or desensitization, thus enhancing the potential for the development of therapy.

## Lipoxin Receptors

LXs can act at their own specific receptors for $LXA_4$ and $LXB_4$, and $LXA_4$ can interact with a subtype of $LTD_4$ receptors. LXs can also act at intra-cellular targets within their cell of origin or after uptake by another cell. The cDNA for the seven-transmembrane-spanning, G protein–coupled $LXA_4$ receptor named ALX/FPR2 (Kd ~ 0.7 nM) has been cloned and characterized. Signaling involves a novel polyisoprenyl-phosphate pathway that regulates phospholipase D.[93] LX actions are cell type specific. The monocyte and neutrophil $LXA_4$ receptors are identical at the cDNA level, but they evoke different responses, and the $LXA_4$ receptor on endothelial cells seems to be a structurally distinct form. $LXA_4$ also binds to the human orphan receptor GPR32, a member of the chemoattractant receptor family. Like $LXA_4$, 15-epi-$LXA_4$ is an anti-inflammatory SPM that binds and activates ALX/FPR2 (Kd ~ 2 nM). Lipoxin $B_4$ ($LXB_4$) and aspirin-triggered 15-epi $LXB_4$ also have anti-inflammatory actions by oral administration and topical application. Stereoselective actions of these compounds indicate they have their own yet-to-be-identified receptors. The resolvins, derived from omega-3 fatty acids, activate the receptors GPR32 and ChemR23.

## Nuclear Receptors

Nuclear receptors are a superfamily of ligand-regulated transcription factors that interact with other transcription factors and with co-regulators that either enhance (co-activators) or inhibit (co-repressors) transcription. The major nuclear receptors involved in regulation of inflammation are the glucocorticoid receptors (GRs), peroxisome proliferator-activated receptors, liver X receptors (LXRs), and the orphan receptor nuclear receptor–related 1 protein (Nurr1). Other members of the nuclear receptor family that contribute to regulation of inflammation include estrogen receptors, vitamin D receptors, and retinoic acid receptors. The clinical efficacy of glucocorticoids is well known, but knowledge of their mechanisms of action has been slow to emerge. The ability of GRs to repress inflammatory responses is due in part to interference with other signal-dependent transcription factors and disruption of activator/co-activator complexes. PPARs are members of the nuclear receptor family of transcription factors, a large and diverse group of proteins that mediate ligand-dependent transcriptional activation and repression. PPARs were first cloned as nuclear receptors that mediate the effects on gene transcription of synthetic compounds called *peroxisome proliferators*. Several mechanisms account for the anti-inflammatory action of PPARγ, including inhibition of NF-κB; inhibition of transcription of genes encoding for chemokines, IL-1β, IL-12, and MMP-9; and promotion of expression of anti-inflammatory mediators, including IL-10 and LXR. PPARγ can be modulated by post-translational modifications (PTMs). Thus regulation of PTMs may be a better approach to disease therapy than the existing PPARγ activators.[94] Interest in PPARs increased dramatically when they were shown to be activated by medically relevant compounds, including NSAIDs and $PGD_2$ and its metabolite 15-deoxy-δ12,14 $PGJ_2$.[95] Most information available on a potential role of PPARs in inflammation relates to PPARγ. One PPARα agonist, palmitoyl ethanolamide, shows promise for relief of chronic pain.[96] PPARα is expressed mainly in tissues that have a high fatty acid catabolism, including liver and the immune system. $LTB_4$ is an activator and natural ligand of PPARα. Activation of PPARα results in induction of genes involved in fatty acid oxidation pathways that degrade fatty acids and derivatives including $LTB_4$. Thus a feedback mechanism that controls inflammation is established. Mechanisms for the anti-inflammatory actions of PPARβ/δ include induction of an anti-inflammatory co-repressor protein, inhibition of NF-κB, and induction of anti-inflammatory mediators. Experiments with PPAR knockout mice indicate that PPARα suppresses $LTB_4$-induced inflammation. PPAR agonists that target PPAR action more selectively are

being developed.[97] Overexpression of Nurr receptors reduces inflammatory cytokine expression, and mutations in the Nurr1 gene are associated with diminished counter-regulation of inflammation.[98]

Several kinases that facilitate co-repressor turnover have been identified. These kinases represent important pharmacologic targets because their inhibition should block gene expression of inflammatory mediators while bypassing the clinically significant adverse events associated with direct targeting of the nuclear receptors. Thus, for example, cartilage matrix degradation is prevented by inhibition of a cyclin-dependent kinase.[98]

## PLATELET-ACTIVATING FACTOR

PAF (1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent mediator of inflammation that causes neutrophil activation, increased vascular permeability, vasodilation, and bronchoconstriction, in addition to platelet activation. PAF is formed by a smaller number of cell types than the eicosanoids, mainly leukocytes, platelets, and endothelial cells. Because of the extensive distribution of these cells, however, the actions of PAF can manifest in virtually every organ system. In contrast to the two long-chain acyl groups present in phosphatidylcholine, PAF contains a long-chain alkyl group joined to the glycerol backbone in an ether linkage at position 1 and an acetyl group at position 2 (Figure 24-9). PAF represents a family of phospholipids (PAF-like lipids: PAF-LL) because the alkyl group at position 1 can vary in length from 12 to 18 carbons. PAF, similar to the eicosanoids, is not stored in cells. Rather, it is synthesized when cells are stimulated, at which time the composition of the alkyl group may change. The immediate effects of PAF are mediated through a cell surface GPCR, PAFR. PAFR is coupled to Gi, Gq, and G12/13. Activation of PAFR results in inhibition of cAMP, mobilization of calcium, and activation of MAPKs, whereas long-term responses depend on intra-cellular receptor activation.[99]

Despite the potent inflammatory effects of PAF, its inhibition in animal models does not lead to marked suppression of inflammatory responses. PAF acetylhydrolase (PAF-AH) circulates in blood in association with lipoproteins, is found in atherosclerotic lesions, and hydrolyzes PAF, and was thus considered to have anti-inflammatory properties and to be a marker for cardiovascular disease. However, PAF-AH also generates compounds that have pro-inflammatory activity, which likely explains why, in three clinical trials, a PAF-AH inhibitor failed to influence C-reactive protein levels, coronary atherosclerosis, or time to cardiovascular death.[100,101] Nonetheless, targeting PAF metabolism, signaling, and the PAF receptor, as monotherapy or with other agents, is a promising approach to suppression of inflammation and carcinogenesis.[102]

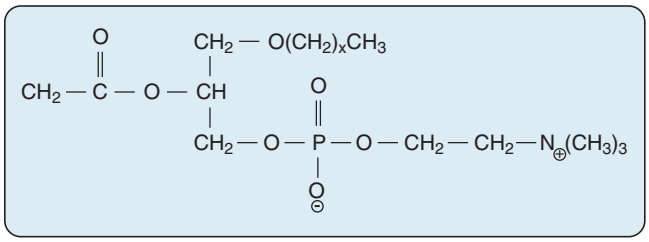



**Figure 24-9** Chemical structure of platelet-activating factor.

## EICOSANOIDS AS REGULATORS OF INFLAMMATION AND IMMUNE RESPONSES

It is clear that in addition to their well-known actions as mediators of inflammation, the stable PGs PGE and $PGI_2$ have anti-inflammatory, inflammatory, and immunomodulatory actions.[103,104] As noted,[68-70] PGJ, LXs, and an array of eicosanoids act as brakes to protect against runaway inflammatory responses. Even $LTB_4$ is capable of modulating inflammation and immune responses.[56] The observations that $PGE_1$ inhibits platelet aggregation and that it suppresses acute and chronic inflammation and joint tissue injury in animal models[105] led to the notion that COX products of AA metabolism might have anti-inflammatory activity. As it became more clear that NSAIDs have anti-inflammatory effects other than interference with COX production and subsequent PG inhibition,[106] consideration was given to the potential protective effects of PGs.

$PGE_1$ has remained an orphan among the eicosanoids, mainly because of a long-held notion that not enough of it is made by human cells to be of use and that its biologic effects are no different from the effects of $PGE_2$ and $PGI_2$. Contrary to popular belief, $PGE_1$ is found in physiologically important amounts in humans. Lost in the vast literature on the "AA cascade" are the early observations of Bygdeman and Samuelsson,[107] who found (using bioassay) that the concentration of $PGE_1$ in human seminal plasma (16 μg/mL) was higher than that of $PGE_2$ (13 μg/mL), $PGE_3$ (3 μg/mL), $PGF_{1\alpha}$ (2 μg/mL), and $PGF_{2\alpha}$ (12 μg/mL). Karim and colleagues[108] found $PGE_1$ to be the sole PGE in human thymus. PG immunoassays usually do not distinguish between $PGE_1$ and $PGE_2$. To identify $PGE_1$, it must first be separated from $PGE_2$ by thin-layer or high-performance liquid chromatography. When such methods have been used, $PGE_1$ has been identified consistently in platelets, leukocytes, macrophages, vas deferens, oviducts, uterus, heart, and skin.[109] Evidence from in vitro and in vivo experiments indicates that PGs, notably PGE compounds, can suppress diverse effector systems of inflammation. PGE can enhance and diminish cellular and humoral immune responses, observations that reinforce a view of these compounds as regulators of cell function. These actions of eicosanoids depend on the stimulus to inflammation, the predominant eicosanoid produced at a particular time in the host response, and the profile of eicosanoid-receptor expression.[110,111]

The "2" series prostaglandins (i.e., E2, D2, and I2) also regulate T cell function and immune responses. $PGE_2$ reduces production of several inflammatory cytokines, including TNF, IFN-γ, and IL-12 and reduces IFN-α production by plasmacytoid dendritic cells (PDCs) from patients with SLE. $PGE_2$-treated PDCs from patients with SLE also induce $CD4^+$ T cell proliferation and skew cytokine production toward a Th2 profile.[112] Another example of an endogenous link between the COX and LO pathways is provided by the observation that $PGE_2$ preserves resolution of inflammation in the murine collagen–induced arthritis model by increasing production of the pro-resolving $LXA_4$.[113]

## MODULATION OF EICOSANOID SYNTHESIS BY ADMINISTRATION OF PRECURSOR FATTY ACIDS

The relationship between essential fatty acids and PGs was discovered simultaneously and independently by van Dorp and colleagues[114] and Bergstrom and colleagues.[115] Both groups reported that AA was converted to $PGE_2$, and shortly thereafter they showed that $PGE_1$ is formed from DGLA.[116] Attempts to modify eicosanoid production have been directed at providing fatty acids other than AA as substrates for oxygenation enzymes in an effort to generate a unique eicosanoid profile with immunosuppressive and anti-inflammatory effects.[63,117] The fatty acids themselves, by virtue of their incorporation into signal-transduction elements, also have effects that are independent of eicosanoid effects on cells involved in inflammation and immune responses.[118]

Experiments directed at suppression of TX synthesis, enhancement of PGI production, and inhibition of platelet aggregation have been done in an effort to limit inflammatory responses. EPA is not found in appreciable amounts in cells from persons who eat a Western-style diet. Fish oil lipids, which are rich in EPA (20:5 n-3), inhibit formation of COX products (e.g., $TXA_2$ and $PGE_2$) derived from AA, and the newly formed $TXA_3$ has much less ability than $TXA_2$ to constrict vessels and aggregate platelets. Production of $PGI_2$ (prostacyclin) by endothelial cells is not reduced appreciably by increased EPA content, and the physiologic activity of newly synthesized $PGI_3$ is added to that of $PGI_2$. Administration of fish oil to humans leads to reduced production of $LTB_4$ by means of 5-LO in stimulated neutrophils and monocytes and induces EPA-derived $LTB_5$, which is far less biologically active than $LTB_4$. Fish oil also reduces production of IL-1β, TNF, and PAF by activated blood monocytes. In randomized controlled trials of administration of fish oil to patients with RA, tender joint counts and duration of morning stiffness are reduced, and use of NSAIDs is decreased. Fish oil treatment of patients with early RA (<12 months) reduces the triple disease-modifying anti-rheumatic drug (DMARD) failure rate and increases the American College of Rheumatology (ACR) remission rate.[119] Because NSAIDs confer an increased risk for cardiovascular disease and increased mortality from cardiovascular disease occurs in patients with RA, an added benefit of fish oil for patients with RA may be reduced risk of cardiovascular disease directly and by virtue of less use of NSAIDs.[120] Another potential benefit of an increase in dietary omega-3 fatty acids might be greater formation, from EPA and DHA, of resolvins, protectins, and maresins, all of which participate in resolution of inflammation.[66,68-70]

The other "alternative" eicosanoid precursor fatty acid, DGLA (20:3 n-6), is also increased by administration of certain plant seed oils, notably oils extracted from the seeds of *Oenothera biennis* (evening primrose) and *Boragio officinalis* (borage), which contain relatively large amounts of GLA. GLA is converted to DGLA, the immediate precursor of $PGE_1$, an eicosanoid with known anti-inflammatory and immunoregulating properties.[103] Administration of GLA to volunteers and patients with RA results in increased production of $PGE_1$ and reduced production of the inflammatory eicosanoids $PGE_2$, $LTB_4$, and $LTC_4$ by stimulated peripheral blood monocytes. In addition to competing with AA for oxidative enzymes, DGLA cannot be converted to inflammatory LTs. Rather, it is converted by means of 15-LO to a 15-hydroxy-DGLA, which has the capacity to inhibit 5-LO and 12-LO activities. DGLA should have anti-inflammatory actions because of its capacity to reduce synthesis of oxygenation products of AA through the COX and LO pathways.[117,121]

In addition to their roles as precursors of eicosanoids, essential fatty acids are important for maintenance of cell membrane structure and function, and they protect the gastric mucosa from NSAID-induced injury. DGLA can also modulate immune responses in an eicosanoid-independent manner. DGLA suppresses IL-2 production by human peripheral blood monocytes in vitro, suppresses proliferation of IL-2–dependent human peripheral blood and synovial tissue T lymphocytes, and reduces expression of activation markers on T lymphocytes directly in a manner that is independent of its conversion to eicosanoids. Oral administration of oils enriched in GLA, but not administration of oils enriched in linoleic acid (the parent n-6 fatty acid) or α-linolenic acid (the parent n-3 fatty acid), reduces proliferation of human lymphocytes activated through the T cell receptor complex.[122]

Addition of GLA to peripheral blood mononuclear cells in vitro or oral administration of GLA to volunteers reduces production of IL-1β and TNF from stimulated cells and reduces autoinduction of IL-1β, preserving the protective effects of IL-1β, while suppressing excessive production of the cytokine.[123] GLA suppresses acute and chronic inflammation, including arthritis, in several animal models, and randomized, double-blind, placebo-controlled trials of GLA in patients with RA and active synovitis indicate that GLA treatment results in statistically significant and clinically relevant reduction in signs and symptoms of disease activity compared with baseline and placebo. GLA also reduces the need for NSAID and corticosteroid therapy.[124,125]

EPA suppresses conversion of DGLA to AA, and a combination of EPA-enriched and GLA-enriched oils exhibits synergy in its capacity to reduce the reduction of synovitis in animal models.[126] In addition, administration of black currant seed oil, which contains the n-3 fatty acid α-linolenic acid (which is converted to EPA) and the n-6 GLA, suppresses active synovitis in patients with RA.[127] The synergy observed in the animal models was not reprised in an 18-month double-blind trial of a combination of fish oil and borage oil in patients with RA. However, treatment with each oil alone and with the combination resulted in meaningful clinical responses, including a reduction in DMARD therapy greater than that seen in matched patients from an RA registry. In addition, all three treatment groups exhibited significantly reduced total and low-density lipoprotein cholesterol and triglycerides, increased high-density lipoprotein cholesterol, and an improved plasma atherogenic index.[128,129] Much larger amounts of the polyunsaturated fatty acids (GLA, EPA, and DHA) can be delivered in far smaller capsules than are needed to accommodate the natural oils, a strategy that should be adopted to improve compliance.



The full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 25



# Cell Recruitment and Angiogenesis

Zoltán Szekanecz • Alisa E. Koch

**KEY POINTS**

- Leukocyte recruitment into tissues through the vessel wall into the synovium is a crucial process in the pathogenesis of inflammatory diseases.
- Several classes of cell adhesion molecules are involved in leukocyte extravasation.
- Chemokines and their receptors stimulate chemotaxis of neutrophils, lymphocytes, and monocytes into tissues.
- Angiogenesis, the formation of new vessels, is involved in inflammation and tumor progression.
- A number of soluble and cell-bound factors, including chemokines and adhesion receptors, may stimulate or inhibit angiogenesis.
- Specific targeting of leukocyte adhesion, chemokines, and/or angiogenesis might be useful for the future management of rheumatic diseases; however, clinical trials have primarily had negative or mixed results to date.

Inflammatory leukocytes, endothelial cells (ECs), synovial fibroblasts, as well as soluble mediators and cell surface–bound adhesion molecules (CAM), are involved in cell trafficking in inflammatory rheumatic diseases[1-6] (Figure 25-1). Leukocyte entry into inflamed tissues occurs by leukocyte adhesion to ECs, followed by transendothelial migration.[3-6] Transendothelial migration or diapedesis may be the point of no return during the inflammatory response.[6] The chemotaxis of inflammatory cells is mainly regulated by chemokines and their receptors.[7-11] The formation of new capillaries from pre-existing vessels, termed *angiogenesis*, is a key event underlying inflammation that perpetuates leukocyte adhesion and migration.[8,12-18] On the other hand, new vessel formation from endothelial progenitor cells (EPCs), termed *vasculogenesis*, is impaired in inflammatory arthritides.[16-20] Inflammatory sites contain an existing network of cells, CAMs, chemokines, and angiogenic factors. Rheumatoid arthritis (RA) is an excellent prototype of chronic inflammatory diseases; thus the inflammatory processes previously described will be discussed mainly in light of the data obtained from the study of this disease. First, the key characteristics of endothelial biology, CAMs, chemokines, angiogenesis, and vasculogenesis will be presented, followed by a description of leukocyte trafficking. Next, the targeting of CAMs, chemokines, and neovascularization will be discussed.

## ENDOTHELIAL PATHOPHYSIOLOGY IN INFLAMMATION

### Endothelial Permeability

ECs line the lumina of blood vessels, and thus they separate and connect the blood and the extra-cellular matrix (ECM) of the vessel wall. The endothelium is involved in numerous homeostatic mechanisms, as well as in pathologic states such as inflammation.[21-24]

ECs express a variety of cell surface molecules and synthesize and release soluble mediators. In inflammatory states, such as arthritis, ECs interact with other cell types, including leukocytes, fibroblasts, and smooth muscle cells. ECs express numerous CAMs, adhere to ECM components, secrete a number of inflammatory mediators, such as cytokines, nitric oxide (NO), prostaglandins, endothelin 1 (ET-1), and proteases, and thus regulate inflammation in the surrounding tissue. On the other hand, ECs are active responders to external stimuli because they may become targets of inflammatory mediators produced by leukocytes.[21-23,25-30]

Under inflammatory conditions, the vascular endothelium undergoes morphologic changes that include vasodilatation and increased permeability (leakage). These morphologic alterations may result from several mechanisms, including EC contraction and retraction, leukocyte- or anti-EC antibody (AECA)-mediated vascular injury and regeneration.[21,26,31,32] ECs and leukocytes release vasodilatory mediators, including prostacyclin ($PGI_2$), NO, and platelet-activating factor (PAF).[21,22,27,33] Factors that trigger endothelial leakage include, among others, histamine, serotonin, complement factors, bradykinin, leukotrienes, PAF, and AECA. EC adhesion, accompanied by cytoskeletal reorganization, occurs upon EC activation by pro-inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF) or interferon (IFN)-γ.[21,33-35]

### Endothelial Injury and Regeneration

In inflammatory states, endothelial injury is caused by inflammatory cells, as well as soluble mediators.[23,36-38]

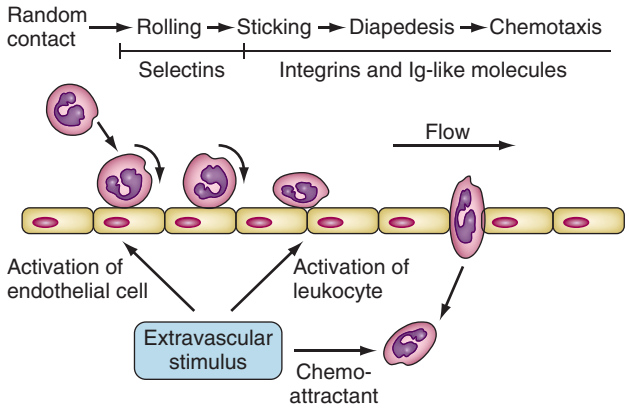



**Figure 25-1** The process of leukocyte extravasation into the synovium. Ig, Immunoglobulin.

Leukocyte-EC interactions, described later in more detail, may cause EC injury and thus increased vascular leakage. EC injury primarily involves reactive oxygen intermediates (ROI) and matrix metalloproteinase (MMP) enzymes produced by inflammatory leukocytes.[23-25,38,39] In addition to leukocytes, various mediators produced by ECs themselves and other cells lead to endothelial injury. ECs synthesize NO, prostaglandins, ET-1, and other substances.[21-23,28,30,39,40] During inflammation, ECs and leukocytes produce high amounts of NO,[23,41] and NO interacts with ROI.[23,39,40] Asymmetric dimethylarginine (ADMA) is a naturally produced amino acid that circulates in the plasma. Increased ADMA production has been associated with both vascular injury and inflammatory rheumatic diseases.[23,40,42,43] ET-1, a vasoconstrictor peptide, activates leukocyte adhesion and is also involved in ECM remodeling and vascular injury.[23,44]

Vascular regeneration occurring after inflammatory injury is linked to EPCs and vasculogenesis and therefore will be discussed later in this chapter. Apart from naturally occurring processes that lead to regeneration, there have been several attempts to prevent endothelial injury, among others, by organic nitrates (isosorbide-dinitrate and isosorbid-mononitrate) that increase NO production, thrombomodulin, tissue factor, prostacyclin (PGI), adiponectin, sirtuins (Sirt-1), or ligustrazine.[45]

## CELL ADHESION MOLECULES

CAMs are involved in cell adhesion and migration underlying inflammation, malignancies, tissue development, and repair. CAMs have been classified into integrin, selectin, immunoglobulin (Ig), and cadherin superfamilies based on their structural features[3,4,6,46] (Table 25-1).

### Integrins

Integrins are αβ heterodimers, in which each of the common β chains is associated with one or more α subunits.[46,47] Cell adhesion to ECM is mostly mediated by $\beta_1$ and $\beta_3$, whereas intercellular adhesion is facilitated through $\beta_1$ and $\beta_2$ integrins.[2,46,47] $\beta_1$ and $\beta_3$ integrins are expressed on ECs, whereas $\beta_2$ integrins are leukocyte CAMs.[47] The $\alpha_1$-$\alpha_6$, $\alpha_V$, $\alpha_L$, $\alpha_M$, $\alpha_X$, and $\beta_1$-$\beta_7$ integrin subunits have all been detected in RA.[3-5,48] Only the most important integrins will be discussed further.

Among $\beta_1$ integrins, the $\alpha_4\beta_1$ heterodimer, also known as very late antigen 4 (VLA-4), plays an important role in lymphocyte-endothelial interactions. This molecule recognizes at least two alternative ligands, such as vascular cell adhesion molecule-1 (VCAM-1), a member of the Ig superfamily, and the CS-1 region of fibronectin. $\alpha_4\beta_1$ is present on mononuclear leukocytes.[3,46,47] The $\alpha_4\beta_1$ integrin, as well as VCAM-1 and CS-1 are expressed on various cell types within the RA synovium.[3,4] Other $\beta_1$ integrins also exert increased expression on lymphocytes under inflammatory conditions.[3,4,48] For example, synovial lining cells also express the $\beta_1$ integrin subunit, whereas ECs express $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$.[3]

All members of the $\beta_2$ (leukocyte) integrin family, namely, the lymphocyte function-associated antigen 1 (LFA-1) ($\alpha_L\beta_2$ dimer), the Mac-1 ($\alpha_M\beta_2$), and the p150,95

**TABLE 25-1** Some Relevant Adhesion Receptor-Ligand Pairs in Inflammation

| Adhesion Receptors | Ligands |
|---|---|
| **Integrins** | |
| $\alpha_1\beta_1$ (VLA-1) | Laminin, collagen |
| $\alpha_2\beta_1$ (VLA-2) | Laminin, collagen |
| $\alpha_3\beta_1$ (VLA-3) | Laminin, collagen, fibronectin |
| $\alpha_4\beta_1$ (VLA-4) | Fibronectin, VCAM-1 |
| $\alpha_5\beta_1$ (VLA-5) | Fibronectin |
| $\alpha_6\beta_1$ (VLA-6) | Laminin |
| $\alpha_L\beta_2$ (LFA-1, CD11a/CD18) | ICAM-1, ICAM-2, ICAM-3, JAM-A |
| $\alpha_M\beta_2$ (Mac-1, CD11b/CD18) | ICAM-2, iC3b |
| $\alpha_X\beta_2$ (CD11c/CD18) | iC3b, fibrinogen |
| $\alpha_E\beta_7$ | E-cadherin |
| $\alpha_4\beta_7$ | Fibronectin, VCAM-1, MadCAM-1 |
| **Immunoglobulin Superfamily** | |
| ICAM-1 (CD54) | LFA-1, Mac-1 |
| ICAM-2 | LFA-1 |
| ICAM-3 | LFA-1 |
| VCAM-1 | $\alpha_4\beta_1$, $\alpha_4\beta_7$ |
| MadCAM-1 | $\alpha_4\beta_7$, L-selectin |
| CD2 | LFA-3 |
| PECAM-1 (CD31) | PECAM-1, $\alpha_V\beta_3$ |
| **Selectins** | |
| L-selectin (CD62L, LAM-1) | Sialylated carbohydrates, GlyCAM-1 |
| E-selectin (CD62E, ELAM-1) | ESGL (Sialyl-Lewis-X) |
| P-selectin (CD62P) | PSGL (sialyl-Lewis-X, other carbohydrates) |
| **Cadherins** | |
| E-cadherin (Cadherin-1) | E-cadherin |
| N-cadherin (Cadherin-2) | N-cadherin |
| Cadherin-11 | Cadherin-11 |
| **Others** | |
| CD44 | Hyaluronic acid, fibronectin |
| Endoglin | TGF-β1, TGF-β3 |
| JAMs | JAMs |

ELAM, Endothelial leukocyte adhesion molecule; ESGL, E-selectin glycoprotein ligand; GlyCam, glycosylation-dependent cell adhesion molecule; ICAM, intercellular adhesion molecule); JAM-A, junctional adhesion molecule-A; LAM, leukocyte adhesion molecule; LFA, lymphocyte function–associated antigen; Mac-1, macrophage antigen-1; MadCAM, mucosal addressin cell adhesion molecule; PECAM, platelet-endothelial adhesion molecule; PSGL, P-selectin ligand; TGF, transforming growth factor; VLA, very late antigen; VCAM, vascular cell adhesion molecule.

($\alpha_X\beta_2$) molecules are present on neutrophils and monocytes. In contrast, only LFA-1 is present on lymphocytes. All three intercellular adhesion molecule (ICAM) ligands for LFA-1, namely ICAM-1, ICAM-2, and ICAM-3 belong to the Ig superfamily. Mac-1 binds ICAM-1 and some non-CAM ligands. All $\beta_2$ integrins are expressed on synovial leukocytes.[3-5]

The $\beta_3$ integrins (gpIIb/IIIa molecule, $\alpha_g$pIIb$\beta_3$, and the vitronectin receptor, $\alpha_V\beta_3$) have been detected on macrophages, lining cells, and fibroblasts in the RA synovium.[3,48] Among other integrins containing the $\alpha_V$ subunit, the fibronectin-vitronectin receptor $\alpha_V\beta_5$ is expressed by most, whereas $\alpha_V\beta_1$ and $\alpha_V\beta_3$ have been detected on some synovial lining cells. Their ligands, fibronectin and vitronectin are also present in the synovial lining layer.[3,48] Another $\alpha_4$ subunit–containing integrin, $\alpha_4\beta_7$, also binds to VCAM-1 and the CS-1 peptide.[47] The $\alpha_4\beta_7$ integrin is highly expressed in tissues belonging to the mucosa-associated lymphoid tissue (MALT), such as the gut and skin, as well as the synovium. Therefore this integrin may link arthritis to inflammatory bowel disease or psoriasis.[49]

## Immunoglobulin Superfamily Members

The Ig superfamily is a group of transmembrane glycoproteins that contain one or more Ig-like motifs.[46,47] VCAM-1, already mentioned previously, is constitutively expressed on ECs, however, its expression is upregulated by pro-inflammatory cytokines.[3,46] There is abundant VCAM-1 expression in the inflamed synovium.[3-5]

ICAM-1, the counter-receptor for the $\beta_2$ integrins, is expressed on both EC and leukocytes.[3,46,47,50] ICAM-2 is constitutively expressed on EC and may not be involved in inflammatory transendothelial migration.[50] ICAM-3 is a leukocyte CAM; however, it is also present on some ECs.[50] ICAM-1 and ICAM-3 expression is upregulated under inflammatory conditions, such as in RA.[3,4,50] Both ICAM-1 and VCAM-1 are clustered to the front of the cell membrane during diapedesis. Actin-rich docking structures are formed before transendothelial migration.[6]

Other members of the Ig superfamily include CD2 and LFA-3. These CAMs bind to each other.[3,46,47] Although CD2 is a T cell marker, LFA-3 is present on ECs and synovial lining cells, as well as macrophages and fibroblasts.[3,4]

Platelet-endothelial adhesion molecule 1 (PECAM-1) mediates homotypic adhesion by binding to another PECAM-1, as well as heterotypic adhesion by recognizing the $\alpha_V\beta_3$ integrin.[3,46,51] PECAM-1 is a marker of ECs,[46,51] and its expression is upregulated on RA synovial lining cells and interstitial macrophages.[3,48,51]

Interestingly, some sialylated and glycosylated CAMs, also known as tumor-associated antigens, such as members of the carcinoembryonic antigen (CD66) family, also belong to the Ig superfamily. Structurally, CD66a-e molecules are closely related to PECAM-1 and CD66. Similar to PECAM-1, these molecules exert abundant expression on myeloid cells in the RA synovium.[1,3]

## Selectins

Selectins contain a lectin-like extra-cellular N-terminal domain, an epidermal growth factor (EGF)-like motif, and two to nine moieties related to complement regulatory proteins.[47,52] E- and P-selectin are expressed by ECs, whereas L-selectin is mostly expressed by leukocytes.[52] E-selectin is a marker for cytokine-induced EC activation.[52] E-selectin ligand-1 (ESL-1) and P-selectin ligand-1 (PSGL-1) contain sialylated glycan motifs such as sialyl Lewis-X ($sLe^x$).[52] P-selectin is constitutively present on the membrane of EC Weibel-Palade bodies.[52] L-selectin serves as a lymphocyte homing receptor, where it mediates the physiologic recirculation of naïve lymphocytes through specialized high-endothelial venules (HEVs).[46,52] However, L-selectin is also involved in inflammatory leukocyte recruitment.[3,52]

Increased expression of E- and P-selectin occurs in RA compared with noninflamed synovial ECs. Whereas E- and P-selectin can be induced upon EC activation, the cellular expression of L-selectin is decreased by shedding after cytokine treatment.[3,4,48,53]

## Cadherins

Cadherins are calcium-dependent CAMs. N-, P- and E-cadherin are primarily involved in embryogenesis and tissue development; however, synovial fibroblast cadherin-11 has been implicated in arthritis as well.[3,4,46,47]

## Other Adhesion Receptors

CD44 exerts a proteoglycan-like structure. It is a receptor for hyaluronate and is expressed on activated ECs in inflammatory sites.[3,4,46,48,53,54] CD44 is present on most synovial lymphocytes, as well as lining cells, leukocytes, ECs, and fibroblasts.[3,4,53]

Vascular adhesion protein 1 (VAP-1) is isolated from synovial ECs. VAP-1 is a marker of activated endothelium, because its expression is increased in inflammation.[55]

Endoglin has been identified as a receptor for transforming growth factors (TGF)-β1 and -β3. This molecule contains the RGD (arginine-glycine-aspartate) sequence, a motif found in a number of ECM ligands for integrins. Thus endoglin may be involved in intercellular adhesion. Endoglin was detected on most ECs; however, endoglin expression is increased on RA synovial lining cells and macrophages.[1,3]

Junctional cell adhesion molecules (JAMs) are also involved in transendothelial migration of leukocytes. JAM-A and JAM-C have been implicated in adhesive events underlying inflammatory conditions, such as RA.[2,6,56]

Other CAMs involved in leukocyte transendothelial migration in various inflammatory conditions include poliovirus receptor (CD155), MUC18 (CD146), CD166, and integrin-associated protein (CD47).[6,57-59]

# CHEMOKINES AND CHEMOKINE RECEPTORS

## Chemokine Superfamilies and Their Receptors

Chemokines have been classified into the CXC, CC, C, and $CX_3C$ supergene families.[60-63] The traditional names of chemokines have been replaced by a unique designation of CXCL, CCL, XCL and $CX_3CL$, considering all chemokines as ligands of their respective receptors.[61-63] In CXC

chemokines, two conserved C residues are separated by one unconserved amino acid. These mediators chemoattract neutrophils, lymphocytes, and monocytes. The underlying molecular mechanisms include leukocyte integrin expression and L-selectin shedding, cytoskeletal reorganization, neutrophil degranulation, and phagocytosis, as well as the production of MMPs and other inflammatory mediators.[10,11,61,62,64] CC chemokines include two adjacent C residues in their structure. These CC chemokines are chemotactic for monocytes and lymphocytes.[61-63] The C chemokine family contains two members, XCL1 (lymphotactin), and XCL2 (single C motif 1β; SCM-1β). The single member of the $CX_3C$ family is $CX_3CL1$ (fractalkine). This chemokine is chemotactic for mononuclear cells, mediates T cell adhesion and cytokine production, regulates the cytoskeletal structure, proliferation, and migration of synovial fibroblasts. $CX_3CL1$ is also an angiogenic mediator[65,66] (Table 25-2).

Chemokine receptors have seven transmembrane domains and are expressed on target cells. They have been termed *CXCR*, *CCR*, *CR*, and *$CX_3CR$*.[60-63] Some chemokine receptors, such as CXCR2, CCR1, CCR2, CCR3, or CCR5 have multiple ligands, whereas others, including CXCR4, CXCR5, CXCR6, CCR8, or CCR9, are specific receptors for one single ligand[10,11,61] (see Table 25-2).

## CXC Chemokines in Arthritis

The most relevant CXC chemokines involved in the pathogenesis of arthritis are CXCL1 (groα), CXCL4 (platelet factor 4; PF4), CXCL5 (epithelial cell–derived neutrophil attractant 78; ENA-78), CXCL6 (granulocyte chemotactic protein 2; GCP-2), CXCL7 (connective tissue–activating protein III; CTAP-III), CXCL8 (IL-8), CXCL9 (Mig), CXCL10 (IFN-γ–inducible 10 kDa protein; IP-10), CXCL12 (stromal cell–derived factor 1; SDF-1), CXCL13 (B cell–activating chemokine 1; BCA-1), and CXCL16. All are abundantly expressed in the sera, synovial fluids, and tissues of RA patients.[60,67]

During the last decade, it has become clear that anti-citrullinated peptide antibodies (ACPA) are a harbinger of RA.[68,69] Given this important finding, we have recently shown that alteration of just one amino acid from arginine to citrulline in CXCL5 (ENA-78) changes its function from a nonmonocyte-recruiting to a monocyte-recruiting chemokine.[70]

Synovial macrophages are the major source of most CXC chemokines.[67,71] CXCL12, 13, and 16 are more peculiar CXC chemokines in many ways. First, although the other CXC chemokines described previously have common receptors, CXCL12, 13, and 16 are specific ligands for CXCR4, 5, and 6, respectively.[67,71] CXCL12 promotes angiogenesis despite lacking the glutamyl-leucyl-arginyl (ELR) motif, which is usually required for this function.[8,72,73] CXCL12 induces CXCR4-dependent integrin-mediated lymphocyte and monocyte adhesion and migration, as well as osteoclastogenesis, bone resorption, and thus radiographic progression in RA.[74-80] CXCL13 is also expressed by synovial fibroblasts, T cells, and ECs in RA.[81,82] CXCL16 is secreted by synovial macrophages and fibroblasts and is involved in mononuclear cell recruitment into the RA synovium.[83-86]

**TABLE 25-2** Chemokines and Chemokine Receptors Relevant for Arthritis

| Chemokine Receptor | Chemokine Ligand |
|---|---|
| **CXC Chemokine Receptors** | |
| CXCR1 | CXCL8, CXCL6 |
| CXCR2 | CXCL8, CXCL5, CXCL1, CXCL7, CXCL6 |
| CXCR3 | CXCL10, CXCL4, CXCL9 |
| CXCR4 | CXCL12 |
| CXCR5 | CXCL13 |
| CXCR6 | CXCL16 |
| CXCR7 | CXCL11, CXCL12 |
| **CC Chemokine Receptors** | |
| CCR1 | CCL3, CCL5, CCL7, CCL14, CCL15, CCL16 |
| CCR2 | CCL2, CCL7, CCL16 |
| CCR3 | CCL5, CCL8, CCL7, CCL15 |
| CCR4 | CCL17, CKLF1 |
| CCR5 | CCL3, CCL5, CCL8, CCL14 |
| CCR6 | CCL20 |
| CCR7 | CCL21 |
| **C Chemokine Receptors** | |
| XCR1 | XCL1 |
| **C-X3-C Chemokine Receptors** | |
| CX3CR1 | CX3CL1 |

Chemokines are classified according to the positions of the first two cysteines (C) of the four conserved N-terminal cysteines: CC chemokine ligand (CCL), first two cysteines are adjacent; CXC chemokine ligand (CXCL), first two cysteines are separated by amino acid X other than cysteine; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor.

## CC Chemokines in Rheumatoid Arthritis

CCL2 (monocyte chemotactic protein [MCP]-1), CCL3 (macrophage inflammatory protein 1; MIP-1α), CCL5 (Regulated upon Activation, Normal T cell Expressed and Secreted [RANTES]), CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4), CCL14 (hemofiltrate CC chemokine [HCC]-1), CCL15 (HCC-2), CCL16 (HCC-3), CCL17 (thymus- and activation-regulated chemokine; TARC), CCL18 (PARC), CCL19 (EBL-1–ligand chemokine [ELC]), CCL20 (MIP-3α), and CCL21 (synovial lining cells [SLC]) have all been detected in RA sera and synovia.[67,71,87]

Among these chemokines, CCL20 preferentially recruits Th17 cells,[88] induces both osteoblast proliferation and osteoclast differentiation, and collaborates with the RANK ligand system in the uncoupling between new bone formation and bone resorption in RA.[89,90] CCL13 has been associated with the RA cartilage and is released by articular chondrocytes.[91,92]

## C and $CX_3C$ Chemokines in Rheumatoid Arthritis

XCL1 (lymphotactin) is involved in T cell accumulation in the joint affected with RA.[93,94] CX3CL1 expression is upregulated in the joint affected with RA.[65] This chemokine has also been associated with accelerated atherosclerosis that leads to increased cardiovascular morbidity in RA.[66,95]

## Chemokine Receptors in Arthritis

In general, most CXCRs have been implicated in the pathogenesis of arthritis. CXCR1 and CXCR2 are the most

relevant inflammatory and angiogenic CXC chemokines described previously.[10,61,96] CXCR3 may be the most important receptor in leukocyte homing into Th1-type inflammatory sites, such as the RA synovium.[97,98] CXCR4 is involved in CXCL12-dependent lymphocyte migration.[78] CXCR4, CXCR5, and CXCR6 are involved in synovial lymphoid neogenesis.[8,87]

Among CCRs, CCR1, 2, 3, 4, 5, 6, and 7 are abundantly expressed in the RA synovium and on synovial cells.[8,67] CCR5 may be the most prominent CCR to characterize Th1 inflammatory infiltrates.[98,99] In some studies, a single nucleotide polymorphism leading to the production of the truncated Δ32-CCR5 nonfunctional receptor allele was found to protect against RA.[100-103] CCR6 is involved in the ingress of Th17 lymphocytes into the joint.[88,104] CCR7 has been associated with synovial lymphoid neogenesis.[105]

XCR1 is expressed on RA synovial lymphocytes, macrophages, and fibroblasts, whereas $CX_3CR1$ has been detected on macrophages and dendritic cells.[61,65,106] $CX_3CR1$ has been implicated in the recruitment of Th1-type lymphocytes into the joint.[106]

## ANGIOGENESIS IN INFLAMMATION

### Angiogenic Process

Synovial angiogenesis is a cascade of multiple events in which new capillaries develop from existing blood vessels. First, angiogenic mediators, which are described later in the text, activate ECs. This activation is associated with the production of proteases. These enzymes degrade the EC basement membrane and the ECM, thus enabling endothelial outgrowth. The migration of loose ECs results in the formation of capillary sprouts; followed by lumen formation within the sprout. Two sprouts anastomose with each other, thus forming capillary loops. New basement membrane is then synthesized. The continuous emigration of ECs results in further generation of new vessels. The outcome of angiogenesis is based upon the imbalance between angiogenic mediators and angiostatic factors described later in the text.[14,107-110]

### Mediators of Angiogenesis in Rheumatoid Arthritis

Several cytokines, growth factors, chemokines and their receptors, certain CAMs, as well as other mediators, can modulate neovascularization in RA[1,14-16,109,110] (Table 25-3).

Among growth factors, vascular endothelial growth factor (VEGF) and the hypoxia-hypoxia–inducible factor (HIF)–VEGF-angiopoietin (Ang)/Tie axis are of outstanding importance in RA-associated angiogenesis (Figure 25-2). VEGF is induced by hypoxia and HIFs (HIF-1 and HIF-2) in RA. Significant hypoxia is characteristic of RA joints. This low oxygen tension has been associated with inflammatory activity and increased expression of angiogenic VEGF. Hypoxia enables the outgrowth of immature, unstable vessels. HIFs are the master regulators of the adaptive responses to hypoxia. HIF is a heterodimeric transcription factor composed of HIF-α and HIF-β. The VEGF gene that contains hypoxia responsive elements (HRE), is a target for HIFs. High oxygen tension leads to hydroxylation and thus degradation of HIF-α, whereas hypoxia inhibits HIF hydroxylation, allowing HIF-1α stabilization and dimerization with HIF-1β (see Figure 25-2). However, hypoxia may also act via HIF-independent regulatory pathways, including peroxisome proliferator-activated receptors (PPAR). The Ang1/Tie2 complex interacts with VEGF during the stabilization of newly formed blood vessels. In contrast, Ang2, an antagonist of Ang1, inhibits vessel maturation. VEGF, HIF-1α, HIF-2α, Ang1, and Tie2 have been detected in the RA synovium even in the very early phase of the disease. Toll-like receptors (TLR) such as TLR2 may also induce VEGF-dependent angiogenesis, and this is mediated through Tie2 signaling in RA.[111] Survivin, an apoptosis inhibitor, is also involved in VEGF-dependent angiogenesis. Hypoxia also stimulates the production of CXCL12 (a major angiogenic chemokine described previously) by RA synovial fibroblasts.[14,112-117] Other angiogenic mediators, such as IL-6, IL-17, IL-18, nitric oxide (NO), monocyte/macrophage migration inhibitory factor (MIF), ET-1, and others may also stimulate angiogenesis via VEGF-dependent mechanisms.[14,112,115]

Placenta growth factor (PIGF) is a member of the VEGF family, and this mediator also binds to the VEGF receptor. There is abundant expression of PIGF in the arthritic synovial tissue. Other growth factors, including fibroblast (FGF-1

**TABLE 25-3** Some Angiogenic and Angiostatic Factors in Rheumatoid Arthritis

| | Mediators | Inhibitors |
|---|---|---|
| Chemokines | CXCL1, CXCL5, CXCL7, CXCL8, CXCL12, CCL2, CCL21, CCL23, CX3CL1 | CXCL4, CXCL9, CXCL10, CCL21 |
| Matrix molecules | Type I collagen, fibronectin, laminin, heparin, heparan sulphate | Thrombospondin, RGD sequence |
| Cell adhesion molecules | $\beta_1$ and $\beta_3$ integrins, E-selectin, P-selectin, CD34, VCAM-1, endoglin, PECAM-1, VE-cadherin, $Le^y$/H, MUC18 | RGD sequence (integrin ligand) |
| Growth factors | VEGF, bFGF, aFGF, PDGF, EGF, IGF-I, HIF-1, TGF-β* | TGF-β* |
| Cytokines | TNF, IL-6, IL-15, IL-18 | IL-4, IL-6,* IFN-α, IFN-γ |
| Proteases | MMPs, plasminogen activators | TIMPs, plasminogen activator inhibitors |
| Others | Angiogenin, substance P, prolactin | DMARDs, infliximab, etanercept, angiostatin, endostatin |

*Mediators with both proangiogenic and antiangiogenic effects.
aFGF, Acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; DMARDs, disease-modifying anti-rheumatic drug; EGF, endothelial growth factor; HIF-1, hypoxia-inducible factor-1; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; MMPs, matrix metalloproteinases; MUC18, mucin-like protein 18; PDGF, platelet-derived growth factor; PECAM, platelet-endothelial adhesion molecule; RGD, arginine-glycine-aspartate; TGF, transforming growth factor; TIMPs, tissue inhibitors of metalloproteinase; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

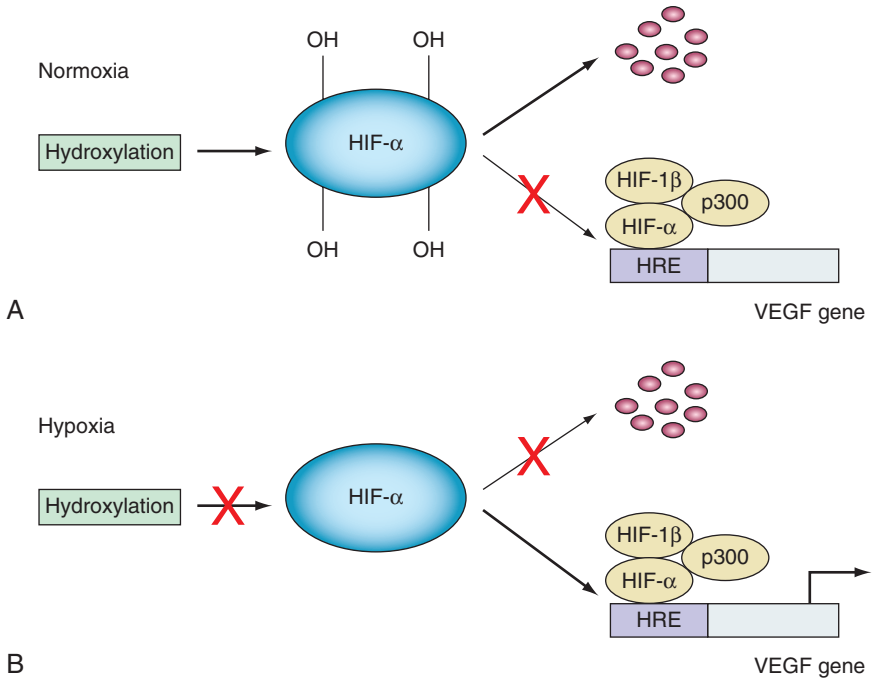



**Figure 25-2** Regulatory events in the hypoxia-hypoxia–inducible factor (HIF)–vascular endothelial growth factor (VEGF) cascade. Under a normoxic state, HIF-α is heavily hydroxylated, which leads to HIF degradation. Under a hypoxic state, HIF-α is dehydroxylated, translocates to the nucleus, and binds to HIF-1β. This results in binding of the HIF complex to hypoxia responsive elements (HRE) found in the VEGF gene and other angiogenic mediators.

and -2), epidermal (EGF), hepatocyte (HGF), keratinocyte (KGF), insulin-like (IGF-I), connective tissue (CTGF), platelet-derived (PDGF), and TGF-β, have been implicated in synovial angiogenesis. Many of these growth factors are bound to heparin and heparan sulfate proteoglycans in the synovial ECM, and they are mobilized by proteases during neovascularization.[1,14-16,109,110]

Pro-inflammatory cytokines may exert direct angiogenic activity or may act indirectly via VEGF-dependent pathways. Among these mediators, TNF, IL-1, IL-6, IL-15, IL-17, IL-18, oncostatin M, MIF, granulocyte colony-stimulating (G-CSF) and granulocyte-macrophage colony-stimulating factors (GM-CSF) have been implicated in synovial angiogenesis.[14,110,118-120]

Numerous chemokines and chemokine receptors described previously have been implicated in angiogenesis underlying RA. The angiogenic nature of most CXC chemokines has been associated with the ELR amino acid motif within their structure. The most relevant ELR-containing CXC chemokines that mediate angiogenesis include CXCL1, 5, 7, and 8.[13,16,64,121] In contrast, the ELR-lacking CXCL4, 9, and 10 inhibit angiogenesis.[8] CXCL12 is also angiogenic despite the fact that this chemokine lacks the ELR motif.[7] Among CC chemokines, CCL2 induces EC chemotaxis in vitro and angiogenesis in vivo. CCL23 has been implicated in the migration of vascular ECs and MMP production. $CX_3CL1$ is involved in both angiogenesis and atherosclerosis underlying inflammatory rheumatic diseases. Among chemokine receptors, CXCR2 may be the most relevant to ELR-containing angiogenic CXC chemokines, such as CXCL1, 5, and 8 on ECs. CCR2-CCL2 and CCR7-CCL21 interactions are also involved in synovial neovascularization.[8,116]

Several ECM components, CAMs, and proteases have been implicated in adhesive and angiogenic processes in RA. Type I collagen, fibronectin, laminin, vitronectin, tenascin, and proteoglycans mediate, whereas thrombospondin 1 (TSP-1) inhibits EC adhesion and neovascularization. As described previously, some growth factors bind to proteoglycans during angiogenesis. Among CAMs, most $\beta_1$ and $\beta_3$ integrins, E-selectin, the L-selectin ligand CD34, selectin-related glycoconjugates, including Lewis$^y$/H and MUC18, VCAM-1, PECAM-1, endoglin, and JAMs are expressed on the EC surface and promote angiogenesis. MMPs, ADAM, and ADAMTS proteases digest the ECM, release growth factors and other angiogenic mediators, and thus promote neovascularization in RA.[3,4,14,107,108,122,123]

Lewis$^y$/H, as well as fucosyl-transferase 1 (FUT1) that synthesizes Lewis$^y$/H have been implicated in angiogenesis, synovial fibroblast proliferation, and monocyte recruitment.[124-126] Lewis$^y$/H and its analog H-2g stimulate endothelial CAM expression and the release of angiogenic growth factors.[125] In the K/BxN serum transfer model, a mouse model for inflammatory arthritis, FUT1 gene–deficient mice were resistant to development of arthritis and also exhibited decreased angiogenesis and leucocyte ingress into inflamed joints.[127] Moreover, FUT1 is also responsible for the fucosylation of a number of human synovial RA antigens. For instance, RA synovial tissue ECs exhibit high expression of α.[1,2] linked fucosylated proteins compared with normal synovial tissue ECs. Depletion of these proteins in RA synovial fluid reduced the ability of these proteins to induce EC migration.[128] These findings suggest that FUT1 may be a novel therapeutic target in RA.

Further angiogenic factors relevant to RA include, without mentioning further details, serum amyloid A

(SAA), ET-1, members of the cyclooxygenase-2 (COX-2)-$PGE_2$ network, angiogenin, angiotropin, pleiotrophin, platelet-activating factor (PAF), substance P, erythropoietin, adenosine, histamine, prolactin, thrombin, sphingosine-1-phosphate (S1P), and many others.[1,14-16,109,115]

### Vasculogenesis in Arthritis

EPCs are hematopoietic stem cells that express, among other antigens, CD34, CD133, type 2 VEGF receptors, and CXCR4. During vasculogenesis, EPCs differentiate into mature ECs. Defective vasculogenesis due to impaired EPC numbers and functions has been described in RA. Effective control of inflammation with corticosteroids and TNF inhibitor agents may stimulate EPCs and thus may restore vasculogenesis. In addition, the induction of vasculogenesis may also suppress premature atherosclerosis in RA.[16,129,130]

## CELLULAR AND MOLECULAR REGULATION OF LEUKOCYTE RECRUITMENT AND ANGIOGENESIS

### Process of Leukocyte Extravasation in Inflammation

Adhesion of peripheral blood leukocytes to endothelium leads to the process of leukocyte transendothelial migration into inflammatory sites, such as the RA synovium.[2-4] High endothelial venules (HEV), primarily found in lymphoid organs, are also present at sites of lymphoid neogenesis in the synovial tissue.[3,131,132] Lymphocytes recirculate through HEVs during homing; thus inflammatory leukocyte recruitment may be considered as pathologic homing.[3,4,132]

During leukocyte adhesion and transendothelial migration, an early, weak adhesion termed "rolling" occurs first. This step involves selectins and their ligands and leads to leukocyte activation. Activation-dependent, firm adhesion involves mostly integrin-dependent interactions, primarily ICAM-1 and VCAM-1, as well as the secretion of numerous chemokines. Chemokines preferentially attract endothelium-bound leukocytes. Transendothelial migration may be the point of no return in inflammation.[2,6,133,134]

### Interactions among Chemokines, Adhesion Receptors, and Angiogenic Factors

The molecular mechanisms and signaling pathways of chemokine-induced CAM expression have been described. Treatment of cells with chemokines induces an atypical protein kinase C (PKC-ξ) through its interaction with PI3K. This leads to increased cell surface integrin expression via further signaling steps.[1] Another example for chemokine-CAM interactions is the regulation of $\beta_3$ integrin expression by CCL2 through the Ets-1 transcription factor. This pathway is involved in both inflammation and angiogenesis.[135] The CCL21-CCR7 interaction results in the stimulation of LFA-1- and ICAM-1–dependent adhesion.[136] Stimulation of CXCR1- and CXCR2-dependent pathways in arthritis models resulted in increased neutrophil adhesion to endothelium.[137] Thus various chemokines and chemokine receptors are involved in the process of leukocyte transendothelial migration.

As described previously, numerous ELR-containing CXC chemokines, CC chemokines, $CX_3CL1$ (fractalkine), and their receptors have been implicated in angiogenesis underlying RA.[8,13,15,121] Much less evidence has become available regarding the role of CC chemokines in angiogenesis. CCL2-induced angiogenesis may occur via $\beta_3$ integrins.[135] CCL23 has been implicated in the migration of vascular ECs.[48] Finally, $CX_3CL1$ (fractalkine) is involved in both angiogenesis and atherosclerosis underlying inflammatory rheumatic diseases.[8,37,38]

Both CAMs and their ECM ligands may mediate adhesive interactions during inflammatory neovascularization.[4,15,138] As discussed previously, most ECM components promote angiogenesis.[14,138] Vasculogenesis is stimulated by the laminin matrix Matrigel (Corning Life Sciences, Tewksbury, Mass.).[139] Among endothelial CAMs, soluble E-selectin; soluble P-selectin; the L-selectin ligand CD34; soluble VCAM-1; some endothelial $\beta_1$, $\beta_3$, and $\beta_5$ integrins; PECAM-1; endoglin; and some cadherins have been implicated in angiogenesis.[14,15,140-143] The $\alpha_V\beta_3$ integrin, as well as the *ITGAV* gene, play a critical role in angiogenesis. This integrin has become a major target for specific therapy.[135,142-144] Other angiogenic factors, such as chemokines, may act via integrin-dependent pathways.[135] Focal adhesion kinases (FAK) are involved in the $\alpha_V\beta_3$ integrin signaling that underlies synovial inflammation and angiogenesis.[145] Among glycoconjugates with adhesive properties, blood group antigens $Lewis^y$ and H promote neovascularization.[124] JAMs have also been implicated in the adhesive processes underlying RA, as well as in synovial angiogenesis.[56,12] Vasculogenesis also involves many integrins, including $\alpha_V\beta_3$, as well as E-selectin.[1,146-149]

## TARGETING CELL ADHESION, CHEMOKINES, ANGIOGENESIS, AND VASCULOGENESIS IN INFLAMMATORY RHEUMATIC DISEASE

Leukocyte recruitment inhibition may be a result of nonspecific anti-inflammatory therapeutic strategies. Numerous traditional and biologic disease-modifying drugs (DMARDs) and immunosuppressive agents may, in addition to other effects, suppress leukocyte recruitment, chemokine production and angiogenesis. Inhibition of cell adhesion and migration, angiogenesis, chemokines, and chemokine receptors with the use of specific antibodies or purified ligands has provided an important perspective on the molecular pathogenesis of RA. In addition, some of these strategies may be included in the therapy of arthritis.[3,4,8,15,16,150-153]

### Inhibition of Cell Adhesion Receptors

Certainly, corticosteroids and other immunosuppressive drugs may have modulatory effects on CAM expression and inflammatory cell adhesion[3,4,154]; however, these nonspecific therapies will not be further discussed.

Regarding specific anti-CAM targeting in humans, first an antihuman ICAM-1 antibody (enlimomab) was used to treat refractory RA. Repeated administration of this antibody resulted in diminished efficacy and frequent adverse events, therefore further development of enlimomab in RA was terminated.[3] Two anti-integrin strategies, (1) the

anti-LFA-1 antibody efalizumab, and (2) the LFA-3–Ig fusion protein alefacept, have been registered for the treatment of psoriasis. Alefacept also yielded a moderate effect in psoriatic arthritis.[155] Efalizumab has recently been withdrawn from the market due to severe side effects. Various other anti-integrin and anti-CD44 antibodies have been tried in animal models of arthritis, but not in human disease.[3,4,53] RGD and other peptides that block CAMs have also been tried in arthritis models.[156] The high number of CAMs and the redundancy of the adhesion network and multiple regulatory pathways may account for the notion that targeting of one CAM may be technically feasible but often yields disappointing results in human trials.

## Chemokine and Chemokine Receptor Targeting

Chemokines and chemokine receptors may be targeted by both indirect and nonspecific and direct, chemokine-specific approaches. Again, only specific targeting will be discussed in more detail; however, some nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and traditional and biologic DMARDs may, in addition to their other anti-inflammatory effects, inhibit chemokines and chemokine receptors.[10,61,67,157-159]

Some other synthetic compounds, as well as natural products, may also influence chemokine secretion. For example, antioxidants such as *N*-acetyl-L-cysteine and 2-oxothiazolidine-4-carboxylate, the bioflavonoid quercetin, and the lipid-lowering simvastatin inhibited the expression of CXCL8 and CCL2 by activated, cultured human synovial fibroblasts.[160-162] Epigallocatechin-3-gallate (EGCG), a compound derived from green tea, as well as green tea extracts, suppressed the production of multiple chemokines, including CXCL1, CXCL5, CCL2, and CCL5, by cultured RA synovial fibroblasts, bone cells, and in a rat model of arthritis.[163-165] Activation of peroxisome proliferator-activated receptor γ (PPARγ) suppresses CCL2 expression in monocytes[166]; thus PPARγ agonists, such as glitazones, may inhibit chemokine production. Some traditional Oriental medicines, such as triptolide, lingzhi, curcumin, tongbiling, honokiol, cool-cool, and others exert antiarthritic effects, which may, in part, be explained by chemokine and chemokine receptor inhibition.[158,167-173]

With respect to specific molecular targeting, neutralizing antibodies to CXCL1, 5, 8, and 16; CCL2, 3, 5, and 24, and $CX_3CL1$ blocked arthritis both therapeutically and preventatively in various RA animal models.[85,174-180] Again, because of the complexity of and several regulatory loops in the chemokine network, most of these approaches failed in human RA. There has only been a very limited number of human antichemokine trials. An anti-CCL2 antibody, ABN912, was evaluated in a randomized, placebo-controlled study. A total of 33 patients received the active compound, whereas 12 received a placebo. Serial arthroscopic biopsies were performed. ABN912 treatment was well tolerated, but there was no detectable clinical benefit or significant changes in synovial biomarkers.[181] This trial was completed in 2006 and not followed by others, suggesting that targeting of a single chemokine may not be effective in arthritis.

Targeting of chemokine receptors that recognize multiple ligands may be more feasible. Some CXCR antagonists have been used in animal models but have not been tested in human RA.[182-189] To our knowledge, no clinical trial results obtained with any CXCR antagonists in arthritis have been published.

CCR1, 2, and 5 bind multiple CC chemokine ligands that have been implicated in the pathogenesis of RA,[10,11,61,87,157] including CCL3, 5, 7, 8, 14, 15, and 16. Therefore numerous synthetic or biologic CCR1, 2, and 5 antagonists have been developed in recent years.[157,190-198] CCR1 is a receptor for CCL3, 5, 7, 14, 15, and 16. Among CCR1 antagonists, in early pre-clinical studies, J-113863 diminished synovitis and joint destruction in murine collagen-induced arthritis (CIA).[199] Met-RANTES, a dual CCR1/CCR5 antagonist, inhibited both murine CIA and rat adjuvant-induced arthritis (AIA).[200,201] This was followed by the development and introduction of two oral CCR1 antagonists, CP-481,715, and MLN3897 to human RA trials.[157,195] In a phase I trial, CP-481,715 was administered to 78 healthy individuals in escalating doses as high as 3000 mg. This drug was well tolerated.[176] CP-481,715 has also been evaluated in a 2-week phase Ib, proof-of-concept study in RA patients with a dose of 300 mg every 8 hours. A total of 16 RA patients were randomly assigned 3 : 1 with active placebo treatment for 14 days. Treatment decreased the number of total and intimal macrophages, as well as $CCR1^+$ cells in the synovium. Approximately one-third of the patients also fulfilled the American College of Rheumatology response criteria (ACR20) for clinical improvement.[202] In a subsequent phase IIa study, RA patients with active disease despite methotrexate treatment received either 10 mg of MLN3897 or a placebo orally once daily with concomitant methotrexate therapy. Although MLN3897 was well-tolerated, no difference in ACR20 was found between the active and placebo group. Interestingly, MLN3897 was associated with a relatively high degree (≥90%) of CCR1 occupancy throughout the trial as determined by CCL3 internalization assays.[191]

CCR2 recognizes CCL2, CCL7, and CCL16. Some CCR2 inhibitors have also been tried in animal studies and then clinical trials.[203] In a phase IIa clinical trial with a CCR2 blocking antibody MLN1202, 32 patients with active RA received three infusions of either placebo or anti-CCR2 antibody at 0.5 mg/kg or 1.5 mg/kg during a period of 6 weeks. The antibody reduced the levels of free CCR2 on $CD14^+$ monocytes; however, no clinical benefit could be demonstrated.[190]

CCR5 binds CCL3, 5, 8, and 14. As discussed previously, in earlier studies, Met-RANTES and other small-molecule antagonists showed some efficacy in pre-clinical arthritis studies.[200,201,204] A small-molecule CCR5 antagonist, AZD5672 has been studied in pre-clinical, phase I and IIb studies. In the phase IIb trial, 371 patients with active RA received various doses of oral AZD5672 once daily, a placebo, or open-label etanercept, 50 mg subcutaneously (SC), once weekly. There was no significant difference in the number of patients receiving the active compound or placebo. Furthermore, etanercept was more effective than AZD5672 or placebo.[196] A phase Ib trial was conducted with SCH351125, a small-molecule oral CCR5 inhibitor. Among 32 patients with active RA, 20 received the active compound and 12 received placebo. No synovial, magnetic resonance imaging, or clinical efficacy could be proved.[197]

Another CCR5 inhibitor, maraviroc, has been examined in a phase II trial in RA.[205] Thus CCR2 and CCR5 blockade yielded disappointing results in RA, and results for CCR1 blockade have been variable.

## Angiogenesis Targeting: Use of Angiostatic Compounds

Angiogenesis can be inhibited by either blocking the action of angiogenic mediators or by using angiostatic compounds[14,112,115,152,153] (see Table 25-3). Currently used antirheumatic agents, such as rofecoxib, dexamethasone, chloroquine, sulfasalazine, methotrexate, azathioprine, cyclophosphamide, leflunomide, thalidomide, tacrolimus, minocycline, and anti-TNF biologics, apart from their other important anti-inflammatory effects, nonspecifically suppress angiogenesis, including VEGF and/or Ang1/Tie2.[14,152,153] For example, TNF blockade by infliximab reduced VEGF expression and vascularity within the RA synovium. Infliximab also reduced synovial Ang1 and Tie2 expression. Certolizumab pegol inhibits TNF-dependent angiogenesis. The anti–IL-6 receptor antibody tocilizumab also decreased serum levels of VEGF in RA.[14,115,118,206] IL-17 has been implicated in inflammatory angiogenesis, therefore, anti–IL-17 blockade may also be feasible in this respect.[120] RGD and other peptides that inhibit angiogenesis have also been tried in animal models of arthritis.[156]

VEGF inhibitors have been used in both arthritis trials and cancer studies. These inhibitors may include antibodies to VEGF or VEGF receptors (VEGFR), soluble VEGFR constructs, small-molecule VEGF and VEGFR inhibitors, or inhibitors of VEGF and VEGFR signaling. The VEGF tyrosine kinase inhibitor vatalanib (PTK787) and an anti-VEGFR1 antibody exerted significant angiostatic and antiarthritic effects in animal models of arthritis. A soluble VEGFR1 chimeric protein inhibited synovial endothelial proliferation.[1,14,112,115,152,153]

The involvement of PPARs in hypoxia-induced VEGF production is discussed previously. PPARγ ligands rosiglitazone and pioglitazone have been found to inhibit VEGF-induced angiogenesis.[14] HIFs may also be targeted in arthritis. For example, YC-1, a superoxide-sensitive stimulator of soluble guanylyl cyclase and an HIF-1 inhibitor, may potentially be used to suppress inflammatory angiogenesis.[114,152] As far as the Ang1/Tie2 system is concerned, a soluble Tie2 receptor transcript, delivered via an adenoviral vector to mice, attenuated the incidence and severity of CIA.[14,152] An Ang2-targeting peptide was genetically fused to the anti-TNF antibody adalimumab. This novel bispecific antibody enhanced anti-TNF efficacy.[156]

Antibiotic derivatives, including minocycline, fumagillin analogues, deoxyspergualin, roxithromycin, and clarithromycin, also inhibit the release of VEGF and other angiogenic mediators, and thus inhibit neovascularization. Synthetic fumagillin derivativesTNP-470 and PPI-2458 inhibit VEGF and VEGF-dependent angiogenesis. These have been used in human RA trials, in which minocycline, roxithromycin, and clarithromycin were found to exert moderate but significant clinical effects.[14,109,152,153]

Among other inhibitors of angiogenic mediators, ET-1 antagonists currently used in the treatment of pulmonary hypertension may also exert antiangiogenic effects.[207] S1P and also angiogenic and S1P inhibitors, such as fingolimod (FTY720), are in clinical trials for various autoimmune diseases. FTY720 also acts as an inhibitor of FAKs as described previously.[14,123,152,153] Peptides have been isolated from libraries of phages that preferentially home to inflamed joints of AIA rats. Some of these peptides that bind to synovial endothelial cells were found to inhibit arthritis in addition to VEGF signaling and angiogenesis.[156]

In recent years, an emerging number of compounds in traditional Chinese and Korean medicine have been implicated in angiogenesis research. A number of these compounds may have angiostatic effects in arthritis,[14] including scopolin, a coumarin derivative found in *Erycibe obtusifolia* Benth; celastrol, an active ingredient of *Tripterygium wilfordii*, also known as "Thunder God Vine"; and fisetin, the active ingredient of *Rhus verniciflua* Stokes.

## Restoration of Impaired Vasculogenesis

Corticosteroids and TNF inhibitor agents may stimulate EPCs and thus restore vasculogenesis in RA. The CXCL12 (SDF-1) inhibitor bicyclam (AMD3100) has been found to mobilize EPCs in mice. Although the number of EPCs correlate with the improvement of DAS28 in RA, the suppression of systemic inflammation and disease activity by any means would improve vasculogenesis.[16,130]

# CONCLUSION

Leukocyte-endothelial adhesion, CAMs, chemokines, chemokine receptors, angiogenesis, and vasculogenesis in leukocyte recruitment underly many inflammatory diseases. The presence of various CAM pairs and interacting chemokines may account for the diversity and specificity of leukocyte recruitment. A number of soluble and cell-bound factors may stimulate or inhibit angiogenesis. The outcome of inflammatory and other "angiogenic" diseases, such as various forms of arthritis, depends on the imbalance between angiogenic and angiostatic mediators. Some CAMs and chemokines, as well as growth factors, proteases, antibiotics, and other agents are also involved in neovascularization. Impaired EPC function and vasculogenesis have been associated with active arthritis. Several attempts have been made to therapeutically interfere with the cellular and molecular mechanisms described in this chapter. Specific targeting of leukocyte adhesion, CAMs, chemokines, chemokine receptors and/or angiogenesis, as well as the restoration of defective vasculogenesis, have been studied in animal systems; however, more human trials are necessary.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 26



# Cytokines

Iain B. McInnes

**KEY POINTS**

- **Cytokines are peptides that have a fundamental role in communication within the immune system and in allowing the immune system and host tissue cells to exchange information.**
- **Cytokines act via binding to a receptor that in turn sends a signal to the recipient cell, leading to a change in function or phenotype. Such signal cascades are complex and integrate a variety of environmental factors.**
- **Cytokines exist in broad families that are structurally related but exhibit diverse function (e.g., the TNF/TNF receptor superfamily, interleukin [IL]-1 superfamily, and IL-6 superfamily).**
- **Cytokine targeting has proven effective in many rheumatic diseases, particularly therapeutics that inhibit TNF or IL-6. Many more cytokines are currently under investigation as therapeutic targets or as therapeutic agents.**

Immune function depends on the biologic activities of numerous small glycoprotein messengers termed *cytokines*. Originally discovered and defined on the basis of their functional activities, cytokines are now designated primarily by structure. Typically, cytokines exhibit broad functional activities that mediate not only effector and regulatory immune function but also have wider effects across a range of tissues and biologic systems. As such, cytokines play a role not only in host defense but also in a variety of normal physiologic and metabolic processes. By this means they integrate de facto host defense and host metabolic function. The Human Genome Project has assisted with the discovery of numerous cytokines, posing considerable challenges in resolving their respective and synergistic functions in complex tissues in health and disease. However, such understanding is essential with the increasing application of cytokine-targeted therapies in the clinic. This chapter reviews general features of cytokine biology and the cellular and molecular networks within which cytokines operate; the focus is on the effector functions of cytokines that are important in chronic inflammation and in rheumatic diseases.

## CLASSIFICATION OF CYTOKINES

In the absence of a unified classification system, cytokines are variously identified by numeric order of discovery (currently interleukin [IL]-1 through IL-38); by a given functional activity (e.g., tumor necrosis factor [TNF] and granulocyte colony-stimulating factor); by kinetic or functional role in inflammatory responses (e.g., early or late, innate or adaptive, and pro-inflammatory or anti-inflammatory); by primary cell of origin (e.g., monokine = monocyte derivation and lymphokine = lymphocyte derivation); and, more recently, by structural homologies shared with related molecules. Superfamilies of cytokines share sequence similarity and exhibit homology and some promiscuity in their reciprocal receptor systems (Figure 26-1). They do not necessarily exhibit functional similarity. Cytokine superfamilies also contain important regulatory cell membrane receptor-ligand pairs, reflecting evolutionary pressures that use common structural motifs in diverse immune functions in higher mammals. The TNF/TNF receptor superfamily[1] contains immunoregulatory cytokines including TNF, lymphotoxins, and cellular ligands such as CD40L, which mediates B cell and T cell activation, and FasL (CD95), which promotes apoptosis. Similarly, the IL-1/IL-1 receptor superfamily[2] contains cytokines including IL-1β, IL-1α, IL-18, IL-33, and IL-36 (α, β, γ), receptor antagonists including IL-1RA, IL-36 receptor antagonist, IL-38, and an anti-inflammatory cytokine, namely IL-37, which mediate physiologic and host-defense function, but this family also includes the Toll-like receptors (TLRs), a series of mammalian pattern-recognition molecules with a crucial role in recognition of microbial species early in innate responses.

## ASSESSING CYTOKINE FUNCTION IN VITRO AND IN VIVO

Although they were originally identified by bioactivity and quantified by bioassay, most cytokines are now identified via homologous receptor binding or sequence homology in gene databases. They are quantified in biologic solutions by enzyme-linked immunosorbent assay, multiplex technology, or meso platform techniques, with the latter allowing many cytokines (25 to 360) to be measured in single, small sample volumes (~20 μL). Cytokines are also assessed in experimental systems at the messenger RNA (mRNA) level, often using quantitative polymerase chain reaction (PCR) or Taqman low-density array (TLDA)-based approaches; the latter allows many cytokines to be identified in small samples simultaneously. Post-transcriptional regulation of cytokines is common, however, suggesting cautious interpretation of mRNA data alone. Function is thereafter assessed by

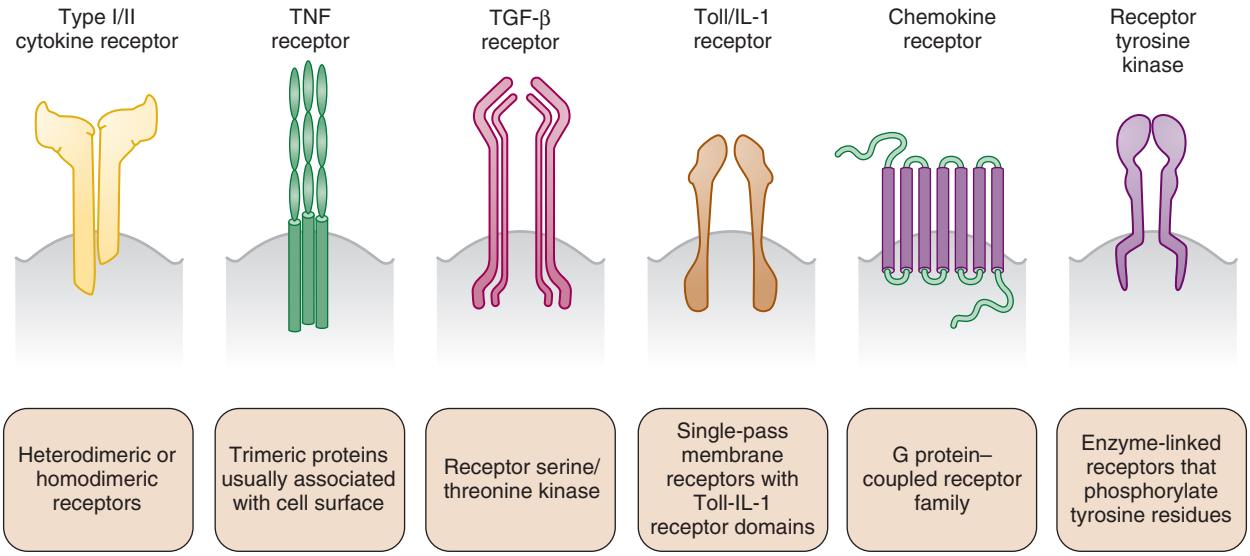



**Figure 26-1** Cytokine receptors. Cytokines, chemokines, and growth factors bind to many different types of surface receptors in the cell membrane. The figure shows several distinct families and representative ligands that are critical. Each receptor type is associated with distinct signaling mechanisms that orchestrate and integrate the cellular response after ligand binding. IL-1, Interleukin-1; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor.

identification of the cellular source of cytokine, determination of native stimuli, characterization of receptor distribution, and determination of function in target cells. Experimental in vivo models use the addition of neutralizing cytokine-specific antibodies or soluble receptors (often as fragment crystallizable fusion or pegylated proteins to enhance half-life and modulate functional interaction with leukocytes) to modulate cytokine function. Genetically modified knockout and knockin mice (cytokine or receptor modified by embryonic stem cell technology) or transgenic mice (tissue/cell lineage–specific overexpression) have proven particularly useful. Conditional gene-targeting approaches (e.g., using the Cre system) facilitate circumvention of embryonic lethal deficiencies or allow kinetic evaluation of the relative contribution of a cytokine throughout a response. Moreover, recent multiphoton microscopic techniques have allowed the additional evaluation of cytokine contributions in three-dimensional tissue orientation and in real time in vivo. Cytokine function is normally assessed in vitro in primary or transformed cell lines stimulated in the presence or absence of recombinant cytokine or specific anti-cytokine antibody or soluble receptor. Gene knock-down approaches with use of small interfering (si)RNA or antisense oligonucleotides are also increasingly employed. Recently CRISPR (clustered regularly interspaced short palindromic repeats) and TALEN (transcription activator-like effector nucleases)-based technologies have facilitated highly specific cytokine and cytokine receptor knock-down cell work.

This general approach has been crucial in rheumatic disease research. Studies in which cytokine addition and neutralization occur in synovial tissue explants or disaggregated cell populations, chondrocyte explants, bone culture models, skin, and renal tissue explants and cell lines have been informative. Ex vivo methodologies now include intra-cellular fluorescence activated cell sorter methods, confocal and laser scanning microscopy, and quantitative histologic evaluation using automated image analysis. Such modalities, particularly when used in human therapeutic cytokine neutralization studies in which inflammatory tissues are obtained throughout therapeutic interventions, advance the understanding of basic and pathogenetic cytokine function. Analysis of synovial biopsy specimens obtained before and after infliximab, adalimumab, abatacept, rituximab, tocilizumab, IL-1RA, IL-10, and interferon (IFN)-β administration in persons with rheumatoid arthritis (RA) provides the strongest evidence for the success of this approach.[3,4]

## CYTOKINE RECEPTORS

Cytokine receptors exist in structurally related superfamilies and comprise high-affinity molecular signaling complexes that assist in cytokine-mediated communication (see Figure 26-1). Such complexes often include heterodimeric or heterotrimeric structures that use unique, cytokine-specific recognition receptors together with common receptor chains shared across a cytokine superfamily. Examples include the use of the common γ chain receptor by IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, and glycoprotein (gp)130 by members of the IL-6 family.[5,6] Alternatively, distinct receptors may use shared signaling domains. Homologous death domains are found in many TNF-receptor family members. Similarly, the IL-1 signaling domain is common to not only IL-1R but also other IL-1R superfamily members, including IL-18R, IL-33R, and the TLRs.[2] It has been recognized more recently that unrelated cytokine receptor systems exhibit close cross-communication on the cell membrane, allowing a cell to integrate a variety of external stimuli to optimize signaling pathways and the cellular response in real time in a changing environment. Although best elucidated in the epidermal growth factor receptor system, this mechanism also has been identified for members of the common γ chain signaling family. Signaling pathways that subserve these responses are discussed in detail elsewhere (see Chapter 27).

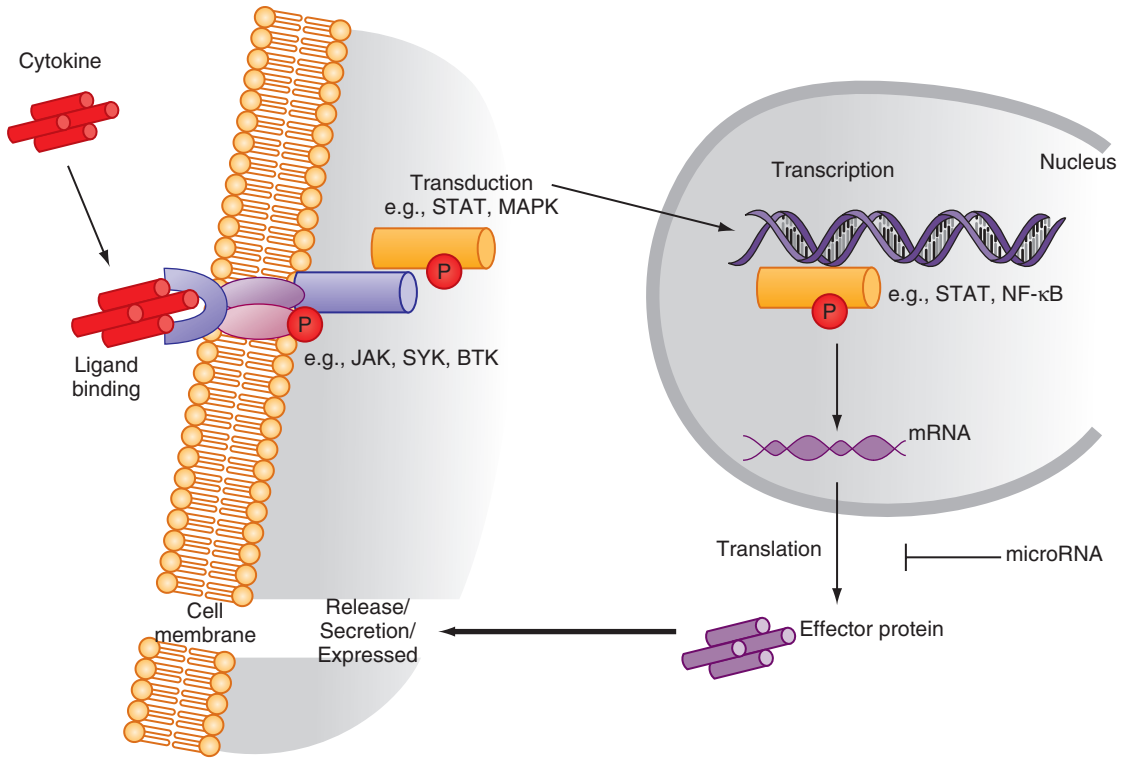



**Figure 26-2** Cytokine signaling and regulation. After ligand binding, cytokine receptors activate a series of signaling molecules that are associated with the cytoplasmic portion of the receptor or the plasma membrane. In this figure, Janus kinase (JAK) or spleen tyrosine kinase (SYK) is activated, which in turn phosphorylates additional cytoplasmic molecules (signal transducer and activator of transcription [STAT]; mitogen-activated protein kinase [MAPK]) that then can migrate to the nucleus and either directly or through additional intermediaries activate gene transcription. Messenger RNA (mRNA) levels can also be regulated after transcription by microRNAs. Ultimately, the translated proteins can be processed and released by the cell into the microenvironment or presented on the plasma membrane to other cells. BTK, Bruton's tyrosine kinase; NF-κB, nuclear factor-κB.

Cytokine receptors can operate via several mechanisms. Membrane receptors, with intra-cellular signaling domains intact, can transmit signals to the target cell nucleus after soluble cytokine binding and promote effector function (Figure 26-2). Membrane receptors may bind cell membrane cytokines assisting cross-talk between adjacent cells. Membrane-bound and soluble cytokines may promote distinct receptor function. Useful exemplars exist relevant to the rheumatic diseases. Thus TNF binds TNF-RI and TNF-RII with similar affinity, but it has a slower rate of dissociation from TNF-RI. Soluble TNF may dissociate rapidly from TNF-RII to bind TNF-RI, promoting preferential signaling by the latter (ligand passing).[1] In contrast, during cell-cell contact, stable TNF/TNF-RI and TNF/TNF-RII complexes form, allowing for differential signaling contribution by TNF-RI and TNF-RII.

Cytokine receptor/cytokine complexes also may operate in trans, whereby component parts of the ligand-receptor complex are derived from adjacent cells. IL-15/IL-15Rα complexed on one cell may bind IL-15Rβ/γ on another.[7] Receptors also exist in soluble form, derived either from alternative mRNA processing to generate receptor-lacking transmembrane or intra-cellular domains or from enzymatic cleavage of receptor from the cell surface (e.g., soluble TNF-R [sTNF-R] and soluble IL-1R1 [sIL-1R1]). Soluble receptors may act to antagonize cytokine function, thus regulating responses. Soluble receptors also may pre-form complexes with cytokine to promote subsequent ligand-receptor assembly on the target cell membrane and enhance function. Soluble receptors can deliver cytokine to the cell membrane via ligand passing. IL-6 provides a particularly important example given its core role in a range of rheumatic disorders. IL-6 binds to a heterodimeric receptor (IL-6R and gp130) and provokes cell activation thereby via conventional signal pathways that involve signal transducer and activator of transcription (STAT)-3. Thus IL-6 may activate a cell expressing the combination of IL-6R and gp130 by conventional (cis) signaling. In addition, however, circulating soluble IL-6R may form functional gp130/IL-6R effector complexes on any cell expressing membrane gp130 and by this means confer on circulating IL-6 the ability to exert broad functional effects (trans signaling). Finally, it is now recognized that some cytokines with the capacity to be retained in the membrane may themselves function as signaling molecules (reverse signaling).

## REGULATION OF CYTOKINE EXPRESSION

Cytokines are synthesized in the Golgi apparatus and may traffic through the endoplasmic reticulum to be released as soluble mediators, or they may remain membrane bound, or they may be processed into cytosolic forms that can traffic intra-cellularly, even returning to the nucleus, where they can act as transcriptional regulators. Cytokines mediate autocrine function either through release or membrane expression and immediate receptor ligation on the source

cell or intra-cellularly within the source cell. Alternatively, cytokines operate in a paracrine manner, allowing cellular communication beyond that assisted by local cell-cell contact. The distance and kinetics for effective function may be limited,[8] however, by numerous factors, including physicochemical considerations of the peptide structure itself, extra-cellular matrix binding (e.g., to heparan sulfate), enzymatic degradation (e.g., serine protease degradation of IL-18), or the presence of soluble receptors (e.g., TNF/soluble TNF-RI and TNF-RII and IL-2/soluble IL-2Rα) or novel cytokine-binding proteins (e.g., IL-18/IL-18 binding protein) in the inflammatory milieu.

Numerous factors promote cytokine expression in vivo (Figure 26-3), including cell-cell contact, immune complexes/autoantibodies, local complement activation, microbial species and their soluble products (particularly via TLRs and nucleotide oligomerization domain [NOD]-like receptors [NLRs]), reactive oxygen and nitrogen intermediates, trauma, shear stress, ischemia, radiation, ultraviolet light, extra-cellular matrix components, DNA (mammalian or microbial), heat shock proteins, electrolytes (e.g., $K^+$ via $P_2X7$ receptors), and cytokines themselves operating in autocrine loops. Commonly used in vitro stimuli include many of these factors and chemical entities including phorbol esters, calcium ionophores, lectins (e.g., phytohemagglutinin), and receptor-specific antibodies such as anti-CD3 and anti-CD28 for T cell activation or anti-immunoglobulin and anti-CD40 for B cells.

Cytokine regulation within the cell can be usefully considered at several levels (see Figure 26-2). Transcriptional regulation depends on the recruitment of discrete transcription factors to the cytokine promoter region. Transcription factor binding allows for numerous signal pathways to regulate cytokine expression across a range of stimuli. Several transcription factors (e.g., nuclear factor-κB [NF-κB], activator protein [AP]-1, and nuclear factor of activated T cell) are crucial in cytokine production. Inhibition of NF-κB activity using either chemical inhibitors or adenoviral delivery of regulatory peptides leads to amelioration of inflammatory synovitis in vivo and in vitro.[9] Sequence polymorphism within cytokine promoters offers potential for differential cytokine expression between individuals that could confer selective advantage against infection but could also increase susceptibility to, or progression of, autoimmunity. This potential is best exemplified in the TNF and IL-1 promoters.[10,11] Single nucleotide polymorphisms in the TNF promoter region (e.g., −308) are associated with altered TNF release on leukocyte stimulation in vitro. Similarly, homozygotes for the A2 allele at +3954 in the IL-1β gene produce more IL-1β with lipopolysaccharide stimulation. Polymorphisms also exist in the *IL-1RA* gene, rendering the functional significance of individual single nucleotide polymorphisms on IL-1 protein release difficult to interpret. In general, the net effect of haplotypes may be more important at the functional level or only play a role in the context of networks where multiple minor polymorphisms can synergize, particularly when their relevance to disease entities is considered.

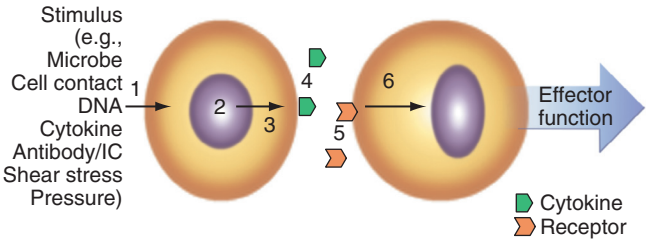



**Figure 26-3** Overview of cytokine regulatory function. Numerous and diverse stimuli *(1)* promote cytokine expression arising either from novel gene expression *(2)* or from activation of pre-formed cytokine *(3)*. Cytokine proteins are thereafter expressed in the cytosol, on the cell membrane, or in soluble form in the extra-cellular environment *(4)*. Cytokines bind to reciprocal receptors that reside either on the membrane of a target cell or in the soluble phase *(5)*. Membrane receptors, on cytokine ligation, signal to the recipient cell nucleus *(6)* and drive novel gene expression to promote effector function. Each phase of cytokine function offers rich therapeutic potential. IC, Immune complexes.

Post-transcriptional regulation is important in determining longevity of cytokine expression. This regulation may operate by promoting translational initiation, mRNA stability, and polyadenylation. AU-rich elements (AREs) within the 52 or 32 untranslated regions (UTRs) of cytokine mRNA are crucial for stability; 32 UTR AREs downregulate TNF expression such that transgenic knockin mice that lack TNF AREs develop spontaneous inflammatory arthritis and bowel disease.[12] Regulatory proteins bind AREs to mediate such effects. HuR and AUF1 exert opposing effects, stabilizing and destabilizing ARE-containing transcripts.[13] TIA-1 and TIAR have been identified as RNA recognition motif family members[14] that function as translational silencers. Macrophages from TIA-1–deficient macrophages produce excess TNF, whereas TIA-1–deficient lymphocytes exhibit normal TNF release, suggesting distinctions in mRNA regulation in discrete cell types.[15] Alternatively, cytokines may generate stable mRNA a priori to assist with subsequent rapid response in tissues. The IL-15 mRNA 52 UTR contains 12 AUG triplets that significantly reduce the efficiency of IL-15 translation. Deletion of this sequence permits IL-15 secretion. IL-15 mRNA can produce a 48 amino acid signal peptide that allows IL-15 release and a shorter 21 amino acid signal peptide that targets intracellular distribution. IL-15 forms thus generated exhibit discrete functions.[16]

Post-translational regulation also modulates cytokine expression via several mechanisms. Patterns of glycosylation are important for cytokine function and may regulate intracellular trafficking.[16] Modified leader sequences can alter intra-cellular trafficking of cytokines. Some cytokines are translated without functional leader sequences. Their secretion depends on nonconventional secretory pathways that are poorly understood. IL-1β employs, among other pathways, a purine receptor–dependent pathway ($P_2X_7$) for cellular release.[17] Enzymatic activation of cytokines is common, whereby nonfunctional promolecules are cleaved to generate functional subunits. Examples include the cleavage by caspase 1 of pro-IL-1β to generate active IL-1β and, similarly, of pro-IL-18 to generate an active 18 kDa species.[18] This organized process occurs both sequentially (in time) and by orientation within the cell (in space). IL-1 processing occurs in a protein complex within the cytosol termed the *inflammasome*. The latter has attracted considerable interest as a therapeutic target in conditions such as

crystal-induced arthritis and in diseases that arise from mutations in certain inflammasome genes (e.g., cryopyrin; see Chapters 27, 94, 96, and 97).

Alternative processing pathways for cytokines include the serine proteases, proteinase 3 and elastase, and adamalysin family members. Enzyme cleavage pathways operate within and outside cells, providing for extra-cellular cytokine activation. Similarly, cell membrane enzymes serve to cleave membrane-expressed cytokine. Members of the adamalysin family regulate TNF release; TNF-converting enzyme cleaves and mediates the release of TNF and its receptors.[19] Extensive molecular machinery exists to regulate tightly not only the production and stability of cytokine mRNA but also its translation and cellular expression and distribution. At each level, opportunities exist for intervention and therapeutic cytokine modulation.

## EFFECTOR FUNCTION OF CYTOKINES

Cytokines possess pleiotropic and potent effector function in acute and chronic inflammatory responses. The identity, receptor specificity, and key effects of cytokines understood to have particular importance in the pathogenesis of human autoimmunity and chronic inflammation are summarized in Tables 26-1 through 26-8.

### Cytokines in Acute Inflammation

Cytokines operate at every stage in the crucial early events that promote acute inflammation. Cells that make up the innate immune response, including neutrophils, natural killer (NK) cells, macrophages, mast cells, and eosinophils, all produce and respond to cytokines generated within seconds of tissue insult. Cytokines prime leukocytes for response to microbial and chemical stimuli, upregulate adhesion molecule expression on migrating leukocytes and endothelial cells, and amplify the release of reactive oxygen intermediates, nitric oxide, vasoactive amines, and neuropeptides, as well as the activation of kinins and arachidonic acid derivatives, prostaglandins, and leukotrienes, which regulate cytokine release. Similarly, cytokines regulate the expression of complement processing and membrane defense molecules, scavenger receptors, NLRs, and TLRs. Cytokines, particularly IL-1, TNF, and IL-6, are crucial in driving the acute-phase response. Some moieties are designated "alarmins" on the basis of their very rapid induction or prior expression and critical early role in initiating inflammation (e.g., IL-33 and high mobility group box 1 [HMGB1]). Tables 26-1 through 26-8 provide descriptions of the function of cytokines expressed within the acute inflammatory response.

**TABLE 26-1** Interkeukin-1 Superfamily Cytokines with Roles in Rheumatic Disease

| Cytokine | Size (kDa) | Receptors | Major Cell Sources | Key Functions |
|---|---|---|---|---|
| IL-1β | 35 (pro)<br>17 (active) | IL-1RI<br>IL-1RAcP<br>IL-1RII (decoy) | Monocytes; B cells; fibroblasts; chondrocytes; keratinocytes | Fibroblast cytokine, chemokine, MMP, iNOS, PG release ↑<br>Monocyte cytokine, ROI, PG ↑<br>Osteoclast activation<br>Chondrocyte GAG synthesis ↓; iNOS, MMP, and aggrecanase ↑<br>Endothelial adhesion-molecule expression |
| IL-1α | 35 (pro)†<br>17 (active) | IL-1RI<br>IL-1RAcP<br>IL-1RII (decoy) | Monocytes; B cells; PMNs; epithelial cells; keratinocytes | Similar to IL-1β<br>Autocrine growth factor (e.g., keratinocytes) |
| IL-1Ra | 22 | IL-1RI<br>IL-1RAcP<br>IL-1RII | Monocytes | Antagonize effects of IL-1β and IL-1α |
| IL-18 | 23 (pro)<br>18 (active) | IL-18R<br>IL-18Rβα | Monocytes; PMNs; dendritic cells; platelets; endothelial cells | T cell effector polarization (Th1 with IL-12/Th2 with IL-4)<br>Chondrocyte GAG synthesis ↓; iNOS expression<br>NK activation; cytokine release; cytotoxicity<br>Monocyte cytokine release; adhesion molecule expression<br>PMN activation; cytokine release; migration<br>Endothelial cells—proangiogenic |
| IL-36 (αβγ) | 35 | IL-1Rrp2<br>IL-1RAcP | Macrophages, DC, lymphocytes, epithelial cells (skin, bronchial), FLS | Innate immune cell activation, cytokine production<br>Keratinocyte proliferation |
| IL-37 | 35 | IL-18Rα | Ill-defined | General anti-inflammatory function: transgenic mice protected from colitis, ischemia reperfusion injury |
| IL-33 | 30 (pro)<br>18 (active) | ST2L<br>IL-1RAcP | Epithelial cells; monocytes; smooth muscle cells; keratinocytes | Promote Th2 cell activation, mast cell activation, and cytokine production |

*Pro forms cleaved to active moieties by proteases including caspase-1, calpain, elastase, and cathepsin G.
†Pro-IL-1α retains bioactivity before cleavage.
DC, Dendritic cell; FLS, fibroblast-like synoviocyte; IL, interleukin; GAG, glycosaminoglycan; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; NK, natural killer; PG, peptidoglycan; PMN, polymorphonuclear neutrophil; ROI, reactive oxygen intermediates; Th, T helper.

**TABLE 26-2** Tumor Necrosis Factor Superfamily Cytokines* with Potential Role in Rheumatic Disease

| Cytokine | Size (kDa) | Receptors | Major Cell Sources | Selected Functions |
|---|---|---|---|---|
| TNF | 26 (pro) | TNF-RI (p55) | Monocytes; T, B, NK cells; PMNs; eosinophils; mast cells; fibroblasts; keratinocytes; glial cells; osteoblasts; smooth muscle | Monocyte activation, cytokine, and PG ↑ |
| | | TNF-RII (p75) | | PMN priming, apoptosis, oxidative burst ↑<br>Endothelial cell adhesion molecule, cytokine release ↑; fibroblast proliferation and collagen synthesis ↓<br>MMP and cytokine ↑<br>T cell apoptosis; clonal (auto)regulation; TCR dysfunction<br>Adipocyte FFA release ↑<br>Endocrine effects—ACTH, prolactin ↑; TSH, FSH, GH ↓ |
| LTα | 22-26 | TNF-RI | T cells; monocytes; fibroblasts; astrocytes; myeloma; endothelial cells; epithelial cells | Peripheral lymphoid development |
| | | TNF-RII | | Otherwise similar bioactivities to TNF |
| RANK ligand | 35 | RANK | Stromal cells; osteoblasts; T cells | Stimulates bone resorption via osteoclast maturation and activation<br>Modulation of T cell–DC interaction |
| OPG | 55 | RANKL | Stromal cells, osteoblasts | Soluble decoy receptor for RANKL |
| BLyS† | 18-32 | TACI<br>BCMA<br>BLyS-R | Monocytes; T cells; DCs | B cell proliferation, Ig secretion, isotype switching, survival<br>T cell co-stimulation |
| APRIL | – | TACI<br>BCMA | Monocytes; T cells; tumor cells | B cell proliferation<br>Tumor proliferation |

*Additional members of importance include TRAIL, TWEAK, CD70, FasL, and CD40L. At least 18 members of the family are now described.
†Also called B cell activating factor belonging to the TNF family (BAFF).
ACTH, Adrenocorticotropic hormone; APRIL, a proliferation inducing ligand; BCMA, B cell maturation protein; BLyS, B lymphocyte stimulator protein; DC, dendritic cell; FFA, free fatty acid; FSH, follicle-stimulating hormone; GH, growth hormone; Ig, immunoglobulin; LT, lymphotoxin; MMP, matrix metalloproteinase; NK, natural killer; OPG, osteoprotegerin; PG, peptidoglycan; PMN, polymorphonuclear neutrophil; RANKL, receptor activator of NF-κB ligand; TACI, transmembrane activator and calcium modulator and cyclophilin ligand; TCR, T cell receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TSH, thyroid-stimulating hormone; TWEAK, TNF-like weak inducer of apoptosis.

**TABLE 26-3** Cytokines Associated Predominantly with Effector Function for T Cells*

| Cytokine | Size (kDa) | Receptors | Major Cell Sources | Key Functions |
|---|---|---|---|---|
| **Type II Interferon** | | | | |
| IFN-γ | 20-25 | IFNγR | Th/c1 cells; NK cells; γδT cells; B cells; macrophage/DCs | Macrophage activation, DC APC function ↑<br>Endothelial adhesion molecule ↑<br>Class II MHC expression ↑<br>T cell growth ↓; opposes Th2 responses<br>Bone resorption ↓; fibroblast collagen synthesis |
| **4α-Helix Family** | | | | |
| IL-2 | 15 | IL-2Rα<br>IL-2/15Rβ γ chain | Th/c cells; NK cells | T cell division; maturation; cytokine release; cytotoxicity<br>NK cell cytokine release; cytotoxicity; monocyte activation<br>Lymphocyte apoptosis ↓ |
| IL-4 | 20 | IL-4Rα/γ chain<br>IL-4Rα/IL-13R1 | Th/c cells (Th2); NK cells | Th2 differentiation, maturation, apoptosis ↓<br>B cell maturation; isotype switch (IgE)<br>Eosinophil migration, apoptosis ↓<br>Endothelial activation; adhesion molecule expression |
| IL-5 | 25 monomer | IL-5Rα | Th/c2 cells; NK cells; mast cells; epithelial cells | B cell differentiation; immunoglobulin production (IgA) |
| | 50 homodimer | IL-5Rβ | | Eosinophil differentiation and activation<br>Th/c maturation |
| **IL-17 Family†** | | | | |
| IL-17A/F | 20-30 | IL-17R | T cells (Th17); fibroblasts | Chemokine release, fibroblast cytokine release, MMP release ↑<br>Osteoclastogenesis; hematopoiesis<br>Chondrocyte GAG synthesis ↓<br>Leukocyte cytokine production ↑ |
| IL-25 (IL-17E) | 20-30 | IL-17R | Th2 cells | Th2 cytokine release; B cell IgA and IgE synthesis; eosinophilia; epithelial cell hyperplasia |

*Additional T cell–derived cytokines of potential interest include IL-13 from Th2 and NK2 cells.
†IL-17 family also contains IL-17B and IL-17C, the distinct functions of which are currently unclear.
APC, Antigen presenting cell; DC, dendritic cell; GAG, glycosaminoglycan; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NK, natural killer; Th/c, T helper/cytotoxic.

**TABLE 26-4** Cytokines Described Initially with Primary Role in Regulation of T Cells*

| Cytokine | Size | Receptors | Major Cell Sources | Key Functions |
|---|---|---|---|---|
| IL-12 | IL-12/23p40<br>IL-12p35 | IL-12Rα<br>IL-12Rβ1<br>IL-12Rβ2 | Macrophages; DCs | Th1 cell proliferation, maturation<br>T cell cytotoxicity<br>B cell activation |
| IL-15 | 15 kDa | IL-15Rα<br><br>IL-2/15Rβ γ chain | Monocytes; fibroblast; mast cells; B cells; PMNs; DCs | T cell chemokinesis, activation, memory maintenance<br><br>NK cell maturation, activation, cytotoxicity<br>Macrophage activation, suppression (dose dependent)<br>PMN activation, adhesion molecule, oxidative burst<br>Fibroblast activation<br>B cell differentiation and isotype switching |
| IL-21 | 15 kDa | IL-21R γ chain | Activated T cells; others (?) | B cell activation |
| IL-23 | IL-12/23p40<br>IL-23p19 | IL-23R<br>IL-12Rβ1 | Macrophages; DCs | Th17 cell expansion and activation; IL-17 release |

*Cytokines included in this table are now understood to exhibit considerable functional heterogeneity as shown. Other T cell regulatory cytokines have been described, including IL-27, the functions of which are currently under investigation.
DC, Dendritic cells; IL, interleukin; NK, natural killer; PMN, polymorphonuclear neutrophil; Th, T helper.

**TABLE 26-5** IL-10 Superfamily Cytokines*

| Cytokine | Receptors | Cellular Sources | Key Functions |
|---|---|---|---|
| IL-10 | IL-10R1<br><br>IL-10R2 | Monocytes; T cells; B cells; DCs; epithelial cells; keratinocytes | Macrophage cytokine release, iNOS, ROI ↓; soluble receptor ↑<br><br>T cell cytokine release, MHC expression ↓; anergy induction<br>Treg cell maturation; effector function (?)<br>DC activation, cytokine release ↓<br>Fibroblast MMP, collagen release ↓; no effect on TIMP<br>B cell isotype switching enhanced |
| IL-19 | IL-20R1/IL-20R2 | Monocytes; others (?) | Monocyte cytokine and ROI release; monocyte apoptosis |
| IL-20 | IL-22R/IL-20R2<br>IL-20R1/IL-20R2 | Keratinocytes; others (?) | Autocrine keratinocyte growth regulation |
| IL-22 | IL-22R/IL-10R2 | Th17 cells; CD8 T cells; γδ T cells; NK cells | Acute-phase response, keratinocyte activation proliferation ↑ |
| IL-24 | IL-22R/IL-20R2<br>IL-20R1/IL-20R2 | Monocytes; T cells | Tumor apoptosis; Th1 cytokine release by PBMC |

*Additional members include IL-26, IL-28, and IL-28A. Many functions of IL-10 superfamily are as yet poorly understood, but they likely reside beyond the immune system.
DC, Dendritic cell; IL, interleukin; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; NK, natural killer; PBMC, peripheral blood mononuclear cells; ROI, reactive oxygen intermediates; Th, T helper; TIMP, tissue inhibitor of metalloproteinase; Treg, regulatory T cell.

**TABLE 26-6** IL-6 Superfamily Cytokines*

| Cytokine | Size (kDa) | Receptors | Major Cell Sources | Key Functions |
|---|---|---|---|---|
| IL-6 | 21-28 | IL-6R† gp130 | Monocytes; fibroblasts; B cells; T cells | B cell proliferation; immunoglobulin production<br>Hematopoiesis, thrombopoiesis<br>T cell proliferation, differentiation, cytotoxicity<br>Hepatic acute-phase response<br>Hypothalamic-pituitary-adrenal axis<br>Variable effects on cytokine release by monocytes |
| Oncostatin M | 28 | OMR gp130 | Monocytes; activated T cells | Megakaryocyte differentiation<br>Fibroblast, TIMP, and cytokine release<br>Acute-phase reactants, fibroblast protease inhibitors ↑<br>Monocyte TNF release ↓; IL-1 effector function ↓<br>Hypothalamic-pituitary axis ↑; corticosteroid release<br>Modulatory effect on osteoblast (?)<br>Pro-inflammatory effects in some models (?) |
| Leukemia inhibitory factor | 58 | LIFR gp130 | Fibroblasts; monocytes; lymphocytes; mesangial cells; smooth muscle cells; epithelial cells; mast cells | Acute-phase reactants ↑<br>Hematopoiesis, thrombopoiesis<br>Role in neural development, neural effector function, implantation<br>Bone metabolism; extra-cellular matrix regulation<br>Leukocyte adhesion molecule expression<br>Eosinophil priming<br>Mixed pro-inflammatory versus anti-inflammatory effects in models |

*Additional members of potential importance include IL-11, cardiotropin-1, and ciliary neurotrophic factor. Note overlapping effects within family.
†Membrane or soluble form can dimerize gp130 to promote signaling, which promotes signal transduction.
gp130, Glycoprotein 130; IL, interleukin; LIFR, leukemia inhibitory factor receptor; OMR, oncostatin M receptor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor.

**TABLE 26-7** Growth Factors Relevant to Rheumatic Diseases

| Cytokine | Receptors | Cellular Sources | Key Functions |
|---|---|---|---|
| TGF-β* | Type I TGFβR | Broad—including fibroblasts, monocytes, T cells, platelets | Wound repair, matrix maintenance, and fibrosis |
| Isoforms 1-3† | Type II TGFβR<br>Others | | Initial activation then suppression of inflammatory responses<br>T cell (Treg and Th17) and NK cell proliferation and effector function ↓<br>Early-phase leukocyte chemoattractant, gelatinase, and integrin expression ↑<br>Early macrophage activation then suppression, reduced iNOS expression |
| BMP family (BMP2-15) | BMPRI<br>BMPRII | Varied (e.g., epithelial and mesenchymal embryonic tissues); bone-derived cell lineages | Regulate critical chemotaxis, mitosis, and differentiation processes during chondrogenesis and osteogenesis, tissue morphogenesis (e.g., heart, skin, eye) |
| PDGF | PDGFRα<br>PDGFRβ | Platelets; macrophages; endothelial cells; fibroblasts; glial cells; astrocytes; myoblasts; smooth muscle cells | Local paracrine or autocrine growth factor for variety of lineages<br>Wound healing |
| FGF family | FGFR (various)<br>Basic FGF<br>Acidic FGF | Widespread | Growth and differentiation of mesenchymal, epithelial, and neuroectodermal cells |

*Members of TGF-β superfamily include BMP, growth and differentiation factor, inhibinA, inhibinB, müllerian inhibitory substance, glial-derived neurotrophic factor, and macrophage inhibitory cytokine.

†Bound to latency-associated peptide to form small latency complex and to latent TGF-β binding protein to form large latent complex; activated by proteolytic and nonproteolytic pathways.

BMP, Bone morphogenetic protein; FGF, fibroblast growth factor; iNOS, inducible nitric oxide synthase; NK, natural killer; PDGF, platelet-derived growth factor; TGF, transforming growth factor; Th, T helper; Treg, regulatory T cell.

**TABLE 26-8** Miscellaneous Cytokines with Potential Roles in Rheumatic Diseases

| Cytokine | Size (kDa) | Receptors | Cellular Sources | Key Functions |
|---|---|---|---|---|
| MIF | 12 | Unclear | Macrophages; activated T cells; fibroblasts (synoviocytes) | Macrophage cytokine release, phagocytosis, NO release ↑<br>T cell activation; DTH<br>Fibroblast proliferation; COX expression; $PLA_2$ expression<br>Intrinsic oxidoreductase activity ("cytozyme") |
| HMGB1 | 30 | RAGE, dsDNA<br>Others (?) | Widespread expression; necrotic cells; macrophages; pituicytes | DNA-binding transcription factor<br>Necrosis-induced inflammation<br>Macrophage activation—delayed pro-inflammatory cytokine<br>Smooth muscle chemotaxis<br>Disrupts epithelial barrier function<br>Bactericidal (direct) |
| GM-CSF | 14-35 | GM-CSFRα<br>GM-CSFRβ | T cells; macrophages; endothelial cells; fibroblasts | Granulocyte and monocyte maturation; hemopoietic effects<br>Leukocyte PG release; DC maturation<br>Pulmonary surfactant turnover |
| G-CSF | 19 | G-CSFR | Monocytes; PMNs; endothelial cells; fibroblasts; various tumor cells; stromal cells | Granulocyte maturation; promotes PMN function |
| M-CSF | 28-44 | M-CSFR | Monocytes; fibroblasts; endothelial cells | Monocyte activation, maturation |
| IL-32α-δ | Unknown | Unknown | Monocytes; T cells; NK cells; epithelial cells | Promotes pro-inflammatory cytokine release from variety of cells |
| Type I interferons IFNα/β family | Various | IFNαβR | Widespread | Anti-viral response<br>Broad immunomodulatory effects (promotes MHC expression)<br>Macrophage activation; lymphocyte activation and survival<br>Anti-proliferative, cytoskeletal alteration, differentiation ↑ |

COX, Cyclo-oxygenase; DC, dendritic cell; dsDNA, double-stranded DNA; DTH, delayed-type hypersensitivity; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HMGB, high mobility group box chromosomal protein; IFN, interferon; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MIF, macrophage inhibitory factor; NO, nitric oxide; PG, prostaglandin; PLA, phospholipase A; PMN, polymorphonuclear neutrophil; RAGE, receptor for advanced glycation end products.

## Cytokines in Chronic Inflammation

Cytokines critically modulate the cellular interactions that characterize chronic inflammation. Studies using real-time image analytic techniques such as two-photon microscopy and confocal scanning suggest continuous cellular motility during inflammation. Inflammatory lesions might properly be considered fluid states in which individual cells under cytokine control transiently contribute to organized functional subunits—such as the ectopic germinal center, synovial lining layer, or renal interstitial nephritis—yet remain competent to migrate thereafter under the influence of chemotactic gradients on the extra-cellular matrix. Cytokines also may promote cell death (apoptosis) either by withdrawal (e.g., IL-2, IL-7, IL-15, and type I IFNs) or by binding cytokine receptors containing death domains (e.g., TNF-R1). Cytokines contribute at every stage of inflammatory lesion development in a dynamic equilibrium, rather than in a static, linear manner. Chronic inflammation in rheumatic disease usually contains cytokine activities reminiscent of innate and acquired immune responses. For convenience, cytokines can be considered by their effect on cell subsets and cellular interactions (Figure 26-2 describes the role of cytokine activity in a developing and chronic lesion). It is critical to recall that cytokines can contribute both pro- and anti-inflammatory effects dependent on the time and context of their expression.

### T Cell Effector Function in Chronic Inflammation

T cells depend on cytokine function at every developmental stage from bone marrow stem cell maturation, through thymic education, to functional determination and maturation after primary or secondary antigen exposure (see the detailed overview in Chapter 12). The latter is of prime importance because re-education of phenotypic T cell responses may be achieved through alteration of the ambient cytokine milieu. T cell receptor–peptide–major histocompatibility complex (MHC) interactions during T cell–dendritic cell interaction rely on co-stimulatory molecule and local cytokine expression to determine functional outcome (see Tables 26-3 and 26-4). IL-12, in the presence of IL-18, promotes type 1 phenotypic development, characterized ultimately by IFN-γ producing T helper (Th) type 1 effector cells.[20] IFN-γ drives macrophage priming and activation and adhesion molecule expression and promotes granuloma formation and microbial killing. IFN-γ has a complex role in tissue destruction, however, with contradictory data obtained in inflammation models in IFN-γ-deficient and IFN-γ receptor–deficient mice. IFN-γ ultimately may retard tissue destruction, perhaps by suppressing osteoclast activation.[21]

A T cell subset has been defined that secretes IL-17A predominantly (Th17 effector cells), together with IL-22 that is increasingly recognized to be critical in a number of autoimmune diseases. Th17 cells are generated in the presence of IL-6 and transforming growth factor (TGF)-β, expanded by IL-1β and IL-23, and antagonized by IL-25 (IL-17E), IL-10, and, paradoxically, by IFN-γ. IL-17A provides a direct and rapid route to tissue damage via such means as neutrophil recruitment and activation, chondrocyte activation, keratinocyte activation, osteoclast activation, or fibroblast-like synoviocyte (FLS) activation.[22] The precise contribution of Th17 cells in human autoimmune disease is emerging; other lineages, such as innate lymphoid cells, likely also contribute to pathogenesis. Persuasive data from rodent models indicate that Th17 cells may be of primary importance as initiator and effector cells. Clinical trials that target IL-17A are ongoing in various rheumatic diseases, and phase II and III data now clearly demonstrate a pivotal role in psoriasis, psoriatic arthritis, and spondyloarthritis. The role for IL-17A in RA is much less clear because selective inhibitors have limited benefit in that disease.

IL-4 dominance during T cell–dendritic cell interactions in the presence of IL-18 leads to type 2 responses, which promote humoral immunity driven by Th2 cells synthesizing primarily IL-4, IL-5, IL-10, and IL-13. Resulting pathogenesis more likely may be B cell mediated. Cytokines that predispose to regulatory T cell development are unclear, although high levels of IL-10 or TGF-β have been suggested in this context.[23] Effector T cells can operate via secretion of cytokines to patterns determined by their prior activatory conditions.

### Cell-to-Cell Interactions

In many inflammatory lesions, there is relative paucity of inducer T cell–derived cytokines (especially IL-17A or IFN-γ), despite abundant pro-inflammatory cytokine expression. Cell-cell membrane interactions between leukocyte subsets and between tissue cells and leukocytes have emerged as a dominant mechanism sustaining chronic inflammation. Cytokines contribute to these interactions at several levels, including directly as membrane-expressed ligands, indirectly via activating cells, and synergistically by enhancing their subsequent cognate activities. The importance of cytokine–cell contact interactions is best studied in synovial tissues but applies to many inflammatory lesions. Many data now show that cognate interactions between T cells and adjacent macrophages constitute a major pathway driving cytokine release and that cytokines sustain this pathway. Such interactions do not depend on T cell receptor–mediated T cell activation and provide a route to expansion of inflammation by T cells independent of local autoantigen recognition.

Vey and colleagues[24] first observed monocyte activation via cell contact with mitogen-stimulated T cells. Freshly isolated synovial T cells activate macrophages by this mechanism, confirming that contact-induced cellular activation is a fundamental property of inflammatory T cells.[25] Antigen-independent, cytokine-mediated bystander activation confers this capacity on human $CD4^+$ memory T cells.[26] Studies using synovial T cells from RA and psoriatic arthritis tissues reveal that exposure of memory T cells to synergistic combinations of cytokines are most potent in this respect, particularly IL-15, TNF, and IL-6.[27,28] Cytokine activities also operate directly on macrophages to synergize with T cell contact. IFN-γ and IL-18 are most potent in this respect, acting via increased adhesion molecule expression.

Activated memory $CD4^+$ and $CD8^+$ T cells promote cytokine release from macrophages via diverse membrane ligands including leukocyte function-associated antigen-1 (LFA-1)/ intercellular adhesion molecule-1 (ICAM-1),

CD69, and CD40/CD154.[27,29] After contact with T cells, macrophages release increased concentrations of TNF and IL-1, but not IL-10, and they exhibit reduced levels of IL-1Ra. Th1 cells promote relatively greater pro-inflammatory cytokine release than do Th2 cells after co-culture. This finding suggests that their functional phenotype extends beyond cytokine secretion to include a differential membrane receptor array.[30] This suggestion is borne out further in the relative phenotypic distinctions between Th1 (CD40L, CCR5, and IL-18Rα) and Th2 (IL-33R/ST2 and CXCR3) cells. Recent data suggest that Th17 cells can also participate in similar cell-cell interactions. Signaling pathways engaged in monocytes after such T cell–membrane interactions are distinct from the pathways activated by conventional cytokine-inducing agents. Distinct use of phosphatidylinositol 3-kinase, NF-κB, and p38 mitogen-activated protein kinase (MAPK) pathways is observed.[31] Similarly, discrete macrophage signals follow contact with cytokine-activated T cells (which resemble synovial T cells) compared with T cell receptor–activated T cells.[32] Such distinctions offer therapeutic potential in targeting cytokine-activated, T cell–driven pathways, leaving antigen-driven responses relatively intact. The activation state of memory T cells necessary for the previously discussed interactions to proceed remains controversial. Purified resting T cell subsets activate synovial fibroblasts to release IL-6, IL-8, matrix metalloproteinase 3 (MMP-3), and prostanoids, in synergy with IL-17.[33]

It is likely that T cells are activated by interactions with diverse moieties, including extra-cellular matrix components and, potentially, autoantigens. Nevertheless, it is now clear that cytokines can promote chronicity by activating T cells to promote inflammation regardless of local (auto) antigen recognition and that this mechanism has enormous therapeutic potential.

## Agonist/Antagonist Cytokine Activities in Chronic Inflammation

Complex regulatory interactions exist to suppress ongoing inflammatory responses. This suppression is often achieved via parallel secretion of antagonistic cytokines and soluble receptors to regulate cytokine effector pathways. Th1 responses are suppressed partly by cytokines of Th2 type (e.g., IL-4 and IL-10), and consequently, exaggerated Th1 responses arise in models in which the Th2 response is deficient.[20] Th1 and Th2 cells similarly limit Th17 cell expansion.[22] Similar regulatory loops operate for other leukocytes, exemplified by the yin-yang effects of TNF and IL-10 on macrophage cytokine release and effector function.[34]

Inhibitory cytokine activities are usually defined with respect to a pro-inflammatory cytokine, and in other contexts they may have quite distinct functions, rendering prediction of their net contribution to an inflammatory response difficult. IL-10 opposes many of the pro-inflammatory effects of TNF and IL-1β (e.g., it reduces adhesion molecule expression, MHC expression, and MMP release), but it potently activates B cell activation and immunoglobulin secretion.[34] Similarly, TNF, which is normally considered a pro-inflammatory moiety, may have an important role in regulating T cell function because T cells removed from sites of chronic inflammation exhibit suppressed capacity to signal via their T cell receptor that recovers on TNF neutralization.[35] Such regulation is complicated further by the precise ratio of cytokine to soluble receptor such as TNF to sTNF-R or IL-10 to sIL-10R within the local environment. Commensurate with this, administration of anti-inflammatory cytokines such as IL-4, IL-10, and IL-11 has generally proved disappointing in the context of clinical inflammatory diseases. Suppressed IL-10 production might be one unanticipated effect of p38 MAPK inhibitors, with efficacy of these agents and underscoring the importance of considering the complex interplay between pro-inflammatory and anti-inflammatory cytokines. An important caveat is the potential requirement of combinations of cytokines to suppress inflammation optimally (e.g., combinations including IL-4, IL-10, and IL-11). Further functional antagonism is exemplified in the antagonistic activities of IL-1β and IL-1Ra and of IL-18 and IL-18 binding protein in regulating macrophage activation.

The role of cytokines in regulating cognate interactions between leukocytes has emerged more recently. Although anti-inflammatory pathways are poorly induced after cell contact, cytokine-activated T cells can induce IL-10 release by monocytes.[32] RA synovial membrane IL-10 release, which is partially T cell dependent, feeds back to regulate TNF release. Cytokine production from adjacent cell lineages within an inflammatory lesion may also be suppressive. IFN-β reduces mitogen-activated, T cell–induced macrophage release of TNF and IL-1, whereas IL-1Ra release is enhanced,[36] thus providing a mechanism whereby type I IFNs could modify pro-inflammatory cytokine production. Regulation extends beyond conventional cytokine activities. Prostaglandins and lipoprotein moieties, particularly high-density lipoprotein, can suppress cytokine-mediated, T cell–macrophage interactions.[37]

## Disease-Modifying Anti-rheumatic Drugs

Conventional disease-modifying anti-rheumatic drugs can also act via modulation of cytokine production. Methotrexate modulates release of various cytokines in vitro, in part mediated via the adenosine–cyclic adenosine monophosphate pathway.[38] The active metabolite of the dihydroorotate dehydrogenase inhibitor leflunomide, A77 11726, reduces TNF, IL-1β, IL-6, and MMP-1, but it does not reduce IL-1Ra release by monocytes after mitogen-activated T cell contact.[39,40] Leflunomide may mediate these activities through modulation of inhibitor of NF-κB (IκB)α phosphorylation and degradation and AP-1 and c-Jun N-terminal protein kinase activation.[41] Sulfasalazine is an inhibitor of pro-inflammatory cytokine-induced NF-κB. Biologic agents also potently modify cytokine expression in a variety of disease states. Signal transduction inhibitors such as agents that inhibit Janus kinase (JAK) or spleen tyrosine kinase (SYK) not only inhibit cytokine action but also the subsequent production of additional cytokines that can exacerbate disease.

## Cellular Interactions Across Diverse Tissues

Cytokines promote cognate cellular interactions across a range of tissues. In contrast to T cell–macrophage and T

cell–dendritic cell interactions, in which adhesion molecule and co-stimulatory pathways are often implicated, cell-cell membrane communications apart from leukocyte-leukocyte interactions are often mediated through membrane cytokine expression. T cell contact–mediated activation of fibroblasts operates via membrane TNF and IFN-γ to enhance fibroblast cytokine release and MMP—but not tissue inhibitors of metalloproteinase—expression, favoring tissue destruction.[29] The source and activation status of the fibroblast are vital; fibroblast-like synoviocytes, but not cutaneous fibroblasts, are potently activated by this route. Other studies have shown T cell contact–mediated activation of neutrophils, keratinocytes, mesangial cells (via a combination of membrane cytokine and CD40L expression), platelets, and renal tubule epithelial cells. Cytokine-activated macrophages (via IFN-γ and sCD40L) may interact via cell contact with mesangial cells to activate adhesion molecule and chemokine release by the latter. Cell-cell contact between cells of the immune system and beyond likely represents a ubiquitous mechanism whereby perpetuation of chronic inflammation is potently influenced by local production of cytokines.

### B Cells and Cytokine Release in Chronic Inflammation

Cytokines are crucial to B cell maturation, proliferation, activation, isotype switching, and survival (see Chapter 14). Cytokine-mediated B cell activation is important in immune complex generation, B cell antigen presentation, B cell–T cell interactions, and germinal center formation. Particular importance has been placed on the TNF superfamily cytokines, B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL). These cytokines are crucial for B cell development, survival, and optimal activation. However, their hierarchical place is now disputed at least in terms of RA biology because clinical targeting of both using atacicept (which neutralizes both BLyS and APRIL) has proven inefficacious in RA. Neutralization of BLyS with a monoclonal antibody has been similarly disappointing in RA, although more promising data have emerged in the treatment of systemic lupus erythematosus (SLE). B cells in turn represent a potent source of cytokines such as IL-6 and IL-10. The latter has now attracted much attention with the identification of B regulatory cells delineated by their dominant IL-10 expression. B cells also have been considered important inducers of macrophage-derived cytokine release. This process may operate primarily via immune complex formation[42] or through regulation of T cell activation (with B cell help). Complex regulatory feedback loops involving cytokine expression and B cells are likely important in a range of rheumatic diseases in which B cells are of paramount pathophysiologic importance. This may be one core mechanism whereby B cell depleting strategies in rheumatic diseases (e.g., via use of rituximab) are mediating their effects.

### Innate Cell Lineages in Chronic Inflammation

Cytokines potently activate innate response cells that contribute to the chronic inflammatory lesion of a variety of rheumatic diseases. Tables 26-1 through 26-8 document relevant examples in which neutrophils, NK cells, eosinophils, and mast cells may be recruited and activated by the presence of appropriate cytokine combinations.

### Growth Factors in Chronic Inflammation

Many data document the importance of growth factor families in chronic inflammation. TGF-β superfamily members including TGF-β isoforms and bone morphogenetic protein family members warrant particular reference. TGF-β is critically involved in processes of cell proliferation, differentiation, inflammation, and wound healing.[43] Bone morphogenetic proteins, in addition to regulating inflammatory responses, are paramount in determining cartilage and bone tissue development and remodeling.[44] As such, they are of increasing interest in the pathogenesis of several rheumatic diseases.

## CYTOKINE EFFECTS BEYOND IMMUNE REGULATION

A striking feature of the cytokine field concerns the broad functional pleiotropy exemplified in the effects of cytokines in normal physiologic and adaptive processes. Cytokine activities are found in muscle, adipose tissue, central nervous system, and liver, mediating normal regulation of metabolic pathways and modulation imposed by altered tissue conditions. Examples are found not only in the release of adipokines that regulate adipose metabolic pathways but also in the release of conventional cytokines by fat pads in inflammatory synovitis. Because cytokines thereby likely mediate normal and pathophysiologic activities in many tissues, it is increasingly recognized that they may underlie the co-morbidity that is observed in vascular, central nervous system, and bone tissues in several rheumatic diseases. Thus cytokines or their receptors arising from the primary target tissues (e.g., joint and kidney) may "leak" into the circulation and promote additional pathology in other tissues. Commensurate with this targeting, such cytokines may modulate this co-morbid risk, which is now exemplified in the reduction of vascular morbidity in patients receiving TNF blockers. However, paradoxical effects occur—for example, tocilizumab inhibits IL-6R, and in so doing it elevates total and low-density lipoprotein cholesterol. The long-term implications of this effect for vascular risk are not yet clear but provide abundant evidence for complex interactions between cytokine and metabolic processing.

## CONCLUSION

Cytokines represent a diverse family of glycoproteins that are active across a broad range of tissues. Their pleiotropic functions and propensity for synergistic interactions and functional redundancy render them intriguing therapeutic targets. Thus far, single cytokine targeting has proved useful in several rheumatic disease states. Further elucidation of the biology and functional interactions within this expanding family of bioactive moieties is likely to prove informative in resolving pathogenesis and in generating novel therapeutic options. In particular, biologic agents that target cytokines will increasingly unravel a novel molecular taxonomy for the rheumatic diseases.[45-47]

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 27



# Principles of Signaling

Vaishali R. Moulton • Abel Suarez-Fueyo • Sean Bradley • George C. Tsokos

**KEY POINTS**

- Cells of the immune system sense environmental stimuli via receptors, which may be expressed on the cell surface or may be intra-cellular.
- Interaction of ligands with receptors initiates signaling cascades, which relay the extra-cellular stimuli within the cell and alter cellular function.
- Signaling pathways typically involve phosphorylation (kinases) and dephosphorylation (phosphatases) of molecules.
- Signaling eventually leads to cellular responses such as changes in growth, activation, proliferation, and differentiation.

Immune cells respond to a vast variety of stimuli to carry out their role in maintenance of the immune system. Physiologic and innocuous and foreign or dangerous signals must be recognized and communicated within the cell, where they culminate into cellular responses such as changes in shape, motility, growth, activation, differentiation, or production of effector molecules. Distinct cascades of interacting molecules connect the perceived stimuli and relay information into the cytosol and/or nucleus to initiate these effector functions either directly or through initiation of gene transcription and protein translation programs. Signaling pathways can be categorized based on the mechanisms by which environmental stimuli are sensed, such as cell surface receptor-mediated interactions or intra-cellular detection of lipid-soluble molecules. Receptor-mediated signaling can further be classified by the presence or absence of enzymatic activity. This chapter focuses on fundamental concepts of signaling based on these groups of receptors and their intra-cellular signaling pathways.

## RECEPTORS WITH ENZYMATIC ACTIVITY

Many ligands are water soluble and therefore impermeable through the lipid bilayer of the plasma membrane, and interact with cognate receptors on the cell surface. These ligands include antigens, immune complexes, chemokines, cytokines, and microbial components. Interactions of ligands with their receptors initiate downstream catalytic activity and recruitment of similar signaling molecules or adaptor molecules. Receptors with enzymatic activity comprise extra-cellular ligand-binding domains, membrane-spanning domains, and intra-cellular signaling domains. Intrinsic enzymatic activity within the receptor includes kinase, phosphatase, or guanylate cyclase activity.

The receptor tyrosine kinase (RTK) family is a common class of these receptors. The human RTK family consists of 20 subfamilies that detect ligands such as stem cell factor, insulin, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), colony-stimulating factor (CSF), and fibroblast growth factor (FGF).[1] These receptors are generally inactive until bound by their ligands, which induce clustering and dimerization of the receptors and autophosphorylation of their kinase domains (Figure 27-1A). Upon activation, other kinases or cytoplasmic molecules are recruited for activation and transmission of signals downstream. In addition to receptor binding, ligands, along with accessory molecules, may also mediate receptor dimerization. FGF, for example, partners with heparin sulfate proteoglycans to cross-link and dimerize FGF receptors (FGFR).[2] Some receptors may dimerize even in the absence of ligands, such as the disulfide-linked insulin receptor and the insulin-like growth factor (IGF)-1 receptors. Ligand binding induces structural changes to activate these receptors and signaling by diverse mechanisms.[1]

Phosphorylation occurs at tyrosine, serine, or threonine residues. The phosphotyrosine is recognized by proteins containing Src homology (SH)2 and phosphotyrosine-binding (PTB) domains. These proteins may be kinases or phosphatases or lack enzymatic activity and act as intermediates known as *adaptor proteins*. SH2 domain proteins include phospholipase Cγ (PLCγ), phosphatidyl inositol-3-OH kinase (PI3K), and SHP phosphatases. Grb-2 and SHP2 are examples of adaptor proteins, of which Grb-2 recruits the guanine nucleotide exchange factor (GEF) SOS. SOS activates the small G protein Ras to activate the mitogen-activated protein kinase (MAPK) pathway. In addition, RTKs can activate PI3K, which activates the formation of phosphatidylinositol-3,4,5-triphosphate ($PIP_3$). PLCγ is also phosphorylated and activated by RTKs, leading to hydrolysis of phosphatidylinositol-4,5-$P_2$ ($PIP_2$) to generate inositol-1,4,5-triphosphate ($IP_3$) and diacylglycerol (DAG).

Transforming growth factor-beta (TGF-β) is an anti-inflammatory cytokine, which binds to receptors similar to the RTK family and regulates a variety of cellular processes.[3] The TGF-β ligand superfamily includes bone morphogenetic proteins, growth and differentiation factors (GDFs), activin, and TGF-β1, 2, and 3. TGF-β binding to a type II receptor that bears a serine threonine kinase mediates phosphorylation of a type I receptor to form a heterotrimeric complex with the ligand. This complex recruits and phosphorylates the intra-cellular SMAD proteins. The SMAD proteins include the receptor Smads 1, 2, 3, 5, and

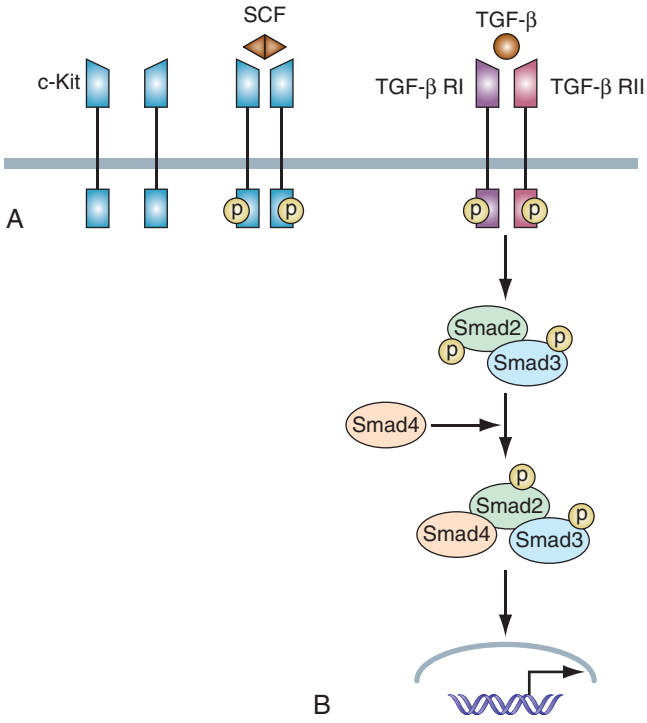



**Figure 27-1** **A,** Receptor tyrosine kinase c-kit, the receptor for stem cell factor (SCF) is depicted in the inactive form as monomers *(left)*. Upon binding to its ligand, the receptor dimerizes and the kinase domains phosphorylate each other *(right)*. **B,** Schematic depicting the transforming growth factor (TGF)-β–TGFβR signaling pathway. P, Phosphate.

9. Once phosphorylated, the r-Smads 2 and 3 oligomerize with a common mediator, Smad4, and translocate to the nucleus to regulate gene transcription (Figure 27-1B).[4]

Some receptors have phosphatase activity, such as protein tyrosine phosphatases (PTP). For example, the Src family kinases contain an activating and an inhibitory tyrosine residue. C-terminal Src kinase (Csk) phosphorylates the inhibitory tyrosine, whereas the transmembrane tyrosine phosphatase CD45 removes this phosphate, which is required for initiation of signaling in lymphocytes.

## RECEPTORS THAT RECRUIT MOLECULES WITH ENZYMATIC ACTIVITY

### Cytokine Receptors

Cytokines are soluble peptide regulators with profound effects on immune homeostasis and autoinflammatory disease.[5] Cytokine receptors are organized into four classes on the basis of their domain and structure.

1. Class I receptors are characterized by a WSXWS motif and can be subdivided on the basis of structural homology or the use of shared signaling subunits. Examples include receptors for interleukin (IL)-2, IL-6, IL-7, IL-15, and also for hormones such as erythropoietin and prolactin.[6]
2. Class II receptors include those for type I and II interferon (IFN), IL-10, IL-20, IL-22, IL-26, IL-28, and IL-29.[7]
3. Tumor necrosis factor (TNF) receptors share structures with some noncytokine receptors, such as CD95.[8]
4. IL-1 receptors can be considered analogous to Toll-like receptors (TLRs).[9]

These receptor complexes present two or more single-pass transmembrane subunits that include domains that recruit signaling proteins upon activation, and subunits with extra-cellular binding domains. Whereas class I and II cytokine receptors use the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, TNFRs use adaptor molecules called *TNFR-associated factors* (TRAFs) to recruit complexes with different functions.

### JAK/STAT Pathway

The mammalian JAK family includes four tyrosine kinases: JAK1, JAK2, JAK3, and tyrosine kinase 2 (Tyk2) (Figure 27-2). All four proteins have a conserved kinase domain and a related pseudokinase regulatory domain. JAK1 and JAK2 are involved in a wide range of cellular functions in growth and development, hematopoiesis, and inflammation, whereas JAK3 and Tyk2 are primarily important in the immune response. JAKs are controlled predominantly by post-translational mechanisms. More than 30 class I cytokine receptors and approximately 12 class II cytokine receptors activate the JAK family members. JAK structure is distinct from other tyrosine kinases. The N-terminal of these proteins contains two domains: an SH2 domain and a band 4.1 ezrin radixin moesin (FERM) domain. Interestingly, however, the SH2 domains do not function as phosphotyrosine residues but instead act as scaffolds. Upon cytokine-receptor binding, JAKs are reciprocally activated by phosphorylation, and phosphorylate receptor cytoplasmic tail regions. This leads to recruitment and activation of SH2 domain-containing proteins known as the *STAT*s (STAT1, 2, 3, 4, 5A, 5B, and 6). The STAT proteins share a high homology in their SH2 domain: a DNA-binding domain and a transactivation domain. STATs bind to the phosphorylated receptor through their SH2 domain and are activated in response to phosphorylation by JAKs. Upon phosphorylation, STATs undergo conformational changes, detach from the receptor, and dimerize with another STAT molecule. Dimers may be homo- or heterodimers, which subsequently translocate to the nucleus to regulate gene expression.[10,11]

Different STATs are important for signaling of distinct cytokines and/or growth factors/hormones (see Figure 27-2). In addition to the STAT proteins, JAK activation also leads to recruitment of SHP2 and Shc, p85, and SOCS3, which leads to regulation of Ras/MAPK and PI3K cascades. STAT proteins continually traffic between the cytoplasm and nucleus to mediate their function as transcription factors.[12] However, not all STATs travel in the same manner. STAT1 and STAT4 entry into the nucleus depends on their tyrosine phosphorylation and, importantly, dimerization, which creates a nuclear localization signal. Unphosphorylated STAT2 is constantly imported into the nucleus in complex with interferon regulatory factor (IRF)-9, but is shuttled back into the cytoplasm because of its dominant nuclear export signal. After phosphorylation, it can dimerize with STAT1 to import the complex into the nucleus. STAT3, 5, and 6 are continually imported to the nucleus regardless of their tyrosine phosphorylation state.

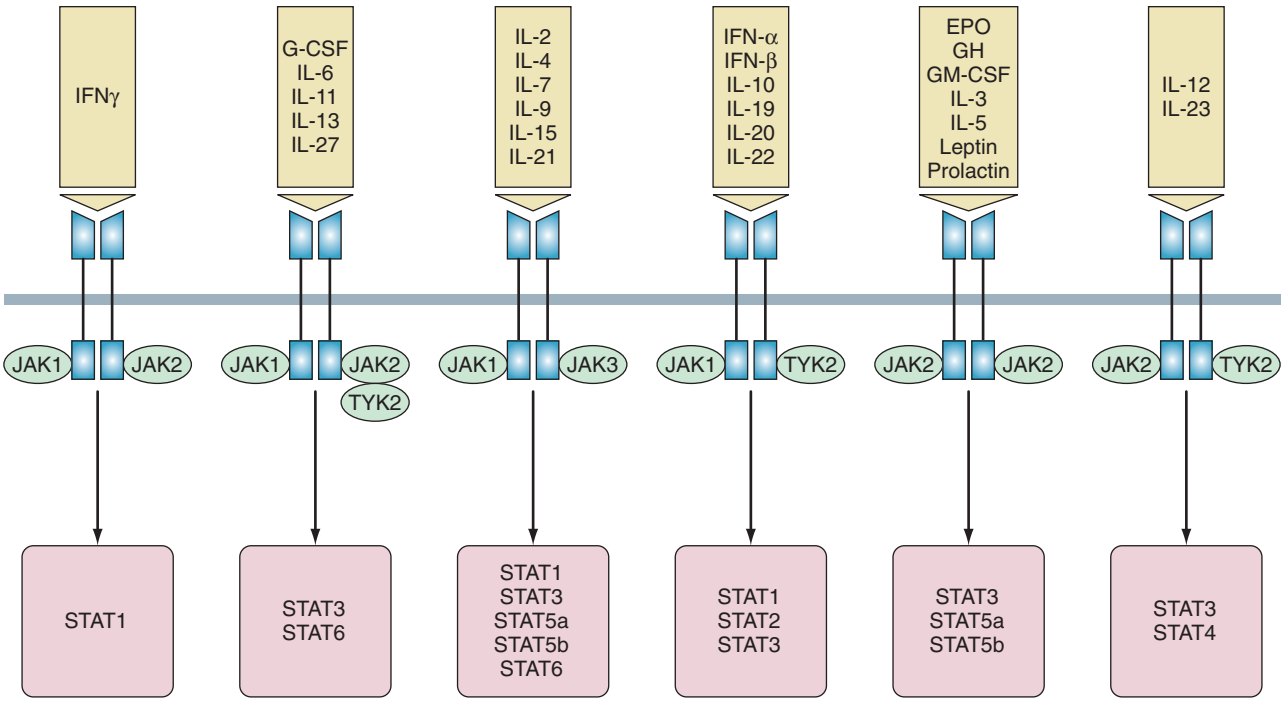



**Figure 27-2** Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. Activation of different JAK/STAT combinations by major cytokines. Cytokine receptors recruit and activate specific pairs of JAK kinases, which induce STAT phosphorylation and dimerization. STAT dimers translocate into the nucleus, where they act as transcription factors. EPO, Erythropoietin; G-CSF, granulocyte-colony stimulating factor; GH, growth hormone; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin.

Cytokine responses and the JAK-STAT pathway are under tight control of molecules, including protein tyrosine phosphatases (PTPs), protein inhibitor of activated STATs (PIAS), and suppressor of cytokine signaling (SOCS) proteins.[13] PTPs include SH2-containing PTP1 (SHP1), SHP2, CD45, and T cell PTP (TCPTP). The PTP and PIAS proteins, although not exclusive regulators of the JAK-STAT pathway, also regulate other cellular functions. PIAS inhibit the STATs by interfering with their DNA binding function and also, likely, by recruiting histone deacetylases. The suppressor of SOCS family consists of eight members: SOCS 1-7 and cytokine-induced STAT inhibitors (CISs), which block STAT activation. SOCS3 acts mainly by binding to the JAK and the cytokine receptor, which leads to inhibition of STAT3 activation, Besides STAT3, SOCS3 can also regulate signaling through other STATs. SOCS3 can either inhibit JAK activation or lead to ubiquitin-proteasome–mediated degradation of the receptor.

## Tumor Necrosis Factor Receptor Signaling

The TNF superfamily consists of 19 ligands and 29 receptors, which play varied roles in inflammation, apoptosis, and proliferation.[14] TNF members generally have pro-inflammatory functions mediated in part through the nuclear factor-κB (NF-κB) pathway. TNF and lymphotoxin-α (also known as *TNFβ*) were the first two members identified, and share approximately 50% homology in their protein sequence. Other members of this superfamily include lymphotoxin-β, CD40L, FasL, CD30L, 4-1BBL, CD27L, OX40L, TNF-related apoptosis-inducing ligand (TRAIL), lymphotoxin-like ligand competitive with gD-1 HSV for HVEM expressed on T cells (LIGHT), receptor activator of NF-κB ligand (RANKL), TNF-related weak inducer of apoptosis (TWEAK), a proliferation-inducing ligand (APRIL), B cell–activating factor (BAFF), vascular endothelial cell-growth inhibitor (VEGI), ectodysplasin A (EDA)-A1, EDA-A2, and glucocorticoid-induced TNF receptor (TNFR) family ligand (GITRL) .

TNF binds to two distinct receptors: TNFR1 and TNFR2 (Figure 27-3). Receptors of the TNF superfamily can be categorized based on the presence or absence of an intracellular death domain (DD), of which the D-containing receptors are ubiquitous in expression. TNFR1 contains a DD and is expressed in virtually all cell types, whereas TNFR2 is expressed mainly on immune cells, endothelial cells, and nerve cells. TNF is expressed both in soluble form and as a transmembrane protein on the cell surface, whereas lymphotoxin-α is only expressed as soluble protein. TNF signaling induces several signaling pathways, including activation of NF-κB, MAPK, and apoptosis. Upon TNF binding to the TNFR1, the DD recruits the TNFR-associated death domain (TRADD) protein, which subsequently recruits the Fas-associated protein with death domain (FADD). This triggers activation of caspase 8 and caspase 3, leading to apoptosis. TNF may also activate apoptosis through the mitochondrial pathway, cytochrome C release, and activation of caspase 9 and caspase 3. On the other hand, TNF can activate NF-κB, leading to cell survival and proliferation. This pathway involves TNF binding to TNFR1 and recruitment of TRADD, followed by TNFR-associated factor 2 (TRAF2/TRAF5), receptor-interacting protein

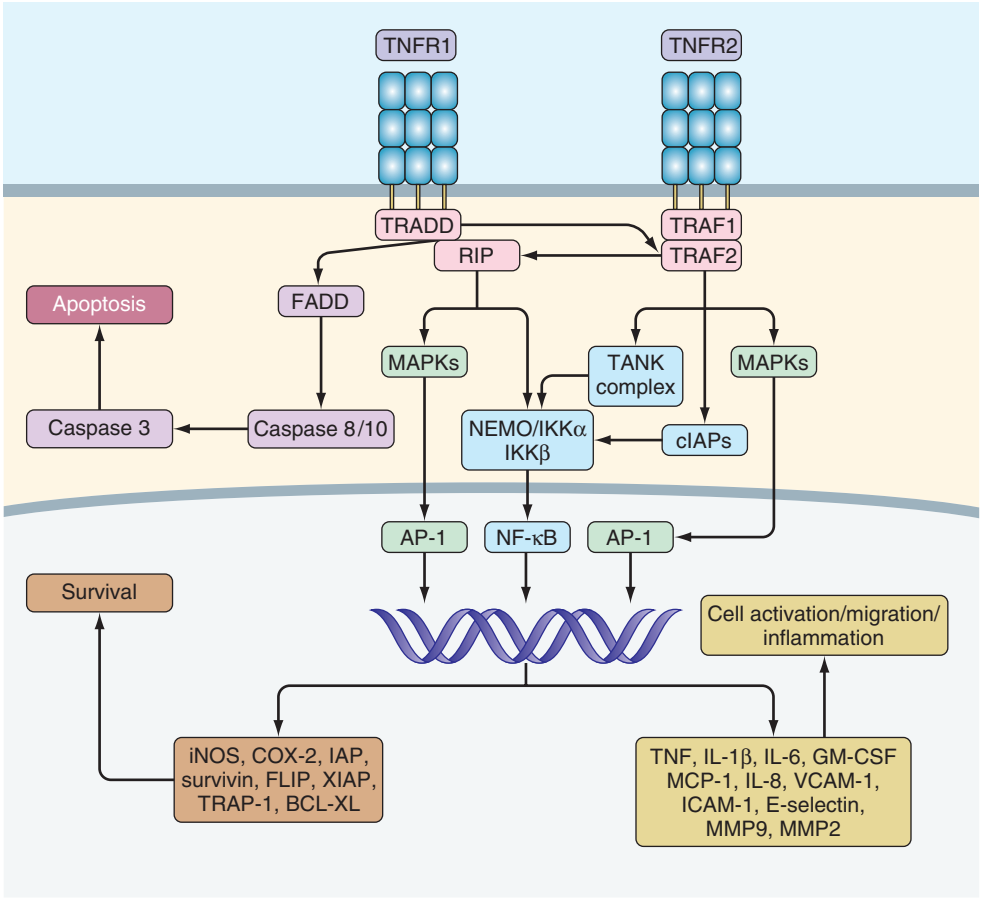



**Figure 27-3** Tumor necrosis factor receptor 1 (TNFR1) and TNFR2 signaling pathways. AP, Activator protein; BCL, B cell lymphoma; cIAPs, cellular inhibitors of apoptosis; COX, cyclooxygenase; FADD, Fas-associated protein with death domain; FLIP, FLICE-like inhibitory protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IAP, inhibitor of apoptosis; ICAM, intercellular adhesion molecule; IKK, IκB (inhibitor of κB) kinase; IL, interleukin; iNOS, inducible nitric oxide synthetase; MAPK, mitogen-activated protein kinases; MCP, monocyte chemotactic protein; MMP, matrix metalloproteinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; RIP, receptor-interacting serine/threonine-protein kinase 1; TANK, TRAF family member-associated NF-κB activator; TRADD, tumor necrosis factor receptor type 1–associated death domain; TRAP, TNF receptor-associated protein; VCAM, vascular cell adhesion protein; XIAP, X-linked inhibitor of apoptosis protein.

(RIP), TAK1, and IκB kinase (IKK). IKK, which is the activator of NF-κB, is composed of two catalytic subunits and one regulatory subunit known as *NF-κB essential modulator* (NEMO). NF-κB activation leads to the production of pro-inflammatory cytokines, such as IL-6, IL-18, chemokines, cyclooxygenase 2 (COX2), and 5-lipoxygenase (5-LOX). TNF–TNFR1 signaling regulates proliferation through TRAF2 to activate MEKK1, MKK7, and JNK. p38 MAPK is activated through TRAF2 and MKK3.[14,15] TNFR2 lacks a DD; therefore, how it mediates signaling is not well understood. It can bind TRAF2 directly, indirectly recruit TRAF1, TRAF-associated NF-κB activator (TANK), and cellular inhibitors of apoptosis (cIAPs) and thereby activate the NF-κB pathway. In addition, TNFR2 signaling can also activate the MAPK pathways efficiently. Despite the general belief that TNFR1 signaling induces apoptosis and the TNFR2 pathway is prosurvival, cross-talk exists between the two pathways, and TNFR2 signaling may also lead to apoptosis in some cells, despite the lack of a DD.[16] TNF signaling in addition induces pro-inflammatory cytokines such as IL-6, and extra-cellular matrix-degrading enzymes known as *matrix metalloproteinases* (MMP), which function in cellular activation and migration.

## G Protein–Coupled Receptors

G protein–coupled receptors (GPCRs) are a large family of approximately 350 members that bind a variety of ligands, including hormones, lipids, chemokines, and leukotrienes. They are seven-transmembrane domain-bearing proteins that associate with an intra-cellular trimeric G protein (α, β, or γ) and are organized into five families: rhodopsin, secretin, glutamate, adhesion, and Frizzled/Taste2 (Table 27-1). Ligand binding promotes the exchange of GDP for GTP and dissociation of the Gα and β subunits from the receptor subunit.

Based on their α-subunits, G proteins are divided into four subfamilies: Gαs, Gαi/o, Gαq/11, and Gα12/13. Each Gα has specific targets: Gαs and Gαi/o activate or repress adenyl cyclase (AC), regulating cAMP levels to impact several ion channels and the activation of protein kinase A (PKA), whereas Gαq/11 activates phospholipase C (PLC)-β to generate $IP_3$ and DAG from $PIP_2$, ultimately leading to $Ca^{2+}$ and protein kinase C (PKC) pathway activation. The targets for Gα12/13 are three RhoGEFs that activate the small GTPase Rho, critical for cytoskeleton regulation via the stress-activated MAPK pathway. Alternatively, the

**TABLE 27-1** Examples of G Protein–Coupled Receptors (GPCR) and Their Ligands

| GPCR Superfamily | GPCR Family | GPCR | Ligands |
|---|---|---|---|
| Rhodopsin | Adenosine receptor | $A_{2A}R$ | Adenosine |
| | Chemokine receptor | CXCR4 | SDF1 |
| | | CCR2 | CCL2 |
| | | CCR3 | CCL5<br>CCL7<br>CCL11<br>CCL13<br>CCL26 |
| | | CCR4 | CCL2<br>CCL4<br>CCL5<br>CCL17<br>CCL22 |
| | | CCR5 | CCL3<br>CCL4<br>CCL5 |
| | Bradykinin receptor | $B_2R$ | Bradykinin |
| | Anaphylatoxin receptor | C5aR | C5a |
| | S1P receptor | S1P1 | S1P |
| | Protease-activated receptor | PAR-1 | Thrombin |
| | | PAR-2 | Trypsin |
| | Prostaglandin receptor | $EP_2/EP_4$ | Prostaglandin E2 |
| Adhesion | | CD97 | CD55 |
| Secretin | | GCG-R | Glucagon |
| Glutamate | | Kainate GluR | Glutamate |
| Frizzled/Taste2 | | FZ5 | Wnt5a |

Gβγ-complexes regulate several ion channels, specific isoforms of AC, PLC, and PI3K (Figure 27-4).[17] Gβγ dimers activate the Rho family G proteins to regulate actin filament reorganization and therefore induce cytoskeletal changes and impact cellular trafficking. These changes are important in response to chemokines, anaphylatoxins, or histamine. Sphingosine-1-phosphate (S1P) is a lipid-signaling molecule present in blood and lymph. Its receptor is abundantly expressed on endothelial cells and is important for lymphocytes exiting from the thymus and lymph nodes.

## Immunoreceptors: T Cell Receptor, B Cell Receptor, and FcRs

Immune cells express antigen recognition receptors that are generated and selected for in a complex fashion.[18,19] T cells and NKT cells express T cell receptor (TCR) and recognize peptides or lipids associated with the major histocompatibility complex (MHC), or CD1d, respectively. B cells, through the B cell receptor (BCR), recognize soluble antigens. Lymphoid and myeloid cells, such as NK cells and macrophages, express receptors of the FcR family, which cause activation upon detection of antibodies in complex with antigen. These receptors are heteromultimeric complexes, with a subunit of one or two chains that recognize antigen, (TCRα and TCRβ) and CD3 subunits that have from one to six chains that contain immunoreceptor tyrosine-based activation motif (ITAM) domains (see Figure 27-4).

Antigen recognition triggers the activation of membrane proximal early kinases, including Src, Syk, and Tec. The Src family phosphotyrosine kinases (PTK) comprise eight kinases (Fgr, Fyn, Src, Yes, Blk, Hck, Lck, Lyn). These kinases associate with different receptors in a variety of cells. Upon activation, they undergo autophosphorylation and phosphorylate tyrosines of the ITAM domain. Their function is regulated by the kinase Csk and the protein tyrosine phosphatase CD45.[20-22] ITAM phosphorylation leads to recruitment and activation of Syk family kinases recruited through SH2 domains. BCRs recruit Syk, whereas ZAP-70 binds to phospho-ITAMs of CD3 zeta within the TCR. Both kinases are implicated in the signaling of Fc receptors because Syk associates with their common gamma chain, although some incorporate CD3 zeta to recruit ZAP-70. These then undergo autophosphorylation to create sites for interaction with SH2 proteins.[23]

The Tec family consists of five different kinases (Bmx, Btk, Itk, Tec, and Txk/Rlk), which translocate to the cell membrane through their pleckstrin homology (PH) domain to bind $PIP_3$. Alternatively, they are recruited via their SH2 domains, which recognize ITAM motifs on adaptor proteins (e.g., BLNK in B cells, or SLP-76 and LAT in T cells). The principal downstream target of Tec kinases is PLCγ.[23,24]

Adaptor proteins are noncatalytic and act as docking sites for other proteins via SH2 and SH3 domains. Linker for activation of T cells (LAT) is a transmembrane protein, whereas SH2-domain–containing leukocyte protein-76 (SLP-76) and BLNK are cytoplasmic. In their absence, activation of Src and Syk kinases fail to stimulate the Ras pathway and calcium flux induced by PLCγ.[25]

### PI3K/Akt Pathway

The PI3K family is divided into three classes on the basis of structure, regulation, and lipid substrate specificity. The focus of this discussion will be class I because it controls the Akt/mammalian target of rapamycin (mTOR) pathway which is involved in proliferation, differentiation, motility, and intra-cellular trafficking (see Figure 27-4).[26] Class I PI3Ks are composed of four heterodimeric proteins, including a catalytic (p110) and a regulatory (p85) subunit, and generate $PIP_3$ upon activation. The group is further divided into subclasses on the basis of the regulatory subunit they bind. Class IA is composed of p110 α, β, and δ catalytic subunits, which bind isoforms of p85, and are activated by tyrosine kinase–associated receptors. Phosphorylation of different ITAMs causes recruitment of PI3K through the SH2 domain and, eventually, generation of $PIP_3$ from $PIP_2$.[27] Akt is a serine/threonine kinase that is recruited to the plasma membrane through its PH domain and binds $PIP_3$. Akt is activated by phosphorylation by phosphoinositide-dependent kinase 1 (PDK-1) and mTOR complex 2. Active Akt phosphorylates a number of targets such as FOXO1, 3A and 4, GSK3α/β, RAF1, TSC2, and PRAS40 (both of which are then inhibited, allowing mTOR 1 function), IKKα (leading to activation of NF-κB), p21CIP1, BAD, or caspase 9.

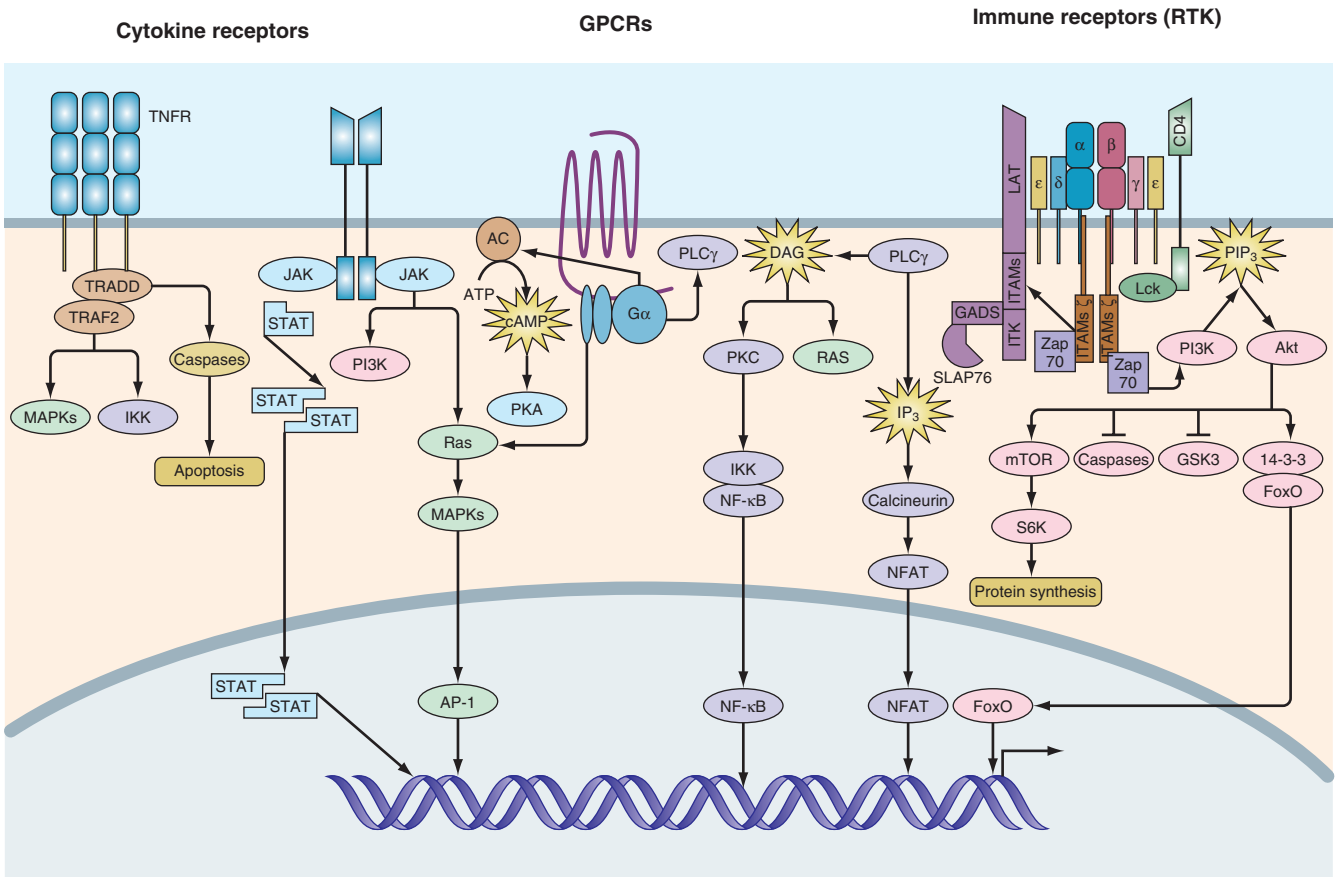



**Figure 27-4** Schematic depicting signaling pathways through cytokine receptors, G protein–coupled receptors (GPCRs) and immune receptors. AC, Adenyl cyclase; AP-1, activator protein-1; GADS, growth factor receptor-bound protein-2-related adaptor protein-2; ITAM, immunoreceptor tyrosine activation motif; JAK, Janus kinase; LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; PI3K, phosphatidyl inositol-3-OH kinase; $PIP_3$, phosphatidylinositol-3,4,5-triphosphate; PKA, protein kinase A; PLC, phospholipase C; SLAP, Src-like adaptor protein; STAT, signal transducer and activator of transcription; TNFR, tumor necrosis factor receptor; TRAF, TNFR-associated factor; TRADD, TNFR-associated death domain.

## Phospholipase C Signaling: Calcium Flux and Protein Kinase C Activation

Activated PLC leads to generation of $IP_3$ and DAG from $PIP_2$. $IP_3$ is a soluble second messenger, which leads to release of stored $Ca^{2+}$ into the cytoplasm. This second messenger activates calmodulin and other $Ca^{2+}$-binding proteins. Calmodulin activates Ras/MAPK via indirect activation of Ras, or directly through activation of Raf-1.[28] In addition, calmodulin activates the serine/threonine phosphatase calcineurin, which dephosphorylates nuclear factor of activated T cells (NFAT). This exposes a nuclear localization signal (NLS) leading to nuclear import and transcription regulation.[29] On the other hand, DAG acts as a second messenger to stimulate PKC. These proteins are also related to the control of NF-κB (via phosphorylation of IKK) and activator protein (AP)-1 transcription factors.[30]

## MAP Kinase Pathway

A wide range of extra-cellular signals, including cytokines, stress, and growth factors, can activate a series of MAP kinases to ultimately regulate gene expression, and diverse cellular processes, including growth, proliferation, survival, and inflammatory immune responses. The MAPK signal cascades involve three tiers of activation (Figure 27-5). The first level of activation is the MAP kinase kinase kinase (MAPKKK or MAP3K), which includes activated Raf, apoptosis signal-regulating kinase (ASK)1, MEKK1-4, MLK, and TGF-β–associated kinase (TAK)1. MAPKKK phosphorylates and activates the next tier MAPKK, examples of which are MEK1/2, and the MKKs. MAPKK then phosphorylates the third tier of MAPK molecules, which include three families, namely, the extra-cellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinase (JNK), and p38 (α, β, γ, and δ), the latter two of which are stress-activated MAPKs. Finally, ERK, JNK, and p38 activate transcription factors, which include Ets, Elk-1, c-Myc activating transcription factor (ATF)2, p53, CREB, NF-κB, and AP-1. Activation of transcription factors may occur through direct phosphorylation or via modulation of ribosomal S6 kinase activity in the case of CREB, and via IKK in the case of NF-κB. JNK also regulates activity of non-transcription factors, such as the apoptosis-related Bcl-2 molecules.

Distinct signals activate the three families of MAPKs (although there can be considerable cross-talk)—ERK1/2, p38, and JNK. ERK1 and 2 are serine/threonine kinases that are components of the Ras-Raf-MEK-ERK cascade of the

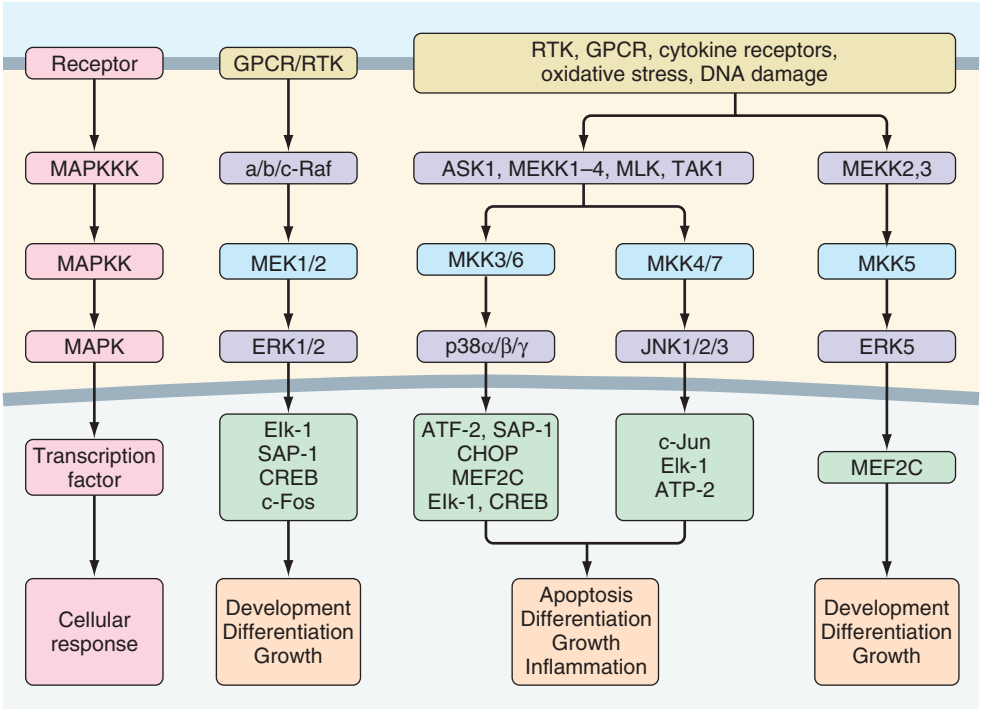



**Figure 27-5** Mitogen-activated protein kinase pathway (MAPK). A general schematic of the MAPK signaling pathway is shown on the left. On the right are specific receptors and three tiers in the MAPK cascades. Transcription factors and cellular responses activated by the different pathways are indicated. ASK, Apoptosis signal-regulating kinase; ATF2, activating transcription factor 2; C/EBP, CCAAT enhancer binding protein; CHOP, C/EBP homologous protein; CREB, cyclic adenosine monophosphate (AMP) response element–binding protein; ERK, extra-cellular signal-regulated kinase; GPCR, G protein–coupled receptor; JNK, c-Jun N-terminal kinase; MEF2C, myocyte-enhancer factor 2C; MLK, mixed lineage kinases; RTK, receptor tyrosine kinase; SAPK, stress-activated protein kinase; TAK, TGF-β–associated kinase.

MAPK pathways.[31] Whereas Raf and MEK kinases have limited substrate specificity, ERK1/2 phosphorylate and activate a vast number of cellular targets, including a large number of transcription factors. ERK1/2 are activated by a variety of cellular stimuli, including bradykinin, epidermal growth factor, FGF, insulin IGF1, PDGF, cytokines, and osmotic stress. Activation of this pathway regulates cell adhesion, migration, survival, proliferation, and differentiation. The p38 α and β isoforms are ubiquitous in their distribution; the γ isoform is mainly expressed in skeletal muscle; and the δ isoform is expressed in the testes, pancreas, and the small intestine. p38 MAPK is activated via pro-inflammatory cytokines and lipopolysaccharide (LPS). The p38 MAPK is critically important for the production of IL-1, TNF, and IL-6 pro-inflammatory cytokines. The JNK proteins are encoded by *JNK1*, *JNK2*, and *JNK3* genes, and alternative splicing results in additional isoforms and complexity. JNK1 and JNK2 are ubiquitous in their tissue distribution, whereas JNK3 is expressed mostly in the brain, heart, and testis. Pro-inflammatory cytokines, such as IL-1 and TNF, and UV radiation lead to JNK phosphorylation. The JNKs are involved in modulation of the extra-cellular matrix by regulating production of metalloproteinases.[32] A specific subgroup of protein tyrosine phosphatases that inactivate the MAPKs are called *MAP kinase phosphatases* (MKPs) and include MKP1, 3, 5, and 7, which can inactivate JNK and provide a means to regulate MAPK activity.

Therefore the MAPKs integrate signals from different receptors to regulate transcription factors to control a variety of overlapping genes and to ultimately regulate cellular survival, proliferation, and differentiation under physiologic and pathologic conditions.[33-35]

## Co-stimulatory Receptors

T cell activation requires two signals, one mediated through the T cell receptor and a second signal called *co-stimulation*. This signal is provided by a family of receptor-ligand molecules that mediate interactions between T cells and antigen-presenting cells. The B7/CD28 is one of the most well-studied pathways of costimulation. B7-1 and B7-2 are expressed by APCs and bind CD28 on T cells. This interaction triggers downstream signaling to amplify the primary signal and initiate effector responses. CD28 is phosphorylated by Lck and activates the PLCγ and PI3K/Akt pathways. An important consequence of this signaling pathway is to enhance mRNA stability of IL-2, which is responsible for the dramatic increase in IL-2 secretion. Cytotoxic T lymphocyte antigen (CTLA)4 is closely related to CD28 in structure, can bind to B7 molecules but with much higher avidity, and curbs the activation and proliferation of T cells.[36] In addition to the B7-CD28 pairing, other co-stimulatory molecules include the inducible co-stimulator (ICOS), which is induced on activated T cells and interacts with B7-H2/ICOSL on activated dendritic cells, monocytes, and B cells. Another class of co-stimulatory molecules is the signaling lymphocyte activation molecule (SLAM) family, a subtype of the immunoglobulin superfamily consisting of nine transmembrane proteins. SLAMs are important for multiple cell types, including T, B, and NK cells, and mediate

their effects via homophilic and heterophilic interactions. The SLAM proteins bear a tyrosine-based switch motif through which they bind SH2-bearing proteins SLAM-associated protein (SAP) and EAT2 with high affinity.[37]

Once activated, T cells express other co-stimulatory molecules, such as CD40L and the programmed cell death (PD)1 receptor. CD40L interacts with CD40 on APCs, and this induces signals, which play an important role in effector functions, such as activation of B cells, to produce antibodies. A co-stimulatory inhibitory pathway involves PD1 on T cells binding to B7-H1/PDL1 and B7-DC/PDL2 expressed on APCs.[38] PD1 belongs to the immunoglobulin superfamily, and its intra-cellular domain bears an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). The tyrosine in the ITSM binds to the phosphatases SHP1 and SHP2, which act to inhibit the PI3K/Akt pathway. PD1 signaling inhibits induction of the antiapoptosis molecule BcL-xL and transcription factors such as T-bet, GATA-3, and Eomes, which are important for T cell differentiation.

Thus co-stimulatory signals not only activate cells to promote proliferation and effector functions required in the immune response but also are inhibitory to curb excessive activation and are important for tolerance induction.

### Adhesion Molecules

A variety of cellular functions, including lymphocyte activation, migration, and cell-cell interaction, involve adhesion molecules. These include selectins, integrins, and immunoglobulin superfamily molecules. L-selectin (CD62L) is important for lymphocyte homing to lymphoid tissues. Activated lymphocytes downregulate L-selectin and upregulate other adhesion/migratory molecules, such as CD44, a transmembrane protein, which recognizes hyaluronic acid and is critical for migration into peripheral sites of inflammation. CD44 lacks intrinsic kinase activity, but the cytoplasmic domain associates with Src family kinases Lck and Fyn. In addition, the cytoplasmic tail binds to the phosphorylated ezrin/radixin/moesin (pERM) proteins, which cross-link actin cytoskeleton proteins to CD44.[39] CD44 signaling also activates PI3K/Akt cell survival pathways.[40]

Integrins are heterodimeric cell surface proteins that provide interactions with other cells and the extra-cellular matrix, allowing for migration to effector sites. Various chemokines can activate integrins to mediate migration into lymphoid or nonlymphoid tissues. T lymphocyte integrins include leukocyte function-associated antigen (LFA)-1, LPAM-1, and very late activation antigen (VLA)-4, which bind immunoglobulin superfamily members ICAM-1, MAdCAM-1, and VCAM-1, respectively. Integrin activation results in inside-out signaling, which regulates the affinity of the integrin receptor to the extra-cellular ligands. Elements of this signaling pathway include the small GTPase RAP1 and its GEFs talin and kindlin3.[41-43]

## INNATE RECEPTOR SIGNALING

TLRs and nucleotide-binding oligomerization like receptors (NLRs) constitute the major pathogen sensors of innate immunity and work in a cell autonomous fashion to initiate anti-microbial responses, including inflammation and apoptosis (Figure 27-6). The major downstream effect of the cascades initiated by pathogen-associated molecular pattern (PAMP) recognition is IRF transcriptional activation of IFN genes that mediate inflammation and tissue repair. PAMPs are detected in endosomes and on the surface of cells by a variety of TLRs. These receptors have leucine-rich repeat domains to detect ligands, a single-pass membrane domain, and an intra-cellular Toll–interleukin-1 receptor (TIR) motif for downstream signaling. Their activities are most relevant in macrophages, monocytes, and dendritic cells.

TLR1, 2, 4, and 6 recognize varieties of endotoxin lipopolysaccharides from gram-negative bacteria, whereas TLR3, 7, 8, and 9 respond to nucleic acids that may also derive from viruses. TLR11 detects profilin protein, and the ligand for TLR10 remains uncharacterized. Response to ligand binding includes dimerization of receptors, which promotes docking of a variety of adaptor proteins, including myeloid differentiation factor (MyD)88, TIR-domain–containing adaptor protein-inducing IFN-β (TRIF), TIR-associated protein (TIRAP), and TRIF-related adaptor molecule (TRAM). A common downstream target of these cascades is NF-κB, which stimulates the transcription of inflammatory cytokines and IFN.

NLRs detect cytosolic PAMPs and endogenous inflammatory signals. Common motifs in these receptors include the nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR). There are more than 20 versions of these receptors in mammals. The best-characterized is NOD1/CARD4 and NOD2/CARD15. NOD1 senses meso-diaminopimelic acid, found in gram-negative strains, whereas the NOD2 ligand is muramyl dipeptide, present in all bacteria.

A common downstream effect of pathogen detection is generation and release of IFNs. These glycoproteins are divided into three classes on the basis of the receptors that they activate. Type I IFNs bind the IFNA receptor (IFNAR), and this group includes IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω. IFN-γ is the sole member of type II in humans, and it binds IFNGR. Type III IFNs activate a receptor complex consisting of IL-10R2 and IFNLR1, and these are IL-29, IL-28A, and IL-28B. Activation stimulates JAK-STAT signal cascades and transcriptional changes made in cooperation with IRF proteins. The expression of so-called *interferon signature genes* (ISG) promotes anti-viral activities, increased MHC presentation, and apoptosis.

## INTRA-CELLULAR RECEPTOR SIGNALING

Steroid hormones synthesized from cholesterol in the adrenals and gonads, and vitamin D from dietary sources or produced by photochemistry in the skin, exert direct effects on gene expression via interaction with nuclear receptors. Because they are lipid soluble, these hormones can diffuse through plasma membranes to the nucleus to interact with receptors that directly modulate transcription, making them potent and common drug targets. In addition to endogenous molecules, many xenobiotics, such as endocrine disruptors, modulate these receptors. Almost half of the roughly 50 mammalian nuclear receptors have poorly characterized or multiple low-affinity ligands and are therefore known as *orphan receptors*.

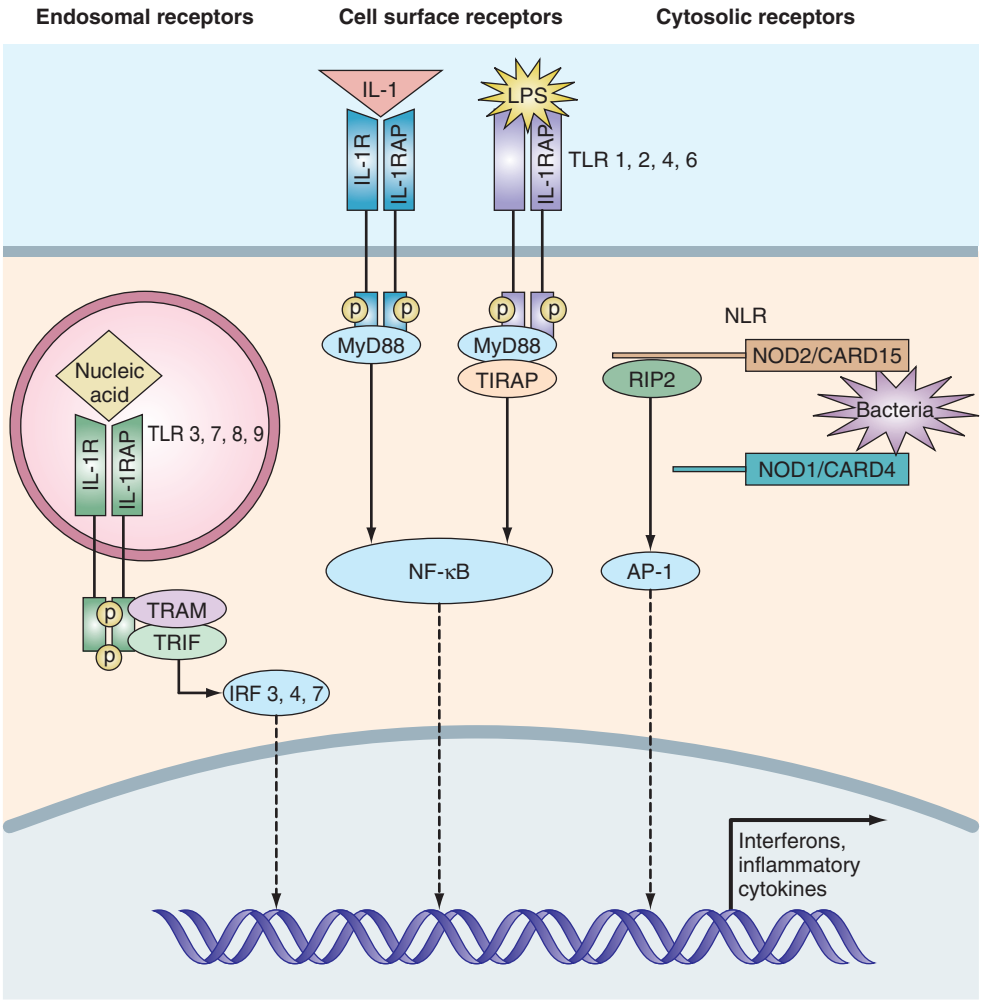



**Figure 27-6** Schematic depicting signaling through endosomal receptors, cell surface Toll-like receptors (TLRs) and cytosolic nucleotide binding oligomerization domain (NOD)-like receptors (NLRs). AP-1, Activator protein-1; CARD, caspase recruitment domain; IL-1RAP, IL-1 receptor accessory protein; IRF, interferon regulatory factor; LPS, lipopolysaccharide; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; TIRAP, Tir-domain–containing adaptor protein; TRAM, TRIF-related adaptor molecule; TRIF, Tir-domain–containing adaptor-inducing interferon-β.

Nuclear hormone receptors are broadly divided into two classes. Type I are retained in the cytoplasm until ligand binding drives a conformational change that releases binding partners, such as heat shock proteins, after which they translocate to the nucleus. Examples include the glucocorticoid, androgen, and estrogen receptors. Glucocorticoid agonists, such as dexamethasone and prednisolone, are important immunosuppressants. Activation not only drives gene expression changes following nuclear translocation but also transrepression of pro-inflammatory transcriptional complexes, such as AP-1 and NF-κB.

Another type I nuclear receptor of importance in immune signaling is the RAR-related orphan receptor gamma (RORγ). Derived from the *RORC* gene, mRNA for this protein is detectable in a variety of tissues, but its physiologic role is largely restricted to the RORγt isoform, critical for the development and maintenance of Th17 cells. This T helper is the primary source of pro-inflammatory IL-17, which makes RORγt a target for novel drug antagonists.[44]

Type II nuclear receptors generally reside on deoxyribonucleic acid and are inhibited by co-repressor proteins until ligand detection. Examples include thyroid hormone receptor (TR), retinoic acid receptor (RAR), and vitamin D receptor (VDR). They act as heterodimers in conjunction with cor-receptors, the most common of which is the retinoid X receptor (RXR). Although its major action is to regulate calcium absorption in the intestine and bone remodeling, vitamin D has increasingly been recognized as an immune modulator. Macrophages within granulomas convert vitamin D into its active form as part of an innate immune regulatory cascade.[45] Activated monocytes also generate this metabolite, and its effects are potentiated by IFNγ. In contrast, IL-4 generated by Th2, attenuates vitamin D effects, perhaps by stimulating its degradation.

## CONCLUSION

Discovery of these signaling pathways has provided valuable insights into immune pathogenesis of autoimmune and immunodeficiency diseases. Many receptors and/or signaling pathways are potential therapeutic targets for rheumatologic diseases. Because many of the signaling pathways and molecules are often widely expressed, carefully balancing risk and benefit of perturbing them will be a critical component of a drug development program.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 28



# Immunologic Repercussions of Cell Death

Helen M. Beere • Douglas R. Green

**KEY POINTS**

- **Apoptosis is a form of cell death induced by multiple stimuli and mediated by the activity of a group of cysteine proteases known as caspases. This type of cell death is characterized by the degradation of nuclear DNA and is generally non-inflammatory.**
- **Apoptosis and caspase activation can be triggered extracellularly by engagement of death receptors or intrinsically by mitochondrial damage and release of cytochrome-*c* and second mitochondrial-derived activator of caspase (SMAC)/DIABLO that function to activate caspases at the apoptosome.**
- **The B cell lymphoma 2 (BCL-2) family includes both pro- and anti-apoptotic proteins that control cell death by regulating the mitochondrial outer membrane potential.**
- **Necrosis and necroptosis are death processes that involve swelling of cells and rupture of the plasma membrane, resulting in inflammation.**
- **Necroptosis is a specialized form of necrosis that is initiated by death ligands and regulated by signaling pathways that are also important for the regulation of apoptosis.**
- **NOD-like receptor molecules act as cell stress sensors to trigger inflammasome assembly and activation of caspase-1 to mediate the processing and secretion of the pro-inflammatory cytokines IL-1β and IL-18.**
- **Autophagy is a normal cellular response to nutrient deprivation that is characterized by the degradation and recycling of cellular components.**

Cells are induced to die in response to a variety of stressors, signaling cues, and damaging events of both intra- and extra-cellular origin. Just as there are multiple types of death-inducing triggers, there too are several distinct cell death pathways, each of which is characterized by specific phenotypic and molecular features. Effective and efficient deletion of a damaged or dysfunctional cell necessitates engagement of self-destruction by different types of stress, be it DNA damage, metabolic disruption, or infection. But why evolve the capacity to eliminate cells by several different pathways, each with a unique phenotypic and molecular fingerprint? Once a cell is destined to die, why does it matter how the cell engages this death?

The answer to that question has proven more interesting than one might have predicted. As it turns out, not all cell deaths are created equal—some pathways are more equal than others, at least from the perspective of immune function. A dead or dying cell can impart signals to its neighboring cells, and these signaling events in turn determine critical physiologic responses essential for host survival. It is the type of cell death that occurs and the pathway by which a cell dies that determine the nature and identity of those signals, and these specific signaling events ultimately determine whether that cell death process is non-inflammatory or pro-inflammatory—or even in some cases actively suppresses inflammation. In this review, we will introduce several different types of cell death and discuss the defining features of each. The impact of these cell death pathways on immune function will be discussed in the context of disease pathologies associated with aberrant immune regulation and defects in cell death pathways.

## TYPES OF CELL DEATH

### Apoptosis

Apoptotic cell death or apoptosis (type I cell death) is perhaps the best characterized of the cell death pathways. The most prominent phenotypic characteristics of apoptotic cell death include perturbation and blebbing of the plasma membrane from which small membrane-bound vesicles, so-called "apoptotic bodies," are released. The plasma membrane maintains its integrity but undergoes lipid reorganization and "scrambling" to externalize phospholipids on the cell surface. As we will see, this event is particularly important with regard to how apoptotic cells are detected by the immune system. Within a cell dying by apoptosis, chromatin condenses and internucleosomal DNA cleavage generates the hallmark DNA "ladder" when separated on an agarose gel. The dying cell then begins to shrink, and if it is adherent, it detaches from neighboring cells and surrounding stroma and matrix.

The phenotypic changes associated with apoptotic cell death are mediated by the collective activities of a family of cysteine proteases, the caspases. It is the enzymatic activity of these ordinarily inactive zymogens that mediate, coordinate, and execute the apoptotic demise of the cell. As

such, caspase activation represents one of the most universal and informative descriptive biochemical features of apoptotic cell death. Of course it is not that simple—several different caspases exist, each of which is activated and regulated by different signaling pathways so that apoptotic cell death can be initiated in response to multiple types of damage or stress.

Once caspases have cleaved their target substrates, the now apoptotic cell is rapidly removed from the body via phagocytosis. Therefore, in contrast to other types of cell death, apoptosis generally, but not always, fails to evoke an inflammatory response. Even so, the term *immunologically silent*, which is often used to describe apoptosis, is somewhat misleading because an apoptotic cell, even when engulfed by a phagocyte, remains a source of signals that regulate components of immune function.

## Necrosis—Classical or Noncanonical?

Necrosis (type III cell death) is probably familiar as a nonprogrammed, disordered type of death that is characterized by swelling of both the cell and its intra-cellular organelles, rupture of the plasma membrane, and release of intracellular contents. Such death can be triggered by stresses such as altered tonicity, heat, or physical trauma and by intra-cellular events such as $Ca^{2+}$ efflux or a rapid decrease in adenosine triphosphate (ATP) levels. Other forms of necrosis, termed *necroptosis* or *programmed necrosis*, are also characterized by rupture of the plasma membrane but are fundamentally distinct from "classical" necrosis in that they are mediated by specific signaling events.[1,2] Cellular rupture, irrespective of its association with "classical" or programmed necrosis, releases numerous immune activators or danger-associated molecular patterns (DAMPs) into the immediate extra-cellular environment. Necrosis is therefore typically immunologically reactive because it generates a potent inflammatory response.

Several of the features that we typically associate with apoptosis can also be detected in cells undergoing necrotic death, including external exposure of phosphatidyl-serine, chromatin condensation, and their uptake by phagocytic cells of the immune system. The requirement for caspase activity is generally used to discriminate apoptosis from necrosis. However, not only are caspases dispensable for necrotic cell death, but programmed necrosis may actually require that their activity be inhibited.

Programmed necrosis can be induced by death receptors of the tumor necrosis factor (TNF) superfamily, members of the immune sensing Toll-like receptors (TLRs), and as part of the interferon (IFN) response. Accordingly, a close and in some cases overlapping functional relationship between signaling components of the immune system and those of programmed necroptosis is emerging. Furthermore, several components of the upstream death receptor signaling machinery are common to both programmed necrosis and apoptosis. How do we reconcile the apparent contradiction of common upstream signaling components with divergent biologic outcome? The answer is the selective activities of specialized death pathway–specific adapters that are able to integrate multiple signaling events to engage the effector molecules appropriate to the death pathway. In the case of programmed necrosis, the critical effector molecules appear to be receptor-interacting protein kinase 3 (RIPK3) and its substrate, mixed lineage kinase domain-like (MLKL). The precise mechanism underlying MLKL function to induce cell lysis remains incompletely understood, although several possibilities have been proposed.

## Pyroptosis

Pyroptosis, which is also a form of regulated necrosis, is a lytic type of cell death inherently associated with inflammation. It is mediated by the catalytic activity of the hence-named "inflammatory caspases" that include caspase-1 and, under some circumstances, caspase-5 (and caspase-11 in rodents).[3]

Beyond the defined requirement for caspase-1 activity to induce pyroptosis, how its downstream substrates mediate this type of cell death remains poorly defined. What we do know is that the morphologic characteristics of pyroptosis include rupture of the cell membrane and release of its intra-cellular contents into the immediate cellular milieu. This event provides a localized pool of potential pro-inflammatory molecules that include both direct activators of immune cells, such as cytokines and chemokines, as well as other signaling molecules or so-called "danger signals" able to trigger the production of inflammatory molecules from a variety of cell types. Rupture of a cell dying by pyroptosis is preceded by a marked increase in cell size that has been attributed to a caspase-1–dependent formation of membrane pores. It is the consequent disruption of ionic gradients across the cell membrane and an increase in osmotic pressure that causes water influx, cell swelling, and, ultimately, membrane rupture. Pyroptosis is also associated with DNA cleavage and nuclear condensation, although, in contrast to apoptosis, nuclear integrity is maintained. Although it is a caspase-1–dependent process, the identity of the nuclease responsible for DNA cleavage during pyroptosis remains unknown.

## Autophagy

Autophagy is a catabolic process of self-digestion in which components of the cell are enclosed within a double membrane to form an autophagosome that is then targeted to lysosomes for degradation. Autophagy is generally considered important for the maintenance of cell survival by providing energy and metabolites during times of starvation and for the removal of damaged organelles and long-lived proteins. It is also suggested that autophagy may engage cell death (type II cell death), particularly under conditions in which autophagy is enforced. However, the lack of stringent experimental discrimination between autophagy-induced cell death and autophagy arising as a consequence of cell death has proved to be a rich source of controversy. In an effort to formally determine if autophagic cell death is a bona fide type of cell death, a strict definition has been proposed—that the term *autophagic cell death* must only be applied from a functional perspective and only to scenarios in which cell death can be inhibited by the suppression of key regulators of autophagy. Nevertheless, our understanding of autophagic cell death is extremely limited, with very little insight into the physiologic conditions under which it is engaged.

## MOLECULAR MECHANISMS OF CELL DEATH

### Apoptosis

Apoptotic cell death is executed by one or more caspases, a family of cysteine proteases with selectivity for aspartate (Asp/D) residues, that cleave their target substrates after tetrapeptide sequences containing aspartic acid at the P1 position—for example, DEVD.[4] All caspases, although synthesized as inactive monomeric zymogens, require dimerization, in addition to other events, to acquire catalytic competence. The basic structure of the inactive procaspase comprises an N-terminal prodomain and a C-terminal protease domain, consisting of a large and a small subunit and the catalytic cysteine residue.

Caspases are broadly categorized according to their general function. The executioner caspases—caspase-3, -6, and -7—are the "do-ers"; that is, they are responsible for the cleavage of specific downstream targets to effect cellular destruction and clearance of the dead cell. In contrast, the initiator caspases, including caspase-8 and -9, provide the molecular "ignition" to initiate the proteolytic cascade and facilitate executioner function. Initiator caspases also include the so-called inflammatory caspases, caspase-1 and -5/11, and although they too initiate apical events to facilitate multiple downstream signaling events, their physiologic role is largely involved in immune regulation. However, emerging findings also implicate several roles in cell death. Caspase-2, despite displaying significant sequence homology to caspases found in primitive organisms, remains somewhat of a mystery. Although the molecular mechanism for its activation has been well characterized, the precise physiologic role for caspase-2 and the identity of its critical target substrates remain to be determined.

### Caspase Substrates of Note

Numerous caspase substrates have been identified, although only relatively few have been assigned functional significance. A critical caspase cleavage event is that mediated by the initiator caspases, which undergo autocleavage to stabilize the active dimer (in the case of caspase-2, -8, and -10) or destabilize it (in the case of caspase-9). Initiator caspases are also responsible for the necessary cleavage and activation of the executioner caspase-3 and -7 (another executioner caspase, caspase-6, is subsequently cleaved and activated by these executioner caspases). Once rendered enzymatically active, executioner caspases then cleave their target substrates to effect the phenotypic and biochemical characteristics of apoptosis. These target substrates include inhibitor of caspase-activated deoxyribonuclease (iCAD) to generate active CAD,[5,6] an endonuclease that cleaves between nucleosomes to generate the classical "DNA ladder"; poly-ADP-ribosyltransferase (PARP), an enzyme critical for DNA repair[7]; components of the cytoskeleton and matrix; and targets responsible for phosphatidylserine externalization to generate a critical extra-cellular signal used by phagocytic cells to remove dead cells.

Although the role of caspase-2 remains unclear, it can cleave BH3 interacting domain death agonist (BID)[8,9] to generate an active BH3-only protein that is able to induce mitochondrial outer membrane permeabilization (MOMP; discussed in more detail later). BID is also targeted by other caspases, including caspase-8[10] and -1.[11] Another substrate of caspase-2 is Golgi 160, although the precise role for this cleavage event is unclear.[12]

The inflammatory caspases, of which caspase-1 is the most well-characterized, directly target and cleave two pro-inflammatory cytokines, interleukin (IL)-1β and IL-18, to generate bioactive cytokines competent for secretion. Additional caspase-1 substrates include components of the actin cytoskeleton, the cellular inhibitor of apoptosis proteins (cIAPs), and metabolic enzymes, although the relevance of these cleavage events to caspase-1–dependent cell death remains poorly characterized. Unlike caspase-1, caspase-5/11 does not cleave the proforms of these cytokines, and the mechanism by which the latter causes cell death currently is not known.

### Caspase Regulation: Activation and Inhibition

In simple terms, there are two types of caspases, denoted "initiators" and "executioners," that require different mechanisms to achieve catalytic competence. Initiator or apical caspases have long prodomains and exist as inactive monomers that require dimerization to stabilize the catalytic site and to generate two catalytically active cysteines, one per monomer. This activation step is achieved by the assembly of oligomeric signaling complexes, comprising the apical caspase, specialized adapter proteins, and additional accessory regulatory proteins, and represents one example of the so-called "proximity" model of caspase activation. In contrast, the executioner caspases harbor minimal prodomains and exist as pre-formed inactive dimers that are rendered active via enzymatic cleavage of a DEVD site within an intradomain linker region between the large and small subunits. The resulting conformational change generates catalytically active caspase dimers. Therefore, dimerization of an apical caspase, enforced by recruitment to its specific activation complex, generates an active enzyme that, in turn, cleaves and activates downstream executioner caspases to mediate proteolysis of specific target substrates (as previously discussed) and, ultimately, cellular destruction.

### Caspase Inhibitors (Inhibitors of Apoptosis Proteins)

Although a critical event in engaging apoptosis, the irreversible consequences of caspase-mediated substrate cleavage might be, under some circumstances, undesirable for a cell. To that end, inhibitor of apoptosis proteins (IAPs), characterized by one or more copies of a motif called the baculovirus IAP repeat (BIR), can bind to and suppress active caspases, thereby inhibiting their destructive path.[13] These caspase inhibitors were first identified in viruses and function to maintain host cell survival, thereby ensuring sufficient time for viral replication.[14-16] Subsequently, based on BIR domain homology, a family of BIR-containing proteins was identified in vertebrates that includes X-linked inhibitor of apoptosis protein (XIAP),[17] c-IAP1, c-IAP2,[18] neuronal apoptosis inhibitory protein (NAIP),[19,20] and survivin.[21]

Although several members of this family have been implicated in the regulation of apoptosis, it is only XIAP that directly does so by interacting with active caspases to halt their activity.[17] XIAP is characterized by three BIR domains, with BIR3 and the adjacent RING domain mediating the inhibition of caspase-9 and BIR2 and the adjoining linker region suppressing the executioner caspase-3 and -7.[22] XIAP and other IAPs, including c-IAP1 and c-IAP2, can function as E3 ligases by virtue of their RING domain to promote ubiquitination and proteosomal degradation of their target protein substrates.[23] In this way, XIAP can also halt caspase activity by inducing protein degradation.

Evidence supporting a critical role for XIAP in the context of normal physiology is limited, particularly because XIAP-deficient mice have no obvious developmental defects.[24] However, in certain circumstances XIAP expression may be a critical determinant of cellular sensitivity to certain apoptotic pathways (discussed in more detail later).

## Caspase Recruitment Domains and Death Effector Domains—Activation Platforms of the Initiator Caspases

One of the critical events necessary for the activation of apical caspases is their recruitment to large protein complexes or signaling platforms. This step promotes dimerization and activation of the caspase. Complex specificity is determined by the identity of the specialized adapter molecules within these complexes and the reciprocal binding domain within the caspase itself. The prodomain of the initiator caspases contain one of two specialized binding domains, the caspase recruitment domain (CARD), present in caspase-1, -2, -4, -5 -9, -11, and -12, and the death effector domain (DED), found in caspase-8 and -10. The DED and CARD domains display no sequence homology, although structurally they are similar in that they form so-called "death folds." Additional protein domains discussed in the review, although not structural features of caspases themselves, are found in other molecules that participate in caspase activation. These include the death domain (DD) and pyrin (PYD) domains. It is these interaction domains that facilitate the recruitment of the caspases to their respective activation complexes (CARDs and DEDs) and/or are involved in accessory signaling events involved in their regulation (DDs and PYDs).

## The Inflammasome-Activation Platform for Procaspase-1 and -11

Caspase-1 is the prototypical human inflammatory caspase, although analogous roles have also been defined for caspase-5 (caspase-11 in rodents). As an initiator caspase, caspase-1 exists as an inactive monomer and requires dimerization to achieve catalytic competence. This is achieved by its CARD-dependent recruitment to a multiprotein complex, the "inflammasome," thereby triggering its activation and autocleavage. It appears that unlike the other caspases, caspase-1 is a much-desired binding partner, with multiple potential suitors or adapter proteins vying for its company. Presumably the functional diversity and availability of multiple adapter proteins ensures that upstream signaling events arising from multiple types of stresses can all converge on that one critical component of the pathway—the activation of caspase-1.

As previously discussed, caspase-1 is essential for the processing of the inactive proforms of IL-1β and IL-18 to generate active cytokines. Release of these bioactive cytokines from the cell occurs via a poorly defined noncanonical secretory pathway that also requires caspase-1 function. Because they are critical regulators of inflammatory responses to multiple stimuli of both self-derived and pathogenic origin, it is essential that biologically active IL-1β and IL-18 be readily available. To meet this need, adapter proteins must integrate signals arising from different origins and of diverse nature with the machinery required for caspase-1 activation.

Initiation of caspase-1–dependent IL-1β/IL-18 release can be triggered by numerous stimuli derived from both host and foreign sources, although is most often associated with components of pathogenic organisms. Triggers of caspase-1 activation are collectively termed DAMPs or "danger signals," and they function by engaging members of the NOD-like receptor (NLR) family. These intra-cellular sensors are collectively capable of responding to multiple stressors to engage the caspase-1–dependent processing of IL-1β/IL-18. Details of NLR-mediated caspase-1 activation and the activation of caspase-5/11 are discussed in more detail later.

## The PIDDosome Activation Platform for Procaspase-2

Caspase-2, despite being the most highly conserved caspase across all species, remains somewhat of a mystery in terms of its function, perhaps in part because only a very few of its target substrates have been identified.[25] Activation of caspase-2 is detectable in response to a range of different stresses including heat stress, metabolic disruption, and DNA damage, and although it can engage mitochondria-dependent apoptosis by cleaving BID, albeit poorly, it is not necessary, suggesting that the primary role of caspase-2 is not to induce cell death.

Although its precise role is unclear, the molecular determinants of caspase-2 activation have been characterized and, much like the other initiator caspases, it requires the regulated assembly of a large protein complex, termed the *PIDDosome*, to mediate caspase dimerization and activation.[26] The specific adapter protein involved in this process is receptor-interacting protein (RIP)-associated ICH-1/CED-3 homologous death protein with a death domain (RAIDD), a CARD-containing protein that interacts with the CARD domain of procaspase-2. RAIDD also contains a DD, which, by association with the DD of another accessory protein, P53-induced protein with a death domain (PIDD),[27] generates multimers of procaspase-9-RAIDD-PIDD. This process in turn leads to the dimerization and activation of procaspase-2, autocleavage, and stabilization of the active caspase.[25,28] What happens next remains a bit of a "black box," but disparate activities attributed to caspase-2 and its regulatory proteins, including a role in tumor suppression,[29,30] suggest that there is a lot more to the activity of this caspase that we have yet to understand.

## The Death-Inducing Signaling Complex Activation Platform for Procaspase-8

Activation of caspase-8 and -10 (not found in rodents) can arise from multiple signaling pathways emanating from a small group of so-called death receptors, a subset of the TNF receptor family, when they are ligated by their cognate death ligand, which are also TNF-like molecules. Depending on the precise identity of the accessory proteins in the activating signaling complex, procaspase-8 cleavage and/or activation can then participate in several biologic pathways. Nonetheless, a few key mediators are involved in the recruitment and activation of caspase-8 via the adapter domains, the DD, and the DEDs, common to the activation of these initiator caspases.

Constitutively trimerized death receptor is activated by trimers of death ligand to expose an intra-cellular DD that is then available for multiple homotypic DD-DD interactions with one of several adapter molecules that includes the Fas-associated protein with a death domain (FADD). This interaction triggers the exposure of the FADD DED domain and enables assembly of the mature signaling death-inducing signaling complex (DISC) by the recruitment of procaspase-8 or -10 via one of the two DED domains of these initiator caspases. This enforced dimerization enables formation of an active catalytic site that then promotes autocleavage and stabilization of an active caspase dimer, which then cleaves and activates the effector caspases 3 and 7—that is, unless procaspase-8 forms heterodimers with a catalytically inert homolog, FADD-homologous ICE/CED-3-like protese (FLICE)-like inhibitory protein (FLIP), which is a negative regulator of procaspase-8 activity. FLIP-procaspase-8 heterodimers, although lacking the ability to promote apoptosis, retain sufficient catalytic activity to inhibit an alternative form of cell death, necroptosis, that is discussed in more detail later.

## The Apoptosome-Activation Platform for Procaspase-9

Procaspase-9 is rendered catalytically active by its recruitment to a large protein complex called the apoptosome, comprising procaspase-9, apoptotic protease activating factor-1 (APAF-1), and additional regulatory proteins.[31,32] APAF-1 is a specialized adapter protein containing an N-terminal CARD domain and a C-terminal protein-binding domain consisting of multiple WD repeats. APAF-1, which is important for development,[33,34] is a cytosolic molecule that is triggered to undergo self-oligomerization by the binding of cytochrome-*c* once it is released from the mitochondrial intermembrane space.[32] Association with cytochrome-*c*, together with the binding and hydrolysis of deoxynucleotides (deoxyadenosine triphosphate [dATP]), induce the conformational changes in the APAF-1 molecule to unmask the C-terminal CARD domain. This process then enables the recruitment of procaspase-9 to the complex via homotypic CARD-CARD interactions[35] to facilitate the dimerization and generation of catalytically active caspase-9.[36] The mature caspase-9–activating complex or apoptosome consists of an APAF-1 septamer, with each APAF-1 molecule able to recruit and activate dimers of caspase-9. Once activated, caspase-9 cleaves and activates the executioner caspases 3, 6, and 7. Autocleavage of caspase-9 causes its release from APAF-1, and the caspase is now inactive. This action liberates APAF-1 to recruit additional procaspase-9, resulting in a "tick-over" of active caspase-9 until sufficient active executioner caspase is produced to propagate the apoptotic signal.

## Mitochondria and Apoptosis—Intrinsic Apoptosis

### Release of Intermitochondrial Regulators to Activate Caspases

Caspase-dependent apoptosis is broadly defined as two pathways that, for the most part, proceed independently of one another—the mitochondria-dependent "intrinsic" pathway and the "extrinsic" pathway that is mediated by death receptor/death ligand interaction (discussed in further detail later). The former pathway, which is dependent upon the initiator caspase-9, is characterized by permeabilization of the outer mitochondrial membrane to release cytochrome c, an intermembrane component of the electron transport chain, into the cytosolic space.[37-39] Once released, cytochrome-*c* binds to APAF-1 to facilitate assembly of the apoptosome and activation of procaspase-9 (previously described). Several additional proteins are also released that include second mitochondrial-derived activator of caspase (SMAC), also known as DIABLO; OMI/high temperature requirement A2 (HTRA2); and apoptosis-inducing factor (AIF).[40-42] SMAC and OMI participate indirectly in caspase activation through binding and antagonism of XIAP (discussed in more detail later).

## Mitochondrial Outer Membrane Permeabilization and Apoptosis

MOMP is a regulated process that permits the release of proteins critical for the induction and regulation of apoptosis.[38-41] Although the outer mitochondrial membrane is endowed with transporters, such as translocase of the inner membrane (TIM) protein complex,[40] that regulate the import and export of proteins, the release of mitochondrial proteins involved in cell death is mediated by an entirely independent mechanism that is engaged only in response to apoptotic stimuli. So how do the specific signals arising from multiple types of apoptosis-inducing stimuli and differing vastly in nature all culminate in disruption of the mitochondrial outer membrane to release cytochrome-*c*?

## Regulation of Mitochondrial Outer Membrane Permeabilization

MOMP is one of the primary features of the intrinsic apoptotic pathway and is positively and negatively regulated by the collective activity of the B cell lymphoma 2 (BCL-2) family of proteins (Figure 28-1; reviewed in references 43 and 44).[43,44] BCL-2 proteins are functionally categorized into those that promote and those that suppress cell death by directly or indirectly regulating MOMP according to specific structural features.[45] BCL-2 proteins are characterized by one or more 20 amino acid stretches of conserved residues corresponding to α-helical segments and constituting the

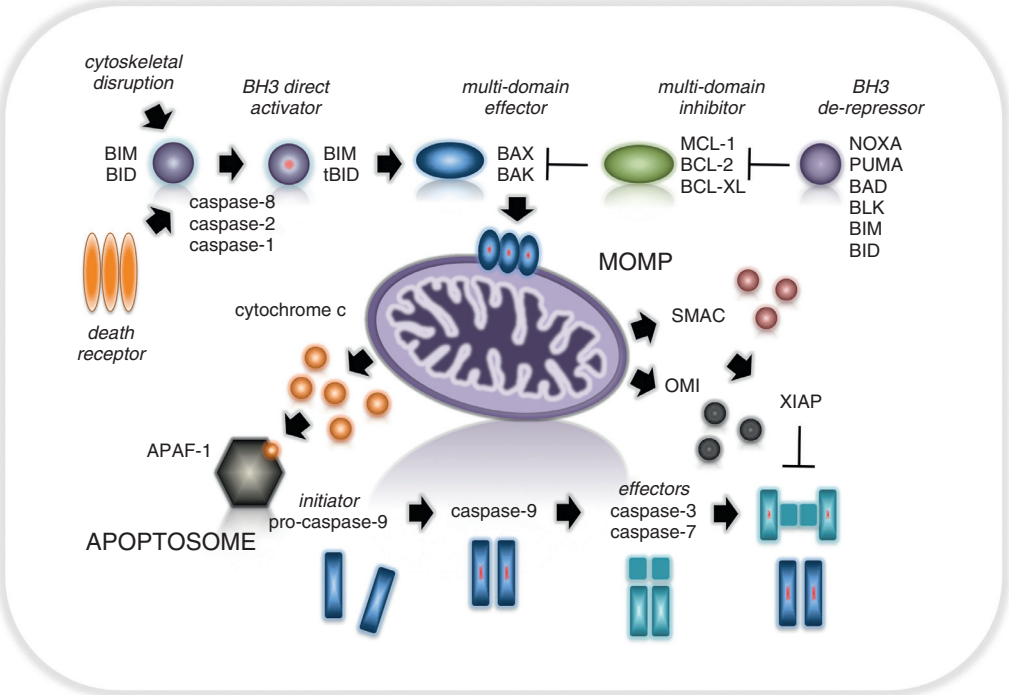



**Figure 28-1** B cell lymphoma 2 (BCL-2) protein-regulated mitochondrial outer membrane permeabilization (MOMP) is a critical event in the intrinsic apoptosis pathway. BCL-2 proteins regulate MOMP in response to a multitude of signals to engage the intrinsic apoptotic pathway. The multidomain proapoptotic effector molecules BAX and BAK undergo several modifications, including conformational change and oligomerization to induce MOMP. The multidomain anti-apoptotic BCL-2 proteins including BCL-2, BCL-$X_L$, and MCL-1 inhibit BAX/BAK–induced MOMP. The proapoptotic BH3-only effector proteins activate BAX and BAK either by direct activation (direct BH3 activators include BIM and BID) or indirectly by displacement of inhibitory anti-apoptotic BCL-2 proteins (de-repressor BH3 proteins include NOXA, PUMA, and BAD). BH3 proteins act as sensors to different types of stress and can be modified by several mechanisms. For example, BID is cleaved and activated by caspase-8 in response to death receptor–mediated signaling events, whereas BIM activation can be mediated by disruption of the cytoskeleton. MOMP permits the release of several intermembrane space proteins, including cytochrome-*c*, second mitochondrial-derived activator of caspase (SMAC), and OMI, all of which regulate the apoptotic pathway. Cytochrome-*c* binds to the adapter protein apoptotic protease activating factor-1 (APAF-1) to trigger the recruitment of inactive procaspase-9 monomers and assembly of the apoptosome complex. This action results in the self-cleavage of procaspase-9 to generate catalytically active caspase-9 dimers, which then cleave and activate the executioner caspase-3 and -7. X-linked inhibitor of apoptosis (XIAP), an E3 ligase, negatively regulates the activity of caspase-9, -7, and -3 either by blocking of the catalytic site or by promoting their ubiquitin-dependent degradation. SMAC and OMI bind to or cleave XIAP, respectively, to inhibit its activity and promote apoptosis. *(From Tait SW, Green DR: Mitochondrial regulation of cell death.* Cold Spring Harb Perspect Biol *5, 2013.)*

so-called BH (BCL-2 homology) domains, numbered 1 to 4 (BH1, -2, -3, and -4). Both the anti-apoptotic and effector BCL-2 proteins are characterized by multiple BH motifs, whereas, as the name suggests, BH3-only BCL-2 proteins are characterized by a single BH3 domain.

The proapoptotic multidomain effector proteins, BAX and BAK, directly permeabilize the outer mitochondrial membrane, whereas the anti-apoptotic family members, including BCL-2 itself, BCL-$X_L$, A1, BCL-B, BCL-w, and MCL-1, function primarily to prevent MOMP. The so-called proapoptotic BH3-only proteins, including BID, BIM, PUMA, NOXA, HRK, BIK, BMF, and BAD, also promote MOMP, but they do so indirectly, either by activating the BAX and BAK effectors or by inhibiting the anti-apoptotic BCL-2 proteins.[46]

Although BAX and BAK are the primary mediators of MOMP, the precise mechanism by which they achieve membrane permeabilization remains only partially understood. In their respective inactive states, monomeric BAX is found in the cytosol, whereas BAK resides on the outer mitochondrial membrane, anchored by its carboxy-terminal region. Conversion to their active state requires transient interaction with one or more of the BH3-only proteins to induce a change in their conformation, multimerization into large homo-oligomers, and, in the case of BAX, translocation to the outer mitochondrial membrane. The subsequent release of intermembrane space proteins clearly requires membrane permeabilization, but whether BAX and BAK oligomers achieve this effect directly, by membrane insertion and forming pores themselves, or indirectly, by triggering the formation of lipid pores, has yet to be resolved. Nonetheless, after permeabilization is achieved, cytochrome-*c*, SMAC (DIABLO), and OMI (HTRA2) are released from the mitochondrial intermembrane space into the cytosol to activate caspases and mediate apoptotic cell death.

A third BCL-2 family member, BOK, is also grouped with the effectors BAX and BAK because it too contains BH domains 1 to 3.[47] BOK can induce cell death when overexpressed,[48-50] but whether it functions to promote membrane permeabilization and MOMP has yet to be convincingly demonstrated. Neither the ablation of *Bok* expression nor its co-deletion with *Bax* or *Bak* revealed any overt developmental phenotype,[51] suggesting that if BOK does have a role in cell death, it is either minor or developmentally redundant. Interestingly, recent in vitro findings describe a

BAX/BAK-dependent prodeath function of BOK, resulting from its predominant localization at the endoplasmic reticulum (ER) and/or Golgi membranes.[31] However, the role of BOK remains somewhat of a mystery for now, although given the pace at which this field has advanced, it is likely only a matter of time before its physiologic role is revealed. Interestingly, *Bok*, unlike both *Bax* and *Bak*, is frequently deleted in cancers,[52] adding further intrigue to its as yet unknown functions.

Despite extensive structural and functional characterization of the BCL-2 proteins and overwhelming evidence that the anti-apoptotic BCL-2 proteins suppress BAK- and BAX-mediated MOMP, exactly how this process occurs remains obscure. What we do know is that the BCL-2 proteins interact with BAX and BAK to prevent their oligomerization. This interaction is mediated by the BH3 domain of BAX and BAK and a binding groove, common to all the anti-apoptotic proteins, composed of multiple α-helices containing BH domains 1 to 3. Because, under constitutive conditions, the BH3 domain of BAX and BAK is "buried" and therefore unavailable for binding, evidence indicates that insertion into the outer mitochondrial membrane is accompanied by a conformational change to reveal the BH3 domain in these proteins. Accordingly, exposure of the BH3 domain of BAK or BAX renders it competent for either self-oligomerization to induce MOMP or, alternatively, interaction with an anti-apoptotic molecule to inhibit it. Interestingly, BAX and BAK display selectivity for different anti-apoptotic proteins in that BAK preferentially interacts with BCL-$X_L$ and MCL-2, whereas BAX "prefers" to associate with BCL-2 and BCL-$X_L$ (and less so with MCL-1). Paradoxically, however, each of the three anti-apoptotic BCL-2 proteins is equally effective at suppressing apoptotic cell death driven by either BAX or BAK. Thus, although the model by which the anti-apoptotic proteins suppress the activity of BAX and BAK to inhibit MOMP is well substantiated, its applicability to both BAX and BAK and all anti-apoptotic BCL-2 proteins is limited. To fully understand the complexity of BAX and BAK regulation by the anti-apoptotic BCL-2 proteins, the activity of BH3 proteins also must be considered.

The proapoptotic BH3-only proteins serve as upstream sensors to integrate the signals arising from intra-cellular stresses to engage apoptosis via BAX and BAK. Although BAX and BAK act as the terminal effectors of MOMP and are negatively regulated by their direct interaction with the BCL-2 proteins, association between the BH3-only and anti-apoptotic BCL-2 proteins can also have an impact on BAX- and BAK-mediated MOMP. Sequestration of the anti-apoptotic BCL-2 proteins by the BH3-only proteins serves to neutralize their inhibitory activity and increase the likelihood of unimpeded BAX and BAK activation to induce MOMP. Importantly, under these conditions, the sensitivity of cells to BAK- and BAX-mediated apoptosis is ultimately determined by both the availability of a given BH3-only protein and its binding specificity for a particular anti-apoptotic protein.[53,54]

We have described how the activation of BAX and BAK can be inhibited by association with anti-apoptotic BCL-2 proteins and how they in turn can be neutralized by interaction with certain BH3-only proteins. How, then, are BAX and BAK, which we know to exist in a constitutively inert state, activated to exert their proapoptotic activities? The solution to this particular conundrum lies in the remarkable ability of certain of the BH3-only proteins to directly trigger BAX and BAK activation. This group of so-called "direct activators" remains somewhat exclusive, with only BID, BIM, and possibly PUMA thus far fulfilling membership requirements. Unlike interactions between anti-apoptotic BCL-2 proteins and proapoptotic multidomain effectors or the BH3 proteins, both of which can be isolated as hetero–BCL-2 protein complexes, activation of BAX or BAK by the direct activators is instead mediated by a transient "hit and run"–type interaction. Interaction between the BH3 activators and effector molecules elicits exposure of the BH3 domain of BAK or BAX, thereby rendering it available for homo-oligomerization, pore formation, and MOMP.

Although the exact mechanism by which BH3 proteins activate BAX and BAK remains a source of discussion, it would seem that the two models proposed are not necessarily mutually exclusive and in due course may prove to be equally valid, depending upon the circumstances.[55] The "neutralization" model argues that BH3-mediated disruption of interaction between a BCL-2 effector molecule and an anti-apoptotic BCL-2 protein both liberates BAX or BAK *and* renders it functionally competent to induce MOMP and apoptosis. The logical extension of this model is that whether a cell lives or dies is determined by the relative ratio of proapoptotic BCL-2 proteins to anti-apoptotic BCL-2 proteins. The alternate "de-repression" model also proposes that interaction between the BH3-only and anti-apoptotic BCL-2 proteins relieves BAX and BAK from the restraints of BCL-2–mediated inhibition. However, the model further suggests that the release of BAK and BAX is not synonymous with pore-forming activity. Instead, it proposes that once BH3-mediated disruption of interaction between BAX or BAK and anti-apoptotic Bcl-2 proteins has released the effector molecule, MOMP can only proceed *if* BAX or BAK is first rendered active by a "direct activator" such as BID or BIM.

Although these two models are not at odds with one another, the primary distinction between the two—that BH3 proteins displace active BAX or BAK from anti-apoptotic proteins versus the idea that both the displacement and activation of BAX and BAK by two different but complementary BH3-dependent mechanisms is required—is of significance in the context of the design of pharmacologic regulators of BCL-2 protein function.

Although it remains to be seen if, in addition to BID, BIM, and PUMA, other BH3 proteins might also harbor the ability to directly activate BAX and/or BAK, it is clear that most cannot. Interestingly, though, the identification of cytosolic p53 as a direct activator of multidomain BCL-2 effectors[56] raises the possibility of additional as yet unidentified non-BH3 proteins capable of directly activating BAX and BAK.

## Post-Translational Regulation of Bcl-2 Protein Function

BCL-2 proteins can also be regulated by several post-translational modifications. Post-translational modification of BCL-2 proteins serves as a general mechanism by which upstream signaling events arising from different types of

stress can selectively engage one or more downstream BCL-2 proteins to regulate cell death. Modifications include phosphorylation, ubiquitin-mediated degradation, protease-mediated cleavage, myristiolyation, and neddylation. Although the precise functional consequences of these modifications are not clear in most cases, a few exceptions exist.

BH3 proteins act as the upstream sensors of multiple types of stresses to then engage the downstream proapoptotic effector molecules, BAX and BAK. In many instances this effect is achieved by the stress-specific activation of kinases that in turn modify BH3 proteins via site-specific phosphorylation. Nutrient withdrawal and the consequent reduction of intra-cellular energy stores is a potent activator of cell death that, in many instances, is attributed to the proapoptotic activities of the BH3-only proteins BIM and BAD. At least in part, kinase-mediated phosphorylation of specific residues within these BH3-only proteins are critical for the integration of upstream signaling events associated with growth factor withdrawal and activation of downstream apoptotic effectors. Activation of AKT, a serine-threonine kinase, by growth factor receptors leads to the phosphorylation and cytosolic sequestration of the transcription factor FOXO3a by 14-3-3 proteins.[57] However, disruption of this signaling pathway under conditions of nutrient withdrawal permits translocation of FOXO3a to the nucleus, where it induces the transcription of a number of genes, including *Bcl2-like11 (Bcl2l11)*.[58] Phosphorylation of BIM at serine-69 by extra-cellular-signal regulated kinase (ERK), a mitogen-activated protein kinase (MAPK) activated in response to growth factors, promotes the proteosomal degradation of BIM to facilitate cellular survival.[59] In contrast, the phosphorylation of BIM by the MAPK, JNK, stabilizes the protein to facilitate cell death in a BAX-dependent manner.[60] Phosphorylation of BAD at residues Ser-112 and Ser-136 by AKT renders it inactive by promoting association with and sequestration by 14-3-3,[61] whereas the dephosphorylation of BAD in response to nutrient deprivation neutralizes its proapoptotic activity by triggering its interaction with BCL-$X_L$ or BCL-2.[62-64] Protein kinase A (PKA)-mediated phosphorylation of BAD at serine-112 also inhibits apoptosis by maintaining BAD in the cytosol, thereby inhibiting activation of BAK/BAX at the mitochondrial membrane.[65] Whereas BIM is a direct activator of BAX and BAK, BAD is a de-repressor protein that promotes apoptosis by neutralizing the anti-apoptotic BCL-2 proteins, BCL-2 and BCL-$X_L$.

Multiple phosphorylation and ubiquitylation events also contribute to the regulation of myeloid cell leukemia 1 (MCL-1) stability.[66] Disruption of AKT activity under conditions of nutrient withdrawal relieves the inhibition of the kinase, glycogen synthase kinase 3 (GSK3), which in turn is then able to phosphorylate MCL-1. This phosphorylation event promotes the degradation of MCL-1, thereby reducing its anti-apoptotic effects to facilitate apoptosis.[67] Also involved in the regulation of MCL-1 protein turnover is MCL-1 ubiquitin ligase E3 (MULE), which is somewhat unusual in that it harbors a BH3 domain. Interaction with MCL-1 via the BH3 domain of MULE limits its enzymatic activity, but once displaced by a proapoptotic BH3-only protein such as NOXA, it is then able to ubiquitlylate MCL-1, rendering it susceptible to degradation.[68] Other proteins that control the stability of MCL-1 include FBW-7,[69] a tumor suppressor frequently deleted in cancers, and the deubiquitinase USP9X,[70] which is expressed in many tumor types. The turnover of the anti-apoptotic proteins BCL-2 and BCL-$X_L$ are similarly subject to regulation by post-translational modification.[71,72] For example, ERK1/2-mediated modification of a serine residue (Ser-70) within the flexible loop between the BH3 and BH4 domains[73] or on serine-87 of BCL-2 both exacerbate its anti-apoptotic activity, at least in part, by maintaining protein stability.[74] Dephosphorylation by protein phosphatase 2A (PPA2) opposes this activity and neutralizes the anti-apoptotic activity of BCL-2.[75] In contrast, the anti-apoptotic activity of BCL-$X_L$ is disrupted by the deamidation of two asparagine residues in the unstructured loop between BH4 and BH1.[76]

Specific cleavage events can also contribute to the function of certain BCL-2 proteins. The BH3-only protein BID is unique among its BH3 relatives in that in its native state, its BH3 domain is buried within the tertiary structure of the molecule. Without exposure of this domain, BID cannot interact with other BCL-2 proteins and is therefore biologically inert. However, cleavage of a flexible linker within BID by one of several proteases, including caspase-8[77] and -2,[8] granzyme B,[78] lysosomal proteases (cathepsins),[79] and the calcium-activated protease, calpain,[80] generates an active cleavage product, tBID, rendering it able to associate with anti-apoptotic proteins or function as a direct activator of BAX and BAK. In this way, upstream cytosolic events leading to the activation of these proteases can engage mitochondria-dependent apoptosis by the direct cleavage and activation of the BH3 protein, BID. Calpain-mediated cleavage of BAX to enhance its pro-apoptotic activity has also been described,[81] although the physiologic significance of this event has yet to be fully delineated. Like BIM, active BID is capable of directly activating BAX and BAK.

Regulation of gene expression is also a mechanism by which the levels of BCL-2 proteins can be modulated to affect the overall susceptibility of cells to apoptosis. Multiple transcription factors have been implicated in the regulation of both pro- and anti-apoptotic *Bcl-2* gene expression, including p53, nuclear factor-κB (NF-κB), and FOXO3a. Several microRNAs (miRNAs), including miR-17-92, miR-15, and miR-16, have also been shown to specifically reduce the levels of *Bim* and *Bcl-2* RNAs, respectively. Several of the BCL-2 proteins exist as multiple splice variants, including BCL-$X_L$, BAX, and PUMA, although their specific roles are poorly defined. However, BIM, found in multiple isoforms, including $BIM_S$, $BIM_L$, and $BIM_{EL}$, appear to harbor differing proapoptotic potencies as a consequence of the presence or absence of a dynein light chain–binding region. Anoikis, which is apoptotic cell death arising from perturbation of the cytoskeleton and detachment of cells from the basement membrane, is mediated by BIM and another BH3 protein, BCL-2 modifying factor (BMF). BIM is anchored to the microtubule complex, whereas BMF is sequestered by the actin cytoskeleton, each by their respective dynein light chain–binding regions. Disruption of the cytoskeleton induces the release of BIM and BMF to promote MOMP and engage apoptosis.

## Alternative/Additional Roles of Bcl-2 Proteins

Our discussion of BCL-2 function has focused on their role in the regulation of cell death in the context of MOMP regulation. Although this role is critically important, it is becoming apparent that the collective activity of BCL-2 proteins is not restricted to the regulation of cell death but that it likely has a significant impact in several additional signaling pathways, including the regulation of mitochondrial dynamics,[82] the response to DNA damage,[83,84] metabolic bioenergetics,[85-87], $Ca^{2+}$ homeostasis,[88] ER function, and autophagy.[89]

## Is MOMP Really the End of It All?

MOMP is clearly a critical event in apoptotic cell death and is regulated by the collective activity of the BCL-2 protein family. However, the induction of MOMP is an equally important event for caspase-independent cell death (CICD), and as in apoptosis, it is dependent upon BCL-2 protein function. The engagement of cell death in the context of caspase inhibition can promote a type of cell death that is characterized by MOMP—so-called CICD. This type of cell death is perhaps most commonly observed during development and particularly under conditions in which certain pathways of caspase activation are inactivated, such as the *Apaf-1*–deficient model. In this case, cells die as a result of a bioenergetic catastrophe as mitochondrial function is lost upon MOMP. The release of intermembrane space proteins upon MOMP, including AIF, has also been proposed to participate in this form of cell death.

Although MOMP is generally considered the point at which the cell is irreversibly committed to cell death, specific circumstances exist in which this may not be the case. Tumor cells are able to circumvent the terminal consequences of MOMP by both inhibiting caspase activity and by preserving a small pool of intact mitochondria capable of regeneration and restoration of cellular bioenergetics. Suppression of caspase activity can be achieved via several means, including the harnessing of XIAP activity and by the downregulation of critical components of the apoptotic machinery by mechanisms such as epigenetic silencing and ubiquitin-mediated degradation. Therefore, MOMP does not always lead to cell death. However, this is the least likely consequence that is most often associated with a variety of disease pathologies and not normal cellular function, perhaps with the exception of postmitotic cells such as neurons.

## Death Receptor–Associated Signaling Events—Extrinsic Apoptosis

The so-called "extrinsic" pathway of apoptosis is initiated by the interaction of extra-cellular "death ligands" with their respective extra-cellular "death receptors" on the cell surface, in response to a variety of different types of stress and developmental signals. The obligate caspase for death ligand–mediated apoptosis is caspase-8, which, like other initiator caspases, exists as an inactive monomer and requires homodimerization to generate enzymatic activity. The death ligands and their receptors represent subsets of the TNF and TNF receptor (TNFR) superfamilies, respectively. The receptors include TNFR1, CD95 (also known as Fas or Apo-1), death receptor (DR) 3 (DR3), the TNF-related apoptosis-inducing ligand (TRAIL) receptors, TRAIL receptor 1 (or DR4), TRAIL receptor 2 (also known as DR5 in humans), and DR6. Their corresponding activating ligands are TNF itself, CD95 ligand (or FasL), and TRAIL, all of which engage apoptosis. Neither DR3 nor DR6 (the ligand for which remains unidentified) promotes apoptosis.

Both the DRs and their ligands exist as pre-formed trimers. Upon interaction of a DR with the appropriate ligand, a conformational change exposes a specialized protein-association domain in the receptor, the DD (mentioned previously). The unmasking of this DD then renders it available for interaction with one of several adapter proteins that contain DD.

Interaction between FASL (CD95 ligand) and its receptor FAS (CD95) reveals the intra-cellular DD-containing receptor region, with which FADD then interacts via its own DD. This interaction is accompanied by a conformational change in FADD to reveal a second specialized binding domain, the DED. Multimerization of the FAS-FADD complex is attributed to the ability of DDs to associate at three different interfaces and has been shown to be necessary for the subsequent recruitment and activation of caspase-8. Assembly of the mature signaling complex or CD95 DISC is completed by the recruitment of procaspase-8 via interaction between one of two DEDs in its prodomain and the DED of FADD. The consequent dimerization of procaspase-8 permits self-cleavage and stabilization of the active caspase, leading in turn to the proteolysis and activation of the effector caspase-3 and -7. DISC formation is an extremely efficient process that is negatively regulated by the dimerization of caspase-8 with its catalytically inactive homolog, FLIP. Although this interaction prevents the dimerization and cleavage of caspase-8, it does not completely inhibit its catalytic activity, which results in the cleavage of FLIP but, importantly, not the induction of apoptosis. Recently, studies have further characterized the complexity of binding between FADD, FLIP, and caspase-8, demonstrating it to be an important determinant of the type of cell death that proceeds, apoptosis or necroptosis (discussed in detail later).

TRAIL ligation to its receptor engages caspase-8 activation and subsequent apoptotic cell death in a similar manner to that described for FAS and its ligand FASL. Negative regulatory mechanisms for TRAIL-induced death are poorly characterized but likely, at least in part, involve FLIP-mediated events.

The signaling events elicited by activation of the TNFR1 are complex, subject to a variety of regulatory mechanisms, and lead to cellular survival, apoptosis, or programmed necrosis, depending upon the specific identity of the proteins recruited to the TNFR1 receptor-signaling complex, termed complex I, IIa, and IIb (Figure 28-2). TNFR1 activation can be mediated by one of two ligands, TNF itself or lymphotoxin, both of which trigger conformational changes within the intra-cellular domain of the receptor to expose the DD, as well as an additional binding site for an E3 ligase, TNF receptor–associated factor 2 (TRAF2). Subsequent recruitment of TNFR1-associated death domain (TRADD) to TNFR1 via their respective DD domains serves to both

stabilize the TRAF2-TNFR1 interaction and recruit RIPK1. Additional components of this so-called complex I include cIAP1, cIAP2,[90] and linear ubiquitin chain assembly complex (LUBAC), itself a complex consisting of HOIL-1, HOIP, and SHARPIN. Ubiquitination of components within this complex by one or more of the E3 ligases, TRAF2, cIAP1, cIAP2, and HOIL-1, are essential for the recruitment and activation of additional signaling proteins (Figure 28-2A). RIPK1 is a functionally versatile molecule that is able to contribute to the activity of several TNFR1 signaling complexes, some of which require its kinase activity and others that do not. In complex I, RIPK1 acts to recruit additional signaling components to the TNFR1 complex in a kinase-independent manner once modified by TRAF2-mediated lysine 63–linked polyubiquitylation. One of these components is NF-κB essential modulator (NEMO), a component of the IκB kinase (IKK) complex that stimulates the phosphorylation, ubiquitylation, and degradation of IκBα to reveal the transcriptional activity of NF-κB. It is important to note that under these conditions, cell death does not proceed; instead, NF-κB induces the transcription of genes that contribute to cellular survival, including those that encode cIAP1, cIAP2, FLIP, and others that participate in inflammatory responses.

The critical physiologic importance of this signaling complex is evidenced by the rescue of embryonic lethality in NF-κB–deficient mice by the co-deletion of TNFR1. However, the embryonic lethality induced by the deletion of NF-κB can be distinguished from that associated with TRAF2, cIAP1, and cIAP2 disruption, indicating that TNFR1-mediated survival may also proceed in an NF-κB–independent manner.

Under some conditions the TRADD-TRAF2-RIPK1 signaling complex is released from TNFR1, with the DD domain of TRADD, which is available after detachment from TNFR1, now recruiting FADD. This scenario is then compatible with the formation of one of two signaling complexes, termed complex IIa and IIb (Figure 28-2B). Recruitment of the FLIP long form ($FLIP_L$) to form heterodimers with caspase-8 prevents the assembly of catalytically active caspase-8 homodimers to inhibit apoptosis, but in a manner that retains the ability of caspase-8 to cleave and inactivate RIPK1 and RIPK3 (involved in necroptosis; described later). However, in the absence of $FLIP_L$, the catalytic activity of caspase-8 is unrestrained and therefore able to promote apoptosis. In contrast, inhibition of caspase-8, or its requisite adapter, FADD, promotes assembly of complex IIb, also termed the *necrosome*, by allowing the recruitment and stabilization of RIPK1 and RIPK3 to instead trigger necroptosis (Figure 28-2C). In this way, $FLIP_L$ provides a critical point at which TNFR1 signaling events are integrated to determine the mode of cell death that is engaged.

### Molecular Interaction with the Mitochondrial Pathway—Caspase-8/BID

For the most part, the apical signaling events of the intrinsic and extrinsic apoptotic pathways proceed independently of one another, converging only at the terminal executioner phase of caspase-3 activation. However, MOMP, although typically associated with the intrinsic death pathway, can also participate in death receptor–mediated apoptosis by virtue of caspase-8–mediated cleavage and activation of the BH3 protein, BID (Figure 28-1). The functional importance of this mitochondrial component of the death signaling pathway differs between cell types, leading to the classification of type I and type II cells—those that engage death receptor–mediated cell death without the involvement of MOMP and those in which apoptosis induced by death ligands requires mitochondria as evidenced by their susceptibility to BCL-2–mediated inhibition, respectively. XIAP appears to be the critical determinant of the relative involvement of BID-mediated MOMP in the extrinsic apoptosis pathway.[90a] XIAP binds to and blocks the proteolytic activities of caspase-9, -3, and -7 but not caspase-8—that is, unless displaced by SMAC and OMI, both of which are released from the mitochondria upon MOMP. The ability of MOMP to promote death receptor–mediated apoptosis can occur independently of APAF-1, indicating that the antagonism of the function of XIAP to permit caspase-8–mediated activation of the executioner caspases represents the major role for MOMP in death receptor–mediated apoptosis in type II cells.

## NONCANONICAL FORMS OF CASPASE-MEDIATED CELL DEATH

### Cell Death, Inflammasomes, and Caspase-1 Activation

In addition to apoptosis, additional types of cell death are also mediated by the activity of caspase proteases, including caspase-1–dependent pyroptosis, as previously introduced. Recruitment of inactive procaspase-1 to a multiprotein complex called the inflammasome forces a self-cleavage event to generate catalytically active caspase-1. The most well-characterized role of bioactive caspase-1 is the processing and activation of its substrates IL-1β[91] and IL-18.[92,93] Release of these cytokines, in addition to other proteins lacking a signal peptide, including IL-33,[94] pro-IL-1α, and fibroblast growth factor (FGF)-2, occurs via a poorly understood noncanonical secretory pathway that also requires caspase-1 activity.[95]

Assembly and activation of the inflammasome complex to initiate the production of the pro-inflammatory cytokines, IL-1B and IL-18, represents a critical component of the host innate immune system to protect against infection and sterile insults. The initiating event for inflammasome assembly is the detection of a "stressor" by one of several sensor molecules or nucleotide-binding domain, leucine-rich repeat–containing (NBD-LRR), or NLRs. Activators of NLRs, collectively termed DAMPs, can be of endogenous, exogenous, self, or foreign origin, although most are commonly associated with components of infectious agents or pathogen-associated molecular patterns (PAMPs).

NLRs are among the so-called pattern recognition receptors (PPRs) that encompass TLRs, C-type lectin receptors (CLRs), the absent in melanoma 2 (AIM2)-like receptor (ALR) families, and retinoic acid inducible gene I (RIG-I)-like receptors (RLRs). NLR members include NLRP1 (NOD-, LRR-, and pyrin-containing domain 1), NLRP3, -6, -7, and -12 (also named IPAF) and are characterized by three distinct domains. The N-terminal region that confers the functional classification of the NLR contains a pyrin,

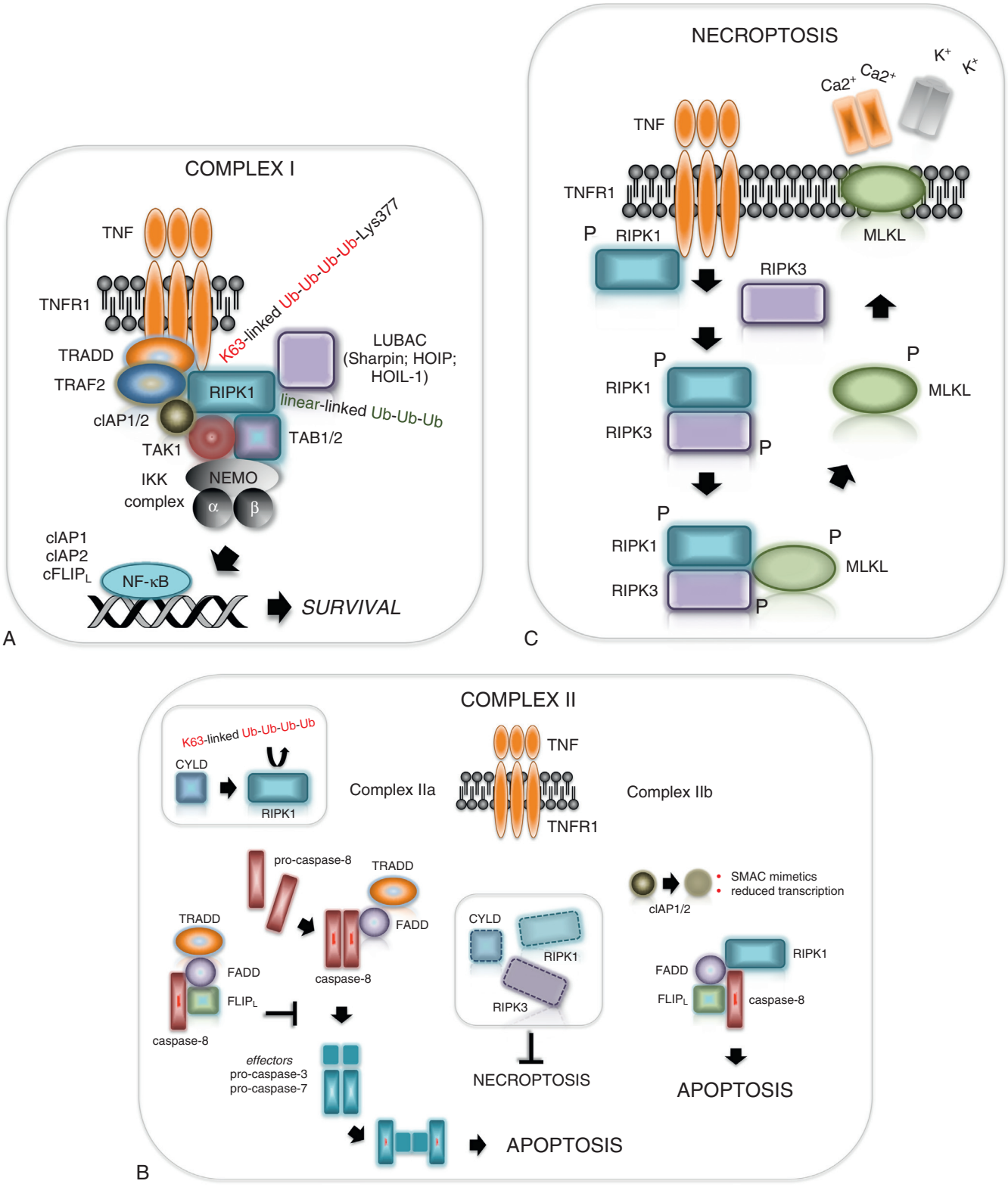

COMPLEX I
TNF
TNFR1
K63-linked Ub-Ub-Ub-Ub-Lys377
TRADD
TRAF2
RIPK1
LUBAC (Sharpin; HOIP; HOIL-1)
linear-linked Ub-Ub-Ub
cIAP1/2
TAK1
TAB1/2
IKK complex
NEMO
α
β
cIAP1
cIAP2
cFLIP$_L$
NF-κB
SURVIVAL
A
NECROPTOSIS
Ca2+
Ca2+
K+
K+
TNF
TNFR1
P
RIPK1
MLKL
RIPK3
P
RIPK1
RIPK3
P
P
MLKL
P
RIPK1
RIPK3
P
P
MLKL
C
COMPLEX II
K63-linked Ub-Ub-Ub-Ub
CYLD
RIPK1
Complex IIa
TNF
TNFR1
Complex IIb
pro-caspase-8
TRADD
FADD
caspase-8
TRADD
FADD
FLIP$_L$
caspase-8
effectors
pro-caspase-3
pro-caspase-7
CYLD
RIPK1
RIPK3
NECROPTOSIS
SMAC mimetics
reduced transcription
cIAP1/2
FADD
RIPK1
FLIP$_L$
caspase-8
APOPTOSIS
APOPTOSIS
B

**Figure 28-2** Multiple tumor necrosis factor (TNF)-TNF receptor 1 (TNFR1) complexes can be formed, each leading to a different biologic outcome. **A,** Complex I: Binding of TNF to its trimeric receptor TNFR1 triggers assembly of complex I that comprises TNFR-associated death domain (TRADD), receptor-interacting protein kinase 1 (RIPK1), and the E3 ubiquitinases, cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, TNFR-associated factor 2 (TRAF2), and HOIL-1 of the linear ubiquitin chain assembly complex (LUBAC). cIAP1/2 mediates the K63-linked polyubiquitylation of RIPK1 on lysine 377 to promote RIPK1-dependent stabilization of complex I and recruitment of the (1) TAB-TAK complexes and (2) nuclear factor-κB (NF-κB) activation of the IκB kinase (IKK) complex. The E3 ligase activity of the LUBAC further contributes to RIPK1 ubiquitination, complex stability, and NF-κB activation. NF-κB–dependent transcription of cellular FLICE-like inhibitory protein long form ($cFLIP_L$) and cIAP1 and -2 contribute to cellular survival by the stabilization of complex I and inhibition of proapoptotic complex II assembly. **B,** Complex IIa: The formation of complex IIa is triggered by TNF-TNFR1 interaction and occurs in the cytosolic compartment under conditions in which complex I is destabilized. One way this can occur is via the activity of CYLD, a deubiquitylase that removes K63-linked polyubiquitin from RIPK1 to promote the destabilization of complex I and dissociation of RIPK1 to participate in alternative TNF-induced signaling complexes. Complex IIa assembly involves the indirect recruitment of Fas-associated protein with death domain (FADD) to TNFR1 via TRADD. This activity promotes the association of procaspase-8 monomers to form catalytically active caspase-8 dimers. Active caspase-8 can then cleave and activate the downstream executioner caspase-3 and -7 to mediate apoptotic cell death. $FLIP_L$ can also bind to FADD and/or procaspase-8. Under these conditions, caspase-8 in dimerization with $FLIP_L$ is catalytically active but not fully processed, displays altered substrate specificity, and cannot engage apoptosis. Complex IIb (ripoptosome): Under conditions in which cIAPs are reduced or inhibited, stable RIPK1-dependent complex I formation is less favored and an alternative complex IIb can assemble. This cytosolic complex is TRADD-independent and comprises RIPK1, FADD, and $FLIP_L$–caspase-8 heterodimers. Under these conditions the proapoptotic RIPK1 function is favored. $FLIP_L$ can also bind to FADD and acts as a competitive inhibitor of caspase-8-FADD association to prevent apoptosis. Changes in the levels of RIPK3, mixed lineage kinase domain-like (MLKL), and caspase-8 activation can alternatively lead to necroptosis. **C,** TNF-induced necroptosis: induction of necroptosis in response to TNF-TNFR1 ligation. Oligomerization of RIPK1 and RIPK3 induces the autophosphorylation of RIPK3 to recruit and phosphorylate MLKL in an RIPK3-dependent manner. How MLKL then induces rupture of the plasma membrane remains unclear, but suggested mechanisms include direct permeabilization by MLKL itself or MLKL-dependent recruitment of $Ca2^+$ and/or $K^+$ ion channels to effect changes in ion homeostasis, cellular swelling, and eventual rupture of the plasma membrane. NEMO, NF-κB essential modulator; SMAC, second mitochondrial-derived activator of caspase. *(From Weinlich R, Green DR: The two faces of receptor interacting protein kinase-1.* Mol Cell *56:469–480, 2014, and Pasparaki, M, Vandenabeele P: Necroptosis and its role in inflammation.* Nature *517:311–320, 2015.)*

CARD, or baculovirus inhibitory domain, the central domain, an NBD or NACHT domain (nucleotide-binding domain) that mediates deoxyribonucleotide triphosphate (dNTP) hydrolysis and multimerization, and the C-terminal LRR domain. It is the LRR domain, which is ascribed autoinhibitory function in several inflammasome complexes, that promotes structural stability and a variety of protein-protein interactions.

Despite significant advances in our understanding of inflammasome biology, several major questions remain unanswered. The precise manner by which inflammasome assembly is triggered after receptor activation remains somewhat of a mystery because no direct binding between the NLR and its cognate ligand has been identified. Furthermore, the precise circumstances governing the formation of one type of inflammasome in preference to another and their specific physiologic role(s) are also largely unclear. Regardless, it is clear that caspase-1 activities play a pivotal role in immune regulation. Later we discuss aspects of inflammasome biology in the context of caspase-1–mediated cell death and how their dysregulation may mediate the underlying pathophysiology of several autoinflammatory conditions.

The inflammasome, although structurally analogous to the caspase-8 activating DISC and the caspase-9 activating apoptosome in general terms, differs from these caspase-activation complexes in one significant way. The inflammasome complex can vary in its molecular composition by virtue of different adapter molecules that are themselves characterized by one of several different protein-binding domains (Figure 28-3). Despite the compositional variations of inflammasome complexes, their primary role is to induce caspase activity via direct or indirect interaction with the CARD domain of caspase-1. One of the most common adapter molecules required for inflammasome assembly is apoptotic speck-forming CARD (ASC), which mediates IL-1 and IL-18 processing via homomultimeric interactions between its CARD domain and that of caspase-1. ASC also contains a PYD, a DD that mediates interaction between the PYD domain of several members of the NLR family, including NLRP1, -3, -6, -7, and -12 and NLRC4, to promote inflammasome assembly. Activating ligands are derived from multiple sources and include endogenous ligands such as glucose and amyloid-β, environmental stressors including asbestos and ultraviolet irradiation, and those derived from infectious agents of bacterial, viral, fungal, and protozoan origin.

## Canonical NLRP3 Inflammasome

In most but not all cases,[96,97] assembly of an active NLRP3-containing inflammasome requires two independent signaling events. The first event primes the cells for inflammasome assembly and arises from NF-κB activation mediated by TLR or TNFR1 activation to induce NLRP3 expression.[98] The second event is mediated by activation of NLRP3 via one of several DAMPs or PAMPs. Whether NLRP3 is a receptor *per se* remains unclear, but inflammasome formation is induced in an NLRP3-dependent manner in response to multiple signaling molecules including asbestos,[99] amyloid-β,[100] uric acid,[101] muramyl-dipeptide,[102] and calcium pyrophosphate dihydrate (CPPD),[103] as well as bacterial RNAs[104] and viral components derived from multiple pathogens. In response to these triggers, NLRP3, via its PYD domain, associates with the PYD-containing adapter ASC. The CARD domain of ASC then interacts with that of caspase-1 to promote inflammasome formation and the processing of IL-1β/IL-18.

The promiscuous nature of NLRP3 activation is likely to represent a common activation mechanism rather than mul-

tiple factors directly interacting with NLRP3 to promote inflammasome assembly. However, despite this assertion, a unifying mechanism has yet to emerge, although many potential triggers have been proposed (Figure 28-2A). These potential triggers include several mitochondrially localized events such as reactive oxygen species (ROS) production,[105] oxidation of mitochondrial DNA,[106] translocation of cardiolipin,[107] and direct association between K63 ubiquitinated NLRP3[108] and mitochondrial anti-viral signaling (MAVS) proteins[109] or mitofusin 2.[110] Molecules shown to physically interact with NLRP3 to promote inflammasome assembly include double-stranded RNA-dependent protein kinase (PKR)[111] and guanylate binding protein (GBP).[112] Several additional regulatory factors include $K^+$ efflux,[113,114] $Ca^{2+}$ influx,[115,116] ATP-triggered puraminergic signaling events,[117] and interaction with chaperone proteins including HSP90 and SGT1 via the LRR domain of NLRP3 to maintain a stable but inactive complex.[118]

Recently, a completely independent signaling pathway has been implicated in the regulation of NLRP3-mediated

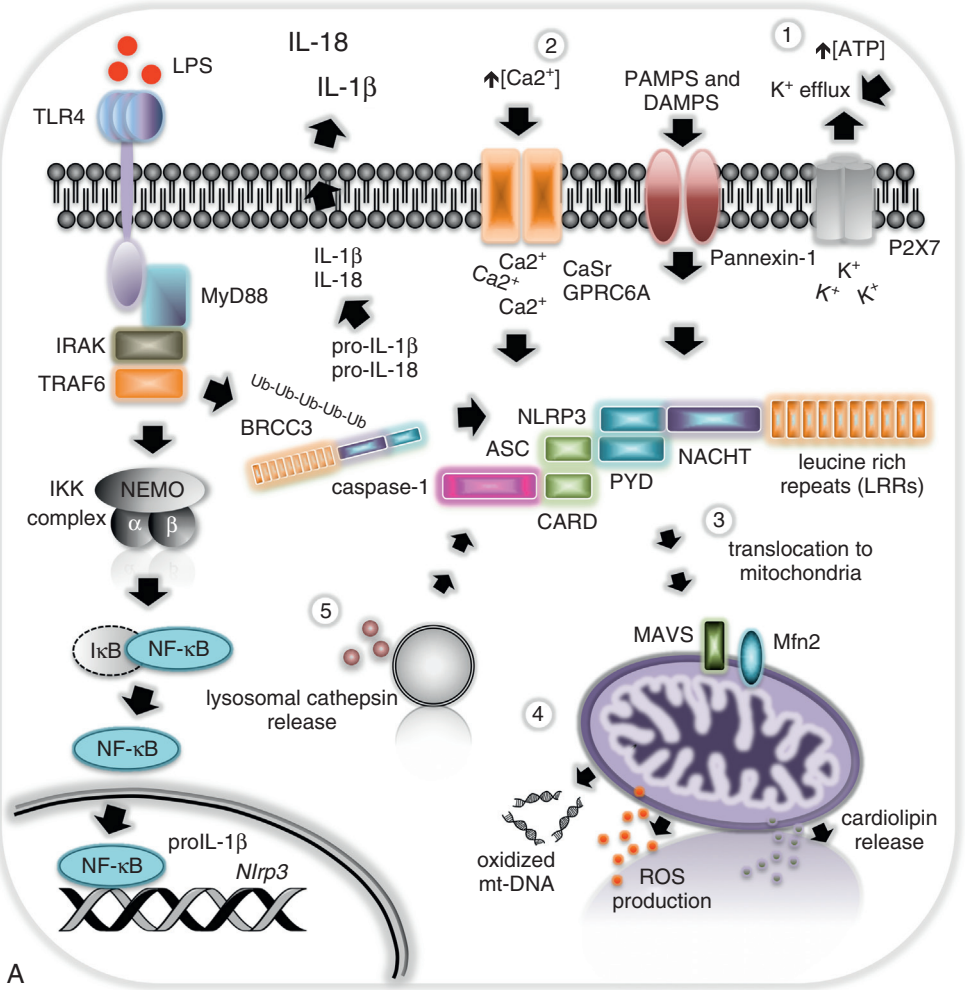



**Figure 28-3** The inflammasome is the activation platform for caspase-1 and is represented by different complexes according to the identity of the specific NLR and adapter proteins. **A,** NLRP3 (NALP3 canonical inflammasome). NLRP3 inflammasome assembly is induced by viral and bacterial pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) arising from environmental and self-derived origins. Examples include pore-forming toxins, amyloid aggregates, extra-cellular adenosine triphosphate (ATP), hyaluronan, monosodium urate (MSU) crystals, asbestos, silica, alum, and ultraviolet irradiation. NLRP3 inflammasome assembly is mediated by interaction between the PYD domains of NLRP3 and apoptotic speck-forming CARD (ASC) and a CARD-CARD interaction between ASC and caspase-1. This results in the activation of caspase-1, cleavage of pro-IL-1β and pro-IL-18, and their subsequent release as bioactive molecules. How NLRP3 is activated in response to all these different stimuli is unclear, although several mechanisms have been proposed: (1) elevation in extra-cellular ATP triggers $K^+$ efflux via the ATP-gated $K^+$ channel, P2X7, and assembly of pannexin-1 pores, through which PAMPs and DAMPs may enter the cytosol; (2) increased availability of $Ca^{2+}$ ions from endogenous stores and extra-cellular sources also activates NLRP3 inflammasome assembly, and several mechanisms have been proposed, including entry of extra-cellular calcium via the G-protein coupled calcium-sensing receptors (CaSRs); mitochondria may function as a source of several activating stimuli; (3) interaction of NLRP3 with mitochondrial anti-viral signaling (MAVS) proteins or mitofusin 2 (Mfn2) or (4) in response to the mitochondrial release of cardiolipin, reactive oxygen species (ROS), or oxidized DNA; and (5) lysosomal rupture and release of cathepsins. In addition to one or more of these activating stimuli, a nuclear factor-κB (NF-κB) priming signal, triggered via Toll-like receptors (TLRs) or tumor necrosis factor receptor 1 (TNFR1; lipopolysaccharide [LPS]-mediated TLR4 activation is shown) induces the transcription of NLRP3 and pro-IL-1β. Deubiquitination of NLRP3 via BRCC3 is also required to permit assembly of the NLRP3 inflammasome.

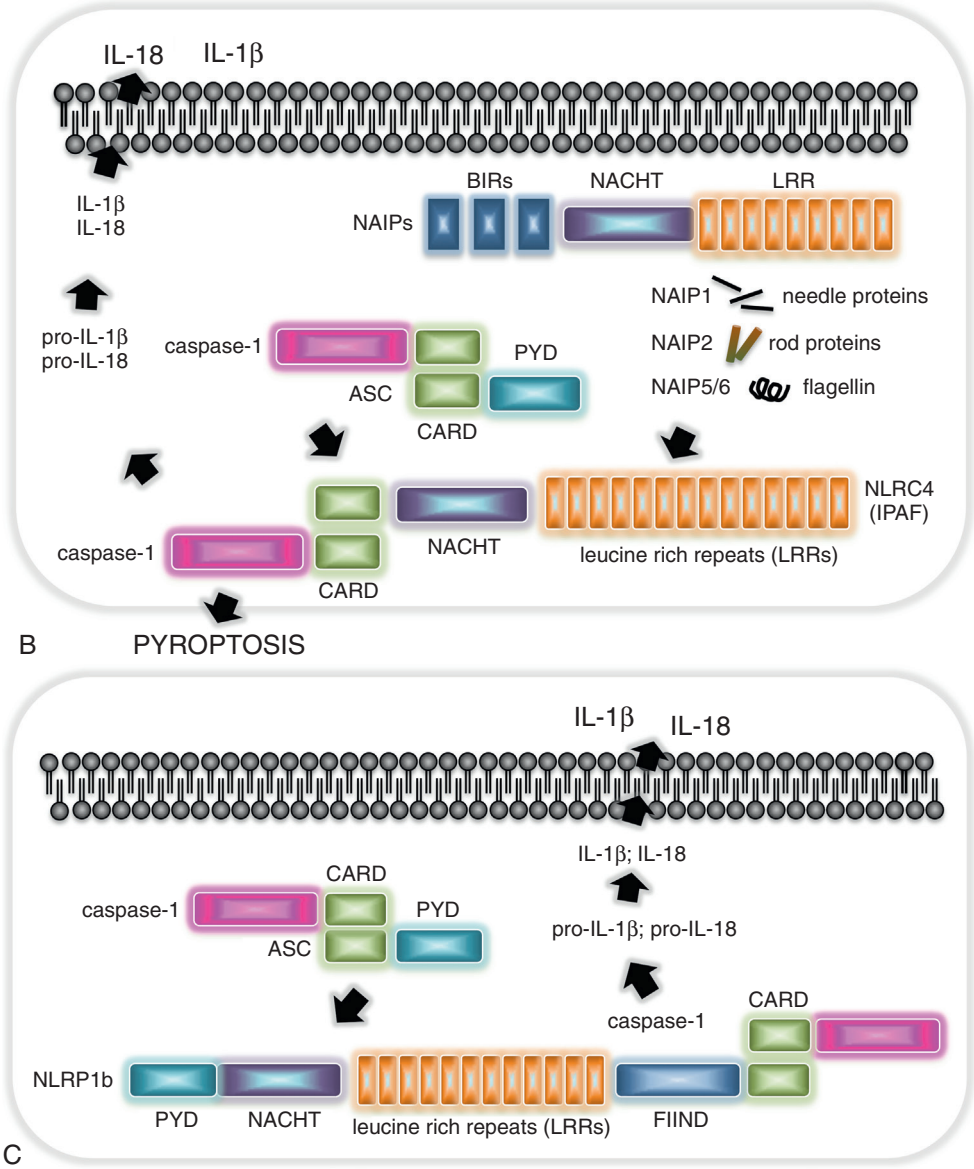
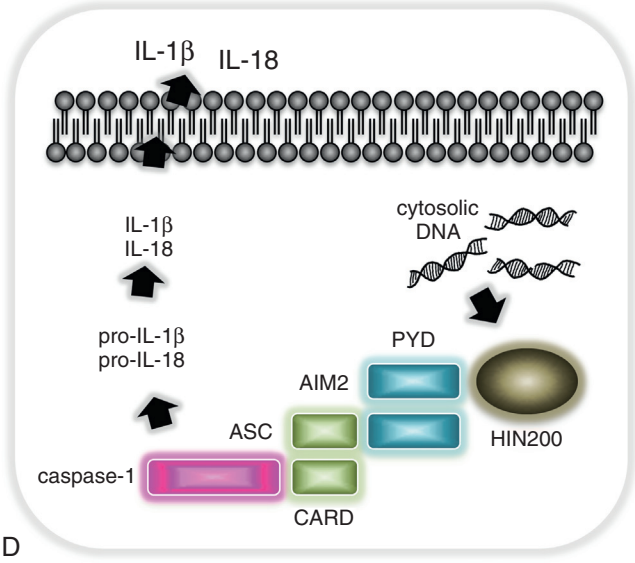



**Figure 28-3, cont'd** **B,** NLRC4 inflammasome. Flagellin and components of the type III and type IV bacterial secretion machinery trigger NLRC4 inflammasome assembly via the NACHT domain of the neuronal apoptosis inhibitory proteins (NAIPs). NAIP1 acts as the upstream sensor of needle proteins, those of NAIP2 sense rod proteins, and NAIP5/6 BIRS are triggered by flagellin. Interaction between the NIAPs and NLRC4 may then trigger oligomerization of NLRC4, and direct interaction between the caspase recruitment domains (CARDs) of NLRC4 and caspase-1 then complete assembly of the inflammasome and IL-1β/IL-18 secretion. Under some circumstances, ASC can also play a role. **C,** NLRP1b inflammasome. Activators include ATP, bacterial peptidoglycan dipeptide (MDP), and lethal toxin from *Bacillus anthracis*. Caspase-1 CARD can directly interact with NLRP1b CARD to form the inflammasome. ASC may stabilize this interaction and potentiate inflammasome activity. Cleavage of the FIIND domain of NLRP1b may be required for its activity.

inflammasome assembly that directly couples it to the regulation of cell death. The functional integration of recognized components of cell death signaling with inflammasome regulation was first uncovered by the demonstration that lipopolysaccharide (LPS; signal 1) in combination with a SMAC mimetic to promote the degradation of cIAP1 and -2 regulated IL-1β processing. Significantly, IL-1β maturation and secretion was mediated in both an NLRP3–ASC–caspase-1–dependent and caspase-8–dependent—caspase-1–independent manner.[119] RIPK3 and ROS generation was required under both conditions. Subsequently, both FADD[120] and caspase-8[120,121] have been shown to be essential for TLR-initiated NLRP3 inflammasome assembly. However, this may not be the case in all cell types.[122]

Multiple inherited and de novo mutations in the *NLRP3* gene have been identified, many clustered within the NBD domain, rendering a genetic predisposition to increased formation of the NACHT, LRR, and PYD domain-containing protein 3 (NALP3)-containing inflammasome. Increased IL-1β production contributes to the pathophysiology of a family of cryopryinopathies or cryopyrin-associated periodic




D

**Figure 28-3, cont'd** **D,** Absent in melanoma 2 (AIM2) inflammasome. Activators of the AIM2 inflammasome include DNA from intra-cellular bacteria and viruses, as well as self-derived double-stranded DNA. Cytosolic DNA binds to the HIN200 domain of AIM2 to trigger its interaction with ASC via their respective PYD domains. Caspase-1 is then recruited via its CARD domain to bind with the CARD domain of ASC. Activation of caspase-1 then processes pro-IL-1β and pro-IL-18 to generate the mature proteins. IKK, IκB kinase; IL, interleukin; IRAK, interleukin-1 receptor-associated kinase; LRR, leucine rich repeat; mt-DNA, mitochondrial DNA; NEMO, NF-κB essential modulator; TRAF, TNFR-associated factor. *(From Wen H, Miao EA, Ting JP: Mechanisms of NOD-like receptor-associated inflammasome activation.* Immunity *39:432–441, 2013, and Lamkanfi M, Dixit VM: Modulation of inflammasome pathways by bacterial and viral pathogens.* J Immunol *187:597–602, 2011.)*

fever syndromes (CAPS) including familiar cold-induced autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal onset multisystem inflammatory disorder or chronic infantile neurologic cutaneous and articular syndrome (NOMID/CINCA).[123] Genetic characterization of a contributory role for NLRP3 in CAPS was identified using murine knockin models harboring specific NLRP3 mutations associated with MWS and FCAS.[124] These NLRP3 mutations, when introduced into cells of the hematopoietic compartment, manifested in several clinical features of the human syndromes, including neutrophil infiltration into the skin, periodic bouts of fever, and elevated Th17-associated cytokines. These symptoms were largely alleviated by anti–IL-1 treatment, suggesting that the underlying pathophysiology can be attributed to increased IL-1β secretion as a consequence of the enhanced formation of NLRP3-containing inflammasomes.

Several other pathophysiologies associated with exuberant production of pro-inflammatory cytokines, including IL-1β, have also implicated NLRP3 function. NLRP3-dependent inflammasome formation can be triggered by the mislocalization of endogenous molecules, including those typically associated with gout, an inflammatory disease arising from deposition of monosodium urate (MSU) crystals in the joints, cholesterol deposits in atherosclerotic plaques, and insoluble aggregates of β-amyloid, a feature of Alzheimer's disease. Tissue damage arising from ischemic-reperfusion injury or that associated with excessive inflammation in the lung during pulmonary fibrosis or asthma is characterized by the release of multiple DAMPs, including extra-cellular ATP, uric acid, the purinergic receptor P2X7, and hyaluronan, a component of the extra-cellular matrix—all of which can directly induce the formation of an NLRP3-dependent inflammasome. Furthermore, lung inflammation arising from environmental factors such as asbestos and silica may well be attributable to the reported NLRP3-dependent production of IL-1β and IL-18 triggered by these agents. Although a direct role for NLRP3 in all of these aberrant inflammatory scenarios remains to be fully tested, a contributory role in the associated pathophysiologies seems likely.

The NLRP3-containing inflammasome is an essential component of the host immune response to numerous bacterial, fungal, and viral pathogens. Examples include *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri*, *Candida albicans*, and *Saccharomyces cerevisiae*.[125] Several of these organisms express pore-forming toxins that have been shown to induce membrane rupture and NLRP3-dependent inflammasome activation and/or pyroptosis. These include toxins A and B from *Clostridium difficile*,[126] pneumolysin from Streptococcus pneumonia,[127] nigericin from various strains of Streptomyces,[128] α-, β- and γ-hemolysins, the virulence factors from *S. aureus*,[129] and listeriolysin from *L. monocytogenes*.[130]

Host-mediated immunity against some DNA viruses including influenza,[131,132] herpes,[133,134] and sendai[135] may also involve activation of the NLRP3 inflammasome.[136] However, identification of AIM2 as the primary sensor of nucleic acids to trigger ASC-dependent inflammasome activation[137] may limit the general role of NLRP3 under some conditions. Nevertheless, clearly NLRP3 is a critical component of effective host defense against multiple bacterial pathogens and DAMPs of self-derived origin.

## NLRC4 (IPAF or CARD12 Inflammasome)

In contrast to the requisite ASC-dependent activation of caspase-1 in the NLRP3 inflammasome, NLRC4 itself contains a CARD domain that is capable of direct interaction with the CARD of caspase-1, leading to its processing and the cleavage and secretion of IL-1β and IL-18.[138,139] However, under certain conditions, NLRC4 and ASC may interact via their respective CARD domains in a manner that does not prevent the interaction of ASC with caspase-1. Such a scenario makes provision for an alternate biologic outcome based on ASC activity. For example, ASC-dependent inflammasome formation is required for IL-1β/IL-18 generation, whereas pyroptosis can proceed in its absence.[140]

Cytosolic assembly of the NLRC4 inflammasome is triggered by components of the type III or type IV secretion systems or by flagellate bacteria including *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. NLRC4 is a unique member of the NLR family in that the mechanism mediating its response to pathogens is known. Members of the NLR-apoptosis inhibitory protein (NAIP) family, 6 paralogs in mice, function as the upstream sensors to then directly engage NLRC4 inflammasome assembly and IL-1β maturation.[141] NAIP proteins are characterized by an N-terminal domain containing three BIR repeats, a

nucleotide-binding oligomerization domain (NOD) harboring multiple α-helices, and a C-terminal LRR. NAIP1[142,143] responds to the needle proteins and NAIP2 responds to the rod proteins[144-147] of the bacterial type III secretion system, whereas NAIP5 and -6 are sensors for bacterial flagellin.[147] Association of the NAIP with its respective ligand is mediated by the NBD and not the BIR domains as previously thought and triggers subsequent interaction with NLRC4 to promote inflammasome assembly.[148] The precise details of the NAIP-NLRC4 interaction remain unclear but may involve permissive phosphorylation by protein kinase C (PKC)-δ,[149] at least under some circumstances.[150]

Two independent NLRC4 gain-of-function point mutations were recently described, both of which rendered the NLRC4 inflammasome constitutively active and were associated with periodic bouts of fever.[151-153] Functional analyses revealed aberrant IL1-β/IL-18 production and an increased susceptibility to pyroptosis within macrophage populations isolated from patients.

## NLRP1b Inflammasome

NLRP1, the first NLR to be identified as an inflammasome mediator,[154] is encoded by a single gene in humans but by three orthologs in the mouse, denoted *Nlrp1a*, *Nlrp1b*, and *Nlrp1c*.[155] Unlike human NLRP, which contains a pyrin domain, the murine paralogs do not contain this domain and are composed of an NBD, an LRR region, a FIIND ("function to find" domain) and a C-terminal CARD. The *Nlrp1b* locus is most frequently associated with inflammasome formation and activation of caspase-1, although aspects of its regulation and function remain unclear. Muramyl dipeptide (MDP), a minimal bioactive peptidoglycan peptide common to both positive and negative bacteria, was initially identified as a ligand for NLRP1b-mediated inflammasome formation. However, whether NLRP1b-mediated inflammasome formation and caspase-1 processing requires pyrin domain–dependent association with ASC or instead occurs via a direct interaction between NLRP1 and caspase-1 via their respective CARD domains remains unclear.[156] Conflicting studies, using cells lacking NLRP1 expression, report that MDP-mediated caspase-1 processing was instead dependent upon NLRP3, either alone or in cooperation with NOD2.[157,158]

Lethal toxin (LT), the major virulence factor of *Bacillus anthracis*, is a potent ligand for NLRP1b-dependent inflammasome formation and triggers IL-1β and IL-18 processing and extensive pyroptosis of macrophages. LT promotes the cleavage of NLRPb1,[159,160] an event that is reportedly both necessary and sufficient[161] for inflammasome assembly. Although autoproteolysis of NLRP3 also correlates with susceptibility to LT-induced inflammasome assembly, it is insufficient for pro-IL-1β activation.[162-164] Additional parameters that contribute to the susceptibility of cells to LT, inflammasome formation, and pyroptosis include extra-cellular ATP release and reduced p38-mediated survival.[165]

The physiologic significance of NLRP1b inflammasome activity in response to LT is supported by the observed resistance to *B. anthracis* infection in mice expressing an LT-sensitive allele of NLRP1b.[166] However, other studies have noted that resistance to *B. anthracis* and IL-1β production does not necessarily correlate with NLRP1b activation,[155] suggesting the contribution of additional factors.

NLRP1a is also associated with inflammasome formation and the consequent secretion of IL-1β and IL-18 in an ASC-independent manner.[167] Regulation of NLRP1a activity is effected at multiple levels, including induction of its expression via NF-κB promoter elements, activation by post-translational autoprocessing. and inhibition via LRR-mediated association with the anti-apoptotic BCL-2 family members, BCL-2 and BCL-$X_L$. Interestingly, an activating mutation of NLRP1 and consequent predisposition to inflammasome formation, although associated with lethal systemic inflammation via IL-1β, also led to caspase-1 activity and extensive pyroptosis in the hematopoietic compartment independently of IL-1β. NLRP1a-mediated inflammasome formation and pyroptosis therefore may have additional roles independent of that in IL-1β–mediated inflammation.

## NLRP6 Inflammasome

NLRP6 is expressed predominantly in gut epithelial cells and can mediate ASC-dependent assembly of the inflammasome, caspase-1 processing, and enhanced secretion of IL-1β and IL-18. However, until recently, the physiologic significance of NALP6 remained elusive. Mice lacking NLRP6, ASC, or caspase-1 exhibited a marked exacerbation in colitis and an enhanced susceptibility to damage-associated gut tumorigenesis.[167a,167b,167c,167d] This outcome was attributed to the disruption of epithelial cell turnover and indicates that localized inflammation is critical for tissue repair. In contrast, the amelioration of colitis in mice lacking NLRP6 and IL-1β secretion aligns instead with the destructive consequences of the inflammatory response.[167e] One intriguing reconciliation of these opposing findings may lie in the observation that NLRP6, by promoting ASC-dependent inflammasome assembly and IL-18 secretion, regulates the gut flora to maintain integrity of the epithelial barrier and reduce the incidence of colitis.[167f] Therefore NLRP6 deficiency negatively regulates inflammasome assembly, promotes aberrant colonization of the gut by damage-inducing microbiota, and leads to a localized inflammatory response.

In contrast to what we believe to be the primary pro-inflammatory role of the other NLRs, NLRP6 instead negatively regulates inflammatory signaling to retard pathogen clearance. Accordingly, mice lacking *Nlrp6* are highly resistant to *L. monocytogenes*, *Salmonella typhimurium*, and *E. coli* that is attributed to NLRP6-mediated suppression of TLR-generated MAPK and NF-κB signaling events.[167g] Whether this finding is representative of a subclass of NLRs that function as critical immune suppressors remains to be determined. It does, however, provide tantalizing evidence of regulatory cross-talk between the NLRs and members of the TLR family.

## AIM2 (Pyrin) Inflammasome

AIM2, although not an NLR, is a pyrin-containing protein that is responsive to cytosolic DNA[168] of either pathogen or host origin and is able to mediate inflammasome formation.[169,170] AIM2, via its HIN-200 domain,[171] directly

associates with its trigger, DNA, to recruit the adapter ASC in a PYD-dependent manner to promote caspase-1 activation.[172,173]

AIM2, by regulating the levels of IL-1β and IL-18, plays a critical role in antipathogen immunity to several cytosol-replicating bacteria such as *Francisella tularenis*[137,174,175] and *L. monocytogenes* and the DNA viruses, vaccinia and cytomegalovirus.[137] The mechanism(s) via which AIM2 can sense cytosolic DNA has/have yet to be fully elucidated, although type I IFN signaling events can contribute to the response to bacterial DNA, although not to that of viral origin.[175,176] AIM2 inflammasome assembly is subject to negative regulation by factors of both host and pathogen origin, including the PYRIN domain-only proteins (POPs)[177] and HIN-200–containing proteins,[178] both of which compete with ASC for binding to AIM2.

The ability of AIM2 to recognize and respond to cytosolic DNA of host origin is thought to mediate the pathophysiology of self-driven inflammatory responses, including systemic lupus erythematosis[179] and psoriasis.[180] Several studies have also linked susceptibility to certain tumor types and AIM2-dependent inflammasome formation,[181,182] although a direct causative role has yet to be shown.

### Noncanonical Inflammasome

NLRP3, in addition to its critical role in canonical inflammasome assembly to activate caspase-1 (previously described), also participates in caspase-11–dependent noncanonical inflammasome formation.[183] The family of inflammatory caspases, in addition to caspase-1, includes murine caspase-11 and human caspase-4, -5, and -12. Caspase-11 can induce IL-1β–dependent pyroptosis in a caspase-1–dependent manner.[183a] Several pathogenic bacteria, including *E. coli*, *Vibrio cholera*, *S. typhimurium*, and *Legionella pneumophila* induce the activation of procaspase-11 via assembly of a noncanonical NLRP3 inflammasome. Constitutively low expression of caspase-11 is induced by several pro-inflammatory stimuli, including TLR4–TIR-domain–containing adapter-inducing IFN-β (TRIF) activation via LPS, IFNs, IFN-γ, and IFN-β that is at least partially dependent on NF-κB and signal transducer and activator of transcription (STAT) signaling. Lipid A, a core structural moiety of LPS, was identified as the requisite trigger for caspase-4, -5, and -11 activation and pyroptosis after challenge with Gram-negative bacteria. Recent observations demonstrate that the activation of these caspases may occur by a direct interaction between LPS and the CARD of the caspase itself.[183b] The initiation of a process by the very same molecule responsible for its execution introduces an unexpected twist in our understanding of caspase biology and to the fundamental regulation of innate immunity. Whether this observation is just the first to define caspases as a novel class of receptors for pathogen-derived PAMPs, as well as an additional pathway by which caspases can be activated, remains to be seen.

However, not all IL-1β production is mediated by activation of the cytosolic NLRs. In a recent study, caspase-8, in a complex with mucosa-associated lymphoid tissue (MALT)-1 and ASC, mediated the processing of IL-1β in a SYK-dependent manner via extra-cellular dectin-1 in response to fungi and mycobacteria.[184] It remains to be determined if this noncanonical caspase-8 inflammasome can be engaged in response to other extra-cellular pathogens in a dectin-1–dependent or dectin-1–independent manner or, indeed, if the activity of other apical caspases can be similarly co-opted for host immune defense.

## NONAPOPTOTIC CELL DEATH SIGNALING PATHWAYS

### Necrosis and Secondary Necrosis

As we discussed briefly in the introduction, necrosis is typically characterized by cellular swelling, rupture of the plasma membrane, and the consequent spilling of the intra-cellular contents into the surrounding milieu. Necrosis can be induced directly by multiple different stimuli, including ATP depletion, nutrient withdrawal, and excessive levels of ROS. However, necrosis can also occur as a consequence of apoptosis and is hence termed *secondary necrosis*. This type of necrotic cell death is most commonly observed in vitro, as most often, cells dying by apoptosis in vivo are rapidly removed by the activities of professional phagocytes. However, disruption of the normal function of these "garbage collectors" can instead lead to secondary necrosis. Cells undergoing secondary necrosis are characterized by chromatin condensation, a feature typically associated with apoptosis, and plasma membrane permeabilization, one of the hallmarks of necrosis. Several other forms of necrosis can proceed entirely independently of apoptosis and can be identified by several distinctive characteristics that can reveal the pathway by which the cell died.

### Necrosis and the Mitochondrial Permeability Transition

In some forms of caspase-independent cell death, including necrotic death as a result of ischemia/reperfusion injury, MOMP may occur as a result of activation of the permeability transition pore (PTP), a channel in the inner mitochondrial membrane that is susceptible to regulation by a number of stimuli, including high concentrations of calcium ions, ROS, and fluctuations in cellular pH, as well as in response to certain drugs.

The precise identity and, for that matter, the role of the PTP in cell death remains somewhat controversial. What appears to happen is that once it is open, the PTP induces a rapid loss in the permeability of the inner mitochondrial membrane and consequent dissipation of the transmembrane potential, or mitochondrial permeability transition (MPT). This process in turn triggers the influx of solutes and then water into the mitochondrial matrix, causing swelling and, ultimately, rupture of the outer mitochondrial membrane. Importantly, although this phenomenon results in the spilling of mitochondrial components into the cytosol, no strong evidence exists to indicate that it activates apoptotic signaling, as would be the case if MOMP were mediated in a BAX/BAK-dependent manner.

The PTP has been proposed to be composed of several components, including the F0F1 adenosine triphosphatase (ATPase) in the inner mitochondrial membrane. Other components may include an adenosine nucleotide transporter (ANT) that mediates the transfer of adenosine

diphosphate (ADP) and ATP across the inner mitochondrial membrane, and the outer mitochondrial membrane voltage-dependent anion channel (VDAC). However, the deletion of ANT or VDAC components fails to have an impact on PTP activity. In contrast, mitochondria lacking cyclophilin D, a matrix peptidylprolyl isomerase, display an abrogated MPT response to calcium. However, this defect does not extend to aberrant apoptosis or developmental abnormalities.

## Poly-ADP-Ribose Polymerase and Necrosis (Parthanatos)

The generation of ATP to provide the necessary energy to sustain normal cellular function is mediated by the collective activities of glycolysis, the Krebs cycle, and oxidative phosphorylation. Disruption of these pathways or an enhanced demand for intra-cellular energy consumption can lead to bioenergetic failure and the consequent engagement of necrosis. PARP is an essential enzyme for DNA repair, an energy-costly process that utilizes large amounts of nicotinamide adenine dinucleotide ($NAD^+$), thereby limiting mitochondrial ATP generation. As a consequence, cellular damage that demands excessive PARP-dependent DNA repair can disrupt oxidative phosphorylation, leading to necrotic cell death, also known as parthanatos (*thanatos*—personification of death).

Of course, nothing in biology is that simple. PARP can also regulate the sensitivity of cells to DNA damage–induced apoptosis via its direct interaction with p53. Under these circumstances, PARP-dependent ribosylation of p53 negatively regulates the function of p53 to direct DNA repair and inhibit p53-dependent apoptotic cell death. However, under conditions of irreparable DNA damage, the interaction between p53 and PARP may actually promote apoptosis. In a reciprocal manner, p53 function can positively regulate PARP activity to facilitate necrosis in response to DNA damage imposed by excessive production of ROS. Consistent with this, the resistance of $p53^{-/-}$ murine embryonic fibroblasts (MEFs) to $H_2O_2$-induced DNA damage correlates with DNA repair and the recovery of intra-cellular $NAD^+$ levels. Furthermore, loss of p53 activity, or disruption of the downstream mediators of p53-mediated apoptosis, BAK and BAX, correlate with a reduction in PARP activity. Therefore, the functional reciprocity between PARP and p53 regulates the cellular response to DNA damage to determine if DNA repair is compatible with survival or if cell death ensues. Furthermore, the type of death that is engaged—p53-dependent apoptosis or PARP-dependent necrosis—is directed by the complex interaction between these two proteins.

## Excitotoxicity and Necrosis

Excitotoxic death or excitotoxicity-induced necrosis is specific to neuronal cells and is induced by excessive levels of the primary excitatory neurotransmitter, glutamate or glutamate mimics, such as N-methyl-D-aspartate (NMDA) and kainic acid. The activity of glutamate is mediated by calcium-permeable ionotropic receptors that include the NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (NMDAR and AMPAR). Glutamate triggers $Ca^{2+}$ influx that, under normal physiologic conditions, is buffered by a variety of sequestration and extrusion pathways to maintain normal $Ca^{2+}$ homeostasis. Mechanisms include sodium-calcium exchanger (NCX), which, after glutamate stimulation, extrudes calcium to help restore normal calcium levels, as well as mitochondria that both facilitate ATP-dependent calcium extrusion and act as a calcium sink to help reduce intracellular calcium levels.

Although glutamate is essential for a number of functions in neural tissues, the accumulation of excessive glutamate induces a massive influx of calcium that can trigger neuronal cell death or excitotoxicity. Glutamate also induces several morphologic changes, including neuronal swelling, a feature typically associated with necrosis. However, inhibition of neuronal swelling by the removal of extra-cellular $Na^+$ does not suppress glutamate-induced $Ca^{2+}$-dependent excitotoxic death, suggesting that this event is independent and not causative. Glutamate receptors consist of several subtypes, of which NMDAR is the most calcium permeable and most often associated with excitotoxic neuronal death. However, NMDAR activation is not always cytotoxic and under some conditions is reported to promote neuronal survival. This outcome may be attributable to the suggestion that only extrasynaptic NMDARs, activated by excessive levels of glutamate that spill beyond the synaptic space, are associated with the $Ca^{2+}$ signaling that triggers excitotoxicity.

Although $Ca^{2+}$ clearly plays a critical role in glutamate-mediated neurotoxicity, the precise molecular pathways that mediate this type of death remain to be fully delineated. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a membrane-bound enzyme that catalyzes the transfer of one electron from NADH or NADPH to oxygen to generate superoxide, a species of ROS. Unlike the analogous mitochondrial NADPH oxidase reaction that functions as part of the electron transport chain to generate energy, the production of superoxide by the nonmitochondrial form of NADPH instead generates protons. To prevent their accumulation, protons are pumped from the cell, but in so doing, a high transmembrane potential is generated. It is this large charge difference across the plasma membrane that is detected as an excitotoxic signal in glutamate-exposed neurons. The generation of superoxide by NADPH oxidase triggers PARP activation that, in turn, depletes intra-cellular ATP and induces necrotic cell death (discussed previously). Loss or attenuated production of ATP also limits the energy required for maintaining essential electrochemical gradients, including those that support the function of glutamate transporters. This limitation not only prevents extra-cellular glutamate uptake but also promotes the reversal of transporter function, further increasing the concentration of extra-cellular glutamate and exacerbating excitotoxic death.

Uptake of calcium by mitochondria during excitotoxic glutamate signaling can also trigger the generation of ROS, causing opening of the PTP, mitochondrial depolarization, $Ca^{2+}$ efflux, and neuronal cell death. $Ca^{2+}$ is required for the activation of calpain proteases, which are able to cleave and inactivate various target proteins. Calpain substrates suggested to contribute to glutamate-induced neuronal cell death include the NCX (previously described), p35, a regulator of CDK5, and the NMDAR.

Because NMDAR permeability is specifically associated with the $Ca^{2+}$ fluxes, promoting excitotoxic neuronal death, it is proposed that a close functional and/or physical association likely exists with death-signaling proteins. Consistent with this proposal, the cytoplasmic tail of the NMDAR can directly bind to death-associated protein kinase 1 (DAPK1), CDK5, or phosphatase and tensin homolog (PTEN) to regulate neuronal sensitivity to glutamate signaling. In addition, PSD95, a membrane-associated guanylate kinase, associates with both NMDAR and neuronal nitric oxide synthase (nNOS) to mediate glutamate-induced $Ca^{2+}$-dependent generation of NO. Peroxynitrite ($ONOO^-$), arising from a reaction between the free radical form of (NO*) and the superoxide anion, $NO^-$, is highly toxic, inducing damage to an array of biomolecules, including lipids and DNA. Significantly, ONOO-mediated DNA damage can engage PARP activity to promote neuronal necrosis, as previously discussed.

Excess levels of extra-cellular glutamate can arise from the disruption of its uptake, breakdown, and/or release. The aberrant accumulation of glutamate and resulting excitotoxic neuronal cell death is implicated in the cause of several pathologic conditions, including cerebral ischemia or stroke that is characterized by irreparable glutamine-dependent neuronal damage. Under these conditions, the release of glutamate from damaged neurons can stimulate additional glutamate release and accumulation, a scenario further exacerbated by additional factors associated with ischemic injury such as reduced availability of oxygen and glucose. The glucose dependence of glutamine breakdown can also manifest in hypoglycemia-associated neuronal cell death in persons with diabetes. Glutamine-induced cytotoxicity has also been implicated in several neurodegenerative diseases, including multiple sclerosis, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS).

## Ferroptosis

Ferroptosis or iron-dependent cell death is a relatively recently described type of nonapoptotic cell death that remains to be fully characterized.[185] Nonetheless, several characteristics of this type of cell death have been described, together with its clinical potential for the treatment of multiple types of disease, particularly those associated with aberrant iron accumulation.[186,187] Iron and iron derivatives are essential for the regulation of a number of ROS-producing enzymes, including lipoxygenases, subunits of the mitochondrial respiratory chain, NADPH, and the antioxidant, catalase. The identification and characterization of ferroptosis as a unique form of necrotic cell death arose from the identification of erastin, a small molecule specifically toxic to oncogenic RAS transformants, and hence termed *RAS-selective lethal* (RSL). Specific features of RSL-induced death include the accumulation of ROS independently of mitochondrial function and chelation of iron.[186,188] Ferrostatin-1, an inhibitor of ferroptosis, can also block glutamate-induced cell death in neurons (previously discussed), suggesting a similar underlying mechanism.[185,187]

Further characterization of ferroptosis has revealed that it is specific for iron and not other metals and can be distinguished from other forms of death, both apoptotic and necrotic, via morphologic and metabolic criteria. Cells undergoing ferroptosis are characterized by mitochondria that are smaller in size but with an increase in the density of their membranes. However, contrary to other forms of necrotic cell death, ferroptosis cannot be attributed to a drastic reduction in ATP levels and bioenergetic failure. The identification of several genes required for ferroptosis, including citrate synthase and an acyl Co-A synthase family member (ACSF2), both of which are involved in mitochondrial fatty acid metabolism, suggests that lipid synthesis could represent one pathway through which ferroptosis may be regulated. Further characterization indicates that ferroptosis is induced by iron-dependent production of lipid-derived ROS via the activities of several enzymes, including members of the NADPH oxidase family.

Additional scenarios in which aberrant regulation of iron levels are associated with cell death include hereditary hemochromatosis (HHC), a spectrum of diseases characterized by excess iron accumulation and diseases that include liver cirrhosis and heart failure. One suggested mechanistic link between excess iron and the associated tissue damage observed in HHC include iron-dependent ROS production and mitochondrial dysfunction, which both are features aligning with the characteristics of ferroptosis. Intriguingly, several neurodegenerative conditions, including Parkinson's disease, can be alleviated by the blocking of iron uptake, which also is suggestive of a causative role for ferroptosis.

## Programmed Necrosis or Necroptosis

Until relatively recently, our understanding of necrosis was somewhat limited, fueled largely by the presumed absence of any underlying molecular mechanism. However, the identification of regulated or programmed necrosis (necroptosis) has invigorated research efforts into determining the molecular basis for this type of death, how it contributes to host biology, and whether its regulatory pathways may be exploited for clinical benefit.[2,189]

Although several stimuli can induce programmed necrosis, TNF and activation of its death receptor, TNFR1, has provided most insight into the molecular basis of this type of necrotic cell death. As we have already discussed, TNFR1 signaling is complex, with TNFR1-induced NF-κB activation promoting cellular survival and death receptor–induced caspase-8 activation critical for the induction of apoptosis. However, long before we had defined its molecular basis or had a cool name for it, an alternative form of death receptor–induced death was also described that proceeded independently of caspase activation and required RIPK1 activity.[190-192] Recent genetic studies have characterized this form of TNF-induced caspase-independent cell death as necroptosis and defined key components of death receptor signaling, including caspase-8, FLIP, and FADD, as essential regulators.

## Were We Missing Something?

The tantalizing suggestion of an unidentified death pathway involving caspase-8, FADD, and FLIP arose from apparent contradictory findings that were not easily reconciled with their known role in apoptosis. These findings included the

unexpected embryonic lethality associated with the deletion of caspase-8 or FADD, two proteins that prior to these studies were exclusively associated with promoting apoptotic cell death and therefore were predicted to phenocopy the cellular accumulation observed in embryos lacking *Apaf-1* or *Caspase-9*.[193-195] These findings were reconciled by the genetic rescue of the early lethality of caspase-8 or FADD deficiencies by the co-deletion of either of two related kinases, RIPK1 and -3.[196-198] Programmed necrosis, or necroptosis, is therefore a death pathway that is mediated by RIPK1 and -3 but only in the absence of caspase-8 activity.

The overlapping requirement for several of the signaling components required for both TNF-induced apoptosis and TNF-induced necroptosis begs the question as to how the activity of caspase-8 is regulated under physiologic conditions to engage cell death via apoptosis or necroptosis, and even, under some circumstances, to sustain cellular survival. Presumably multiple checkpoints determine not only the makeup of the TNFR1 signaling complex but also the activity of these components to ensure an appropriate biologic response.

## Molecular Regulation of Programmed Necrosis

The realization that RIPK3 was required for TNF-induced RIPK1-dependent necrosis provided a critical piece of a mechanistic puzzle[199-201] that was completed by the subsequent identification of MLKL as the target of RIPK3 and terminal effector of necroptosis.[202,203] Interaction between catalytically active RIPK1 and RIPK3 is mediated by their respective receptor interacting protein (RIP) homotypic interaction motifs (RHIMs).[199,200,204] Neither the homodimerization of the N termini of RIPK1 lacking a RHIM domain (RIPK1$^{\Delta RHIM}$) nor heterodimerization of RIPK1$^{\Delta RHIM}$ and RIPK3$^{\Delta RHIM}$ is able to trigger necroptosis. However, enforced dimerization of either the N termini of RIPK1 or RIPK1$^{\Delta RHIM}$ and RIPK3 are both able to do so, as are homo-oligomers, but not dimers of the C termini of RIPK3$^{\Delta RHIM}$, as well as dimers of the N termini of RIPK3$^{\Delta RHIM}$.[205]

RHIM-dependent interaction between RIPK1 and RIPK3 kinases triggers the feed-forward self-propagation of large hetero-oligomeric RIPK complexes that are essential for both kinase activity and execution of necroptosis.[199,200,204] Characterization of these high-order RIPK1 and -3 oligomers revealed strikingly similar characteristics to amyloid aggregates and an underlying mechanism by which kinase activation and necrosome assembly mutually reinforce each other.[206] Amyloid-like structures can be toxic in and of themselves. However, acquisition of kinase activity as a consequence of RIPK1 and -3 complex assembly is more compatible with the requirement for specific RIPK substrates to mediate necroptosis, rather than the complexes themselves causing toxicity. Recently identified is the RIPK-dependent phosphorylation of the pseudokinase, MLKL, to induce its activation, oligomerization, and translocation to the plasma membrane.[202,203,207] The swelling and loss of membrane permeability in cells dying by necroptosis is attributed to the ability of MLKL to disrupt ion homeostasis. Whether this disruption occurs by the direct binding of MLKL to phospholipid components of the plasma membrane or by its association with $Ca^{2+}$ or sodium channels remains to be determined.[208-211]

## RIPK1—Apoptosis, Necroptosis, or Survival—Take Your Pick

Although the activity of RIPK1 is critical for TNFR1-mediated activation of RIPK3 and execution of necroptosis, it is now apparent that its ability to regulate cell death mechanisms also encompasses roles in TNF-induced apoptosis under certain conditions, as well as suppressing the aberrant inflammation associated with RIPK3 and caspase-8 activities. RIPK1 therefore clearly represents a critical signaling nexus within several pathways, including those induced by TNF, TLRs, DNA sensing pathways, and IFNs. The diverse consequences of its regulatory activities are evidenced by its impact on multiple downstream signaling pathways and their biologic consequences.[212]

Insight into the mechanisms by which RIPK1 activity can differentially regulate multiple signaling pathways are beginning to emerge. Deletion of RIPK1 is associated with perinatal lethality characterized by multiorgan inflammation and widespread cell death.[213] However, mice harboring one of several homozygous point mutations that eliminate RIPK1 kinase activity are viable.[214-216] Recent genetic studies have begun to elucidate not only the molecular basis for the postnatal lethality associated with RIPK1 deletion but also the identity of signaling pathways regulated by RIPK1 and the mechanisms mediating its effects. Although the individual deletion of FADD, RIPK3, or caspase-8 all fail to affect the lethal phenotype observed in *RIPK1*$^{-/-}$ mice, the ablation of both RIPK3 and caspase-8 or RIPK3 and FADD in the context of *RIPK1*$^{-/-}$ completely rescues postnatal lethality.[217-219] Development also proceeds normally in mice lacking both RIPK1 and RIPK3 and the requisite adapter molecule for caspase-8 activation, FADD.[217] The unambiguous conclusion from these studies is that RIPK1 is an inhibitor of two independent pathways associated with embryonic lethality—one that is mediated by FADD/caspase-8 and the other, a lethal RIPK3-mediated pathway. This conclusion is consistent with roles for RIPK1 in the inhibition of both apoptosis and necroptosis. However, have we not just discussed the requirement for RIPK1 in *promoting* cell death? In fact, this assertion also is supported by unambiguous genetic data—specifically, that the embryonic lethality associated with ablation of caspase-8 or FADD is completely rescued by the co-deletion of RIPK3 or RIPK1.[197,198] The simple conclusion, then, is that RIPK1 must drive or mediate the lethal signal resulting from the ablation of caspase-8 or FADD. Must we therefore reach the "not so simple" conclusion, then, that RIPK1 can both promote and inhibit cell death? As we will now discuss, that is indeed the case.

Homodimerization and activation of caspase-8 can be triggered by the ligation of TNFR1 via the adapter proteins TRADD and FADD to promote apoptosis.[194,220,221] NF-κB, which is also activated by TNFR1 ligation, can inhibit caspase-8–dependent cell death by inducing transcription of FLIP, a catalytically inactive form of caspase-8. FLIP, by forming heterodimers with caspase-8, prevents the homodimerization of caspase-8 to inhibit apoptosis. RIP1K, by acting as a scaffold protein within this signaling complex,

may provide a critical activation signal for NF-κB[222] or facilitate amplification of NF-κB–mediated transcription.[223-225] Either way, RIPK1 activity can contribute to cellular survival under conditions of TNFR1 ligation. MEFs deficient for RIPK1, but not those expressing a kinase-dead version of RIPK1, are more sensitive to TNF-induced apoptosis.[223] Furthermore, intestine-specific ablation of RIP1K in adult mice is associated with a postnatal lethality, characterized by extensive apoptosis of the gut epithelium and generalized inflammation. This RIPK1 null phenotype can be rescued by the tissue specific co-deletion of caspase-8 or FADD but not by the introduction of catalytically inactive RIPK1.[226,227] The observed accumulation of cIAP1 and TRAF2 in tissues lacking RIPK1 is consistent with a model in which the alleviation of cIAP1/TRAF2–mediated stabilization of NF-κB–inducing kinase (NIK) leads to increased NF-κB activity that in turn promotes TNF transcription to induce apoptosis. This model is supported by the rescue, albeit partial, of the excessive apoptosis associated with gut-specific ablation of RIPK1, as well as *Ripk1*$^{-/-}$/*Ripk3*$^{-/-}$-induced lethality, by the co-deletion of TNFR1.

RIPK1 can also mediate FADD-dependent caspase-8 activation to promote apoptosis in response to several stimuli, including depletion of cIAPs, TNF, and TLR activation.[228-230] Homotypic interaction between the DDs of FADD and RIPK1 to recruit and activate procaspase-8 constitutes the so-called "ripoptosome." TLR-induced ripoptosome formation is mediated by interaction of the respective RHIM domains of TRIF and RIPK1.[231-233] This prodeath role of RIPK1 is substantiated by several genetic studies in which the absence of RIPK1 (or caspase-8) can rescue the embryonic lethality associated with catalytically inactive RIPK3$^{D161N}$.[215] In a similar manner, ablation of MLKL, which is the target of RIPK3 and is required for necroptosis, instead can promote apoptosis via an RIPK3/RIPK1/caspase-8–dependent pathway.[234]

The death-inducing role of RIPK1 is also evident in RIPK3/MLKL–dependent necroptosis, but only under circumstances in which caspase-8 activity is disrupted. RHIM-mediated interaction between the RIPK1 and RIPK3 is essential for RIP3K activity and the induction of necroptosis by ligation of TNFR1. For this, RIP1K absolutely requires its kinase activity, although the precise reason remains unclear, because RIPK1-mediated phosphorylation of itself, but not RIPK3, can be detected. Potentially, therefore, autophosphorylation and a consequent conformational change of RIPK1 may mediate its interaction with RIPK3, or alternatively, additional RIPK1 substrates that participate in the activation of RIPK3 to promote necroptosis remain to be identified.

## RIPK1-Independent Necroptosis

Whereas the ubiquitous activity of RIPK1 can be utilized for cellular survival, apoptosis, or programmed necrosis, RIPK3/MLKL–dependent necroptosis can also proceed in the absence of RIPK1[235] (Figure 28-4A). TRIF, the signaling adapter associated with activation of TLR3 or -4, can directly bind and activate RIPK3 to promote necroptosis in the absence of RIPK1.[236] TRIF-independent TLR signaling can also induce necroptosis by autocrine or paracrine-mediated TNF activities.[237] The DNA sensor, DNA-dependent activator of IFN-regulatory factors (DAI), is activated by viral infection and contains an RHIM domain that can also directly engage RIPK3 function to promote necroptosis in the absence of RIPK1.[238-240] Type I (α/β) and II (γ) IFNs can also engage necroptosis under conditions in which either caspase-8 or FADD are inactivated. Although IFN-induced necroptosis is mediated via a RIPK1-RIPK3 complex, the kinase activity of RIPK1 is not required. Instead, the RNA-responsive kinase (PKR) interacts with RIPK1 to induce the formation of RIPK1-RIPK3 heterodimers and necroptosis.[235,241] If RIPK1 is present under any of these circumstances, necroptosis can only proceed if the proapoptotic FADD–caspase-8–FLIP axis is disrupted.

These observations are consistent with RIPK1-dependent inhibition of RIPK3/MLKL–mediated necroptosis. RIPK1 can suppress RIPK3-mediated necroptosis via two known pathways. The first requires its association with FADD to assemble the FADD–caspase-8–FLIP complex to facilitate the formation of FLIP–caspase-8 heterodimers (Figure 28-4B). These heterodimers, although unable to cleave and activate caspase-8 to promote apoptosis,[242-244] are sufficiently active to cleave components of the necrosome and its regulators,[198,245] including both RIPK1 and RIPK3, and suppress MLKL-mediated necroptosis.[246-248] In an alternative scenario, necroptosis initiated by caspase inhibition and TLR ligation or IFN treatment is inhibited by the disruption of RIPK1 activity with the RIPK1 inhibitor necrostatin (Figure 28-4C).[236,237,249] Furthermore, a catalytically inactive RIPK1 mutant, or RIPK1 bound to necrostatin, can still associate with RIPK3 when triggered by TLR signaling and caspase inhibition, but it does so in a manner that precludes interaction between RIPK3 and MLKL.[217]

## Pro-inflammatory Effects of Programmed Necrosis

Insight into the molecular mechanisms of programmed necrosis, although informative, is fueling the need for constant reevaluation of the critical factors required for programmed necrosis. As yet, a complete understanding of the role of programmed necrosis in regulating immune function remains to be delineated. However, with the validation of shared signaling components in necroptosis and pro-inflammatory pathways, it seems reasonable to speculate that we are only at the very beginning of appreciating the ramification of programmed necrosis in the context of immune regulation and how disruption of these pathways may underlie the etiology of numerous pathophysiologies.

Murine models in which the expression and/or function of RIPK3 or MLKL are disrupted have provided at least some insight into the in vivo role of necroptosis in inflammation. Deletion of RIPK3 prevents the spontaneous inflammation associated with tissue-specific deletion of FADD in the intestinal epithelial compartment[250] and skin keratinocytes,[251] consistent with a pro-inflammatory role of necroptosis. Similarly, the inflammatory consequences of tissue-specific deletion of caspase-8 in either the gut or the skin are abrogated by the co-deletion of RIPK3.[252] Deletion of RIPK3 or MLKL also ameliorated necroptosis of keratinocytes and immune infiltration into skin deficient for epidermal RIPK1.[226] Necrostatin has also been a useful tool with which to interrogate the significance or necroptotic

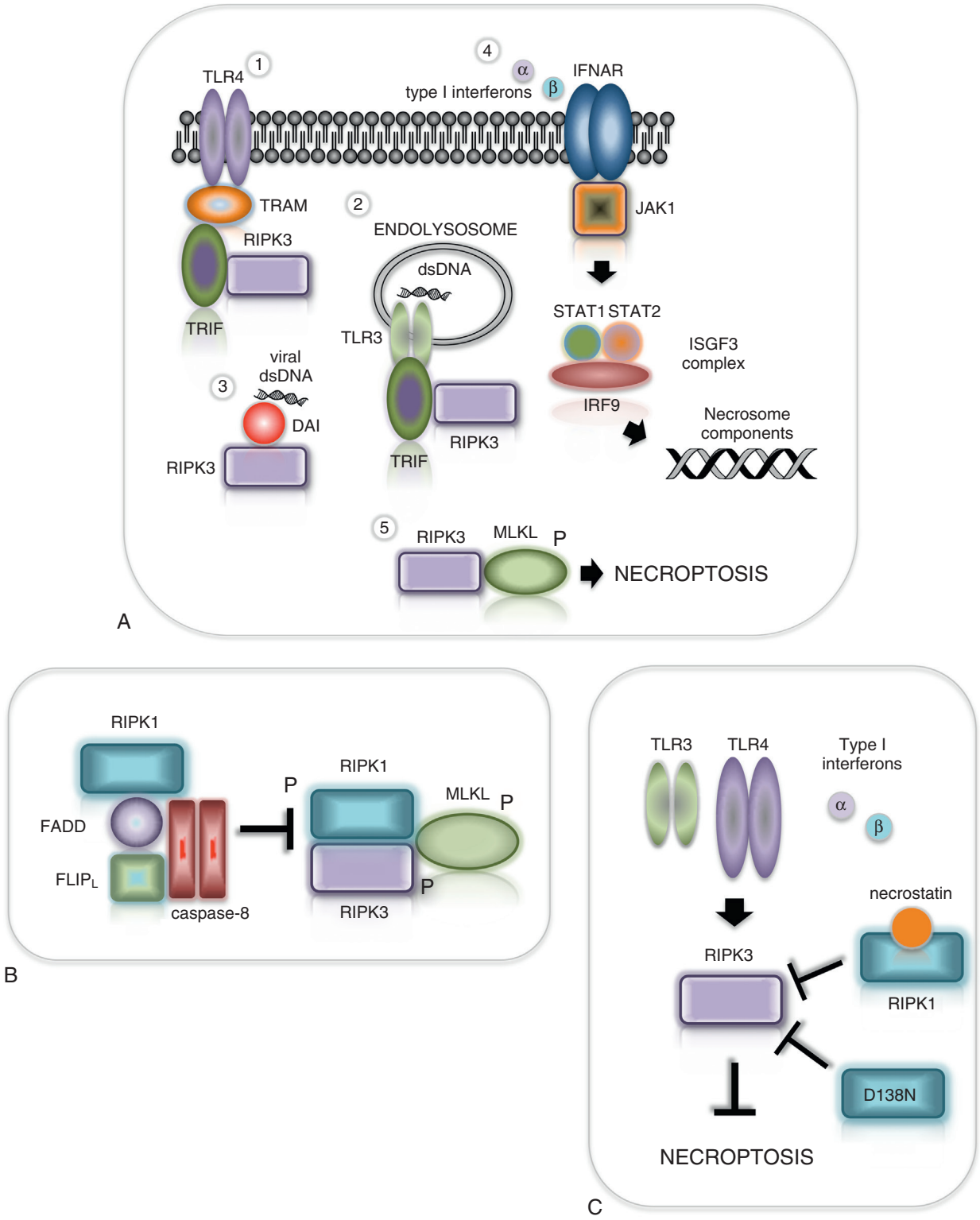



**Figure 28-4** "Signal 2"–dependent necroptosis. **A,** Necroptosis can be engaged in a receptor-interacting protein kinase 1 (RIPK1)-independent manner in response to Toll-like receptor 4 (TLR4) (1), TLR3 (2), DNA-dependent activator of IFN-regulatory factor (DAI) (3), and type I interferons (4). Each of these pathways result in RIPK3-dependent recruitment and activation of mixed lineage kinase domain-like (MLKL) to promote necroptosis (5). RIPK1 can actively suppress RIPK3 via two pathways, shown in **B** and **C**. **B,** RIPK1–death domain (DD) interacts with FAS-associated protein with death domain (FADD)-DD to recruit FLICE-like inhibitory protein long form ($FLIP_L$) and caspase-8, resulting in the inhibition of caspase-8–induced apoptosis, but with retention of sufficient caspase-8 activity to cleave and inactivate RIPK1 and RIPK3 to disrupt the MLKL-activating complex. **C,** Alternatively, kinase inactive RIPK1 or its inhibition by necrostatin also suppress TLR-induced necroptosis. Therefore, kinase inactive RIPK1 can block RIPK3-MLKL–dependent necroptosis. dsDNA, Double-stranded DNA; dsRNA, double-stranded RNA; IRF9, interferon regulatory factor 9; ISGF3, interferon-stimulated gene factor 3; STAT, signal transducer and activator of transcription; TRAM, translocation-associated membrane; TRIF, TIR-domain–containing adapter-inducing interferon-β. *(From Weinlich R, Green DR: The two faces of receptor interacting protein kinase-1.* Mol Cell *56:469–480, 2014, and Pasparakis M, Vandenabeele P: Necroptosis and its role in inflammation.* Nature *517:311–320, 2015.)*

signaling events under a variety of physiologic conditions and to determine if the inhibition of necroptosis may be therapeutically advantageous.[253] The realization, however, that RIPK1 function is dispensable for RIPK3-MLKL–dependent necroptosis under certain conditions should be factored into any conclusion that relies solely on the use of necrostatin. Nonetheless, in several models that mimic a variety of pathophysiologic conditions, including ischemic brain injury, myocardial infarction, and kidney ischemia-reperfusion injury, necrostatin was shown to provide significant projection against tissue damage and, in some cases, offset detrimental immune cell infiltration.[189]

The mechanism(s) by which necroptosis triggers inflammation has/have yet to be fully defined but likely involve(s) the associated release of DAMPs to trigger a myriad of inflammatory signaling events[254] or act indirectly by compromising the integrity of epithelial barriers in tissues such as the skin or gut.[255] Perhaps more intriguingly, however, is the recent delineation of RIPK-3–dependent pro-inflammatory pathways that proceed in the absence of necroptosis.[119,122]

## AUTOPHAGY

Autophagy is a cellular garbage disposal mechanism that is engaged in response to multiple stresses including protein aggregation, metabolic dysfunction, and cancer chemotherapeutics to induce degradation of long-lived proteins and damaged organelles. Three distinct pathways can mediate autophagic degradation: microautophagy, chaperone-mediated autophagy, and that most commonly described and discussed here, macroautophagy (herein, "autophagy" will be used to mean macroautophagy). Autophagy is largely regarded as a catabolic survival mechanism that is initiated during periods of stress, such as nutrient deprivation, to generate an alternative intra-cellular energy source and maintain bioenergetic balance. However, autophagy-induced cell death (ACD) is now emerging as a bona fide pathway, triggered by excessive activation of the pathway.[256-259] Autophagy is associated with several diseases including type II diabetes, neurodegeneration, and Chiron's disease, rendering it an attractive target for potential clinical intervention.[260] As we learn more about the underlying mechanisms of autophagy, it is becoming apparent that one of its primary roles is in the context of immune function and that numerous immunoregulatory events intersect with the autophagy signaling network to provide protection against disease and microbial infection.[261,262]

### Molecular Mechanisms of Autophagy

The process of autophagy, in general terms, comprises the inclusion of cytosolic components within the phagophore or isolation membrane, which then matures into a double membrane–bound vesicle, termed the *autophagosome*. The source of lipids to generate the double membrane remains somewhat obscure but likely arises from multiple sources, including the ER membrane. The autophagosome then fuses with lysosomes or endosomes to generate the autolysosome, in which digestion of the vesicular cargo proceeds to generate macromolecules for reuse in cellular metabolic processes. The molecular details of autophagy are complex but for the most part are fairly well characterized (Figure 28-5A, B). The extensive family of autophagy-related genes (ATGs) includes those constituting the critical core machinery necessary for assembly of the autophagosome—the ATG1/UNC51-like kinase (ULK) complex; the ATG12 and ATG8 (LC3) conjugation system; the class III phosphatidylinositol-3-kinase (PI3K)/VPS34 complex; and the transmembrane proteins ATG9 and vacuole membrane protein 1 (VMP1).

This multistep ATG-dependent autophagic process is under the master control of the mammalian target of rapamycin (mTOR) complex.[263,264] mTOR, a serine-threonine kinase, is responsive to growth factor signaling, changes in nutrient availability, and energy status. It functions within the RAPTOR-containing mTORC1 complex[265] to integrate signals arising from the AKT pathway under conditions of nutrient availability to suppress autophagy.[264] However, when nutrients are limited, mTOR activity is inhibited and the autophagic process is initiated. The downstream mediator of mTOR activity is the ULK complex, comprising several proteins that are required for autophagosome formation.[266,267] In mammals, there are three ULK members, ULK1, ULK2, and ULK3, all of which are reportedly involved in the autophagic pathway. However, it remains unclear whether their respective roles differ mechanistically and/or are associated with different stress-inducing stimuli and/or cell type.[259] ULK1 and ULK2 are part of a large complex containing ATG13 and the scaffold protein FIP200.[266-268] Importantly, although this complex is assembled independently of nutrient availability, it is regulated in a nutrient-dependent manner. Nutrient sufficiency stimulates the incorporation of mTORC1 into the ULK1 complex to facilitate mTOR-mediated phosphorylation and inhibition of ULK1, ULK2, and ATG13. However, under conditions of starvation, leucine deprivation, and/or rapamycin-mediated suppression of mTOR, mTORC2 dissociates from the complex and the activity of ULK1 and -2 is regained, resulting in the phosphorylation of FIP200 and ULK1 in a ULK1-dependent manner.[266,267]

Beclin-1 (Bcl-2 interacting coiled coil protein) (mATG6) is essential for mammalian autophagy[269] and forms three independent complexes that collectively regulate the next phase of autophagy to generate the mature autophagosome. These three complexes comprise (1) Beclin-1, VPS34 (a PI3K that generates phosphatidylinositol triphosphate [$PIP_3$]), VPS15, and ATG14L; (2) Beclin-1, VPS34, VPS15, and ultraviolet irradiation resistance-associated gene (UVRAG); and (3) Beclin-1, VPS34, VPS15, UVRAG, and Rubicon (RUN domain and cysteine-rich domain containing Beclin 1–interacting protein).[270,271] The first complex initiates autophagy and is thought to generate the requisite $PIP_3$ and "mark" the phagophore membrane for the subsequent recruitment of additional ATG proteins, such as ATG16L and LC3. UVRAG, a component of the second complex, competes with ATG14L for binding to Beclin-1 to promote autophagosome formation via the stimulation of VPS34 activity. Rubicon opposes this activity by direct interaction with VPS34 to suppress $PIP_3$ generation and autophagosome assembly.

After initiation of phagophore formation by the VPS34-Beclin-1 complex, expansion of the vesicular membrane is completed by two ubiquitination-like conjugation

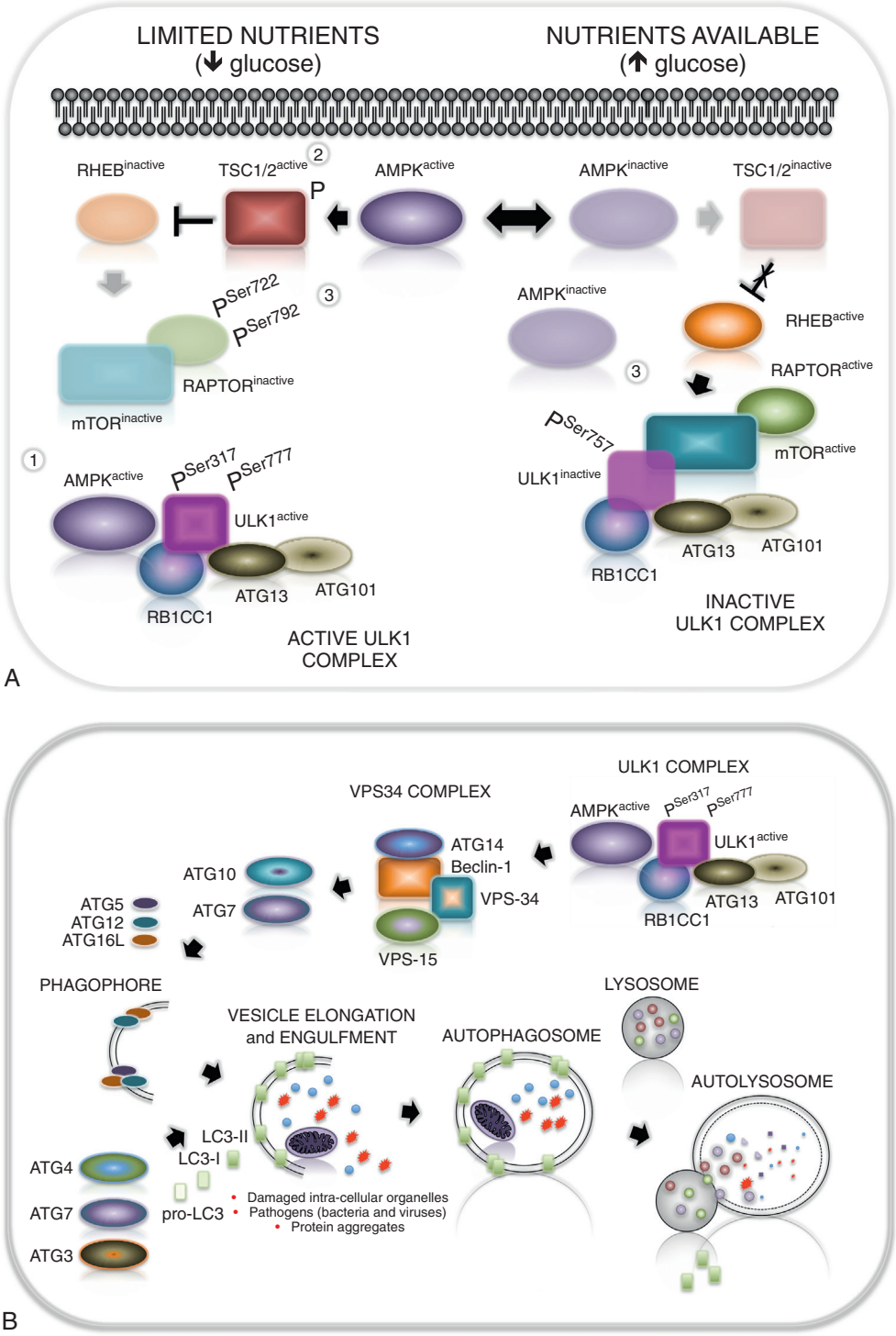



**Figure 28-5** Autophagy is engaged by multiple stresses including protein aggregates, intra-cellular pathogens, metabolic dysfunction, and cancer chemotherapeutics to induce degradation of long-lived proteins and damaged organelles. **A,** Upstream signaling: The mammalian target of rapamycin (mTOR) and UNC51-like kinase (ULK) complexes function in an opposing manner, with mTOR acting to inhibit autophagy and the ULK complex acting to initiate it. During times of glucose restriction or other stresses, adenosine monophosphate-activated protein kinase (AMPK) phosphorylates and activates ULK1 (Ser317 and Ser777) (1) to initiate assembly of the ULK complex comprising ULK1, autophagy-related gene (ATG)13, ATG101, and RB1CC1 (or FIP200). This complex is critical for the initiation of autophagy. AMPK negatively regulates the mTOR pathway by phosphorylating both tuberous sclerosis (TSC) (2) to inhibit Ras homolog enriched in brain (RHEB) and RAPTOR (Ser722 and Ser792) (3), a protein necessary for mTOR activity. Conversely, under conditions of nutrient sufficiency, AMPK activity is suppressed, TSC is rendered inactive, and RHEB, now active, phosphorylates ULK1 (Ser757) to disrupt its interaction with AMPK and activates mTOR. **B,** Downstream events: Activation of the ULK complex in response to stresses such as nutrient deprivation initiates assembly of the phosphatidylinositol 3 (PI3) kinase VPS34 complex, which in turn generates PI3P, an important component of the autophagosome membrane. The initiator membrane or phagophore, the precise source of which remains unclear, is then decorated with the ATG5-ATG12-ATG16L complex together with lipidated LC3 (LC3-II) via the combined activities of two ATG conjugation complexes—ATG10 and -7 and ATG3, -4, and -7. The double membrane then undergoes elongation to encapsulate the cargo destined for disposal including pathogens, dysfunctional organelles, and large insoluble protein aggregates. This action forms the autophagosome, which fuses with a lysosome to form the autolysosome. The lysosomal proteases then mediate proteolytic destruction of the cargo, thereby inactivating pathogens and/or providing nutrients for recycling and restoration of bioenergetic potential during conditions of limited nutrient availability. *(From Yang Z, Klionsky DJ: Mammalian autophagy: core molecular machinery and signaling regulation.* Curr Opin Cell Biol *22:124–131, 2010.)*

pathways, both of which require the E1 ligase activity of ATG7. In one, the small protein ATG12 is conjugated to ATG5 using ATG7 and ATG10 as the E1 and E2 ligases, respectively. The ATG5-ATG12 conjugate then interacts with ATG16L to form a large complex. In the second pathway, pro-LC3 is first cleaved by ATG4 to generate cytosolic LC-1, which is then conjugated to the lipid, phosphatidylethanolamine, using ATG7 and ATG3 as the E2 ligase. The lipidated form of LC3, LC3-II, is inserted in the autophagosomal membrane. Although the conversion of LC-I to its lipidated LC3-II form is commonly used as a marker for autophagy, LC3-associated phagocytosis (LAP), a process that shares common signaling components with autophagy, is also characterized by the formation of membrane-bound vesicles decorated with LC3-II.[272] Fusion of the mature LC3-II$^+$ autophagosome with lysosomes to form autolysosomes then initiates proteolysis and digestion of the vesicular cargo.

The biologic significance of autophagy is evident in the context of tumor growth. Heterozygous deletion of Beclin-1/ATG6 promotes tumor formation in several murine models, and monoallelic loss in several human tumor types is also frequently observed. Furthermore, several Beclin-1-interacting proteins have been implicated in tumorigenesis and the regulation of proliferation including BIF-1 or Endophilin B1, AMBRA or activating molecule in beclin-regulated autophagy, and BCL-2. The role for Beclin-1 and autophagy in tumor suppression presumably reflects its ability to effectively remove potentially toxic protein aggregates and dysfunctional organelles to limit the harmful consequences of ROS production and genomic lesions. However, cell death is also a potent tumor suppressor mechanism, as evidenced by the pro- or antitumorigenic activities of numerous regulators of apoptosis, including BCL-2 and p53. It is therefore conceivable that the tumor suppressive activity of Beclin-1 may instead reflect its role in promoting autophagic cell death.

However, a direct role for autophagy in promoting cell death has proven difficult to substantiate. Historically, delineation between the detection of autophagic markers arising as a consequence of or coincidentally with the destruction of dead cells and cell death arising directly from autophagy-generated signals has confounded the unambiguous identification of ACD. However, the availability of genetic tools has now provided definitive characterization of a form of autophagic cell death that can be triggered by overactivation of the autophagy pathway. Accordingly, under some circumstances, the deletion of one more component of the autophagy pathway is sufficient to inhibit cell death—a criterion that must be applied to determine the authenticity of cell death driven by autophagy.

## Overeating Is Good for You—Autophagy and the Immune System

Autophagy regulation via NLRs—that is, inhibition of inflammasome formation via deletion of some NLRs—can promote autophagy, suggesting that the inflammasome may negatively regulate autophagy, or perhaps that suppression of pyroptosis engages autophagy to promote an alternative type of cell death. Conversely, evidence suggests that autophagy inhibits the inflammasome and that defects or polymorphisms in autophagy are associated with inflammatory disease. For example, systemic lupus erythematosus and Crohn's disease are associated with polymorphisms in *Atg5* and *Atg16*, respectively.

## Mitophagy

Mitophagy is a specialized autophagic process that deletes dysfunctional organelles to maintain overall mitochondrial integrity and to provide the necessary components for mitochondrial regeneration. The removal of damaged mitochondria is mediated by their selective targeting to the LC3$^+$ phagophore to promote engulfment and degradation by the autophagosome. Mitochondrial effector proteins can initiate one of two mitophagy pathways—ubiquitin- or receptor-mediated—triggered by ubiquitin-tagged proteins or those containing LC3-interacting regions (LIRs), respectively.

The outer mitochondrial membrane LIR-containing proteins, NIX1, also known as BNIP3L, BNIP3 (BCL-2 and adenovirus E1B 19-kDa-interacting protein 3), and FUNDC1 (FUN14 domain containing 1), trigger mitophagy under different physiologic conditions. NIX1, for example, mediates mitochondrial removal in the reticulocyte, which is a critical step during the development of mature erythrocytes, whereas both BNIP3 and FUNDC1 are important for the removal of mitochondria as a consequence of hypoxia. Serine phosphorylation adjacent to the LIR domain of BNIP3 and dephosphorylation/phosphorylation of FUNDC1 regulate their interaction with LC3 under conditions of reduced oxygen. Externalization of cardiolipin, normally localized on the inner mitochondrial membrane, also facilitates binding to LC3 and the removal of damaged mitochondria in neuronal cells.

Ubiquitin-dependent mitophagy is mediated by the coordinated activities of the serine-threonine kinase PTEN-induced putative kinase 1 (PINK1) and Parkin, an E3 ligase. Accumulation of PINK1 on the outer surface of mitochondria triggers the kinase-dependent recruitment and activation of Parkin to promote the attachment of ubiquitin to several proteins within the outer mitochondrial membrane (OMM). The constitutive turnover of PINK1 prevents the inappropriate deletion of mitochondria and is regulated by transporter outer membrane (TOM)-dependent translocation to the inner mitochondrial membrane and proteolysis by the rhomboid protease, presenilins-associated rhomboid-like protease (PARL). However, upon loss of outer membrane potential or in response to the accumulation of excess unfolded proteins in the mitochondrial matrix, the import of PINK1 is inhibited, proteolysis is prevented, and instead it accumulates on the surface of the mitochondria. PINK1-mediated phosphorylation of a serine residue within both the ubiquitin domain of Parkin and ubiquitin itself triggers a conformational change in Parkin and acquisition of its E3 ligase activity. Parkin-mediated Lysine 48–linked ubiquitination of several substrates, including the fusion proteins mitofusin-1/mitofusin-2, encoded by the *Mfn1/Mfn2* genes, promotes their degradation to facilitate mitophagy. A number of additional accessory proteins appear to be critical for Parkin-mediated mitophagy, although their precise roles remain unclear. These include several additional Parkin substrates and

the autophagy adapter proteins ULK1, ATG14, and ATG16L1, all of which translocate to mitochondria during mitophagy.

The physiologic significance of ubiquitin-mediated autophagy is exemplified by the frequent mutation of PINK and Parkin and accompanying mitochondrial dysfunction in certain autosomal recessive forms of Parkinson's disease. Mitophagy also shares many signaling components with xenophagy, an autophagy-dependent process that mediates the destruction of intra-cellular bacteria. Indeed, Parkin has been implicated in the ubiquitination and clearance of *Mycobacterium tuberculosis* and in the susceptibility of mice and *Drosophila* to several intra-cellular infections. Intriguingly, polymorphisms in the human Parkin gene, *PARK2*, correlate with an increase in susceptibility to *Mycobacterium leprae* and *Salmonella enterica*.

## Mitophagy and Immune Function

The role of mitochondria in various types of cell death clearly has implications in immune regulation. However, additional mitochondria-dependent activities, including their role in MAVS-mediated signaling and as a source of DAMPs such as ROS and mitochondrial DNA underscore the importance of mitochondrial integrity and the role of mitophagy in innate immune responses.

Clearly, sustaining mitochondrial function is essential not only for maintaining oxidative phosphorylation, a critical source of ATP, but also for regulating cellular oxidation via ROS production. Damaged mitochondria are a rich source of ROS, requiring efficient removal by mitophagy to limit potential oxidative damage. Mitochondria-mediated ROS production as a consequence of defective mitophagy and accumulation of damaged mitochondria is a potent mediator of NLRP3-dependent inflammasome activation and IL-1β production. RIPK2-dependent phosphorylation of ULK1 to promote mitophagy similarly limits aberrant NALP3 inflammasome assembly and inflammation during viral infection. Effective anti-viral immunity also includes a role for the mitochondria-localized MAVS, an adapter protein that promotes inflammation in a RIG-1–dependent manner. Negative regulators of MAVS signaling include the ubiquitin ligases, SMURF1 (SMAD ubiquitination regulatory factor 1), MUL1 (mitochondrial ubiquitin ligase 1), and Gp78, all of which have been implicated in the regulation of mitophagy. Furthermore, dissipation of mitochondrial membrane potential severely limits MAVS-mediated signaling. Maintaining mitochondrial fidelity is therefore an essential component of immune regulation.

## "Like All Successful Relationships, It's Complex"—Interaction between Cell Death and Immune Function

A complementary relationship between the immune system and cell death exists. As we have discussed at points throughout this chapter, components of inflammatory responses, such as TNF and IL-1β, can induce cell death and, in a reciprocal manner, dead or dying cells can have a significant impact on immune function. Cell death plays critical roles in development of the immune system, maintenance of normal homeostasis, and in regulating immune function in response to pathogenic invasion. However, the specific type of cell death, how it is induced, and the signaling and molecular events associated with it can differentially alter the inflammatory response.

TLRs and NLRs are vital for the detection pathogens by responding to PAMPs, pathogen-derived components that trigger TLR and NLR signaling events to engage inflammation. At the heart of these responses in most cases is the inflammatory caspase, caspase-1, which is activated by inflammasome assembly to cleave, activate, and secrete IL-1β and IL-18. The efficiency with which this pathway can detect, respond, and eliminate pathogens is matched only by the equally effective mechanisms expressed by pathogens to subvert detection, disrupt critical pro-inflammatory signals, and evade elimination. Pathogens are master manipulators, evolved to avail of beneficial host-derived pathways and to disrupt the mechanisms detrimental to their replication and survival. It is therefore no surprise that both viruses and bacteria have evolved multiple ingenious mechanisms to evade host-mediated elimination by expressing proteins that regulate components of the apoptotic and necroptotic signaling machinery.

Caspase-1 is at the epicenter of inflammatory responses and host immune defense by virtue of its ability to cleave and activate pro-IL-18 and pro-IL-1β, both of which have diverse roles in immune regulation and protection against bacterial and viral infection. It is therefore no surprise that pathogens have evolved to express multiple virulence factors able to disrupt inflammasome assembly and/or its downstream pro-inflammatory effects (reviewed in references 273-275).[273-275] A few examples are provided.

Poxvirus family members express one of several so-called "serpins," including cowpox virus protein cytokine response modifier A (CRMA), SPI-1, and SPI-2 that directly inhibit the enzymatic activity of caspase-1 and caspase-8.[276-278] CRMA acts as a pseudosubstrate for caspase-1, forming a covalent bond with the active site cysteine in caspase-1 to render it catalytically inert.[279,280] Additional mechanisms through which virulence factors can disrupt the pro-inflammatory activity of inflammasomes include the secretion of soluble scavenger proteins that neutralize host-derived cytokines such as an IL-1β receptor able to bind to and inactivate host-derived IL-1β[281] and soluble IL-18 inhibitory binding proteins to neutralize host-derived IL-18.[282] Several virulence factors can also directly disrupt inflammasome formation, primarily by the expression of decoy proteins. Examples include the herpes virus–derived protein, Orf63, an NLRP1 homolog that lacks both the CARD and pyrin interaction motifs that, by associating with host NLRP1 or NLRP3, prevents caspase-1 recruitment and activation.[283] Virally derived POPs also act as decoys to sequester PYD-containing host proteins involved in inflammasome assembly, including the adapter protein ASC.[284,285]

Bacterial factors that function to dysregulate the inflammasome–IL-1β/IL-18 axis include the *Yersinia* Yop proteins, negative regulators of Rho guanosine triphosphatases that inhibit caspase-1 activation[286] and mask recognition by the NLRs, NLRP3 and NLRC4, to prevent inflammasome assembly.[287] Several other bacterial-derived proteins with a diverse array of activities negatively regulate the assembly of different types of inflammasome or directly inhibit caspase-1 activity, although the precise mechanisms

mediating these effects remain unclear. Examples of these proteins include ExoU, a protein that harbors phospholipase A2 activity, the ADP-ribosyl transferase, ExoS,[288,289] Zmp1, a $Zn^{2+}$ metalloprotease, and the lipid flippase *mviN*.

Apoptotic caspases orchestrate the demise of a cell and therefore by definition would appear as very vulnerable targets to those unscrupulous pathogens intent on survival. As we have already discussed, mammalian cells also express their own caspase inhibitor, XIAP. But so too do pathogens—in fact, the first IAP to be identified was isolated from baculoviruses and provided the basis for their subsequent identification in vertebrates based on BIR domain homology. CRMA, expressed by pox viruses and a potent inhibitor of caspase-1 function (previously discussed) can also inhibit the catalytic activity of caspase-8 function. Baculovirus also expresses p35 protein, which, once cleaved and processed, functions as a pseudosubstrate for caspases, thereby binding to and inhibiting the catalytically active cysteine and preventing proteolysis of target substrates.

Herpes viruses, including murine cytomegalovirus (MCMV), can circumvent death of the infected host cell by expressing the viral inhibitor of caspase-8 processing (vICA). Clearly, although this activity may effectively suppress caspase-8–dependent apoptosis and limit viral replication, it coincidentally triggers necroptosis–an alternative cell death pathway that also has a critical role in anti-viral immunity. However, cells infected by MCMV neither succumb to apoptosis nor necroptosis because it also expresses the RHIM-like domain containing protein, vIRA, product of the *M45* gene that prevents interaction between RIPK3 and DAI, a sensor of viral infection. The viral protease NS3/4a binds to mitochondria where it cleaves an important signaling adapter, MAVS, thus preventing viral clearance by endogenous IFN production. Viral infection also triggers TNF production as part of the host defense mechanism to induce necroptosis. Both $Ripk1^{-/-}$ and $Ripk3^{-/-}$ cells are refractive to TNF-induced programmed necrosis as evidenced by abrogation of necrosis and inflammation in mice deficient for RIPK3. However, the failure to induce necroptosis manifests as uncontrolled viral replication, an inability to clear infection, and, ultimately, death of the host. Thus it is possible that necroptosis may act as a form of defense against viral infection.

Bacteria have also evolved multiple mechanisms to regulate cell death and inflammatory pathways, both of which can be detrimental to their survival (reviewed in references 274 and 275). TNF-induced signaling is frequently targeted by components of pathogenic bacteria to prevent bacterial elimination induced by either TNF-induced inflammation or cell death. Cell death also serves as a rich source of PAMPS to trigger inflammation and limit bacterial replication. NF-κB, a component of TNF-induced signaling, is a critical regulator of inflammation via the transcriptional induction of several cytokines. NleB and various homologs, expressed by *Salmonella* and other pathogenic *E. coli* strains, are potent inhibitors of TNF-induced NF-κB activation. Several mechanisms exist, including that mediated by the unusual ability of NleB proteins to modify arginine residues via N-acetylglucosamine transferase activity. Strikingly, NleB can modify a highly conserved arginine residue critical for intermolecular interactions in the DD of multiple components of TNF-induced signaling, including TRADD, FADD, TNFR1, RIPK1, FAS/CD95, DR3, and the TRAIL receptors.[290] Translocated-intimin receptor (Tir), a bacterial protein characterized by ITIM-like motifs (immunoreceptor tyrosine-based inhibition motifs), is also an effective inhibitor of NF-κB. Although the precise mechanism by which this process is mediated remains unclear in mammalian cells, ITIMs are present in the cytoplasmic signaling domains of the T cell and B cell receptors and function to negatively regulate proimmune signaling events and limit effector function, suggesting that bacterial Tir may function in an analogous manner.

As we have discussed, ubiquitylation of several components of the TNF-R-signaling complex can profoundly alter biologic outcome and determine if a cell lives or dies. Accordingly, several mechanisms by which bacterial proteins can disrupt ubiquitylation events to alter the stability of signaling intermediates in the TNF-R complex have also been identified. For example, NleE and related proteins in several bacteria, including *Shigella* and *Salmonella*, function to modify critical cysteine residues in proteins necessary for the coordination of ubiquitylation events required for NF-κB activation. Other bacterial proteins can disrupt NF-κB activation by inhibiting the degradation of IκB, either by directly interacting with ubiquitin conjugates or by targeting NEMO for proteasomal destruction. The subunits of NF-κB, p65, and p50 can also be directly cleaved and inactivated by a bacterial zinc metalloprotease NleC.[291]

As previously discussed, hetero- and homodimerization of pro- and anti-apoptotic BCL-2 proteins regulate mitochondrial events, specifically MOMP, that are required for the activation of caspase-9 to promote or suppress apoptosis. As we might predict, the significance of this event does not escape the wily virus. To that end, several viral BCL-2 proteins have been identified that function to mimic many of the activities of mammalian prosurvival proteins to neutralize the effector function of BAX and BAK and maintain cellular survival. Although in many cases v-BCL-2 proteins display very limited sequence homology to their mammalian orthologs, by virtue of their structural plasticity and ability to adopt the conformations required to mimic BCL-2 function, they are able to disrupt the function of proapoptotic BCL-2 proteins via association with the effector molecules BAX and BAK to inhibit their MOMP-inducing activity or, alternatively, associate with and sequester the BH3-only proteins. Although v-BCL-2 proteins can effectively inhibit cell death, the affinity for proapoptotic proteins is much reduced compared with mammalian BCL-2. It is intriguing to speculate that perhaps the "suboptimal" performance of v-BCL-2 proteins may be supplemented by their abilities to regulate additional signaling pathways important for cell death induction. Indeed, recent evidence suggests that the vaccinia BCL-2 ortholog, F1L, can both inhibit caspase-9 activity and disrupt inflammasome assembly. With much to learn about the function of mammalian BCL-2 proteins, it remains only a matter of time before new findings instruct our understanding of v-BCL-2 proteins or, as is often the case, viruses teach us something new.

## CONCLUSION

As we uncover the molecular characteristics and regulatory components of different cell death pathways and their

relevance to specific physiologic scenarios, the more likely it is that pharmacologic intervention may be utilized to recover normal function under pathophysiologic conditions. To that end, several pharmacologic regulators of cell death are currently under development and/or in the clinic for their potential application to a range of pathologies, including those characterized by aberrant cell death and immune dysfunction.

## Caspase Inhibitors

Currently, no caspase inhibitors are in clinical trials. Several pharmacologic inhibitors used experimentally include the peptide-based inhibitors z-VAD-fmk and qVD-oph, and the small molecule inhibitor, IDN-6556.

## Necroptosis Inhibitors

The role of the RIP kinases in necroptosis has prompted the search for inhibitors of these kinases. The RIPK1 inhibitor Necrostatin-1, widely used experimentally, also inhibits the enzyme IDO, which has immunologic consequences. Another RIPK1 inhibitor, Necrostatin-1s, does not have this cross-reactivity. RIPK3 inhibitors are also described, including GSK2399872B. Necroptosis can also be inhibited by targeting MLKL. Necrosulfonamide inhibits human but not murine MLKL, and as yet, no rodent inhibitors have been identified. Currently, no inhibitors of necroptosis are approved for human use.

## BH3 Mimetics and BCL-2/MCL-1 Inhibitors

Several inhibitors of anti-apoptotic BCL-2 proteins are described, including Obatoclax (GX15-070), Gossypol (AT-101), a natural phenolic compound and the Gossypol derivative, Sabutoclax (ONT-701). However, none of these agents have been shown to have specificity for their targets. Of more potential utility are the BH3 mimetic compounds, which include the experimental compounds ABT-737 (inhibitor of BCL-$X_L$, BCL-2, and BCL-w), WEHI-539 (a BCL-$X_L$ selective inhibitor), and UMI-77 (an MCL-1 selective inhibitor) and the drugs navitoclax (ABT-263) (inhibitor of BCL-$X_L$, BCL-2, and BCL-w), venetoclax (ABT-199) (a BCL-2 selective inhibitor), and A-1155463 (a BCL-$X_L$ selective inhibitor). The BH3 mimetics show exquisite specificity for the anti-apoptotic Bcl-2 proteins and are in clinical trials for a number of human malignancies.

## IAP Inhibitors

As previously discussed, ubiquitination is a powerful modulator of multiple pathways, including the TNFR1 signaling complex, to regulate the selective engagement of cellular survival or the death pathways, apoptosis and necroptosis. These include the NF-κB inhibitory complex IκB to induce its degradation, thereby promoting NF-κB transcriptional activation. RIPK1 is also regulated by IAP-mediated ubiquitination, and it is this activity that helps to divert TNFR1-mediated signaling toward survival by accelerating RIPK turnover. The use of SMAC mimetics (SMs), which function as antagonists of IAP function and promote the auto-ubiquitination and degradation of IAPs, can overcome cellular survival and increase the sensitivity of cells to RIPK-mediated necrosis. This approach could be of clinical importance by circumventing the commonly observed resistance of cancer cells to apoptotic death and instead evoke death in an RIPK-dependent manner. As we begin to understand how RIPK-mediated death may intersect with immune function, it may also be possible to utilize SMs to prime immune function against tumor-derived antigens. Harnessing or unleashing the hosts' own defense mechanism via activation of the immune system to directly target tumor cells, which are often able to evade immune detection, is an approach receiving an enormous amount of focus. It would seem feasible to suggest that tumor cells, frequently characterized by mutation and inactivation of apoptotic pathways, may be rendered susceptible to SMAC-mediated sensitization to induce programmed necrosis, in the hope that the associated activation of immune function will further exacerbate tumor regression.

## Autophagy Regulators

Autophagy is negatively regulated by TORC1, and therefore TORC1 inhibitors are an effective way to engage autophagy in cells under nutrient-replete conditions. The inhibition of the final step of lysosomal degradation can be inhibited by chloroquine and related compounds, and the combined use of TORC1 inhibitors and chloroquine has been investigated as therapeutics for human malignancies. Inhibition of the earlier steps in autophagy has not been reported, although broad PI3K inhibitors, including Wortmannin and 3-methyladenosine, can inhibit canonical autophagy. Effective inhibition of autophagy by such treatments as a means to disrupt autophagic cell death has not shown promise, however.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 29

# Experimental Models for Rheumatoid Arthritis



Rikard Holmdahl

**KEY POINTS**

- **Animal models are tools to understand the basic mechanisms believed to cause rheumatoid arthritis (RA).**
- **Many different animal models exist for RA, some of which are standard, such as collagen-induced arthritis (CIA), collagen antibody–induced arthritis (CAIA), and adjuvant (pristane)-induced arthritis (PIA).**
- **Arthritis can be induced in animals by immunization with cartilage components, adjuvants, bacterial or viral components, or genetic modification.**
- **Animal models have a defined disease course and are useful for analysis of the three stages that lead to RA: priming, onset, and chronicity.**
- **Animal models allow controlled experiments with specific variations of environment and genetics.**
- **Animal models provide direction for novel approaches to treatment, such as cytokine inhibition.**

For a deeper understanding of the complexity of the pathogenesis of rheumatoid arthritis (RA), the use of animal models is a necessity. Obviously, a disease identical to RA in humans cannot develop in animals because they are of different species with different genetics and environments.

The three main advantages of using animal models are as follows:

- Inbred animal strains can be genetically and environmentally controlled.
- Manipulative experiments can be performed. The genome of inbred strains can be changed by mutations, insertions, and deletions. The environment can also be changed in a controlled way; they can be immunized or infected which may lead to arthritis. Controlled experiments can be performed.
- It is more ethical to use animals instead of humans.

To evaluate and select proper animal models for RA, it is of value to reproduce some of the basic features of RA. The following are hallmarks of RA.

- *The disease starts before the clinical diagnosis*. An autoimmune and inflammatory process precedes the clinical onset by several years.
- *Tissue-specificity*. RA is characterized by a tissue-specific inflammatory attack affecting diarthrodial, peripheral, and cartilaginous joints. Although systemic immune responses as well as manifestations are usually present, the inflammation is mainly directed towards peripheral joints.
- *Chronicity*. The disease is chronic and occurs in tissues in which no causative infectious pathogens have so far been demonstrated. Acute joint affections are common manifestations in both physiologic responses to infections and in connection with other inflammatory disorders, but in RA chronicity is an essential characteristic. The disease course may proceed with identifiable relapses, but there is usually a steady progression of joint destruction.
- *Autoantibodies*. The development of RA is preceded and associated with elevated levels of autoantibodies in serum. Anti-citrullinated protein antibodies (ACPA) have the highest specificity and sensitivity followed by antibodies to immunoglobulin (rheumatoid factors), but antibodies to other antigens do also occur in subsets of patients, such as antibodies to type II collagen (CII) and to hnRNP-A2.
- *Class II major histocompatibility complex (MHC) association*. The genetic influence is significant but complex. The by far largest genetic contributions are class II genes in the major histocompatibility complex. In particular certain structures in the peptide-binding pocket of human leukocyte antigen (HLA)-DR4 molecules are highly associated with RA. Several other loci confirm an involvement of adaptive immunity *(PTPN22, CTLA4, IL21)* strengthen the view that RA is an autoimmune disease.

Accumulating data suggest that RA develops in three discrete stages: priming, onset, and chronicity (Table 29-1). The disease priming process starts several years before clinical onset, with enhanced levels of autoantibodies (ACPA and RF) and a higher level of inflammation markers. Thus, it is likely that the etiologic factors are operating at this early time. Some authors have suggested that smoking and various chronic infections such as periodontitis are associated with the early disease process.[1] This process, however, is not joint specific, and a further spreading of the autoimmune reactivity towards joint structures is likely to occur before the onset of arthritis. To be classified as RA, the first appearance of arthritis needs to progress with chronic inflammation in the joints to establish the disease state that has been extensively described by clinical research. Animal models, however, have not yet been used to describe the established chronic stage but, usually, only the second-onset phase (see Table 29-1).

In all three phases, it has been considered that an infectious agent plays a critical role, but with different mechanisms. In the first priming phase, it is believed that an

**TABLE 29-1** Three Stages of Rheumatoid Arthritis Development in Human and Animal Models

| Disease Stage | Studies in Humans | Studies in Animal Models | Stage-Suitable Models |
|---|---|---|---|
| Pre-RA | Only few retrospective studies | Many studies but not mimicking pre-RA | No models mimicking pre-classic RA with both class II MHC association and ACPA response. The most promising models involve induction of autoimmunity by various adjuvant components. |
| Clinical onset | Only few studies | Most studies | Most models useful. Each model may involve specific pathways leading to subtypes of arthritis. |
| Chronic arthritis | Almost all studies | Almost no studies and only few models | DA rat or C57Bl mouse strains may show chronic relapsing models with adjuvant (pristane) or cartilage/antigen induced arthritis.<br>Several spontaneous arthritis models show chronic progressive arthritis. |

RA develops in three discrete steps, but the focus on the different stages has been different in human RA and animal models.
ACPA, Anti-citrullinated protein antibody; MHC, major histocompatibility complex; RA, rheumatoid arthritis.

infectious agent triggers an inflammatory response involving citrullination that the immune system responds to, such as *Porphyromonas gingivalis* causing chronic gingivitis. In the onset phase, infections of joints can trigger a joint inflammation such as with *Borrelia*, which could persist in the joints and explain the chronic relapsing pattern. Alternatively, the initiating immune reaction could be caused by triggering of chronic inflammation by noninfectious agents that could give rise to citrullination and the breakdown of tolerance to endogenous autoantigens. The inflammatory targeting of the joints, and the chronic relapsing pattern, could also be explained by breakdown of tolerance to joint antigens.

It is likely that the adaptive immune system is critical for the initiation and onset of disease, in particular because these stages seem to be strongly associated with class II MHC and lymphocyte activation genes; whereas no strong evidence exists that the chronic relapsing stage is driven by the adaptive immune system. Chronically activated fibroblasts and, perhaps, macrophages could drive the disease.

Thus the causes and driving forces are not yet clarified and are likely to operate differently in different diseases leading to the syndrome we call *rheumatoid arthritis*.

Animal models are excellent tools for understanding the basic mechanisms of the immune system and how it may develop disease, and disease models are excellent for analysis of distinct pathways and are likely to reflect distinct subsets of human disease. Recent advancement of different animal models mimicking different aspects of human diseases as well as the improvement in genetic techniques has dramatically increased their usefulness. The present overview will include not only models for RA, but will briefly add models with related disease pathways such as psoriasis arthritis, Reiter's disease, ankylosing spondylitis, Lyme disease and septic arthritis (summarized in Table 29-2). However, there is a focus on the classic models for RA, currently commonly used in both industry and academia; the adjuvant arthritis models in the rat, the collagen induced-arthritis (CIA) and the collagen antibody-induced arthritis (CAIA).

## ARTHRITIS CAUSED BY INFECTIOUS AGENTS

Several infectious agents may invade joints where they persist and cause arthritis. Similar to most persistent infectious agents, a balance between the parasite and the host is usually achieved. Thus inflammatory consequences may not only be caused directly by the parasite but also by an aberrant inflammatory response of the host. When microorganisms are present in the target tissue, chronic autoimmunity could be maintained by different mechanisms such as superantigen-mediated T cell activation, a cross-reactive immune response, or the presence of adjuvant material enhancing autoantigen presentation. Several such arthritogenic agents have been described in experimental animals, and some of these mimic a corresponding infectious disease in humans.

### Mycoplasma Arthritides

Arthritis associated with mycoplasma infection is endemic among farm animals. It is also possible to induce arthritis in rodents after inoculation with *Mycoplasma arthritidis*. However, *Mycoplasma* bacteria are not easily found in joints with RA, although it may cause arthritis in individuals with severe B cell deficiency. Inoculation of mice induces a mild chronic arthritis in conjunction with the persistence of the microorganism, but this is driven by immune activation by a mycoplasma-derived superantigen.[2] In accordance with observations in humans, B cell-deleted mice are more susceptible to *Mycoplasma*-induced arthritis.

### Lyme Arthritis

*Borrelia* is a spirochete that may persist in joints and cause arthritis. The clinical picture is chronic, resembles RA, and is genetically associated with class II-DR4 MHC, as is RA. Live bacteria persist in the joints, but in many patients it has been difficult to identify the spirochete in the arthritic joints. Mice infected with *Borrelia* develop arthritis similar to the human version of the disease. Similar to humans, MHC controls susceptibility to arthritis, and the immune response associated with human class II MHC expressed in mice is directed to *Borrelia*-derived antigens.[3] The persistence of the spirochete seems to be a requirement for the development of the arthritis, although some mouse strains do not develop arthritis in spite of high levels of bacteria in the joints.

### Staphylococcal Arthritis

Septic arthritis is most commonly caused by a persistent infection of *Staphylococcus aureus*. The bacteria tend to be

**TABLE 29-2** Overview of Rodent Arthritis Models

| Model | Species | Genetics | Disease Characteristics | Reference |
|---|---|---|---|---|
| **Arthritis Caused by Infection** | | | | |
| Mycoplasma-induced arthritis | Rats and mice | More pronounced in B cell-deficient mice | Mild chronic arthritis | 2 |
| *Borrelia*-induced arthritis | Mice | Major histocompatibility complex (MHC) | Severe and erosive arthritis with spirochetes in the joints | 62 |
| *Staphylococcus*-induced arthritis | Rats and mice | MHC | Severe arthritis | 4 |
| *Yersinia*-induced arthritis | Rats and mice | LEW and SHR but not DA and BN rats | Severe arthritis with bacteria in the joints | 63 |
| **Arthritis Caused by Bacterial Fragments** | | | | |
| *Mycobacterium*-induced arthritis ("adjuvant arthritis" [AA]) | Rats | MHC, non-MHC genes (LEW >F344) | Acute and generalized inflammatory disease including erosive arthritis | 6 |
| Streptococcal cell wall-induced arthritis | Mice and rats | Non-MHC genes (LEW >F344), (DBA/1=Balb/c >B10) | Severe and erosive arthritis | 64 |
| **Arthritis Induced by Adjuvant** | | | | |
| Avridine-induced arthritis (AvIA) | rats | MHC (f) | Very severe, erosive, and chronic arthritis | 65 |
| Oil (mineral oil)-induced arthritis (OIA) | DA rats | non-MHC loci on chr 4, 10 | Acute and self-limited inflammation of peripheral joints | 10 |
| Pristane-induced arthritis (PIA) | Rats | MHC, non-MHC loci on chr 1, 4, 6,12, 14 | Chronic and erosive arthritis in peripheral joints<br>Can be passively transferred with ab T cells | 11 |
| PIA | Mice | MHC (q, d)?<br>Balb/c, DBA and C3H gene backgrounds | Chronic and generalized inflammatory disease also affecting joints | 17 |
| Mannan-induced psoriasis and psoriasis arthritis | Mice | B10.Q, B10.RIII.<br>Arthritis enhanced by mutation in Ncf1. | Both psoriasis and psoriasis arthritis developing a few days after injection mannan. Macrophage, gd T cell, and neutrophil dependent. | 20 |
| **Arthritis Induced By Cartilage Protein Immunization** | | | | |
| CII (heterologous or homologous CII in mineral oil)-induced arthritis (CIA) | Rats | MHC (a, l, f and u), non-MHC loci on chr 1, 4, 7, 10 | Chronic and erosive arthritis in peripheral joints | 66 |
| CII (heterologous or homologous CII in CFA) induced arthritis (CIA) | Mice | MHC (q and r), non-MHC loci on chr 1, 2, 3, 6, 7, 8, 10, 15 | Erosive arthritis in peripheral joints | 27,67 |
| CXI (rat CXI in IFA) induced arthritis | Rats | MHC (f, u) | Severe, chronic arthritis | 24 |
| Human proteoglycan (in complete Freund's adjuvant-induced arthritis) | BALB/c mice | MHC (d), several non-MHC loci | Chronic arthritis | 23 |
| Cartilage oligomeric matrix protein (COMP) (in mineral oil)-induced arthritis | Rats, mice | MHC (RT1u, H2q) | Severe but self-limited arthritis | 25,44 |
| G6PI induced arthritis | Mice | MHC (H2q), C3HQ>DBA/1>B10.Q | Severe but self-limited arthritis | 18,76 |
| **Antibody-Induced Arthritis Models** | | | | |
| Collagen antibody-induced arthritis (CAIA) | Mice | Balb/c>B10.RIII>B6 | Acute arthritis | 77 |
| KXBN serum-induced arthritis (SIA) | Mice | | Severe acute arthritis | 68 |
| **"Spontaneous" Arthritis Models** | | | | |
| HLA-B27 transgenic animals | Mice and rats | B27 heavy chain transgene | Ankylosing spondylitis, colitis, balanitis, arthritis | 5 |
| The MRL/lpr mouse (mutation in the *fas* gene controlling apoptosis) | Mice | lpr | Generalized inflammation as a part of lupus disease, which also affects joints | 53 |
| Stress-induced arthritis | DBA/1 mice | Non-MHC genes | Enthesopathic response with no evidence for immune involvement. A model for psoriasis arthritis. | 52 |

*Continued*

**TABLE 29-2** Overview of Animal Arthritis Models—cont'd

| Model | Species | Genetics | Disease Characteristics | Reference |
|---|---|---|---|---|
| Tumor necrosis factor (TNF) transgenic mice (overproduction of TNF) | Mice | TNF transgene | Erosive arthritis as well as generalized tissue inflammation | 54 |
| IL-1R antagonist-deficient mouse | Balb/c mice | IL-1Ra deficiency | Arthritis | 57 |
| Gp130 IL-6R mutant mouse | C57 Black mice | IL-6R mutation | Arthritis | 58 |
| KxBN | | TCR transgenic specific for a glucose-6-phosphate isomerase (G6PI)-derived peptide | Severe arthritis with autoreactivity to G6PI | 68 |
| *Zap70* mutation | Balb/c mice | Spontaneous mutation in *Zap70* | Severe arthritis with autoreactivity | 19 |
| Severe combined immunodeficiency (SCID) mouse | Mice | Local injection of fibroblasts into immunodeficient SCID mouse | Sustained destructive arthritis | 60 |

encapsulated in tissues including joint synovia and persist for years. Inoculation with certain *Staphylococcus aureus* strains induces septic arthritis in many mouse and rat strains.[4] Severe and prolonged arthritis develops in infected joints, mimicking human disease. Interestingly, protection of the host is critically dependent on the innate defense, such as neutrophils and complement, whereas the adapted immune response is not effective. Instead, the apparently aberrant adapted immune response promotes arthritis.

### Arthritis and Ankylosing Spondylitis Induced by Intra-cellular Bacteria

Some bacteria with the capacity to invade cells upon infection (e.g., *Yersinia*) are known to be related to postinfectious arthritides such as reactive arthritis and ankylosing spondylitis. These diseases are genetically associated with HLA-B27, a class I MHC allele of the B locus. It has been possible to reproduce the human disease to a large extent in HLA-B27 transgenic mice and rats.[5] In B27-transgenic rats, ankylosing spondylitis, balanitis, colitis, dermatitis, and arthritis occur spontaneously. However, if the rats are made germ-free, the joint manifestations are no longer present. This phenomenon indicates the importance of a so far unknown infectious agent. Importantly, the development of arthritis is most likely related to the triggering of inflammation by adjuvant fragments from the pathogen.

## ARTHRITIS CAUSED BY BACTERIAL FRAGMENTS

Postinfectious arthritis may develop after bacterial infections. The occurrence of arthritis can be dependent on several different bacteria-derived compounds, such as cell wall fragments, DNA, and heat shock proteins. Bacterial cell wall fragments are difficult to degrade and may cause prolonged activation of macrophages, also synovial macrophages. The first animal model for RA to be described was the so called *adjuvant arthritis* (AA) induced in rats after injection of mycobacterium cell walls suspended in mineral oil (i.e., complete Freund's adjuvant [CFA]).[6] Only rats (and not mice or primates) develop arthritis after mycobacterium challenge, although joint-related granuloma formation may occur also in humans treated with mycobacterium-containing vaccine. CFA is a potent adjuvant that activates a multitude of pattern recognition receptors, activating antigen-presenting cells and enhancing T cell immunity. Subcutaneous injection of CFA in rats leads to granulomatous inflammation in many organs, for example, the spleen, liver, bone marrow, skin, and eyes, and causes profound inflammation in peripheral joints. AA is severe but self-limited, and the inflammation subsides after 5 to 7 weeks. The mycobacterium cell wall fragments are most likely disseminated throughout the body and engulfed by tissue macrophages, which have difficulties in degrading the bacterial cell wall structures and are therefore transformed into an activated state, which trigger inflammation.

AA can be abrogated by elimination of the classic alpha/beta type of T cells, and spleen-derived T cells can transfer the disease. The specificity of such T cells has, however, not been reproducibly demonstrated, although some possibilities have been suggested, including bacterial structures and cross-reactive self-components. Although a role for heat shock proteins as T cell antigens has not been confirmed, they play a regulatory role for the development of arthritis. A minimal arthritogenic structure in the mycobacteria cell wall is the peptidoglycan component muramyl dipeptide. Interestingly, T cells do not recognize this structure, but it has potent adjuvant capacity because it activates the inflammasome by stimulating innate immune receptors (NOD2) and antigen-presenting cells. In addition, the unmethylated DNA of bacteria independently triggers arthritis in mice and contributes to arthritis severity of AA in rats. The bacterial DNA triggers Toll-like receptors on both antigen-presenting cells and inflammatory macrophages and will, therefore, interact with both T cell–dependent and inflammatory pathways. Another T cell–dependent arthritogenic pathway is triggered by the mineral oil in which the mycobacteria is suspended, as is discussed later in more detail. Thus, this classic "adjuvant-induced arthritis" is mediated by different and interacting pathways, dependent on both different mycobacterium cell components as peptidoglycans, DNA and heat shock proteins but also dependent on adjuvant activity mediated by the oil used to suspend the mycobacteria.

Postinfectious arthritis has also been observed to occur after streptococcal infections. A rapidly developing form of arthritis has been observed after systemic inoculation of

streptococcal cell wall fragments in rats and mice but not in primates.[7] Parenterally injected peptidoglycans rapidly disseminate throughout the body, including the joints. These structures are difficult to degrade for the macrophages and, as a consequence, synovial macrophages are persistently activated. T cells are necessary for the initiation and perpetuation of the arthritis.

Chronic parodontitis caused by *P. gingivalis* has been suggested to be involved in the initiation of RA,[8] but so far no arthritis has been induced in animal models by using live bacteria or by immunization with *P. gingivalis*-derived components.

## ADJUVANT-INDUCED ARTHRITIS

The induction of arthritis in rats was found to be not only dependent on the mycobacteria but also the oil into which the mycobacterium fragments were suspended (reviewed in[9]). Interestingly, some oils were found to support the induction of arthritis whereas others did not. Many years later it was noted that the mineral oils that supported the induction of arthritis were in fact arthritogenic by themselves.[10] It was also found that subcutaneous administration of nonbacterial adjuvant compounds, such as pristane, hexadecane, and squalene, were highly effective in inducing arthritis.[11] These adjuvant compounds in most cases produce inflammation confined to the joints and offer more appropriate experimental models for RA than the earlier commonly used AA (i.e., mycobacterium in oil-induced arthritis) model.

Mineral oil-induced arthritis (OIA), avridine-induced arthritis (AvIA), pristane-induced arthritis (PIA), hexadecane-induced arthritis, and squalene-induced arthritis in the rat share many common features but differ by the degree of chronic development (Figure 29-1). They are induced with adjuvant compounds that lack immunogenic capacity (i.e., no specific immune responses are elicited). Instead they are rapidly spread throughout the body after a single subcutaneous injection, penetrate through cell membranes into cells, and interact with as yet unknown cell surface receptors and intra-cellular proteins. One or two weeks after injection, arthritis suddenly develops. The arthritis appears in the peripheral joints, with a similar distribution as seen in RA. Occasionally other joints are involved, but systemic manifestations in other tissues have so far not been reported. In certain rat strains, especially in the PIA model, the arthritis proceeds as a chronic relapsing disease. Interestingly, a systemic immune response occurs that leads to production of antibodies to RA33 and rheumatoid factors but not ACPA, whereas an immune response to cartilage proteins appears in the more chronic development of the disease.[12] A role for cartilage proteins in regulation of disease activity is seen because the disease can be prevented and, in fact, therapeutically ameliorated by nasal vaccination with various cartilage proteins. Interestingly, the first acute phase of the disease is class II MHC–dependent and αβ T cells play a critical role in all stages of the disease. T cell transfer of the disease seem to be oligoclonal rather than monoclonal, and so far antigen-specific T cells have not been identified.[13] A role for environmental infectious agents is not likely because no difference in disease susceptibility could be seen in germ-free rats, although only conventional rats respond to heat shock proteins.[14] There is so far no evidence for recognition by lymphocyte receptors or receptors involved in the innate immune system. Surprisingly, some of the arthritogenic adjuvants are in fact components already present in the body before injection. For example, pristane is a component of chlorophyll and is normally ingested by all mammals including laboratory rats. Pristane is taken up through the intestine and spread throughout the body. However, they all share the capacity to penetrate into cells where they could change membrane

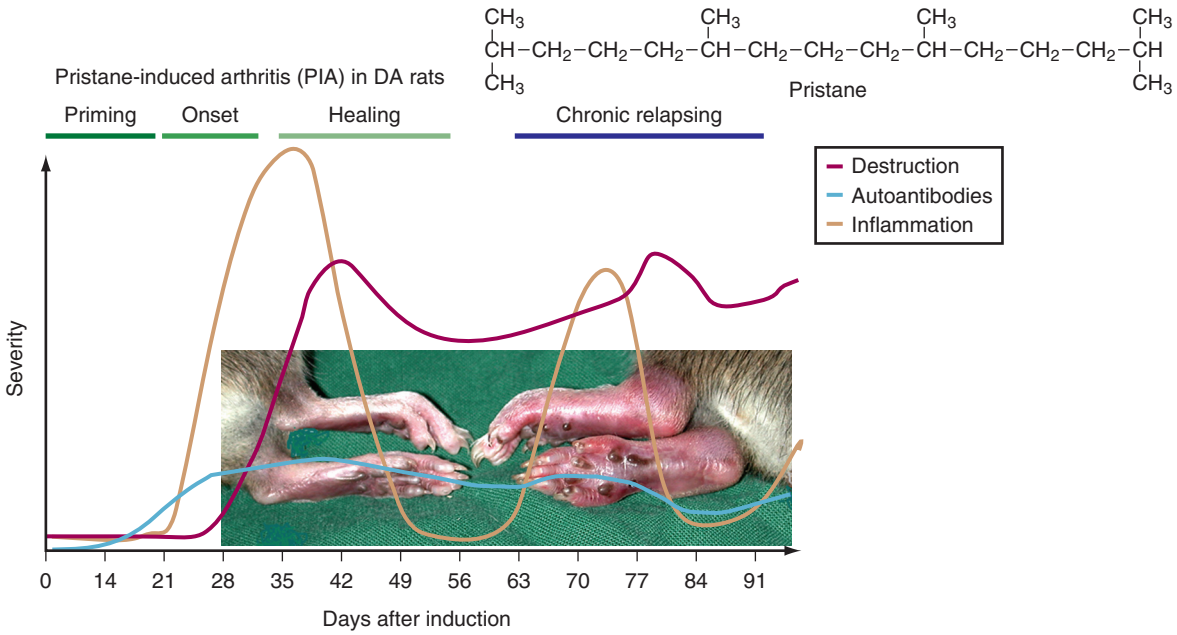



**Figure 29-1** Pristane-induced arthritis in dark agouti rats; induced with pristane subcutaneously. Development of severe and chronic relapsing arthritis starting 10 to 14 days after injection

fluidity and modulate transcriptional regulation and in higher doses induce apoptosis. The injection route and dose is critical (i.e., it is decisive for which cell is first activated and to what extent).

The PIA model has been subjected to genetic analysis showing that the disease is polygenic,[15] and the identified loci control distinct phases of the disease such as arthritis onset, clinical severity, joint erosion, and chronicity. Some of the underlying genes have been identified. The strongest effect is mediated through a polymorphism of the *Ncf1* gene of both inbred and wild rat populations.[16] The *Ncf1* gene codes for the p47phox component of the NOX2 complex and controls the oxidative burst. Surprisingly, a higher oxidative burst capacity was associated with more severe arthritis. The effect was found to operate before T cell activation, and therefore it also controls the degree of autoimmunity, linking innate and adaptive immunity. Importantly, the class II MHC region, controlling the adaptive T cell response, and a C-type lectin gene cluster *(APLEC)*, which are likely to have importance in uptake of antigen to antigen-presenting cells also control PIA.

AA is not easily inducible in species other than rats. Of the above mentioned AIA models, only PIA has been described in mice.[17] However, the induction of PIA in mice requires repeated intraperitoneal injections of pristane, which triggers a widespread inflammatory disease with a late and insidious onset. The induced disease mimics systemic lupus erythematosus (SLE) rather than RA.[18] The disease is clearly different from PIA in the rat; the same inducing protocol does not induce disease in the rat, and the disease course and characteristics are different. Another adjuvant-related model is the induction of mild arthritis after intra-articular injection of agents that activate macrophages, such as unmethylated DNA. It has recently been found that several mouse models previously believed to be spontaneous are critically dependent on adjuvants and should therefore be classified as AIA. From the observation that a BALB/c mouse substrain in Japan spontaneously developed arthritis a causative amino acid replacing mutation in the *ZAP70* gene was identified.[19] The mutation *(W163C)* caused a weaker TCR mediated signalling and the development of arthritis was preceded by increased levels of IL-17 producing autoreactive T cells. The *Zap70* mutation led to defective positive selection and the emergence of autoreactive T cells that attached the joints. As a result, both rheumatoid factors and CII reactive antibodies were detected in the mice. The arthritis, however, did not develop in specific pathogen-free conditions and it could be shown that injection of beta-glycan or mannan induced the arthritis. Thus this model seems to be an AIA in the mouse. The resulting arthritis may however differ depending on the inducing agent and the genetic background. For example, injection of mannan in certain mouse strains with a deficiency in reactive oxygen species production results in a disease mimicking psoriasis arthritis rather than the classic RA.[20]

Also some other spontaneous arthritis models, the KxB/N model or the IL-1R deficient mouse, are mediated by a likely adjuvant component because arthritis was strongly attenuated under germ-free conditions.[21,22] The causative arthritogenic effect was mediated by intestinal bacterias, segmented filamentous bacteria (SFB),[21] and lactobacillus,[22] respectively.

Taken together, there is today a set of useful adjuvant type of arthritis models in both mice and rats.

## CARTILAGE PROTEIN-INDUCED ARTHRITIS

Arthritis is inducible with several different cartilage-proteins, such as aggrecan,[23] type XI collagen CXI,[24] cartilage oligomeric matrix protein (COMP),[25] and CII.[26] These various models have different characteristics and genetics. CIA, induced with CII, is today the most commonly used model for RA; it was first demonstrated in the rat[26] and was later reported using other species, such as mice[27] and primates.[28] Today it is the most commonly used model for RA.

### Collagen (II)-Induced Arthritis

Immunization with the major collagen in cartilage, type II collagen (CII), leads to an autoimmune response and, as a consequence, a sudden onset of severe arthritis. It is usually necessary to emulsify the CII in adjuvant, such as mineral oil in the rat and complete Freund's adjuvant in the mouse, but the disease can be distinguished from the various forms of AA. The susceptibility of the CIA model varies considerably depending on the experimental animal strain, the adjuvant used, and on whether CII used is of self or nonself origin.

In both rats and mice immunized with heterologous CII, a severe, erosive polyarthritis develops 2 to 3 weeks after immunization but usually subsides within 3 to 4 weeks (Figure 29-2). The most commonly used DBA/1 strain thus develops a severe but only an acute disease. However, a genetic influence is obvious because mice on C57Bl backgrounds develop a milder arthritis, which later may develop into a more chronic relapsing disease course (Figure 29-3). In all of the models, the erosive inflammatory phase is followed by a healing phase, with pronounced formation of new cartilage and bone that clinically can be difficult to distinguish from active inflammation. The disease is critically dependent on both a T and a B cell response to CII, and pathogenic antibodies play a role in the inflammatory attack on the joints.[29] The CAIA is inducible with certain CII-specific monoclonal antibodies[30] (Figure 29-4). These CII-specific antibodies bind to the cartilage and destabilize the cartilage matrix. Subsequently, the inflammatory response is triggered with infiltration of antibodies into cartilage matrix, fixation of complement attraction of neutrophilic granulocytes, and activation of FcR expressing inflammatory cells in a process independent of the immune system.[31] Interestingly, the epitopes recognized on CII contain arginines that potentially can be citrullinated. The major CII epitopes are citrullinated and monoclonal antibodies specific for the citrullinated peptide induce arthritis, showing an important link to RA.[32]

Mice and rats are less susceptible to immunization with homologous CII as compared with heterologous CII, but once started it could be more severe and is often more chronic. The pathogenic events in the chronic disease phase are largely unknown but are most likely dependent on both autoreactive B and T cell activity. Nevertheless, the CIA model is the most extensively investigated model for RA and has given valuable insights into the genetic control of the arthritic process and of the autoimmune

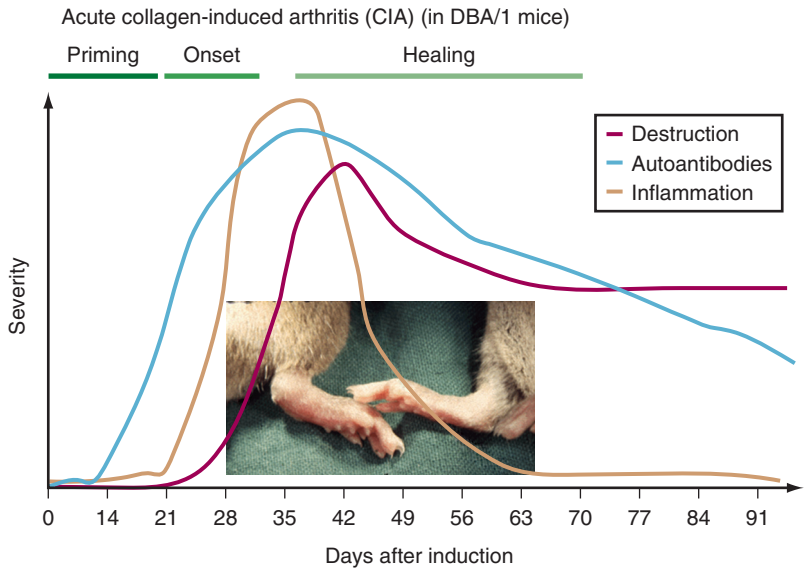



**Figure 29-2** Collagen-induced arthritis in DBA/1 mice. Induced after immunization with heterologous (bovine, chicken, rat) type II collagen emulsified in complete Freund's adjuvant. Severe arthritis starts 3 to 4 weeks after immunization. Although the arthritis is severe and causes dramatic erosions and bone remodeling, there is no chronic relapsing inflammatory disease course.

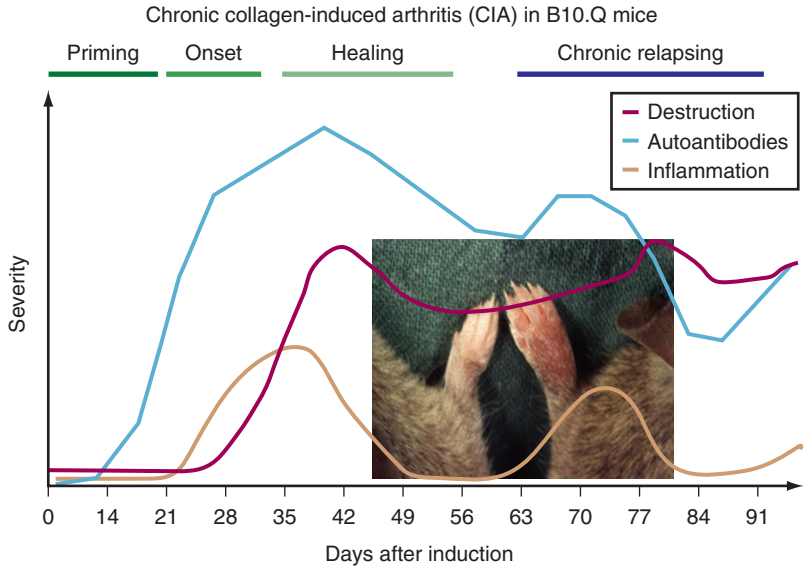



**Figure 29-3** Collagen-induced arthritis in B10.Q mice; induced after immunization with rat type II collagen emulsified in complete Freund's adjuvant. Mild arthritis starts 3 to 5 weeks after immunization. The arthritis is mild but causes inflammatory joint erosions and bone remodeling. It is, however, often followed by a chronic disease with inflammatory relapses.

interactions with cartilage. It has also been useful for the development of new therapeutic approaches and for drug screening.

## Genetic Basis of Collagen-Induced Arthritis

Susceptibility to CIA varies dramatically between different inbred strains. The CIA is a complex, polygenic disease, in similarity with the AA models described in the preceding text. In the CIA model, the autoimmune process is already determined by the induction through immunization with a defined antigen. Not surprisingly, the class II MHC polymorphism is important for determining susceptibility, but there is also a major influence by a large number of genes outside the MHC region. The major gene regions have been identified through genetic segregation experiments in both mice and rats, which have given an overall picture of the genetic inheritance of the susceptibility.[33] As in other complex diseases, these genes operate in concert and can only by identified through isolation in a controlled genetic and environmental context. Class II MHC genes *(Aq)*, *Ncf1*, and complement C5 have until now been positioned from genetic analysis. The *Ncf1* gene was defined through analysis of PIA and CIA in rats.[34] A spontaneous mutation in the mouse *Ncf1* gene, when combined with the CIA-susceptible class II MHC allele Aq in the C57Bl/10 mouse, causes a chronic relapsing form of CIA.[35] In addition, these mice tend to develop a severe form of chronic arthritis in

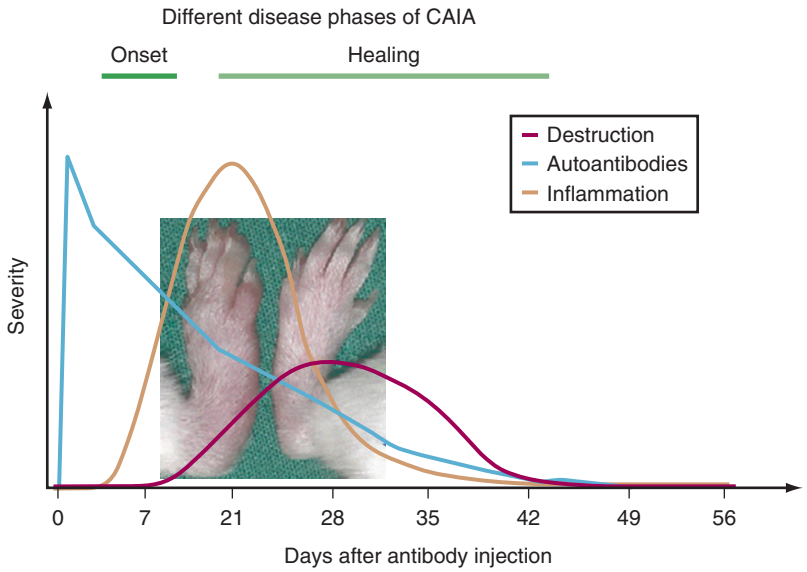



**Figure 29-4** Collagen antibody -induced arthritis in Balb/c mice; induced after intravenous injection of a defined set of monoclonal antibodies to type II collagen. Mild arthritis starts 48 hours after injection, and severe arthritis starts after an additional boost with injection of LPS intraperitoneally. The arthritis is acute with mild joint erosions but with no bone remodeling. The disease is acute and resolves within a few weeks.

the postpartum period, with the spontaneous development of autoimmunity to CII. Another genetic polymorphism of importance is the complement C5, which is deficient in many mouse strains. The deficiency leads to a relative resistance to CIA, suggesting a role for complement pathways in arthritis, which in fact is the opposite of its role in mycoplasma-induced arthritis. A role for alternative complement pathways as well as Fc receptor-mediated pathways have been demonstrated by using both CIA and the CAIA models.[36,37] The rapid progress of genome-wide association analysis of large human cohorts today gives direct information on involved genes and gene clusters in common disease such as RA. However, animal models are needed to understand their functional relevance, and for this the genes that control the corresponding disease in the animals need to be identified.

## Autoimmunity Associated with the Major Histocompatibility Complex in Collagen-Induced Arthritis

Early observations with use of the CIA model in both mice and rats indicated a role for the MHC region. In the mouse, CIA induced with either heterologous or homologous CII is most strongly associated with the $H2^q$ and $H2^r$ haplotypes, whereas most other haplotypes, such as b, s, d, and p, are relatively resistant.[38] The major underlying gene within the $H2^q$ haplotype has been identified as the $A^q$ beta gene.[39] Moreover, the immunodominant peptide derived from the CII molecule bound to the arthritis-associated q variant of the A ($A^q$) molecule has been found to be located between positions 259 and 271 of CII.[40] This peptide can be glycosylated on the central lysine side chain and is recognized by most of the CII-reactive T cells. Interestingly, the peptide is also bound by DR4 (DRB1*0401/DRA) and DR1 molecules (i.e., the shared epitope, which is associated with RA). Mice that transgenically express DR4 or DR1 are susceptible to CIA and respond to CII259-271 peptide and CII-reactive T cells from RA patients.[41] These findings suggest a model for studies of RA that not only mimic some basic pathogenic events but might also share some critical structural similarities.

Arthritis is also inducible in mice strains that do not express q or r. A commonly used model is to induce arthritis with high doses of chicken or bovine CII emulsified in mycobacterium tuberculosum-containing CFA. T cell autoreactivity to CII, however, is not reproducibly demonstrated and there is no verified T cell response to CII. It is likely that the T cell reactivity is directed to a contaminant in the preparation such as another matrix protein or the pepsin used for the preparation. Such T cell reactivity could help B cells produce antibodies to CII, which would explain the development of arthritis.[42]

It is important to emphasize that the structural interaction between class II $MHC^+$ peptide complexes and T cells does not give us the answer to the pathogenesis of CIA (or RA), but rather, a better tool for further analysis. An important question is how the immune system interacts with the peripheral joints (i.e., how autoreactive T and B cells are normally tolerized and what happens in the pathologic situation after their activation by CII immunization). Most of the T cells reactive with the rat CII259-271 peptide do not cross-react with the corresponding peptide from mouse CII. The difference between the heterologous and the homologous peptide is position 266, in which the rat has a glutamic acid (E) and the mouse an aspartic acid (D), which leads to a weaker binding of the mouse peptide to $A^q$. The importance of this minor difference was demonstrated in transgenic mice expressing CII mutated to express a glutamic acid at this position.[43] When mutated CII was expressed in cartilage, the T cell response to CII was partially but not completely tolerized. The mice were susceptible to arthritis, but the incidence was low, similar to what is seen in mice immunized with homologous CII. This finding shows that a normal interaction between cartilage and T cells leads to the activation of T cells, but with less capacity to induce

arthritis or with regulatory properties. These CII autoreactive T cells may under extreme circumstances (such as CII immunization) be pathogenic. In contrast, B cells reactive with CII are not tolerized, and as soon as the T cells are activated, even in a partially tolerized state, they may help B cells to produce autoreactive and pathogenic antibodies. It is possible that a similar situation may exist in humans that could explain the difficulties in isolating CII reactive T cells, compared with the relative ease in which CII-reactive B cells can be detected in the joints.

## Induction of Arthritis with Other Cartilage and Joint-Related Proteins

### Type XI Collagen-Induced Arthritis

The type XI collagen (CXI) is structurally similar to CII and is to a large extent co-localized. CXI is a heterotrimer with three different α chains where one is shared with CII (the a3 chain). Both heterologous and homologous CXI induce arthritis in rat strains.[24] Interestingly, the induction with homologous CXI gives a chronic relapsing disease, which is distinctly different from the heterologous CXI-induced disease and CII-induced CIA.

### Cartilage Oligomeric Matrix Protein-Induced Arthritis

Another cartilage protein is cartilage oligomeric matrix protein (COMP). Homologous COMP induces arthritis in both rats and mice.[25,44]

### Proteoglycan (Aggrecan)-Induced Arthritis

Other major components of joint cartilage are proteoglycans, of which the largest is aggrecan. Immunization of Balb/c mice with foetal human aggrecan induces chronic arthritis.[23] Both B and T cells are involved in the pathogenesis. Autoreactive T cells have been isolated and respond to the G1-domain of aggrecan in which neoepitopes are created.[45] T cell receptor transgenic mice spontaneously develop arthritis at old age. The disease has been genetically mapped and share many gene regions in common with CIA.

### Antigen-Induced Arthritis

Antigen-induced arthritis is a classic model of RA that is induced by immunizing animals with a foreign antigen, usually bovine serum albumin, and subsequently injecting the same antigen into a joint. A pronounced T cell-dependent immune complex-mediated and destructive arthritis develops in the injected joint as a result. The model is very well controlled and has been used to understand the effector phase of joint inflammation and cartilage destruction.[46]

### Glucose-6-Phosphate Isomerase-Induced Arthritis

The successful induction of arthritis after immunization with recombinant glucose 6 phosphoisomerase in adjuvant[47] stems from the identification of the K/BxN model in which transgenic T cells specific for glucose-6-phosphate isomerase (GPI) leads to spontaneous arthritis in NOD mice.[47a] The GPI-induced arthritis is MHC dependent and associated with the H2q haplotype (as CIA), and GPI peptides with an Aq-binding capacity can induce arthritis.[48] GPI has a unique affinity for cartilage, because it binds with high affinity to cartilage proteoglycans[49]; spontaneous immune activation in the K/BxN mouse seems to primarily arise in lymph nodes draining joints.[50] Thus, although GPI is ubiquitously expressed, the immune system may recognize its presence in the joints. The GPI-induced arthritis is T and B cell dependent. Interestingly, depletion of FoxP3-positive Tregs leads to a chronic disease course.[51]

# SPONTANEOUS ARTHRITIS

Many of the classic inbred mouse and rat strains tend to spontaneously develop arthritis, in particular under certain environmental influences (Table 29-3). In some strains, such as DBA/1, the grouping of males easily induces intermale aggressiveness; such stress seems to be associated with development of severe arthritis.[52] This stress-induced arthritis is very different from inflammatory arthritis models such as CIA and have less inflammatory synovial infiltrate, but with enthesopathy and new cartilage and bone formation, more similar to psoriasis arthritis than RA. There are also a number of genetic mutations that strongly enhance arthritis development. One such mutation occurs in the *Fas* gene, of critical importance for apoptosis. In the MRL mouse background, arthritis develops with a severe lupus disease.[53] Other examples are the previously mentioned mutations in the *Ncf1*[35] or *ZAP70*[19] genes.

## Spontaneous Arthritis in Genetically Modified Strains

Importantly, models that develop spontaneous arthritis have been possible to create by genetically modifying mouse strains. The prime example of this is the demonstration that overexpression of tumor necrosis factor (TNF) leads to severe arthritis.[54] This model has been extremely useful in delineating the role of TNF in mediating arthritis. Other genetic mutations leading to overexpression of TNF, such as deletion of an upstream regulatory element that controls TNF secretion in fibroblast[55] or the deletion of DNasII,[56] which leads to aberrant secretion of TNF by chronically stimulated macrophages, also lead to spontaneous development of severe arthritis. Mice overproducing TNF develop

**TABLE 29-3** Some Environmental Effects on Arthritis Models

| Environmental Effect | Effect on Arthritis | Reference |
|---|---|---|
| Cage Grouping | + | 52 |
| Noise Stress | ++ | 69 |
| Predator Stress | – | 70 |
| Pregnancy | – | 71 |
| Postpartum | + | 72 |
| Estrogen | – | 73 |
| Darkness | + | 74 |
| Infections | –/+ | 14,21,75 |

+, Increased arthritis; –, decreased arthritis.

arthritis through activation of the innate immune system and not dependent of adaptive T and B cell immunity. Another important lesson from the TNF overproducing mice was that it primarily led to the development of arthritis, although in some situations colitis and encephalomyelitis also develop.

Subsequently, several other genetically modified mouse strains developing arthritis have been reported. A mouse deficient for the IL-1 receptor antagonist[57] developed arthritis that not only affected the downstream effector functions but could also be dependent on T cell activation.

A mutation in the gp130 IL-6 receptor was observed to lead to arthritis in elderly mice.[58] Interestingly, the mutation led to an accumulation of polyclonal autoreactive $CD4^+$ T cells that secreted inflammatory cytokines, indicating a regulatory role of the IL-6 receptor of the adaptive immune system.

Another type of spontaneous arthritis was observed in a T cell receptor transgenic mouse in which the TCR recognizes a peptide derived from the ubiquitously occurring protein glucose-6-phosphoisomerase (G6PI) bound to the class II MHC protein Ag7, the KxBN mouse.[59] However, as discussed in a preceding section, the arthritis requires SFB bacteria in the intestinal flora, indicating that this disease is not strictly spontaneous but rather is induced by a bacterial adjuvant stimulation. The pathogenic effector pathway in this model is dependent on antibodies reactive with G6PI. This serum-induced arthritis (SIA) can be transferred with sera from K/BxN mice and the antibodies bind to precipitated GPI on the cartilage surface, mimicking the pathogenesis of anti-CII antibodies in the CAIA model. The use of the G6PI antibody-induced arthritis has been instrumental in finding early inflammatory steps in the joint attack, which involves complement activation through the alternative pathway, and neutrophil infiltration. Clearly, the joints are specifically targeted in the disease and it remains to be determined how T cells and antibodies recognize this systemically expressed autoantigen in a joint-specific context.

Another type of model is induction arthritis after transplantation of human synovial fibroblasts into immune-deficient SCID mice.[60,61] The same type of arthritis develops after transfer of murine fibroblast cell lines. This model is likely to reflect inherent properties of fibroblast-mediated mechanisms showing different features compared with other arthritis models like CIA and PIA.

These models most likely represent various aspects of the processes leading to arthritis, which will be determined by the transgene or defective gene, or due to transplantation of specific cells.

## USE OF ANIMAL MODELS

### Increasing the Knowledge of Disease Pathways

An ideal model for human RA should mimic the complexity of the human disease in being polygenic and dependent on environmental factors. The animal models have the advantage that both genetics and environment can be better controlled. There are many forms of arthritis, and the different clinical varieties are likely to continue to be subdivided just as has happened with ACPA+ RA and seronegative RA, whereas animal models represent very divergent pathways and diseases that lead to arthritis.

Ideally, animal models should mimic the various subtypes of RA. With increasing knowledge of RA, such as the identification of ACPA as a predictive biomarker, and the identification of new genes associated with RA, there will also be new demands on the animal models. Thus, an animal model does not yet exist that reflects the production of ACPA nor has a properly humanized mouse strain been developed that pathophysiologically mimics the genetic polymorphism identified in humans. To introduce new genes (e.g., human class II MHC) or environmental factors (e.g., smoking), a proper and well-controlled genetic and environmental context is critical. For example, the introduction of the human class II MHC as transgenes in the mouse has led to a number of artifacts, some of them related to the non-physiologic interactions between human and mouse genes. However, as long as the mouse models have been studied with a well-controlled genetic and environmental context, they have and will contribute detailed information on the molecular pathogenesis that leads to arthritis. For this work, the possibility to genetically modify both mice and rats is a powerful tool, and the possibility to make controlled experiments is a critical advantage.

### Developing New Therapeutic Strategies

To test new drugs and therapies, it will be necessary to select from the different models available. Obviously there is no optimal model for RA, and there will never be one. The models described, however, are useful because they represent different aspects of RA pathogenesis. Thus, depending on the questions to be asked or symptoms to be treated, different models may be used. The most common model used today to test new therapeutic approaches is the CIA model, which should be included as a reference model. The usefulness of this model has been confirmed with the anti-TNF treatment, which was subsequently introduced in RA.

Recapitulating the three stages through which RA develops—priming, onset, and chronicity—reasonable criteria are that the animal models should be useful for studies of specific pathways of importance in these stages. A common mistake is to only use acute models and to only use disease prevention and not established chronic disease as a read out. It is also of critical importance to be aware of the specific environmental influences on arthritis development in rodents. Of particular importance are stress effects which are easily produced by mixing mice from different litters in the same cage and which will lead to cage-dependent effects. Other important factors are sex hormones and, most likely, neurohormones, which play an important role in modulating disease activity—seen as effects by oestrus cycling, pregnancy, and light effects. Clearly, not only environmental effects need to be controlled, but also genetic effects. The control of genetics is usually achieved by testing standardized inbred strains. The problem is that these vary considerably between different colonies, mainly because of genetic contamination. In spite of these problems, there is no question that both environment and genetics can be better controlled in experimental animal models than in studies directly involving the human population.

## ETHICAL CONSIDERATIONS

One important drawback of using experimental models for RA is suffering of animals. However, in the light of various human activities that use animals, the use of them in research seems to be the easiest to defend. In fact, it would be unethical not to use them for research because it would prohibit further understanding of human diseases, thereby letting humans suffer from diseases that will be possible to cure or prevent. It should also be emphasized that the recent development of animal models for RA has refined them to be of more specific use, which has decreased animal suffering. For example, the historically most commonly used model for RA, the mycobacterium-induced AA, is a systemic and severe inflammatory disease, whereas pristane-induced arthritis and collagen-induced arthritis are more specific disease of the joints.

## CONCLUSION

Experimental animal models are essential tools not only for investigating the basic mechanisms leading to RA but also for the development of new therapies. Many models have been described, and each represents different aspects of the disease; it is therefore important to use different models. It has been emphasized that the models used should reflect essential clinical hallmarks of RA, and they should reflect the fact that RA is a polygenic disease triggered by unknown and multifactorial environmental factors.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 30

# Neuronal Regulation of Pain and Inflammation



Camilla I. Svensson • Linda S. Sorkin

**KEY POINTS**

- Pain signaling is amplified by inflammation.
- Peripheral sensory neurons have efferent functions that contribute to inflammation, including axon and dorsal root reflexes.
- Neurogenic inflammation results in flare, edema, and increased pain.
- The sympathetic nervous system modulates (enhances or attenuates) many immune cell types via adrenergic receptors.
- Vagal nerve stimulation attenuates disease activity in models of inflammatory arthritis.
- Neural control of inflammation involves multiple feedback loops involving somatic, sympathetic, and parasympathetic nervous systems and the hypothalamic-pituitary-adrenal axis.

Pain historically was viewed as a symptom and not a contributing factor to the inflammatory process. Nonetheless, in the early 1900s, papers began indicating that stimulation of dorsal roots and dorsal root ganglia (DRG) neurons results in peripheral vasodilation,[1] suggesting that sensory neurons not only conduct information to the spinal cord but also have an efferent function. Thus the foundation was laid for future investigators to show that the four cardinal signs of inflammation—erythema and warmth (secondary to vasodilation), swelling (secondary to plasma extravasation), and hypersensitivity (pain secondary to alterations in the excitability of nearby sensory neurons)—can result from neural activation and that thermal, mechanical, and chemical damage to skin is sufficient to trigger these reflexes.

These "so-called" axonal and dorsal root reflexes lead to release of bioactive substances from the peripheral nerve endings, which in turn act on target cells in the periphery, such as mast cells, leukocytes and lymphocytes, and vascular smooth muscle cells. This phenomenon is referred to as "neurogenic inflammation." Thus a bidirectional relationship exists between the inflammatory process and sensory activation. The nervous system is activated by inflammatory mediators, which leads to increased excitability of peripheral nociceptive sensory fibers, causing inflammatory pain. Conversely, the nervous system feeds back on the peripheral inflammatory process. This effect is achieved by output systems at different levels, including primary afferent fibers (axonal reflex), spinal cord (dorsal root reflexes), and the brain (neuroendocrine functions). Our view of the interaction between the inflammatory process and the nervous system has expanded to include both the sympathetic and parasympathetic efferent nervous systems.

A 1903 report concluded that the sympathetic nerve influenced the course of inflammation by nervous functions other than vasoconstriction and vasodilatation.[2] Since then, several interactions between the sympathetic nervous system and the immune system have been identified. Lymphoid tissue is highly innervated by sympathetic nerve fibers, and in primary and secondary lymphoid tissues, sympathetic nerve terminals are found in close proximity to immune cells.[3] Whereas multiple neuromediators are released from sympathetic terminals, the majority of research has focused on the role of norepinephrine. Both pharmacologic and molecular biologic investigations have shown that most immune cells express functional adrenergic receptors, which translate neuronal signals into immune cell signals. The immune system can be influenced (enhanced or attenuated) by various adrenergic receptor agonists or antagonists.[4] A role for the parasympathetic nervous system, in particular the vagus nerve, in immune regulation has also been demonstrated,[5] although the efferent linkage is a matter of heated debate.[4]

The importance of intact communication between brain and inflamed tissue has long been clinically recognized. In the early 1960s, it was known that disturbing this linkage—for example, by a stroke—resulted in reduced inflammation on the paralyzed side in patients with rheumatoid arthritis (RA).[6] It soon became accepted that an intact innervation was permissive for peripheral inflammation. However, protection of the affected limb from inflammatory disease was present after paralysis as a result of stroke and loss of upper motor neurons, as well as after loss of lower motor neurons and peripheral innervation.[6-9] Because these two mechanisms are very different, it is difficult to ascertain the common means of protection.

Observations in which normally symmetric inflammation was confined to the innervated/normal limb and was absent or greatly reduced in the mirror-image, paralyzed limb have been confirmed in animal experiments, substantiating this viewpoint.[10] However, some investigators have suggested that the protective effect was due to immobilization rather than denervation. In the past few years, the intriguing possibility that the protective effect of denervation is not strictly neural but rather is the result of a somatotopic-specific inhibition of vascular leakage induced by inflammation has started to penetrate the literature.[11] In summary, major advances have been made in deciphering the details of how the nervous system modulates the immune

system, and although it is clear that important two-way interactions occur between these systems, it is important to note that many mechanisms of that communication need to be unraveled.

## PRIMARY AFFERENT FIBERS

Nociceptors—that is, peripheral receptors that signal pain—are classically thought to transmit information regarding real or impending tissue damage from external sources. Evidence is accumulating that, in addition to sensing changes in the interface between self and the outside environment, many nociceptive fibers signal variations in acidity, temperature, carbon dioxide, and the metabolic state of the internal environment from a predetermined set point. Thus nociceptors can also be thought of as homeostatic sensors and are elements of a feedback loop.[12,13] During chronic inflammation and pathologic pain states, these feedback mechanisms frequently go awry and become players in the pathology.

Nociceptors are called "free nerve endings" because they are not encapsulated or associated with traditional receptor structures, although they are frequently in close contact with small distal blood vessels, lymphatic vessels, and mast cells.[14] This anatomic proximity suggests a functional relationship (see later section "Sensitization"). Cutaneous nociceptors are associated with Aδ (finely myelinated) and C (unmyelinated) axons. A different nomenclature is employed for afferent fibers from joint and muscle, which are known as group I-IV fibers. Group III and IV fibers are very similar to Aδ and C fibers, respectively, in that group III fibers are finely myelinated and group IV fibers are unmyelinated, and both classes have a large proportion of nociceptors. All primary afferent fibers are glutamatergic, and many contain one or more neurotransmitters, notably peptides, including substance P (SP), calcitonin gene–related peptide (CGRP), somatostatin, and galanin. Chemically sensitive afferents are activated and/or sensitized by a wide variety of tissue injury products, including inflammatory mediators such as pro-inflammatory cytokines, eicosanoids,[15-17] glutamate,[18,19] and protons (acidity).[17]

### Skin

Many Aδ and C fibers are polymodal nociceptors—that is, they respond to tissue-damaging levels of mechanical, thermal, and/or chemical stimulation.[20,21] Others are specific sensors for mechanical mechanisms, heat, or cold.

### Joint Afferents

Although most joint structures are innervated, importantly, they do not have equivalent innervation density or signal pain. Nociceptors are not found in cartilage and innervate only the outer third of the menisci; however, nociceptors do innervate synovium, fibrous capsules, ligaments, and bone. Only 20% of fibers in articular nerves are myelinated, with the preponderance being nociceptive finely myelinated group III fibers. Unmyelinated fibers are equally distributed between sympathetic efferent and group IV sensory fibers, with nociceptors comprising almost half of the total population.[22] Many group IV nociceptive fibers are normally quiescent and do not respond to normal range of movement. Some fire action potentials (APs) only after tissue damage and therefore are referred to as silent nociceptors (see later discussion). During inflammation, these fibers may become sensitized (see later discussion) by mediators such as prostaglandin $E_2$, whereupon even movements in the normal range can elicit APs. Other group IV nociceptors are activated predominantly or selectively by noxious levels of pressure or joint hyperrotation.[23] Sympathetic fibers regulate the blood flow in the joint and, via direct or indirect mechanisms, regulate articular vessel permeability.[24]

### Fascia

Fascia are innervated by group III and IV fibers[25-27] concentrated in the outer layers next to the skin, with only sparse innervation of the inner layer overlying the muscle. Collagen fiber bundles do not appear to be innervated by nociceptors.[27] Group III fibers are exclusively mechanoreceptors, whereas the group IV fibers include a large percentage of polymodal nociceptors that respond to heat/cold and chemical factors, as well as to pinch and pressure.

### Muscle

Among the nociceptive afferents that respond to high intensities of local pressure, almost all are also chemoreceptive.[28] Intraneural microstimulation of single group III and IV muscle afferent fibers in human volunteers produces sensations of well-localized muscle cramping that is clearly distinguishable from the sharp pain produced by stimulation of tendon afferents.[29,30]

### Mechanically Sensitive Afferents—"Silent Nociceptors"

Nociceptors of the skin and joints include a class called *mechanically insensitive afferents* or silent nociceptors.[31,32] These nociceptors are normally unresponsive, but once they are sensitized by inflammation or tissue damage, they have greatly reduced thresholds. Their chemical activators are released from injured tissue, from the vasculature via plasma extravasation, from activated resident cells such as mast cells and keratinocytes,[33] and/or from infiltrating immune cells. After sensitization they respond to both normally innocuous and noxious stimulation, frequently including light touch and normal joint movement.

### Sensitization

Sensitization is defined as increased responsiveness and reduced threshold to stimulation, and it occurs at all levels of the neuraxis. It can occur instantaneously or have longer latency and duration. Short-term sensitization can result from receptor phosphorylation or dephosphorylation or trafficking of receptors and channels into or out of the plasma membrane. Longer-lasting sensitization can occur after increased mRNA translation and synthesis of receptors and ion channels.[34]

In the primary afferent, protein synthesis may occur in DRG and protein transport to the terminal may be required[35]; alternatively synthesis may occur in the terminal

itself, obviating the necessity for transport time.[36] Sensitization may include activation of satellite cells in DRG and of astrocytes and microglia in the central nervous system (CNS), with a resultant release of cytokines and other proinflammatory mediators. Release of cytokines within the CNS contributes to pathologic pain and illness behavior.[37] After chronic injury, macrophages and other immune cells may also infiltrate into the DRG and CNS.[38,39]

## EFFERENT FUNCTIONS OF PRIMARY AFFERENT FIBERS

### Neurogenic Inflammation

In the absence of actual tissue injury, activation of peripheral nerves or their terminals results in peripheral release of the same neurotransmitters as are released from their central (spinal) terminals. Action potentials in nociceptors going toward the spinal cord (orthodromic) result in pain sensations. However, action potentials traveling away from the spinal cord (antidromic) lead to a peripheral release of neurotransmitters throughout all branches of that fiber, a process called the *axon reflex* (Figure 30-1). Peripheral release of the neuropeptides SP and CGRP induces increased blood flow and edema, respectively, and gives rise to neurogenic inflammation.[40-43] This "injury free" inflammation can manifest as low levels of redness (flare) from increased blood flow and vasodilation, edema secondary to increased plasma extravasation, and nociception/pain after activation of additional nociceptive terminals.

Electric stimulation of a nerve at Aδ strength induces vasodilation, but not plasma extravasation, whereas stimulation at C fiber intensity results in both.[43] Importantly, in the absence of previous inflammation or direct activation of the immune system, immune cells do not infiltrate in response to nerve stimulation or intradermal SP.[44,45] However, reports have been made of mast cell degranulation after electrical nerve stimulation, indicating that neurogenic inflammation can activate these resident immune cells.[46] In addition, in joint synovium, peptidergic C fibers and sympathetic efferents have trophic effects on mast cell density, providing one specific avenue by which the CNS could influence neurogenic inflammation and other inflammatory states.[47]

One important aspect of neurogenic inflammation is that there is an active spinal cord contribution. Dorsal root rhizotomy eliminates sensory traffic between the spinal cord and the periphery while maintaining the integrity of the sympathetic nervous system and the peripheral terminal (Figure 30-2). Capsaicin activates the vanilloid receptor subtype 1 (TRPV1) expressed on peptide-containing C fibers, elicits a sensation of burning pain, and causes neurogenic inflammation. Significantly, rhizotomy of lumbar sensory roots reduces intradermal capsaicin-induced increases in blood flow and paw edema.[40,48-51] Loss of peptidergic C fibers, depletion of their neurotransmitters, or

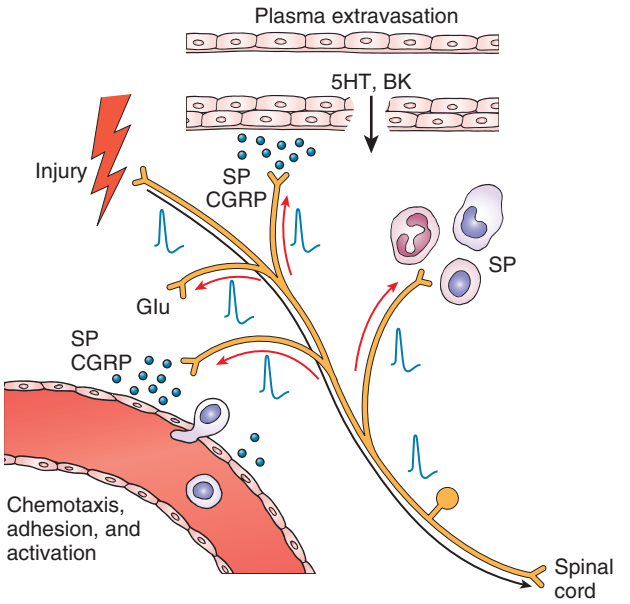



**Figure 30-1** The axon reflex is initiated by nociceptor activation in peripheral tissue by injury and inflammation. When noxious stimuli activate primary afferent fibers, action potentials (APs) are conveyed toward the spinal cord *(black arrows)*. However, at each fiber branch point, APs travel in every possible direction, including anterogradely *(red arrows)*. Thus antidromic axon reflexes are generated and induce peripheral release of neuropeptides. Neurotransmitter release signals to vascular endothelial cells to increase blood flow and induces plasma extravasation (leakage of vascular proteins, 5-hydroxytryptamine [5HT] and bradykinin [BK], and edema), which degranulates mast cells and activates other nerve fibers. Although the axon reflex by itself is not sufficient to recruit immune cells, in a primed system it facilitates recruitment of inflammatory leukocytes. Taken together, these actions produce the flare around an injury site. CGRP, Calcitonin gene–related peptide; Glu, glutamate; SP, substance P.

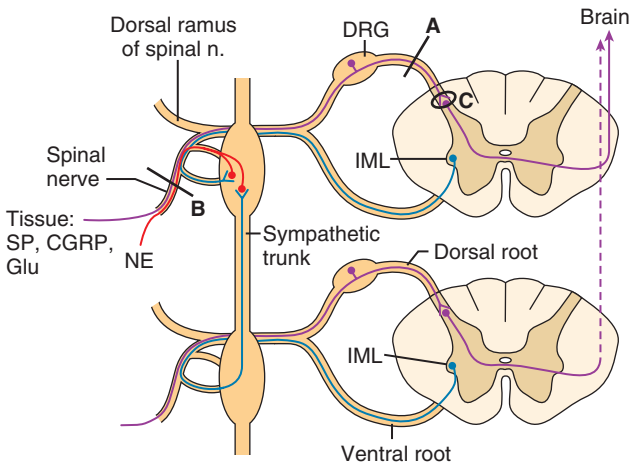



**Figure 30-2** Wiring diagrams of lesions used to assess pathways of neural control of inflammation. Cross-sections of two sequential segments of thoracic spinal cord are illustrated. At each level, a sensory neuron *(purple)*, sympathetic preganglionic neuron (*blue*, located in the intermediolateral cell column [IML]) and a sympathetic postganglionic fiber *(red)* are illustrated. Preganglionic fibers synapse in sympathetic ganglia at their entry level and also send collaterals to sympathetic ganglia along the chain. Dorsal rhizotomy (lesion A) severs only the sensory afferent fibers. Spinal nerve transection (lesion B) severs the sensory fibers and the efferent sympathetic postganglionic axons, including those innervating the vasculature. Intrathecal capsaicin destroys (bilaterally) the central terminals of the peptidergic C fiber (lesion C); importantly, the cell bodies in the dorsal root ganglia (DRG) and peripheral terminals are left intact. If capsaicin is given neonatally, all of the peptidergic fibers are lost. CGRP, Calcitonin gene–related peptide; Glu, glutamate; n., nerve; NE, norepinephrine; SP, substance P.

administration of SP-receptor antagonists also blocks or reduces neurogenic inflammation-induced vascular changes.

## Neurogenic Anti-Inflammation

A neurogenic component exacerbates the inflammation; however, efferent activity in the peripheral nerve also has anti-inflammatory influences. Under conditions of bacterial infection, complete Freund's adjuvant–induced inflammation, or the K/BxN mouse model of arthritis, nociceptors also release neuropeptides (CGRP, somatostatin, and galanin) that modulate draining lymph nodes and can reduce the amount of pro-inflammatory cytokines released by infiltrating macrophages. Somatostatin and galanin also inhibit peripheral release of sensory neuropeptides from peptidergic terminals and thus inhibit plasma extravasation.[52,53] Not surprisingly, animals with a genetic alteration that reduces nociceptor activity or animals with a loss of peptidergic C fibers have reduced pain behavior. Unexpectedly, under the inflammatory conditions previously noted, these animals display increased edema and immune cell infiltration and lymphadenopathy of the draining lymph node.[54-56] The decreased release of anti-inflammatory neuropeptides from the deleted or altered nociceptors could be responsible. Certainly, an anti-inflammatory role for somatostatin is reasonable because treatment with a long-lasting somatostatin analogue has been shown to elicit improvement in patients with refractory RA.[57] Factors controlling the final pro-inflammatory and anti-inflammatory balance of neurogenically released neurotransmitters remain undefined.

## Dorsal Root Reflex

The dorsal root reflex (DRR) is a strictly somatic positive feedback mechanism that engages neurogenic inflammation after intense peripheral activation of nociceptive afferent fibers (Figure 30-3A). Orthodromic action potentials in Aδ and C fibers arising from peripheral tissue activate ascending spinal nociceptive pathways and γ-aminobutyric acid (GABA)–containing inhibitory neurons within the spinal cord via glutamatergic linkages. The inhibitory neurons have collaterals synapsing on the central terminals of the Aδ and C fibers.[58,59] Low-frequency firing in this system elicits pre-synaptic inhibition and a decrease in spinal neurotransmitter release via a process known as primary afferent depolarization (PrAD).[60] In marked contrast, substantial tissue inflammation or injury leads to excessive PrAD and induces enhanced central release of GABA,[61,62] which results in generation of APs in afferent fibers that travel anterograde from the spinal cord to the injured tissue. Neurogenic inflammation triggered by the peripheral release of neurotransmitters, including glutamate, SP, and CGRP, then occurs.

Significantly, DRRs have been recorded in nerves contralateral to the initiating tissue insult.[63-65] This occurrence is consistent with the concept that inflammation in one limb results in inflammation on the mirror-image contralateral limb.[63,66,67] This appears to be a neuronal polysynaptic pathway, and it is not mediated by humoral factors.[66] Importantly, experimental monoarthritis can produce a cellular infiltrate and increased temperature that is specific to the

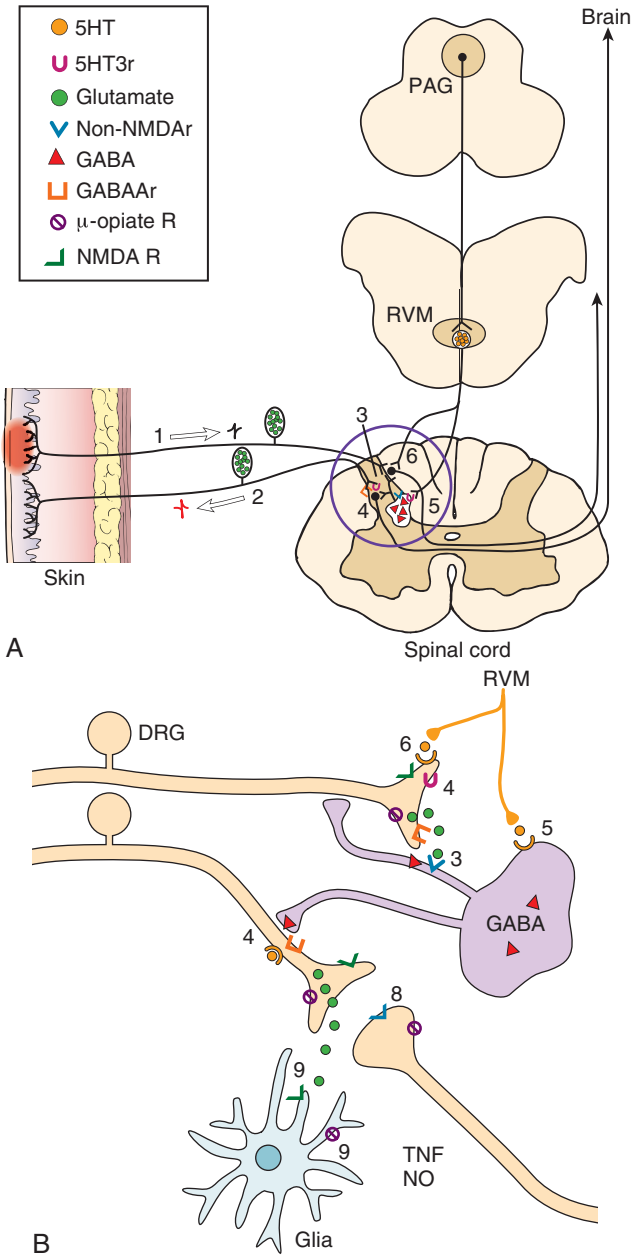



**Figure 30-3** **A,** The dorsal root reflex (DRR). A DRR is induced when (1) action potentials (APs) reach the spinal cord and then (2) anterograde propagation back to the tissue occurs. It is dependent on the central terminals of the afferent fibers being stimulated at an intensity that causes primary afferent depolarization (PrAD) at a level that exceeds action potential (AP) threshold. As pictured, the afferent action potential activates (3) a non–*N*-methyl-D-aspartate (NMDA) linkage on a γ-aminobutyric acid (GABA)ergic neuron. (4) Excess GABA acts on a $GABA_A$ receptor on the central terminal to elicit PrAD. Antagonism of GABA and/or non-NMDA receptors blocks DRR. (5) The system may also be triggered via 5-hydroxytryptamine (5HT) release from the raphe-spinal system acting on 5HT-3 receptors on the inhibitory interneuron or (6) on the terminal endings. **B,** Enlargement of inset in **A**. Activation of spinal opiate receptors and NMDA receptor antagonism is also effective in some longer-lasting models. These agents may work by (7) blocking pre-synaptic release of neurotransmitters, either by direct inhibition or removal of glutamatergic positive feedback. Alternatively, they can (8) act postsynaptically on pain transmission neurons or (9) by blocking glial activation and the resultant central release of pro-inflammatory agents such as nitric oxide (NO) and tumor necrosis factor (TNF). DRG, Dorsal root ganglia; PAG, periaqueductal gray; RVM, rostral ventromedial medulla.

mirror-image contralateral joint.[68] These studies also postulate a role for sympathetic efferent fibers on the noninjured side. Although anatomic details of this pathway remain undefined, DRRs along (group IV) C fibers leading to neurogenic inflammation provide one explanation of a final output pathway.

## Pain Transmission in the Dorsal Horn

The nociceptive sensory primary afferent fibers traverse from the peripheral tissue to the spinal dorsal horn, irrespective of their innervated structure. Not surprisingly, spinal nociceptive projection neurons reflect the local termination pattern of the primary afferents. Thus Aδ and C (groups III and IV) fibers project monosynaptically to superficial dorsal horn,[69-71] the area with the largest concentration of nociceptive-specific neurons. These neurons are subdivided into those having predominant input from Aδ or C fibers and convey information regarding sharp or burning pain, respectively.[72] The lateral deep dorsal horn (lamina V) receives nociceptive input from cutaneous and articular Aδ afferents[69] and also has low-threshold Aβ input. Neurons in this location predominantly have a wide dynamic range, meaning that they respond over the range from innocuous to nociceptive input but have higher-frequency firing in response to the higher-intensity stimulation. Pain projection neurons in the intermediate zone and central ventral horn have convergent input from muscle and cutaneous afferent fibers, including proprioceptors and nociceptors.[69]

Projection neurons receiving information concerning pain and inflammation convey this information to a wide variety of supraspinal structures. These neurons contribute to sensory discriminative, homeostatic, and affective components of pain, as well as to the autonomic responses resulting from the nociceptive stimuli. The classic spinothalamic tract (STT) projects to the ventral posterior lateral thalamus (VPL) and on to the somatosensory cortex. This pathway plays a prominent role in sensory discrimination of nociceptive stimuli. A second STT tract (only lamina I neurons) projects to the central portion of the medial thalamus (MDc) and then to the anterior cingulate, which contributes to the affective but not the sensory component of pain. A third STT pathway to the posterior portion of the ventral medial thalamus (VMpo) thalamus also receives an exclusive lamina I projection and projects rostrally to posterior insula, as well as to area 3a of the cortex. This area is thought to be the cortical processing area for homeostatic input, describing the extra-cellular milieu of the chemodetecting nociceptive C fibers and inflammatory pain, as well as some level of both sensory discrimination and affect.[73]

The spinomesencephalic and spinoreticular tracts project throughout the brain stem reticular formation. In addition to a strong lamina I contribution to the midbrain periaqueductal gray (PAG) and the surrounding parabrachial region, these two tracts provide some input from deeper spinal laminae. The parabrachial region has numerous two-way interconnections with autonomic control centers throughout the brain stem[74] and projects to limbic structures, including the amygdala. There is also a lamina I dorsal horn projection to medullary noradrenergic nuclei that integrate autonomic input and project back down to the preganglionic neurons of the intermediate horn, and thus participate in important sympathetic spino-bulbo-spinal feedback loops. Importantly, the vagal nucleus solitarius is also a termination site for lamina I neurons, thus involving the parasympathetic nervous system as well. Neurons in the deeper laminae (VII-VIII) have a strong bilateral projection to the brain stem (Figure 30-4A).

## Descending Modulation

Although in the early literature it was suggested that pain-selective descending modulation was exclusively inhibitory,[75] it is now appreciated that important excitatory descending pathways exist. As joint inflammation develops and activity in articular nociceptive afferents increases, descending inhibition to spinal nociceptive neurons increases in parallel.[76] This inhibitory feedback loop ameliorates the effects of ascending nociceptive activity. Under other conditions, positive feedback exacerbates it.[77] The backbone of the best-studied descending inhibitory modulation includes a linkage from the PAG to the spinal cord by way of the raphe nuclei, or via the brain stem noradrenergic nuclei (Figure 30-4B). The PAG processes indirect input from ascending pain pathways, the limbic system, and cortical pain-processing centers. Stimulation of ventrolateral PAG in animals and humans is strongly analgesic. The raphe nuclei have a prominent serotonergic projection to the superficial dorsal horn and, indeed, are the only source of dorsal horn serotonin. Serotonin (5-hydroxytryptamine [5HT]) released in the spinal cord can be anti-nociceptive if (1) inhibitory 5HT-1 or 5HT-2 receptors on pain projection neurons, such as in the spinothalamic tract, are involved or (2) if binding is to the excitatory 5HT-3 receptor found on GABAergic inhibitory interneurons or terminals of nociceptive afferents in the superficial dorsal horn.

Although activating an inhibitory pathway is analgesic, serotonin acting on 5HT-3 receptors is proalgesic because (1) in the presence of inflammation, additional 5HT-3 receptor activation of the inhibitory neuron acts just like excessive GABA and results in DRR generation, enhanced inflammation, and pain[63] and (2) some nociceptive projection neurons also have 5HT-3 receptors.[78] Enhanced pain associated with joint and tissue inflammation, but not with surgical incision, is subject to facilitatory influences from the raphe region.[79-81] The parallel PAG-spinal noradrenergic pathway has similar facilitatory and inhibitory feedback components. Inflammation in hairy but not glabrous skin is subject to an α2-adrenergic–mediated descending inhibition via this pathway.[82] Clinically, intrathecal administration of α2-adrenergic agonists produces analgesia. However, much like the receptor dichotomy seen for the serotonergic system, α1-adrenergic antagonists produce pain facilitation.

The amygdala is noteworthy not only because it appears to be important for pain processing but because of its unusual laterality. After induction of a unilateral monoarthritis, neurons in the right but not the left central nucleus of the amygdala develop increased nociceptive responses, irrespective of the site of inflammation.[83] Even more intriguingly, locally administered antagonists of second-messenger pathways to the right amygdala in animals with inflammation result in reduced/blocked enhanced pain behavior resulting from stimulation anywhere in the body.

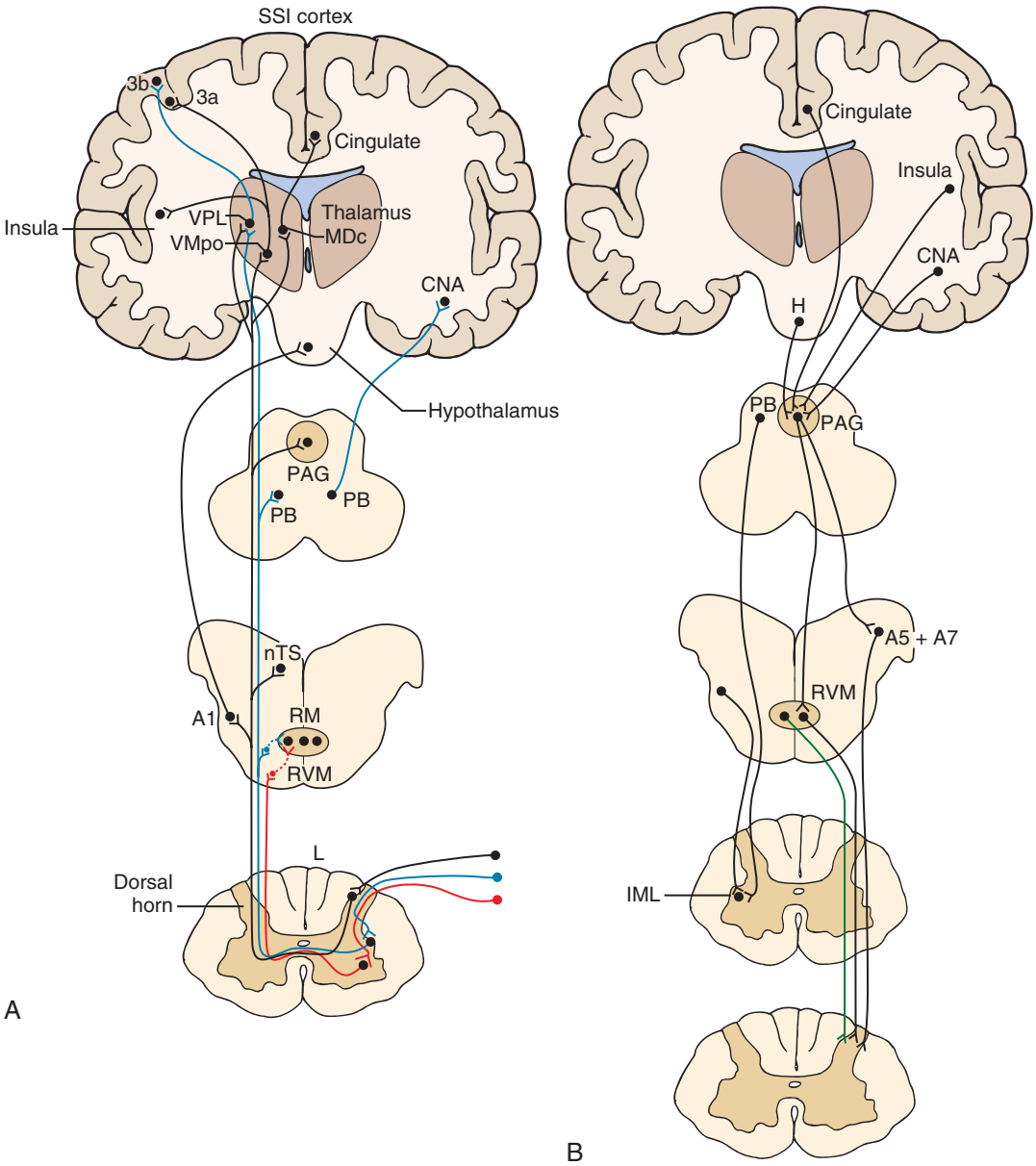



**Figure 30-4** **A,** Ascending pain pathways. Nociceptive afferent fibers enter the spinal cord through the dorsal root and activate neurons in laminae I *(black),* V *(blue)* and VII-VIII *(red)*. Lamina I neurons have strong monosynaptic C fiber input (slow, burning pain) and project directly to the vagal nuclei, periaqueductal gray (PAG), parabrachial nucleus (PB) and thalamic nuclei, mediodorsal thalamic nucleus (MDc) and posterior ventral medial nucleus (VMpo), with a weaker projection to ventral posterior thalamus (VPL). Importantly, the spinovagal connection is triggered by visceral but not cutaneous nociceptive stimulation. Lateral PAG projects onto the amygdala, whereas the VMpo projects further to the insula cortex and area 3a in the deep primary somatosensory cortex (SSI). Thus area 3a has predominantly C fiber input, perhaps accounting for its association with burning pain and inflammation. The MDc projects onto the cingulate gyrus (area 24c). There is also a lamina I projection to several of the catecholamine cell groups in the ventrolateral medulla, including the nucleus of solitary tract (nTS), and A1, a relay to the hypothalamus, which participates in nociceptive stimulation-induced release of adrenocorticotrophic hormone (ACTH). Lamina V-VIII neurons also project to the brain stem reticular formation and PAG and onto the VPL thalamus. Whereas all of the other spinal projections are primarily contralateral, the spinoreticular tract is bilateral (not illustrated). *Dotted lines* indicate indirect connections. **B,** Descending modulatory pathways. Several cortical areas project to the ventrolateral PAG, including the insula, cingulate, frontal lobe, and amygdala. There is also a prominent connection from the hypothalamus. PAG, in turn, projects to several of the noradrenergic brain stem nuclei (including A5 and A7) and to nuclei of the rostral ventral medulla (RVM). Importantly, these RVM neurons contain substance P and other neurotransmitters, as well as serotonin, and wield bidirectional (excitatory and inhibitory) control over ascending pain impulses. Descending control over preganglionic neurons in the intermediolateral cell column (IML) come from the lateral PB and ventrolateral medulla. CNA, Central nucleus of the amygdala; H, hypothalamus; L, lumbar.

## NEURONAL REGULATION OF INFLAMMATION IN ACUTE INFLAMMATORY MODELS

Acute inflammation can be induced by injection of kaolin and carrageenan into the knee joint. In this model, DRRs were recorded from group II, III, and IV fibers, indicating that they are generated in the large myelinated (group II) fibers, as well as the finely myelinated and unmyelinated nociceptive afferents.[84] If acute inflammation has a DRR component, then spinal blockade of either the glutamate receptor (non-*N*-methyl-D-aspartate [non-NMDA]) on the spinal inhibitory neuron or antagonism of the pre-synaptic $GABA_A$ receptor located on the central terminal of the nociceptive afferent fiber should reduce DRR reflexes and peripheral signs of inflammation.

Pre-treatment with either of these agents is effective in blocking not only the actual anterogradely transmitting DRRs[85] but also in reducing edema and temperature increase in the inflamed joint or tissue and pain behavior referred to the paw.[86-88] NMDA or $GABA_B$ receptor antagonists are without effect. These data are consistent with involvement of the DRR originating in the articular afferents, as previously outlined. These data argue against a requirement for activated spinal glia, whose activation is more closely linked to NMDA receptor–mediated spinal sensitization, playing a prominent role in this reflexive activity. Unexpectedly, spinal post-treatment with the non-NMDA and $GABA_A$ antagonists partially reversed already established knee joint swelling but did not reduce the elevated joint temperature,[86] indicating perhaps that different aspects of the inflammation are under separate controls and that, even in acute models, these paradigms change after initiation of the inflammation.

Experiments were repeated with use of capsaicin injection into the skin rather than co-injection of kaolin and carrageenan into the joint. Capsaicin also induced anterograde DRRs in Aδ and C fibers, but unlike the joint afferents, not in Aβ fibers.[89,90] Pre- and post-treatment with spinal $GABA_A$ and non-NMDA glutamate receptor antagonists was effective in this model, but in contrast to the previous paradigm, spinal NMDA receptor antagonists also blocked the enhanced DRRs, as well as reducing the surrounding flare and edema.[90] It is not known if NMDA receptor dependence is due to the location of the insult (joint vs. skin) or to another variant in the experimental design.

The anatomic substrate of the DRR theory requires involvement of sensory fibers running through the dorsal root for both the afferent and efferent legs of the reflex in addition to spinal inhibitory neurons. Theoretically, it does not require the autonomic nervous system, supraspinal centers, or the adrenohypothalamic axis. Both DRRs and edema in the acute inflammatory phase of the kaolin/carrageenan model are not blocked by surgical sympathectomy or high spinal cord transection but are readily interrupted by rhizotomy, lidocaine application to the peripheral nerve, or nerve crush.[91,92] However, reduction in peripheral inflammation due to the functional loss of primary afferent fibers does not indicate whether the nerve fibers are a necessary part of the afferent, the efferent, or both legs of the reflex. A corollary to the need for GABA release from the inhibitory interneuron for DRRs is that anything that activates the interneuron will increase neurogenic inflammation and factors that inhibit inhibitory interneuron decrease the peripheral effect. As previously stated, serotonin, acting on postsynaptic 5HT-3 receptors on the interneuron, facilitates GABA release.[93] Thus, although supraspinal circuitry is not necessary for DRR-induced neurogenic inflammation, activation of either the PAG or the raphe nuclei enhances the phenomena.[63,94]

Another model of acute knee inflammation induced by injection of lipopolysaccharide (LPS) into a knee joint previously primed with carrageenan generates a reactive monoarthritis with leukocyte infiltration into synovial fluid and joint swelling as indices of inflammation. This model is fundamentally different from the previous kaolin/carrageenan one in that spinal administration of glial inhibitors prevented signs of inflammation.[95] This outcome is consistent with the observed dependence of joint damage and inflammation on NMDA receptor activation.[96]

Pre-treatment with spinal tumor necrosis factor (TNF) antagonists or neutralizing antibody also reduced vascular leakage into the knee and infiltration of polymorphonuclear leukocytes into the synovial fluid.[95,97] A spinal PrAD inhibitor (furosemide) also successfully blocked signs of peripheral inflammation in this model.[95] Co-administration of furosemide with either glial inhibitor did not produce an additive effect, indicating, perhaps, that they are in series within a common pathway. Thus, in this third intermediate duration model, DRRs are linked to spinal modulation of peripheral inflammation in tandem with a requirement for glial activation. These results were unaffected by corticosteroid synthesis inhibitors, indicating a lack of participation of the hypothalamic-pituitary-adrenal (HPA) axis.

Spinal administration of morphine also prevented knee swelling and preserved joint integrity in the monoarthritis model with a nonsignificant trend toward fewer immune cells infiltrating into the joint space.[96] The opiate could be acting via (1) pre-synaptic inhibition of the nociceptive afferent signal, reduction of afferent transmitter release and blockade of GABAergic interneuron activation, GABA release, and the DRR or (2) by postsynaptic inhibition of glia or neurons along the nociceptive transmission pathway. Activated glia release TNF and nitric oxide (NO) and further drive and sensitize the system (Figure 30-3B).

In a fourth type of model, cutaneous acute inflammation was induced by intradermal or subcutaneous injection of paw carrageenan, and either vascular leakage (edema) or neutrophil infiltration was measured. Intrathecal pretreatment with a nonsteroidal anti-inflammatory drug, morphine, or agents that inhibit the NO/cyclic guanosine monophosphate (cGMP) pathway all blocked edema without reducing neutrophil infiltration.[98,99] Mechanistically, these agents collectively reduce spinal neurotransmitter release from excited nociceptors. In similar experiments, spinal pre-treatment with either 5HT-1 receptor agonists or 5HT-2 receptor antagonists reduced paw swelling.[98,100] This outcome would fit with a blockade of DRRs, but not of other elements, that elicit immune cell chemotaxis.

In marked contrast, spinal administration of adenosine A1–specific (A1) but not adenosine A2–specific (A2) agonists inhibited neutrophil accumulation due to intradermal carrageenan.[101,102] The adenosine effect was mimicked by a spinal NMDA antagonist and reversed by spinal NMDA

agonists. This latter experiment demonstrated that the NMDA linkage is downstream of the adenosine effect.[44] Inhibition of cellular infiltrates highlight that the spinal A1 protective effect goes beyond merely blocking the DRRs through direct inhibition of the spinal terminals. Interestingly, paw carrageenan elicits a massive reduction in peripheral adenosine, which is time linked to neutrophil infiltration. Adenosine loss, as well as the increase in neutrophil accumulation, is blocked by spinal pre-treatment with an NMDA antagonist.

Peripheral adenosine acting on A2 receptors inhibits peripheral neutrophil infiltration,[103,104] and thus maintenance of peripheral adenosine is thought to be the basis of the antiallodynic activity of both the spinal adenosine A1 agonists and NMDA receptor antagonists.[101] Surprisingly, although both SP and TNF increased in the inflamed skin, neither spinal adenosine A1 agonists nor NMDA antagonists affected these substances in this acute model. Spinal adenosine A1–mediated modulation of inflammation is also dependent on an intact dorsal root (sensory fibers) but is not dependent on the sympathetic efferent fibers.[102] Unlike the other cutaneous models, intradermal carrageenan-induced inflammation was not affected by spinal inhibition of non-NMDA glutamatergic receptors or by functional elimination of capsaicin-sensitive C fibers.

## NEURONAL REGULATION OF INFLAMMATION IN CHRONIC MODELS OF INFLAMMATION

Animal models with joint inflammation that lasts for weeks or more are used to study spinal cord control of peripheral inflammation in RA and include adjuvant-induced arthritis (AIA) in rats, antigen-induced arthritis (AA) in mice, and collagen-induced arthritis (CIA) in mice. As with the acute inflammatory models, section of major lumbar nerve trunks greatly diminished the development of inflammation in the denervated limb.[105,106]

Upon first contact with locally injected capsaicin, afferent neurons discharge action potentials, which in turn evokes burning pain. Higher doses or repeated application of capsaicin desensitizes sensory neurons, and eventually nerve conduction through the treated segment is selectively blocked in capsaicin-sensitive (some peptidergic C) fibers. When capsaicin is injected at high doses intrathecally, it leads to destruction of axon terminals in the primary afferent terminal regions of the spinal cord (see Figure 30-2). Systemic capsaicin administration to neonatal rats destroys subpopulations of DRG cells, reflected by loss of SP and CGRP immunoreactivity and sensory deficits. When intrathecal or systemic neonatal capsaicin pre-treatment was used to eliminate only the central peptidergic sensory fibers, or both their central and peripheral terminals, respectively, severity of the inflammation in the AIA model was consistently ameliorated, indicating that this subpopulation of sensory afferent fibers, and presumably some element that they activate in the spinal cord, contributes to the inflammation.[66,107-109] Interestingly, capsaicin pre-treatment, at doses sufficient to reduce sensory activity, also reduced AIA-induced T cell infiltration into the synovium.[107]

For unclear reasons, lumbar dorsal rhizotomy prior to induction of AIA decreased the time to presentation of clinical signs in limbs innervated by sectioned nerve roots.[66] The commonality among the three lesions is loss of the peptidergic afferent fiber connection. Both capsaicin and rhizotomy preserve the sympathetic efferent fibers. Although there is a loss of the entire peptidergic fiber after systemic neonatal capsaicin, it is thought that both rhizotomy and intrathecal capsaicin preserve all of the DRGs and peripheral peptidergic fibers.[110] Additionally, motor function is preserved after rhizotomy and capsaicin (see Figure 30-2). Interestingly, in a combined lesion study in which animals were pretreated with capsaicin (bilaterally) in conjunction with a unilateral rhizotomy, the joint injury was reduced on the capsaicin-only side, whereas the lesion-plus-capsaicin side displayed increased disease severity.[66] These results show that the effect of peripheral denervation on the severity of joint inflammation in the rat is not dependent only on loss of a particular subset of neurons but rather reflects changes in the activity of both afferent and efferent peripheral neurons. In addition to the complexity of the wiring, it is necessary to involve additional autonomic and endocrine feedback loops to explain the system.

In accordance with the effect of spinal A1 adenosine (Ado) receptor agonists on dermal neutrophil infiltration in an acute inflammation model, continuous spinal administration of an adenosine A1 receptor agonist greatly ameliorated AIA severity. Spinal treatment was beneficial even if it was started as late as 8 days after immunization when animals first presented with clinical signs, but it had a much smaller, nonsignificant effect on paw swelling when the course of treatment began after clinical signs were well established (day 14).[111] Noxious stimulation of afferent fibers results in an increase in c-Fos expression in the nuclei of neurons in the spinal dorsal horn, which is thought to reflect increased neuronal activity levels.[112] Despite the more than 80% decrease in AIA-induced presentation of clinical signs observed with intrathecal adenosine A1 agonist treatment, simultaneous reduction of the AIA-induced c-Fos expression in the superficial dorsal horn was only 22%.[111]

Reduction of c-Fos expression in other spinal laminae was not affected by the A1 agonist. Taken together, this implies that pain is only marginally reduced by the spinal adenosine agonist. Similar anti-inflammatory results were observed with either intrathecal administration of a TNF-neutralizing antibody or SB203580, a p38 mitogen-activated protein kinase (MAPK) inhibitor. In addition, these agents also suppressed synovial infiltration of immune cells and expression of the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and TNF and matrix metalloproteinase (MMP)-3.[113,114]

Arthritis induces a relative increase in sympathetic tone compared to tone of the parasympathetic nervous system; these changes in tone are also prevented by blocking the effects of spinal TNF.[114] Continuous spinal administration of morphine or the NMDA antagonist ketamine throughout a 3-week course of AIA caused a major decrease in joint swelling and synovial infiltration of inflammatory cells.[96] This outcome is likely the result of a loss of DRRs due to pre-synaptic actions on μ-opioid and NMDA receptors, respectively. The opioid-associated reduction in swelling was maintained over the entire period, and tolerance did not develop.

## SYMPATHETIC EFFECTS ON PERIPHERAL INFLAMMATION ARE TIME DEPENDENT

When activated, most sympathetic postganglionic neurons release norepinephrine, which activates adrenergic receptors expressed on the peripheral target tissue. There are two adrenergic receptor types (α and β) that have several receptor subtypes (α1, α2, α3, β1, and β2). Cells in the innate immune system appear to express α1, α2, and β2 receptors, whereas those of the adaptive immune system primarily express the β2 subtype.[4] Importantly, stimulation of the receptor subtypes can elicit different functional effects. For example, macrophages are activated through α2-receptor stimulation, whereas β2-receptor stimulation has a suppressive effect.[4] Thus the role of the sympathetic nervous system in immune cell functioning is complex, and directed pharmacologic studies show mixed results in animal models of arthritis, both ameliorating and exacerbating disease severity. It has been suggested that during the early phase of inflammation, there is "a shift in the autonomic balance toward a sympathetically dominated state."[114]

Neuroactive agents are released from the sympathetic terminals, which contribute to increases in joint and tissue swelling. Mechanisms can vary from increases in blood flow to redistribution, migration, and chemotaxis of leukocytes toward the site of inflammation and β2-mediated release of interleukins and other algesic agents from resident keratinocytes.[115,116] Thus conflicting data with regard to the effect of adrenergic drugs might, in part, be explained by timing of treatments relative to arthritis induction and the onset of disease.

Sympathectomy prior to or at induction of AIA, CIA, and AA, with a loss of the sympathetic terminals in the inflamed joint, results in amelioration of bone damage and a delay in onset of the clinical arthritis.[66,117-119] In contrast, when the sympathetic nervous system was interrupted after the early phases of inflammation, it either did not alter joint swelling and histopathologic scores or exacerbated the disease.[118-121] These studies suggest that the sympathetic nervous system has immunomodulating effects in arthritis models: Prior to the immunization phase or at the early stage of inflammation, it has pro-inflammatory effects, and after the onset, it may have anti-inflammatory effects. This change may be due to a functional loss of sympathetic innervation of the inflamed tissue over time; this loss has been observed in patients with RA and in animal models of RA.[116,122,123] Indeed, an inverse relationship exists between tyrosine hydroxylase (TH)–positive nerve fibers (a catecholaminergic marker of sympathetic neurons) and both the inflammation index and levels of released IL-6 in synovial tissue of patients with RA.[124] This reduction in adrenergic nerve endings is specific and may be due to increased secretion of mediators of sympathetic axon repulsion, such as semaphorin 3c; peptidergic sensory nerve fibers are not affected.[125]

The biphasic nature of the sympathetic system is most prominent in polyarthritis models where swelling and inflammatory signs are present throughout the full duration of the experiment, usually about 2 months. In the acute monoarthritis model mentioned previously, joint swelling presents on day 1 and resolves in 7 to 10 days.[119] As in the polyarthritis model, sympathectomy prior to swelling presentation in the monoarthritis model ameliorated the disease, and post-treatment had no effect. However, if a second flare is induced by a second injection of antigen into the joint after the first flare has resolved, preinjection sympathectomy is once again effective. This outcome implies that in this model and, perhaps, in clinical RA, the sympathetic terminals return and become functional between flares.

Examination of adrenergic receptor subtypes in more detail suggests a prominent role for β2-adrenergic receptors in the sympathetic nervous system's time-dependent, immunomodulatory effect. Rats with continuous administration of a catecholamine reuptake inhibitor or a β2-adrenergic receptor antagonist that began prior to disease induction showed a delayed presentation of clinical signs and less severe joint damage than did control subjects.[126] Smaller, yet significant, protective effects of these agents on joint injury were obtained when treatment was confined to the period either before or after presentation of clinical disease, indicating that reduction of endogenous catecholamines was beneficial throughout the entire 28-day time course.

Subsequent studies from other groups have confirmed the role of early systemic β2-receptor activation as contributory to joint damage and report that administration of β2-agonists at or shortly after disease onset increased the severity of the disease.[120] In vivo studies on the function of α-adrenergic receptor subtypes in experimental arthritis have shown that administration of a nonselective α1/2 antagonist prior to immunization had no effect, whereas administration at the time of immunization increased severity and at the time of disease onset reduced severity.[120,126] Administration of an α1- or α2-selective antagonist prior to immunization had no effect.[126,127] When administered prior to immunization, α2-selective agonists reduced arthritis severity.[128] Thus, the significance of different adrenergic receptor subtypes at different times is unclear, although it seems that both α- and β-adrenergic receptors contribute to inflammation. Beyond these adrenergic-dependent mechanisms are adrenergic-independent components of the inflammation. Local production of inflammatory agents such as bradykinin act directly on sympathetic terminal varicosities to release prostaglandins and adenosine.[129] Many of the complexities concerning sympathetic effects arise from the fact that both adenosine and norepinephrine have concentration-dependent preferences for different receptor subtypes. Different outcomes (pro-inflammatory or anti-inflammatory) result from the subtypes activated.

## PARASYMPATHETIC EFFECTS ON PERIPHERAL INFLAMMATION

Parasympathetic, preganglionic neurons are found in the brain stem and in the caudal spinal cord (S2 through S4 segments). The vagus nerve is the main parasympathetic interface between the brain and body. One body of evidence indicates that vagal stimulation dampens inflammation via activation of a homeostatic "cholinergic anti-inflammatory reflex."[130] The hypothesis is that activation of high-threshold sensory and/or vagal afferents in response to peripheral inflammation provides input to the vagal nuclei in the brain stem and subsequent activation of parasympathetic efferent

pathways. This process culminates in peripheral release of acetylcholine (ACh), which binds to nicotinic ACh receptor subunit 7 (α7nAChR) expressed on immune cells. However, the exact anti-inflammatory efferent linkage is uncertain, and questions remain to be answered. One model suggests that the efferent vagus nerve signals to the celiac plexus and activates the splenic nerve, resulting in local release of norepinephrine. Norepinephrine activates splenic T cells, which promotes local production and release of ACh that acts on macrophages and other immune cells and suppresses cytokine release (Figure 30-5A). However, details of this efferent anti-inflammatory pathway are still a matter of debate. The vagus nerve does not innervate lymphoid tissues, and it has been questioned whether vagal efferents have functional connections to the splenic nerve in the celiac plexus.[4,131]

An alternative explanation is that the vagus nerve has an as yet unidentified target, where it stimulates and mobilizes ACh-synthesizing T cells in lymphoid tissues. Once mobilized, a subset of these T cells is sequestered in the spleen. In this hypothesis, ACh activates α7nAChR on the peripheral terminals of the splenic sympathetic nerves, resulting in noradrenaline release, which then acts on β-adrenergic receptors on splenic macrophages to suppress their production of cytokines[131] (Figure 30-5B). Of note, afferent nerve signals also activate the HPA axis, which promotes glucocorticoid release from the adrenal glands (Figure 30-5).

Vagal involvement has been extensively studied in experimental colitis and endotoxemia models, but several studies have demonstrated effects in animal models of peripheral inflammation. Injection of CNI-1493, a pro-inflammatory mediator synthesis inhibitor, into the cerebral ventricles 1 hour before injection of carrageenan into the paw prevented the expected edema. This anti-inflammatory effect is mimicked by vagal stimulation and attenuated by either bilateral cervical vagotomy or by systemic atropine, thus solidly implicating the vagus nerve.[132,133] Similar benefits of vagal stimulation were observed in CIA (decreased edema, pannus formation, and bone erosion).[134] Systemic treatment with nicotine or an α7nAChR-specific agonist also ameliorated disease activity, whereas unilateral cervical vagotomy exacerbated disease severity.[135] In addition, α7-deficient mice showed a marked increase in synovial inflammation compared with wild-type littermates.[136] Vagal stimulation or direct activation of α7nAChR inhibits inflammasome activation[137] and release of extra-cellular high mobility group box-1 (HMGB1),[138] which has been linked to joint disease in persons with CIA.[139] Interestingly, intrathecal administration of the p38 MAPK inhibitor SB203580 acutely increases vagal outflow and the resultant peripheral cholinergic activity.[140] It is possible that the joint protection offered by p38 MAPK inhibition in arthritic animals is due to increased vagal outflow rather than decreased DRR activity.

## ROLE OF PAIN IN INFLAMMATORY DISEASE

The impact of CNS activation in regulation of inflammation may not be inherently obvious. In addition to generating pain sensations, activation of nociceptors leads to local axon reflexes that cause release of neurotransmitters from peripheral sensory nerve terminals. This mechanism regulates not only small blood vessels but also chemotaxis and activation of immune cells, mainly in a pro-inflammatory fashion, and importantly, it is spatially confined. Activation of nociceptors can also generate dorsal root reflexes, which, in addition to driving neurogenic inflammation at the site of peripheral stimulation, can cause similar responses on the mirror image body site. In contrast, autonomic stimulation has both localized and systemic effects on immunity.

Sympathetic outflow might affect the inflammatory process in specific sympathetically innervated tissue or have a more general impact by driving systemic release of catecholamines from adrenal medulla and thus affect immune cells, or it can modulate immune organs that are innervated by sympathetic efferents, such as lymph nodes and spleen. Although linkages delineating the vagal anti-inflammatory pathway are still being defined, evidence for a parasympathetic influence on the immune system is strong and points to non-neuronal linkages between vagal efferents and the effector site. It is becoming clear that somatosensory and autonomic neuroimmune interactions are much more intricate than previously thought. Furthermore, it is quite likely that it is not individual neural processes and molecules but rather specific combinations of activating and suppressing neuronal signaling that influence different stages and types of immune responses.

Currently, preventing or reducing the activity in the sensory nervous system is a therapeutic target, primarily when it comes to controlling pain as a symptom. However, because activation of nociceptors drives neuronal release of factors that trigger neurogenic inflammation, treatment of immune disorders may therefore need to include targeting of nociceptors, as well as of immune cells. Thus an increased understanding of the molecular interplay between the sensory nervous system and the immune system and the ability to regulate excitability of sensory neurons may have important therapeutic consequences in a number of diseases, including (but not limited to) migraine, arthritis, asthma, and inflammatory bowel disease.

The role of the autonomic nervous system in immunoregulation has led to the proposal of new anti-inflammatory therapeutic approaches for chronic inflammatory conditions. Based on the animal models previously discussed, it can be envisioned that regulating the activity in the sympathetic or parasympathetic nervous system can control inflammation. No clinical study of adrenergic agents as a treatment strategy for patients with chronic inflammatory joint disease has been published. Because data targeting adrenergic receptors obtained from animal models are somewhat variable, additional research is needed to fully understand how catecholamines influence the functional state of specific immune cell populations; without that information, it is difficult to draw definitive conclusions as to which treatment would be beneficial for which patient groups.

With regard to the parasympathetic nervous system and treatment, the two major options are pharmacologic activation of the α7nAChR or vagal stimulation. Nicotine is a potent α7nAChR agonist, but the therapeutic value of nicotine is limited because of its lack of specificity and toxicity. More specific α7nAChR agonists are well tolerated in healthy volunteers and in patients with schizophrenia.[141,142] However, the LPS-induced innate immune

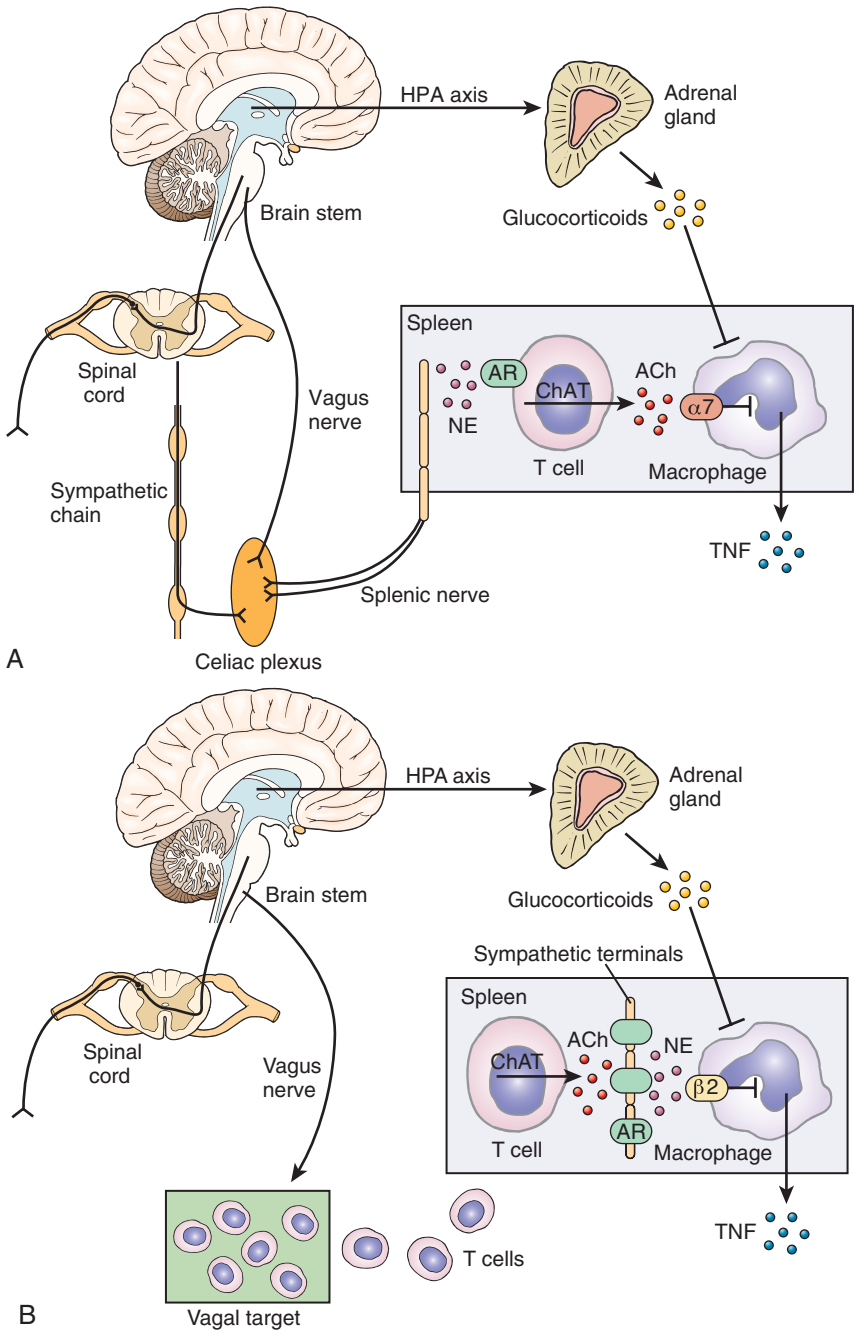



**Figure 30-5** Stimulation of the efferent vagus nerve inhibits tumor necrosis factor (TNF) production in the spleen. The illustrations depict two models for this anti-inflammatory pathway. **A,** Stimulation of the efferent arm of the vagus nerve signals to the celiac plexus, where the splenic nerve is activated. The splenic nerve releases norepinephrine (NE) in the spleen, which activates adrenergic receptors (AR) on choline acetyltransferase (ChAT)-positive T cells. T cells then produce and release acetylcholine (ACh), which acts on α7nACh receptors (α7) on macrophages and other immune cells and suppresses cytokine release. **B,** This model is based on the absence of a direct neuronal link from the vagus to the spleen and suggests a non-neural connection. The vagus nerve targets a (as yet unidentified) structure, where it is in close contact with ChAT-positive T cells, which are mobilized and sequestered in the spleen. ACh released by the T cells activates α7nAChR on the peripheral terminals of the splenic sympathetic nerves, which release norepinephrine. Norepinephrine then acts on β-adrenergic receptors on macrophages and suppresses their production of TNF. Last, following sensory input to the brain stem, the signals are also relayed to the nuclei controling the function of the hypothalamic-pituitary-adrenal (HPA) axis. This results in an increased release of glucocorticoids from the adrenal gland, also connecting the networks to humoral anti-inflammatory mechanisms.

response (release of TNF, IL-6, IL-10, and IL-1 receptor antagonist) in humans was not reduced by the highest tolerable dose of GST-21, an α7nAChR agonist, compared with placebo.[143] Ongoing studies exploring the potential of vagal nerve stimulation in patients with RA are under way. Vagal stimulation is already in use clinically for treatment of refractory epilepsy and depression.[144,145] Intriguingly, long-term vagal stimulation in patients with epilepsy caused a normalization of plasma cytokines and cortisol levels compared with the control group.[146] However, further preclinical and clinical studies are needed to further explore the potential and safety of using vagal stimulation as therapy for patients with inflammatory disorders.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 31

# Clinical Research Methods in Rheumatic Disease



Yvonne M. Golightly • Joanne M. Jordan • Kenneth G. Saag

**KEY POINTS**

- Epidemiology is the study of the distribution of disease and its determinants in populations. Epidemiologic methods can be used to describe the frequency or development of disease and to determine underlying risk factors.
- Prevalence is the frequency of disease in the population at a given time, including both existing and new cases. Incidence measures the development of disease with time in an initially disease-free population.
- The odds ratio (OR) compares the odds of disease in an exposed population to that in a population without the exposure or risk factor under study. The relative risk (RR) is the risk of development of disease with time in an exposed population compared with an unexposed population. In instances in which the disease is rare, the OR can estimate the RR.
- Threats to the validity of a study include chance, systematic bias, and confounding. Confounding occurs when an extraneous factor, associated with both the exposure of interest and the disease, but which is not part of the causal pathway between exposure and disease, is superimposed on the true risk factor/disease relationship.
- Case-control studies examine exposures in a population that already has the disease, and compares them with exposures in otherwise comparable but disease-free individuals derived from the same source population. This study design may be subject to recall bias, in which individuals with disease are more likely to recall exposure to risk factors than those without the disease, but it may be the design of choice for rare diseases.
- Cohort studies follow groups of individuals with and without an exposure of interest to measure the development of disease with time. Because the exposure assessment precedes the disease, time can help determine causation.
- Randomized controlled trials (RCTs) most closely resemble formal experiments in which the exposure is manipulated by the investigator and the response upon disease is compared between groups that receive the active intervention and those that receive a placebo or other comparator. A pragmatic clinical trial provides evidence of real world effectiveness.

Epidemiology is the study of the distribution of disease and its determinants in populations.[1] Its purpose is to describe the frequency of disease and to determine risk factors and causes responsible for variation in disease occurrence. Comparison of the relative strengths of risk factors and assessment of their generalizability can allow truth to be inferred to a broader population. Causality is often difficult to discern in observational investigations, particularly based on the results from a single study. The criteria proposed to assess the likelihood of a cause-effect relationship include consideration of the strength of the association, biologic credibility, consistency with other investigations, the temporal sequence, and a dose-response relationship.[2] This chapter will explain basic epidemiologic concepts and definitions; describe the major study designs, their strengths and weaknesses, and their usefulness in inferring causality; and demonstrate specific applications of these principles to the study of rheumatic diseases. The term "disease" will be used to represent a disease, death, or other health outcome of interest, and the term "exposure" will be used to represent a risk or protective factor examined for its association with disease.

## MEASURES OF DISEASE OCCURRENCE

The frequency of a disease in a population at any given time is referred to as its *prevalence*. Measured at one point in time, prevalence is the proportion of individuals with a disease of the total population under study. Importantly, the numerator makes no distinction between new and established cases of disease. Multiple estimates of disease prevalence during a period of time are commonly used to determine trends in disease occurrence or the need for health services.

### Incidence

To determine the likelihood that disease will develop with time, repeated observations of the same people are required to determine in whom disease develops and in whom it does not. Incidence proportion, or risk, is the frequency of new cases during a specified time, out of those at risk for, but without, the disease at baseline. During the observation period, a person may manifest the disease in question, die

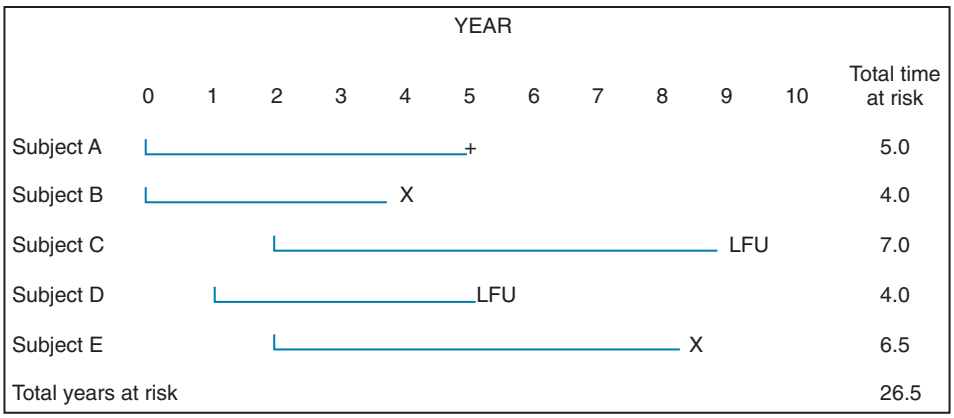



**Figure 31-1** Hypothetical calculation of person-time at risk for study of incidence of systemic lupus erythematosus during 10 years. |, Beginning of observation period; LFU, lost-to-follow-up; +, died; X, developed disease.

from competing risks, or be lost to follow up. All of these situations result in that person no longer contributing time at risk to the denominator. The concept of person-time includes the actual time at risk contributed by each individual. For example, consider the hypothetical example of incidence of systemic lupus erythematosus (SLE) during a 10-year period (Figure 31-1). The disease may not develop in Person A during the observation period, but he or she may die at year 5 of a competing cause; this person contributes 5 person years to the denominator. SLE might develop in Person B 4 years after the study begins, when he or she is thus no longer at risk; this person contributes 4 person years of time at risk to the denominator. Person C joins the study at year 2 and is lost to follow-up at year 9, contributing 7 years of time at risk. Person D joins the study at year 1 and is lost to follow-up at year 5, for a total of 4 years of person time at risk. Person E joins the study at Year 2 and manifests SLE halfway between year 8 and year 9, contributing 6.5 person years at risk. Incidence rate is defined by the following[2]:

$$\text{Incidence rate} = \frac{\text{new cases developing during time period of observation}}{\text{total person-time at risk for each individual without the disease at study entry}}$$

In the example, two new cases of SLE are observed and the total person time from persons A through E at risk equals 5.0 + 4.0 + 7.0 + 4.0 + 6.5 = 26.5 person years. Thus, the incidence rate is 2 cases per 26.5 person years, or 1 case per 13.25 person years. This may be expressed as 0.0754 cases per person year or 7.54 cases per 100 person years.

## MEASURES OF EFFECT

The association between the disease and exposures or risk factors is more important than the description of the frequency of disease or its development. One way to examine this association is to compare the prevalence or incidence of disease in groups with a given exposure with those without that exposure. To assess the potential exposure/disease association, it is critical that the exposed and unexposed groups are comparable. Measures to delineate this relationship between disease occurrence and exposure vary according to study design. Cross-sectional surveys and case-control studies utilize the odds ratio (OR), which is a measure of the odds (prevalence odds = prevalence / 1-prevalence; incidence odds = incidence / 1-incidence) of disease in the exposed group compared with the unexposed group. Longitudinal designs can calculate a RR (ratio of risks) of the incidence of disease in the exposed compared with the unexposed groups.

## CLINICAL RESEARCH STUDY DESIGNS

Clinical research study designs include ecological studies, cross-sectional surveys, case-control studies, cohort studies, and randomized controlled clinical trials. The latter is frequently considered the most rigorous study design and the one most closely representing a formal experiment. Each study design has its own inherent strengths and weaknesses (Table 31-1), and the choice of study design depends upon the research question, the rarity of the disease under study, the availability of appropriate study and comparable control populations, resources available to conduct the study, and logistics.[3,4]

### Observational Studies

In observational studies, the exposure is not randomly distributed in a population. The investigator observes the exposure rather than selects the exposure status of an individual.[5] Types of observational studies include ecological, cross-sectional, case-control, and cohort.

Disease registries may be a data source for some cross-sectional surveys and cohort studies. A disease registry is typically formed when individual occurrences of a disease are obtained from multiple sources within a defined geographic area. The data from these sources are linked to avoid duplication of cases. Registries may be population-, hospital-, or clinic-based. Hospital- and clinic-based registries may identify potential participants for clinical research.[5,6] Examples of registries that collect longitudinal population-based data on rheumatic diseases include the National Data Bank for Rheumatic Diseases (http://www.arthritis-research.org/) and the Arthritis Internet Registry (https://www.arthritis-research.org/join-research/arthritis-internet-registry).

**TABLE 31-1** Common Epidemiologic Study Designs and Their Strengths and Weaknesses

| Study Design | Definition | Measure of Effect | Strengths | Weaknesses |
|---|---|---|---|---|
| Ecological | Aggregate data on exposures and disease; unit of analysis is a group, not an individual | Odds ratio | Inexpensive<br>Short duration<br>Hypothesis-generating | Susceptibility to confounding<br>Ecological fallacy |
| Cross-sectional survey | Data on exposures and disease obtained at one time from all individuals in an area (or a sample thereof) with and without disease | Prevalence<br>Odds ratio | Can study several outcomes<br>Short duration<br>Can generate population prevalence estimates of disease and risk factor distributions | May not be able to determine whether disease preceded exposure<br>Not practical for rare diseases<br>Cannot produce incidence or relative risk estimates |
| Case-control | Study of exposure/disease relationship in cases with a disease and controls without that disease, who are selected from source population from which the cases arose | Odds ratio | Best for studying rare conditions or those with long latency<br>Short duration<br>Small sample*<br>Inexpensive*<br>Odds Ratio can approximate Relative Risk | Inefficient for rare exposures<br>Potential bias from sampling cases and controls separately<br>May not be able to determine whether exposure preceded disease<br>Potential recall bias<br>Potential survivor bias<br>Cannot produce prevalence or incidence estimates |
| Cohort | Individuals without disease are followed during a period of time to determine which characteristics predict who will get the disease and who will not. | Incidence<br>Relative risk | Can determine sequence of events<br>Less susceptibility to survivor bias and bias in measuring predictors<br>Can study multiple outcomes<br>Can generate population incidence, relative risk | Frequently requires large samples<br>Not feasible for rare outcomes<br>More expensive<br>Long duration |
| Prospective | Study sample selected by investigator and followed forward in time for development of disease. | Incidence<br>Relative risk | Investigator control over selection of participants and measures | Increased expense<br>Long duration |
| Retrospective | Study sample and measurement of exposures and disease during a period of time have already occurred. | Incidence<br>Relative risk | Less expense<br>Short duration | Less control over selection of participants and measures |
| Nested case-control and case-cohort | Case-control study within the context of a prospective or retrospective cohort | Incidence<br>Relative risk | Underlying cohort design<br>Relatively inexpensive, compared to measurement on entire cohort | May require bank of samples that can be assayed at later date until or after outcomes occur |
| Randomized clinical trial | Exposure (pharmaceutical, non-pharmacologic device, educational intervention) manipulated by investigator | Relative risk<br>Hazard ratio | Most closely emulates an experiment<br>Strongest design to produce evidence for cause and effect<br>Random assignment of intervention minimizes confounding<br>May be faster and cheaper for some study questions than observational studies | Costly in time and money<br>Some research questions not suitable because of rare disease or ethical barriers<br>May not be generalizable if highly controlled environment does not reflect "real world" common practice<br>May have narrow scope and study question |
| Pragmatic clinical trial | Effectiveness of interventions evaluated in usual clinical practice conditions | Relative risk<br>Hazard ratio | Tests whether intervention works in real-life practice setting<br>Participants more likely to represent most individuals with the condition of interest<br>Results may be more generalizable to clinical care and more relevant to patients than efficacy trial | Participants and providers may be unblinded so important that assessors are blinded to group assignment |

*Relative to cohort study design.

Modified from Hennekens CH and Buring JE: *Epidemiology in medicine*, Little Brown and Company, 1987, and Hulley SB, Cummings SR: *Designing clinical research: an epidemiologic approach*, Williams & Wilkins, 1988.

## Ecological Studies

In this study design, the unit of observation is a group, rather than an individual.[6] Aggregate data on rates of disease and risk factors are compared to examine associations between disease frequencies and exposures. The ecological study is frequently a design of expediency, and can generate hypotheses for more rigorous testing in studies by using individual-level data.[4] One of its chief drawbacks is its high susceptibility to confounding. This occurs when an extraneous factor, not on the causal pathway, masks the true relationship between exposure and disease, by virtue of its association with both.[7] Further, associations in the aggregate may not necessarily hold true for the individual.[4] This concept is termed the *ecological fallacy*. As a hypothetical example, rates of specific kinds of cancers may be higher in countries in which cigarette sales are also high. Whether the individuals who are buying, and presumably smoking, the cigarettes are the same individuals in whom cancer develops is not known from this study design.

## Cross-Sectional Surveys

The goal of this study design is usually descriptive, including all individuals, with and without the disease under study, in the population, or a representative sample of them, at one point in time with no follow-up period. Surveys can estimate prevalence of a particular disease in the population and determine need for health services and resource allocation.[4] Typically, information about risk factors is obtained simultaneously. Such risk factor data may or may not represent the most relevant time of exposure, nor can it be determined whether the exposure preceded or resulted from the disease.[3]

An example of a cross-sectional survey, conducted approximately once per decade in the United States, is the National Health and Nutrition Examination Survey. This survey samples a proportion of the residents in the contiguous 48 states and measures various health outcomes and habits, such as blood pressure, serum lipids, height, weight, smoking, and dietary intake. These surveys have been used in rheumatology to determine the prevalence of radiographic knee and hip osteoarthritis (OA) in various age, sex, and race/ethnicity subgroups.[8]

## Case-Control Studies

Much maligned by the uninitiated because of its susceptibility to bias, the case-control study can be the study design of choice—or sometimes the *only* appropriate study design—in certain situations, particularly when the disease under study is rare. This type of study usually includes fewer individuals and is much less costly and more efficient than cohort studies. These advantages stem from the fact that it is composed of individuals who already have the disease under study, compared with the cohort study in which researchers must wait for the disease to develop in a small proportion of a large cohort with time. Most important in the design of a case-control study are (1) the choice of the control group, which must be comparable to the cases, and (2) recognition of potential biases that may threaten validity.

Strictly defined, the case-control study compares a group of individuals with the disease under study with a control population without the disease. Both groups are drawn from the same source population.[1,7] The source population may be the residents of a particular geographic area or a hospital's referral base. The control group serves as an estimate of the distribution of the exposure in the source population, and consequently, the control group must be sampled independently of exposure status.[1,7] For example, if researchers are interested in examining the possible association between smoking and progressive systemic sclerosis (PSS), the controls must be from the same source population that generated the cases of patients with PSS, if this can be determined, and must be sampled without regard to their smoking status.

### Selection of Controls for Case-Control Study

If the source of the cases is a well-defined population, the controls can be sampled directly from that population. If the source population is too large to allow a complete enumeration, controls may be matched to each case by their residence in the same neighborhood. Random-digit dialing can be used to select controls, but this labor-intensive method omits those without telephones or those who cannot be reached.[1] If the cases are drawn from a particular hospital or clinic, then the source population should represent people who would be treated in that hospital or clinic if they developed the disease under study, but frequently, this source population can be difficult to identify and is influenced by referral practices.[1] Hospital or clinic controls can be used, but this method can have particular pitfalls, because the controls might not be selected independently of the exposure in the source population. For instance, in a hospital-based study of smoking in SLE, individuals hospitalized for other diseases, such as myocardial infarction or pneumonia, might have exposures different from the source population in general, especially if the exposure, in this case smoking, causes or prevents the "control" disease selected. One way to avoid this is to exclude diseases known to be associated with the exposure under study, but this may create other biases. Another tactic could be to select hospital controls with diseases thought to be unrelated to the disease or exposures under study, such as traumatic leg fractures,[1] or to use several control groups selected by different methods.[4] For instance, sample controls might be chosen from hospitalized patients with diseases other than that under study, nonhospitalized patients in the same medical care system, or nonhospitalized individuals in the general population, comparing each control group separately with the diseased group.

### Weaknesses of the Case-Control Design

It is not possible to derive incidence or prevalence estimates from a case-control study. The greatest threat to validity is the inherent susceptibility to bias that can exist in this study design, because the cases and controls are sampled separately, and the assessment of exposure variables is retrospective.[4] Matching the cases and controls on factors such as age, sex, or race/ethnicity, can help ensure comparability of cases and controls to a degree. As mentioned above, more than one control group, selected in different ways, can be

used to see whether findings are consistent across control groups with different sampling biases. A nested case-control design (described later), in which a case-control study is performed within a larger cohort study, has the advantage of minimizing sampling bias because the cases and the controls would have been previously sampled in identical fashion into the parent cohort study.[4]

The other chief source of bias in the case-control study is recall bias, which occurs when exposures predating the disease are differentially reported by the controls and the cases, the latter of whom may have incentive to remember and report exposures. This can be partially prevented by using exposure data measured before the disease occurred, if available, and by blinding the observer and the participant to the exposure under investigation, or if possible, blinding them even to the specific disease under study and therefore, to case or control status. For example, in a case-control study examining racial/ethnic variation as the exposure variable of interest in SLE, race/ethnicity is immutable and thus not subject to recall bias. In contrast, if study participants know or suspect that prior exposure to hair dye, for instance, is the exposure of interest in the same case-control study, those with disease may be more prone to "remember" their exposure than those without disease. Investigators can obtain information about multiple potential exposures or even include several "dummy" exposures to mask the real hypothesis to try to minimize this type of bias.[9]

## Cohort Studies

Cohort studies follow groups of individuals without the disease in question during a period of time to describe the development or incidence of disease and to compare the incidence of disease between groups with different risk factors or exposures. Cohorts can be prospective or retrospective.[1,4]

### Prospective Cohort Study

Prospective cohorts are characterized by the selection of the cohort and measurement of risk factors or exposures before the outcome has occurred, thereby establishing time sequence or temporality, an important factor in determining causality. This design provides a distinct advantage versus the case-control study, in which exposure and disease are assessed simultaneously.

The primary disadvantage to the prospective cohort study is its expense. It requires large numbers of individuals to be followed up, potentially for long periods of time. Biases can creep in, particularly if there is significant loss to follow up. This study design is highly inefficient and inappropriate for study of rare diseases, but its efficiency increases while the frequency of the disease in the population increases.[4] For example, a prospective cohort study would be inappropriate to study PSS, because of its rarity, but excellent to study a common condition such as OA.[10,11]

### Retrospective Cohort Study

In a retrospective cohort study, individuals are followed during a period of time, but the cohort selection and collection of data have already occurred, sometimes for a different purpose than the current disease under study. For example, a cohort of individuals with small vessel vasculitis seen at a particular hospital between 1990 and 1992 could be identified, and data abstracted regarding baseline serologies, physical examination findings, and biopsy results when the patients were first evaluated. Then, examination of outcomes such as stroke or development of dialysis-dependent renal disease could be ascertained in 2000, by medical record review or by re-contact with the individuals so identified. Because exposure or risk factor assessment precedes assessment of outcome, this study design can establish temporality, as in a prospective cohort, and is less subject to recall bias that can hinder case-control studies. By selecting the cases and controls from the same source population, this study design also avoids some of the selection biases of case-control studies, in which the cases and controls are sampled separately. The retrospective cohort design is cheaper and more efficient than a prospective cohort, but because the data collection has already occurred, inferences from such a study are highly dependent upon the quality, completeness, and appropriateness of the original risk factor assessments to study their association with the disease in question.[4]

### Nested Case-Control and Case-Cohort Studies

These studies are case-noncase studies that occur within the context of a prospective or retrospective cohort, and are particularly useful to assess risk factor variables that would be too expensive to measure among all members of the cohort, such as biologic or genetic measurements.[4] In these designs, all members of a cohort who have experienced a particular outcome during the observation period (cases) are selected and compared with a subset of individuals within that same cohort. Full exposure or risk factor information is then collected. Sampling of these noncases differs between the nested case-control and case-cohort designs. In nested case-control studies, controls are sampled from individuals at risk for the outcome (i.e., they do not yet have the outcome but might have it in the future) at the same time that cases are identified. Controls may be selected to match cases on potential confounding variables (see Confounding), forming matched sets of cases and controls. In the nested case-control sample (i.e., cases and sampled controls for each case), exposure variables and covariates are then observed. In case-cohort studies, controls are derived from a subcohort sampled from the baseline cohort.[12] This subcohort is a random sample of the entire cohort, which means that selection of this subcohort is independent of whether or not an individual becomes a case; the subcohort may contain cases by chance. Data on exposures and covariates from the cases and subcohort sample are then collected and observed.

## Clinical Trials

### General Principles of Clinical Trial Design

The study designs described previously in this chapter are all observational designs that include no experimental manipulation of the exposure or outcome. Experimental

study designs or interventions include clinical trials, field trials, and community intervention trials.[6] Inferences from such trials of treatments assigned randomly to a large enough sample are much less likely to suffer from biases and other threats to validity than observational designs. In theory, randomization should eliminate most confounding, although some variation in risk factors between the intervention and control groups may occur by chance. This is more likely if the study size is small, and this possibility should always be ascertained and addressed in the analysis if necessary. The validity of conclusions from a RCT depends in part upon the avoidance of loss to follow up or participant dropout.

RCTs can be conducted for pharmacologic or nonpharmacologic interventions, such as dietary, physical activity, assistive devices, or educational interventions. Trials can include single or multiple dosages of the study intervention, placebo controls, active comparator controls in which the intervention of interest is compared to another agent whose efficacy is known, and combinations of interventions. For example, the Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) compared glucosamine hydrochloride alone, chondroitin sulfate alone, and the combination of glucosamine and chondroitin with placebo and with an active comparator, celecoxib, for their effects on symptoms of OA of the knee.[13-16] The Intensive Diet and Exercise for Arthritis (IDEA) trial was a nonpharmacologic rigorous weight loss intervention in which intensive dietary restriction, exercise, and the combination of diet and exercise were compared.[17] Such nonpharmacologic trials may include an "attention-control" in which the control group does not get the specific intervention of interest, but does get at least a minimal amount of attention from the investigator, because it is known that even minimal contact with the participants in a study can improve outcomes.[18] Optimally, to minimize bias, the study should be double-blind, in which the assignment of treatment is unknown to the participant and to the data collector evaluating the participant's response. A cross-over design is a within-patient design which allows each participant to be his/her own control and receive either the active intervention followed by a "washout" period in which no active or inactive treatment is given, and then the control treatment, or vice-versa. This design has some advantages, particularly in sample size requirements, but can be biased if there is a significant carry-over effect of the active treatment into the "control" observation period.[6] Response to treatment may also differ depending upon whether the active treatment is received before or after the placebo or other comparator.[19,20]

Other important considerations in RCTs are the selection and means of assessment of primary and secondary outcomes, which must be pre-specified. Outcomes can include measures of disease modification, symptom modification, and frequency of side-effects or other poor outcomes. Symptom modification trials are frequently of short duration and less expensive than disease modification trials, which generally are interested in longer-term outcomes. In RCTs of biologics for rheumatoid arthritis, for instance, effects on symptoms can frequently be measured in weeks to months, whereas effects on prevention or healing of radiographic erosions may require longer follow-up times.[21] Disease modification trials in OA that use radiographs generally require large numbers of individuals followed up for at least 2 years, predominantly because responsiveness to change in minimal joint space width is improved in studies of 2 years duration.[22,23] Outcomes based on magnetic resonance imaging exhibit moderate evidence for construct and predictive validity and good evidence for reliability and responsiveness, likely allowing for smaller sample sizes and shorter observation periods to demonstrate an effective response.[24] The Osteoarthritis Research Society International made recommendations to the Federal Drug Administration about study design, imaging modalities of choice, and other issues regarding structure modification in OA in 2011.[24]

Other trial designs can apply interventions to entire communities or to health care workers with measurement of outcome in their patients. An example of the latter would be the Patient and Provider Interventions for Managing Osteoarthritis in Primary Care (PRIMO) study,[25] in which patient-specific treatment recommendations for behavioral and clinical treatment of knee and hip OA (e.g., weight management, physical activity) are given to providers at the time of care. The physicians receive the intervention, but whether a particular intervention is prescribed and whether it improves patient symptoms, is measured by assessing the patient.

Although RCTs represent the "ultimate" study design closest to a controlled experiment, there are significant potential threats to its validity. Importantly, not all conditions can be subject to randomization. For example, in studying the association between cigarette smoking and rheumatoid arthritis, it would be unethical to randomly assign participants to smoking, thus only observational studies are possible. Another challenge of RCTs is that they generally are too short in duration to adequately assess therapeutic safety for many drugs and devices for which longer term outcomes are of public health interest. One of the most important biases can occur when there is large loss to follow up. To minimize this type of bias, every effort should be made to continue to obtain outcome information on all participants, even those who otherwise discontinue study assignment to therapy. Because all predictors of dropout cannot be known, and because dropouts may differ from individuals who remain in a study in ways that cannot be controlled, conventional analyses of treatment status are likely confounded.[6] Data may be analyzed in an intention-to-treat fashion, in which all randomly assigned participants are analyzed as a member of the group to which they were initially randomized, regardless of whether they actually adhered to the group assignment, but this analytical method can be biased by noncompliance leading to a misclassification of treatment status.[6] One method for dealing with treatment noncompliance suggested by Mark and Robins[26] includes making the assigned treatment a fixed covariate and received treatment a time-dependent exposure in a structural failure-time model. Completer, or "according to protocol," analyses are often also performed, in which only those who adhered to their assigned group treatment are included in the analysis. Per protocol analyses are generally not the primary analysis for efficacy in therapeutic trials, but they may be the most important analysis to examine for safety outcomes. Pre-randomization screening and run-in periods before randomization can help to avoid randomly

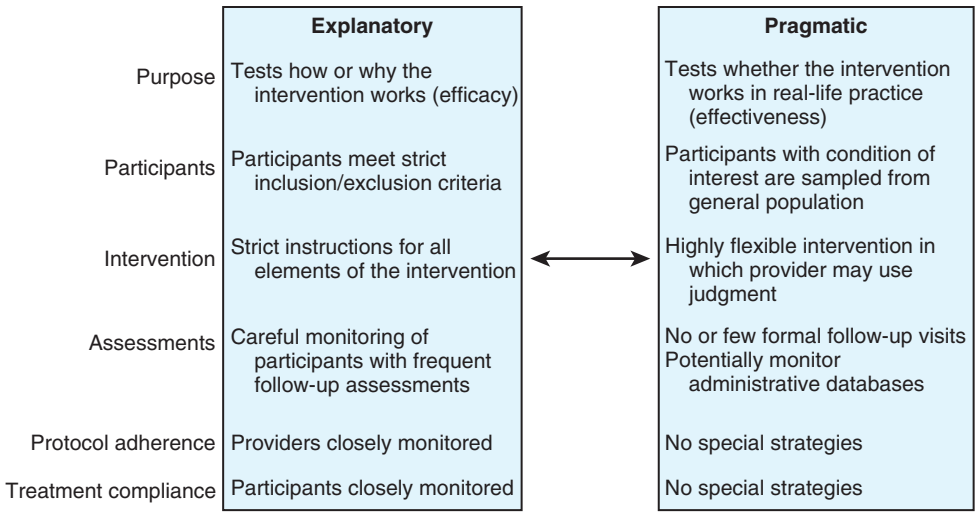



**Figure 31-2** Clinical trials: explanatory to pragmatic continuum.

assigning those unlikely to adhere to or complete the protocol, thereby minimizing expense and dilution of effects.[27,28] Other issues to consider in the interpretation of results of RCTs are generalizability and the difference between efficacy in a controlled environment and effectiveness in the real world of everyday practice (see section on Pragmatic Clinical Trials). Post-marketing observations can often reveal side-effects or unintended consequences of interventions that may not be apparent within the context of highly regulated trials.

## Noninferiority Trials

The most common type of RCT is the superiority trial, in which investigators determine whether a new treatment is more effective than placebo, no treatment, a lower dose of the test treatment, or an established treatment that is widely used or has known effectiveness. Noninferiority trials, on the other hand, are used to determine whether the effect of a new treatment is no worse than a reference treatment.[29-31] This differs from an equivalence trial, which aims to demonstrate that the effect of a new treatment is similar to the effect of the reference treatment.[29]

Designing and interpreting noninferiority trials can be challenging because of several weaknesses of this study compared with superiority trials. Intention-to-treat analysis (a commonly used approach in superiority trials, in which not all participants may have completed the treatment protocol) is not possible in noninferiority trials. Intention-to-treat tends to bias results towards the null (treatment equivalence), which, in a noninferiority trial, would result in an inferior treatment being incorrectly labeled as noninferior.[29,30] Additionally, an inferiority margin must be predetermined, and this margin may be subjectively based on the expectation of a minimally important effect or, more objectively, on the effect of the reference treatment in prior studies.[30,31] For the latter, the assumption is that the effect of the reference treatment in the noninferiority trial is similar to its effect in prior trials, which may not be true if the current and prior trials differ based on critical factors (i.e., study population).[29,30]

## Pragmatic Clinical Trials

Pragmatic trials evaluate the effectiveness of interventions in usual clinical practice conditions.[32] This study design differs from efficacy trials (Figure 31-2), which test interventions in ideal conditions among participants meeting strict inclusion and exclusion criteria. A continuum exists between pragmatic and efficacy trial designs,[33] and a purely pragmatic trial design is rare. Efficacy trials examine whether the intervention works among patients with specific demographic and clinical characteristics under certain conditions, whereas pragmatic trials examine whether the intervention works in real-life practice settings in a way that is relevant to the patients and generalizable in routine conditions. Thus, pragmatic trials offer higher external validity, whereas efficacy trials offer higher internal validity. Because exclusion criteria intentionally are less rigorous (e.g., only excluding individuals for whom there are significant safety concerns that preclude study participation), participants selected for pragmatic trials are likely to represent most individuals with the condition or disease of interest. For example, in the Benefits of Effective Exercise for Knee Pain (BEEP) study,[34] the investigators purposely chose to not use radiographic diagnosis of knee OA as a criterion for study inclusion because they wanted study participants to represent patients typically seen in primary care.

## Cluster Randomized Implementation Trials

A cluster-randomized implementation trial does not randomly assign at the individual-level, but rather by group (e.g., clinical practices or hospital sites). This type of trial design is especially useful in comparative effectiveness and implementation studies, particularly if the intervention is implemented at the cluster level,[35] such as among teams of healthcare providers. Also, the cluster-randomized design may help prevent treatment group contamination by separating groups by location or time.[35] However, contamination can occur in cluster-randomized trials if there is spillover of the exposure to treatment and control conditions between the clusters. For example, an educational

intervention might be administered at the patient level that aims to "activate" patients in an effort to influence the patient physician communication about effective osteoporosis treatment prescribing. Because prescribing occurs by doctors, random assignment is performed at the doctor level and patients are nested within clusters of doctors. If a doctor is randomly assigned to the control cluster, but some of her patients are inadvertently exposed to the educational intervention, this contamination could affect her management of her other patients in the control arm of the study and adversely impact the internal validity of the study findings. To reduce the risk of recruitment bias, participants or recruiters of participants should be blinded to cluster assignment at time of enrollment,[36] or random assignment of clusters can occur after enrollment of groups. Standard approaches to calculating sample size would provide an underestimate. To account for the lack of independence among individuals within the same cluster, the average cluster size and degree of correlation within clusters must be considered and the "design effect" (inflation factor) should be included in sample size calculations.[32] Power is influenced by the number of clusters rather than the size of the cluster.[37] Furthermore, analytic approaches should account for the unit of analysis (the cluster),[37] such as mixed linear models, hierarchical linear modeling, and generalized estimating equations.

## Comparative Effectiveness Research and Patient-Centered Outcomes Research

Evidence of effectiveness of treatments is necessary for informing medical decisions by clinicians, patients, and caregivers, reducing healthcare costs, and improving outcomes. Comparative effectiveness research (CER) generates evidence on the effectiveness of treatments with the goal of determining which treatment is best for particular groups of people with certain conditions to improve the quality of treatments and outcomes.[38] Systematic reviews may be conducted in which all results from existing studies are compiled and the benefits and risks of the treatments are evaluated across different populations. Alternatively, new studies may be conducted to examine the effectiveness of a treatment, including its benefits, its side effects, and costs compared to other available treatments for a given outcome in a specified population.

The quality of health care has continued to improve during the past decade in the United States, yet the increasing costs and financial burden of health care, as well as regional differences in treatment use, cost, and outcomes, are concerning.[38,39] For example, Fisher et al.[40] report that individuals in the highest-spending regions received 60% more healthcare than the lowest-spending regions, but this additional care did not result in improved mortality rates, functional status, or patient satisfaction. The rising healthcare costs and distressing regional differences have given rise to a push for focused efforts on CER nationally. Under the American Recovery and Reinvestment Act (ARRA) of 2009, Congress allocated $1.1 billion to spark the advancement of CER nationally to improve healthcare quality while reducing costs.[38] As required by the ARRA, the Institute of Medicine (IOM) Committee on Comparative Effectiveness Research Prioritization selected 100 health topics requiring CER, including osteoarthritis (musculoskeletal disorders) and rheumatoid and psoriatic arthritis (immune system, connective tissue, and joint disorders), based on the input of public and private stakeholders.[38] For additional information on the national priorities of CER, the IOM's Initial National Priorities on Comparative Effectiveness Research is available at the National Academies Press website (www.nap.edu).

### Patient-Reported Outcomes

Central to Patient-Centered Outcomes Research (PCOR) is measuring the outcomes that matter most to patients. International efforts, supported and endorsed by organizations such as United Kingdom's National Institute for Health and Care Excellence (NICE) and German Institute for Quality and Efficiency in Health Care (IQWiG),[41] are advancing the promotion of patient engagement to define and improve quality of care criteria for specific conditions, and great strides have occurred in rheumatology around patient-reported outcomes (PROs). These are discussed in Chapter 33, and both older PROs such as the Health Assessment Questionnaire (HAQ) and somewhat newer ones such as Routine Assessment of Patient Index Data (RAPID 3) are critical to the study of many of our disease states. The PRO field is moving toward computer adaptive testing (CAT) using item response theory (IRT).[42] The principle of this newer form of PROs is to use the response of an initial question to guide the next PRO question. For example, a patient who can run a mile is not asked about their ability to do activities of daily living, but is next given survey items selected based on their performance level noted. Population normative data are growing for these approaches, and one initiative that uses the Patient-Reported Outcomes Information System (PROMIS)[43] has gained international recognition as a newer state-of-the-art PRO measure.

# BIASES IN STUDY DESIGN

Error in a study may be random (chance) or systematic (bias). Bias includes errors in the selection of participants, errors in measurement of a variable, or confounding. Bias may produce an incorrect conclusion about the association between an exposure and disease.

## Selection Bias

The procedures used to select participants for a study or factors related to study participation may result in a different exposure-disease association between participants and nonparticipants. Selection bias may occur in any study design, most notably in retrospective or case-control studies where the exposure and outcome both occur before selection of participants. Differential participation may arise in cohort studies or clinical trials with loss to follow up, particularly if participants leave the study for reasons related to the exposure or the disease.

## Information and Recall Bias

Errors in the measurement or collection of information may occur. If a variable is measured categorically, information

about a participant may be placed in the wrong category, or misclassified. Nondifferential misclassification of an exposure occurs when misclassification is not related to the presence of disease.[1] Misclassification is differential if exposure differs by disease status.[1] Similarly, misclassification of a disease is nondifferential if it does not differ by exposure status and differential if it varies by exposure status. Nondifferential misclassification biases an association between exposure and disease towards the null, except if the association is the null. Differential misclassification may bias the association in either direction.

In case-control studies, those who are cases may have a different recall of their exposure history than those who are noncases. This difference in recall can introduce a bias that inflates the estimate of an association between exposure and disease. Recall bias is differential misclassification because the exposure is misclassified differently among cases and controls.[1] Methods for reducing recall bias include structuring questions to improve recall for both groups, selecting a control group that would be more likely to have good recall of exposure history, or use of information other than interviews, such as medical records.[1]

## Confounding

Confounding occurs when there is a "mixing of effects" between the exposure, outcome, and a third factor.[44] Specifically, a confounding variable is a risk factor for the disease, is associated with the main exposure, and is not an intermediate step on the casual pathway from exposure to disease.[6] For example, when examining leg length inequality as a risk factor for lower extremity OA in a cohort study, a likely confounding variable would be injury to the lower limb. Injury is a risk factor for OA, it is associated with leg length inequality (a severe injury to a lower limb can result in a shortening of that limb), and it precedes both OA and leg length inequality. Methods used to control confounding include stratifying data by the confounding variable or including the variable as a covariate in multivariable statistical models. Matching may reduce confounding in case-control studies. In experimental studies, random assignment is a strategy to reduce confounding.

The amount of confounding is an important consideration in determining whether one should control for it in analyses.[6] If the estimate of an association minimally changes after adjusting for a potential confounding variable (e.g., unadjusted odds ratio [OR] = 2.62, adjusted OR = 2.58), the inclusion of the variable as a covariate in a multivariable model may not be necessary. However, if the estimate changes profoundly (e.g., unadjusted OR = 2.62, adjusted OR = 1.05), then methods to control confounding should be used to reduce bias in the association.

### Confounding by Indication and Channeling in Observational Studies of Therapeutics

In observational studies of therapeutics, delivery of treatment is not randomized, and the reason for treatment may be based on health risks.[45,46] Because the risk profiles between treated and comparison groups differ, results may be biased. Channeling occurs in observational studies when drugs are prescribed ("channeled") to patients differently based on prognostic characteristics or disease severity.[47] Channeling can make study outcomes related to a drug appear better or worse than they truly are, incorrectly attributing health benefits or adverse events to the drug. Confounding by indication is a type of channeling bias that occurs when the indication for which a drug is prescribed (drug exposure) is an independent risk factor for the outcome.[45,46] There has been considerable controversy in rheumatoid arthritis, for example, about the role of low-dose glucocorticoids and their association with adverse outcomes such as infections and cardiovascular disease. It is clear that considerable confounding is present by indication around glucocorticoid use, whereby patients with more active/severe RA are more likely to be prescribed glucocorticoids, and these same patients, in turn, are also the group most likely to experience an adverse outcome that may or may not be fully attributable to the low-dose glucocorticoid, but instead to their underlying disease state.[48]

### Analytic Methods to Address Confounding by Indication

Methods available for accounting for confounding by indication in observational studies include propensity scores, instrumental variables, and marginal structural models. The propensity score is the probability that a participant received a treatment.[49] In a randomized controlled study, the propensity score for each participant would be 0.5 if assignment was determined by a coin toss. In observational studies, the propensity score is unknown and is estimated based on baseline participant characteristics. The propensity score can be used analytically in different ways to assist with balancing the study groups to make them analogous. By considering the propensity score when comparing groups, the observational study becomes similar to a randomized block study in which each block represents a group of participants with the same propensities. The use of instrumental variable is another approach to control for confounding by indication. The overall goal is to select a factor, or instrument, that is strongly associated with the treatment but has no association with the potential outcome or possible confounders (co-variates). Including this factor in statistical models in many ways mirrors a RCT. An example in rheumatology is an observational study of the association of cyclo-oxygenase (COX)-2 selective NSAIDs (also known as coxibs) with gastrointestinal (GI) bleeding.[50] In this study, the last NSAID prescription written by a given physician (to either a traditional nonselective vs. a coxib) was used as an instrument to adjust for confounding by indication associated with the preferential choice of coxibs for patients at higher risk of GI bleeding. Last, an even newer method involving marginal structural models with inverse probability weighting affords a sophisticated analytic approach to partially address this same issue.[51]

## EFFECT MEASURE MODIFICATION

Two factors are considered to be independent if the combination of their effects is equal to their joint effects. If the effect of one factor depends on the effect of another, effect measure modification exists. This concept is also known as *statistical interaction*. Examining effect measure modification

allows researchers to investigate whether the association between exposure and disease differs across subgroups. For example, Krishnan and colleagues.[52] reported a strong association between past history of smoking and RA in men (OR, 2.0; 95% CI, 1.2-3.2), but not women (OR, 0.9; 95% CI, 0.6-1.3). Upon further exploration, this association was only seen among men with rheumatoid factor-positive RA. If effect measure modification is not considered, results could be biased or important groups for targeting interventions could be missed.

## SCREENING

Screening is an important public health strategy in reducing morbidity and mortality.[44] Screening tests classify a person who is asymptomatic as likely or unlikely to have the disease. This differs from diagnostic tests, which determine whether a person with signs or symptoms of a disease truly has the disease. If a screening test suggests a high likelihood of disease, then further diagnostic evaluation may occur to confirm disease presence. Although not applicable in all diseases, the early detection of disease when a person is asymptomatic is felt to result in more effective treatment than if disease detection occurs later when symptoms develop with advanced disease.[44] To determine the validity of a screening or diagnostic test, one must establish the sensitivity or specificity of the test. Often, a new test may be compared with a "gold standard" of the definition of a disease, although this standard may not encompass all signs and symptoms of that disease. For example, biologic markers may be useful screening tools for detecting early changes in joint health that lead to OA. The presence of radiographic features of OA may be used as a comparative "gold standard," yet this definition of OA lacks other components used to diagnose the disease, such as pain, aching, or stiffness in the joint.

### Sensitivity

*Sensitivity* is the probability that a test will correctly classify a case. This is expressed as a proportion of the number of cases identified by the test out of the total number of individuals with the disease. In screening, sensitivity is the probability of correctly classifying an individual as a detectable, pre-clinical case. If a test correctly provides a positive test result for 37 out of 43 people with disease, the sensitivity of the test is 86% (Table 31-2).

### Specificity

*Specificity* is the probability that a test will correctly classify a noncase. This is expressed as a proportion of the number of individuals without disease identified by the test out of the total number of individuals without disease. If a test correctly provides a negative test result for 62 out of 66 people without disease, the specificity of the test is 94% (see Table 31-2).

**TABLE 31-2** Hypothetical Distribution of Patients by Disease and Test Result.

| | Disease | No Disease | Total |
|---|---|---|---|
| Positive Test | 37 | 4 | 41 |
| Negative Test | 6 | 62 | 68 |
| Total | 43 | 66 | |

### Predictive Value

Predictive values are used to interpret the results of a test by examining the correct classification of individuals by the test. This measure is valuable because whether a person is truly a case or noncase is difficult to know (for determining sensitivity or specificity), but a positive or negative result of a test is known. A positive predictive value is a proportion of the number of cases identified out of all positive test results. If 37 people truly have disease out of 41 with a positive test result, the positive predictive value is 90% (see Table 31-2). A negative predictive value is a proportion of the noncases identified out of all negative test results. If 62 people truly do not have disease out of 68 with a negative test result, the negative predictive value is 94% (see Table 31-2).

## CONCLUSION

Epidemiologic methods can be used to measure frequency or development of disease and evaluate risk or protective factors in disease occurrence. Choice of study design depends on multiple factors, including the research question, disease under study, availability of appropriate study populations, and resources available. Each study design has its own set of advantages and disadvantages, with the clinical trial considered the most rigorous.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 32



# Economic Burden of Rheumatic Diseases

Edward Yelin

**KEY POINTS**

- Studies of the economic burden of diseases help allocate health care resources for musculoskeletal conditions in which mortality rates are less prominent.
- The economic burden is determined by summing expenditures for medical care, called "direct costs," and earnings losses and value of productivity losses in other activities, called "indirect costs." Per person direct costs of musculoskeletal conditions have risen by more than 61% in the United States during the past two decades, to $7768 in 2011 dollars.
- Much of this increase is fueled by a 60% increase in the United States in the number of prescription medications used each year by people with musculoskeletal conditions and by a 68% increase in the price per prescription.
- Per person earnings losses among individuals with musculoskeletal conditions have risen by almost 105% during the same time, to $1224 in 2011 dollars.
- Insured people with musculoskeletal conditions experienced dramatically higher medical care costs than people without insurance, suggesting that before health legislation in the United States, those without insurance did not receive the care they needed.
- The aggregate economic impact of musculoskeletal conditions in the United States has increased by approximately 120% during the past two decades, from the equivalent of 3.4% to 7.6% of the gross domestic product.
- The increasing use of biologic agents for rheumatoid arthritis has led to a rapid increase in the direct costs associated with this condition. The costs of these agents alone exceed the total costs of rheumatoid arthritis from the prebiologics era, including direct and indirect costs.

Cost-of-illness studies are used to provide a measure of the impact of medical conditions, and can overcome the natural tendency of policymakers to focus attention on those conditions with high mortality rates, to the exclusion of those with a high impact on quality of life.

Studies of the economic impact of musculoskeletal conditions as a whole in the United States have been conducted since the early 1960s by using a mélange of publicly available data sources. Such studies indicate that during three decades, the economic impact of musculoskeletal conditions increased from the equivalent of approximately half a percent of the U.S. gross domestic product (GDP) to more than 2.5%. During the past two decades, the availability of a well-designed individual data source in the United States, the Medical Expenditures Panel Survey, has permitted researchers to estimate the economic impact with greater precision and to show that during this time frame, the economic impact increased by the equivalent of approximately 4 percentage points of GDP, to slightly less than 8%. In the Great Recession that began in 2008, GDP declined by approximately 7%, so the economic impact of musculoskeletal conditions can be said to approximate the impact of the Great Recession, although the effect is chronic, not acute.

A substantial literature now exists on the economic impact of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and osteoarthritis (OA). The studies in this literature are largely based on clinical samples, most from tertiary care centers, and indicate that there are substantial costs associated with all three conditions. This was true for RA even before the development of biologic agents. The most recent studies of RA indicate that, with the advent of these agents, the costs of RA have skyrocketed, with costs from biologic agents alone eclipsing the total costs of RA before their development, including all other medical care costs and all wage losses.

The economic burden of musculoskeletal conditions is rapidly increasing in developing nations. Each discipline may use different tools to assess the burden of disease. Rheumatology health professionals may measure the impact of these conditions by estimating both the discomfort and disability they cause and to what extent professionals may alleviate these factors by providing health care. Psychologists may measure the impact of musculoskeletal conditions on the achievement of mental equipoise, or its opposite, mental discomfort. Economists take the measurements provided by other disciplines and turn them into monetary equivalents to describe the magnitude of the burden associated with musculoskeletal conditions. Thus economists are concerned with the monetary value of the resources used to produce health and with the return on the use of those resources in terms of reduced impacts of illness on pain and functioning. Cost of illness is an accounting of the resources spent in pursuit of health and of the residual amount of discomfort and disability left after those resources are spent[1-4] (Table 32-1).

Economics is far more than a positive science, that is, a discipline that observes how things are rather than seeking to change them. The normative part of the discipline has dual and sometimes competing goals.[5,6] The first of these is to ensure that resources are allocated equitably (i.e., making sure that they are distributed fairly across individuals). The second is to make sure that they are used efficiently (i.e., as well as contemporary technology permits to produce goods).[7]

**TABLE 32-1** Principal Methods to Assess Costs of Illness

There are two principal methods to assess the costs of illness.

1. The human capital approach, developed by Dorothy Rice when she was at the Social Security Administration and, later, the National Center for Health Statistics,[1,4] and
2. The willingness-to-pay approach.[5]

The two methods do not differ in the way that they assess the direct costs of medical care. For the indirect costs associated with loss of function and the intangible impacts of disease, the human capital approach uses the market value of the labor to reduce the impacts (e.g., by hiring a replacement worker).

In a variant of the human capital approach called the friction method,[3] the losses are estimated from the perspective of the employer and only last until the replacement worker is hired and then achieves the same productivity as the worker who left as a result of disease.

At that point, an employer would be said to incur no additional costs from the onset of the prior incumbent's disease. The willingness-to-pay approach values the loss of function as the amount the affected individual would pay to restore the function, which may be more, the same, or less than the amount it would take to replace the worker in the labor market.

The human capital approach is more reliable in estimating the economic impact of the lost productivity of affected individuals because the cost of labor is well established in all advanced societies and, therefore, easy to estimate.

The human capital approach, however, only enumerates the intangible impacts of disease (e.g., the burden associated with the experience of intense pain) but does not translate them into economic terms. The willingness-to-pay approach can theoretically incorporate all of the costs of disease in those terms, although as a practical matter there are problems associated with attempting to do so.[6]

In health, resources would be distributed equitably if people with similar levels of health received relatively equal access to health care services that reduce the burden of disease and which, based on a thorough knowledge of the treatment options, they chose for themselves. Similarly, in health, resources would be used efficiently if they produced the health outcomes people wanted while leaving the maximum amount to be used for other purposes, what economists refer to as "the opportunity cost" of those resources.

The pursuit of equity and efficiency may work in harmony. If resources are used efficiently to provide health care, then more can be allocated to ensure that everyone has access to the same set of services they desire. But the opposite is also true: if resources are used inefficiently, then the "wastage" cannot be redistributed to ensure that everyone has equal access.

The evidence that the distribution of health care resources in the United States is inefficient has been increasing for several decades and stems from studies conducted within the United States[8,9,10] and from those that compare the United States with other nations.[11-13] The inequitable distribution, however, has also recently become a central focus of analysis and policy. The signposts for inefficiency might be said to include the use of any test or procedure for which evidence has emerged suggesting that the test or procedure does not work or does not work as well as alternatives that are available. Every comparative trial that does not result in a decreased use of the comparator that worked less well is, thus, evidence in support of the argument that our system of care is inefficient. However, the most compelling evidence comes from studies that compare populations of regions for the use of select tests and procedures and fail to find that those areas that use more of one achieve better health outcomes.[14] Similarly, international comparisons often show that the extra usage within the United States fails to improve health-related quality of life, let alone longevity in comparison with other nations.[11] Such studies also fail to show that the extra usage engenders greater satisfaction with most aspects of care.

The methods and data sources to estimate the economic burdens of disease, the positive side of health economics, have certainly improved during the years since the first cost-of-illness studies for the nation as a whole were completed by Dorothy Rice and colleagues. Subsequent to the development of the methods of cost-of-illness studies by Rice and others, individual investigators used those methods and the availability of clinical samples to provide highly specific estimates of discrete rheumatic conditions, particularly RA.[15] For national estimates, the U.S. federal government has developed an annual survey, the Medical Expenditures Panel Survey (MEPS) to provide reliable estimates of the cost of illness experienced by individuals.[16-18] The prior work by Rice relied on separate data sources about ambulatory care, hospital admissions, and nursing homes. The same individuals were not included in each of these data sources; indeed, the data sources were based on samples of medical care encounters rather than of individuals with specific conditions. MEPS provides estimates on every kind of health care for the same individuals and does so through systematic sampling of the U.S. population. Because of the uniformity of data collected in each year, it provides the opportunity to merge multiple years of data so that sample sizes are sufficient to provide estimates of the cost of fairly rare conditions reliably.[19] Similarly, the batteries of items to measure impact in each domain (e.g., the magnitude of the ambulatory care used) have been vetted through reliability studies comparing survey responses with medical records and billing information. The one aspect of cost-of-illness studies that cannot be replicated by using MEPS concerns the indirect costs associated with mortality. Thus a full accounting of the costs of illness would include an estimate of the discounted present value of lost earnings among those who die prematurely. Although mortality rates are elevated for those with inflammatory rheumatic conditions,[20-23] in general, musculoskeletal conditions are not associated with dramatically higher mortality rates. Therefore the bias in estimates of the costs of these conditions by using MEPS data is relatively small.

The comprehensive sampling and reliability of batteries notwithstanding, the findings from the studies of Rice and colleagues about all kinds of conditions in the nation as a whole and the studies about people with discrete conditions in clinical samples stand the test of time. Thus the early and contemporary studies both highlight the effect of population dynamics, particularly the aging of the population; increase in the cost of care as a function of changes in the kinds of services provided and increase in the unit price of those services; and changing employment circumstances and attendant wage losses on the burden of disease experienced by individuals with musculoskeletal conditions.[19]

The tools of the normative part of economics have also evolved with time (Table 32-2). The tools are normative

**TABLE 32-2** Economic Methods to Assess the Value of Health Interventions

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|---|
| Concise reviews are available of the methods to estimate the relationship between health care expenditures and the returns of these expenditures in terms of health-related quality of life including one of its domains, employment. |
| When alternative levels of health expenditures will result in improved outcomes, attempts are needed to reduce the wastage of health expenditures, the subject of "cost-minimization studies." |
| When alternative treatments for a condition are available, "cost-effectiveness analysis," which shows the relative returns from these alternatives in a common natural metric (e.g., longevity), is used. |
| When comparing alternative investments across conditions, an outcome metric that applies to all conditions equally is needed; often the easiest outcome to measure in common terms is the dollar value of lost wages, the subject of "cost-benefit analysis." However, there are inherent problems in translating outcomes into dollar terms. |
| Economists have developed common metrics as the quality-adjusted life year, which takes into account the value individuals in society place on achieving a common outcome (economists use the term *utility* for these evaluations and use the term *cost-utility analysis* for assessing the returns on alternative health expenditures). |

because they are based on the notion that certain levels of health care expenditures unnecessarily divert resources from other uses, and that society should have allocative mechanisms, through the market or regulatory means that redirect resources away from specific conditions or, more frequently, specific treatments for those conditions. That direct medical care expenditures for RA equal "x billion dollars" is a positive statement; that society should not be spending "y billion dollars" on specific treatments for RA is a normative statement; that a treatment achieves an end (e.g., extending life or reducing disability-adjusted life years, or DALYs) less expensively than another, the product of a cost-effectiveness analysis, is a tool that may provide a guide in reallocating resources from RA care, in putting in place a policy to implement the notion that spending "y billion" on specific treatments for RA is not a good use of those resources.

This chapter summarizes the evidence accumulated on the current burden of musculoskeletal conditions overall, as well as on specific conditions within that overarching rubric. This is similar to indicating the temperature outside but not indicating whether that temperature is hot or cold. Thus it is impossible to determine whether the tools to assist in redirecting resources such as cost-effectiveness analysis should be used. However, although the evidence base that supports individual treatments for musculoskeletal conditions, for example, use of one or more disease-modifying agents in RA, or entire strategies of treatment, for example, the use of both pharmaceutical and behavioral interventions for OA increases, we begin to be able to move from the positive aspects of health economics to the normative.

## STUDIES OF THE COSTS OF ALL FORMS OF MUSCULOSKELETAL DISEASE

In this section, data from the United States and elsewhere about the economic impact of musculoskeletal conditions as an entire category are presented.* Three-year averages smooth out individual discrepant values in any one year caused by small sample sizes. The first triad of years, thus, incorporates data from 1996 through 1998, whereas the last incorporates data from 2009 through 2011. All data represent annualized figures. Between 1996 to 1998 and 2009 to 2011, little change has occurred in the use of physician visits or hospital care for people with musculoskeletal conditions. In each triad of years, approximately 85% of such people have one or more physician visits, and the average number of visits per person has not changed, averaging approximately 5.6 a year in the initial and last 3-year periods. Similarly, the proportion with one or more hospitalizations has remained relatively constant in the range of 11% to 12% a year, with the average number of admissions holding steady at 0.2 per person per year. In contrast to physician visits and hospital care, there has been substantial growth in the use of other ambulatory care (for example, physical therapists, occupational therapists, nurses and nurse practitioners, physician assistants, and chiropractors) and prescription medications. Between 1996 to 1998 and 2009 to 2011, the proportion with any use of ambulatory care other than physicians increased by 30% in relative terms, from slightly less than 40% to approximately 52%, and the number of visits per year to such providers increased by 42%, from 2.6 to 3.7 per year. The growth in the use of prescription medications was substantial. Although the proportion with one or more prescription inched up from approximately 81% to 84%, the average number of medications per person increased by approximately 60%, from 13.1 to 20.8.

The unit price of ambulatory visits, including both physician visits and those to nonphysician providers, increased from $186 in 1996 to 1998 to $281 in 2009 to 2011, or by approximately 51% in real terms. The unit price of hospital admissions also substantially increased, from $8792 in 1996 to 1998 to $11,184 in 2009 to 2011, or by more than a quarter in relative terms. The unit price associated with prescription medicines increased the most, 68%, from $51 in the 1996 to 1998 period to $85 in 2009 to 2011. The third set of columns, per-person costs, shows the product of the average number of units of services used and the unit prices. Total per-person costs increased by approximately 60% between 1996 and998 and 2009 to 2011, from $4832 to $7768. The overall increase was driven overwhelmingly by increases in prescription drug costs, but with smaller contributions from ambulatory visits and hospital admissions. Prescription drug costs increased by 167% between 1996 to 1998 and 2009 to 2011 in constant dollars, from $665 to $1778. Ambulatory visit costs increased by slightly more than 70% during this time frame, from $1522 to $2614, hospital costs increased by approximately a quarter, from $1758 per person in 1996 to 1998 to $2237 in 2009 to 2011. As a result of the growth in the costs of prescription drugs, the proportion of all medical care costs attributable to drugs increased from 14% to 23% of the total, whereas the total attributable to ambulatory care increased only

*The results are based on the author's analysis of MEPS data. The methods were described in a 2001 publication[24] and replicated in subsequent publications.[25,26]

slightly to 34%, and the shares attributable to hospital admissions and the residual category actually decreased. Another way to assess the cost of illness is to estimate the increment in total costs beyond those that would be incurred on behalf of people similar to those with musculoskeletal conditions but who do not actually have such conditions. There are two ways to make these estimates: by asking individuals to apportion health care episodes to various causes and by using regression techniques that take into account the health and demographic characteristics of the individuals, because the attributions made by the individuals may not be reliable. With use of the second method, approximately 30% of the costs incurred by individuals with musculoskeletal conditions can be attributed to those conditions.

The aggregate medical care costs associated with musculoskeletal conditions are the product of the costs per case and the number of persons with the conditions at any one time. The aging of the population is resulting in a substantial increase in the number of persons and percentage of the population with musculoskeletal conditions in the United States. Between 1996 to 1998 and 2009 to 2011, the number reporting one or more musculoskeletal conditions increased from 76 to 102.5 million, or by more than a third in relative terms, whereas the percentage of the population with such conditions rose by approximately a fifth in relative terms, from 28% to 33.2%. When multiplied by the cost per person in each triad of years covered by the analysis, the aggregate costs rose from $367.1 to $796.3 billion in constant dollars, or by 117% in relative terms. The dual importance of population aging and increases in the cost per case in the dynamics in the aggregate costs of musculoskeletal conditions can be seen by the fact that the increase in the former, more than a third, and the percentage increase in the latter, 61%, were both substantial.

The relatively large increase in the cost per case of all musculoskeletal conditions would have been even greater were it not for the fact that the vast majority of cases within the overall musculoskeletal diseases rubric are OA and similar mechanical diseases rather than such autoimmune conditions as RA or SLE. Treatments for OA, for example, have been relatively stable over time. As explained later, costs for individual autoimmune conditions have risen substantially over the past decade as new treatments such as the biologics for RA have taken hold.

Musculoskeletal conditions are more prevalent among females; females with the conditions also experience per-person medical care costs that are slightly higher than males, $8075 compared with $7865. When the higher prevalence and higher per-person costs are combined, aggregate costs are $464.6 billion among females and only $362.5 billion among males. Per-person medical care costs associated with musculoskeletal conditions increase monotonically with age, from a low of $3288 among those younger than age 18 to a high of $11,708 among individuals 65 or older. However, because of the number of persons in the 45- to 64-year-old group, aggregate costs are actually slightly higher in the latter group than among those 65 or older, $348.9 billion versus $305.4 billion. Nonwhites and whites with musculoskeletal conditions experience similar per-person costs, $7949 and $8154, respectively. On the other hand, Hispanics with musculoskeletal conditions incurred medical care costs of $6305 versus $8180 among non-Hispanics, about a quarter lower in relative terms. Per-person costs did not differ systematically by education level. Probably reflecting age and severity, costs were greater among divorced, widowed, or separated individuals ($8849) than among those who were married or living with a partner ($7970) and especially than among those who have never been married ($6612). Of note, people with musculoskeletal conditions reporting public insurance actually incurred higher medical care costs than those with private insurance ($10,142 vs. $7842, a difference of about a quarter), but both groups had substantially higher costs than those without insurance, who averaged only $2479 per person in medical care costs, a difference of more than two-thirds relative to those with any private insurance and 75% relative to those with any form of public insurance. Thus it cannot be said that those without insurance necessarily receive the care they need. The years analyzed, 2009 to 2011, predate full implementation of the recent health care legislation in the United States.

Earnings losses associated with musculoskeletal disorders among the working age population, the component of indirect costs calculable in MEPS, are substantial and growing in real terms. The number of individuals with a work history between ages 18 and 64 increased from 48.5 million in the first triad of years covered to 63.4 million in the last triad, or by about a third. Concurrently, the per-person earnings losses among working age people with musculoskeletal conditions increased from $596 to $1224, or by more than 100% in real terms (although it has declined somewhat in the most recent three-year periods). As a result of the increase in the prevalence of the at-risk population and the per-person earnings losses among such people, aggregate earnings losses among people with musculoskeletal conditions rose from $28.9 billion in 1996-1998 to $128.6 in 2003-2005; it has since decreased to $77.5 billion. The decreased per-person and aggregate earnings losses may reflect relative stasis in wages in the United States, a phenomenon which may affect the population at risk for work loss, that is, those who are unable to continue working with the onset of physical impairments due to musculoskeletal conditions.

Figure 32-1 summarizes the overall economic impact of musculoskeletal conditions since the initiation of the MEPS in 1996. During the less than two decades since the MEPS began, the total costs of musculoskeletal conditions have risen from the equivalent of 3.4% of GDP to 7.6%. The bulk of the costs—and the bulk of the increase—are the result of increases in the medical care costs. Medical care costs among individuals of all ages with musculoskeletal conditions have risen from the equivalent of 3.2% of GDP to 6.9%, or by about 117% in relative terms, while the earnings losses, estimated here only for those aged 18 through 64, have increased from the equivalent of 0.3% to 0.7% after the recent decrease.

A recession is said to occur when the economy contracts by 1% of GDP or more for two quarters or more. In the deep recession that began in 2007, GDP fell by about 7%[27]; on an annualized basis. The economic impact of musculoskeletal diseases can therefore be said to be the equivalent of a severe recession, although to judge by the increases over time, the onset occurs more slowly. However, the

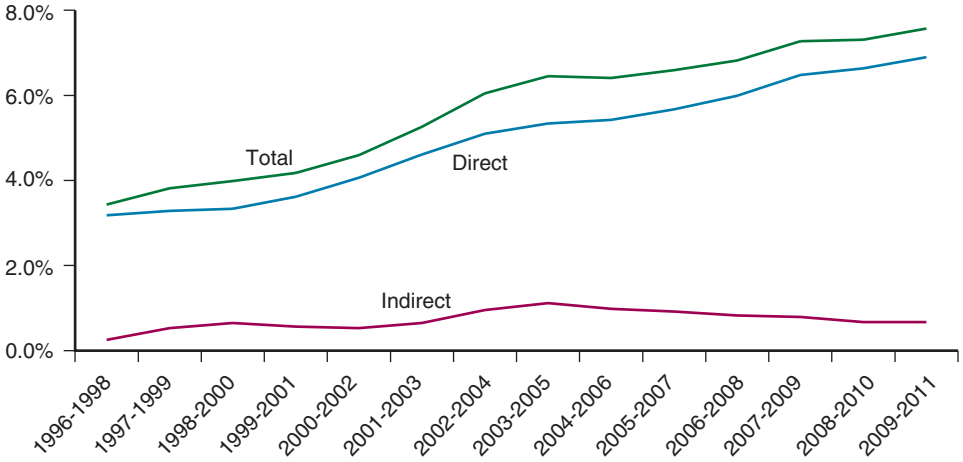



**Figure 32-1** Direct, indirect, and total costs of musculoskeletal conditions as a percentage of gross domestic product, United States, select years. *(Data [pre-1995] from Rice D, Hodgson T, Kopstein A: The economic costs of illness: a replication and update.* Health Care Fin Rev *7:61-80, 1985; Badley E: The economic burden of musculoskeletal disorders in Canada is similar to that for cancer, and may be higher.* J Rheumatol *22:204-206, 1995; Odonnell S LC, McRae L, Bancej C: Life with arthritis in Canada: a personal and public health challenge.* Chronic Dis Inj Can *31:135-136, 2011; Arthritis Foundation of Australia: Cost of arthritis to the Australian community.* Industry Commission Report *14-22, 1994; and Arthritis and Osteoporosis Victoria:* A problem worth solving, *2013. Data [1996 and later] from author's analysis of Medical Expenditures Panel Survey [references 16 and 17].)*

impact is permanent, at least until medical care expenditures either result in improved functionality and concomitant reduced earnings losses or, without that impact, are reduced by decreases in the amount of health care used and/or the unit prices of the services used to treat this group of conditions.

## OTHER NATIONS

Studies on the economic impact of musculoskeletal conditions have been conducted in Canada,[28,29] Australia,[30,31] the United Kingdom,[32] Sweden,[33] and the Netherlands.[34,35] Systematic reviews, incorporating both methodological considerations[36] and comprehensive review of evidence across countries, have also been published.[37] All use methods broadly similar to the studies in the United States conducted by Rice and colleagues.[38] The most recent Canadian study[29] using 2000 data but published in 2011, showed that the economic impact of musculoskeletal conditions was approximately $22.3 billion CDN, or roughly 2.1% of that country's GDP for that year, similar to the most recent of Rice's studies in the United States. In the Canadian study, indirect costs associated with earnings losses were much greater than direct medical care costs for these conditions. The initial study in the Netherlands is particularly instructive. In that nation, it was found that musculoskeletal conditions ranked second among all major diagnostic groups in medical costs, exceeding coronary and other circulatory conditions and only being eclipsed by mental retardation. In the Australian study,[31] costs were allocated to specific conditions, with the largest costs attributable to back problems and OA. Overall, costs amounted to AUS$55.1 billion.

The demographic structure of the developed nations in which these studies have been conducted are similar, with each facing a pandemic of musculoskeletal disease because of the aging of their populations. Increases are also probably associated with the increased prevalence of obesity in many of the developed nations; obesity is associated with OA. However, we are not aware of systematic studies of the cost of these conditions in the developing world.

## COST OF DISCRETE CONDITIONS

The first studies of the cost of illness for discrete rheumatic diseases were conducted about RA and, reflecting the importance of this condition in the practices of rheumatologists and other rheumatology health professionals, the economic impact of RA continues to be the subject of extensive investigation. However, a sizable literature on the economic impact of SLE and OA exists, and at least a few studies have been published about the costs associated with ankylosing spondylitis, psoriatic arthritis, gout, and fibromyalgia.

### Rheumatoid Arthritis

The costs of RA have been extensively reviewed,[39-46] most recently in a systematic review of 127 articles about all forms of costs[47] and another focused only the impact of productivity losses.[48] Several studies parse the literature for methodological differences among studies, including the perspective of the analysis the individual with the condition, focusing on the share of total costs paid out of pocket; payer, focusing on the share paid by health insurance plans or national health insurance; and society, focusing on all of the resources devoted to health care for the condition, regardless of who pays for those resources, as well as the forgone productivity; type of case studied (prevalent or incident); and effect of specific treatments, including but not limited to the biologic agents.

Among studies of RA not of recent onset and preceding the development of the biologic agents, direct costs in most studies were in the range of $5000 to $7000 per year, whereas indirect costs averaged between two and three times direct costs. Within direct costs, approximately two-thirds were attributable to hospital admissions, even though less than

10% of people with the condition are admitted to the hospital in any one year. Among those hospitalized, the vast majority received surgical interventions, primarily joint replacement surgery.

Among the approximately 90% of people with RA not admitted to the hospital in a year, costs in the prebiologic era could be quite low. The modal costs for RA in that era were $685 per year in one study, whereas the median was only $2715. At the 90th percentile, however, costs were higher than $8000 per year, while at the 95th percentile, costs were in excess of $30,000.[49]

The high indirect costs of RA are a function of the prevalence of work disability, with rates varying from a low of 34% in one study to a high of 59% in another.[50] One study reported on the duration of time from onset of the condition until work disability occurred. Of individuals with RA, 10% stopped working in the first year after onset, approximately half had stopped working in the first decade after onset, and 90% left work before the usual age of retirement.[51]

The impact of the biologic agents on costs of RA has been so profound that studies should be divided into those that predate and postdate the availability and diffusion of these agents. In some series, the proportion of people with RA receiving biologic agents has reached a third or more.[52] At approximately $20,000 per year per user, medical care expenditures for RA for biologic agents alone now exceed estimates of the total costs of the disease, including indirect costs, from the prebiologics era. Several studies quantify the incremental costs associated with the use of the biologics beyond what would be expected for people with similar health status. Michaud and colleagues[53] report that medical care costs for RA were more than $19,000 per year for patients taking biologic agents, but only approximately $6000 for those who were not, even after accounting for differences in the two populations. Fautrel and colleagues[54] reported that the incremental costs associated with etanercept and infliximab were approximately $23,000 to $25,000 per year, or more than three times the annual direct costs of RA in the prebiologics era. Sorensen and Anderson[55] studied the potential impact of the biologics on the Danish health care service, reporting that under varying scenarios, the biologics would raise the cost of treating RA from 50% to 500%. In a study of elderly people with RA, Weycker and colleagues[56] noted that RA care averaged between $12,000 and $23,000 in the biologics era, or more than twice the average from the prebiologics era.

However, perhaps the best way to gauge the impact of the biologic agents on the costs of RA is merely to note that, in this disease with a prevalence of less than 1% of the population, pharmaceutical companies find it profitable to advertise the agents on national television shows in the United States. Assuming that the prevalence was 0.5% of the approximately 250 million adults in the United States, or 1.25 million, that about a quarter of these used the biologic agents, and that the agents cost $20,000 per year, then biologic agents would account for more than $6 billion a year in health care expenditures for RA.

The costs of RA may be substantial early after onset. Merkesdal and colleagues[57] observed annual indirect costs resulting from lost wages within the first 3 years of disease. Of note, however, they observed that short-term sick leave costs were higher in the first year than the next two, but that permanent work disability costs increased to partially offset the decrease in sick leave. Similarly, Hallert and colleagues[58] reported that direct costs in the first year after diagnosis averaged almost $5000, whereas indirect costs were more than twice as high. Among individuals with seropositive RA of approximately 6 months' duration, Newhall-Perry and colleagues[59] reported that direct and indirect costs were approximately $200 and $300 per month, respectively. The relatively high indirect costs were a result of the high rate of work cessation (>10% among those employed at diagnosis) and sick leave (>5% in the same group).

The high medical costs associated with RA that manifest even among those of recent onset, present a paradox for choice of therapy at the level of the individual patient-provider dyad and at the level of the payer and society. On the one hand, there is ample opportunity to reduce much of the nonmedication cost of treatment for joint replacement surgery and for indirect costs resulting from lost wages by timely use of the newly discovered and highly efficacious biologic agents. On the other hand, iron clad evidence that they can reduce the frequency of joint replacement and work loss lacks information needed to prove cost-effectiveness. This lack of evidence stems in part from methodological problems in the studies conducted, including lack of head-to-head trials and uncertainty about the results of the studies that have been done.[60] Thus we know that the costs of RA are high and growing because of the use of biologic agents, but we do not know whether using them can reduce the indirect costs and the functional losses that lead to joint replacement surgery.

Although the bulk of the now vast literature on the economic impact of RA derives from clinical samples, it is possible to make an estimate for the United States as a whole by using MEPS data, with the caveat that there may be individual respondents to the MEPS surveys who are unaware of the specific form of arthritis they have, even though a physician would diagnose them with RA and, on the other hand, some who inaccurately state that they have RA. The advantage is that MEPS samples are from the community at large, eliminating the bias from sampling in tertiary care centers or select health plans. The MEPS estimates were made by merging five annual waves of data, from 2008 to 2011. In the United States, direct costs among individuals with RA averaged slightly more than $17,000 a year, whereas earnings losses among those aged 18 to 64 years totaled approximately $14,000 per year. In aggregate, direct costs and earnings losses associated with RA averaged $30.1 billion during the years the analysis was conducted, or approximately 0.2% of the nation's average annual GDP in those years.

## Systemic Lupus Erythematosus

The first studies of the costs of RA appeared in the mid-1970s; the first studies of the costs of SLE did not appear until almost two decades later. Nevertheless, there is now a substantial literature that spans three continents[61,62] and goes beyond studies of undifferentiated SLE to focus on the impact of specific levels of disease activity[63] and specific organ manifestations[64-67] on direct and indirect costs. A

comprehensive review of the literature on the economic burden of SLE has recently been published.[68] As we have seen, the costs of RA have been historically driven by work disability and joint replacement, but more recently expenditures for biologic agents have come to play an important role. SLE is also associated with high rates of work loss, and certain manifestations, particularly renal failure and neuropsychiatric impairment, result in high medical care costs because of hospitalization. However, the use of biologic agents in SLE is now only a prospect, so biologic agents are not yet a factor in the costs of SLE. The work loss costs may be profound in SLE, both because a high proportion of people with this condition experience temporary or permanent reduction of employment, and also because the age of onset is, on average, a decade earlier than in patients with RA.[69]

In the studies reviewed by Meacock and colleagues,[68] medical care costs ranged from a low of US$2214 to a high of US$14,604, with an average among the studies of approximately US$9000 per year. In an important study, Clarke and colleagues compared costs in the United States, United Kingdom, and Canada.[61] To ensure that prices for services did not affect their estimates, they used the same unit prices for each country. They observed that costs of SLE in Canada and the United Kingdom were of similar magnitude, but both were approximately 10% to 15% less than in the United States. This extra level of expenditure does not, however, result in better outcomes.

Indirect costs are measured by using a greater heterogeneity of methods than for direct costs, but for those using similar methods, indirect costs exceed direct costs by an average of approximately 2 : 1.[70] In the recent Meacock et al review,[68] the ratio of indirect to direct costs was approximately 1.25, with average indirect costs of approximately US$11,000 in 2010 terms. Indirect costs are high in SLE because of high rates of work loss; prevalence of work loss among those employed at onset of disease is estimated to be 15% within the first 5 years of diagnosis and 63% within the first two decades.[71] Even though these rates of work loss are certainly worthy of concern in their own right, the labor market outcomes of people with SLE may be worse than that of people with some other diseases of similar severity because symptoms such as fatigue, pain, and neurocognitive deficits may not be as obvious to the untutored eye and may affect the ability to work.[69]

In studies of the impacts of specific levels of disease activity and specific organ manifestations on direct and indirect costs, there is mounting evidence of the profound effect of lupus nephritis; other measures of renal damage; neuropsychiatric manifestations including memory impairment; and global measures of disease activity and severity and disease flare on costs. For example, in one study, Zhu and colleagues[62] observed that SLE patients experiencing flares incurred twice the total costs of SLE as those without flares, with those experiencing renal or neuropsychiatric flares having the highest levels of costs. Similarly, a study showed that work loss was more common among those with incident manifestations and higher levels of disease activity.[72] Clearly, effective treatment to prevent damage accrual to specific organs or to reduce the frequency and severity of flares may result in a substantial reduction in the costs of SLE.

## Osteoarthritis

Surprisingly few studies of the costs of OA have been completed. In part, this is because relatively few formal diagnoses are made of this condition relative to its "true" prevalence because treatment modalities would not change if a diagnosis were made. As a result, many of the costs of OA are subsumed within the broad groupings of "arthritis" and "musculoskeletal conditions." In addition, much of the costs of OA when they are enumerated are due to the side effects of nonsteroidal anti-inflammatory drugs (NSAIDs), including ulcers, or total joint replacement surgery. Another high-cost item is drugs said to decrease the prevalence of NSAID gastropathy, either gastroprotective drugs in concert with traditional NSAIDs or selective COX2 inhibitors that may be said to lower the frequency of gastropathy.[73]

Recently, Chen and colleagues reviewed the literature on costs of OA and found 11 studies.[74] In those studies, direct costs averaged approximately US$3100 in 2010 terms, whereas indirect costs averaged approximately US$4000. In the literature, it is clear that when hospitalization occurs for the complications of NSAIDs or for surgical intervention, average direct costs rise several-fold. Also, indirect costs enumerated are relatively small, because many people with OA are older than the normal age of retirement and thus are said to have no wage losses from the condition.

In the author's analysis of MEPS data, direct costs among individuals with OA averaged approximately $11,000, and earnings losses among people aged 18 through 64 years averaged approximately $7500. Aggregate direct costs totaled just under $340 billion, whereas indirect costs were about $121 billion. We also estimated the incremental costs experienced by people with OA compared with those of similar age and gender. In this estimate, aggregate direct costs totaled $62 billion and indirect costs totaled about $80 billion.

## Back Conditions

MEPS data are ideal for estimating the economic burden associated with back problems because the overarching category does not require a specific diagnosis from a physician but, instead, can be reported by an individual experiencing a back problem. With MEPS, the authors estimated that the prevalence of self-reported back problems increased by approximately 13% between 1996 to 1998 and 2009 to 2011, from 27.4 to 31.0 million, but the prevalence rate held constant, at 10.1% of the population.

Direct costs per case associated with back problems increased by approximately 68% during the period covered, from $4835 to $8150 in 2011 terms. This increase was fueled by a 167% increase in prescription medicine costs, in turn the result of an increase of 70% in the number of prescriptions filled per case and a 57% increase in the cost of each prescription. Earnings losses among individuals aged 18 to 64 years with back problems are relatively slight because a majority of people with back problems experience temporary rather than permanent disability. It should be noted that back problems are common causes of work loss, but that such loss occurs with relative infrequency compared with the high overall prevalence of back problems in the population.

### Ankylosing Spondylitis

Boonen and colleagues[75] have spearheaded efforts to characterize the burden associated with ankylosing spondylitis in a systematic review of the cost-of-illness studies in this condition and by an article describing the frequency with which various kinds of impacts for the condition are experienced.[76] In the former publication, they note that the total costs associated with ankylosing spondylitis including direct costs ranged between $7243 and $11,840, amounts comparable with the cost of RA in the prebiologic era. Woolf[77] observed that the typical individual incurs relatively high costs for assistive devices and personnel and sustains substantial earnings losses. However, the distribution among cost categories differs among countries. In the United States, coverage for physical therapy is relatively poor and hospital admissions are rarely reimbursed for ankylosing spondylitis, so a larger proportion of total costs of the condition are attributable to wage losses than in other nations.

### Fibromyalgia

Fibromyalgia, a controversial diagnostic entity, is defined by persistent, widespread pain and, perhaps, heightened reactivity to painful stimuli compared with those without the condition. Symptoms related to the condition include sleep disturbance and depressive mood. Fibromyalgia disproportionately affects women, many who are not employed for pay outside the home and others who, if employed, are subject to the normal discrimination in employment that women experience more generally. Annemans and colleagues[78] recently summarized the literature on the economic impacts of fibromyalgia; studies since their article was published corroborate the magnitude of the impacts they enumerated.[79-82] In the literature reviewed, direct medical care costs, averaging between $5000 and $6000, dwarf indirect costs due to earnings losses, which averaged between $2000 and $3000. This occurs despite evidence that a relatively high proportion of those who are employed at onset either stop working, reduce their hours, or at least may be subject to temporary disability. Others may change job tasks or switch jobs to accommodate the symptoms. Of note, the studies reviewed show that direct costs associated with fibromyalgia often spike before diagnosis because diagnostic testing accounts for a substantial burden on society but may be reduced while the diagnostic phase of care passes and/or the person with fibromyalgia accommodates to the condition or responds to the combination of pain medications and antidepressives that are often prescribed.

### Gout

Trieste and colleagues[83] recently reviewed the literature on the economic impact of gout, finding ten studies from the past decade. Since they finished their literature review, another study has been published,[84] the results of which are consistent with the summary from Trieste and colleagues. In the studies reviewed, gout is associated with an increment of between $100 and $1000 a year in direct medical costs beyond what would be expected of those of similar age. The strongest predictors of increased costs are increases in ambulatory and hospital utilization, but not prescription medications because most of the latter are not expensive drugs. Although the average increment in expenditures may be relatively small, one study of employees indicated that expenditures on behalf of those with gout were two times as great as those among a matched population. One study not included in the review by Trieste and colleagues reported on the costs associated with new cases of gout and observed that such cases had aggregate direct costs of $27 million in the United States in 2003.[85]

## CONCLUSIONS

Researchers use cost-of-illness studies to describe the impact of conditions on individuals and society (the positive function) and to assist policymakers in allocating resources to redress those burdens (the normative function). Some have criticized the development of such measures of impact because it deflects attention from assigning relative values to interventions that might alleviate the impacts (i.e., from calculating the cost-effectiveness of interventions regardless of the condition for which they are to be used).[86] In essence, they argue that the fact of presenting large estimates of burden may mean that resources may be diverted from using highly cost-effective interventions on conditions of small prevalence even when no effective interventions exist for the conditions of high prevalence and impact, in effect, wasting money.

However, an effective counterargument is that allocation decisions will still be made on criteria other than cost-effectiveness. For example, Verbrugge[87] long ago noted that fatal conditions tend to garner more attention from policymakers. This puts at a disadvantage groups with conditions with apparent low fatality rates but which are severely disabling. She observed that men tend to have higher rates of many fatal conditions, particularly cardiovascular disease, whereas women have higher rates of musculoskeletal and neurologic conditions that may have severe impacts but which are not commonly considered to cause mortality.

Certainly societies should allocate services on the basis of the return from the investment, but we must also be certain that we do not unduly discriminate against those with conditions that do not always garner attention proportional to their effect on people's lives. At the least, we must acknowledge the positive aspect of cost-of-illness studies in describing how resources that are diverted by disease could be used for other purposes were the disease to be eradicated, to provide a measure of the potential returns from effective interventions as they arise.

This debate about the role of cost-of-illness studies notwithstanding, there is no debating that musculoskeletal conditions do divert substantial resources from other uses and that the amount has been increasing because of the aging of the population and increases in the amount spent to treat them, especially for medications. Since the U.S. government began collecting MEPS data almost two decades ago, the gross economic impact of musculoskeletal conditions has grown by the equivalent of more than 4% of GDP, to 7.6%. If one accounts only for the increment expected of people of similar age and gender, the impact is smaller, but approximately a third of that total, not small. Musculoskeletal conditions do divert significant economic resources

away from other uses because of the substantial expenditures for medical care and by depriving the economy of the productivity at work and in other activities of those affected by the conditions.

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CHAPTER 33



# Assessment of Health Outcomes

Dorcas E. Beaton • Maarten Boers • Peter Tugwell

**KEY POINTS**

- **Any single health outcome can give you only a particular view of the impact a disease has on a person. Make sure you have the right view for the purpose or question at hand.**
- **Core sets are minimal, but not exclusive, domains of outcomes that professional groups agree are important to include (if available) in all studies of a condition. They are available for several rheumatologic conditions.**
- **Defining and understanding what an investigator needs to measure is the key to choosing the right instrument.**
- **Choosing an instrument requires following a step-by-step process—looking for evidence of practical aspects of using the instruments as well as methodological/statistical properties.**
- **If the selected instrument lacks evidence of a certain property, the study can be performed to create the evidence rather than abandon the instrument.**

In an era of rising health care costs, greater provider accountability,[1] and an increased emphasis on measuring what matters to patients,[2,3] appropriate, high-quality instruments are needed for health outcome assessment. Researchers, industry, regulators, and clinicians are faced with the need to know what health outcomes to measure and how to measure them to be able to use that information in research, resource allocation, policy making, and clinical decision making. Initiatives in patient-centered outcomes research, such as Patient-Centered Outcomes Research Institute (PCORI), have shifted our usual view of health outcomes to focus on those of greater importance[3] to patients and their families so that results of clinical trials and other research will be more relevant and informative,[2,4] both to patients and to the realities of clinical decision making. Often this means including outcomes that are to the right side of frameworks such as the International Classification of Functioning, Disability, and Death (ICF)[5]: elements more attuned to functioning and participation in work and home roles, quality of life as well as symptoms, biomarkers, disease activity, and response.[6] It also means being open to new ways to understand familiar concepts. Hearing patients and their experiences has in the past created new understanding of concepts such as fatigue[7] or new instruments to capture a new understanding of osteoarthritis (OA) pain.[8] The more effectively instruments can capture our patients' experiences, the closer we will be to having the right set of tools in our arsenal.

Although the latter part of the 20th century saw the proliferation of instruments to capture outcomes such as health and quality of life, the last 10 to 15 years have seen advances in the standards for these instruments. Reeve and colleagues found 28 guidance documents associated with the selection of patient-reported outcomes (PROs).[9] Some standards, such as the U.S. Food and Drug Administration (FDA) guidance statement,[10] the European Medicines Agency (EMA) reflection, or the Outcome Measures in Rheumatology (OMERACT) Filter renewal,[11] define what evidence (measurement properties) is needed to be confident in the instrument to perform certain measurements.[12] Other standards focus on the appraisal and synthesis of studies on the performance of instruments, in efforts led by groups such as Evaluation of the Measurement of Patient-Reported Outcomes (EMPRO)[13] and the Consensus-Based Standards for the Selection of Health Status Measurement Instruments (COSMIN),[14] which outline methods for systematic reviews (search, appraisal, and synthesis) of studies of measurement properties. Still other efforts seek to standardize outcome approaches across studies. PROMIS (PRO Measurement Information System, www.promis.org), a federally funded initiative in the United States, has created a large item bank calibrated to the general population and is able to measure different health domains (including pain, function, depression) through computer adaptive testing (CAT). The predictability of patterns of response allow a small number of items to be used to focus on the score for an individual.[15] This has the potential to be a powerful tool to compare diseases or calibrate different scales onto a common metric. All of these efforts aim to put the best instrument in the user's hands and to provide consistent and high-quality data across studies to support the synthesis of findings and their application to clinical care.

In this chapter we will review a way to think about that need for an outcome measure and how to select the instrument to measure it. An outcome instrument is similar to a window in a building or home. It can provide only one view defined by its location, and its size (or, in the case of an outcome instrument, its content and response set). Deciding which instrument to use must logically begin with answers to the questions "What window do you want to look through?" and "What do you want to be able to see?" The clearer we can be about what we want to be able to measure in that situation, the easier and better the selection will be. Simple as it seems, answering these questions is often neglected in the selection of health outcome instruments. Once a good candidate is in hand, we move into a

decision-making process consistent with several other standards in the field but articulated here by OMERACT Filter initiative.[11,16] The Filter (now in version 2.0) is a set of criteria that need to be met to provide confidence that the instrument is the best to measure the target domain/concept. This is judged by accumulation of quality evidence of a set of measurement properties. This chapter addresses three questions: (1) What types of outcomes are measured in arthritis? (2) How do you know what you need to measure? (3) How do you find an instrument that can meet that need?

## WHAT TYPES OF OUTCOMES ARE TYPICALLY MEASURED IN ARTHRITIS?

In reading the rheumatology literature, and in monitoring the clinical care of patients, certain highly relevant outcomes will emerge. A group of these often emerge and are called "core sets" of outcomes.

### Arthritis/Disease Specific Instruments: The Core Outcome Sets

Core outcome sets are the minimal, but not exclusive, set of domains to be measured in a study of arthritis. They are usually recommended by groups such as OMERACT, The European League against Rheumatism (EULAR), the International League of Associations for Rheumatology (ILAR), the American College of Rheumatology (ACR), or groups formed around specific diseases, such as the Assessment for Ankylosing Spondylitis (ASAS) or the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA). Various methods can be used to determine core sets, but the most popular are those based on consensus of key stakeholders (researchers, industry, regulators, patients, and clinicians) (OMERACT, Core Outcomes Measures in Effectiveness Trials [COMET]).[11,17] Core outcome sets (COS) standardize outcomes that should be used in each research study in a field, allowing greater comparability and less risk of bias associated with heterogeneity of outcomes in things like systematic reviews. Table 33-1 shows core outcome sets for clinical trials in nine types of rheumatologic conditions.[18-32] It also shows what each group recommends [R] as additional domains to be considered or that need more research before they become part of a set to be measured in every study. The first column shares the potential core areas and core domains suggested by the most recent OMERACT Filter 2.0.[11] OMERACT has boldly asked groups to ensure at least one outcome domain is covered in each core area (life impact, pathophysiologic manifestations, adverse effects, death and, optionally, resource utilization). The remaining columns show what has been selected for core sets in different rheumatic disease at present. There are many things in common: Most sets contain or recommend inclusion of pain, physical function, patient and clinician global assessments, and markers of inflammation. These should appear in any clinical trial or other research project in these diseases. Many also include disease activity and/or damage indices, which are often an aggregated clinical index of diverse clinical findings (joint count, acute phase reactants, and global ratings of severity) that reflect the activity of the disease at that point in time. Some core sets contain domains reflecting the unique aspects of the disease (e.g., spinal mobility in ankylosing spondylitis),[33] or the unique target of the study (e.g., tophi in measuring response in gout)[31] (see Table 33-1).

Although Table 33-1 focuses on the core outcome sets, in some cases an instrument choice has also been suggested, for example, the Health Assessment Questionnaire (HAQ) for disability in inflammatory arthritis (IA). In other instances, several options are provided. Strand reviewed six disease activity indices in lupus and found that they gave comparable results.[34] In some cases the domains are shared but the measurement technique varies within or by disease. In rheumatoid arthritis, the Disease Activity Scale (DAS) 28 uses 28 joints,[6] whereas in ankylosing spondylitis 44 joints are counted.[33] We will briefly review some of the more commonly encountered instruments used in arthritis, organized by the core areas described previously.

#### Indicators of Pathophysiologic Manifestations of the Disease

Disease activity is one of the most frequently measured concepts in pathophysiologic manifestations of the disease. One of the most commonly used indicators of disease activity (indicators of the inflammatory activity) is the DAS[35] and DAS28[6] in rheumatoid arthritis, in which a set of the core outcomes (i.e., acute-phase reactants, joint counts, and global ratings) are combined to form a weighted score that provides a score of 2 to 10 (DAS) or 0 to 9 (DAS28). Based on these scores, cutoffs were established to define high, moderate, and low disease states. Until recently, the low disease state (DAS28 <2.6) was considered an indicator of remission of arthritis and will be revisited later. Very recently, new criteria for remission in rheumatoid arthritis RA have been proposed. These new criteria recognize that disease activity is not always sensitive enough to perform at extremely low levels of functioning, leading others to refine scales or develop new ones to reach.[36] Other examples of disease activity indices (DAIs) include the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[37] and the six available for lupus.[34] When more than one is available, it is helpful to seek direct comparisons of instruments, such as that performed by Strand, to evaluate comparable information.[34,38] Work is also underway to focus on an evidence-based, consensus-driven definition for a flare-up or worsening of arthritis[39] to use in clinical trials to describe a worsening of the condition.

Damage indices are indicators of structural damage to joints, typically shown by joint space narrowing, erosions, subchondral cysts, or osteophytes. In rheumatoid arthritis, particular attention has been paid to this by Van der Heijde, who reviews three approaches (Sharp, Larsen/Scott, and Van der Heijde) that are used to assess joint damage and progression in joint damage by using change greater than the smallest detectable change around error.[33]

#### Life Impact of the Disease

**General Health Status.** Generic health outcomes provide information on an aspect of health across many conditions. Thus, theoretically, comparisons can be made of the burden of low back pain with that of arthritis or diabetes. This

**TABLE 33-1** Core Areas and Domains Currently Considered in Core Outcome Sets for Different Rheumatologic Disease Groups

| Core Areas and Domains That Could Be Included in a Core Set Wolfe, 1999; Boers, 2014 | CORE OUTCOME SETS (AND INSTRUMENT IF MENTIONED) BY DISEASE GROUP | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rheumatoid Arthritis | Osteoporosis | Osteoarthritis | SLE | Ankylosing Spondylitis | Psoriatic Arthritis | Vasculitis | Fibro-myalgia | Gout | |
| | | | | | | | | | Acute | Chronic |
| | Boers, 1994<br>Felson, 1993<br>Kirwan, 2007<br>Bartlett, 2012 | Cranney, 1997 | Bellamy, 1997 | Smolen 1999<br>Strand, 1999 | van der Heijde, 1999 | Gladman, 2005<br>Gladman, 2007 | Merkel, 2009<br>Merkel, 2011 | Mease, 2009<br>Choy, 2009 | Schumacher, 2009<br>Grainger, 2009 | |
| **Pathophysiologic Manifestations** | | | | | | | | | | |
| Aggregate index | ✓(EULAR DAS) | | | ✓DAI, R: disease severity | | ✓ | ✓BVASv3 | | | |
| Biomarkers | | ✓ Biochemical | Opt | | | | R | CSF (R) | | Serum urate |
| Joint tenderness | ✓ 28 or 68 joints | | | | ✓ 44 peripheral joints | ✓ | | ✓ | ✓ | ✓ |
| Enthesitis | | | | | Enthesitis† | R | | | ✓ | Opt |
| Joint swelling | ✓ | | Opt | | | R | | Stiffness (R) | | ✓ (Tophi) |
| Joint stiffness | | | | | ✓Spinal stiffness<br>✓Spinal mobility | R | | Cognition (Opt) | Opt | Opt |
| Acute-phase reactants | ✓ | | Opt | R | ✓† | ✓ | | | R | R |
| Radiographic or imaging | ✓ >1 yr | ✓ (BMD) | ✓ (> 1 yr) | | ✓ Spine and hip‡ | ✓ Structural | R | R | | Opt |
| Deformity | | | | | | | | | | |
| Organ damage | | R (BL)/✓* Fractures<br>R (BL)/✓* Change height | | ✓ Damage index | | ✓ Skin, nail | ✓ | | | |
| **Life Impact** | | | | | | | | | | |
| Global disease<br>Patient<br>Physician | ✓<br>✓ | | ✓<br>R | | ✓<br>— | ✓<br>✓ | ✓<br>— | ✓<br>— | ✓<br>Opt | Opt |
| Quality of life | R (utility) | R (BL)* | R | ✓ | | ✓ | ✓ | ✓ | | ✓ |
| Symptoms | Pain<br>R (fatigue) | R (back)* | ✓Pain | R (fatigue) | ✓Pain<br>✓Fatigue | Pain<br>Fatigue | | ✓ Pain, Sleep, Fatigue | ✓ Pain | ✓ Pain |
| Physical function | ✓Disability | R* | ✓ | R | ✓ | ✓ | Opt, R | ✓ | ✓ | ✓ |
| Psychosocial | R | | | R | | | | Dep (Opt)<br>Anx (R) | | |
| Role participation | | | | | | | Opt, R | | | |
| **Resource Use** | | | | | | | | | | |
| Costs/HCU (R) | R | R* | | R | | | R | | | Opt<br>Opt |
| Work disability as cost (R) | | | | R | | R | R | | Opt | |
| **Death** | | | | | | | ✓ | | | |
| **Adverse Events** | R | ✓ | | ✓ | | | | | | |

*Shaded rows* reflect core areas, and listed below the core areas are domains currently being considered. Rows are organized according to areas and domains from the Outcome Measures in Rheumatology.

Osteoporosis: Core set depends on focus. *Studies aiming to reduce fracture rates. Data from Cranney A, et al: Osteoporosis clinical trial endpoints: candidate variables and clinimetric properties. *J Rheumatol* 24(6):1222–1229, 1997.

Ankylosing spondylitis: Core set elements that vary depending on focus of study. †Clinical records and symptom modifying; ‡Disease modifying only; remaining = all studies.

Anx, Anxiety; BL, bone loss studies; BMD, bone mineral density; BVASv3, Birmingham Vasculitis Activity Score version 3; ✓, core domain; CSF, cerebrospinal fluid; DAI, disease activity index; Dep, depression; EULAR DAS, European League against Rheumatism Disease Activity Scale (or Revised DAS28, 28 joints in joint count); HCU, health care utilization; Opt, optional outcome; R, recommended for further research and possible inclusion in core set.

depends on how well an instrument captures the burden in each disease group. Generic instruments have the advantage of allowing comparisons across diseases and of covering a broader range of health issues that may otherwise be overlooked in a core set (e.g., mental health). However, generic instruments, because of their breadth, tend not to delve sufficiently into the depth of experience in any one disease. As a result, they are often weaker in their ability to detect specific changes, and their sensitivity to different levels of disease activity may be low. They should, therefore, usually be supplemented with disease-specific instruments.[40] Popular examples of a generic instrument include the Short Form (SF)-36.[41] Direct comparisons of generic instruments have shown a surprising discordance in the scores across instruments.[42-44] Studies or clinical results may not be comparable if they use different health status scales.

**Utilities: Value of Health State.** Utility scales offer an overall score for the value of a health state, setting death at zero and full health at one. The emphasis is not upon describing the state, but on assigning a value, worth, or preference to that state.[45,46] Utilities are needed for economic appraisals in which the value of a health state is weighed against the costs of treatment. Utility states can be obtained by direct or indirect methods. Direct methods, such as standard gamble and time trade-off, involve the respondent working through exercises to elicit the value for his or her own health state against elements such as time, or more/less favorable health situations.[45] Indirect methods capture the state with standardized questions and then apply predetermined weights.[46] Examples include the EQ-5D, which is composed of five items (three response categories) combined to describe a health state. Similarly, the Health Utility Index (HUI) gathers information on six or seven dimensions of health (depending on the version) on five- to six-item response scales, to define a health state.[46] They, along with the increasingly popular SF-Six dimensions (SF-6D),[47] then use weights previously determined in different populations to assign the value to these health states, hence the "indirect" weighting. The absolute value obtained across these different approaches will vary.[45,48]

## Symptoms

Pain is usually measured by using a 10-cm visual analog scale or a 0 to 10 numeric rating scale of the intensity of the pain.[49] This simple instrument has been well tested and is easily understood by patients. However, Hawker led an initiative to better understand pain in hip arthritis, and found patients describing differences in their intermittent and constant pain. This resulted in a new instrument to be considered for pain.[8,50] Fatigue is another important symptom, which many patients feel is quite distinct from being "tired."[7,51] Teams are recommending either global indices or one of several available scales that were reviewed by OMERACT attendees.[52-54] In the area of sleep, work done through OMERACT on the measurement of the problem provides a recent strong example of moving through the concept of impairment of sleep, defining it, and then focusing on the available scales that capture that concept and definition.[55-56]

## Disability Scales

Physical disability is an example of a disease-specific measure of health status in arthritis because it is often affected and warrants a more specific instrument focused on arthritis. This is often measured by using the HAQ Disability Index (HAQ-DI),[57] which covers 20 items that examine different domains of daily functioning. Patients score each item on a 0 to 3 scale, in which 3 represents the greatest disability. Scores are obtained for each domain and then combined for a total score expressed on the same 0 to 3 scale. Scores are adjusted to a worse health state (a 2/3) if a support is used to complete a task. More details on the HAQ-DI are widely available in print and on the Internet. The HAQ is one of the "legacy" instruments that is fully linked with the PROMIS database.[15]

There are other scales or subscales that assess physical function, such as the Arthritis Impact Measurement Scale (AIMS)[58] and the AIMS2.[59] Others measure with even more specific foci, such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[60] or Knee Injury and Osteoarthritis Outcome Scale (KOOS),[61] which are the most commonly used scales for lower limb OA. The AUSCAN (Australian-Canadian) Osteoarthritis Index is an option for hand OA.[62]

**Self-Efficacy/Effective Consumer.** Self-management is becoming a part of programs for persons with multiple chronic conditions. For patients with arthritis, these programs have been shown to improve levels of self-efficacy: the confidence one has in one's ability to manage pain and disease effectively. Lorig's Self-Efficacy Scale is one of the most commonly used outcomes for this type of study.[63] Tugwell's group has developed a complementary "effective consumer scale," which captures the degree to which the patient is effectively managing his or her own health care decisions, interactions with the health care team, and disease monitoring.[64] It is an instrument with demonstrated reliability, validity, and responsiveness in arthritis, and has been a support for consumers with arthritis.[65]

## Work Disability: Looking Beyond Absenteeism

With the shift toward more aggressive management of earlier rheumatic disease, more people with arthritis are working, and outcomes that matter include their ability to manage at work.[26,51,66] Work is hard to measure because it forces us into considering the context of the disability: the job (hours, demands, flexibility) that will impact the presence at or absence from work as well as the difficulties experienced by the patient on the job. In work through OMERACT, more than 20 at-work productivity measures were identified.[67] Through 6 years of work with these tools, researchers winnowed through a review of content and conceptual focus to seven scales, and then reviewed or created evidence on the necessary measurement properties to satisfy the OMERACT Filter.[66,68,69] A slew of evidence was published that reported on multi-item and global scales. Correlations amongst these scales are still only moderate; however, they individually had accumulated a satisfactory amount of evidence to support their properties.

**Nonpaid Work Roles.** Participation in valued nonpaid roles, such as parenting, volunteer work, or leisure activities,

can be very important aspects of the burden of disease.[70] This concept will need to be considered carefully and outcome instruments reflecting this tested or developed in the future.

**Patient-Specific Indices.** Patient-specific scales allow the patient to nominate his or her own items, in effect creating their own outcome scale. Most researchers encourage three to five items to be nominated, often those that are most challenging for the person. A surprising number of these scales have been developed and reviewed by Jolles and colleagues.[71] Each taps very relevant content for patients, and because of this they can be responsive to change.[72] The challenge is in the mathematics and how to analyze the numeric score that has been created by different sets of items for each person. Analysis that focuses on individual-level quantification is likely best, for example, the percentage of people who reach their goal or improve in their selected activities.

### Toxicity/Adverse Events

Medical and nonmedical management of many rheumatic conditions carries a risk of toxicity and adverse events,[73] many of them unexpected. Because patients, clinicians, and policy-makers want to balance benefits versus harms when considering intervention, a comprehensive documentation of a range of adverse events is important in outcomes assessments separate from the treatment benefits.[10,74,75]

### Death

Rheumatologic conditions are associated with increased mortality, and arthritis-specific mortality should be monitored in arthritis research.

### Resource Utilization (Costs)

Costs associated with treatment (drugs, equipment, and physician visits) are all important in considering the benefit of one treatment versus another. Thus OMERACT Filter 2 includes resource utilization as an important but optional domain. Along with direct costs, indirect costs (e.g., lost productivity and caregiving) should also be considered, but can vary considerably. Harmonization is important to get a comparable estimate of cost across studies.[76]

This section has reviewed the standards that have been set to date. Many changes are underfoot with a renewed interest in patient-centered outcomes, and new domains and concepts are likely to emerge. The next two sections will help the reader to address the issue of wanting to measure something new, or deciding between different instruments.

## HOW DO YOU KNOW WHAT YOU NEED TO MEASURE? DEFINING YOUR MEASUREMENT NEEDS

Just as investigators define a research question before embarking on a clinical trial, so should users of health outcomes define a measurement need before choosing an instrument.[12,77] There are three parts to this definition: What, Why, and Who?

### What Do You Want to Measure?

It is important to have a sense of the concept you want to measure before you choose an instrument. This way, you will be sure to choose an instrument that meets your needs, and you will be less swayed by what the developers say is measured. The use of theory or, better yet, patients' own experiences can help clarify the scope and content of an ideal measure of elements such as recovery[78] or fatigue[54] and complement the view of the researcher. This understanding can only help with the selection of the best instrument, scientifically and from the perspective of the relevance of the scores for our patients.[79,80] Groups such as EULAR or the FDA have named the direct involvement of the patient in the development of patient-reported outcomes an essential feature for new scales,[10] (for example, as seen in the development of Rheumatoid Arthritis Impact of Disease [RAID]).[81] Involving patients in this role as research partners may have challenges in communication and research nomenclature,[82] but the benefit in terms of understanding the concept you are trying to measure (the "what") is unmatched.

Defining key concepts or outcomes may also be facilitated through use of a conceptual framework because they define key concepts/constructs and their theoretical relationships. One increasingly popular framework is the International Classification of Functioning (ICF), endorsed by the World Health Organization in 2001.[5,83] It describes three main concepts (Figure 33-1): (1) impairments (symptoms, structural limitations), (2) activity limitations (difficulties while performing tasks), and (3) participation restrictions (social role participation). Around this the ICF adds the importance of environmental factors (job demands, environmental barriers, weather) and personal factors (predispositions, coping strategies). OMERACT made use of the ICF in formulating the core domains of measurement in Filter 2.[11] Choosing a framework that helps you to define and think about the concept you wish to measure will be very helpful down the road when you try to understand your findings. They form the basis for understanding your observations, testing hypotheses, or planning and executing an analysis. Thinking about your concept and defining it carefully and explicitly should be done before any instruments

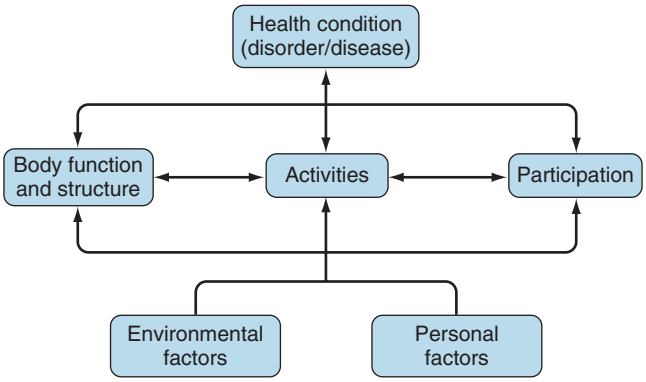



**Figure 33-1** The International Classification of Functioning (ICF) conceptual framework showing the hypothesized relationships between domains of impairment, activity limitations, and participation restrictions and the direct influence of environmental and personal factors on these domains.

or core sets are reviewed. The instrument should meet the need, and not the reverse.

### Why Are You Measuring?

Clarity about how you intend to use the scores from an instrument and define the intended purpose is needed.[12,77] Kirshner and Guyatt describe three purposes: descriptive (measurement of a concept at one point in time, such as the burden of illness [i.e., this group is sicker than that group]), predictive (provide information about the future, such as the HAQ's ability to predict mortality in arthritis), and evaluative (measure change during time, such as the benefit or harm from treatment).[84] This intended purpose will define the measurement properties you need to establish to ensure the instrument is suitable. In this chapter, we focus on the purposes relevant to health outcome assessment: describing an endpoint state in a trial at one point in time (Kirshner descriptive purpose) and evaluating the amount of change experienced during time (Kirshner evaluative purpose[84]).

### Who Comprises the Target Population?

The target population is critical, but often overlooked. A given instrument may, for example, work very well in severe OA of the hip but not be sensitive to the early symptoms of the disease. It is equally important to consider if you want to measure for an individual patient or for describing a group of patients as a whole, for example, in a clinical trial. The former demands much higher levels of things such as reliability (precision), such as a coefficient of greater than 0.90 as opposed to minimum 0.75 to 0.80 for group-level applications.[85]

The measurement need is defined by the concept, the intended use of the instrument scores (the purpose) and the population. All need clear definition before any attempt to decide on an instrument for that concept of interest in that context of use. Much of that decision making will be based on a well-defined need.

## DECISION-MAKING INSTRUMENT FOR SELECTING THE OUTCOME THAT CAN MEET YOUR MEASUREMENT NEEDS

Armed with a well-articulated need, the selection of an outcome is a set of steps. Failure at any one of the early steps would quickly suggest it is time to choose another candidate instrument. Failure of a missing measurement property is a flaw, but is often correctable with additional study. There are many guidelines for selection available, particularly for the acceptable levels of reliability and validity, that offer more detail than can be provided here.[9] What we will describe here is a decision-making process that can be used to assess whether a given instrument fits with the articulated measurement need. This process, depicted in Figure 33-2,

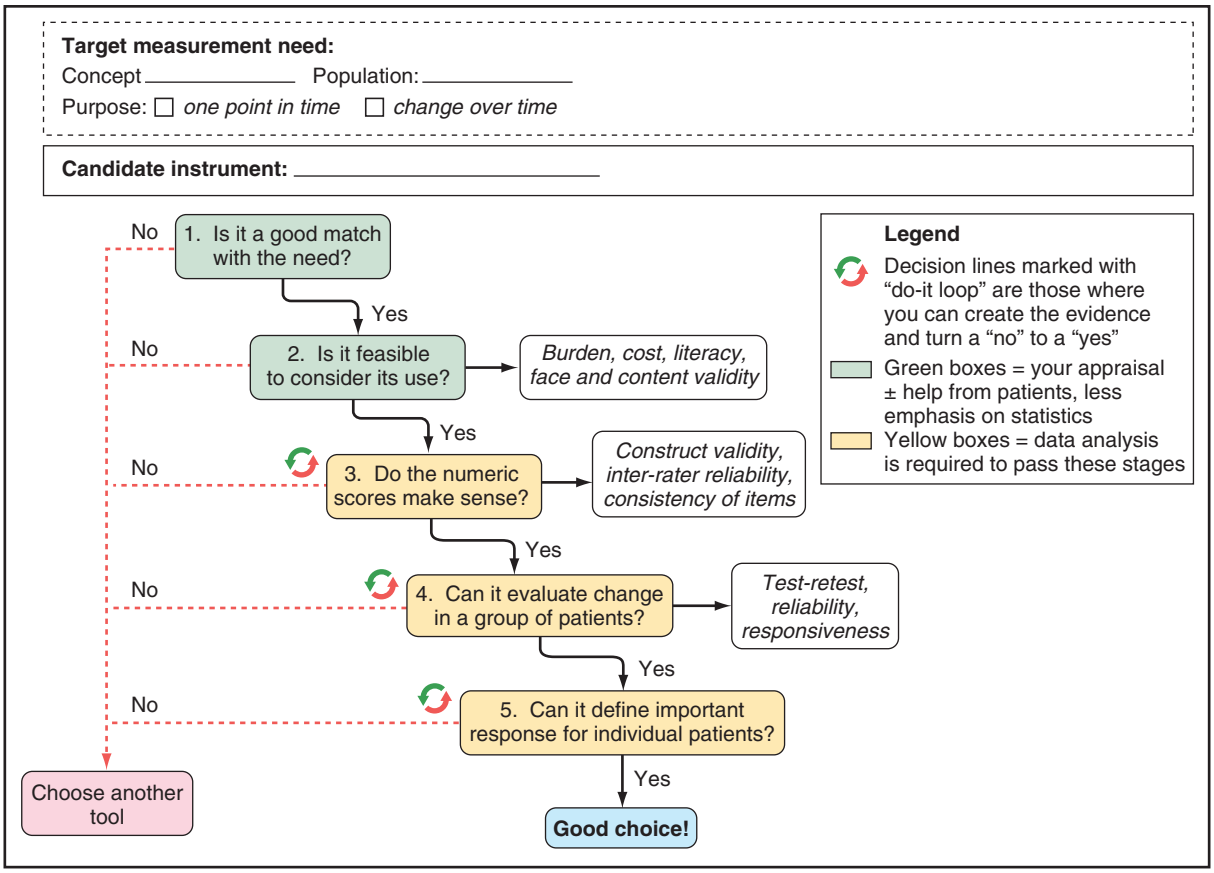



**Figure 33-2** Algorithm showing the decision-making process for the fit of a candidate measure with your target measurement need. The first two *(green)* boxes can be completed by appraisal of the instrument, hopefully with input from respondents. The last three *(yellow)* boxes require data. Many instruments are weeded out as a poor fit in steps 1, 2, and 3; things that cannot be corrected. The *"do-end" loop* denotes stages at which you can pause to create evidence if it is missing and do not necessarily have to abandon the instrument.

builds on the work of Law,[86] Lohr,[87] Mokkink,[14] Reeve[9] and the OMERACT Filter[88] as well as highlighting key concepts in each area from the published works.

This decision-making process has three key features that deserve attention. First, it always starts with a firm statement of the measurement need (and a search for all candidate measures). Second, much work must be done with a user's guided reflections on what they think of the instrument itself, including, if possible, feedback from patients before the correlations and effect-size statistics are viewed.[69,89] A common misconception is that measurement has to do with statistics, whereas much has to do with common sense. Third, the inability to confirm each of the earlier stages indicates an irreconcilable mismatch and suggests that you are better off finding another candidate instrument. Conversely, if you have made it past this prestatistical appraisal and you still have a candidate measure, you are likely holding a good scale, and may want to create any missing evidence on its measurement properties as opposed to abandoning it for their lack (*do-it loops* in the diagram). With these overarching points in mind, let us look at the process.

## Step 1: Is It a Good Match with Your Needs?

Think about your concept and then decide, based on the description of the candidate instrument and the nature of the items, whether there is a match between the instruments concept and the measurement need (concept, population, purpose).[77] An operational definition of the target concept, the applicable populations (specific patient graph or general population), and intended purpose should be articulated by the instrument's developer and should match your current need.[10,87,90] If these elements are not there, or if the instrument is not a good match, start with another candidate instrument.[91] For example, your target might be physical function, and the instrument might only briefly cover physical function and include much more detail on emotional and social health than even is in use in your research community. For you, if the concept is not a clear match with your target, pass it by.

## Step 2: Is It Feasible to Use?

Feasibility covers the practical aspects of using this scale in the intended setting.[86-88] Does it take too much time? Are the licensing costs too high? Does it require special equipment? Is it too burdensome for your patients (e.g., language, literacy, acceptability of questions)? Is it well-formatted, and do the responses and scoring make sense given the target and the question? Are the questions phrased in a clear and simple manner? Are the necessary scoring instructions available? Are the results of the score easily interpretable? A negative response to any of these questions could direct you to another, more feasible instrument. Feasibility often makes or breaks a decision about a candidate instrument.[88,89] Others might call this "sensibility" or "clinical usefulness" of an instrument. Usually the appraisal is completed by the investigator, but additional insight can be gained by including patients' input about the length, difficulty, and burden of the questionnaire or by their response to it (floor/ceiling effects).[89]

### Content Validity

Does it appear that the candidate instrument covers the domains of your concept well, and do the items align in this content and/or domains? It now becomes clear why your measurement need must be well articulated before this point to avoid an exercise in circularity. This, like step 2, is usually done by the clinicians or researchers, but patients can also provide valuable insight into ensuring the content is comprehensive.

Content validity appraises the items and domains of a scale, and whether the authors have covered the breadth and depth of the concept.[85] In other words, are all the important areas, covered, and is there enough depth to capture the range of experience of the patients you wish to administer it to?

## Step 3: Do Its Numeric Scores Make Sense? (Construct Validity)

Having convinced yourself of the content and practical feasibility of this scale, the next step begins the more data-intensive evaluation.

### Item onto Scale (Only for Multi-item Reflective Scales): Structural Validity

The first phase is at an item-to-scale level: The gathering of data on whether the scale at an item level is performing in the manner it should. This is often called *structural validity*, confirming the structure of the items on their subscales against the developer's expectations. It bridges content and construct validity and is often aligned more with content, but is considered here because of its dependence on data and its analysis. It is assessed through approaches such as factor analysis (if the instrument was designed to have multiple items aligned with one underlying trait) or item response theory[92,93] to assess whether the items designed to capture consecutive levels of the trait along the continuum are doing a satisfactory job. It should be noted that factor analysis will favor multiple items that are endorsed in a similar manner across them. These will be highly correlated. The goal of factor analysis is to see whether similar items are sufficiently correlated to suggest they are eliciting consistent patterns of response across people. If there are multiple subscales, then these will separate as different patterns of response, with more correlations between items within its subscale than items across subscales. It all makes logical sense and is summarized mathematically by inter-item correlations, item-to-total correlations, and factor analysis.

If another approach, such as item response theory (IRT), has been used in selection of items for a scale, then a wider range of less highly correlated items will be selected to capture all levels of the latent trait, which would not be summarized well by traditional factor analysis. The approach used needs to match the approach used in development. IRT-based instruments need to have structural validity assessed within modern measurement approaches, using IRT-based methods, perhaps checking the stability of the items and their ranking/weight across disease severity or disease type (differential item functioning). One form of

IRT is the simplest Rasch model. It stands as a template for linear measurement of a single dimension, so when the Rasch fits, those items are confirmed as representing an underlying trait, confirming its structure. In other forms of IRT, the model is adjusted to fit the data rather than changing the scale (as in Rasch), and model fit in a new sample and/or stability across different groups is used as confirmation of the structure of the scale.

### Score Level Analysis: Construct Validity

The next step is to explore data to see whether the instrument's numeric scores make sense and behave in the same way we would expect an excellent instrument of this concept/domain to behave. Confidence with construct validity should be established regardless of the eventual purpose (descriptive or evaluative) because it answers the question of whether or not the tool measures what it says it should measure. Sometimes it is de-emphasized in evaluative instruments in favor of a more detailed evaluation of change and responsiveness; however, others (including these authors) stress the importance of ensuring you know what you are measuring before focusing solely on change scores.

Construct validity is generally measured by comparisons with similar scales or related constructs (i.e., high and low levels of pain and function). Theoretical situations are established *before* analysis, the direction and magnitude of the expected relationship declared, and then the relationship is tested.[77,87,91,93] Ideally, testing with scales expecting high correlations, and also no correlation will provide more confidence in what this tool is measuring. Comparisons should also be made among groups known to differ (high vs. low severity); known groups construct validity. Again, this is based on an a priori theory to check whether the instrument is measuring what it is supposed to measure.[91] If the evidence is not available, or is not available for the intended context of use, you have a choice to abandon the instrument or conduct a study to create that evidence, and then continue to advance.

As well as understanding construct validity, precision of measurement is also important. Again, for more psychometric, reflective multi-item scales, this comes in the form of a check of the consistency of responses across items by using Cronbach's alpha coefficients, or its equivalent Kuder-Richardson Formula 20 if the scale is dichotomous (yes/no). In IRT-based scores, the same idea of precision is reflected in a person separation index (PSI). High cross-sectional precision suggests that the true score lies within a tighter smaller range around the calculated or observed score (margin of error). Internal consistency coefficients lie between 0 and 1. Higher is generally better, although coefficients higher than 0.95 suggest redundancy in the items, and if burden is a concern, it might be desirable to drop some items. The internal consistency reliability can be converted back into the scale score by calculating the precision limits (by using 95% limits, the true score list somewhere within $1.96 \times s[1\text{-}r]^{1/2}$, where r = internal consistency and s = standard deviation). This tells us the range within which the true score for an individual will reside. This is not a feature of a scale containing weighted sums of different attributes, such as disease activity measures.[94]

If more than one person will be gathering the data, interrater/observer reliability should be measured and quantified with an intraclass correlation coefficient (ICC) for continuous measures or a weighted kappa for ordered categories.[95] This would be important for either IRT-based or summed multi-item scales as well as more observer-based tools of clinician-reported outcomes or clinical indices (disease activity indices). There are different types of ICC, depending on the model used for the variance estimates. The type of ICC should always be named.[95] The ICC and weighted kappa assess how well the instrument reproduces the exact same score across observers, and are preferred versus correlation coefficients that look only for trends and not a direct match in numeric values. Cutoffs are always challenging, but, in general, reliability should be at minimum 0.75[85,87] for group-level analyses (in which analysis is always done for a group of patients, as in the mean score comparisons in a clinical trial). For description of an individual patient or making decisions about their care, ICC should be 0.90 to 0.95.[85,87]

## STEP 4: CAN THIS INSTRUMENT EVALUATE CHANGE DURING A PERIOD OF TIME IN A GROUP OF PATIENTS?

After getting a general sense of the construct validity of the instrument, we sometimes wish to know whether this outcome is able to measure change during a period of time. This is only important if you are going to measure and make claims about a *change* in score (i.e., if a change of 50 points was observed). If your goal is to describe an outcome, such as the level of pain after a treatment, you may have enough information at step 3. In the original OMERACT Filter, this was the "discrimination" component of the information need in Filter 2. This is expanded and contextualized to answer the question "Can the instrument discriminate well enough to detect the change you need to be able to detect?" The hallmark of the ability to measure change in a group is twofold: First, do the scores remain the same when the target concept has *not* changed during a period of time (test-retest reliability), and second, when the concept changes, does the score on the instrument change as well (responsiveness or sensitivity to the target construct of change)?

### Test-Retest Reliability

Test-retest reliability requires two administrations of the instrument during a period of time when no change in the target concept has occurred. As a reader of reliability studies, you should feel convinced that no change in the target (e.g., pain, function, or disease activity) would have occurred in these patients in this situation.[14,96,97] Often, people conducting studies of test-retest reliability will establish a clinical situation in which no change should have occurred, or they use an external anchor (e.g., a question about whether the patient's pain is the same as last time) to find patients who have not changed. Similar to interobserver reliability, the ICC is the preferred statistic for continuous scores and weighted kappa, its equivalent, for categorical scores.[95] The cutoffs are the same, and a coefficient can be converted into

a "minimal detectable change"[98] = $1.96 \times s(2[1\text{-}r])^{1/2}$, where s = standard deviation and r = test-retest reliability (ICC).[85,98] Ninety-five percent of people who are stable will have change scores less than this value, hence a change greater than this is not likely to occur in a stable patient. This becomes a lower boundary of meaningful change. Anything below that boundary could be simply day-to-day fluctuations in scores.

Responsiveness is perhaps best thought of as longitudinal construct validity. Similar to construct validity, it depends on an a priori theoretical relationship in which the attribute is changing with time (e.g., change in pain with time). We all too often focus on the amount of change recorded, rather than the match of the change in the instrument's scores with the type or amount of change that has actually occurred and was expected in that testing situation. A large change is not useful if we were expecting a small one; it only suggests error and noise. The amount of change expected in a study of responsiveness should be carefully described and should be directly associated with the intended application (i.e., measurement need).[99] If the goal is to detect change in a clinical trial, then it is important to assess the instrument's ability to detect the difference in change between treatment and control groups. If the goal is to detect change in a cohort, it might be more useful to examine change in a single group, perhaps in a treatment of known efficacy that is close to your intended application (e.g., hip replacement) or in people who rated themselves as improved on an external anchor (e.g., global index of change). Responsiveness is summarized with statistics of signal (change) overnoise (error) such as the standardized response mean (mean change/standard deviation of change), *t* statistic (mean change/standard error), or effect size (mean change over standard deviation of baseline).[95] These statistics on their own have little meaning without an a priori construct of change (magnitude and direction expected).

When the target application is the quantification of the relative change between treatment and control groups, such as in a clinical trial, the ability of a scale to discriminate at that level, and against similar groups as in the target application, is needed. The effect size of statistics described above can be adapted for relative change between two groups[72,100] or a standardized mean difference can be calculated.

Deyo also described the correlational approach (correlate change and another indicator of change) as a direct parallel to cross-sectional construct validity.[101] He also suggested a receiver-operator characteristic curve approach (various change scores against external gold standard that the person change). This offers information on the sensitivity and specificity of different change scores for application to individuals as well as the area under the curve as a summary of discrimination between changed and unchanged groups.[101] All of these approaches are dependent on the external anchor of change having occurred, and this is an important part of the study. Regardless of the approach, the numeric summaries of responsiveness, such as effect sizes or areas under the curve, should correspond to the type of change (magnitude and direction) expected in the a priori theory. A large effect size or area under the curve does not mean an instrument is to be stamped as "responsive." It should correspond with the change anticipated in the study, small or large. Comparisons of the effect sizes are helpful if different instruments are compared in the same study, as was done by Buchbinder[72] or Verhoeven[100] in early IA patients. But caution should be used when comparing instruments across studies. Responsiveness is a highly contextualized property, and the same instrument may not be responsive in another situation (early vs. late disease, OA vs. IA, 2-week vs. 6-month follow-up).[87]

## STEP 5: ARE THRESHOLDS OF MEANING DEFINED FOR THIS SCALE?

The final step, often deemed the most elusive,[102] is the interpretability of the scores. Responder analyses that quantify outcome as the "proportion" improved, recovered, or who have responded to therapy are classifying and counting individuals who have responded or not[10] and although misclassification error might not affect the proportions in one group relative to another, they do impact clinical decision making for one patient. The intended application should be considered.

### Benchmarking States

At the end of a trial, a patient's pain is at 2 of 10 on a pain scale. Is this a good outcome? The meaning of different scores on an outcome assessment is used both for classifying people at the beginning of a trial and at the end (final state). To establish the meaning of the specific values of an outcome scene, comparisons are made with other known health states, such as severity indices, ability to work, or self-rating as mild.[103] Gradually, enough trends might be seen across different scenarios to gain confidence in the meaning of "good" or "mild."[78,104] In rheumatology, we see the emergence of low or minimal disease activity states (LDAS; MDA),[105-107] patient acceptable symptom states (PASS),[108] or remission criteria with the DAS28[38] as thresholds below which people are considered to be in an acceptable state (defined as either tolerable symptoms or disease activity that does not require medication changes). At this point these thresholds are being established, and similar to change thresholds, we may find variability in the values[38] that will need to be sorted out with methodological work and application in clinical practice.

### Changes in State

The second type of interpretability concerns change scores.

#### American College of Rheumatology Response Criteria

The American College of Rheumatology (ACR) took the core-set measures and determined that if one observed an XX% change in joint count, swollen joint count, and in at least three of the following: (1) erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP); (2) physician global, patient global, pain, or (3) physical disability, then one had a clinical response, and the individual would be classified as a responder. The percentage (XX%) is usually 20%, but 50% and 70% have also been considered. The

ACR20 is widely used, defining responses across a wide variety of domains, and discriminates well in clinical trials.[100]

### Minimal Clinically Important Differences/ Improvements

Defining the threshold of change above which a person has had an important shift in outcome is what Kirwan has described as the "elusive crock of gold at the end of the rainbow."[102] Nevertheless, important advances have been made. In 2000, Wells described nine different methods for deriving minimum clinically important differences (MCID) from the literature.[109] Some use distributional cutoffs (e.g., 1/2 standard deviation(s), or effect size of 0.2 or 0.5),[110] which have been criticized as lacking any meaningful anchor. Others are dependent on an external anchor, which indicates that important improvement has occurred, but are sometimes challenged by their dependence on that anchor as well as on the perspective of the person who determines the response (patient, doctor, payor). MCIDs repeatedly have been shown to vary with baseline state[111] and with improvement versus deterioration,[112] mandating that they are looked at separately.[113] In MCID work, one should plan on working with a range of values as a result,[114,115] rather than one absolute. It is also important that the testing situation and the study design (kind of change, anchor for change) are similar enough to your intended use that you can be confident in the results.

### Combined Approaches: Change and State

An attractive, although often overlooked option, is to combine the last two approaches. In 1996, EULAR defined clinical response as a change in DAS28 score of more than 1.2 (change), plus a final DAS28 score of less than 2.4 (final state).[35] Jacobson did the same in defining response to psychotherapy; change greater than error was induced by care (change > error [MDC]), plus a final "normal" state.[116] This combination has been echoed by studies of how patients define being "better."[78,104,117] Both change and landing in a good state would be required.

The approaches described above focus on interpretation at the level of the individual, perhaps for use in clinical practice or in a response-type analysis of a clinical trial or economic appraisal (% responder). Verhoeven has shown that the same instrument may not perform equally well in a responder-type of analysis and for a group-level change.[100]

At each stage of this appraisal, there is an element of judgement. There will likely never be perfect evidence across all stages. The user will need to assess the potential risk of accepting less than ideal evidence, or abandoning the scale. They may also, however, *create* the evidence by doing it themselves. An instrument that makes it through this appraisal is likely a good fit with the measurement need. Anything short of that could lead to error and be prone to misinterpretation. By working from top to bottom on the figure, instruments that are not targeting the right concept or are impractical to use in the intended setting can be quickly eliminated before extensively reviewing the literature for the measurement properties.

## AREAS OF GROWTH IN HEALTH OUTCOME ASSESSMENT

### Systematic Reviews of Measurement Properties

With the growth in the number of articles published on measurement properties for a given tool, we are seeing the growth in the number of reviews and, more recently, systematic reviews of the literature ($n$ >489, www.cosmin.nl). By systematic, we mean standardized methods for searching the literature, selection of articles as relevant, critical appraisal for risk of bias, and synthesis. There are several approaches recommended; however, COSMIN is a valuable resource both for search strategies and suggestions for a scored critical appraisal instrument (not risk of bias). There are many critical appraisal tools available, most focused on quality of reporting and quality of methods versus risk of bias. Similar to a systematic review of intervention studies, a lot of work is involved in systematically reviewing the articles for each measurement property. But this process can be streamlined by returning to the points we have emphasized above: first that you don't need to go that far if you do not have a match for your concept, and second, there are good- and bad-quality studies of any measurement property that we need to weigh differently in our decision making. Unlike systematic reviews of intervention studies, those for measurement properties are actually several parallel reviews, one for each property.

### The Message Could Be in the Subgroups: The Rise of Cluster Analysis to Uncover Patterns of Responses

Clinicians and researchers are always trying to maximize the understanding they can get from their outcome measures to capture the true experiences of patients. In recent years we have seen emphasis placed on looking for patterns of response in patients rather than mean change alone. This type of analysis needs at least three measurement points and a large sample but allows subgroups such as "quick responders" versus "unchanged during a period of time" to be identified. This latent trait clustering of these longitudinal data points allows new insights into types of response, or, likely, ongoing course. It makes a great deal of clinical sense, with clinicians recognizing patterns such as a quick, sustained positive response over time in contrast to a patient with a slowly progressive deterioration in function. These types of patterns can only be captured with longer follow-ups, and this latent class analysis.

### Adaptation to an Ongoing Disease

In this chapter, we have focused on the measurement of health states, and indeed their improvement or deterioration over time. However, people with chronic diseases will successfully adapt to ongoing disease with behavioral strategies, or cognitively reshape their idea of what "good health" means.[118] These are things that are sometimes induced in our patients while therapists try to help them deal with the impact of the disease. In some circles this is called "adjustment,"[117] in others "response shift."[119] The challenge in

health outcomes assessment is that this type of outcome will not be captured well in a pre-post change score. A number of groups are researching how to incorporate adaptation into our health outcome assessments.

## CONCLUSION

Patient-centered care initiatives, such as patient-centered outcomes research, have brought a lot of attention to the topics raised in this chapter. A commitment to outcomes that matter, and increased rigor in the standards these measures must achieve to be used in clinical care or labeling means that the coming years will see even more advances and choices in health outcomes measurement. Standardized sets are not meant to restrict a researcher's outcome choice but rather to open doors to increased comparability across studies, interventions, and patient experiences. Trends in RA mentioned in this chapter show an increased use of core sets since their release, thus allowing for more confidence in the results of a synthesis.

Testing instruments to make sure they are good metrics for a given measurement need (as defined by the target concept, population, and purpose) will become more deliberate and more focused on assessment of whether the tool works. While the research and clinical communities move to this level, our instruments and our ability to make use of their scores will get better and better.

The field is on the brink of deciding the role to be played by item response theory and computer adaptive testing in widespread care settings. Improved access to PROMIS and networking platforms that will arise from PCORI will help to facilitate this. However, despite progress in assigning a numeric value to a complex health state, a healthy struggle continues with the interpretability of scores. What does the numeric score mean in the real world of patient-clinician decision making? It is not always a simple translation from questionnaire score to clinical meaning. Health outcome assessment is well advanced in arthritis care, and we thankfully acknowledge the years of work and commitment of many professional and patient/consumer groups. Advances will continue in the use of technology, the breadth and depth of our outcomes, and the quality of measurement methods while the field of arthritis outcome measurement strives to keep pace with the needs of patients and their families, clinicians, researchers, and policy makers.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 34

# Biologic Markers in Clinical Trials and Clinical Care



David L. Boyle • William H. Robinson

**KEY POINTS**

- Biomarkers can facilitate the diagnosis, stratification, and therapeutic management of rheumatic diseases.
- Biomarker validation requires careful demonstration of utility in multiple independent data sets
- Actionable biomarkers can guide therapy and clinical decision making.
- "Mechanistic" biomarkers are based on disease pathogenesis.
- Predictive and pharmacodynamic biomarkers can facilitate drug development by identifying the individuals most likely to benefit and by providing an assessment of response.
- Surrogate biomarkers can be used in regulatory processes for drug approval.
- Multiplexed biomarkers could offer increased utility compared with individual biomarkers.

Drug development in rheumatic diseases has primarily relied on clinical endpoints, such as mortality, joint damage, or other easily quantified metrics.[1] Clinical trials focused on these parameters often require very large numbers of patients, are underpowered for early "go, no go" decisions and require very long time lines.[2,3] Similarly, patient care in rheumatology typically utilizes a combination of traditional evaluation approaches that integrate standard laboratory tests with clinical assessments.[4,5] A pressing need to integrate laboratory or imaging methodologies in the clinic and drug development has emerged, especially to help stratify patients and individualize therapy.[6,7]

To address this need, a concerted effort to develop novel biomarkers has gathered momentum. Although many definitions of biomarkers exist, one of the broadest and most relevant was provided by the U.S. Food and Drug Administration (FDA): "Biological marker (biomarker): A characteristic that is objectively measure and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[8]

Some biomarkers are used routinely in clinical practice, such as C-reactive protein (CRP) to assess disease activity or liver enzymes to monitor drug toxicity.[9,10] Imaging technology can evaluate synovitis, as with power Doppler ultrasound.[11] Genomics studies could ultimately be integrated into clinical evaluation to help personalize therapy.

Biomarkers are divided into a variety of categories based on their application, including susceptibility, exploratory, proof-of-concept, and many others.[12] One category of biomarker requires true validation, namely, the surrogate biomarker. A surrogate biomarker is a test approved by regulatory authority as an endpoint that can be used in place of a clinical outcome for drug approval.[13] For example, decreases in hemoglobin A1c are sufficient for regulatory approval for a diabetes drug. In rheumatology, changes in radiographic joint damage can be used for a "disease-modifying" claim in rheumatoid arthritis rather than joint replacement surgery.[14] On the other hand, other measures such as magnetic resonance imaging (MRI) or ultrasound, might not be accepted and have not been sufficiently validated for regulatory authorities.[15]

## MOLECULAR BIOMARKERS

Molecular biomarkers are biochemical variables that include measurements of proteins, RNA, lipids, carbohydrates, metabolites, and other biomolecules in the blood, other biologic fluids (e.g., synovial fluid) or tissues. Molecular biomarkers can be objectively measured through a variety of techniques, and can be indicators of normal or pathologic processes, or can be indicators of response to therapy.[16] The advent of large-scale nucleic acid sequencing, other genomics, proteomics, lipidomics, glycomics, and metabolomics technologies have enabled the identification of next-generation molecular biomarkers. Examples of molecular biomarkers related to autoimmunity and inflammation are shown in Table 34-1 and discussed in detail in the following text.

### Characteristics of Biomarker Measurement

Molecular biomarkers are measured using a variety of specialized assay platforms, and the performance characteristics of the specific assay largely determine the utility of the assay. Key characteristics include sensitivity, specificity, predictive value, and assay reproducibility.[17] Whenever possible, transition of candidate biomarkers from a discovery platform to a clinical grade test can significantly improve the performance characteristics of a biomarker. For example, mRNA biomarkers can be initially identified by using DNA microarrays or RNA-sequences that detect very large numbers of RNA transcripts in each experiment but are limited in assay reproducibility. As a result, for use in clinical medicine or in clinical trials, narrower assays such as quantitative polymerase chain reaction PCR (qPCR) assays are frequently developed to enable robust and reproducible measurement of a select set of mRNAs with utility as predictive and/or

**TABLE 34-1** Examples of Molecular Biomarkers of Autoimmune Disease*

| Type of Biomarker | Biomarker | Use of Biomarker | Data Source |
|---|---|---|---|
| Cytokine | IL-7 | Prediction of responsiveness to IFN-β in MS | Retrospective testing in samples from clinical trials |
| Chemokine | CXCL13 | Assessment of disease activity in RA<br>Prediction of disease progression in RA<br>Assessment of response to TNF blockade<br>Prediction of the rate of B cell repopulation after rituximab therapy | Retrospective testing in samples from clinical trials |
| Cell type | Th1 cells, Th17 cells | Prediction of responsiveness to IFN-β in MS | Retrospective testing in samples from clinical trials |
| Autoantibodies | ACPA | Diagnosis of symptomatic RA<br>Diagnosis of asymptomatic RA | Clinical practice<br>Retrospective testing in samples from clinical studies |
| | ANA | Diagnosis of symptomatic SLE | Clinical practice |
| Signaling molecule | Erb2 | Prediction of responsiveness to anti-ErbB2 monoclonal antibodies and to tyrosine kinase inhibitors in breast cancer | Clinical practice |
| Gene expression | Wound–healing signature | Identification of women with breast cancer who do not need adjuvant chemotherapy | Retrospective testing in samples from clinical studies |
| | IFN-α/β-inducible gene signature | Assessment of the efficacy of anti–IFN-α monoclonal antibody therapy in neutralizing IFN-α and downstream signalling in SLE | Prospective testing in clinical trials |
| | Imatinib-responsive gene signature | Identification of systemic sclerosis patients most likely to respond to treatment with the tyrosine kinase inhibitor imatinib | Retrospective testing in samples from clinical trial |

*Outlined are molecular and cellular mechanisms involved in the initiation and progression of autoimmune responses in rheumatoid arthritis (RA) and other autoimmune rheumatic diseases. The molecules, molecular pathways, and cellular responses outlined can be measured as biomarkers for characterization of RA and other rheumatic diseases.

ACPA, Anti-citrullinated protein antibodies; ANA, anti-nuclear antibodies; CXCL13, C-X-C motif chemokine 13; ErbB2, receptor tyrosine-protein kinase ErbB2; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; Th1, T helper 1 cell; Th17, T helper 17 cell; TNF, tumor necrosis factor.[35]

pharmacodynamic (PD) biomarkers. In rheumatology, such an approach is illustrated by the discovery via DNA microarrays and clinical implementation with qPCR of a type I interferon mRNA signature to predict the response to rituximab in rheumatoid arthritis (RA).[18]

Assays have two fundamental characteristics[19]:

- *Accuracy*. Does the result of the assay reflect the true value of the analyte? This requires a "gold standard" reference to compare results.
- *Precision*. Is the assay reproducible? Replicate values are typically required to be within variability.

Biomarkers have three additional characteristics[20,21]:

- *Sensitivity*. Does the assay discriminate between two states (e.g., the presence or absence of RA)?[22]
- *Specificity*. Does the assay discriminate between only the two states and avoid false positives (e.g., diagnosis of RA in a patient with systemic lupus erythematosus [SLE]).[23]
- *Stability*. Are the analyte and assays stable during the period and conditions of collection and measurement? In other words, will repeating the assay multiple times during a period of weeks or months provide the same results?[24]

## Biomarker Validation

Biomarker and assay validation is a complex process that includes (1) technical validation of the assay method and (2) validation of the application of the biomarker to answer or address a clinical question.[25,26] The basic parameters include a reference standard from which an assay working range and precision can be determined.[16] Sample processing is tuned to fit assay values within the assay's linear range, and acceptable precision is defined by the FDA at a coefficient of variation of 15% to 20%.[27] This establishes that an analyte can be measured, but not its utility.

Validation to address a specific clinical question (e.g., does it correlate with changes in disease activity or a clinical endpoint) requires multiple independent studies, consensus building, and perhaps meta-analysis to confirm the conclusions. This is particularly true for surrogate markers, which can replace a clinical endpoint for regulatory purposes.[28] For a biomarker to become a surrogate biomarker, it must meet substantially higher standards of technical validation and utility as a predictor of a traditional endpoint used for drug approval. In practice, few disease-specific surrogate markers have been developed to this level.[29]

A recent concept used to establish intermediate levels of validation applicable to biomarkers not meeting the surrogacy standard is "fit-for-purpose" analytic validation.[30] This approach includes validation of biomarker applications such as pharmacodynamic markers used for dosing and sensitive molecular measures correlated with clinical response used for decision making that are not approved surrogates. These "actionable" biomarkers can be used to stratify patients or monitor therapy and establish the companion diagnostics.

Some assays are discreet such as the presence or absence of a genetic mutation; many others are continuous measures

such as CRP level. However the marker is measured and reported, performance of a biomarker can be thought of in binary terms with respect to sensitivity and selectivity. Sensitivity measures true positive outcomes, whereas selectivity reflects the true negative result. The statistical model used to describe performance parameters of sensitivity and specificity is the receiver operating characteristic (ROC).[31] ROC space plots sensitivity versus specificity and can be thought of as graphical representation of the assay's propensity for type 1 (false positive) or type II (false negative) errors.[32] For example, CRP has good sensitivity for inflammation, but very poor specificity for rheumatic disease, whereas a marker such as anti–double-stranded DNA antibody might have good specificity for lupus, but poor sensitivity. The binary nature of the ROC illustrates that a biomarker is more than the measurement of an analyte. It must discriminate a biologic process, pathologic process, or response to therapy.

## Biomarker Timing

The timing of biomarker measurement is an important parameter in determining the value of the measurement. Although we often think of diurnal variations in serum or urine biomarkers, the utility of a biomarker might also vary during longer periods of time.[33] Different pathogenic processes may predominate at different times during the course of disease. On the other hand, similar final common pathways may drive late-stage diseases with varying initial disease mechanisms. For example, genetic and epigenetic markers in RA might be actionable early in the disease (or even before clinical manifestations), but disease activity markers could be more valuable after clinical signs develop. Biomarkers do not exist in a vacuum: CRP may be of limited value in detecting arthritis in a pre-clinical disease state, but in the context of established disease, it might associate closely with disease activity. Identifying markers that reflect a change in state, such as from early to established disease, could be valuable in selecting therapy or deciding when to start treatment.[34]

## Mechanistic and Descriptive Biomarkers

Actionable biomarkers are tests that could be used to guide the clinical management of disease.[35] For example, a diagnostic biomarker would meet this criterion when the knowledge of a disease diagnosis guides the initiation of a disease-modifying therapy. Mechanistic biomarkers, for which the biomarker is rooted in the biologic mechanisms of disease, provide the greatest potential for guiding clinical decision making.[35] A series of time points exist in the development and progression of RA and other autoimmune rheumatic diseases at which actionable biomarkers can be used to guide clinical decision making, including the presence of rheumatoid factors or anti-citrullinated protein antibodies (ACPA). Mechanistic biomarkers provide the potential to be superior other types of biomarkers.[36]

Descriptive biomarkers include erythrocyte sedimentation rate (ESR) and CRP, and reflect the state of a disease but are not directly involved in disease pathogenesis. For example, ESR and CRP are components of the RA disease activity score (DAS),[37] but these clinical laboratory biomarkers are not specific to RA and are elevated in many infectious and inflammatory diseases. Because descriptive biomarkers do not directly mediate disease pathogenesis, the value of the diagnostic and prognostic information they provide is limited.[35]

Mechanistic biomarkers can provide important advantages compared with descriptive biomarkers. Because the former are directly involved in disease pathogenesis, they are more likely to be disease specific. Further, they offer greater potential to provide utility as predictive and PD biomarkers because they represent the dysregulation of molecular pathways directly involved in pathogenesis.

## Biologic Sample Sources for Biomarker Assays

A variety of biologic samples can be utilized in molecular biomarker assays. The most accessible biologic samples include samples that can be obtained via noninvasive or minimally invasive acquisition and include urine, saliva, and peripheral blood.[38] However, the urine and saliva are likely to be limited to certain omics analyses (e.g., proteomics) and the measurement of certain joint and cartilage breakdown products in urine as biomarkers for osteoarthritis (OA).[39] Based on its accessibility, blood is widely used as a biologic sample for biomarker analysis. Although blood frequently contains the immune cells and molecules involved in rheumatic disease pathogenesis, it is usually not the tissue directly involved in rheumatic diseases. Therefore it does not always provide clear correlation to the dysregulated molecular pathways and molecules involved. Many groups are measuring molecular biomarkers in synovial tissue derived from RA, OA, and other rheumatic diseases. Although synovium provides the greatest access to mechanistic biomarkers that provide insight into the disease pathogenesis and therapeutic mechanism of action, it can be difficult to obtain, and analysis can be complicated by sampling error and the large number of cell lineages within the tissue.[40]

Although the collection of joint tissue has been limited by practical reasons to small clinical proof-of-concept trials, it requires consideration of an additional dimension of biomarker validation.[41,42] Synovium is a complex tissue with multiple functions in health and disease.[43] When inflamed, synovitis can be quite variable in terms of location within the joint as well as between different involved joints. To measure synovial biomarkers, a representative needs to be collected. Biopsies of synovial tissue for biomarker analysis require a minimum of six tissue fragments to minimize intrapatient variation.[44]

Synovial biopsy studies have yielded insights into the disease process and therapeutic mechanisms. One application is identifying soluble factors produced in the joint that can be detected in blood. Recently, synovial biopsies have yielded information on possible patient stratification based on histologic patterns and have been proposed as a source of clinical biomarkers.[6,45,46] When the joint is used as a source of biomarker material, synovial fluid is often considered because of its proximity to joint tissue and ease of collection.[47] However, synovial fluid volume itself is a poor biomarker, and the variability of synovial tissue renders it an unreliable source of biomarkers in many clinical circumstances.

### Biomarker Discovery

Assay systems that measure large numbers of analytes such proteins or metabolites are in theory a rich source of biomarker discovery. National and international efforts that use high-content genetic, epigenetic, and proteomic methods are in use to discover biomarkers in rheumatic diseases. Costs of "omics" assys are decreasing, and informatics methods are improving.[48] Nevertheless, challenges remain regarding how we apply these approaches to complex diseases, and the statistical hurdles are daunting because of the need for corrections for multiple comparisons. These methods are usually best thought of as hypothesis generating, and the analyte(s) selected require subsequent rigorous validation.[49] As such, study size and statistical threshold compromises are appropriate, and any candidate biomarker would require further study in a separate single-analyte assay. Single-measure tests, whether derived inductively from omics assays, or deductively from disease process or pharmacology, require formal validation in multiple independent cohorts.

The source of clinical material for biomarker discovery constrains the type of biomarkers evaluated. Peripheral blood is the most common material for biomarker studies, although rheumatic diseases are often not blood diseases. Any signature or marker present in blood can be either derived from or a response to conditions at the site of disease.[50] The use of joint tissues in arthritis studies is challenging, but it increases the possible markers to include intra-cellular and tissue-specific factors or mediators enriched in the local environment. An inclusive strategy is to start at the site of disease and then identify feasible methods of assay. Ideally, a blood assay detecting the marker directly or its surrogate could be developed. If not, an actionable biomarker might justify performing a synovial or other solid tissue biopsy to provide decision-making clinical material.[51]

### Biomarker Profiles

An alternative approach to biomarker discovery between the unbiased omics assays and the single-analyte assay is the creation of multiplexed panels of analytes. Narrowing the candidates limits the possibilities, but can be both practical and feasible. Many studies measuring blood plasma inflammatory mediators, such as cytokines, chemokines, and growth factors, have been performed without yielding a validated biomarker that is sensitive and specific.[52] However, the approach of using relatively small panels of candidate biomarkers has intrinsic statistical advantages, allowing for the panel itself to be consider a composite biomarker.[53] The performance of individual analytes can be modeled and its performance given a statistical weight within the panel.[54] Some markers receive increased weight; some may receive "0" weight and are removed.[55] Negative weights can also be used. The resulting multiplex assay can be performed in automated assays with good technical quality. One critical component often ignored is the fact that biomarkers in a panel should be independent and not part of a clinical disease assessment score. For example, many analytes, such as CRP and serum amyloid A protein, are regulated by interleukin (IL)-6. Thus any process that decreases IL-6 would decrease the others in a dependent fashion and only serves to amplify the effect of decreasing IL-6 in a composite biomarker assay. Similarly, if change is used to show correlation with the disease activity score (DAS), then interpretation becomes difficult because CRP is included in both the clinical and the biomarker analysis.

In many rheumatic diseases, profiles or panels of biomarkers could provide increased accuracy and utility in guiding diagnosis and therapeutic decision making. In rheumatic diseases, individual biomarkers are frequently associated with diagnosis or disease state at the population level, but might not exhibit sufficient predictive utility to be used at the level of the individual patient. The development of technologies that enable multiplex measurement of candidate biomarkers has greatly facilitated identification of biomarker profiles with increased diagnostic and predictive utility.[56,57] In the future, it will likely be possible to develop candidate panels from higher content unbiased methods.

## APPLICATION OF BIOMARKERS IN CLINICAL PRACTICE AND DRUG DEVELOPMENT

### Diagnosis of Established Disease

A frequent use of an actionable biomarker is diagnosing symptomatic established disease, and thereby establishing the basis for therapeutic intervention. For example, detection of autoantibodies, including rheumatoid factor and ACPA, contribute to the diagnosis of RA; detection of anti-nuclear antibodies (ANA) contribute to the diagnosis of SLE, and detection of anti-neutrophil cytoplasmic antibodies (ANCA) contribute to the diagnosis of ANCA-associated vasculitis. These autoantibodies could contribute to disease pathogenesis[58-61] and thus might be classified as actionable mechanistic biomarkers.

#### Diagnosis of Pre-clinical or Asymptomatic Disease

An important application of biomarkers is to identify individuals with pre-clinical disease. The ability to identify individuals before clinical onset of disease provides an opportunity for therapeutic intervention to prevent the development of symptomatic disease or to reduce the severity of symptomatic disease.[62,63] For example, profiles of specific subsets of ANA are detected in the blood of individuals who ultimately progress to have clinical SLE,[62] and this biomarker could potentially enable treatment to prevent development of SLE. In RA, profiles of autoantibodies and cytokines in blood can be used to identify asymptomatic individuals who will progress to have RA.[56,57,64] Therapeutic intervention in pre-clinical RA patients provides the potential to reduce the incidence or severity of RA in these individuals.[65]

#### Assessment of Disease Activity and Prognosis

Biomarkers that assess disease activity can identify patients with increased likelihood for progression, thereby guiding clinicians to institute a more aggressive therapeutic regimen. Such biomarkers are particularly valuable in diseases, and in individual patients, in which there is discordance between symptoms and disease activity, and can be used to guide selection of an appropriate therapeutic regimen.[66]

Although many biomarkers are imperfect, diseases activity and prognostic biomarkers include the ESR, CRP, complement proteins C3 and C4, and anti-DNA antibodies. Nevertheless, the sensitivity, specificity, and utility of these biomarkers are limited. In recent years a profile of inflammatory cytokines and molecules has been developed to assess disease activity in RA.[67,68] Nevertheless, it remains unclear whether this profile provides increased utility compared with the well-established DAS-28[37] or whether the profile is specific for RA (relative to other autoimmune, inflammatory, and infectious conditions).

## Pharmacodynamic Biomarkers

### Assessing Response to Therapy

PD biomarkers are tests that can be used to monitor response to therapy. PD biomarkers can be mechanistic or descriptive. Mechanistic PD biomarkers are directly related to the biological mechanism underlying the disease process, and this biologic mechanism can also be the target of the therapeutic intervention.[35] Descriptive PD biomarkers frequently represent generic inflammatory or tissue injury biomarkers, and such markers can be used to assess response to therapy but are not directly related to the biologic mechanism(s) of disease.

Examples of mechanistic PD biomarkers used in clinical practice in rheumatology are peripheral B cell and peripheral blood plasmablast analysis following rituximab therapy in patients with RA.[68,69] Flow cytometry is cumbersome and not readily amenable to widespread clinical use, and a PCR assay to measure IgJ transcripts, a marker for antibody-secreting plasmablasts, has been developed as a biomarker to guide clinical decision making.[70] In samples derived from trials of rituximab in inadequate responders to anti-TNF, 25% of treated subjects with elevated baseline mRNA levels of IgJ showed reduced clinical response rates.[70]

Examples of descriptive PD biomarkers used in clinical practice in rheumatology include ESR in polymyalgia and a rheumatic and inflammatory molecule panel reflective of disease activity in RA.[67] In SLE, quantification of proteinuria and analysis of urine sediment can be used to assess response to therapy.[71] Imaging technologies such as MRI[72] and ultrasound,[73] that enable assessment of synovitis in RA and other inflammatory arthritides, represent another biomarker of disease activity.

PD biomarkers play a central role in facilitating therapeutic development, particularly for demonstrating clinical proof-of-concept and assessing toxicity in early stages of drug development. They are prioritized by the FDA's Critical Path Initiative and described as having the potential to substitute for a clinical endpoint that "reflects how a patient feels, functions, or survives."[74-76] PD biomarkers can streamline clinical development and reduce costs by enabling rapid demonstration that a candidate therapeutic modulates pathogenic mechanisms. Although PD biomarkers are used to accelerate and reduce the costs of drug development, clinical endpoints still form the basis of regulatory approval by the FDA and other agencies.

PD biomarkers have been utilized to characterize the response to candidate therapeutics for autoimmune rheumatic diseases for decades. For example, in the 1990s it was demonstrated that decreases in blood levels of IL-1 are associated with clinical responses to methotrexate,[77] and that reductions in blood levels of CRP are associated with response to treatment with infliximab.[78]

Transcription profiles in peripheral blood cells are emerging as a potential biomarker in RA and other rheumatic diseases.[18] For example, reports suggest that peripheral blood RNA transcript profiles can predict the response of patients with RA to rituximab[18] and anti-TNF therapies.[79] Although the correlations are statistically significant, they are not robust enough to be useful for clinical decision making. Further, transcriptional profiling of genes associated with the type I interferon (IFN) pathway in samples derived from blood or skin have provided promising results for monitoring response to therapy in SLE[80] and scleroderma.[81]

Molecular biomarker studies extend to the primary site of disease pathogenesis in RA, the synovium. Decreases in the number of macrophages in the synovial sublining represent a potentially sensitive and early biomarker of therapeutic efficacy in RA, although responses to rituximab, abatacept, and tofacitinib are independent of this biomarker and therefore limit its utility.[82] Analysis of the activated states of the signal transduction molecules signal transducer and activator of transcription (STAT)1 and STAT3 in the synovial lining were demonstrated to correlate with clinical response to tofacitinib in RA (Figure 34-1).[83] The transcription profiles in RA synovium and other tissues involved in rheumatic diseases have also provided PD markers. In systemic sclerosis, transcriptional profiles in skin biopsies identified individuals with cutaneous sclerosis driven by the tyrosine kinases platelet-derived growth factor receptor (PDGF) and Abl, and are therefore predicted to be more likely to respond to treatment with receptor tyrosine kinase inhibitors.[84] In RA, gene expression analysis revealed synovial phenotypes that correlated with distinct responses to rituximab[85] and anti-TNF[86] therapy.

### Assessing Toxicity

PD biomarkers can potentially streamline drug development by enabling early detection of drug-related toxicity, a significant source of the failure of novel investigational drugs in human trials.[87] Examples of PD toxicity biomarkers include clinical laboratory variables, such as liver function and renal function tests, as well as biomarkers of cardiac toxicity, such as prolongation of the QT interval on electrocardiogram, increased blood pressure, and alterations in lipid profiles. Moving forward, identification of new biomarkers that serve as more reliable indicators of organ or tissue toxicity will further accelerate the development of novel therapeutics for RA and other rheumatic diseases.

## Predictive Biomarkers

Predictive biomarkers predict response to therapy before the therapy is initiated. A key challenge in both clinical practice and drug development is selection of the subset of patients in whom the mechanism that the drug targets is active.[88] The ability to identify responders in advance of treatment provides significant benefits to patients, both in the reduction of health care costs and in the acceleration of clinical development of next-generation therapeutics.[89]

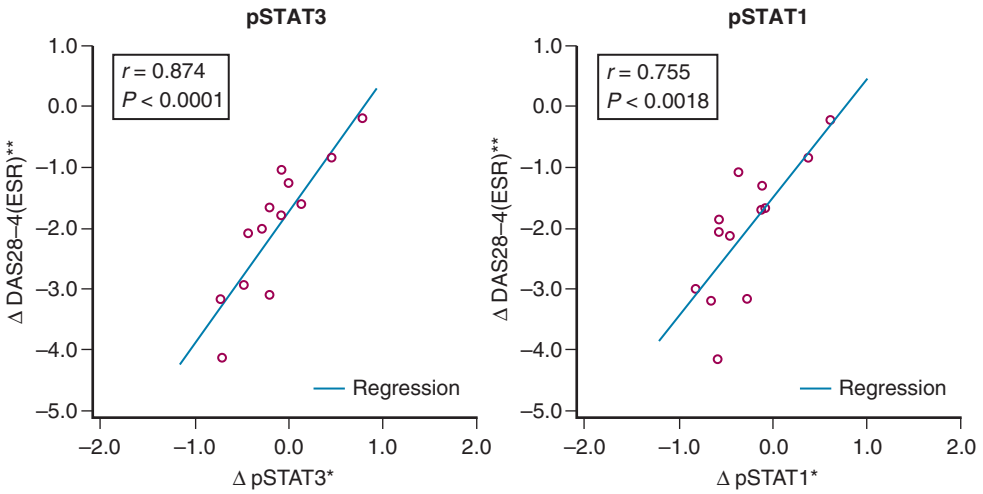



**Figure 34-1** Tofacitinib-induced synovial changes in pSTAT3 and pSTAT1 predict clinical response at month four**.** pSTAT1 and pSTAT3 were measured in serial synovial biopsies obtained from tofacitinib-treated patients with rheumatoid arthritis, and the graphs demonstrate that the changes in pSTAT1 and pSTAT3 correlated with clinical response. *Biomarker data change from baseline (day 7) to day 28; **disease activity scale (DAS)28-4 (erythrocyte sedimentation rate) change from day 1 to month 4. pSTAT, phosphorylated signal transducer and activator of transcription; *r*, correlation coefficient. *(From Boyle DL, Soma K, Hodge J, et al: The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis.* Ann Rheum Dis *74:1311–1116, 2015.)*

In oncology, predictive single-gene biomarkers are routinely used in both clinical practice and drug development. For example, in breast cancer, overexpression of *HER2* indicates responsiveness to anti-Her antibody (trastuzumab, pertuzumab), and in chronic myelocytic leukemia, the *BCR-ABL1* translocation is indicative of responsiveness to imatinib. In contrast, RA and other rheumatic diseases are not driven by single-gene mutations, and both single-gene biomarkers and multiple-gene biomarkers have not provided significant predictive utility to date.[90] Ongoing work is investigating epigenetic biomarkers in RA, and such epigenetic biomarkers hold great potential.[91]

Drug developers and the FDA recognize the opportunity and power of predictive biomarkers, and the FDA has undertaken initiatives to facilitate the development of predictive and PD biomarkers for candidate therapeutics.[74-76] The cost of therapeutic development has skyrocketed in the past decades,[92] and integrated use of predictive and pharmacodynamic biomarkers represents an important strategy to streamline clinical development and thereby reduce the cost of development of next-generation therapeutics.[35]

## Mechanistic Biomarkers

### Cytokines

Rheumatic disease diagnoses, such as SLE and RA, often comprise multiple disease subtypes that may exhibit similar clinical presentations but differ markedly in molecular phenotype. Insight into the pathogenesis of disease provides the basis for developing mechanistic biomarkers to distinguish these molecular subtypes of disease, and thereby guide selection of the most appropriate therapy for each patient. Although targeting TNF, IL-6, IL-1, and other cytokines has provided benefit in some patients with RA and subsets of patients with other autoimmune rheumatic diseases, to date simple measurement of the corresponding cytokine levels in blood, synovial tissue, or synovial fluid has failed to yield predictive biomarkers for therapeutic response.[93]

For RA, a potential cytokine biomarker is CXCL13, also known as B lymphocyte chemoattractant. CXCL13 plays a key role in germinal center formation, and increased levels of CXCL13 mRNA in RA synovium is associated with synovial germinal centers and increased blood CXCL13.[94] In addition to B cells, osteoblasts express the CXCR5 receptor for CXCL13. CXCL13 stimulates osteoblasts to release degradative enzymes,[95] and CXCL13 has been identified as a potential biomarker of erosive progression.[96] Blood levels of CXCL13 decreased with response to anti-TNF therapy. A human anti-CXCL13 monoclonal antibody has been developed and is under evaluation. Further studies will be needed to further determine the sensitivity, specificity, and predictive utility of blood CXCL13 as a candidate mechanistic biomarker in RA. 14-3-3n, a soluble signaling molecule measurable in serum that is associated with joint damage in RA, has been proposed as a potential biomarker.[97]

A more advanced approach compared with simple measurement of blood or synovial fluid cytokines is being undertaken in the search for mechanistic biomarkers for SLE. SLE is associated with increased levels of type I IFNs in blood, and type I IFNs are believed to contribute to the pathogenesis of SLE.[98] Increased levels of type I IFN-induced mRNAs are associated with increased disease activity in SLE,[99] and profiles type I IFNs mRNAs are already used as disease activity biomarkers in clinical trials.[80] The type I IFN-induced transcriptional signature represents an actionable mechanistic biomarker for SLE.

### Autoantibodies

Autoantibodies exemplify mechanistic biomarkers for RA, SLE, and certain other autoimmune rheumatic diseases. In SLE, autoantibodies specific for RNA-, DNA- or chromatin-associated proteins result in the formation of immune complexes that dually stimulate dendritic cells via Toll-like receptors (TLRs) 7 or 9 to produce type I IFNs.[100,101] Such autoantibodies are associated with increased blood IFN-α activity, as assessed by in vitro induction of IFN-induced

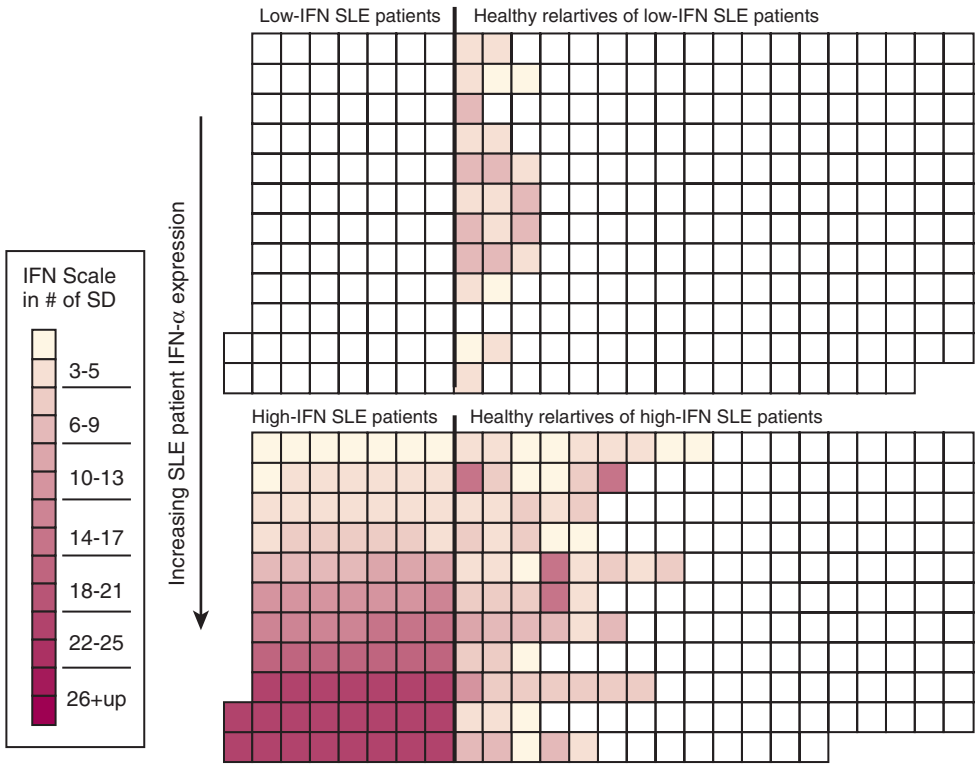



**Figure 34-2** Familial aggregation and correlation of interferon (IFN)-α activity in first-degree relatives of patients with systemic lupus erythematosus (SLE). Each box represents a person, with the *solid bold line* separating patients with SLE from healthy first-degree relatives. *White shading* indicates no significant difference from healthy unrelated donors, and *increasing yellow to red shading* indicates increasing quantitative IFN-α activity. SLE patients are ordered by quantitative IFN-α activity in ascending order, with the lowest at top left, the highest at bottom right. The gap in the middle of the diagram represents the categorical cutoff for high versus low IFN activity in the SLE patients. Healthy relatives are then arrayed on the right side of the bold line, ordered by the IFN-α activity value of their corresponding SLE-affected family to show the variance in familial IFN-α activity as it relates to the SLE affected in the same family. Boxes representing family members with high IFN-α activity are moved centrally within the same row to allow easier comparison of the relative proportion of high IFN-α family members between rows. *(From Niewold TB, Hua J, Lehman TJ, et al: High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus.* Genes Immun *8:492–502, 2007.)*

gene expression (Figure 34-2).[102] RNA-, DNA- and chromatin-associated autoantigens can also dually stimulate autoreactive B cells via membrane Ig and TLR7 or TLR9 to produce autoantibodies.[101]

In RA, ACPA binds citrullinated fibrinogen, vimentin, histones, and multiple other citrullinated antigens.[103,104] Citrullinated fibrinogen binds TLR4 to stimulate macrophage to produce TNF, and citrullinated fibrinogen immune complexes synergistically stimulate macrophages to produce TNF via co-ligation of TLR4 and Fcγ receptors.[60] Thus, in both SLE and RA autoantibodies, the binding of immunologically active autoantigens (such as RNA, DNA chromatin, or citrullinated fibrinogen) drive the activation of inflammatory pathways that contribute to disease pathogenesis. As a result, these autoantibodies represent mechanistic biomarkers.

Autoantibody profiling has been demonstrated to provide utility for early diagnosis and for identifying at-risk individuals likely to progress to SLE or RA. Autoantibodies are present in the blood for years before clinical SLE[62] or RA develops.[105,106] Similarly, increased levels of cytokines[57] and other inflammatory markers[105] are also present in the blood before the onset of clinical RA. Further, epitope spreading and the accumulation of multiple antibody specificities precede the onset of clinical symptomatic RA.[63,103]

For RA, detection of autoantibodies in at-risk individuals or in early-stage disease might also be used to guide treatment. For example, in individuals with undifferentiated arthritis, methotrexate treatment reduced the rate of progression to clinical RA, but this effect was only found in in ACPA$^+$ individuals.[107] Thus, in individuals with undifferentiated arthritis, ACPA represents a potential mechanistic biomarker that could be used to guide therapeutic intervention. In another dimension, in the future detection of autoantibodies targeting immunologically active antigens has the potential to enable section of a therapeutic specific for the relevant downstream inflammatory pathway (e.g., therapies that target Janus kinases, spleen tyrosine kinase, IL-6, TNF, IL-1, and IL-17).

## Epigenetic Biomarkers

Epigenetic markers represent an emerging class of mechanistic biomarkers. Epigenetic regulators include DNA methylation, histone modification, and microRNAs (miRNAs), and each contributes to pathogenic mechanisms in autoimmunity.[91] For example, in RA fibroblast-like synoviocytes (FLS), alterations of imprinted DNA methylation have been identified even in very early RA that can modulate cell migration, matrix regulation, and immune responses.[108]

miRNAs are another subclass of epigenetic factors that regulate rheumatic diseases.[109] An individual miRNA regulates the mRNA stability and translation of multiple genes,

and thus exert broad biologic effects. miR-146a is expressed by activated T cells in which it suppresses apoptosis and IL-2 production,[110] miR-146a is expressed in RA synovium, and synovial expression is associated with increased disease activity. Another candidate miRNA biomarker is miR-155, which induces development of Th1 cells and Th17 cells,[111] and increased expression is observed in peripheral blood mononuclear cells from patients with RA. These observations suggest that miRNAs might prove to be mechanistic biomarkers. Further studies are needed to rigorously assess the predictive value, reproducibility of detection, and utility of miRNAs as biomarkers of RA and other rheumatic diseases.

### Metabolomics

Metabolomics is an emerging area of investigation.[112] Alterations in T cell metabolism have been associated with RA,[113] and future studies will determine whether these alterations provide utility as mechanistic biomarkers. Alterations in RA FLS choline metabolism have also been identified, which could serve as clues to how metabolic enzymes could be targeted in rheumatic disease.[114] Studies characterizing a spectrum of metabolites in RA and other rheumatic diseases are ongoing, and it remains to be determined if any of these will have utility as actionable and/or mechanistic biomarkers.

## Tissue Degradation Product Biomarkers

Joint, cartilage, and other tissue degradation products represent another class of biomarkers. Although cartilage breakdown products are classically used to monitor tissue injury in OA or RA,[115] more recently cartilage and tissue breakdown products have been demonstrated to function as damage-associated molecular patterns (DAMPs)[116] that possess innate immune stimulatory activity, tissue-repair–promoting activity, or both. For example, syndecans are proteoglycans released during joint tissue injury that promote cartilage development and remodeling.[117] In contrast, tenascin-C is a cartilage protein released in RA and OA that binds TLR4 to stimulate innate immune-mediated inflammation.[118] In another example, the cartilage matrix protein fibromodulin has been demonstrated to activate the complement system to produce the membrane attack complex and thereby contribute to the pathogenesis of OA.[119] Urinary measures of α-C-telopeptide of type I collagen (α-CTX) and C-telopeptide of type II collagen (CTX-II) reflect bone turnover and osteophyte activity (Figure 34-3).[120] Thus cartilage and tissue degradation products represent

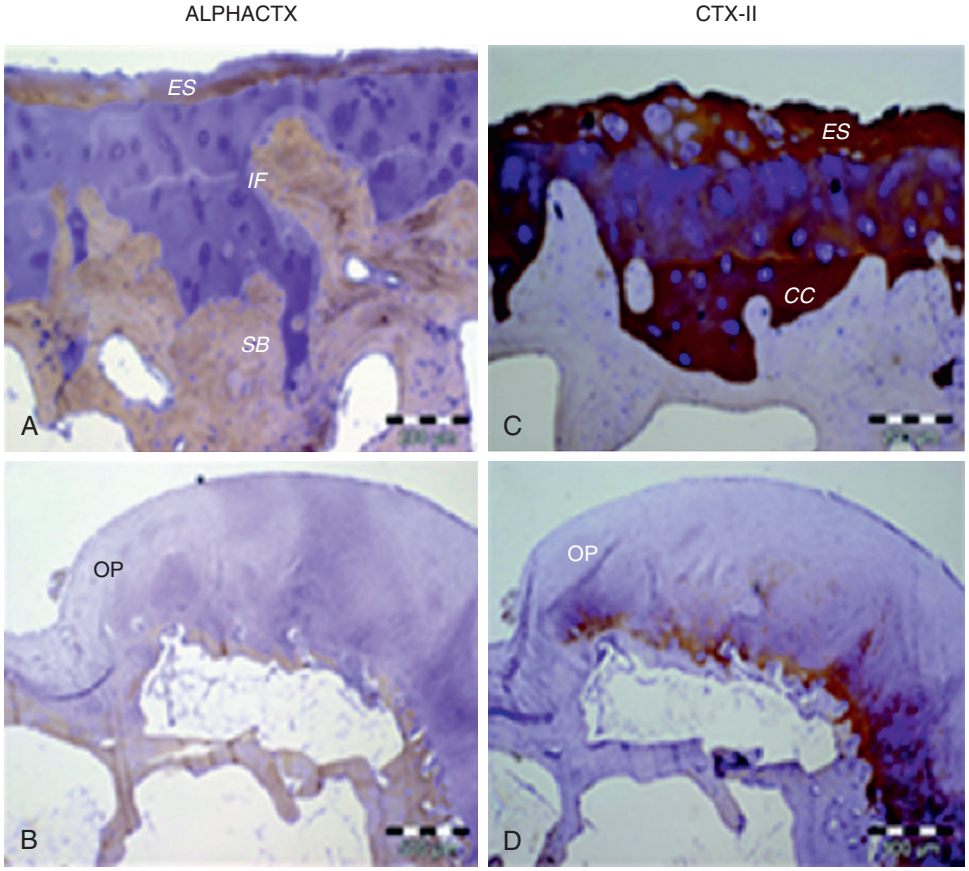



**Figure 34-3** Biomarkers of cartilage and bone damage can correlate with tissue damage. Immunolocalization of α-C-telopeptide of type I collagen (α-CTX) and C-telopeptide of type II collagen *(CTX-II)* in human osteoarthritis cartilage and bone from patients with severe erosions of the cartilage. **A** and **C**, Staining for α-CTX (**A**) and CTX-II (**C**) in sections from a severely eroded area with an eroded surface (ES), thickening of the subchondral bone (SB), and bone invasion fronts (IF) through the calcified cartilage (CC). **B** and **D**, Staining for α-CTX (**B**) and CTX-II (**D**) in sections from an area from the rim of the cartilage where an osteophyte (OP) is forming (i.e., chondrophyte), seen by the diffuse structure of the tissue. No background immunostaining was observed with the immunoglobulin G control (results not shown). Bars = 200 μm in **A** and **C**; 500 μm in **B** and **D**. IFN, Interferon; SLE, systemic lupus sclerosis. *(From Huebner JL, Bay-Jensen AC, Huffman KM, et al: Alpha C-telopeptide of type I collagen is associated with subchondral bone turnover and predicts progression of joint space narrowing and osteophytes in osteoarthritis.* Arthritis Rheumatol *66:2440–2449, 2014.)*

potential mechanistic biomarkers in RA and OA. Furthermore, they have potential to provide early information on the effect of novel therapeutic agents on tissue damage.

## CONCLUSION

Expectations that biomarkers can be used to diagnose and stratify rheumatic diseases must be tempered by evidence that there are no simple answers and no single analyte that will suffice. Complex diseases might require an integrative approach that includes multiple technology platforms and careful assessment of unbiased platforms. Surrogate biomarker discovery remains even more elusive. On the other hand, new standards for biomarker validation have been established and provide application-specific validation methods. Understanding the role and context of a proposed biomarker is critical. Single-analyte biomarkers have increased use in mechanistic studies and as companion diagnostics. Panels of analytes or omics-derived markers could be more sensitive measures of disease activity. The identification and use of fit-for-purpose markers to stratify patients for targeted therapy is a realistic goal.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 35



# Occupational and Recreational Musculoskeletal Disorders

Richard S. Panush

## KEY POINTS

- Some occupational and recreational activities have been linked with musculoskeletal syndromes or disorders that are manifested by neck pain; shoulder, elbow, hand, or wrist pain or tendinitis; carpal tunnel syndrome; and hand-arm vibration syndrome. These associations might be less clear than has been previously thought.
- The intuitive concepts of so-called cumulative trauma disorders and repetitive strain disorders have poor support in the literature. Causal relationships between most occupations or activities and these "syndromes" have not been well established.
- Some activities and mechanical stresses have been associated with osteoarthritis at certain sites—for example, the hips of farmers, the knees of workers whose jobs involved frequent bending of the knees, and the hands of workers who perform repetitive tasks with their hands.
- Certain rheumatic disorders have been related to environmental or occupational risks.
- Putting a normal joint through its physiologic range of motion is not necessarily harmful for an otherwise healthy individual. However, if the joint, motion, stress, or biomechanics are not normal, there may be a risk of harm to the joint.
- Most healthy persons comfortably engaging in reasonable recreational activities can do so without evidence of lasting soft tissue or articular damage. Runners, who have been best studied, exemplify this principle. Conversely, persons who exercise with pain, effusions, underlying joint abnormalities or abnormal or unusual biomechanics, or as professional or elite athletes may be at increased risk of joint injury.
- Performing artists, vocalists, dancers, and musicians have a risk of soft tissue and joint injury analogous to that of athletes.

**"The diseases of persons incident to this craft arise from three causes: first constant sitting, second the perpetual motion of the hand in the same manner, and thirdly the attention and application of the mind. … Constant writing also considerably fatigues the hand and whole arm…."**

—Ramazzini, 1713[1]

**"When job demands … repeatedly exceed the biomechanical capacity of the worker, the activities become trauma-inducing. Hence, traumatogens are workplace sources of biomechanical strain that contribute to the onset of injuries affecting the musculoskeletal system."**

—National Institute for Occupational Safety and Health, 1986[2]

The possible associations of certain occupational and recreational activities with musculoskeletal disorders are not as clear as had once been thought. Conventional wisdom was that "wear and tear" from at least some activities led to reversible or irreversible damage to the musculoskeletal system.[2-5] Despite the intuition that work or recreational activities might cause rheumatic and musculoskeletal syndromes, this putative relationship is controversial and likely seriously flawed. Many of the available data have confounding aspects, as will be discussed in this chapter.

## OCCUPATION-RELATED MUSCULOSKELETAL DISORDERS

Many presumptive work-related musculoskeletal disorders have been described and are presented in Table 35-1.[1-8] Although the appealing and suggestive names invite conclusions of causal association, these have not been demonstrated.[1-8] Work-related musculoskeletal injuries comprise at least 50% of nonfatal injury cases resulting in days away from work.[9] The cost of work-related disability from musculoskeletal disorders has been equivalent to approximately 1% of the United States' gross national product, making these entities of considerable societal interest.[10] Worldwide ergonomic (occupational) disability from low back pain in 2010 was estimated to affect as much as 26% of the population.[11] Industries with the highest rates of musculoskeletal disorders were meatpacking, knit-underwear manufacturing, motor vehicle manufacturing, poultry processing, mail and message distribution, health assessment and treatment, construction, butchery, food processing, machine operation, dental hygiene and dentistry, data entry, hand grinding and polishing, carpentry, industrial truck and tractor operation, nursing assistance, housecleaning, and, worldwide, agriculture. Associations between work-related musculoskeletal syndromes and age, gender, fitness, and weight have been imprecise.[6-8,11]

A number of work-related regional musculoskeletal syndromes have been described. These syndromes include disorders of the neck, shoulder, elbow, hand and wrist, lower back, and lower extremities[7] (Table 35-2); some of these syndromes are discussed in greater detail in other chapters. Neck musculoskeletal disorders are associated

**TABLE 35-1** Reported Occupation-Related Musculoskeletal Syndromes

| | |
|---|---|
| Cherry pitter's thumb | Gamekeeper's thumb |
| Staple gun carpal tunnel syndrome | Espresso maker's wrist |
| Bricklayer's shoulder | Espresso elbow |
| Carpenter's elbow | Pizza maker's palsy |
| Janitor's elbow | Poster presenter's thumb |
| Stitcher's wrist | Rope maker's claw hand |
| Cotton twister's hand | Telegraphist's cramp |
| Writer's cramp | Waiter's shoulder |
| Bowler's thumb | Ladder shins |
| Jeweler's thumb | Tobacco primer's wrist<br>Carpet layer's knee |

From Mani L, Gerr F: Work-related upper extremity musculoskeletal disorders. *Primary Care* 27:845–864, 2000; and Colombini D, Occhipinti E, Delleman N, et al: Exposure assessment of upper limb repetitive movements: a consensus document developed by the Technical Committee on Musculoskeletal Disorders of International Ergonomics Association endorsed by International Commission on Occupation Health. *G Ital Med Lav Ergon* 23:129–142, 2000.

**TABLE 35-2** Selected Literature Describing Regional Occupation-Related Musculoskeletal Syndromes

| Syndrome | No. of Epidemiologic Studies | Odds Ratio/ Relative Risk |
|---|---|---|
| Neck pain | 26 | 0.7-6.9 |
| Shoulder tendinitis | 22 | 0.9-13 |
| Elbow tendinitis | 14 | 0.7-5.5 |
| Hand-wrist tendinitis | 16 | 0.6-31.7 |
| Carpal tunnel syndrome | 22 | 1-34 |
| Hand-arm vibration syndrome | 8 | 0.5-41 |

with repetition, forceful exertion, and constrained or static postures. Shoulder musculoskeletal disorders occur with work at or above shoulder height, lifting of heavy loads, static postures, hand-arm vibration, and repetitive motion. For elbow epicondylitis, risk factors are overexertion of finger and wrist extensors with the elbow in extension, as well as posture. Hand-wrist tendinitis and work-related carpal tunnel syndrome were noted with repetitive work, forceful activities, flexed wrists, and duration of continual effort.[1,7] Hand-arm vibration syndrome (Raynaud-like phenomenon)[12] has been linked to the intensity and duration of exposure to vibration. Work-related lower back disorders are associated with repetition, the weight of objects lifted, twisting, poor biomechanics of lifting, and particularly agriculture.[11,13] Other risk factors for work-related musculoskeletal disorders involving the back include awkward posture, high static muscle load, high-force exertion at the hands and wrists, sudden applications of force, work with short cycle times, little task variety, frequent tight deadlines, inadequate rest or recovery periods, high cognitive demands, little control over work, a cold work environment, localized mechanical stresses to tissues, and poor spinal support.[1]

Rehabilitation for these so-called occupational musculoskeletal disorders requires collaboration by workers, employers, insurers, and health professionals. The process has been divided into phases of protection from and resolution of symptoms, restoration of strength and dynamic stability, and return to work.

Not long ago, the prevailing view was that many musculoskeletal disorders were consistently and predictably work related. That understanding has been questioned and is now perceived more critically.[2,14-20] Much published information (see Table 35-2) about occupational musculoskeletal disorders is now considered flawed. The quality of this information was uneven and perhaps poor in some instances. Definitions of musculoskeletal disorders were imprecise. Diagnoses, by rheumatologic standards, were infrequent. Studies were usually not prospective, and selection and recall biases were present. Inferential observations were made, and investigators had difficulty quantifying activities and defining health effects. Outcome measures varied. The quality of reported observations was uneven. Psychologic influences and secondary gain were often ignored. Claims, anecdotal, and survey data were often used without validation of subjective complaints. Quantification of putative causative factors was difficult. Indeed, a review of this literature concluded that none of the published studies satisfactorily established a causal relationship between work and distinct medical entities.[17] In fact, certain experiences argued powerfully against the notion of work-related musculoskeletal disorders. In Lithuania, for example, where insurance was limited and disability was not a societal expectation or entitlement, "whiplash" from auto accidents did not exist.[16] Similarly, when legislation for compensability was made more stringent in Australia, an epidemic of whiplash and repetitive-strain injuries abated.[18,19] In the United States as well, expressed symptoms correlated closely with the likelihood of obtaining compensation.[21] In other instances it was found that ergonomic interventions had no effect on alleged work-related symptoms, and close analysis of epidemics of work-related musculoskeletal disorders revealed serious inconsistencies.[14] A Japanese study found no relationship between physical activity and musculoskeletal pain.[22] Interestingly, another report described familial linkage to chronic musculoskeletal pain.[23] Thus the Industrial Injuries Committee of the American Society for Surgery of the Hand and the American Society for Surgery of the Hand, the Working Group of the British Orthopaedic Association, and the World Health Organization[2,14,17,18] have all stated that current data do not support a causal relationship between specific work activities and the development of well-recognized disease entities; in addition, they have noted that these had become socio-political problems and urged restraint in considering regulations regarding these so-called entities.[15] Hadler[2,14] also has written particularly forcefully that popular notions about work-related musculoskeletal disorders have been based on inadequate science.

An appreciation of the importance of psychosocial factors influencing work disability has emerged. These factors include lack of job control, fear of layoff, monotony, job dissatisfaction, unsatisfactory performance appraisals, distress and unhappiness with co-workers or supervisors, repetitive tasks, duration of the work day, poor quality of

sleep, perceptions of air quality and ergonomics, poor coping abilities, divorce, low income, less education, poor social support, presence of chronic disease, self-rated perception of poor air quality, and poor office ergonomics.[2,14-21,24,25] This situation is reminiscent of the story of silicone breast implants and their presumed association with rheumatic disease, where—as seems to be the case for work-related musculoskeletal disorders—there was a coalescence of naïvely simplistic assumptions, untested hypotheses, confusion between the repetition of hypotheses and their scientific validation, media exaggeration, and public advocacy intertwined with politics and governmental regulatory agencies, confounding compensatory rewards, litigation, and inadequate science. All these elements perverted the silicone breast implant story[26] and may have confused the interpretation of evidence-based work-related musculoskeletal disorders as well. More good quality, standardized investigation is necessary to learn about work-related musculoskeletal disorders and to clearly identify the circumstances in which they occur. Work-related musculoskeletal disorders exist, but they are less pervasive and less noxious than originally thought.

## OCCUPATION-RELATED RHEUMATIC DISEASES

Associations between occupations and well-defined rheumatic disorders are clearer than those involving musculoskeletal disorders. This discussion also recapitulates the simplistic perception that joints deteriorate with use. However, this notion is neither necessarily logical nor correct.

### Osteoarthritis

Is osteoarthritis (OA) caused, at least in part, by mechanical stress? OA is presented in Chapters 98 to 100; however, brief consideration of the role of certain occupational and recreational activities is within the scope of this discussion. One analytic approach to determining a possible relationship between activity and joint disease is to consider the epidemiologic evidence that degenerative arthritis may follow repetitive trauma. Most discussions of the pathogenesis of OA include a role for "stress."[27-40] Several studies have suggested an increased prevalence of OA of the elbows, knees, and spine in miners[31-33]; of the knees in floor layers and in other occupations requiring kneeling; of the knees in shipyard workers and a variety of occupations involving knee bending; of the shoulders, elbows, wrists, and metacarpophalangeal joints in pneumatic drill operators[34]; of the intervertebral disks, distal interphalangeal joints, elbows, and knees in dockworkers[32]; of the hands in cotton workers,[35] diamond cutters,[31,36] seamstresses,[36] and textile workers[14,37]; of the knees and hips in farmers; and of the spine in foundry workers[39,40] (Table 35-3). Population studies have noted increased hip OA in farmers, firefighters, mill workers, dockworkers, female mail carriers, unskilled manual laborers, fishermen, and miners and have reported increased knee OA in farmers, firefighters, construction workers, house and hotel cleaners, craftspeople, laborers, and service workers.[39,40] Activities leading to an increased risk for premature OA involved power gripping, carrying, lifting, increased physical loading, increased static loading, kneeling, walking, squatting, and bending.[39,40] Recent studies and systematic reviews have confirmed that heavy lifting and crawling and sometimes climbing were associated with knee and hip OA; individual studies were variable, often small, and with interpretive limitations.[41] The effect of body mass index (BMI) in work-related OA appeared to predispose toward the development of knee OA, with primarily valgus malalignment.[42,43] A recent systematic review and meta-analysis concluded that data supported risk of OA from activities involving heavy or manual work (average relative risk [RR], 1.45; range, 1.20 to 1.76), elite sports (RR, 1.72; range, 1.35 to 2.20), kneeling (RR, 1.30; range, 1.03 to 1.63), squatting (RR, 1.40; range, 1.21 to 1.61), lifting/carrying (RR, 1.58; range, 1.28 to 1.94), climbing stairs (RR, 1.29; range, 1.08 to 1.55), standing work (RR, 1.11; range, 0.81 to 1.51), and knee bending/straining (RR, 1.60; range, 1.15 to 2.21).[44] A common theme for occupational activities leading to knee OA was cumulative joint loading.[45] Bending was associated with magnetic resonance imaging abnormalities in cartilage of asymptomatic persons,[46] as was physical activity, assessed by objective measures[47-49]; injuries accelerated progression of knee OA.[50]

Studies of skeletons of several populations have suggested that age at onset, frequency, and location of osteoarthritic changes were directly related to the nature and degree of physical activities.[51] However, not all these studies adhered to contemporary standards, nor have they been confirmed. One report, for example, failed to find an increased incidence of OA in pneumatic drill users and criticized inadequate sample sizes, lack of statistical analyses, and omission of appropriate control populations in previous reports.[33] The investigators further commented that earlier work was "frequently misinterpreted" and that their studies suggested that "impact, without injury or preceding abnormality of either joint contour or ligaments, is unlikely to produce osteoarthritis."[34]

Do epidemiologic studies of OA implicate physical or mechanical factors related to disease predisposition or development? The first national Health and Nutrition Examination Survey of 1971 to 1975 (HANES I) and the Framingham studies explored cross-sectional associations between radiographic OA of the knee and possible risk factors.[38-43,52] Strong associations were noted between knee OA and obesity and occupations involving the stress of knee bending, but not all habitual physical activities and leisure-time physical activities (e.g., running, walking, team sports, racquet sports, and others) were linked with knee OA.[27-29,53-55] (See Chapter 98 for a more detailed discussion of the pathogenesis of OA.)

### Other Occupational Rheumatologic Disorders

Certain rheumatic diseases other than repetitive strain or cumulative trauma disorders have been reported to be associated with occupational risks. Reports have been made of reflex sympathetic dystrophy after trauma; Raynaud's phenomenon with vibration or exposure to chemicals (particularly polyvinyl chloride); autoimmune disease from teaching at a school, farming, occupations with exposure to animals and pesticides, mining, use of a textile machine, and decorating operations[40,56]; scleroderma from exposure to

**TABLE 35-3** Occupational Physical Activities and Possible Associations with Osteoarthritis

| Occupation | Involved Joints | Risk of OA | References |
|---|---|---|---|
| Miner | Elbow, hip, knee, spine | Increased | Lawrence[32] (1955), Kellgren and Lawrence[33] (1958), Felson et al.[39,40] (1997, 1998) |
| Pneumatic driller | Shoulder, elbow, wrist, MCP joint | Increased/ none | Jurmain (1977; cited in reference 47), Burke et al.[34] (1977) |
| Dockworker | Intervertebral disk, DIP joint, elbow, hip, knee | Increased | Lawrence[32] (1955); Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Cotton mill worker | Hand | Increased | Lawrence[35] (1961) |
| Diamond worker | Hand | Increased | Kellgren and Lawrence[31] (1957), Tempelaar and Van Breeman[36] (1932) |
| Shipyard laborer | Knee | Increased | Goldberg and Montgomery (1987; cited in Felson et al.,[39,40] 1997, 1998) |
| Foundry worker | Lumbar spine | Increased | Lawrence et al. (1966; cited in Felson et al.,[39,40] 1997, 1998) |
| Seamstress | Hand | Increased | Tempelaar and Van Breeman[36] (1932) |
| Textile worker | Hand | Increased | Hadler et al.[37] (1978) |
| Manual laborer | MCP joint, hip | Increased | Williams et al. (1987; cited in Felson et al.,[39,40] 1997, 1998); Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998); McWilliams et al.[44] (2011) |
| Occupations requiring knee bending | Knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998); McWilliams et al.[44] (2011) |
| Farmer | Hip, knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Firefighter | Hip, knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Millworker | Hip | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Female mail carrier | Hip | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Fisherman | Hip | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Construction worker | Knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| House and hotel cleaner | Knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Craftperson | Knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Service worker | Knee | Increased | Anderson and Felson[38] (1988); Felson et al.[39,40] (1997, 1998) |
| Heavy lifter | Hip, knee | Increased | Allen et al. (2010)[41] |
| Crawling | Hip, knee | Increased | Allen et al. (2010)[41] |
| Kneeling | Knee | Increased | McWilliams et al.[44] (2011) |
| Squatting | Knee | Increased | McWilliams et al.[44] (2011) |
| Lifting/carrying | Knee | Increased | McWilliams et al.[44] (2011) |
| Climbing stairs | Knee | Increased | McWilliams et al.[44] (2011) |
| Standing work | Knee | Increased | McWilliams et al.[44] (2011) |

*As cited in Greer JM, Panush RS: Musculoskeletal problems of performing artists. *Baillieres Clin Rheumatol* 8:103, 1994.
DIP, Distal interphalangeal; MCP, metacarpophalangeal; OA, osteoarthritis.

chemicals, silica, and solvents and with use of vibrating tools[57,58]; scleroderma-like syndromes from exposure to rapeseed oil and L-tryptophan[58,59]; systemic lupus erythematosus from exposure to the sun, silica, mercury, pesticides, nail polish, paints, dye, canavanine, hydrazine, and solvents[60,61] and with shiftwork and patient contact[62]; lupus, scleroderma, and Paget's disease from exposure to pets[63]; granulomatous vasculitis from exposure to mercury and lead[64]; primary systemic vasculitis from farming, exposure to silica and solvents, and allergy[65]; anti-synthetase syndrome from exposure to dust, gas, and fumes[66]; arthritis in patients with psoriasis from infections requiring antibiotics and in persons who have performed heavy lifting[67]; spondyloarthropathy from stressful events[68]; gout (saturnine) and hyperuricemia with lead intoxication[69]; and rheumatoid arthritis (Caplan's syndrome) with silica exposure, farming, mining, quarrying, electrical work, construction and engine operation, nursing, religious, juridical, and other social science–related work, smoking, traffic and pollution, insecticides, and periodontal disease[70-72] (Table 35-4).

## RECREATION- AND SPORTS-RELATED MUSCULOSKELETAL DISORDERS

Do recreational or sports-related activities lead to musculoskeletal disorders?[72-98] It has been suggested that the risk of joint degeneration is increased by participation in sports that have high impact levels with torsional loading.[72] The presence of prior joint injury, surgery, arthritis, joint instability and/or malalignment, neuromuscular disturbances,

**TABLE 35-4** Other Reported Occupation-Related Rheumatic Diseases

| Disease or Syndrome | Occupation or Risk Factor |
|---|---|
| Reflex sympathetic dystrophy | Trauma |
| Raynaud's phenomenon | Vibration<br>Chemicals (polyvinyl chloride) |
| Autoimmune disease[40,56] | Teaching at a school |
| Scleroderma[57,58] | Chlorinated hydrocarbons<br>Organic solvents<br>Silica |
| Vasculitis[64,65] | Mercury, lead, silica, solvents, allergy |
| Scleroderma-like syndromes[58,59] | Rapeseed oil<br>L-Tryptophan |
| Anti-synthetase syndrome[66] | Dust, gas, fume exposure |
| Systemic lupus erythematosus[61-63] | Canavanine, hydrazine, mercury, pesticides, solvents, shift work, patient contact |
| Lupus, scleroderma, and Paget's disease[63] | Pet ownership |
| Rheumatoid arthritis (Caplan's syndrome)[70,71] | Silica, insecticides, traffic, pollution, smoking, periodontal disease |
| Arthritis in persons with psoriasis[67] | Heavy lifting, infection requiring antibiotic treatment |
| Spondyloarthritis[68] | Stressful events |
| Gout (saturnine)[69] | Lead |

and muscle weakness also predispose to higher risks of joint damage during sports participation.[72] Persons with sports injuries to the anterior cruciate and medial collateral ligaments (such as from downhill skiing and football) frequently experienced the chondromalacia patellae and radiographic abnormalities of OA (20% to 52%).[27-29] Retrospective studies found that OA was associated with varus deformity, previous meniscectomy, and relative body weight.[73,74,99] Both partial and total meniscectomies have been linked with degenerative changes. Early joint stabilization and direct meniscus repair surgery may decrease the incidence of premature OA. Observations like these support the concept that abnormal biomechanical forces, either congenital or secondary to joint injury, are important factors in the development of exercise-related OA.[27-29] Other observations include certain physical characteristics of the participant, biomechanical and biochemical factors, age, gender, hormonal influences, nutrition, characteristics of the playing surface (when applicable), unique features of particular sports, and duration and intensity of exercise participation, as has been reviewed extensively elsewhere.[27-29] It is increasingly recognized that biomechanical factors have an important role in the pathogenesis of OA, as has been presented.

Is regular participation in physical activity associated with degenerative arthritis? Several animal studies (of tentative scientific relevance, but of interest) have suggested a possible relationship between exercise and OA. For example, it has been stated that the husky breed of dog has increased hip and shoulder arthritis associated with pulling sleds, that tigers and lions develop foreleg OA related to sprinting and running, and that racehorses and workhorses develop OA in the forelegs and hind legs, respectively, consistent with their physical stress patterns.[27,29] In rabbits with experimentally induced arthritis in one hind limb, progressive OA did not develop when they exercised on treadmills, but OA did develop in sheep with normal health who walked on concrete. Other studies found that OA did not develop in dogs (beagles) who ran 4 to 20 km a day,[27-29] that lifelong physical activity (running) protected mice from OA,[100] that running 30 km in 3 weeks or 55 km in 6 weeks induced OA in rats,[101] and that running exacerbated induced OA in other rats.[102] Although these observations were not entirely consistent, they suggested that physical activities in some circumstances might predispose to degenerative joint disease.

Human studies have provided pertinent observations[27-29] (Table 35-5). Wrestlers were reported to have an increased incidence of OA of the lumbar spine, cervical spine, and knees; boxers, of the carpometacarpal joints; parachutists, of knees, ankles, and spine, which was not confirmed; cyclists, of the patella; cricketers, of the fingers; and basketball and volleyball players, of the knees..[27-29,42] In addition, athletes involved in sports requiring repetitive overhead throwing such as baseball, tennis, volleyball, and swimming were reported to have an increased incidence of early glenohumeral arthritis[77]; persons with meniscal and anterior cruciate ligament injuries incurred in youth-related sports were reported to have an increased incidence of knee OA[78]; soccer players were reported to have an increased incidence of OA of the talar joint, ankle, cervical spine, knee, and hip[27-29,79-82,99]; and OA of the hips and knees of elite athletes from impact sports.[83] Studies of American football players have suggested that they are susceptible to OA of the knees, particularly those who sustained knee injuries while playing football.[30] Among football players (average age, 23 years) competing for a place on a professional team, 90% had radiographic abnormalities of the foot or ankle, compared with 4% of an age-matched control population; linemen had more changes than did ball carriers or linebackers, who in turn had more changes than did flankers or defensive backs. All athletes who had played football for 9 years or longer had abnormal radiographic findings.[27-30,78] Most of these studies were deficient in several respects: criteria for OA (or "osteoarthrosis," "degenerative joint disease," or "abnormality") were not always clear, specified, or consistent; duration of follow-up was often not indicated or was inadequate to determine the risk of musculoskeletal problems at a later age; intensity and duration of physical activity were variable and difficult to quantify; selection bias toward persons exercising or participating versus those not exercising or participating was not weighted; other possible risk factors and predispositions to musculoskeletal disorders were rarely considered; studies were not always properly controlled, and examinations were not always "blind"; little information regarding nonprofessional, recreational athletes was available; and little clinical information about functional status was provided.[27-29,75,76,99]

A number of studies have examined a possible relationship between running and OA. Uncontrolled observations generally suggested that runners without underlying biomechanical problems of the lower extremity joints did not

**TABLE 35-5** Sports Participation and Alleged Associations with Osteoarthritis

| Sport | Site (Joint) | Risk | References |
|---|---|---|---|
| Ballet | Talus<br>Ankle<br>Cervical spine<br>Hip<br>Knee<br>Metatarsophalangeal | Possibly to probably increased depending on type, intensity, and duration of participation† | Ottani and Betti (1953), Coste et al. (1960), Brodelius (1961), Miller et al. (1975)<br>Washington (1978), Ende and Wickstrom (1982)<br><br><br><br>Washington (1978) |
| Baseball | Elbow<br>Shoulder | | Adams (1965), Hansen (1982)<br>Bennett (1941) |
| Boxing | Hand (carpometacarpal joints) | | Iselin (1960) |
| Cricket | Finger | | Vere Hodge (1971) |
| Cycling | Finger | | Bagneres (1967) |
| American football | Ankle<br>Foot<br>Knee<br>Spine | | Vincelette et al. (1972)<br>Rall et al. (1964)<br>Richmond et al.[99] (2013)<br>Ferguson et al. (1975), Albright et al. (1976), Moretz et al. (1984) |
| Gymnastics | Elbow<br>Shoulder<br>Wrist<br>Hip<br>Knee | | Bozdech (1971)<br><br><br>Murray and Duncan (1971), Richmond et al.[99] (2013)<br>Richmond et al.[99] (2013) |
| Lacrosse | Ankle<br>Knee | | Thomas (1971) |
| Martial arts | Spine | | Rubens-Duval et al. (1960) |
| Parachuting | Ankle<br>Knee<br>Spine | | Murray and Duncan (1971)<br><br>Murray-Leslie et al. (1977) |
| Rugby | Knee | | Slocum (1960) |
| Running (see Table 35-6) | Knee<br><br><br><br><br>Hip<br><br><br>Ankle | Small | McDermott and Freyne (1983), Lane et al. (1986, 1987, 1998), Panush et al. (1986), Cheng et al.[92] (2000), Thelin et al.[97] (2006), Chakravarty et al.[85] (2008), Hansen et al.[104] (2012),<br>Tveit et al.[83] (2012), Williams[91] (2013), Richmond et al.[99] (2013),<br>Miller et al.[98] (2014)<br>Puranen et al. (1975), de Carvalho and Langfeldt (1977), McDermott and Freyne (1983), Lane et al. (1986, 1987, 1998), Panush et al. (1986), Konradsen et al. (1990), Richmond et al.[99] (2013)<br>Konradsen et al. (1990), Marti et al. (1990) |
| Soccer | Ankle-foot<br>Hip<br>Knee<br><br><br>Talus<br>Talofibular | | Pellissier et al. (1952), Pellegrini et al. (1964), Sortland et al. (1982)<br>Klunder et al. (1980), Kuijt et al.[82] (2012). Tveit[83] (2012), Richmond et al.[99] (2013)<br>Pellissier et al. (1952), Solonen (1966), Klunder et al. (1980), Thelin et al.[97] (2006), Kuijt et al.[82] (2012), Tveit[83] (2012),<br>Richmond et al.[99] (2013)<br>Brodelius (1961), Solonen (1966)<br>Burel et al. (1960) |
| Weightlifting | Spine | | Aggrawal et al. (1965), Muenchow and Albert (1969), Fitzgerald and McLatchie (1980) |
| Wrestling | Cervical spine<br>Elbow<br>Knee | | Layani et al. (1960) |

*Cited in Panush RS, Lane NE: Exercise and the musculoskeletal system. *Baillieres Clin Rheumatol* 8:79, 1994; Panush RS: Physical activity, fitness, and osteoarthritis. In Bouchard C, Shephard RJ, Stephens T, editors: *Physical activity, fitness, and health. International Proceedings and Consensus Statement.* Champaign, Ill., 1994, Human Kinetics Publishers, pp 712–723; and Panush RS: Does exercise cause arthritis? Long-term consequences of exercise on the musculoskeletal system. *Rheum Dis Clin North Am* 16:827, 1990.

†This risk level is true of all of the sports listed except running.

develop arthritis at a different rate from a normal population of nonrunners. However, persons who had underlying articular biomechanical abnormalities from a previously injured joint (and perhaps elite athletes, particularly women) appeared to be at greater risk for the subsequent development of OA. Early studies showed that groups of long-duration, high-mileage runners and nonrunning control subjects had a comparable (and low) prevalence of OA and suggested that recreational running need not inevitably lead to OA.[103] These observations have generally now been confirmed by the original authors in long-term follow-up studies[85] and by other investigators[27-29,75,76,79,84-86,91,98,103] and in comprehensive reviews[88,99,104] (Table 35-6). Eight- and 9-year follow-up observations were supportive; most of

**TABLE 35-6** Studies of Running and Risk of Developing Osteoarthritis

| References | No. of Runners | Mean Age (yr) | Mean No. of Years Running | Miles/Wk | Comments |
|---|---|---|---|---|---|
| Minor et al. (1989; cited in references 27-29) | 319 | NA | NA | NA | OA noted more frequently in former runners (with underlying anatomic "tilt" abnormality—epiphysiolysis) than in nonathletes |
| Puranen et al. (1975; cited in references 27-29) | 74 | 56 | 21 | NA | Champion distance runners had no more hip OA than did nonrunners in their sixth decade |
| De Carvalho and Langfeldt (1977; cited in references 27-29) | 32 | NA | NA | NA | Radiographic findings of runners' hips and knees were similar to those of control subjects |
| Marti et al.[94] (1990) | 20 | 35 | 13 | 48 | OA occurred in runners with underlying anatomic (biomechanical) abnormality |
| Sohn and Micheli[88] (1985) | 504 | 57 | 9-15 | 18-19 | No association between moderate long-distance running and future development of OA (of hip and knees) |
| Panush et al.[84] (1986) | 17 | 53 | 12 | 28 | Comparable low prevalence of lower extremity OA in runners and nonrunners |
| Lane et al.[75] (1986) | 41 | 58 | 9 | 5 h/wk | No differences between runners and control subjects in cartilage loss, crepitus, joint stability, or symptoms |
| Lane et al. (1987; cited in references 27-29) | 498 | 59 | 12 | 27 | No differences between groups in conditions thought to predispose to OA and musculoskeletal disability |
| Marti et al.[93,94] (1989, 1990) | 27 | 42 | NA | 61 | More radiographic changes of hip OA in former Swiss national team long-distance runners than in bobsledders and control subjects; few runners had clinical symptoms of OA; no difference in ankle joints |
| Konradsen et al.[89] (1990) | 30 | 58 | 40 | 12-24 | No clinical or radiographic differences in hips, knees, and ankles between runners and nonrunners |
| Vingard et al.[95] (1995) | 114 | 50-80 | NA | NA | Unvalidated questionnaire reported threefold increase of hip arthrosis in former athletes |
| Kujala et al.[90] (1994) | 342 | NA | NA | NA | More former athletes hospitalized with hip OA than expected |
| Kujala et al.[79] (1995) | 28 | 60 | 32 | NA | Women soccer players and weightlifters, nonrunners were at risk of premature OA |
| Panush et al.[103] (1995) | 16 | 63 | 22 | 22 | 8-yr follow-up of original observations made in 1986 still found no differences between runners and nonrunners |
| Lane et al.[76] (1998) | 35 | 60 | 10-13 | 23-28 | Running did not appear to influence the development of radiographic OA (with possible exception of spur formation in women) |
| Cheng et al.[92] (2000) | 16,691 subjects | 40% > 50 | Variable | | >20 miles/wk was associated (2000) with 2.4 hazard ratio for OA in men |
| Chakravarty et al.[85] (2008) | 1/45 | 58 | 18 | 183.5 h/wk | No increased OA in runners |
| Hansen et al.[104] (2012) | NA | NA | NA | NA | Comprehensive literature review of in vitro, animal, and human studies; "Low-and moderate-volume runners appear to have no more risk of developing OA than non-runners. The existing literature is inconclusive (for)… high-volume running…" |
| Williams[91] (2013) | 74,752 | 46 | 13 | <1,8 – >5.4 MET h/day | Fewer hip and knee replacements in runners |

MET, Metabolic equivalent of the task; NA, not available; OA, osteoarthritis.

the original runners were still running, with a prevalence of OA that was comparable with that of the control subjects.[76] Perhaps even more significant was the growing evidence that running and other aerobic exercise protected against the development of disability and early mortality.[84] In another study,[85] former college varsity long-distance runners were compared with former college swimmers; no association was found between moderate levels of running or number of years running and the development of symptomatic OA. Other authors have concluded that running in and of itself does not cause OA; rather, prior injuries and anatomic variances were directly responsible for some of the changes.[27-29,103] Prospective studies have found that runners were not at risk for the development of premature OA of the knees.[85-90] Runners had hip replacements less frequently than did other persons (perhaps related to lower BMIs).[91] However, another recent study found that running 20 miles per week was associated with 2.4 hazard ratio for OA for men younger than 50 years.[92]

Studies examining hip OA in former athletes[93-97] noted that former champion distance runners had no more clinical or radiographic evidence of OA than did nonrunners.[89] However, another study found more radiographic changes due to degenerative hip disease in former national team long-distance runners than in bobsled competitors and control subjects.[105] In all the subjects studied, age and mileage run in 1973 were strong predictors of radiographic evidence of hip OA; for runners, running pace in 1973 was the strongest predictor of subsequent radiographic evidence of hip OA in 1988. These authors concluded that high-intensity, high-mileage running should not be dismissed as a risk factor for premature OA of the hip. Other reports found that former top-level soccer players and weightlifters, but not runners, were at risk for the development of knee OA,[79,96] but it was suggested elsewhere that former athletes seemed to be disproportionately represented in hospital admissions for OA of hip, knee, or ankle.[96] A questionnaire of former elite and track-and-field athletes noted they had increased hip OA.[95] Similarly, radiographic OA of the hip and knee was reported in women who were formerly runners and tennis players.[96] Other investigators reported no correlation between OA and running but rather with other sports, particularly soccer and tennis (where knee injuries were prevalent).[97] It was speculated that peak load per unit distance (stride and short duration of ground contact) may explain the fewer injuries and reduced prevalence of OA in running compared with certain other sports.[98]

Cross-sectional studies on the effect of weight-bearing exercise on the development of OA of the hip, knee, or ankle and foot must be interpreted with caution. The radiographic scoring methods used by each group of investigators differ, and their reliability has not been adequately tested. This information is important when the major end points in the studies are radiographic features of OA.

## PERFORMING ARTS–RELATED MUSCULOSKELETAL DISORDERS

Musculoskeletal problems are common among performing artists. Performing artists—particularly musicians and dancers—have unique medical and musculoskeletal problems that merit special consideration. Injuries that might be trivial to others may be catastrophic to such artists. These injuries are usually associated with overuse—the consequences of tissues stressed beyond anatomic or normal physical limits. Understanding the technical requirements and biomechanics required in the performance of a craft (art), as well as the lifestyle required to pursue a successful career in these fields, should help physicians appreciate causative factors that lead to these injuries.

The following principles are important in treating such patients:

- Musculoskeletal problems constitute the bulk of health issues for these persons.
- Performing artists are usually wary of consulting with physicians and skeptical of their expertise.
- An appropriate evaluation should be carried out by someone knowledgeable about the technical and biomechanical requirements of the patient's craft(s)/art(s). The evaluation should consider instrument(s), instrument usage, travel with instruments, shoes, performance surface and setting, practice and performance routines, repertoire, coaches and training/trainers, and lifestyle and psychological factors, as appropriate.
- Evaluation should include attention to joint laxity and other physical features of the artist, as well as to their relationship to performance, considering the entities encountered as listed in Table 35-7.[105-109] The evaluation should assess muscle tension and fatigue. Patients should demonstrate how they use an instrument while both the actively moving body parts and the relatively immobilized parts are examined.[105,110,111]
- Inquiry should be made about all prescription and nonprescription therapies, nutritional and exercise practices, and non-mainstream (so-called "complementary and/or alternative") treatments.
- The practitioner must have an understanding of and sympathy for the unique expectations of these performers and expertise in assessing their medical problems and developing treatment plans.
- Prevention should be emphasized—ensuring performance ability, promoting endurance and conditioning, facilitating good posture, protecting joints, maintaining proper ergonomics, and establishing appropriate exercise regimens.[110,111]
- Therapeutic interventions will usually be conservative.

### Instrumentalists

The frequency of musculoskeletal problems in musicians rivals the frequency of disability in athletes. Up to 82% of orchestral musicians have experienced medical problems (mainly musculoskeletal) related to their occupation. Up to 76% of musicians have reported a musculoskeletal issue that is grave enough to influence their ability to perform.[105,112] Woodwind players and female instrumentalists seem to be affected more often compared with other types of other instrumentalists and male artists, respectively. Muscle-tendon overuse or repetitive stress injuries, nerve entrapment problems, and focal dystonias are most common (see Table 35-7).[105,106]

The causes of, mechanisms of, and therapies for these musculoskeletal problems are unclear. Overuse, tendinitis, cumulative trauma disorder, repetitive motion disorder,

**TABLE 35-7** Musculoskeletal and Rheumatic Disorders Associated with Overuse in Performing Artists

| Instrument | Affliction (Common Name) | References |
|---|---|---|
| Piano, keyboard | Myalgias | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Tendinitis | Hochberg et al. (1983), Caldron et al. (1986), Knishkowy and Lederman (1986), Newmark and Hochberg (1987) |
| | Synovitis | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Contractures | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Nerve entrapment | |
| | Median nerve (carpal tunnel–pronator syndrome) | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Ulnar nerve | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Brachial plexus | Hochberg et al. (1983), Knishkowy and Lederman (1986) |
| | Posterior interosseous branch of radial nerve | Hochberg et al. (1983), Charness et al. (1985) |
| | Thoracic outlet syndrome | Hochberg et al. (1983), Knishkowy and Lederman (1986), Lederman (1987) |
| | Motor palsies | Hochberg et al. (1983), Schott (1983), Caldron et al. (1986), Knishkowy and Lederman (1986), Merriman et al. (1986), Cohen et al. (1987), Jankovic and Shale (1989) |
| | Osteoarthritis | Bard et al. (1984) |
| Strings | | |
| Violin, viola | Myalgias | Fry (1986b), Hiner et al. (1987), Bryant (1989) |
| | Tendinitis | Fry (1986b), Hiner et al. (1987) |
| | Epicondylitis | Fry (1986b), Hiner et al. (1987) |
| | Cervical spondylosis | Fry (1986b), Hiner et al. (1987) |
| | Rotator cuff tears | Fry (1986b), Newmark and Hochberg (1987) |
| | Thoracic outlet syndrome | Roos (1986), Lederman (1986) |
| | Temporomandibular joint syndrome | Hirsch et al. (1982), Ward (1990), Kovera (1989) |
| | Motor palsies | Schott (1983), Knishkowy and Lederman (1986), Hiner et al. (1987), Jankovic and Shale (1989) |
| | Garrod's pads | Bird (1987) |
| | Nerve entrapment | |
| | Ulnar | Knishkowy and Lederman (1986) |
| | Interosseous | Maffulli and Maffulli (1991) |
| Cello | Myalgias | Fry (1986b) |
| | Tendinitis | Caldron et al. (1986), Fry (1986b) |
| | Epicondylitis | Fry (1986b) |
| | Low back pain | Fry (1986b) |
| | Nerve entrapment | Caldron et al. (1986), Knishkowy and Lederman (1986) |
| | Motor palsies | Schott (1983) |
| | Thoracic outlet syndrome | Lederman (1987), Palmer et al. (1991) |
| Bass | Low back pain | Fry (1986b) |
| | Myalgias | Fry (1986b) |
| | Tendinitis | Caldron et al. (1986), Fry (1986b), Mandell et al. (1986) |
| | Motor palsies | Caldron et al. (1986) |
| Viola da gamba | Saphenous nerve compression (gamba leg) | Schwartz and Hodson (1980), Howard (1982) |
| Harp | Tendinitis | Caldron et al. (1986) |
| | Nerve entrapment | Caldron et al. (1986) |
| Woodwinds | | |
| Clarinet and oboe | First web space muscle strain | Fry (1986b), Newmark and Hochberg (1987) |
| | Tendinitis | Dawson (1986), Fry (1986b) |
| | Motor palsies | Jankovic and Shale (1989) |
| Flute | Myalgias | Fry (1986b) |
| | Spine pain | Fry (1986b) |
| | Temporomandibular joint syndrome | La France (1985) |
| | Tendinitis | Patrone et al. (1988) |
| | Nerve entrapment | |
| | Digital | Cynamon (1981) |
| | Posterior interosseous | Charness et al. (1985) |
| | Thoracic outlet syndrome | Lederman (1987) |
| Brass | | |
| Trumpet, cornet | Motor palsies | Turner (1893), Dibbell (1977), Dibbell et al. (1979) |
| | Orbicularis oris rupture (Satchmo's syndrome) | Planas (1982, 1988), Planas and Kaye (1982) |
| English horn | de Quervain's tenosynovitis | Studman and Milberg (1982) |
| French horn | Motor palsies | James and Cook (1983), Jankovic and Shale (1989) |
| Saxophone | Thoracic outlet syndrome | Lederman (1987) |

**TABLE 35-7** Musculoskeletal and Rheumatic Disorders Associated with Overuse in Performing Artists—cont'd

| Instrument | Affliction (Common Name) | References* |
|---|---|---|
| Percussion | Osteoarthritis | Caldron et al. (1986) |
| Drums | Tendinitis | Fry (1986b), Caldron et al. (1986) |
| | Myalgias | Fry (1986b) |
| | Nerve entrapment | Makin and Brown (1985) |
| Cymbals | Bicipital tenosynovitis (cymbal player's shoulder) | Huddleston and Pratt (1983) |
| Miscellaneous | | |
| Guitar, strings | Tendinitis | Newmark and Hochberg (1987) |
| | Synovitis | Mortanroth (1978), Bird and Wright (1981) |
| | Motor palsies | Mladinich and De Witt (1974), Cohen et al. (1987), Jankovic and Shale (1989) |
| Congas | Pigmenturia | Fenichel (1974), Furie and Penn (1974) |
| Spoons | Tibial stress fracture (spoon player's tibia) | O'Donoghue (1984) |

*As cited in Greer JM, Panush RS: Musculoskeletal problems of performing artists. *Baillieres Clin Rheumatol* 8:103, 1994.

occupational cervicobrachial disorder, and regional pain syndrome have been considered critical risk factors in the development of joint laxity in musicians.[112] Joint laxity declined with age and was associated with gender, starting earlier in men but persisting in women through their mid 40s. The presence or absence of hypermobility at certain sites was associated with musicians' reports of associated symptoms. Hypermobility in musicians might produce advantages or disadvantages, depending on the site of the laxity and the instrument played.[113] Paganini, with his long fingers and reported hyperextensibility, had a wider finger reach on the violin than his contemporaries, but he may have had a predisposition to OA because of this. Of interest and seemingly unexplained was the high frequency of symptoms among women (68% to 84%); perhaps this finding is related to their higher incidence of hypermobility.[112] Stress also likely contributes to motor function problems such as occupational cramps; dealing with this issue often requires the best efforts of a team of physicians and therapists.[112-115]

## Vocal Artists

Musculoskeletal problems among singers have not been addressed extensively. The frequency of musculoskeletal problems was the same in both instrumentalists and opera singers. However, singers had more hip, knee, and foot joint complaints, perhaps reflecting the effects of prolonged standing.[114]

## Dancers

Dance has been viewed as a demanding art form. Classical ballet ranked first in activities generating physical and mental stress, followed by professional football and professional hockey. The dancer and athlete have much in common, but important differences in training and performance technique influence the nature of their injuries. Other important sociocultural differences affect their care. Professional dancers (as well as musicians and vocalists) traditionally have not been convinced that most physicians know how to effectively approach the unique issues of dance and music. Injured dancers seeking care have often been told that the treatment is to stop dancing. Others, seeking assistance with weight control, have been told to gain weight. Dancers frequently underreport their injuries and seek care from nonmedical therapists.

The incidence of reported dance-related injuries ranged from 17% to 95%.[116] The majority of injuries involved the foot, ankle, and knee. It is difficult to generalize about dance injuries because "dance" and its training, performance, and settings are so variable. Most injuries are from overuse and are rarely catastrophic, regardless of the style or setting.[112] The distribution of injuries is strongly influenced by the type and style of dance and the age and sex of the population.[113,117] A better understanding of the technical and aesthetic requirements of a dance, as well as the biomechanics involved to perform these requirements, is necessary to appreciate the type of injuries that can be sustained by dancers. For example, ballet dancers in companies whose choreography emphasizes bravura technique with big jumps and balances are more likely to experience Achilles tendinitis than are those in companies that do not have this emphasis. Men are more likely to have back injuries because of the requisite jumping and lifting, whereas women who dance *en pointe* are more prone to toe, foot, and ankle problems. Also in ballet, the most important physical feature is proper turnout of the hip, which requires maximal external rotation of the lower extremity that can result in hyperlordosis of the lumbar spine, valgus heel with forefoot pronation, and external rotation of the knee.[116,118]

Tendinitis of the flexor hallucis longus tendon, commonly known as dancer's tendinitis, may be confused with posterior tibial tendinitis because of the location of pain at the posteromedial ankle. Other dancer- and environment-related factors that increase the risk of dance-related injuries include nutritional status, improper support from footwear and floors, and their rehearsal and performance schedules.[116,118] Most dance shoes do not have a shock-absorbing sole, and some dances may be performed barefoot.[118] Traditionally constructed with paper, glue, and satin or canvas or leather, ballet pointe shoes tend to soften once broken in, thus contributing to ankle injury. Intensive rehearsals before and during the opening months of a performance season and pressures to return to work quickly after an injury must also be considered in the care of dancers.[112,118] Touring companies may encounter nonflexible surfaces, including concrete, predisposing to shin splints

and stress fractures. Stress fractures may be associated with the pressure to maintain a certain weight, resulting in amenorrhea, disordered eating, and low bone density. Physicians caring for dancers, particularly ballet dancers at any level, must be aware of the aesthetic pressures for extreme leanness and the potential health consequences. Unfortunately, the dance world is not lacking in other serious medical problems including mental illness, drug abuse, and human immunodeficiency virus infection.[112]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 36

# Cardiovascular Risk in Inflammatory Rheumatic Disease



Sherine E. Gabriel • Cynthia S. Crowson

**KEY POINTS**

- For nearly half a century, excess rates of cardiovascular (CV) disease have been reported among patients with inflammatory rheumatic diseases.
- CV mortality and morbidity—in particular, ischemic heart disease and heart failure—are significantly higher among persons with rheumatoid arthritis (RA) and/or systemic lupus erythematosus (SLE), and likely other autoimmune disorders as well, when compared with people in the general population of the same age who are free of these diseases.
- With the exception of smoking, the prevalence of traditional CV risk factors is not significantly elevated in persons with RA.
- Although the prevalence of some traditional CV risk factors is elevated in persons with SLE, these elevations alone are inadequate to explain the excess CV disease risk in persons with SLE.
- Some traditional risk factors, such as dyslipidemia, behave in a paradoxical manner in persons with RA and SLE, which can confuse the clinical picture, particularly for risk assessment.
- The systemic inflammation and immune dysfunction that characterize rheumatic diseases appear to be a major driver of CV disease risk in these patients.
- The relationship between rheumatic drugs and CV disease risk is difficult to disentangle because of confounding by indication/contraindication.

For nearly half a century, excess rates of cardiovascular (CV) disease (CVD) have been reported among people with inflammatory rheumatic diseases.[1-3] More recently, the discovery of the inflammatory and immune mechanisms underlying atherosclerosis has spurred renewed interest in the association between CVD risk and the rheumatic diseases. In this chapter we review biologic mechanisms underlying CV comorbidity in the rheumatic diseases and discuss the risks of these diseases, especially in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). We also discuss the contribution of traditional and nontraditional CV risk factors to the observed excess CVD risk.

## BIOLOGIC MECHANISMS: RELATIONSHIP BETWEEN INFLAMMATION AND CARDIOVASCULAR DISEASE

The role of inflammation in the development of heart disease has only been recognized relatively recently. Rheumatic disease can be viewed as a "natural experiment" in the interplay between chronic inflammation and CVD, which could elucidate the fundamental mechanisms by which inflammation accelerates the development of atherosclerosis and heart disease. RA, SLE, Sjögren's syndrome, systemic scleroderma, inflammatory myositis, and psoriatic arthritis are characterized by chronic inflammation in various body systems, most often the joints, skin, eyes, lungs, and kidneys, but also in the heart and vascular system. RA is the most common and best studied of the autoimmune rheumatic diseases. The immune underpinnings of CVD and of RA share many similarities. Circulating acute-phase reactants, such as C-reactive protein (CRP), are elevated in persons with RA and are risk markers for heart disease in the general population. Emerging evidence suggests that T lymphocytes play a crucial pathogenic role in both RA and CVD.[4,5] The major risk gene for RA, *HLA-DRB1*, predisposes to disease by promoting the selection and survival of autoreactive $CD4^+$ T cells. HLA-DRB1 alleles are also associated with increased risk of myocardial infarction (MI) and various forms of non–RA-associated heart disease. T cells isolated from the joints of patients with RA have enhanced production of IFN-γ and interleukin (IL)-17, which presumably mediate chronic inflammation.[6,7] The proven efficacy of antagonizing T cell co-stimulation is perhaps the most compelling evidence that T cells are pathogenic in RA.[8] Similarly, percutaneous stents that elute T cell inhibiting drugs (e.g., sirolimus) prevent in-stent restenosis and repeat revascularization in people with coronary artery disease (CAD).[9]

In people with either RA or CVD, $CD4^+$ T cells characteristically lose expression of the co-stimulatory molecule, CD28, which ordinarily provides the "second signal" required for T cell activation. So-called "$CD28^{null}$" T cells are believed to have undergone reprogramming, leading to premature senescence.[10,11] Expansion of these senescent T cells among persons with RA is associated with extraarticular inflammatory manifestations, including vasculitis and lung disease, as well as CAD.[10,11] In the setting of heart disease, $CD28^{null}$ T cells are identified in atherosclerotic plaque, where they are believed to contribute to the inflammatory process by producing cytokines and by killing vascular smooth muscle cells.[12] Interestingly, *HLA-DRB1*, the aforementioned RA-risk gene, also predisposes to expansion of $CD28^{null}$ T cells in RA and in CAD.[13]

Premature senescence of T cells in RA appears to be caused by fundamental defects in the hematopoietic system.

$CD34^+$ hematopoietic progenitor cells have accelerated telomere erosion, a sign of senescence.[14] Naïve T cells in persons with RA also are prematurely aged, with increased fragility and damage of their DNA because of insufficient activity of basic DNA repair enzymes.[15] Similarly, telomere shortening in hematopoietic progenitor cells correlates with myocardial dysfunction in persons with CAD.[16] The onset of both RA and CVD coincides with the loss of thymic emigration of naïve T cells in the fifth decade, suggesting that T cell senescence may underlie the pathogenesis of both of these age-associated conditions. In the foreseeable future, rejuvenation of senescent T cells with use of new drugs that restore genomic repair and integrity has the potential to be an effective strategy for the prevention and treatment of CVD.[17]

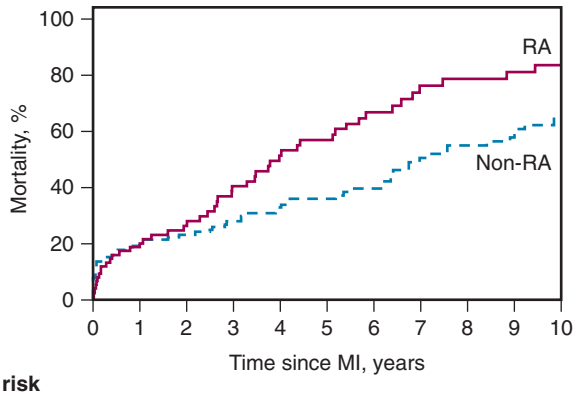
## CARDIOVASCULAR MORBIDITY IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

### Rheumatoid Arthritis

#### Ischemic Heart Disease in Rheumatoid Arthritis

Patients with RA are at increased risk of ischemic heart disease (IHD).[3,18-20] Data from the Rochester Epidemiology Project has shown that, in the 2-year period immediately preceding the fulfillment of the American College of Rheumatology (ACR) 1987 criteria, persons with RA were more likely to experience hospitalization for MI (odds ratio [OR], 3.17; 95% confidence interval [CI], 1.16 to 8.68) and unrecognized ("silent") MI (OR, 5.86; 95% CI, 1.29 to 26.64) than age- and sex-matched control subjects. The increased risk of unrecognized MI persisted after the diagnosis of RA (hazard ratio [HR], 2.13; 95% CI, 1.13 to 4.03). Holmqvist et al.[21] failed to demonstrate a statistically significant elevated increase in MI, angina, or heart failure (HF) prior to the onset of symptoms in two large Swedish cohorts, although trends toward such elevation were reported. As in studies of mortality, these results suggest that accelerated atherosclerosis begins at the onset of RA symptoms, or even earlier, and not at the time of diagnosis or later in the disease course.

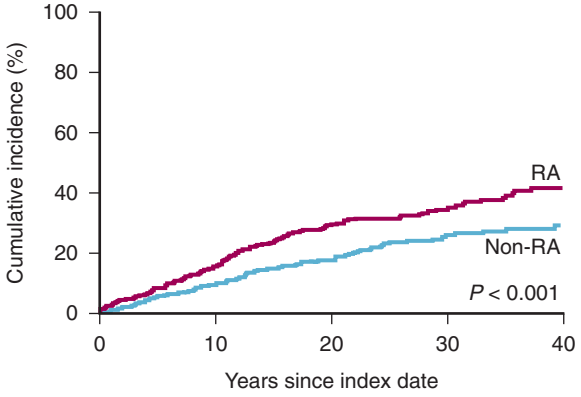
Patterns of clinical care and outcome after MI may vary in persons with RA when compared with the general population. Some evidence suggests that, although patients with RA receive MI care that is similar to that received by patients without RA, they experience higher rates of HF and death after sustaining an MI (Figure 36-1).[20,22,23] However, other investigators have reported that patients with RA who experience acute MI receive acute reperfusion and secondary prevention medications (including β-blockers and lipid-lowering agents) less frequently than do control subjects.[24] Another group recently reported that among patients with MI, those with RA were more likely to undergo thrombolysis and percutaneous coronary intervention (PCI) but were less likely to receive medical therapy and/or coronary artery bypass grafting.[25] That study also suggested that patients with RA may have an in-hospital survival advantage, particularly those undergoing medical therapy and PCI, although potential confounding could not be ruled out.




**No. at risk**

| | | | | | | |
|---|---|---|---|---|---|---|
| RA | 77 | 44 | 26 | 15 | 9 | 7 |
| Non-RA | 154 | 82 | 66 | 47 | 31 | 23 |

**Figure 36-1** Cumulative incidence of mortality after myocardial infarction (MI) among 77 patients with rheumatoid arthritis (RA) and 154 patients without RA (55 deaths in patients with RA; 85 deaths in patients without RA; log-rank $P = 0.036$). *(From McCoy SS, Crowson CS, Maradit-Kremers H, et al: Longterm outcomes and treatment after myocardial infarction in patients with rheumatoid arthritis.* J Rheumatology *40:605–610, 2013.)*




**No. at risk**

| | | | | | |
|---|---|---|---|---|---|
| RA | 575 | 336 | 133 | 51 | 7 |
| Non-RA | 583 | 386 | 189 | 75 | 15 |

**Figure 36-2** Comparison of the cumulative incidence of congestive heart failure in the rheumatoid arthritis (RA) cohort and the non-RA cohort, according to the number of years since the index date, adjusting for the competing risk of death. *(From Nicola PJ, Maradit-Kremers H, Roger VL, et al: The risk of congestive heart failure in rheumatoid arthritis: a population-based study over 46 years.* Arthritis Rheum *52:412–420, 2005. Permission to reprint from John Wiley & Sons.)*

#### Heart Failure in Rheumatoid Arthritis

Persons with RA are at increased risk of developing HF compared with the general population.[26,27] In the Rochester RA cohort, the cumulative incidence of HF (defined according to the Framingham criteria) at 30-year follow-up was 34%, compared with 25% in the non-RA cohort (Figure 36-2).[27] Even after adjustment for demographics, CV risk factors, and IHD, patients with RA had almost twice the risk of developing HF as subjects without RA (HR, 1.87; 95% CI, 1.47 to 2.39). This increased risk of HF appeared to be predominantly in the subgroup of patients with rheumatoid factor (RF) who were $RF^+$; the HR in $RF^+$ patients was 2.59 with a 95% CI of 1.95 to 3.43, whereas the HR in $RF^-$ patients was 1.28 with a 95% CI of 0.93 to 1.78.

The clinical presentation of HF in patients with RA differs from that of HF in patients without RA.[28] Patients with RA who have HF are less likely to be obese or hypertensive or have clinical IHD. Moreover, patients with RA who have HF are less likely to have typical signs and symptoms. Importantly, the proportion of patients with HF who have preserved ejection fraction (>50%) is significantly higher among patients who have RA compared with patients who do not have RA (Figure 36-3).[28]

It has also been shown that patients with RA who have HF tend to undergo less aggressive investigation and be managed less aggressively than patients without RA.[28] Finally, patients with RA who have HF also appeared to have poorer outcomes (Figure 36-4), experiencing approximately twice the risk for death in the period immediately after HF identification compared with patients who do not have RA.[29]

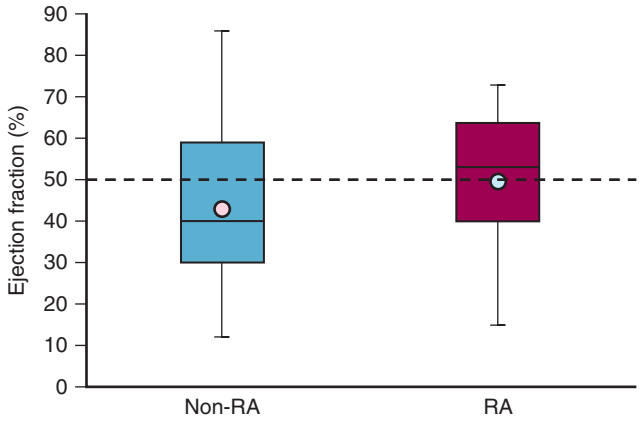



**Figure 36-3** Distribution of ejection fraction (EF) between patients with and without rheumatoid arthritis (RA) at the onset of heart failure. Data are presented as box plots, with the *boxes* representing the 25th to 75th percentiles, the *vertical lines* representing the 10th and 90th percentiles, the *circles* representing the means, the *lines within the boxes* representing the medians, and the *broken line* representing the 50% EF reference. *(From Davis JM, Roger VL, Crowson CS, et al: The presentation and outcome of heart failure in patients with rheumatoid arthritis differs from that in general population.* Am Coll Rheumatol *58:2603–2611, 2008. Permission to reprint from John Wiley & Sons.)*

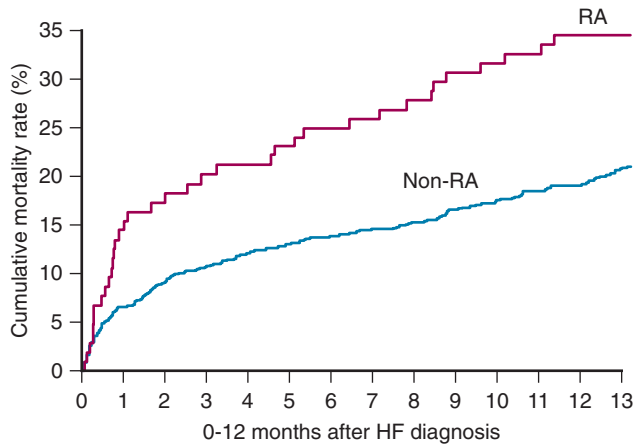



**Figure 36-4** Mortality through 1 year after the onset of heart failure (HF) in the rheumatoid arthritis (RA) and non-RA cohorts. *(From Davis JM, Roger VL, Crowson CS, et al: The presentation and outcome of heart failure in patients with rheumatoid arthritis differs from that in general population.* Am Coll Rheumatol *58:2603–2611, 2008. Permission to reprint from John Wiley & Sons.)*

## Systemic Lupus Erythematosus

### Ischemic Heart Disease in Systemic Lupus Erythematosus

Accelerated atherosclerosis is an established complication of SLE.[30-35] The prevalence of atherosclerotic vascular events varies from 1.8% early in the course of the disease to more than 27% later in the course of SLE.[34] The majority of studies reported increased risk of MI ranging from twofold to greater than 10-fold in various SLE patient groups compared with the general population.[33,34,36] This increased relative risk (RR) of MI is particularly apparent in younger patients with SLE. The most striking example comes from the University of Pittsburgh lupus cohort, in which women with SLE aged 35 to 44 years were more than 50 times as likely to have an MI compared with women without SLE in the Framingham Offspring study (RR, 52.43; 95% CI, 21.6 to 98.5).[34] Furthermore, the majority (67%) of women with SLE were younger than 55 years at the time of their first cardiac event. In addition, a more than twofold increased risk of hospitalizations for MI has been reported in young women with SLE between the ages of 18 and 44 years compared with persons without SLE (OR, 2.27; 95% CI, 1.08 to 3.46).[37,38] As in RA, it appears that the increased risk of CVD among persons with SLE may begin before the diagnosis of SLE (OR, 3.7; 95% CI, 1.8 to 7.9), as recently reported in a population-based study of 70 patients who had SLE compared with 2565 patients who did not have SLE.[39] The risk of CVD and mortality was also shown to be increased 10-fold among women with SLE who had successful deliveries, despite the rarity of CVD among women of reproductive age.[40]

In a recent study of patients undergoing coronary revascularization procedures, no significant differences were found in the mean percentage of coronary stenosis and total occlusion in subjects with and without SLE.[41] Except for the increased likelihood of lesions confined to the left anterior descending artery in subjects with SLE versus subjects without SLE (42% vs. 19%; $P = 0.003$), the pattern of coronary involvement, including artery dominance and prevalence of multivessel disease, appeared similar in subjects with SLE versus subjects without SLE. However, the study reported significantly worse CV outcomes at 1 year after PCI in subjects with SLE versus subjects without SLE, including higher risk of MI (16% vs. 5%; $P = 0.01$) and repeat PCI (31% vs. 12%; $P = 0.009$) in persons with SLE, even after adjustment for important covariates.[37,41] Given the increased vulnerability of atherosclerotic plaque in persons with SLE, which is associated with the risk of occlusive events irrespective of the size of the plaque, these findings suggest an increased risk of unfavorable CV events in persons who had SLE compared with persons who did not have SLE with a seemingly similar pattern of coronary involvement.[42]

A large population-based study from 1996 to 2000 of patients hospitalized in California with acute MI showed that in-hospital mortality and length of stay were essentially

similar in patients who had SLE compared with patients who did not have SLE, adjusting for age, race, ethnicity, type of medical insurance, and Charlson Index. In contrast, data from the 1993-2002 U.S. Nationwide Inpatient Sample showed significantly increased rates of in-hospital mortality (RR, 1.46; 95% CI, 1.31 to 1.61) and prolonged hospitalization (RR, 1.68; 95% CI, 1.43 to 2.04) for acute MI in persons who had SLE compared with control subjects, adjusting for age, sex, race/ethnicity, income, and HF.[37] Some differences in methodology (i.e., a smaller sample size and older age of patients with SLE and control subjects in the earlier study and a shorter observation period in the later study) may at least in part explain these contrasting results. Considering the presence of myocardial involvement, chronic systemic inflammation, vasculitis, and hyperviscosity syndrome in SLE, worse outcomes after acute coronary events and associated interventions can reasonably be expected.[43] However, further study is required.

### Heart Failure in Systemic Lupus Erythematosus

The risk of HF and related hospitalization in persons with SLE appears to be substantially increased.[37,38] In particular, young women with SLE between the ages of 18 and 44 years have more than a 2.5-fold increased risk of hospitalization for HF compared with persons who do not have SLE, even after adjustment for age, race, insurance status, hospital characteristics, and the presence of hypertension, diabetes mellitus, and chronic renal failure.[44] The nature of HF in SLE is likely multifactorial and only partly attributable to atherosclerosis.[45,46] The presentation of HF in persons with SLE may vary from severe overt HF to insidious myocardial involvement.[45-49] Finally, mortality in patients with SLE who have HF is significantly higher than in patients without HF (18% vs. 6%, $P < 0.001$) and approximates 3.5-fold compared with patients who have HF in the general population.[37,45]

## CARDIOVASCULAR MORTALITY IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

### Rheumatoid Arthritis

It is well known that the mortality of patients with established RA is higher than that of the general population. A meta-analysis of 24 mortality studies in persons with RA that were published between 1970 and 2005 reported a weighted combined all-cause standardized mortality ratio (met-SMR) of 1.50 (95% CI, 1.39 to 1.61), with similar increases for IHD (met-SMR, 1.59; 95% CI, 1.46 to 1.73) and stroke (met-SMR, 1.52; 95% CI, 1.40 to 1.67) and for men (met-SMR, 1.45) and women (met-SMR, 1.58).[50-52] Approximately 50% of all deaths in subjects with RA are attributable to CV causes, including IHD and stroke,[53] and CVD appears to occur earlier in persons with RA. The latter observation is consistent with the recent hypothesis of accelerated aging in persons with RA.[54] Moreover, persons with RA frequently experience "silent" IHD and/or silent MI—that is, showing no symptoms at all before a sudden cardiac death. Sudden cardiac deaths are almost twice as common in patients with RA as in the general population (HR, 1.99; 95% CI, 1.06 to 3.55).[18]

The excess CVD mortality related to RA may be confined to, or at least be substantially higher, in subjects who are $RF^+$.[55-57] The link may be even stronger with anti-citrullinated protein antibody (ACPA) positivity.[58] As might be expected, the RR of CVD mortality is highest in younger age groups (persons younger than 55 years) and in women, whereas the attributable risk is highest in the oldest age groups and in men.[19,56,59]

Controversy persists regarding how soon after symptom onset the excess CVD mortality risk becomes apparent and/or whether there is a secular trend toward improving CVD mortality in persons with RA (as is seen in the general population). This controversy may in part be explained by differences in the period of follow-up—that is, follow-up starting from the time of symptom onset, from a physician's diagnosis of RA, or from the date of fulfillment of ACR criteria or other diagnostic criteria. The latter may not occur until some years after the first symptoms appear. In the Norfolk Arthritis Register (NOAR), the excess CVD mortality is detectable beginning around 7 years after symptom onset.[55] In a Dutch inception cohort of 1049 patients with RA recruited between 1985 and 2007, excess mortality became apparent at around 10 years from diagnosis (with all subjects having <1 year symptom duration).[60] Emerging evidence indicates that mortality may be improving among persons with RA, but CV mortality has also improved in the general population, and thus the relative increase in CV mortality has not changed over time. [61] A meta-analysis that included 17 studies (91,916 patients) also showed no trend toward improving CVD mortality with time.[62]

### Systemic Lupus Erythematosus

As first described by Urowitz et al.[2] in 1976, mortality in SLE appears to follow a bimodal pattern, with an early peak in mortality (within 1 year of diagnosis) and a later peak occurring more than 5 years after diagnosis. Reported survival in the first 5 years of SLE has improved considerably from about 50% in the 1950s to greater than 90% in the 1990s.[63] However, there is some question about whether this improvement may be due, at least in part, to earlier diagnosis and improved ascertainment of mild cases. In the Toronto lupus cohort of 1241 patients recruited between 1970 and 2005, the SMR improved from 13.84 (95% CI, 9.78 to 19.76) during 1970-1978 for those who entered the cohort in that decade to 3.81 (95% CI, 1.98 to 7.32) during 1997-2005 in persons who entered the cohort in that period.[64] The SMR during 1997-2005 was very similar for patients regardless of their disease duration, ranging from 3.23 for those who had entered the cohort in 1970-1978 to 3.93 for those who had entered the cohort in 1988-1996. Similarly, evidence from Olmsted County showed an SMR of 2.70 with significant improvement in survival in recent decades.[65] In a study of 434 female patients with SLE from Seoul, Korea, who were followed up from 1992 to 2002, the SMR was 3.02 (95% CI, 1.45 to 5.55).[66] A recent population-based cohort study of 70 patients with incident SLE in 1991-2008 reported nearly twofold increased mortality and increased CVD rates. In addition, increased mortality and

CVD event rates were found perioperatively in women with SLE who were undergoing surgical procedures, even for low-risk procedures (OR, 1.54; 95% CI, 1.00 to 2.37).[67]

## RISK FACTORS FOR CARDIOVASCULAR DISEASE

Evidence for the pivotal role of inflammation in driving increased CVD risk in persons with RA is compelling. Yet, despite improved control of inflammation, CVD risk among people with RA remains elevated. Moreover, the identification of clinically valuable biomarkers of CVD risk in persons with RA remains elusive.[68] In the next sections we highlight recent evidence and discussions regarding the relative contribution of traditional and nontraditional risk factors (e.g., markers of inflammation and RA features) toward the excess CVD risk in persons with RA and suggest directions for future research.[69]

### Traditional Cardiovascular Risk Factors in Rheumatoid Arthritis: Occurrence and Impact

The role of traditional risk factors in the development of CVD in persons with RA is an area of active research. One possible explanation for the increase in CVD seen in persons with RA could be that the traditional CVD risk factors are more common in persons with RA or that they are as common but more deleterious. Alternatively, the excess CVD risk may be explained by the adverse impact of the inflammatory and immune changes of RA on the vessel wall. It is most likely, however, that traditional risk factors and inflammation are intimately interconnected and perhaps act synergistically (Figure 36-5).[70] Indeed, one study showed that progression of a new CV event in subjects with early RA may be predicted by traditional CV risk factors but precipitated by high disease activity.[71]

#### Smoking in Persons with Rheumatoid Arthritis

Smoking is a known risk factor for the development of RA, in particular $RF^+$ and $ACPA^+$ RA.[72] Thus, as expected, there is a higher prevalence of current and ex-smokers among subjects with RA than among the general population. In a meta-analysis of four case-control studies of traditional CVD risk factors in RA including 1415 patients with RA, the prevalence of smoking was found to be significantly higher than in control subjects (OR, 1.56; 95% CI, 1.35 to 1.80).[73] Smokers with RA also appear to have higher RF titers and a worse prognosis in terms of disability, radiographic damage, and treatment response.[74] A known interaction exists between smoking, HLA-DR1 shared epitope (SE) alleles, and the production of ACPA[75] and between smoking, ACPA, and the SE in premature CVD mortality in RA.[76]

#### Hypertension in Rheumatoid Arthritis

Hypertension is common in persons with RA, but it remains unclear whether it is more common than in the general population. Whereas some studies have found hypertension to be an important risk factor for CVD in persons with RA,[77] a recent meta-analysis of seven case-control studies (1053 patients with RA) found the prevalence of hypertension to be the same in persons with RA as in control subjects (OR, 1.09; 95% CI, 0.91 to 1.31).[73] However, some evidence exists for underdiagnosis and undertreatment of

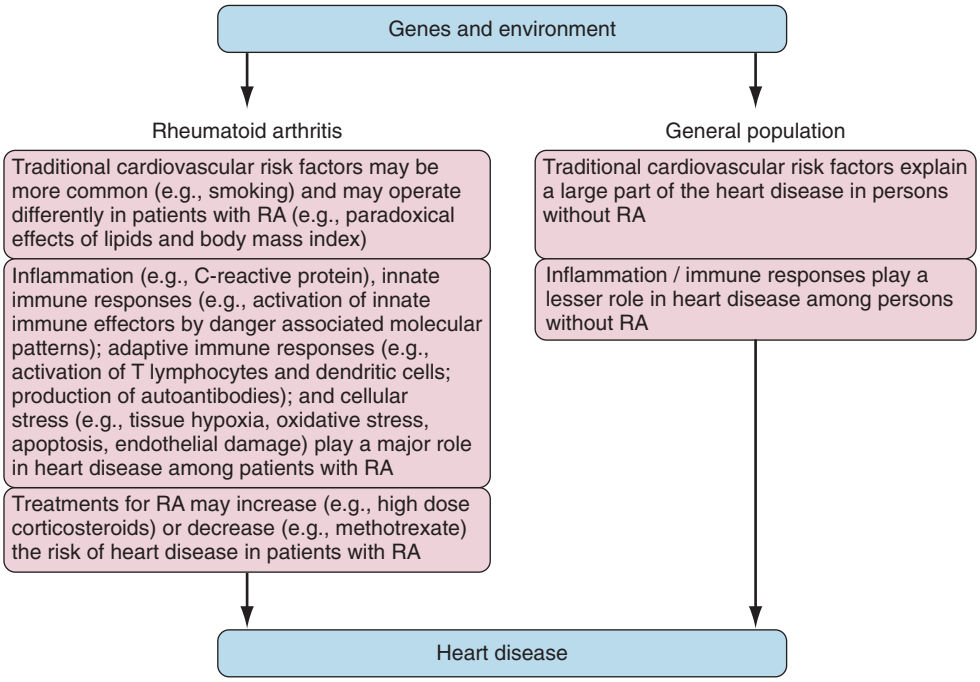



**Figure 36-5** Why do patients with rheumatoid arthritis (RA) develop heart disease? Determinants of heart disease in patients with RA differ from the general population. *(From Crowson CS, Liao KP, Davis JM, et al: Rheumatoid arthritis and cardiovascular disease.* Am Heart J *166:622–628, 2013. Permission to reprint from Elsevier.)*

hypertension in persons with RA.[78] Multiple other factors may influence blood pressure control in persons with RA, including physical inactivity, obesity, specific genetic polymorphisms, and some anti-rheumatic medications, including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, leflunomide, and cyclosporine.

### Dyslipidemia in People with Rheumatoid Arthritis

Although findings have been somewhat inconsistent, hyperlipidemia appears to have a paradoxical effect on CVD risk in persons with RA, whereby decreased lipid levels are associated with increased CVD risk (Figure 36-6).[79,80] Serum levels of total cholesterol and low-density lipoprotein (LDL) cholesterol decline precipitously during the 3- to 5-year period prior to RA incidence,[81] and lower total and LDL cholesterol levels have been shown to be associated with higher CVD risk.[80] Suppression of total and LDL cholesterol levels during acute or chronic high-grade inflammation is well described, as is a proportionately greater suppression of high-density lipoprotein (HDL) cholesterol, resulting in a disadvantageous atherogenic index (total: HDL cholesterol ratio).[82] This phenomenon may explain why hyperlipidemia (high total or LDL cholesterol) appears to be less common in persons who have RA compared with subjects who do not have RA.[79,83,84] Indeed, even among patients who sustained a previous MI, those with RA had significantly lower levels of total and LDL cholesterol than did those without RA.[85] Dyslipidemia (i.e., alterations of individual lipid components and their ratios as defined by specific criteria) may affect up to half of all patients with RA.[86] A recent meta-analysis showed that RA is associated with an abnormal lipid pattern, principally low levels of HDL cholesterol.[87] In vitro, animal model, and in vivo human studies clearly demonstrate that the interplay between inflammation and lipid components is far more complex than simple alterations of their serum levels (Figure 36-7). [88]

Inflammation-induced alterations of the structure and function of lipid molecules require further study, specifically in persons with RA and in the context of disease control through nonbiologic and biologic disease-modifying anti-rheumatic drugs (DMARDs).[89] Several studies suggest anti-rheumatic therapy effects on lipid levels, including glucocorticoids, hydroxychloroquine, gold, and cyclosporine, as well as the biologics anti–tumor necrosis factor (TNF), rituximab, and tocilizumab.[90] However, these studies are of short duration, and more research is needed. Multiple other factors are involved in lipid regulation and function,

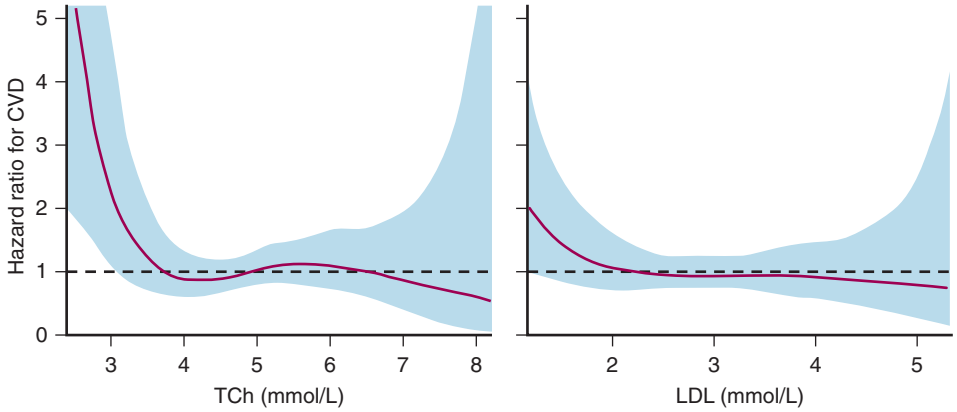



**Figure 36-6** Hazard ratios for cardiovascular disease (CVD) in rheumatoid arthritis (RA; *solid lines*) according to total cholesterol (TCh; *left panel*) and low density lipoprotein (LDL) cholesterol *(right panel)*. Shaded areas represent 95% confidence intervals. *(From Myasoedova E, Crowson CS, Maradit-Kremers H, et al: Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease.* Ann Rheumatic Dis *70:482–487, 2011. Permission to reprint from BMJ Publishing Group LTD.)*

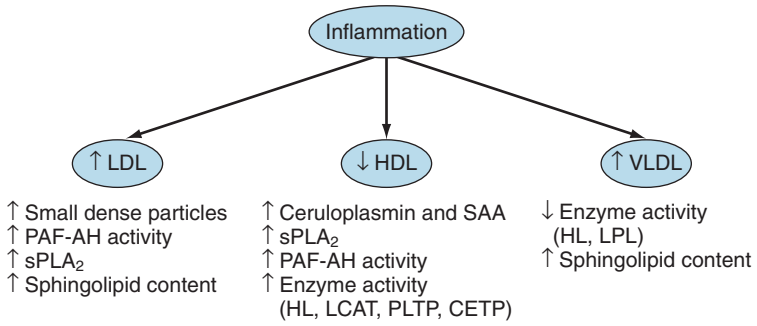



**Figure 36-7** The effects of inflammation on lipid and function. CETP, Cholesterol ester transfer protein; HDL, high-density lipoprotein; HL, hepatic lipase; LCAT, lecithin cholesterol acyltransferase; LDL, low-density lipoprotein; LPL, lipoprotein lipase; PAF-AH, platelet-activating factor acetylhydrolase; PLTP, phospholipid transfer protein; SAA, serum amyloid A; $sPLA_2$, secretory phospholipase A; VLDL, very low density lipoprotein. *(From Toms TE, Symmons DP, Kitas GD: Dyslipidaemia in rheumatoid arthritis: the role of inflammation, drugs lifestyle and genetic factors.* Curr Vasc Pharmacol *8:301–326, 2010. Permission to reprint from Bentham Science Publishers LTD.)*

including physical activity, adiposity, diet, alcohol intake, and smoking. However, their effects have not been assessed in sufficient detail in persons with RA. Similarly, the importance of genetic regulation of lipid metabolism, particularly in the context of gene-environment interactions, has not been addressed in the RA population. This factor may be particularly important because lipid alterations appear to predate the diagnosis of RA.[91] Although the relationships between lipids and CVD in RA are complex, the value of primary lipid screening in persons with RA is undisputed. Unfortunately, evidence suggests an unacceptable low rate of primary lipid screening and of statin use in persons with RA.[86,92,93]

### Diabetes Mellitus and Metabolic Syndrome in Rheumatoid Arthritis

A recent meta-analysis of seven case-control studies (1230 patients with RA) reported that the prevalence of diabetes mellitus was increased in comparison with control subjects (OR, 1.74; 95% CI, 1.22 to 2.50).[73] Likewise, patients with RA were significantly more likely to have metabolic syndrome than were subjects who did not have RA.[94,95] Abdominal obesity, anti-hypertensive medication, disease activity, and use of corticosteroids all affect glucose metabolism in RA.[96] On the other hand, use of hydroxychloroquine has been shown to reduce the risk of developing diabetes mellitus in persons with RA by approximately 77%.[97]

### Body Composition/Obesity in Rheumatoid Arthritis

Escalante and colleagues[98] reported a "paradoxical effect of BMI on survival in persons with RA," demonstrating that as body mass index (BMI) declined, so did survival probability among study subjects with RA. Among persons without RA, low BMI is not associated with increased risk of CV death. However, among patients with RA, low BMI, which may indicate uncontrolled active systemic inflammation, is associated with a threefold increased risk of CV death[99] even after adjustment for cardiac history, smoking, diabetes mellitus, hypertension, and malignancy.

Obesity is associated with the development of RA.[100,101] Obesity is also associated with an increased frequency of traditional CV risk factors in patients with RA.[102] In particular, abdominal fat is associated with insulin resistance and inflammatory load in patients with RA, and new evidence indicates that in such patients, abdominal fat is distributed differently between the visceral and subcutaneous compartments, with visceral fat more strongly associated with cardiometabolic risk.[103,104] Adipose tissue is metabolically active and, through a network of adipocytokines, regulates not only energy intake and expenditure but also inflammation. Interventions to reverse rheumatoid cachexia, control obesity, and regulate insulin resistance, including comprehensive physical exercise programs, in persons with RA have been inadequately studied.

## Nontraditional Cardiovascular Risk Factors in Rheumatoid Arthritis: Occurrence and Impact

Rheological characteristics such as whole blood viscosity, plasma viscosity, erythrocyte deformability, aggregation, and erythrocyte nitric oxide (NO) production have been increasingly linked to CVD risk in the general population.[105-107] A recent study showed that whole blood viscosity and erythrocyte NO production are independently associated with subclinical atherosclerosis in women with RA who did not have previous CV events.[108] The study also documented disturbed and unfavorable hemorheological features in association with disease activity and with traditional CV risk factors. Thus abnormal rheological characteristics of blood might represent important, nontraditional risk factors pointing to a different mechanism that may contribute to atherogenesis in inflammatory rheumatic diseases. Likewise, cytokines such as IL-17, IL-6, and TNF,[109,110] as well as wider cytokine/chemokine profiles, may play a role.[111]

### The Impact of Rheumatoid Arthritis Disease Activity and Severity on Cardiovascular Co-Morbidity

A number of studies have linked risk of CV comorbidity with markers of RA disease activity such as baseline CRP and erythrocyte sedimentation rate (ESR).[18,112] In a study of 231 male veterans with RA, a baseline disease activity score (DAS28) greater than or equal to 5.1 predicted CV events (HR, 1.3; 95% CI, 1.1 to 1.6).[113] Markers of disease severity such as RF, ACPA, physical disability, destructive changes on joint radiographs, rheumatoid nodules, vasculitis, rheumatoid lung disease, and corticosteroid use are statistically significantly associated with increased risk of CV events and/or death even after adjustment for traditional CV risk factors.[58,114-118] Analyses of ESR levels in 172 persons with RA who had HF demonstrated that the proportion with significantly elevated ESR (≥40 mm/h) was highest during the 6-month period prior to HF diagnosis compared with any other time over the entire follow-up period (Figure 36-8).[114] RF and ANA have also been shown to be risk factors for MI, HF, and/or vascular disease, even in subjects without RA, suggesting that immune dysregulation may

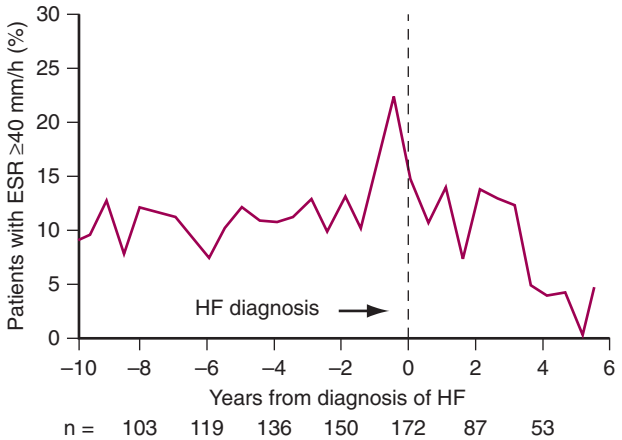



**Figure 36-8** Proportion of patients with rheumatoid arthritis with erythrocyte sedimentation rate (ESR) greater than 40 mm/h during the 6-month intervals before and after the heart failure (HF) incidence date (among 172 patients with rheumatoid arthritis who experienced heart failure). n, the number of patients under observation within the interval. *(Maradit-Kremers H, Nicola PJ, Crowson CS, et al: Raised erthrocyte sedimentation rate signals heart failure in patients with rheumatoid arthritis.* Ann Rheumatic Dis *66:76–80, 2007. Permission to reprint from BMJ Publishing Group LTD.)*

promote CVD risk not only in persons with rheumatic disease but also in the general population.[115]

### Medications as Cardiovascular Risk Factors

Medications used for the treatment of rheumatic diseases may also affect CVD risk. Because of the common use of NSAIDs and concerns surrounding CVD risk with their use, this subject has been extensively studied. Although some evidence indicates that NSAID use[119] is not associated with increased CVD risk in persons with RA, a recent network meta-analysis of 31 trials in 116,429 patients concluded that there is little evidence to suggest that any of the investigated drugs (i.e., naproxen, ibuprofen, diclofenac, celecoxib, etoricoxib, rofecoxib, or lumiracoxib) are safe.[120] In contrast, use of DMARDs (methotrexate in particular) and/or biologic agents has been suggested to decrease CVD risk[121-125] and, perhaps, to improve endothelial function (Figure 36-9).[70,126] This outcome is believed to be due to the effective long-term control of systemic inflammation. Although these findings are intriguing, they cannot be considered definitive evidence that these agents reduce CVD risk because of confounding by indication and/or contraindication. Statins have established effectiveness in the primary prevention of CV events in the general population and at-risk subpopulations (e.g., patients with diabetes mellitus). These agents have both lipid modifying and anti-inflammatory effects. However, their role in RA has only more recently been explored.[86,127]

## Traditional and Nontraditional Cardiovascular Risk Factors in Systemic Lupus Erythematosus

It is well known that traditional risk factors confer important CVD risk in the general population. Patients with SLE have an increased prevalence of several of these risk factors, including sedentary lifestyle, elevated very low density lipoprotein cholesterol and triglycerides, smoking, premature menopause, chronic renal impairment, hypertension, hyper-homocystinemia, metabolic syndrome, and insulin resistance.[128-133] Nonetheless, traditional CV risk factors cannot alone explain the observed excess risk of premature and accelerated CVD in patients with SLE. Recent research clearly demonstrates that nontraditional risk factors are major contributors to this excess risk. The most reproducible nontraditional CVD risk factors in patients with SLE include disease activity, disease duration, and corticosteroid use. The genetics of CVD in patients with SLE have not been clearly elucidated; however, early findings point to predictor single nucleotide polymorphisms, which also confer risk of thrombosis in the general population.[134] Several biomarkers have been associated with CVD risk in SLE, including autoantibodies such as anti-phospholipid antibody (aPL) antibodies, autoantibodies directed against apolipoprotein A, HDL and heat shock protein,[135-138] as well as pro-inflammatory HDL and high-sensitivity CRP. IFNs have received particular attention as mediators of disease pathogenesis and even as treatment targets in SLE. It is now increasingly recognized that by promoting endothelial injury and failed repair during SLE disease flares, IFNs may also have a profound impact on CVD risk.[139,140] As is the case in RA, SLE therapeutics likely have mixed effects on CVD risk. Although it is well known that duration of corticosteroid use is an independent predictor of CVD in patients with SLE, it is also clear that aggressive immunosuppressive and anti-inflammatory therapy correlates with reduced CVD burden.[35] Among all SLE therapeutics, the strongest evidence for a vasculo-protective effect exists with antimalarial agents.[141] These agents have been shown to be associated with reduced vascular stiffness, decreased carotid plaque, and lower cholesterol levels in addition to a protective effect on thrombovascular events.[142,143] Although some evidence suggests that mycophenolate mofetil may also protect against CVD in patients with SLE, these findings are less consistent than those reported with antimalarial drugs.[144] Despite this large and growing body of evidence regarding risk factors for CVD in patients with SLE, no randomized controlled trials (RCTs) have been performed to test preventive CVD strategies in this patient population. Thus management of risk factors must be individualized for each patient who has SLE, with prompt treatment of modifiable traditional risk factors (including use of statins, where indicated), careful monitoring of SLE therapeutics, and aggressive treatment of the underlying disease process.

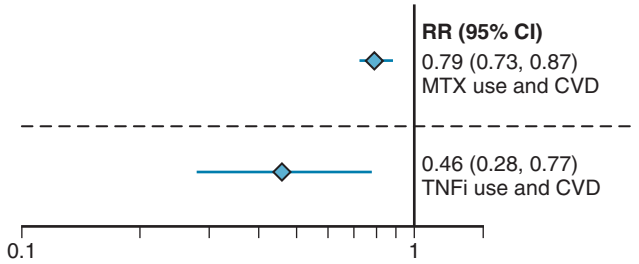



**Figure 36-9** Estimated relative risks (RRs) from meta-analyses on observational data for cardiovascular disease (CVD) risk in patients with rheumatoid arthritis associated with methotrexate (MTX) use and CVD risk associated with TNF inhibitor (TNFi) use. CI, Confidence interval. *(From Crowson CS, Liao KP, Davis JM, et al: Rheumatoid arthritis and cardiovascular disease.* Am Heart J *166:622–628, 2013. Permission to reprint from Elsevier.)*

# CARDIOVASCULAR MORTALITY, MORBIDITY, AND RISK FACTORS IN OTHER RHEUMATIC DISEASES

## Ankylosing Spondylitis

Ankylosing spondylitis (AS) represents the prototype of the spondyloarthropathies and is characterized by axial disease, enthesitis, and oligoarthritis. Although the risk of CVD in persons with AS has not been as well defined as in persons with RA, awareness of the risk of CVD in this population has been heightened.[145-148] As authors of a recent retrospective cohort study using population-based administrative data from 8616 subjects with AS reported, the sex standardized prevalence ratio for IHD was 1.37 (95% CI, 1.31 to 1.44), and for HF it was 1.34 (95% CI, 1.26 to 1.42).[146]

## Psoriasis and Psoriatic Arthritis

Both psoriasis and psoriatic arthritis have been shown to be associated with an increased risk of CVD relative to the

general population. The prevalence of traditional CV risk factors has been demonstrated to be higher among patients with psoriasis, but even after adjusting for these variables, the risk of IHD remains high (OR, 1.78; 95% CI, 1.51 to 2.11), with recent studies suggesting that psoriasis may be an independent risk factor for MI.[149-151] Persons with psoriatic arthritis are more likely to have evidence of pre-clinical atherosclerosis and are at increased risk of CVD relative to the general population.[148,152-154] Among 648 patients with psoriatic arthritis enrolled in the University of Toronto database, the risk of MI (standardized prevalence ratio 2.57; 95% CI, 1.73 to 3.80) and angina (standardized prevalence ratio 1.97; 95% CI, 1.24 to 3.12) was significantly higher than that of the general population, with increased risk seen in patients with more severe psoriasis.[155]

### Giant Cell Arteritis and Polymyalgia Rheumatica

Whether the risk of CVD is increased among patients with giant cell arteritis is less clear. Authors of two population-based studies reported two- to threefold increased risk, and authors of another study reported no increased risk.[156-158] The risk of CVD among patients with polymyalgia rheumatica has not been well studied. Authors of a few studies have reported increased risks for CVD in patients with polymyalgia rheumatica that are similar to the risk levels reported in other inflammatory diseases.[159,160] However, a recent systematic review found heterogeneity between studies that precluded a formal meta-analysis, suggesting that more research is needed to definitively determine whether the risk of CVD is increased among patients with polymyalgia rheumatica.[161]

### Dermatomyositis and Polymyositis

Because large epidemiologic studies of CVD risk in patients with myositis are lacking, the frequency of CVD in patients with myositis is not clear, but cardiac involvement is well recognized as a clinically important manifestation of myositis.[162] A recent report utilizing the Nationwide Inpatient Sample found that one-fifth of dermatomyositis hospitalizations in the United States were associated with an atherosclerotic CV diagnosis or procedure and that patients with dermatomyositis and CVD have twice the risk of in-hospital death compared with control subjects and patients who have dermatomyositis without CVD.[163] In a Canadian study, high rates of CVD were found among patients with dermatomyositis and polymyositis (13 per 1000 person-years for MI); these rates were higher compared with data from the general population of Canada.[164] Of note, use of DMARDs was associated with a reduced risk of arterial events (adjusted RR, 0.5; 95% CI, 0.2 to 1.0), which suggests that controlling systemic inflammation could reduce the risk of CVD.[165]

### Osteoarthritis

Although osteoarthritis is the most common rheumatic disease, very little is known about the link between osteoarthritis and CVD. Osteoarthritis may be associated with an increased risk of CVD because of its associated synovial inflammation and muscle weakness or because it leads to less physical activity and to NSAID use, which are all associated with increased risk of CVD.[166] Rahman et al.[166] found that osteoarthritis increased the risk of IHD (RR, 1.30; 95% CI, 1.19 to 1.42) and HF (RR, 1.15; 95% CI, 1.04 to 1.28). Hawker et al.[167] found that patients with more severe osteoarthritis were at higher risk for CV events than those with milder diseases.

### Pediatric-Onset Rheumatological Diseases

CVD has been difficult to study in patients with pediatric-onset rheumatologic disease, such as juvenile idiopathic arthritis, pediatric-onset SLE, and juvenile dermatomyositis, because CV events are rare at young ages.[168] Surrogate measures of CVD, such as flow-mediated dilatation, carotid intima media thickness, and pulse wave velocity have recently been used to explore CVD in children with pediatric-onset rheumatologic diseases. Studies of these measures of subclinical CVD and of lipids have shown increased levels among children with juvenile idiopathic arthritis, pediatric-onset SLE, and juvenile dermatomyositis.[169-173] It is likely that these patients will experience significant CVD morbidity and mortality in adulthood. More studies are needed to better define the increased risk of CVD among patients with pediatric-onset rheumatologic diseases and to design and test preventive strategies to circumvent this eventuality and improve the long-term outcomes of these patients.

Although data on the increased risk of CVD among patients with less widely studied inflammatory rheumatic diseases is still emerging, CVD has been recognized as an important contribution to increased mortality and morbidity in this patient population and represents an area of ongoing research.[174]

## CARDIOVASCULAR RISK ASSESSMENT

As previously noted, both traditional and nontraditional risk factors affect the development of CVD in persons with RA and SLE. Traditional CV risk factors alone cannot account for the increased risk of CVD in these patients.[33,175] This information, together with the findings that traditional CV risk factors behave differently in patients with RA and SLE compared with the general population, suggests that risk scores based on traditional CV risk factors alone are unlikely to accurately estimate CVD risk in RA and SLE. Indeed, authors of recent studies have reported that such risk scores (e.g., Framingham) underestimate CVD risk by twofold in patients with RA.[176] The SCORE, QRISK II, and Reynolds CVD risk calculators also underestimate future CV events in patients with RA, except for those in the highest risk classes.[174,176,177] In the latest European Society of Cardiology guidelines for CVD prevention,[178] immunologic diseases like RA were for the first time mentioned and categorized as a CV risk factor and have similarly been included in the latest version of the British CV risk calculator, the QRISK II.[179] The European League Against Rheumatism (EULAR) recognizes that patients with inflammatory joint diseases have increased risk for CVD and made recommendations for management of CV risk factors based largely on expert consensus.[174] The EULAR task force group was

well aware of the lack of evidence and made a conservative recommendation for a multiplication factor of 1.5 to the calculated CVD risk by SCORE, if the patient with RA had two or more of the following factors: disease duration greater than 10 years, RF/ACPA positivity, or extraarticular manifestations. Corrales and colleagues[180] report that utilizing the 1.5 multiplication factor reclassifies only 0.03% of the patients into more appropriate CVD risk classes, but even then patients at high risk and those with asymptomatic carotid atherosclerosis were not adequately identified. Thus the multiplication factor does not fully adjust for the increased CVD risk in RA. Further development of accurate CVD risk assessment tools for RA and SLE are needed to allow incorporation of inflammatory and disease-related factors that may help predict CVD in these patients, such as the RA-specific risk calculator that is currently being developed by the A TransAtlantic Cardiovascular risk Calculator for Rheumatoid Arthritis (ATACC-RA) consortium.[80,176-178,181-187]

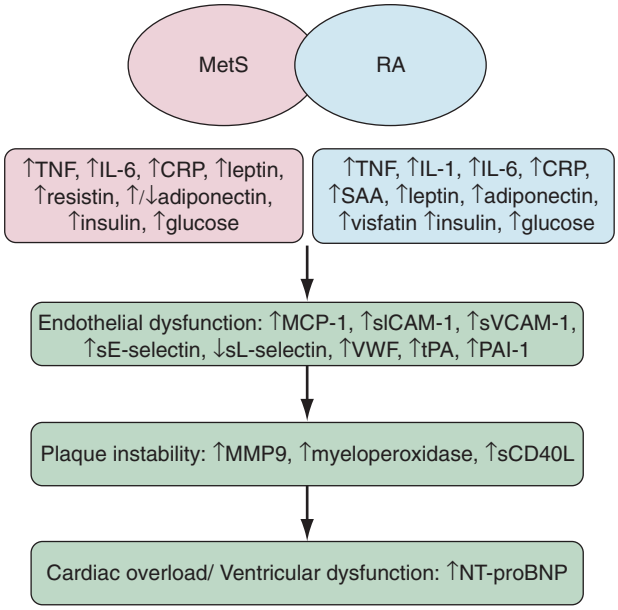



**Figure 36-10** Key soluble biomarkers that are associated with different stages of the atherosclerotic pathway in the metabolic syndrome and rheumatoid arthritis (RA). CRP, C-reactive protein; IL, interleukin; MCP, monocyte chemotactic protein; MetS, metabolic syndrome; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro–brain natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; SAA, serum amyloid A; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; VWF, von Willebrand factor. *(From Kozera L, Andrews J, Morgan AW: Cardiovascular risk and rheumatoid arthritis-the next step: differentiating true soluble biomarkers of cardiovascular risk from surrogate measures of inflammation.* Rheumatology *50:1944–1954, 2011. Permission to reprint from Oxford University Press.)*

## CONNECTION TO THE CLINIC

### Risk Scores and Risk Markers: Biomarkers and Tests for Subclinical Disease

One reason for underestimation of CVD risk in persons with RA may be the high frequency of asymptomatic atherosclerosis,[181-184] which is easily visualized upon ultrasound of the carotid arteries. In addition, carotid artery plaques in patients with RA have been shown to be predictors of a future acute coronary syndrome.[185] Moreover, in the recent European guidelines on CVD prevention,[178] the presence of carotid artery plaques is considered a CVD equivalent, which suggests that including ultrasound of carotid arteries in the CVD risk evaluation would increase the proportion of correct risk stratification.[183,186,187] Other tests for subclinical disease that might be useful for assessing CVD risk in patients with RA include aortic pulse wave velocity and lipoprotein(a).[188] Soluble biomarkers are also appealing because they are typically less costly and have more widespread availability.[68] However, they also may be more difficult to interpret in patients with rheumatic disease because of their dual association with CVD and rheumatic disease (Figure 36-10).[68]

These assessments currently may not be feasible in rheumatology outpatient clinics, but they will provide a great advantage if they are included as a risk evaluation tool in the future. In conclusion, available CVD risk calculators developed for the general population seriously underestimate CVD risk in patients with RA but currently represent the best utility for CVD risk evaluation in these patients.

### Translating Knowledge into Clinical Practice

Despite the large and growing body of knowledge showing increased risk of CVD in persons with RA and other rheumatic diseases even when controlling for traditional CV risk factors,[33,176] little translation into clinical care has occurred. Inflammation and immune mechanisms underlie the atherosclerotic process.[189] Because traditional risk factors alone do not explain the elevated CVD risk in patients with rheumatic diseases, addressing control of inflammation is essential to decrease the CVD risk.[190]

Thus close collaboration between the rheumatologist and the cardiologist is vital.[191-193] Not only does the rheumatologic condition need to be treated aggressively to decrease the inflammatory burden, but the traditional CV risk factors must be identified and appropriately treated. Specialized CV clinics that specifically address the needs of rheumatologic patients allow these patients to be followed up in a more rigorous fashion, with more uniform evaluation and longitudinal follow-up. Examples of such clinics include the Preventive Cardio-Rheuma clinic at the Department of Rheumatology at Diakonhjemmet Hospital in Norway and the Cardio-Rheum clinic at the Division of Cardiovascular Diseases at Mayo Clinic in Rochester, Minnesota. Both models represent deep and ongoing collaborations between the clinical disciplines of rheumatology and preventive cardiology. Integral to both models are (1) extensive patient and provider education regarding CVD risk in the unique setting of autoimmune diseases such as RA; (2) comprehensive CVD risk assessment including history, physical examination, and use of relevant risk assessment scores, as well as noninvasive and (when indicated) invasive measurement techniques; and (3) development and monitoring of an aggressive plan for CVD risk reduction, considering not only traditional CVD risks but also tight control of systemic inflammation and RA disease management. Each program

also includes a clinical research component aimed at continually evaluating and improving the preventive care paradigms.

## Managing CVD Risk in Patients with Rheumatic Disease

Both in Europe and in the United States, patients with RA are significantly less likely to receive CVD preventive measures compared with the general population.[194] A study showed that despite receiving similar treatment, patients with RA who had an MI experienced worse long-term outcomes than did patients with an MI who did not have RA.[23] This deficiency in CVD risk management is amply confirmed in an investigation of CV risk factor control in 836 patients with RA by Primdahl and colleagues.[195] Among the most striking observations is that in the 644 patients without established CVD or diabetes mellitus, inadequate blood pressure and lipid control was documented in 36% and 55% of the participants, respectively. Findings from a preventive cardio-rheuma clinic revealed that 63% of the patients referred were in need of CVD preventive medical intervention.[184]

Compounding the uncertainties surrounding management of CVD risk, no evidence is available regarding CVD preventive treatment effects in patients with RA. To date, there is a lack of published prospective RCTs in RA comparing either different primary prevention strategies (e.g., lipid-lowering or anti-hypertensive medications) or the effects of the different anti-rheumatic drugs using CVD outcomes as the primary endpoint. Such trials require a high number of patients monitored for lengthy periods and have high cost. Promising results from a post hoc analysis of two large statin trials with CVD endpoints showed that effects on lipid reduction and CVD events by statins were comparable in patients with and without inflammatory joint diseases, including RA.[196] A large RCT regarding statin versus placebo (Trial of Atorvastatin for the Primary Prevention of CVD Events in patients with Rheumatoid Arthritis [TRACE RA], including >3000 patients with RA) was recently prematurely terminated because of a low incidence of the primary CVD endpoint and because power was inadequate to be able to reach a clear conclusion.

An important CV risk factor in the general population is lipid levels; however, lipid levels appear to be less important for patients with inflammatory joint diseases.[80] However, meta-analyses of RCTs have shown that patients in the general population benefit from lipid-lowering treatment regardless of baseline lipid levels.[197] Clinical experience regarding use of CVD preventive medications in inflammatory joint diseases is scarce. Experience from a preventive cardio-rheumatology clinic for patients with inflammatory joint diseases revealed that lipid targets were attained using fewer than three consultations in 90% of the patients without serious adverse events.[184] These results indicate that treating patients who have RA with statins and achieving recommended lipid goals is safe. Prospective, longitudinal data are needed to demonstrate whether patients with RA need to achieve the same low lipid targets as persons without RA, or if a relatively smaller or larger reduction in lipids will result in the same CVD protection as in persons without RA.

Modern RA therapy and the multiple comorbidities lead to polypharmacy, thus increasing the possibility of drug to drug interactions with CVD preventive medications such as statins. Interaction between anti-rheumatic treatments as NSAIDs and synthetic or biologic DMARDs with CVD preventive medications is also an area of uncertainty. The influence of statins on clinical response and B cell depletion after rituximab treatment in persons with RA is inconclusive.[198,199] An increased CVD risk associated with NSAID use is recognized in the general population,[120,200] but in a recent Danish nationwide study, the CVD risk associated with use of NSAIDs in persons with RA was modest and significantly lower than in persons without RA.[201] Furthermore, NSAIDs interfere with the thrombocyte inhibitory effect of aspirin, which complicates secondary CVD prevention.[202] Observational studies have provided indications that use of synthetic and biologic DMARDs is associated with reduced CVD risk in persons with RA.[122,124] However, it is unknown whether this effect is related to the reduction in inflammation opposing the increased levels of atherogenic lipids that occur with biologic DMARDs and improvement of HDL function.[203] Studies to clarify these issues are needed.

CVD prevention in patients with RA is fraught with concerns of drug- and disease-related complexities. Therapies of rheumatic conditions have evolved enormously in the past two decades, as have CVD prevention strategies. The available literature suggests substantial underuse of CVD preventive pharmacotherapy in the general population.[204,205] According to the latest DYSlipidemia International Study (DYSIS) report, more than half of all patients were not reaching their LDL treatment goal in general practice.[204] These findings are also supported by the Eurospire III report, which indicates that clinical implementation of secondary prevention treatments remains suboptimal.[205]

Efforts to address the high CVD risk in patients with rheumatic disease should be a focus in the cardiology community and among rheumatologists. It is hoped that such a focus will result in more patients with RA being evaluated for CVD risk and the institution of preventive measures when necessary.

## Patient Awareness of Cardiovascular Disease Risk

Communicating future risk and the need for preventive treatment is one of the most challenging aspects of the patient/health professional interaction. Awareness of patients about their CVD risk is important for successful implementation of preventive strategies related to lifestyle modifiable CV risk factors.[206] Although such programs for assessing CVD risk in persons with RA have been developed, it will take a great effort to fully disseminate them. These strategies include education of health care professionals, written material, and online and group education of patients and their families. Even starting with simple information and brochures as a precursor to implementing a full education program is a step forward. It is of the utmost importance for patients with rheumatic diseases to understand that effective CVD risk management comprises not only adequate treatment of conventional CV risk factors but also tight control of the rheumatic disease activity.

## FUTURE DIRECTIONS

CVD remains a major problem for people with systemic inflammatory diseases. Systemic inflammation and its interplay with traditional and nontraditional CV risk factors appear to have a major role. Future work should focus on further delineating the underlying biologic mechanisms involved, developing and evaluating risk assessment tools and biomarkers, and prevention and treatment strategies specific to rheumatic disease populations. Optimal control of traditional risk factors is imperative but insufficient to reduce CVD risk for people with rheumatic diseases. Tight control of systemic inflammation will be required for optimal results.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 37

# Cancer Risk in Rheumatic Diseases



Eric L. Matteson

**KEY POINTS**

- Risk of malignancy, especially lymphoproliferative malignancy, is increased in autoimmune rheumatic diseases.
- The occurrence of cancer in patients with rheumatic diseases adversely affects quality of life and life expectancy compared with the general population.
- This risk is related to the pathobiology of the underlying rheumatic disease, including the inflammatory burden, immunologic defects such as overexpression of *Bcl*-2 oncogenes, traditional risk factors such as smoking, and, in some cases, associated viral infection.
- Several of the immunomodulatory treatments used in the management of autoimmune disease, especially chemotherapeutic agents, are associated with an increased risk of cancer.
- The decision to use immunomodulating therapies in patients with rheumatic disease must take into account host and environmental risk factors for cancer. Effective screening and monitoring strategies can markedly reduce the risk of cancer in these patients.

Systemic rheumatic diseases have been associated with an increased risk of development of malignancy. This increased risk is the result of fundamental underlying immunologic effects of autoimmunity on cancer risk and on the risk of cancers associated with drug treatments for rheumatic diseases.

Accelerated growth of cancer cells in immunodeficient mice and increased risk of cancer in heavily immunosuppressed transplant patients have shaped the perception of the immune system as a potent barrier against neoplasms.[1,2] It might be expected that immunosuppressive treatment would inevitably result in effects favoring malignant cell growth. However, emerging evidence supports the seemingly paradoxical notion, perhaps formulated first by Rudolph Virchow in 1863, that inflammation is a critical component of cancer initiation and progression, and that reduction of systemic inflammation may reduce cancer risk in these conditions.[3]

Assessment of cancer risk in rheumatic diseases must be weighed against the lifetime risk of developing cancer, which is approximately 20% in Western Europe and North America, with 5% of the general population having current cancer or a history of cancer.[4] Breast cancer will develop in approximately 1 in 10 women , prostate cancer will develop in as many as 1 in 8 men, colorectal cancer in 1 in 25, lung cancer in 1 in 40, and approximately 1 in 100 will develop lymphoma or other lymphoproliferative malignancy.[4]

The combination of increased risk for some and decreased risk for other types of cancers in different rheumatic diseases may result in a neutral effect for malignancies in general, emphasizing why, from a clinical standpoint, it is important to identify risks pertaining to specific cancers, which may be uncommon. The statistical approach for capturing differences in sparse event data, particularly when malignancy is not a prespecified study outcome, and assumptions of proportional hazards models and stable frequencies of events during a period of time for a nonlinear risk such as cancer can lead to major errors in interpretation.

## MALIGNANCY IN AUTOIMMUNE RHEUMATIC DISEASES

Several rheumatic diseases, particularly lymphoproliferative disorders, appear to be associated with increased risk of malignancy. A list of rheumatic diseases that have been associated with malignancy is provided in Table 37-1. A global assessment of susceptibility to Hodgkin's lymphoma that used population-based linked registry data from Sweden and Denmark identified significantly increased risks of Hodgkin's lymphoma associated with a personal history of autoimmune disorders, including rheumatoid arthritis (RA; odds ratio [OR], 2.7; 95% confidence interval [CI], 1.9 to 4.0), systemic lupus erythematosus (OR, 5.8; 95% CI, 2.2 to 15.1), sarcoidosis (OR, 14.1; 95% CI, 5.4 to 36.8), and immune thrombocytopenic purpura (OR, ∞; $P = 0.022$).[5] A statistically significant increase in the risk of Hodgkin's lymphoma was associated with family histories of sarcoidosis (OR, 1.8; 95% CI, 1.01 to 3.1) and ulcerative colitis (OR, 1.6; 95% CI, 1.02 to 2.6).[5]

The occurrence of cancer has a profound effect on the already compromised quality of life of patients with rheumatic diseases and may affect survivorship and influence treatment decisions. A population-based study of cancer survival in patients with inflammatory arthritis from Great Britain suggests decreased survival compared with the general population.[6] Survivorship related to the occurrence of cancer in this population was unrelated to disease-modifying anti-rheumatic drug (DMARD) exposure.[6]

**TABLE 37-1** Rheumatic Diseases Associated with Malignancy

| Connective Tissue Disease | Malignancy | Associated Factors | Clinical Alert |
|---|---|---|---|
| Sjögren's syndrome | Lymphoproliferative disorders | Glandular features: lymphadenopathy, parotid or salivary enlargement<br>Extraglandular features: purpura, vasculitis, splenomegaly, lymphopenia, low C4, cryoglobulins | Clues to progression from pseudolymphoma to lymphoma include worsening of clinical features, disappearance of rheumatoid factor, and decline of IgM |
| Rheumatoid arthritis | Lymphoproliferative disorders | Presence of paraproteinemia, greater disease severity, longer disease duration, immunosuppression, Felty's syndrome | Rapidly progressive; refractory flare in long-standing rheumatoid disease may suggest an underlying malignancy |
| Systemic lupus erythematosus (SLE) | Lymphoproliferative disorders | — | Non-Hodgkin's lymphoma should be considered in SLE patients who develop adenopathy or masses; lymphoma of the spleen is another cause of splenic enlargement in SLE |
| Systemic sclerosis (scleroderma) | Alveolar cell carcinoma<br>Nonmelanoma skin cancer<br>Adenocarcinoma of the esophagus | Pulmonary fibrosis, interstitial lung disease<br>Areas of scleroderma and fibrosis in the skin<br>Barrett's metaplasia | Annual chest radiograph after fibrosis is detected<br>Change in skin features or poorly healing lesions should be evaluated<br>If indicated, esophagoscopy and biopsy of distal esophageal constricting lesions |
| Dermatomyositis | Ovarian, lung, and gastric cancers in Western populations; nasopharyngeal carcinoma in Asian populations | Older age, normal creatinine kinase levels, presence of cutaneous vasculitis; less likely in setting of myositis-specific antibodies | Malignancy evaluation needs to be tailored to individual patient's age, symptoms, and signs |

## RHEUMATOID ARTHRITIS

### KEY POINTS

**Rheumatoid arthritis is associated with a greater than twofold increased risk of lymphoma. This risk is higher in patients with high disease activity and with more severe disease, including extra-articular involvement.**

**The risk of solid malignancies is variable, with an increased risk of lung cancer and likely decreased risk of colorectal cancer, breast cancer, and cancers of the urogenital tract in both men and women.**

A considerable body of evidence supports rheumatoid arthritis as a pathogenic factor in the development of lymphoma. A standardized incidence ratio (SIR) of 2.4 for lymphoma was described in a population of more than 20,000 Danish patients, as was an increased risk of 1.8 (95% CI 1.5-2.2) in 19,591 U.S. patients.[7,8]

With use of a different approach, a pooled SIR of 3.9 for lymphoma was found by using a random effects model in a meta-analysis.[9] Studies that used other methods have found ORs of 1.3 to 1.5 for lymphoma in case-control studies.[10]

Patients with RA may be at higher risk for non-Hodgkin's lymphoma, especially diffuse large B cell type.[9] Large B cell lymphomas represent as much as two-thirds of non-Hodgkin's lymphomas in patients with RA[5,6]—approximately twice the rate of diffuse large B cell lymphoma as a proportion of overall non-Hodgkin's lymphoma in the general population. The immunophenotype, grade, and histology of lymphomas in patients with RA are not different from the general population.

In many studies, the risk of cancer is particularly increased early in the disease course, and cancer risk appears to be greater in patients who have persistently high disease activity, high cumulative disease activity, and more severe disease, and in those who have positive rheumatoid factor (SIR, 3.6; 95% CI, 1.3 to 7.8).[10,11] The unadjusted OR for average disease activity, comparing highest versus lowest quartile, was 71.3 (95% CI, 24.1 to 211.4), and the OR for cumulative disease activity of the tenth decile versus the first decile was 61.6 (95% CI, 21.0 to 181.0) in a case-control registry study from Sweden.[11]

Extraarticular disease of RA, particularly Felty's syndrome and Sjögren's syndrome, confers a further increased risk of non-Hodgkin's lymphoma; one study of 906 men with RA revealed a twofold increase in total cancer incidence among patients with RA who have Felty's syndrome.[12] Large granular T cell leukemia (T-LGL) may, rarely, occur in association with RA.[13] T-LGL in RA usually is chronic and in rare cases becomes aggressive.

Whether patients with RA are at higher risk for lymphoproliferative disorders other than lymphoma is unclear. A meta-analysis of 21 publications from 1990 to 2007 summarized the risk of malignancy in patients with RA.[14] The risk of lymphoma was increased approximately twofold (SIR, 2.08; 95% CI, 1.8 to 2.39), with greater risks of Hodgkin's and non-Hodgkin's lymphoma. The risk of lung cancer was increased, with an SIR of 1.63 (95% CI, 1.43 to 1.87). The risk of colorectal cancer was decreased (SIR, 0.77; 95% CI, 0.65 to 0.90), as was the risk of breast cancer (SIR, 0.84; 95% CI, 0.79 to 0.90). The overall SIR for malignancy was slightly increased, at 1.05 (95% CI, 1.01 to 1.09). The overall increased risk of cancer in patients with RA was largely caused by the increased risk of lymphoproliferative cancers.

The risk of lung cancer appears to be higher among patients with RA. U.S. veterans with RA were 43% more likely (OR, 1.43) to develop lung cancer compared with patients without RA, after adjustments were made for age, gender, race, and tobacco and asbestos exposure. The risk for lung cancer in British patients on nonbiologic DMARDs was found to be higher as well (SIR, 2.39; 95% CI, 1.79, 5.07.[15,16]

Details from the study of 42,262 patients hospitalized with RA from 1980 to 2004 indexed to the Swedish National Cancer Registry found that SIRs for Hodgkin's and non-Hodgkin's lymphoma were increased for upper digestive tract cancers and for squamous cell skin cancer, and detected an excess of nonthyroid endocrine tumors in patients with RA.[17] Colon and rectal and endometrial cancers were less frequent in patients with RA. The decreased risk of colorectal cancer may be attributable to the use of long-term nonsteroidal anti-inflammatory agents in patients with RA.[18]

In summary, cancer is frequent in the general population and is at least as common among patients with RA. Following a diagnosis of rheumatoid arthritis at the typical age of 55 years, one in five patients will be diagnosed with cancer; however, in the great majority of patients, the cancer cannot be linked to RA or to its treatment but rather reflects the background cancer risk.

## SYSTEMIC LUPUS ERYTHEMATOSUS

**KEY POINTS**

**The risk of lymphoma is at least twofold increased in systemic lupus erythematosus (SLE).**

**Risks of solid malignancy, including lung, thyroid, kidney, and skin cancers, are increased overall in SLE, although rates of cervix and prostate cancers appear to be somewhat lower. Breast cancer risk has been reported to be increased in some studies and decreased in others.**

The risk of at least certain malignancies appears to be increased in patients with SLE. Overall SIR ranges from 1.1 to 2.6,[19] with the most increased risk that of lymphomas (SIR, 3.57 for non-Hodgkin's lymphoma and 2.35 for Hodgkin's disease).[19,20] The risk is especially high for diffuse large B cell lymphoma, often of aggressive subtypes.[21,22]

A large multicenter (23 centers) international cohort of 9547 patients with an average follow-up of 8 years confirmed an increased risk of cancer in patients with SLE.[20] For all cancers combined, the SIR estimate was 1.15 (95% CI, 1.05 to 1.27); for all hematologic malignancies, it was 2.75 (95% CI, 2.13 to 3.49); and for non-Hodgkin's lymphoma, it was 3.65 (95% CI, 2.63 to 4.93). The data also suggest increased risks of lung cancer (SIR, 1.37; 95% CI, 1.05 to 1.76) and hepatobiliary cancer (SIR, 2.60; 95% CI, 1.25 to 4.78).[20]

Patients with SLE in a California statewide patient hospital discharge database from 1991 to 2001 were followed up by using cancer registry data to compare observed versus expected numbers of cancers based on age, sex, and specific incidence rates in the California population.[23] A total of 30,478 SLE patients were observed for 157,969 person-years. There were a total of 1273 cancers for an overall significantly increased cancer risk (SIR, 1.14; 95% CI, 1.07 to 1.20). Patients with SLE had higher risks of vagina/vulva (SIR, 3.27; 95% CI, 2.41 to 4.31) and liver cancers (SIR, 2.70; 95% CI, 1.54 to 4.24). Elevated risks of lung, kidney, and thyroid cancers and hematopoietic malignancies were also observed, with lower rates of screenable cancers, including breast cancer, cervix cancer, and prostate cancer. Drug effects were not assessed.[23]

Other studies have reported possibly increased risk of malignancies other than lymphoproliferative cancers in SLE. Patients may be at slightly higher risk of thyroid cancer.[24] An increased risk of squamous cell skin cancer was found in 238 patients with SLE.[25] The risk of breast cancer may be increased by approximately 1.5-fold to twofold compared with the general population, even after consideration of age, parity, family history, and exogenous estrogens.[19,26] The risk of abnormal Pap smears and cervical dysplasia appears to be higher in women with SLE than in those without, although the risk of invasive cervical cancer is not increased.[20]

The origin of any risk of the development of malignant disorders in SLE remains unclear, although it does not appear to be associated with the use of immunosuppressive or cytotoxic agents; most cohorts are too small to allow for detection of a statistically meaningful risk increase in rare events during short periods of observation. Race and ethnicity have not been identified as major factors in cancer risk in SLE.[27] Antimalarial drug use does not appear to affect the relative risk (RR) of malignancy, as was postulated in early studies.[28]

Risk factors for the development of hematologic malignancies may relate to inflammatory burden and disease activity, immunologic defects and overexpression of *Bcl-2* oncogenes, and viruses, especially Epstein-Barr virus (EBV).[29] A nested case-control study that included 6438 patients with SLE linked to the national cancer registry in Sweden found that leukopenia, independent of immunosuppressive treatment, was a risk factor for these leukemias. Bone marrow investigation was suggested for SLE patients with long-standing leukopenia and anemia.[30] Disease characteristics predisposing to non-Hodgkin's lymphoma include longer disease duration and increased disease activity with moderately severe end-organ damage.[31]

Women with SLE, likely out of concern for treatment side effects and effects of pregnancy on disease control, are less often exposed to oral contraceptives and are more likely to be nulliparous, which may affect their malignancy risk. On the other hand, the possible increase in breast cancer risk suggests that other, poorly understood factors may increase this risk in women with SLE, whereas at least one study suggests that patients with SLE are less likely than healthy women to undergo breast cancer screening.[32] Patients with SLE also appear to be less likely to undergo routine Pap testing. Increased prevalence of human papillomavirus infection and immunosuppression have been implicated in the apparent increase in prevalence of abnormal Pap smears and cervical dysplasia in patients with SLE.[33]

Women with SLE may be at higher risk of lung cancer, for which smoking is a predictor.[29] Similar to RA, smoking is a risk factor for both SLE and lung cancer, reflecting a complex interplay of disease susceptibility factors.

## SYSTEMIC SCLEROSIS (SCLERODERMA)

**KEY POINTS**

- Risks of lymphoma, skin cancer, and lung cancer are markedly increased in scleroderma.
- Scleroderma-related risk factors for malignancy include esophageal disease related to Barrett's esophagus and lung cancer related to pulmonary fibrosis.

The risk of malignancy in patients with scleroderma appears to be increased in most reviews and reports, although at least one population-based study failed to detect increased risk.[34,35] Generally, estimates of malignancy risk in scleroderma range from SIRs of 1.5 to 5.1, compared with the general population.[36,37] The highest SIRs for individual cancers are for lung cancer, with an incidence ratio of as high as 7.8, and non-Hodgkin's lymphoma, with an incidence rate ratio (IRR) of 9.6.

A population-based disease registry and cancer registry retrospective cohort linkage study from Sweden following up patients from 1965 to 1983 revealed an SIR of 1.5 for overall cancer, with the highest rates for lung cancer (SIR, 4.9), skin cancer (SIR, 4.2), hepatoma (SIR, 3.3), and hematopoietic malignancies (SIR, 2.3).[36] A cohort study of patients followed up from 1987 to 2002 revealed a similar magnitude of increased overall risk of malignancy (SIR, 1.55) with the observation of markedly increased risks of oropharyngeal cancer (SIR, 9.63; 95% CI, 2.97 to 16.3) and esophageal cancer (SIR, 15.9; 95% CI, 4.2 to 27.6).[36] Esophageal disease associated with systemic sclerosis is the likely reason for the increased incidence of Barrett's esophagus, which has been reported in 12.7% of patients with scleroderma.[37]

A high rate of abnormal Pap tests in women with onset of scleroderma before the age of 50 years has been reported, with a lifetime prevalence by self-report of 25.4% (95% CI, 20.9 to 30.4) compared with a self-reported prevalence of abnormal Pap tests in the general Canadian population of 13.8% (95% CI, 11.6 to 16.4). A significant relationship was found between self-reported abnormal Pap tests and diffuse disease and younger age at disease onset.[38]

Lung cancer has been reported to account for as many as 30% of all cancers in patients with scleroderma; it is thought to be related to fibrosis. The association with smoking is uncertain and studies are contradictory, with some reporting a link and others failing to find a link to smoking.[35,36,39]

The mechanisms of malignancy in scleroderma are largely unexplored. Risk factors for development of malignancy in patients with scleroderma may be related to inflammation and fibrosis of affected organs. The link to smoking is controversial.[35,39] As in some other autoimmune rheumatic diseases, the risk appears higher early in the disease, and patients who are older at the time of diagnosis may be at higher risk as well.[36,40] It is not certain whether the presence of scleroderma-specific antibodies, including topoisomerase I (Scl-70) and RNA polymerase I/III autoantibody, defines an increased risk for development of cancer in these patients.[34,40,41] Some patients with scleroderma harbor autoantibodies to RPC1, an RNA polymerase subunit encoded by the *POLR3A* gene that may drive both cancer and the disease.[41] In contrast to systemic sclerosis, localized scleroderma, including morphea and linear scleroderma, has not been associated with increased risk of cancer.[42]

## IDIOPATHIC INFLAMMATORY MYOPATHY

**KEY POINTS**

- The risk of cancer in patients with idiopathic inflammatory myopathies is approximately five to seven times higher than in the general population.
- Malignancy is strongly associated with dermatomyositis and, if present, is often detectable at disease outset.
- The most common malignancies in inflammatory myositis are adenocarcinomas.
- Suspicion for cancer should be high, especially in patients with active muscle and skin inflammation but normal creatine kinase levels, age older than 50 years, and periungual erythema.

Both dermatomyositis and polymyositis occurring in adults have been associated with malignancies. The link to malignancy in newly diagnosed patients with dermatomyositis is strongest, although in neither condition is the origin of the association well understood. The association between malignancy and polymyositis and inclusion body myositis is less strong.

Assessment of risk is complicated by the temporal relationship between the development of malignancy and cancer. In particular, some cancers predate the onset of inflammatory myopathy, so that the inflammatory myopathy can be better considered a paraneoplastic syndrome (see Chapters 85 and 123); it is also likely that the presence of inflammatory myopathies represents a risk factor for the subsequent development of malignancy.[43]

The incidence of cancer in patients with inflammatory myositis is approximately five to seven times higher than in the general population.[44] The prevalence of malignancy is approximately 25%; cancers are reported more frequently in dermatomyositis, occurring in 6% to 60% of patients, and in 0% to 28% of patients with polymyositis.[45]

In most studies, cancers manifest within 2 years before or after the initial diagnosis of inflammatory myopathy.[46,47] Inflammatory myopathies may initially manifest with the recurrence of a previously diagnosed cancer. A previously diagnosed but inactive inflammatory myopathy may become reactivated with occurrence of a cancer, supporting the hypothesis of autoantigens as drivers of the inflammatory disease.

The strength of the association between malignancy and inflammatory myositis varies. One study done at the Mayo Clinic failed to reveal an increased risk of malignancy in patients with inflammatory myopathy, and a more recent registry study from Sweden of 788 patients diagnosed with dermatomyositis or polymyositis between 1963 and 1987

revealed that 15% of 392 patients with dermatomyositis had cancer diagnosed concurrently with or after the diagnosis of dermatomyositis, with a relative risk of cancer of 2.4 (95% CI, 1.6 to 3.6) for males and 3.4 (95% CI, 2.4 to 4.7) for females.[43,46] Of 396 patients with polymyositis, 9% had cancer at or after the time of diagnosis of polymyositis, with the RR of 1.8 for development of cancer (95% CI, 1.1 to 2.7) in males and 1.7 (95% CI, 1.0 to 2.5) in females.

A population-based retrospective cohort study from Victoria, Australia, of 537 patients with biopsy-proven dermatomyositis and polymyositis reported an RR for malignancy in dermatomyositis compared with polymyositis of 2.4 (95% CI, 1.3 to 4.2), with a higher SIR for dermatomyositis than polymyositis (6.2 vs. 2).[47] Finally, ORs for association of cancer with dermatomyositis from a large meta-analysis were reported to be 4.4, and for polymyositis, 2.1.[48]

A wide range of malignancies are associated with dermatomyositis and polymyositis. The most common malignancies in populations of Northern European descent are adenocarcinomas of the cervix, lungs, ovaries, pancreas, bladder, and stomach, which account for more than two-thirds of these cancers.[43,44,48] In patients from Southeast Asia, a higher proportion of nasopharyngeal cancers are found, followed by lung cancer.[49]

The association of cancer is less well understood for more unusual forms of inflammatory myopathies. Amyopathic dermatomyositis, a rare form of dermatomyositis with typical cutaneous but no muscle involvement, can be associated with the development of cancer, but the frequency of this condition is low, so that no stable estimates of cancer risk are available.[50] Inclusion body myositis has not been well studied for the same reasons, although the overall risk of cancer of 2.4 suggests a possible link.[47]

It is likely that relevant antigens are expressed in the underlying tumor and affected muscle. Myositis-specific antigens develop during the process of regeneration in patients who have myositis; these are the same antigens expressed in some cancers known to be associated with the development of inflammatory myopathies.[51] This relationship is supported by the finding that antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1γ are present in most (83%) patients with cancer-associated dermatomyositis.[52] A link between malignancy and inflammatory myositis is further supported by observations that in many cases myositis improves after removal of the malignancy.[53]

Clinical disease characteristics that may portend higher malignancy risk include active inflammatory disease with normal serum levels of creatine kinase, distal extremity weakness, pharyngeal and diaphragmatic involvement, and leukocytoclastic vasculitis.[54,55] In one study of 92 patients, other independent risk factors for the development of cancer in patients who have dermatomyositis included age at diagnosis older than 52 years (hazard ratio [HR], 7.24; 95% CI, 2.35 to 22.41), rapid onset of skin and/or muscle symptoms (HR, 3.11; 95% CI, 1.07 to 9.02), periungual erythema (HR, 3.93; 95% CI, 1.16 to 13.24), low baseline level of complement factor C4 (HR, 2.74; 95% CI, 1.11 to 6.75), and, possibly, topoisomerase I.[56,57] A low baseline lymphocyte count was a protective factor for malignancy (HR, 0.33; 95% CI, 0.14 to 0.80), although the number of assessable patients was small.[56]

## SJÖGREN'S SYNDROME

**KEY POINTS**

**The risk of lymphoproliferative cancers, especially various types of lymphoma, is at least sixfold increased in patients with primary Sjögren's syndrome.**

**Immunologic perturbations, including *p53* mutations and B cell activation, as well as *Helicobacter pylori*, are likely predisposing risk factors.**

Patients with primary Sjögren's syndrome are at increased risk of lymphoproliferative diseases. The overall cancer risk is increased compared with the general population (pooled RR, 1.53; 95% CI, 1.17 to1.88).[57] The relative risk for the development of lymphoproliferative disorders in these patients ranges from 6 to 44 in individual studies, and a meta-analysis of cohort studies reported a pooled SIR of 18.8.[58] Lymphoproliferative disorders eventually occur in between 4% and 10% of patients with primary Sjögren's syndrome, with a lifetime risk of non-Hodgkin's lymphoma of approximately 5%.[8,58-61]

In addition to non-Hodgkin's lymphoma, forms of lymphoproliferative disease seen in patients with Sjögren's syndrome include low-grade B cell lymphoma and diffuse large B cell lymphoma, including follicular center lymphoma. Less commonly seen lymphoproliferative diseases include lymphocytic leukemia, Waldenström's macroglobulinemia, and multiple myeloma.[60] Except for thyroid cancer (RR, 2.58), the risk of other cancers does not appear to be particularly high in patients with Sjögren's syndrome.[57,60,61] The development of lymphoma and malignancy in patients with Sjögren's syndrome does not appear to affect or cause mortality.[8,61]

The pathoetiology of lymphoproliferative diseases occurring in patients with Sjögren's syndrome is unclear. It is likely that the B cell activation characteristic of Sjögren's syndrome is a predisposing risk factor. Most lymphomas in Sjögren's syndrome appear to arise from lymphoepithelial sialadenitis or benign lymphoepithelial lesions, perhaps associated with *p53* mutations.[62] Infectious agents such as hepatitis C and Epstein-Barr virus have been implicated, although the nature of this relationship remains speculative. *Helicobacter pylori* is associated with MALT (mucosa-associated lymphoid tissue) lymphoma in Sjögren's syndrome.[63]

Appropriate evaluation in symptomatic patients should include diagnostic testing for *H. pylori* in this setting.[64] The link of cancer in Sjögren's syndrome to the proto-oncogene *Bcl-2* translocation is, as yet, not clearly defined but may be helpful for early detection of malignancy.[65,66]

## VASCULITIS

**KEY POINT**

**It is unclear whether the risk of cancer development is increased in vasculitis independent of drug treatment effects.**

Vasculitis as a paraneoplastic syndrome may be present in about 8% of patients with malignancy (see Chapter 123).[67] Not as well studied is the risk of primary malignancy in

patients with vasculitis. Most cases appear to be related to treatment, although one study that used the Danish Cancer Registry suggested an increased risk of nonmelanoma within 2 years of the vasculitis diagnosis in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (granulomatosis with polyangiitis [GPA]) (OR, 4.0; 95% CI, 1.4 to 12).[68]

Current data do not support a link between ANCA-associated vasculitis (GPA) and malignancy as a trigger for vasculitis, or the vasculitis itself as a trigger for malignancy, independent of treatment effects. However, data from several studies suggest a standardized incidence ratio of cancer in AAV of 1.6 to 2.0 compared with the general population and a possibly higher risk in GPA than in microscopic polyangiitis (MPA).[69]

The risk of malignancy among patients with giant cell arteritis was not increased in a population-based study of 204 patients with giant cell arteritis and 407 age- and sex-matched controls.[70]

## SERONEGATIVE SPONDYLOARTHRITIS

### KEY POINT

**The overall risk of cancer does not appear to be increased in seronegative spondyloarthropathies.**

The risk of cancer among patients with spondyloarthropathies is not as well studied as that of patients with RA and other connective tissue diseases. A cohort study from Canada of 665 patients with psoriatic arthritis revealed an SIR for all cancers of 0.98 (95% CI, 0.77 to 1.24) without evidence of increase in cancer type–specific SIR for hematologic, lung, or breast cancer.[71] A U.S.-based study of 2970 patients with psoriatic arthritis and 19,260 patients with RA found that overall malignancy incidence was similar at 0.56 per 100 person years for both diseases, and incidence rates were similar across malignancy subtypes.[72]

No overall increased risk of cancer has been noted among patients with ankylosing spondylitis, as was reported in a Swedish national cohort of patients with ankylosing spondylitis admitted to Swedish hospitals from 1965 to 1995, linked to the Swedish National Cancer Registry and Death Registry. These patients appear to not have increased risk of malignant lymphoma.[73,74] A population-based cohort study of patients with ankylosing spondylitis from Australia suggests a prevalence of malignancy of 6.8%, which parallels baseline population prevalence rates reported in Western populations.[75] In contrast, an increased rate of cancer in Asians with ankylosing spondylitis was reported using national health insurance system data in Taiwan. Matching each of 4133 patients with 4 comparators, the HR for all cancers was 1.38 (95% CI, 1.18 to 1.60).[76]

## CANCER RISKS ASSOCIATED WITH ANTI-RHEUMATIC DRUG THERAPIES

### KEY POINTS

**The effect of therapies used in the treatment of autoimmune rheumatic diseases on cancer can be difficult to distinguish from the underlying risk intrinsic to the diseases.**

**Nonsteroidal anti-inflammatory drugs and glucocorticosteroid have not been associated with increased risk of cancer.**

**The risk of cancer with chemotherapeutic drugs is greatest in patients who have had the highest cumulative exposure to these agents.**

**Chronic active inflammation is related to increased risk of lymphoma in RA. Effective management of the underlying disease may reduce this risk.**

Assessment of risk associated with both nonbiologic and biologic DMARDs is challenging because of the overall generally high burden of cancer in the population, the variable rheumatic disease–related cancer risk, and the potential risk of cancer associated with agents used to treat them. Disease severity may be a risk factor for developing cancer, introducing confounding or channeling bias if patients with severe disease are treated more intensively with immunomodulatory agents. The sequential and combined use of immunomodulatory agents further complicates the assessment of risk related to individual agents. A further concern, as with all immunosuppressive drugs, is the oncogenic potential of immunosuppressive therapies in patients who have a pre-existent or concurrent cancer, and whether such patients should be treated with DMARDs, and if so, which DMARDs should be given. Major guidelines for management of rheumatic diseases lack recommendations for DMARD use in patients with pre-existing malignancies [77,78]

Nonsteroidal anti-inflammatory agents and glucocorticosteroids do not appear to be associated with increased risk of malignancy in patients with RA nor other rheumatic diseases.[79] In a large population-based cohort study from Sweden, a total duration of oral corticosteroid treatment of less than 2 years was not associated with lymphoma risk (OR, 0.87; 95% CI, 0.51 to 1.5), whereas treatment lasting longer than 2 years was associated with a lower lymphoma risk (OR, 0.43; 95% CI, 0.26 to 0.72).[80] The duration of RA at initiation of oral corticosteroids did not affect lymphoma risk. Whether this observed reduction in lymphoma risk may be due to decreased disease activity, is a generic effect of corticosteroids, or is specific to RA, is uncertain.[80]

## NONBIOLOGIC DISEASE-MODIFYING ANTI-RHEUMATIC DRUG THERAPY

### KEY POINTS

**The nonbiologic disease-modifying anti-rheumatic drugs (nb-DMARDs) gold, hydroxychloroquine, penicillamine, and sulfasalazine are not associated with an increased risk of cancer.**

**The risk of lymphoma, especially B cell lymphoma, may be increased with methotrexate. Some patients may experience regression with discontinuation of methotrexate.**

**Use of azathioprine is associated with increased risk of lymphoma, and alkylating agents are particularly associated with increased risk of several types of malignancies, including bladder and lung cancer and leukemia in patients treated with cyclophosphamide.**

The nb-DMARDs sulfasalazine, hydroxychloroquine, gold, and penicillamine do not appear to be associated with an increased risk of cancer. Radiation is no longer used for the treatment of rheumatic diseases and will not be further addressed. Although no increase in cancer occurrence has been reported, few data are available regarding the long-term risks of malignancies occurring with leflunomide.[81]

All of the anti-metabolites used in prevention of transplant rejection and treatment of cancer have tumorigenic potential and, in general, may, or do, confer an increased risk of malignancy. The risk of cancer is increased with chemotherapeutic nb-DMARDs, particularly cyclophosphamide, and the risk of certain cancers, particularly lymphoproliferative disorders, may be increased with the use of other nb-DMARDs, such as azathioprine, methotrexate, and cyclosporine, although data are relatively sparse. Data regarding cancer risk in rheumatic diseases in patients treated with mycophenolate mofetil are few, although cancers clearly do occur in patients treated with this drug.[82]

Overall risk for cancer in patients with RA treated with nb-DMARDs was found to be 28% higher compared with the general population in a review of 3771 patients from the British Society for Rheumatology Biologics Register.[16] The cancer risk appears to be greatest in patients who have had the highest cumulative exposure to DMARDs, compared with patients with less than 1 year of exposure (SIR, 4.82).[83] Exposure to azathioprine, cyclosporin or cyclophosphamide increased the relative risk for cancer 65% in the British study, although only 367 patients had taken one of these drugs.[16]

### Methotrexate

The overall malignancy risk attributable to methotrexate treatment in patients with rheumatic diseases does not appear to be increased, although numerous studies suggest that the risk of lymphoproliferative disease may be increased. This increase may be related to higher weekly doses, but not the cumulative dose of methotrexate.[84]

Most cases of methotrexate-associated lymphomas reported in the literature are B cell lymphomas, often with extranodal involvement.[85] Assays for EBV in one study revealed that 7 of 17 patients (41%) were positive.[85] Further evidence supporting a link between methotrexate use and the development of lymphoma comes from observations of spontaneous remission of B cell lymphoma in 8 of 50 cases, including 4 that were positive for EBV.[85] This suggests that methotrexate may potentiate persistent immunologic stimulation, clonal selection, and malignant transformation of B cells by direct oncogenic action, decreased apoptosis of infected B cells, and decreased natural killer cell activity.[85]

### Azathioprine

The use of azathioprine may be associated with increased risk of lymphoproliferative disorders. Studies from a Canadian azathioprine registry revealed increased rates of lymphoproliferative disorders in patients with RA compared with the general population (SIR, 8.05).[86] A study from Britain also revealed a markedly increased risk of development of lymphoma in RA patients treated with azathioprine, with an estimated 1 case of lymphoma per 1000 patient-years of azathioprine treatment.[87] Risk was highest in patients on higher daily doses of azathioprine of as much as 300 mg per day.

The risk of leukemia in patients with SLE treated with azathioprine has been generally reported as not increased, although one study reported an increase in risk.[88] In as many as 24 years of longitudinal follow-up, 5.4% of patients treated with azathioprine developed malignancies, none of which were lymphomas, compared with 6.7% of patients who had never received azathioprine, three of whom developed lymphoma.[88]

### Cyclosporine

Relatively few patients with rheumatic diseases treated with cyclosporine have been followed for protracted periods, making assessment of malignancy risk in these patients difficult. Similar to methotrexate, cyclosporine has been associated with the development of EBV-associated lymphomas in a few patients with rheumatoid arthritis.[89] A retrospective study aggregating experience from clinical trials of more than 1000 patients with RA treated with cyclosporine failed to demonstrate an increased risk of malignancy, at least beyond that seen with other DMARDs.[90]

### Alkylating Agents

The use of alkylating agents in patients with RA, systemic lupus erythematosus, and vasculitis has been associated with an increase in non-Hodgkin's lymphoma, leukemia, skin cancer, bladder cancer, and solid malignancies.[91,92,93] Considerably more experience with cyclophosphamide than chlorambucil has been documented in these diseases.

Cyclophosphamide use in rheumatic diseases is associated with an overall increased risk of developing malignancy of between 1.5 and 4.1, compared with control subjects. This risk is best studied in AAV (especially necrotizing GPA), in which risk is highest for bladder cancers (SIR, 4.8), leukemia (SIR, 5.7), and lymphoma (SIR, 4.2). Bladder cancer is a particular concern, and patients who have been treated with higher doses over longer periods and those who smoke appear to be at especially high risk.[92] Bladder cancer related to cyclophosphamide use may occur within 1 year of initiation of therapy and as long as 15 years or longer after discontinuation of cyclophosphamide treatment.[92]

Increased risk of hemorrhagic cystitis of the urinary bladder or development of bladder cancer is caused by cyclophosphamide metabolites, especially acrolein. For this reason, current recommendations are to attempt to restrict the use of cyclophosphamide to 6 months or less, and to use it only in life-threatening or organ-threatening disease. The risk of bladder cancer, although probably not the overall malignancy risk, may be less with the use of pulse intravenous cyclophosphamide than with daily oral administration. Some authors advocate concurrent administration of mesna, which inactivates acrolein in the urine. Mesna may be administered intravenously at the time of pulse cyclophosphamide dosing, or by mouth daily, although this is rarely done because of its disagreeable taste.

## BIOLOGIC THERAPEUTIC AGENTS

**KEY POINTS**

**The overall risk of cancer associated with biologics used in the treatment of RA does not appear to be markedly increased from baseline cancer risk in these patients.**

**The risk of skin cancer, especially nonmelanoma skin cancer, does appear to be somewhat increased by approximately 1.5-fold in patients treated with anti–tumor necrosis factor therapy.**

Biologic response modifiers target specific pathways involved in the pathogenesis of some rheumatic diseases such as RA and spondyloarthritis. The term *targeted* should not imply absolute selectivity between physiologic and pathologic processes with these drugs. It is speculative that the risk of malignancy with use of biologics therapy may be related to the specific biologic mechanisms of the drug. However, a review of 63 randomized clinical trials with 29,423 patients on abatacept, adalimumab, anakinra, certolizumab, etanercept, golimumab, infliximab, rituximab and tocilizumab for at least 6 months' duration did not find a significantly increased risk of malignancy.[94] An important aspect of all randomized clinical trials of biologics is the exclusion of patients with a history of cancer, sometimes excepting nonmelanoma skin cancer (NMSC).

### Anti–Tumor Necrosis Factor Agents

Since their introduction, anti–tumor necrosis factor (TNF) agents have been studied over a wide range of indications. The concern regarding cancer arises from animal models of TNF action, in vitro studies, and studies in humans that suggest that TNF is important in cancer initiation and promotion. Indeed, anti-TNF agents may even be beneficial for patients with cancers, although clinical evidence of such a benefit is lacking.[95] An interesting observation of regression of non–small cell lung cancer in a patient receiving anti-TNF therapy has been reported, which adds to the biologic plausibility of a connection between anti-TNF therapies and malignancy in individual patients who may have particular susceptibility.[96]

When comparing patients on anti-TNF agents with patients on nb-DMARDs, cancer does not occur more frequently,[97,98,99,100,] nor does lymphoma.[98-102] NMSC does not occur more frequently,[99,103,104] whereas melanoma may occur more frequently.[105] Table 37-2 contains a list of meta-analyses and cohort studies undertaken to explore anti-TNF treatment and solid cancers, lymphoma, NMSC, and melanoma in RA.

The possibly increased risk of cancer early after the start of anti-TNF therapy may be a factor in meta-analyses of randomized clinical trials, which have suggested a possibly increased risk of cancer in patients with RA treated with these agents. One meta-analysis of nine randomized clinical trials (RCTs) including infliximab and adalimumab found an OR of 2.4 for developing any malignancy for patients receiving infliximab or adalimumab compared with patients receiving placebo.[106] A meta-analysis of RCTs including 15,418 patients on anti-TNF agents and 7486 randomly assigned to comparators could not refute or verify that individual anti-TNF therapies affect the short-term clinical emergence of cancer.[107]

A pooled analysis of RCTs that used etanercept, infliximab, or adalimumab attempted to distinguish between the effects of recommended versus higher doses of anti-TNF on the development of malignancy, excluding NMSC. Exposure-adjusted analysis revealed ORs of 1.21 (95% CI, 0.79 to 4.28) and 3.04 (95% CI, 0.05 to 9.68) in patients treated with recommended and high doses of anti-TNF agents, respectively.[108]

Results of larger observational studies have not replicated the increased risk of malignancy observed with meta-analytical approaches. In the Swedish Biologics Registry, overall cancer risk was similar in anti-TNF–treated patients with rheumatoid arthritis compared with three different control cohorts.[97] In this database, no trend toward increased cancer incidence was noted with longer duration of TNF exposures. Studies from other databases, including the German and British Biologic Registries and North American cohort and administrative databases, have detected no significant safety signals with respect to overall cancer risk.[99,100,109,110]

Use of anti-TNF agents may be associated with an increased risk of NMSC. An OR for nonmelanoma skin cancer of 1.5 (95% CI, 1.2 to 1.8) was reported from the U.S. National Databank of Rheumatic Diseases.[109] A cohort study from this population suggested that the combination of TNF plus methotrexate versus control agent was associated with a higher risk of NMSC (HR, 1.97; 95% CI, 1.51 to 2.58) compared with a HR of 1.24 (95% CI, 0.97 to 1.58) in patients receiving anti-TNF monotherapy versus control subjects, emphasizing the possible potentiating effects of combination therapies for the development of cancers. This point is perhaps underlined by observations from a RCT of 180 patients with ANCA-associated (granulomatosis with polyangiitis) vasculitis. The occurrence of solid and skin malignancies in the group assigned to etanercept was increased above that expected from treatment with cyclophosphamide alone.[111]

Discordant results regarding cancer risk are likely explained by different patient populations and differing drug exposures. Meta-analyses of clinical trials generally reflect relatively short-term effects but offer the advantage of randomization, which should largely neutralize the variability introduced by individual comorbidities and previous drug exposures; long-term observational studies place more emphasis on mid- and long-term results. In either case, if cancer events occur in a nonlinear fashion with early dropout of individuals at risk for cancer, a conclusive analysis, even after several years of follow-up, may be unable to capture such a signal.

A crucial clinical question is whether patients with pre-existent cancers should be exposed to anti-TNF or other immunomodulatory therapies. Patients with pre-existent malignancies are generally excluded from clinical trials, and in clinical practice, clinicians may be reluctant to treat such patients with anti-TNF therapy, resulting in channeling of treatment with these agents toward low-risk cohorts. An analysis from the British Biologics Registry detected no increased risk of recurrent cancer in patients with pre-existing malignancy (IRR, 0.53; 95% CI, 0.22 to 1.26).[110] Also, data from the German Biologic Registry reveal no

**TABLE 37-2** Summary of Observational Studies of Malignancies Occurring In Patients with Rheumatoid Arthritis Treated with Anti–Tumor Necrosis Factor Agents

| Study, Reference Number | Registry | Intervention | Control nb-DMARDs | Control General Population | Adjusted Hazards Ratio (Intervention vs. Comparator/Control) | Adjusted Hazards Ratio (Intervention vs. General Population) | Risk of Bias |
|---|---|---|---|---|---|---|---|
| **All Types of Cancer** | | | | | | | |
| Askling, 2009[97] | ARTIS | 3 TNFi | nb-DMARDs | General population | TNFi vs. pts starting MTX: 1.0 (0.8, 1.2): TNFi vs./ nb-DMARDs combination therapy 1.0 (0.7, 1.4) | 1.1 (1.0, 1.3) | Low |
| Carmona, 2011[98] | BIOBADASER | 3 TNFi | nb-DMARDs | General population | 0.5 (0.1, 2.5) | 0.7 (0.5, 0.9) | Low |
| Haynes, 2013[99] | Claim database | 3 TNFi | nb-DMARDs | N/A | 0.8 (0.6, 1.1); ever-analysis 0.9 (0.8, 1.1) | N/A | Moderate |
| Strangfeld, 2010[100] | RABBIT | 3 TNFi + anakinra | nb-DMARDs | General population | TNFi vs. nb-DMARDs 0.7 (0.4, 1.1); ANA vs. nb-DMARDs 1.4 (0.6, 3.5) | 0.8 (0.5, 1.0) | Low |
| **Patients with History of Cancer** | | | | | | | |
| Dixon, 2010[110] | BSRBR | 3 TNFi | nb-DMARDs | N/A | 0.5 (0.1, 2.2); Censoring after firstcancer 0.5 (0.1, 2.2). | N/A | Low |
| ***Lymphoma*** | | | | | | | |
| Askling, 2009[102] | ARTIS | 3 TNFi | nb-DMARDs | General population | 1.4 (0.8, 2.1) | 2.7 (1.8, 4.1) | Low |
| Carmona, 2011[98] | BIOBADASER | 3 TNFi | nb-DMARDs | General population | N/A | Hodgkin's, 5.3 (0.1, 29.5); non-Hodgkin's, 1.5 (0.31, 4.4) | Low |
| Haynes, 2013[99] | Claim database | 3 TNFi | nb-DMARDs | N/A | 0.8 (0.3, 2.1), ever-analysis 1.3 (0.7, 2.2); any lymphoma or leukemia: 0.7 (0.3, 1.5); ever-analysis (1.0 (0.6, 1.6) | N/A | Moderate |
| ***Non-Melanoma Skin Cancer*** | | | | | | | |
| Amari, 2011[103] | Claim database | 3 TNFi | nb-DMARDs | N/A | 1.4 (1.2, 1.6); TNFi vs. MTX 1.4 (1.2, 1.7) | N/A | Moderate |
| Mercer, 2012[104] | BSRBR | 3 TNFi | nb-DMARDs | General population | BCC 1.0 (0.5, 1.7), SCC 1.2 (0.4, 3.8); 1st cancer per subject BCC 0.8 (0.5, 1.5) | 1.7 (1.4, 2.0) | Low |
| Haynes, 2013[99] | Claim database | 3 TNFi | nb-DMARDs | N/A | 0.8 (0.5, 1.4); ever-analysis 1.1 (0.8, 1.5) | N/A | Moderate |
| ***Melanoma*** | | | | | | | |
| Raaschou, 2013[105] | ARTIS | 5 TNFi | nb-DMARDs | N/A | 1.5 (1.0, 2.2) | N/A | Low |

ANA, Anakinra; ARTIS, Swedish Biologics Register; BCC, basal cell carcinoma; BIOBADASER, Spanish Biologics Register; BSRBR, British Society of Rheumatology Biologics Register; nb-DMARDs, conventional, synthetic nonbiologic disease-modifying anti-rheumatic drugs; MTX, methotrexate; N/A, not available; pts., patients; RABBIT, German Biologics Register; SCC, squamous cell carcinoma; TNFi, tumor necrosis factor inhibitor.

Modified from Ramero, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2013 update of the EULAR recommendations for management of rheumatoid arthritis. *Ann Rheum Dis* 73:529–535, 2014.

significantly increased risk of recurrence in patients with a previous malignancy treated with anti-TNF agents (IRR, 1.4; 95% CI, 0.5 to 5.5).[100] However, very few events were included in these analyses, so definite conclusions about overall or cancer-specific risks in individual patients cannot be drawn.

## Rituximab

B cells appear to be involved in generation of antitumor responses and are important in maintaining inflammatory states, which promote carcinogenesis and tumor growth. The absence of B cells, for example, in hypogammaglobulinemia, has not been associated with increased susceptibility to cancers, and B cell depletion with rituximab has been shown to slow the growth of solid nonhematopoietic murine tumors.[112]

Pooled analysis of safety data from patients with RA treated with rituximab in RCTs with more than 5000 patient-years of exposure revealed an incidence of malignancy, excluding NMSC, of 0.84 per 1000 patient-years (SIR, 1.05; 95% CI, 0.76 to 1.42).[113] The incidence appeared

to be stable over multiple courses of rituximab, and no unusual pattern of malignancy type was observed.

### Abatacept

Abatacept is a fusion protein consisting of the extracellular domain of human cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) linked to the Fc portion of human immunoglobulin. CTLA-4–mediated T cell suppression has been suggested to be important in the pathogenesis of several types of malignancies, especially malignant melanoma.[114] Because abatacept blocks the same signal that new anticancer treatments based on this interaction are intended to enhance, theoretically a potentially increased risk of malignancies may be seen with the use of this biologic. So far, however, no signal for increased malignancy risk, including lung cancers, has been noted in patients treated with this agent.[94,115]

### Tocilizumab

Tocilizumab is a humanized monoclonal antibody to the interleukin (IL)-6 receptor. IL-6 has an important role in inflammation and in the promotion of various types of malignancies, including suppression of apoptosis, promotion of angiogenesis, and induction of genes that mediate cell proliferation.[116]

Data from studies of rheumatoid arthritis, the disease in which experience with this agent is most extensive, so far have not revealed any signals of an increased incidence of cancer.[94] Labeling information for tocilizumab contains a general statement that "treatment with immunosuppressants may result in an increased risk of cancer," but no specific warnings are included.

### Anakinra

The IL-1 receptor antagonist, anakinra, has been best studied in RA. As is the case for most studies of biologic response modifiers, methotrexate usually has been administered concomitantly. The case rate for the development of malignant lymphoma with anakinra is 0.12 cases per 100 patient-years, with 8 cases of lymphoma observed among 5300 patients with rheumatoid arthritis treated with this drug in clinical trials for a mean of 15 months.[117] This represents a 3.6-fold higher than expected rate of lymphoma compared with the general population. The SIR for lymphoma in this study (3.71; 95% CI, 0.77 to 11.0) is consistent with odds ratios from other studies of patients with RA. A number of solid tumors have also been reported with this agent.[94]

### Tofacitinib

Similar to most other disease-modifying anti-rheumatic drugs, the Janus kinase 3 inhibitor tofacitinib carries a U.S. Food and Drug Administration black box warning regarding the potential risk for malignancy. Malignancies have occurred in RCTs of tofacitinib, but there are thus far no safety signals regarding malignancy risk, including data from a systematic review.[118]

## CANCER SCREENING IN PATIENTS WITH RHEUMATIC DISEASE

Evidence of cancer risk in patients with rheumatic diseases forms the basis for clinically useful recommendations regarding cancer screening. First, with respect to management of the inflammatory disease, it is imperative to achieve optimal disease control and the lowest level of clinical disease activity possible using the least intensive treatment regimen available. Second, patients for whom immunomodulatory therapy, including nb-DMARDs and biologic DMARDs, is considered should undergo routine cancer screening that is appropriate to their age, sex, familial cancer burden, and risk factors such as smoking. Third, because cancers may develop at an accelerated rate in the first few months to the first year or so of treatment, patients should be seen at frequent intervals and closely questioned and examined for signs and symptoms of malignancy, especially during this initial treatment period, and throughout the course of their disease.

Routine blood counts and differential blood cell counts should be performed at the initiation of treatment and as appropriate for the specific drug therapy used. Age- and gender-appropriate cancer screening for colorectal cancer, prostate cancer, breast cancer, and cervical cancer is advisable. In patients with particularly high cancer risk, such as dermatomyositis, assessment for tumor markers such as CA-125 and radiographic imaging of chest, abdomen, and pelvis may be appropriate yearly in the first year or two of the disease, and then as clinically indicated.[119]

Patients taking alkylating agents such as cyclophosphamide especially may be at particularly high risk of cancer. These patients certainly should undergo routine cancer screening Pap smears and urinalyses for at least 15 years from cyclophosphamide therapy. With these considerations, the morbidity and mortality experienced by patients with rheumatic disease can be favorably managed.

## CONCLUSION

Assessment of risk for malignancy in patients with rheumatic diseases is complex. Some rheumatic diseases such as dermatomyositis and Sjögren's syndrome appear to confer a particularly high risk of cancers, particularly lymphoproliferative disorders. Many of these diseases are relatively rare, so that large patient cohorts required for more precise assessment of risk are not available or must be studied over long periods of time to develop stable risk estimates.

Malignancy and perineoplastic syndrome should be considered when patients present with musculoskeletal symptoms caused by an underlying malignancy, or noted as symptoms and signs associated with the presence of an underlying malignancy in a patient with a pre-existent autoimmune disease.

Most of the agents used in the treatment of these diseases are purposefully employed to modulate the immune response, and some, including the alkylating agents, are known carcinogenics. Others may modulate immune response to decrease tumor surveillance. Further complicating the assessment are the individual susceptibility host factors, including the presence of oncogenic genes such as

*Blc-2*, and family history and environmental factors such as viruses, which may enhance the carcinogenic potential of the treatments.

Recommendations for treatment must include a general understanding of the disease and treatment-related malignancy and individualized discussion with the patient regarding risks and benefits of treatment as they relate to disease activity and severity and, most important, patient preference.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 38



# Introduction to Physical Medicine, Physical Therapy, and Rehabilitation

Maura D. Iversen

**KEY POINTS**

- The goals of rehabilitation are to maximize function and participation and to minimize activity limitations and impairments.
- The International Classification of Functioning, Disability, and Health provides the framework for rehabilitation.
- Modern rehabilitation is interprofessional and uses a patient-centered approach.
- Exercise is consistently effective in the treatment of various rheumatologic diseases.
- Physical modalities may be used as an adjunct to active therapies.
- Rehabilitation includes the training of individuals with impairments and disabilities, as well as adaptations of the environment to enhance participation.

Rehabilitation is a specialized medical field that combines medical therapy and nonpharmacologic interventions with the goals of maximizing function, independence, and participation, and ameliorating symptoms. The rehabilitation community has embraced the International Classification of Functioning, Disability, and Health (ICF)[1] as a framework to develop interventions to address the consequences of disease, communicate about strategies that enable individuals to engage fully in society, and formulate rehabilitation research[2-7] (Figure 38-1).

Rehabilitation interventions are multimodal and diverse and are designed to help individuals with arthritis live with their disease.[8-15] Exercise is the most studied and best proven intervention.[15-17] Physical modalities such as ultrasound, transcutaneous electric nerves simulation (TENS), and heat are adjuncts to exercise and show modest benefits with respect to pain relief, extensibility of tissue, and relaxation.[8-10,13-15] Orthotics, ambulatory devices, splints, and adaptive devices enable patients to navigate barriers and to fully participate in society.[18-20]

Musculoskeletal rehabilitation programs are based on the disease state and severity, the use and type of medications, and social, psychological, and environmental factors. This chapter provides an introduction to physical medicine and rehabilitation with an emphasis on nonpharmacologic interventions. Key principles of rehabilitation will be discussed, and management strategies for specific rheumatologic conditions will be highlighted.

## BRIEF HISTORY OF REHABILITATION IN RHEUMATOLOGY

Early rheumatology rehabilitation focused on bed rest, splinting, and range-of-motion (ROM) exercises. Physicians and rehabilitation specialists believed that physical activity and strenuous exercise would produce pain, increase joint swelling and temperature, and accelerate joint damage. In the 1940s, with the introduction of steroids, highly efficient and potent anti-inflammatory drugs, the rehabilitation focus shifted toward splinting and mobilizing patients with assistive devices to promote function. Surgical approaches such as joint replacements were used in the 1960s and 1970s to "stop" disease progression, and rehabilitation interventions were focused on post-operative protocols to enable patients to regain function and maximize independence. Throughout the 1970s and 1980s, with the proliferation of disease-remitting agents, such as Myochrysine, methotrexate, and sulfasalazine, rehabilitation regimens began to incorporate dynamic exercises and functional activities earlier in the disease process. Researchers also began to evaluate the impact of isometric and low-intensity isotonic exercise on immune response and function. Early data from these trials suggested positive effects on disease activity and strength.[21,22] While more medications entered the market and the prevalence of disease-modifying antirheumatic drugs (DMARDs) expanded in clinical practice, rehabilitation research shifted to the evaluation of various intensities, frequencies, and modes of exercises on patient outcomes. From the mid-1990s onward, studies of aerobic exercise illuminated the benefits of aerobic conditioning for cardiovascular function without deleterious effects on joints and soft tissue.[23-25] In the 21st century, with the use of advanced radiologic techniques and the advent of biologic therapies, researchers are investigating the impact of weight-bearing activities of various intensities on joint integrity[26] and are embracing a public health perspective through the promotion of physical activity designed to improve the quality of life, function, and participation.[27,28]

## GOALS OF REHABILITATION, REHABILITATION TEAM MEMBERS, AND MODELS OF TEAM CARE

Rehabilitation interventions address all aspects of the patient's condition with the aim of maximizing function, independence, and participation in society. As such, interventions range from the prescription of adaptive devices,

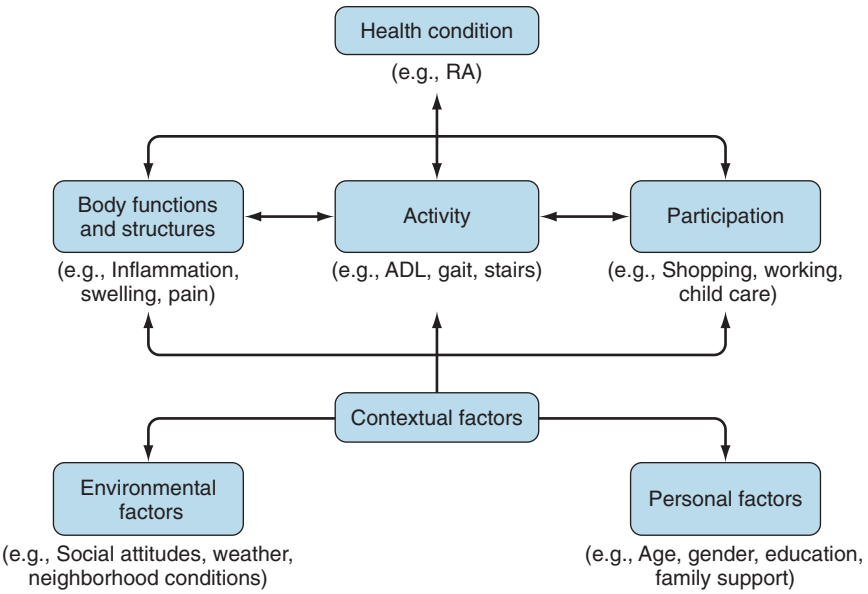



**Figure 38-1** Application of the International Classification of Functioning, Disability, and Health in rheumatoid arthritis (RA). ADL, Activities of daily living.

such as splints, orthotics, and ambulatory devices, to the use of physical modalities and instruction in exercise and self-management strategies (e.g., relaxation and proper rest). Rehabilitation specialists are educated to assess environmental factors so that they can best address barriers and facilitators that affect their patients.[29]

The initial phase of the rehabilitation process involves a comprehensive assessment of all dimensions of the patient's life and condition. Given the fluctuating course of many rheumatologic conditions, a coordinated effort by a variety of skilled rehabilitation specialists is required. The structure, function, and resources of the medical system in which the patient resides influence the team composition.[30,31] Physiatrists, or rehabilitation medicine physicians, are educated in medicine and rehabilitation to treat patients and refer to, and/or supervise, other skilled rehabilitation professionals.[29] These physicians typically are responsible for the care of patients with arthritis. However, in some circumstances, the rheumatologist may be the only physician involved in the patient's care. Other professionals involved in patient care are primary care physicians, nurse practitioners/nurse specialists, nurses, physical therapists, occupational therapists, social workers, nutritionists, psychologists, podiatrists, and vocational rehabilitators, who work with the patient's family.[30]

Comparative studies of team care delivery versus individual practitioner models indicate that coordinated team efforts yield better outcomes.[32-34] In all cases, the patient is the focal point of the team. Traditional models of team care are categorized as interdisciplinary/interprofessional, multidisciplinary, or transdisciplinary. In the interdisciplinary/interprofessional model, each professional conducts an independent patient evaluation and shares this information during team meetings to facilitate integrated team goals. Negotiation, communication, and collaboration are prevalent principles. Multidisciplinary team care does not foster intercommunication between professionals in a coordinated manner.[35] Rather, each professional conducts an examination and develops separate goals for care in concert with the patient, and documents findings. The transdisciplinary model allows for the transference of professional roles by crossing professional boundaries (e.g., a physical therapist can perform some functions of a nurse).[36] Any of these models of care may occur in inpatient or outpatient settings.

Although team care has been widely used in Europe,[37] this is not the case in North America, in part due to budgetary constraints and shortages of health care professionals, especially in rural areas. Consequently, innovative care models such as the clinical nurse-specialist model,[37,38] the primary therapist model,[36] and telemedicine have emerged.

## INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY, AND HEALTH: A FRAMEWORK FOR REHABILITATION MANAGEMENT

The ICF provides a framework for the development and implementation of rehabilitation interventions[1] by systematically organizing various aspects of an individual's health condition. The term *functioning* describes body functions, activities, and participation; the term *disability* refers to impairments, activity limitations, and participation restrictions. The ICF also considers environmental (e.g., stairs and curbs) and personal factors (e.g., attitudes and beliefs) that interact with body functions, body structures, activities, and participation.[1] This framework integrates aspects of the biopsychosocial medical model with an ecological perspective. The equal emphasis on ecological factors, such as personal and environmental contextual factors, helps rehabilitation specialists identify and address elements that may facilitate or present barriers to obtaining independence. The ICF definitions serve as a common language for providers to communicate about patients' health conditions, interventions, performance of activities and participation in society[1-8] (Figure 38-1). Researchers use the ICF to solidify and examine core constructs relevant to clinical studies of

rehabilitation.[3,6,7] These core sets have been compared with well-validated outcome measures to identify the most salient measures for rehabilitation clinical trials.[3]

## ASSESSMENT TOOLS AND THE REHABILITATION CYCLE

The first step in rehabilitation is problem identification based on a comprehensive history and physical examination using reliable and valid health measurements. These data are used to develop interventions and to evaluate outcomes. Rehabilitation health professionals use a variety of assessments, including the following:

- Technical measures: electrophysiologic, biomechanical, and computerized devices
- Clinical tests: ligament laxity tests, ROM, and strength testing
- Performance measures: gait velocity, mobility and balance tests
- Patient-centered measures: patient and proxy self-reports on health status, quality of life, and health preferences

Because the primary goal in rehabilitation is to diminish impairments, restore function, and facilitate engagement in social activities, the emphasis of a rehabilitation examination is on assessment of functioning and societal participation.[35]

Body structures and function elements, such as joint ROM, may be assessed subjectively through simple observation, goniometry, or high-speed cinematography. Goniometry, measurement of joint ROM by using a device called a *goniometer*, is often used in clinical settings because it is inexpensive, easy to perform, and psychometrically sound. Muscle strength is frequently assessed by manual muscle testing procedures, although its reliability and validity varies by joint location and by disease. Quantitative methods of maximal isometric strength measurement with hand-held dynamometers are reliable in patients with inflammatory arthritis[35,39,40] and in those with degenerative lumbar spinal stenosis.[41] Hand-grip strength can be measured reliably with a hydraulic hand dynamometer.[42]

Gait velocity, calculated as the time needed to walk a specific distance, is measured to assess walking mobility[43] and serves as a test of lower extremity function. The "Timed Up and Go" test[44] is a basic mobility test that measures the time it takes a patient to rise from an arm chair (seat height of 46 cm and arm height of 65 cm), walk 3 m, and return to and sit down in a chair. This performance measure has established normative values for different age groups.

Patient self-report measures are widely implemented in the core-set measures for various rheumatologic conditions. In inflammatory arthritis, the Health Assessment Questionnaire (HAQ) assesses dimensions of health, including disability, pain, medication effects, and costs of care.[45] A modified version of the HAQ (MHAQ) uses fewer items and maintains good psychometric properties, although differences in scores for disability are evident between the HAQ and the MHAQ.[46] The Katz Index of activities of daily living and the Macmaster Toronto Arthritis Preference Disability Questionnaire (MACTAR) also measure general activities of daily living. The MACTAR is unique in that it allows patients to choose which activities are important to them.[35]

A range of patient-oriented, disease-specific measures of function and symptoms may be used to assess pain, psychological status, well-being, fatigue, sleep, and quality of life.[12] Clinicians and researchers may have difficulty selecting the most appropriate measure for their purpose. To address this problem, one can refer to the ICF framework, which provides a clear picture of which health domains are addressed by each measure. There are also linking rules to relate technical and clinical measures, health status measures, and interventions to the ICF.[4]

## WHAT DO CURRENT GUIDELINES SUGGEST FOR REHABILITATION MANAGEMENT OF SELECT RHEUMATOLOGIC CONDITIONS?

Clinical guidelines for management consistently promote the use of exercise, physical activity, and physical therapy.[47-58] Recommendations are based on substantial evidence from randomized controlled trials (RCT) of exercise[59-63] indicating moderate effect sizes for the benefits of exercise for pain relief, function, and muscle strength. However, specific exercise prescriptions are not provided. Lack of specificity regarding exercise prescriptions originates from inconsistency in information regarding the intensity, frequency, mode, and duration of exercise in early clinical trials.[64] More recent studies adhere to standardized reporting criteria, such as the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) framework,[65] thus providing detailed information about exercise interventions to enable better data synthesis. Information regarding accepted clinical rehabilitation is provided in the section addressing specific diagnoses and interventions.

## NONPHARMACOLOGIC INTERVENTIONS TO MANAGE RHEUMATOLOGIC CONDITIONS

Nonpharmacologic interventions used to manage rheumatologic symptoms include rest (total body or local), massage and trigger point techniques, exercise, assistive devices, orthotics and splints, counseling, education and self-management, manual therapy/mobilization, gait training and instruction in ambulatory devices, mobility devices, ergonomic modifications, and vocational rehabilitation. Table 38-1[8,13,66-76] provides a description of the interventions and their role in management. Among these, exercise, patient education programs, and self-management interventions are the best studied and the most effective. Across diseases, exercise interventions, whether dynamic or static, yield moderate improvements (effect sizes of 4 to 6) in strength, pain, and function, and produce small to moderate improvements in mood, quality of sleep, sleep patterns, and psychological well-being.[15-17,62-64,77] Exercise improves endurance and reduces inflammation.[78-80]

Patient education is an integral component of rehabilitation management. Patient education programs provide structured information about disease, medications and their use, when and how to perform exercise, disease management strategies (e.g., energy conservation and rest), and community resources. These programs are distinct from self-management interventions in that they are often provider driven and formulated, and focus on information dissemination versus behavioral interventions.[81] For example,

**TABLE 38-1** Definitions, Descriptions, and Purposes of Common Rehabilitation: Interventions to Manage Symptoms of Arthritis

| Intervention | Description |
|---|---|
| Energy conservation/ total body rest | Important with inflammatory rheumatic diseases to have intermittent rests during the day (half hour) and 8-10 hours of rest per night.[83] |
| Local rest/resting splints | Resting splints (worn at night or during periods of total body rest to prevent joint movement) provide local rest to joints and maintain joint alignment. In rheumatoid arthritis (RA) and other systemic inflammatory disorders, resting wrist splints are well tolerated and effective.[14] |
| Manual therapy/joint mobilization/ manipulation | Performed as low- or high-velocity, small- or large-amplitude passive movement techniques. Flexibility and strengthening exercises follow manual therapy/mobilization to gain full benefit. Evidence is strongest in treating hip and low back disorders, but effects are small.[64,103,105] |
| Trigger point therapy | Used for muscle pain. Consists of ischemic compression of trigger points, followed by isotonic contractions or muscle tissue stretching. Additional techniques include myofascial release, trigger point injections, trigger point dry needling, and intramuscular manual therapy. A local twitch response, provocation of referred pain, and subsequent relaxation of the taut band indicate successful application.[35] |
| Massage | Increases local blood and lymph flow, facilitates muscle relaxation, and reduces muscle stiffness, pain, and spasm. Techniques include gliding, kneading, deep friction, and percussion. Gliding and kneading reduce muscle tension, improve circulation, and decrease edema. Friction breaks up adhesions. Lymph drainage increases lymph flow and decreases edema. Massage is contraindicated for malignant tumors, open wounds, thrombophlebitis, and infected tissues. Lymph drainage should be avoided in patients with congestive heart failure.[35,67] |
| **Exercise** | |
| Range-of-motion (ROM) and flexibility exercises | Maintains joint movement and function and may be performed passively (by therapist) or may require active patient participation. In active-assisted exercises, the patient exerts some force with joint movement but is assisted by the therapist. Active ROM exercises require the patient to exert muscle effort to achieve the desired ROM.[35,83] Flexibility or stretching exercises enhance extensibility of muscle tissue. Stretching is best performed by using gentle, smooth movement, and then holding the stretch for 2 to 15 sec. |
| Isometric (static) exercise | Muscle contraction performed without a change in joint range or muscle length. These exercises produce less strain on joints than is produced by dynamic exercise.[83] |
| Isotonic/dynamic/ isokinetic exercise | Isotonic exercises require changes in muscle fiber length by elongating (eccentric) or shortening (concentric). These exercises involve movement through a fixed ROM at a fixed rate (velocity) against variable resistance. A machine provides resistance and rate of movement that matches exactly the force generated by the patient at any point in the range. This equipment can calculate the torque developed during the exercise activity.[35] *Note:* Avoid in the presence of inflammation, popliteal cysts, and joint derangement.[83] |
| Aerobic conditioning or endurance exercise | Provides cardiovascular pulmonary benefits, improves muscle strength, and reduces inflammation and weight. Modest effect sizes for these outcomes are achieved when exercises are performed at moderate levels of intensity for extended periods. Modes of aerobic exercise include walking, running, hiking, cycling, swimming, and stair climbing.[23-25] |
| Aquatic exercise, spa therapy, balneotherapy | Based on physical properties of water (i.e., buoyancy, molecule adhesion, temperature), provide physiologic benefits such as muscle relaxation and ease of movement. Exercises performed in water allow buoyancy to support body weight and unload joints. Water adhesion properties may provide resistance when exercising. Diuresis and hemodilution are physiologic effects experienced with aquatic exercise. The recommended water temperature is 33°C to 34°C (92°F to 94°F). A systematic review of randomized controlled trials investigating balneotherapy has reported positive findings. However, the evidence was insufficient to allow formal conclusions about the efficacy of balneotherapy for patients with arthritis.[10] |
| **Physical Modalities** | |
| Superficial heat/cold therapy | Radiation (infrared light) and conduction (hot packs, paraffin, or water) are mechanisms for superficial heat/cold generation that are applied for 20 minutes. Superficial heat increases the pain threshold, decreases muscle spasms, and produces analgesia by acting on free nerve endings. *Note:* Avoid heat therapy in the presence of acute inflammation or in people with altered sensation. Cold therapy may be applied using ice packs, ice massage, vapocoolant sprays, or cold water baths. Cold therapy induces superficial and intra-articular tissue vasoconstriction, reduces local metabolism, and slows nerve conduction, thereby reducing pain and inflammation. Complications of cryotherapy include frostbite, cold-induced urticaria, and nerve damage. Use cryotherapy with caution in patients with Raynaud's phenomenon or with cryoglobulinemia.[35] Although evidence for physiologic benefits of heat and cold therapy indicates small effects,[9] patients report psychological benefits. |
| Electrotherapy | Uses electricity to stimulate nerves and muscles and to alleviate pain. Surface electrodes are the common transfer medium. Only electroacupuncture and dorsal horn stimulation use needle electrodes percutaneously. Electrotherapy uses direct continuous galvanic currents and modulated direct currents. Galvanic currents decrease pain conduction in slow unmyelinated nerve fibers (C fibers) to reduce pain. Modulated middle-frequency electrotherapy results in inhibition of pain-related potentials at spinal and supraspinal levels. Electrical stimulation of fast-conducting myelinated nerve fibers can partially decrease pain through inhibition of pain impulses carried more slowly by unmyelinated fibers. Faster impulses arrive at the level of the dorsal horn first and "close the gate." Transcutaneous electrical nerve stimulation (TENS) is used for musculoskeletal pain, post-traumatic or post-surgical pain, peripheral nerve injury, neuropathic pain, and sympathetically mediated pain.[12,35] Electrotherapy is contraindicated in patients with cardiac pacemakers or implanted cardiac defibrillators, and is used with caution in patients with atrophic skin.[35] A systematic review of randomized controlled trials of TENS in arthritis reports inconsistent results in managing RA symptoms[12] and low back pain, and some benefits for pain and knee stiffness in patients with knee osteoarthritis (OA). |

**TABLE 38-1** Definitions, Descriptions, and Purposes of Common Rehabilitation: Interventions to Manage Symptoms of Arthritis—cont'd

| Intervention | Description |
|---|---|
| Deep tissue heating/ ultrasound/ diathermy | Parameters for application vary by the device; the device must be applied by a skilled provider owing to the risk of deep tissue burns of the skin, fat, muscles, and bones. Heating occurs mostly at tissue interfaces (e.g., bone–soft tissue interfaces). Ultrasound may penetrate 7 to 8 cm of fat, but less than 1 mm of bone, depending on the energy level and frequency chosen. In practice, ultrasound with frequencies of 0.1 to 1 MHz can increase temperature by 4°C to 5°C at depths of 7 to 8 cm.[35] Two systematic reviews of ultrasound[8,71] reported small benefits for pain, knee and hand stiffness, grip strength, and tender and swollen hand joints.[8] *Note:* Avoid deep heating in patients with altered sensation, implants, or history of cancer. Ultrasound must be applied using continuous movement over joints and bony surfaces or in a water bath to avoid heating of bone. |
| **Devices to Stabilize and Protect Joints** | |
| Orthotics/braces | Orthotic devices include braces, splints, corsets, collars, and shoe modifications. Orthotic devices restore or maximize function by altering biomechanics through stabilizing, realigning, and/or maximizing joint position, thereby reducing pain. Orthotics provide some pain relief but inconclusive evidence of improved function long term.[18,19] Successful prescription and use of orthotics require the identification of functional limitations (on all International Classification of Functioning, Disability, and Health levels) and patient collaboration to adjust the orthotic. Devices can be simple and inexpensive, but may be specially designed and consequently expensive.[35] Bracing is prescribed to stabilize joints. The most common are knee braces typically prescribed for OA.[19] |
| Dynamic splints | Dynamic splints maintain joint alignment and reduce pain during functional activities. The most common dynamic splints used in RA are functional wrist splints, although many others are available for the small hand joints. In OA, thumb splints are common. A Cochrane review from 2003 concluded that dynamic wrist splints significantly increase grip strength but do not impact pain, morning stiffness, pinch grip, or quality of life. No evidence suggests that resting splints changed pain, grip strength, or the number of painful or swollen joints. Patients preferred using these splints to nonuse.[20,35] |
| Collars/corsets | Cervical collars include soft collars, Philadelphia collar, and sterno-occipitomandibular plaster immobilization. These provide varying levels of stability. None prevent subluxation or displacement.[69] Patients with night pain resulting from a cervical disk syndrome may profit from wearing a soft collar at night. Corsets and abdominal binders provide feedback but limited stability to patients in terms of body position.[35] |
| Assistive devices | A multitude of devices may be used to assist function and reduce barriers to independence. Examples include button hooks, long-handled reachers, sock aids, modified eating utensils (padded handles), bottle openers, and modified container lids. A physical or occupational therapist may recommend modified door handles, a raised toilet seat, a commode, safety bars on the bathroom wall, or a lift in the bath to ensure safety and maximize independence with hygiene activities. |
| Mobility devices | Mobility devices, such as canes, crutches (axillary, forearm, and platform), wheeled walkers, and wheelchairs, are used when walking is limited by lower extremity joint instability, pain, weakness, and fatigue or balance problems. These devices are easily accessible and provide immediate assistance, but they require more physical effort than is required for normal ambulation. The choice of a mobility device is based on impairments and resulting disability (e.g., patients with RA with bilateral upper extremity involvement and leg weakness may be safer ambulating with platform than axillary crutches). Patients with balance disturbances and leg weakness may be better suited for walkers. The device type also impacts the weight-bearing status. For example, a single-point cane can bear about 25% of body weight, axillary or forearm crutches approximately 50%, and a wheeled walker more than 50%.[70] Wheelchairs are necessary if patients are unable to walk with devices. In these cases, a wheelchair can improve the quality of life by maintaining some mobility. Individuals unable to navigate a manual wheelchair can profit from an electrical chair. Accidents can occur while traversing ramps, sidewalks, and streets.[35] |
| Vocational rehabilitation | Vocational rehabilitation is an interdisciplinary approach that enables patients to acquire and maintain gainful employment and varies among countries. A common feature consists of problem assessment at work and the development of individual solutions. Studies demonstrate that vocational support prevents or delays work disability and improves fatigue and mental health.[71] |
| Work hardening/ functional restoration programs | These highly structured, goal-oriented, individually tailored programs are provided by an interdisciplinary professional team to address functional, physical, behavioral, and vocational needs to facilitate return to work.[71] Work hardening programs bridge the gap between initial injury and return to work. Most programs include a formal worksite ergonomic to address workplace design issues and to reduce injury risk while improving function, as well as social interventions. The Commission on Accreditation of Rehabilitation Facilities defined and developed standards for work hardening practice to standardize program content and implementation.[71] Work hardening programs integrate real or simulated work activities to model appropriate behaviors and assess functional, biomechanical, neuromuscular, cardiovascular/metabolic, behavioral, attitudinal, and vocational performance.[71] A systematic review of these programs provides evidence for significant reductions in sick days. |
| Self-management and patient education | Patient education is defined as a set of planned educational activities used to improve a patient's health behaviors and/or health status. Most patient education programs are provider driven and developed. Self-management programs are patient oriented and patient active, and combine education with cognitive-behavioral strategies to influence patients' attitudes toward disease and disease management.[81,82] |
| Cognitive-behavioral therapy (CBT) | CBT acknowledges that pain and its resulting disability are influenced by somatic pathology, and by psychological and social factors. In general, three behavioral treatment approaches are distinguished: operant, cognitive, and respondent. The primary focus is to reduce disability. A review of 21 studies found no significant differences between the various types of CBT; also, CBT did not differ from exercise in its benefits. A conclusion regarding whether clinicians should refer patients with chronic low back pain to behavioral treatment programs or to active conservative treatment was not possible. |

ankylosing spondylitis education programs teach patients about their disease, medications, importance of good posture, daily activities that promote spinal extension, and coping skills.

Self-management programs are patient focused, patient driven, and action oriented.[81] They combine education, behavioral interventions, and cognitive strategies to enable patients to problem-solve, cope, and develop strategies to maximize function and independence. These programs assess and influence patients' attitudes and beliefs about disease and their ability to manage their disease. Self-management programs demonstrate benefits with respect to disease outcomes[81,82] and have proved cost-effective.[82]

Evidence of the benefits of physical modalities, therapeutic relaxation techniques (massage, trigger point therapy), splints, orthotics, and gait interventions varies across intervention type and disease. Overall, the evidence is weak.[8-10,12,83] However, it is important to note that relaxation techniques, alternative therapies (acupuncture), and physical modalities are adjunct therapies designed to prepare patients for dynamic exercise, flexibility training, or gait training and are not stand-alone interventions.[12,83,84] Additionally, many patients report satisfaction with and preference for these therapies.[84]

## PRINCIPLES GUIDING REHABILITATION IN PEOPLE WITH RHEUMATOLOGIC CONDITIONS

Research and clinical management outcomes suggest the following guiding principles:

- Primary, secondary, and tertiary programs are integral to patient care. Primary prevention focuses on preventing disease. Secondary prevention refers to detection of disease before symptoms arise. Tertiary prevention, the most common in arthritis, focuses on interventions for people already experiencing symptoms to ameliorate these and to maximize function, independence, and participation.
- Exercise prescriptions for people with systemic inflammatory diseases are developed based on the disease state (active vs. inactive), disease severity, patient preferences and goals, medications used and their corresponding side effects, and latency periods.[83]
- Persons with arthritis are less physically active than their healthy counterparts.[85-87]
- Asymptomatic heart disease may be disproportionately present in people with systemic inflammatory disease, and their response to exercise may be altered; therefore careful monitoring of cardiovascular pulmonary response is needed before, during, and after exercise testing and participation.[83]
- Dynamic resistance strengthening exercises should be avoided in the presence of joint derangement and popliteal cysts.[83]
- During periods of active myositis and elevated creatine phosphokinase (CPK) levels, activities should be limited to ROM exercise and functional activities of daily living, and rest should be encouraged.

The Centers for Disease Control and Prevention (CDC) in conjunction with the American College of Sports Medicine (ACSM) has established physical activity guidelines to promote the health of all Americans, including people with rheumatologic diseases (Table 38-2).[88,89,90] The CDC deliberately framed these recommendations simply reporting a range of minutes of exercise versus traditional physiologic parameters to enable consumers to interpret activity to meet these recommendations. Presently, physical inactivity among people with doctor-diagnosed arthritis in the United States is a major public health issue; only 17.2% of people with arthritis meet the CDC recommendations for moderate physical activity.[88]

**TABLE 38-2** Centers for Disease Control and Prevention and American College of Sports Medicine Guidelines for Physical Activity

| |
|---|
| 30 minutes of moderate* physical activity, 5 days per week |
| *or* |
| 20 minutes of vigorous activity, three times per week |

**Moderate activity* is defined as an increase in breathing or heart rate; perceived exertion in the range of 11 to 14 out of 20 on a perceived exertion scale[90] or 3 to 6 metabolic equivalents of task, or any activity that burns 3.5 to 7 kcal/min.[88,89]

## REHABILITATION OF SELECT RHEUMATOLOGIC CONDITIONS

In this section, select conditions are discussed, along with the evidence for rehabilitation interventions. Diseases that have similar attributes are grouped together, although they are distinct entities. In this manner, the rationale for interventions based on similar impairments, functional limitations, and activity participation can be delineated.

### Rheumatoid Arthritis and Inflammatory Arthritis

Both rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are chronic systemic inflammatory rheumatologic disorders affecting connective tissues and joints. Of the two conditions, RA is more prevalent. Although the origin and prevalence of RA and PsA differ, the two disorders have similar symptoms. For example, patients are seen with polyarticular symmetric joint involvement, malaise, morning stiffness, pain relievable with activity, muscle weakness, enthesitis and/or plantar fasciitis, and cardiovascular pulmonary involvement. Both diseases are characterized by exacerbations and remissions. Differences between these diseases are noted with respect to the joints involved, integumentary involvement, dactylitis,[91] and predominant pathology. The primary pathology in PsA is enthesitis, and the axial skeleton is chiefly involved. Chronic inflammation eventually may lead to spinal ankylosis, significantly impacting trunk ROM and rib cage excursion. RA is a disease of the synovium that typically involves the hands, feet, wrists, and weight-bearing joints. Although RA does not affect most of the spine, subluxation of the atlantodental joint can occur.[83]

Systemic features lead to impairments such as fatigue, malaise, fever, sarcopenia, reduced aerobic capacity, restricted joint range of motion, postural instability, cardiovascular pulmonary dysfunction, and depression.[91,92] Both conditions are associated with significant disability and early death. Medical management includes the use of

**TABLE 38-3** Physical Therapy Interventions for Various Stages of Disease Activity Inflammatory Arthritis

| Acute Inflammation | Subacute Inflammation | Inactive/Chronic |
|---|---|---|
| Rest and continue physical activity as tolerated | Moderate physical activity (4-5x/week for 30 minutes) | Vigorous physical activity (3x/week for 20 minutes) or moderate physical activity (5x/week for 30 minutes) |
| Passive and active range-of-motion (ROM) | Active ROM | Active ROM |
| Isometric exercises | Dynamic exercises with resistance, aerobic exercise<br>70%-80% heart rate | Dynamic exercises with resistance, aerobic exercise<br>70%-80% heart rate |
| Modalities for pain and inflammation | Ergonomic interventions, work accommodations | Ergonomic interventions, work accommodations |
| Resting splints, dynamic splints, orthoses, and assistive/ambulatory devices as needed | Resting splints, dynamic splints, orthoses, and assistive/ambulatory devices as needed | Dynamic splints, orthoses, and assistive/ambulatory devices as needed |

biologics, DMARDs, steroids, nonsteroidal anti-inflammatory agents and biologics. Rehabilitation professionals must be aware of medication indications, potential side effects, and the latency period to effectiveness if they are to develop treatments that adjust as responsiveness to medications increases.

Given the fluctuating nature of both diseases, the rehabilitation interventions selected vary by disease activity. Table 38-3 provides an intervention algorithm that illustrates the different modes of exercise and interventions used to manage these conditions during acute, subacute, and chronic disease stages. When RA or PsA is highly active, patients may report low-grade fever, fatigue, or malaise, and may be seen with warm, swollen, and tender joints. Adaptive shortening of soft tissues, joints, and tendons may occur secondary to inflammation and as the result of protective joint splinting in response to pain. In RA, metacarpophalangeal and interphalangeal joint subluxation, as well as ulnar deviation of the fingers and radial deviation of the wrists, is prevalent, whereas dactylitis may be present in PsA.[91] Soft tissue inflammation may lead to atrophy of the foot intrinsic muscles and loss or subluxation of plantar fat pads in both, resulting in plantar fasciitis and enthesitis. Eventually, loss of cartilage may result, with severe disease, joint subluxation, and derangement. These joint changes may lead to functional limitations, gait abnormalities, and deconditioning.[83,91]

Interventions used to manage acute inflammatory symptoms include patient self-management strategies, ROM exercises, isometric (static) exercises, physical modalities, total body rest, and splints. Exercise frequency and intensity is generally set at three to five repetitions, twice a day. The greatest evidence for benefits from interventions is for exercise regardless of mode of exercise. To enhance adherence, patients are instructed to conduct daily joint checks, a simple check of each joint to determine which are most limited and can serve as the focus of exercises for that day. Energy conservation strategies are important. Patients are advised to sleep 8 to 10 hours per night and to rest frequently during the day. Resting and dynamic splints can help maintain joint alignment, reduce pain, and control inflammation. Resting splints are used when the patient is sleeping to hold joints in a neutral position, restricting all movement. Dynamic splints allow joint movement to accommodate functional activities. During the acute inflammatory stage, cold therapy (cold packs, ice massage) is preferred versus heat to reduce swelling and alleviate pain. However, thermal modalities (heat/cold) are contraindicated in people with altered sensation and vascular conditions.[83] Assistive/adaptive and ambulatory devices (e.g., long-handled reachers, crutches) may be used to maximize function and participation. Ambulatory devices and orthotics are particularly important in the presence of foot involvement and deformities to redistribute weight and alter lower extremity biomechanics. Orthotics may also improve function and reduce pain.[18] When wrists and hands are involved, platform attachments to ambulatory devices are warranted. In all cases, patient preferences need to be considered when ambulatory devices are selected.

When symptoms subside (e.g., subacute stage), interventions may be modified to include increased repetitions of ROM exercises, with progression from isometric to dynamic exercises (five to ten repetitions of each exercise, three times per day). However, if popliteal cysts are present, active resistive exercise should be avoided. Flexibility exercises are initiated to reduce soft tissue tightness and contractures. The use of heat therapy and/or massage before stretching may reduce muscle spasm and enhance tissue flexibility. Clinical trials of thermotherapy report small effects for relief of muscle spasm, pain, and extensibility of soft tissue.[9] TENS, laser, massage, and trigger point therapy provide inconsistent but small benefits.[8,9,13,93-95] Core stability and balance exercises may be used to address muscle imbalances. RCTs of exercise at low to high intensity demonstrate moderate effects in improving strength, function, and mood state, and in reducing pain, stiffness, and inflammation, without deleterious effects on joints.[12,14,,21,94,95] Aquatic exercises are encouraged to promote flexibility, improve gait patterns, maximize strength, and increase independence. The buoyancy of the water facilitates movement and reduces joint loading.

With stable disease, patients should incorporate dynamic strengthening exercises with resistance (eight to ten repetitions a day). Studies suggest that dynamic exercises can effectively increase muscle strength, physical capacity, and aerobic capacity.[21] It is imperative to initiate aerobic exercises (30 minutes, three to five times per week) to address cardiovascular risk, improve aerobic performance, and increase muscle strength.[96,97] Patient counseling regarding physical activity and active lifestyles should be emphasized

to prevent secondary effects of inactivity.[85-87] The most common modes of physical activity and exercise include low-impact exercise, such as walking programs, aquatics, dance, and cycling, and dynamic exercises with resistance. Recent clinical trials of high-impact physical activities report significant improvements in aerobic capacity, grip strength, physical activity, anxiety, and depression.[26] High-intensity exercises appear to be safe and effective, except in the presence of joint changes in large weight-bearing joints, joint derangement, and cartilage loss before initiation of weight-bearing activities.

## Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a systemic inflammatory disease that predominantly affects women in their childbearing years and is more common among African-Americans. Its cause is unknown. SLE affects multiple organ systems, including the joints, skin, kidneys, heart, lungs, brain, and gastrointestinal tract. Fatigue is prevalent and may arise from myositis, anemia, depression, pulmonary disease, or deconditioning. Joint involvement is not uncommon. If present, the small joints of the hands, especially the proximal interphalangeal joints, may be involved, and recommendations similar to those for RA apply. Aseptic necrosis of the hip may occur. With unilateral hip disease, use of an ambulatory device on the contralateral side reduces acetabular loading and hip abductor muscle activity, thereby decreasing joint hip load and pain. Eventually, the patient may require a total joint arthroplasty.[83]

Asymptomatic coronary heart disease is a major cause of mortality. Thus a comprehensive cardiovascular pulmonary system review is needed along with exercises modes to enhance cardiovascular performance, such as biking, walking, and dynamic exercises at moderate intensity (50% to 70% one repetition maximum). For safety, vital signs are assessed at baseline and at regular intervals during the exercise session.[98] Patients who are severely deconditioned may benefit from interval training (short bouts of 10 to 15 minutes of exercise twice a day) to enhance aerobic conditioning.

Studies of exercise in SLE are limited. In an earlier trial of endurance training that used stationary cycles with individually tailored resistance settings based on submaximal exercise testing, patients with SLE demonstrated small to modest effects on function but no significant impact on $VO_2$max (maximal oxygen consumption).[99] A review of exercise for patients with SLE concluded that patients with mild to moderate SLE benefit from exercise of moderate to high intensity, yielding significant benefits for aerobic capacity, fatigue, physical function, and depression.[100]

## Osteoarthritis

Osteoarthritis (OA) is a progressive, multifaceted disease of the cartilage that can lead to cartilage and bone loss. Typical joints involved include hips, knees, spine, and hands (distal and proximal interphalangeal joints, first carpometacarpal, and first metatarsal phalangeal). Intrinsic and extrinsic factors, such as joint injury, malalignment, trauma, obesity, and quadriceps weakness, are associated with the development of OA. Patients typically note short-term morning stiffness (<30 minutes) or stiffness after prolonged inactivity. The combination of excess weight and poor joint biomechanics increases OA risk approximately threefold.

Patients typically report specific joint pain, muscle weakness, and restrictions in ROM. Osteophytes and bone fragments may produce radicular pain if a nerve root is compressed and can limit ROM. Heberden's nodes at the medial and dorsolateral aspects of the distal interphalangeal joints and/or Bouchard's nodes at the proximal interphalangeal joints may be present. Hip pain is frequently located at the joint line but may radiate to the groin, buttocks, anterior thigh, or knee. Reduced hip ROM impacts function and hygiene. Knee osteoarthritis (KOA) may appear as knee instability, restrictions in ROM, crepitus, pain with loading activities, or buckling of the leg with transfers and ambulation.

Common interventions to manage OA include dynamic strengthening exercises (both open and closed chain exercises), core stability exercises, education, balance activities, aerobic exercises (biking, swimming), joint mobilization and manipulation, bracing (severe cases), gait training, use of orthotics, and appropriate footwear.[58] Thermotherapy, including deep heat applications (ultrasound), may alleviate pain, enhance tissue extensibility, and reduce stiffness.[14] Paraffin is useful for hand treatment because it provides a medium for dispersing heat over small joints. Exercise can effectively improve function, reduce stiffness, and maximize independence. Recent reviews and synthesis of exercise literature for KOA and hip OA, provided at varying intensities, frequencies, and durations, report significant moderate gains in muscle strength and function (effect sizes from 0.3 to 0.6).[16, 62-65,75,101-103] The emphasis of patient education is on joint protection techniques, activity modification, weight reduction, and strategies to unload the joint.

Manual therapy techniques for patients with KOA include tibiofemoral and patellofemoral joint mobilization. Manual therapy is followed by stretching of the quadriceps, hamstrings, tensor fascia, iliotibial band (ITB), hip flexors, and gastrocnemius soleus, and with soft tissue mobilization techniques. These combined interventions yield the greatest benefits for joint range and function.[75,81,101-103] Open-chain isometric exercises, such as quadriceps exercises, are used with progression to closed-chain weight-bearing exercises, such as mini-squats and step-ups.[64] Balance activities, such as single-leg stance and hop, are designed to facilitate co-contraction, and coordination of muscle firing useful for promoting knee stability. Sensorimotor interventions may provide necessary proprioceptive input, but their efficacy is still unknown.[104] Lateral wedge orthoses in patients with medial compartment KOA assist with altering biomechanical knee loading and are well tolerated.[19] With progressive disease and instability, knee braces are used to decrease medial compartment pressures resulting from varus positioning. Systematic reviews of knee bracing report limited benefits for pain relief and function. However, it is important to note that adherence to these devices is problematic.[19]

Hip OA often results in restrictions in internal hip rotation and impacts gait. Manual techniques for the hip, such as joint mobilizations and manipulations, aim to improve the elasticity of the joint capsule and surrounding muscles. The combination of mobilization and exercise appears to be

more effective than exercise alone.[16,64,105] Ambulatory aids (canes, crutches) help unload the joint and allow patients to maximize independence.

## Ankylosing Spondylitis

Ankylosing spondylitis (AS) is a systemic inflammatory disease that targets the sacroiliac and axial joints. Systemic features include fatigue, malaise, and sarcopenia, resulting in reduced physical activity engagement. Enthesopathy is common and impacts gait and transfers. With persistent inflammation, ankyloses of the spine can occur,[106] limiting trunk ROM and rib cage excursion. AS may also involve the hip and shoulder joints. Cardiovascular pulmonary manifestations may be seen; when they are coupled with trunk and rib cage restrictions, as well as the potential for costochondritis, aerobic capacity may be dramatically impacted.[83]

Rehabilitation interventions for patients with AS include physical modalities, such as thermal therapy to relax soft tissues and prepare the patient for flexibility exercises, manual therapy, assistive and ambulatory devices, orthoses, and functional activities. The principles of physical modalities for people with AS are similar to those for those with PsA. Flexibility exercises are used to promote extension and maintain a neutral spine. Incorporating posture and flexibility exercises into daily routines can enhance adherence and maintain joint ROM. For example, patients can be encouraged to read the newspaper while lying prone or to take stretch breaks during the day to avoid long periods of time sitting. Given the potential for reduced rib cage excursion, deep breathing exercises are used, although their efficacy is unproven. Evidence for dynamic progressive strengthening exercises of the postural muscles (at moderate intensity) and motor control activities demonstrate these exercises are useful for maximizing strength and function. A Cochrane review of three RCTs reported greater improvements in spinal mobility and patient global assessment among patients participating in supervised group exercise.[107] Aquatic exercises provide a nice alternative, and the buoyancy of the water can facilitate movement. However, studies of aquatic therapy for individuals with AS are limited and, at best, show small effects.[108] Ambulatory and assistive devices may be warranted as the disease progresses or to unload a painful hip. Patient education typically focuses on smoking counseling, joint protection, energy conservation, exercise, and coping strategies.

## Strategies to Increase Adherence to Exercise and Rehabilitation

Rehabilitation interventions require active patient participation and a commitment for benefits to be acquired. The team must always maintain a patient-centered focus and engage the patient in mutual goal setting and problem solving. Clear, concise communication about treatment expectations and the establishment of small, progressive, and achievable milestones are important. Potential barriers to exercise, such as fear of joint damage or exacerbation of pain, lack of social support for exercise, fatigue, lack of transportation, or cost, must be considered in the development of appropriate and acceptable interventions. With systemic inflammatory diseases, disease activity primarily influences the mode, frequency, and intensity of exercises prescribed, although other factors that influence intervention selection include disease severity, systemic manifestations, other comorbidities, and patient preferences. Self-management programs promote adherence to exercise and active lifestyles and are highly effective.[81] Referring patients to community resources such as Arthritis Foundation exercise programs (e.g., *Walk with Ease* and *Fit and Strong!*) allows them to develop social networks, to share their experiences in living with arthritis, and provides venues for exercise. Mobile applications to promote physical activity available free or with a modest cost, are currently undergoing testing as mechanisms to support an active lifestyle and promote social networking. Their effectiveness is unknown.

# CONCLUSION

Rehabilitation interventions comprise a vast array of interventions. Physical modalities are adjuncts to exercise and self-management and provide small benefits with respect to pain relief. Evidence for orthotics and bracing is weak, although adherence is a factor that influences results. Exercise is the most intensively studied intervention; it provides modest benefits for pain relief, strength, function, mood state, and joint stiffness across most disease entities. Selection of rehabilitation interventions for systemic inflammatory diseases is influenced primarily by disease state but is also dependent on disease severity, medication latency periods, comorbidities, disease severity, and patient preferences.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 39



# Pregnancy and Rheumatic Diseases

Lisa R. Sammaritano • Bonnie L. Bermas

**KEY POINTS**

- With careful planning and a team approach, most women with rheumatic diseases can have successful pregnancies.
- For best maternal and fetal outcome, patients with rheumatic diseases should conceive in periods of low disease activity and while on medications considered low risk for pregnancy.
- Rheumatology patients should undergo pre-pregnancy assessment to assess disease severity and activity, medication safety, and relevant autoantibodies.
- Not all anti-rheumatic medications can be used during pregnancy or breastfeeding, but recent data provide guidance for many commonly used medications.
- Use of effective and safe contraception is critical for rheumatic disease patients, especially those with severe disease, active disease, or those on teratogenic medications.

The predominance of many rheumatic diseases in women during their reproductive years makes management of pregnancy an important component of the comprehensive care of these patients. Advancements in disease therapies and identification of risk factors for poor pregnancy outcome allow many more patients to pursue pregnancy.

## INTERPLAY OF RHEUMATIC DISEASE AND PREGNANCY PHYSIOLOGY

**KEY POINTS**

- Normal pregnancy induces multiple physiologic changes that often impact manifestations of rheumatic diseases.
- Hypertensive diseases of pregnancy may complicate pre-existing rheumatic disease and be difficult to differentiate from active rheumatic disease.

Understanding potential interactions between pregnancy and rheumatic disease requires a basic knowledge of pregnancy physiology. Pregnancy affects the maternal immune system in multiple ways to ensure fetal survival. In brief, cell-mediated immunity decreases, immunoglobulin secretion increases, and pregnancy-specific proteins act to suppress lymphocyte function. Complement levels rise as a result of increased synthesis. The dominant T helper 2 cell cytokine profile, possibly important in sustaining pregnancy, may have varying implications for different autoimmune diseases.[1]

During pregnancy, a majority of the organ systems experience some degree of change. Intravascular volume increases by 30% to 50% and may be poorly tolerated by patients with significant renal or cardiac compromise. The glomerular filtration rate (GFR) increases by 50% in a normal pregnancy; as a result, patients with pre-existing proteinuria experience increased urinary protein excretion, which may raise concerns about active renal disease. Pregnancy induces a prothrombotic state, and the combination of estrogen-induced hypercoagulability, venous stasis, and compression by the gravid uterus elevates the risk of venous thromboembolism in a normal pregnancy by a factor of five. Red blood cell mass increases to a lesser extent than plasma volume, resulting in anemia that is secondary to hemodilution. Thrombocytopenia occurs in 8% of uncomplicated pregnancies. The white blood cell (WBC) count is elevated and the erythrocyte sedimentation rate (ESR) also rises, making both tests less useful as indictors of inflammation. Elevated progesterone levels in pregnancy decrease gastrointestinal motility and sphincter tone: in conjunction with uterine compression, these changes result in gastric reflux in as many as 80% of pregnant women. Slowed intestinal transit time contributes to an exacerbation of reflux and constipation in illnesses such as systemic sclerosis. Pregnancy-related rashes may occasionally be confused with autoimmune disease skin manifestations. A more common problem is facial and palmar erythema caused by pregnancy-induced vasodilatation mimicking inflammatory rash. Chloasma gravidarum, an estrogen-induced facial hyperpigmentation, may similarly suggest a malar rash. Hormone-induced ligamentous laxity often causes arthralgias that may be attributed to joint inflammation. Finally, reversible bone loss occurs with both pregnancy and lactation; this is especially worrisome for patients with pre-existing osteoporosis caused by rheumatoid arthritis (RA) or long-term corticosteroid use.[2]

Hypertensive disorders complicate as many as 10% of pregnancies in the general obstetric population and are more common in patients with rheumatic disorders. They are a major cause of maternal, fetal, and neonatal complications. Gestational hypertension and pre-eclampsia are both more common among women with systemic lupus erythematosus (SLE) and among women with renal disease of any etiology. The hypertension, proteinuria, renal insufficiency, and edema associated with pre-eclampsia can mimic lupus nephritis, scleroderma renal crisis, or vasculitis flare. Eclampsia, which includes seizures and rarely stroke, may be confused with central nervous system lupus or vasculitis.

HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, a variant of severe pre-eclampsia, is characterized by the presence of hemolysis, elevated liver enzymes, and low platelets.

## GENERAL PRINCIPLES OF PREGNANCY AND RHEUMATIC DISEASE: PRE-PREGNANCY ASSESSMENT

**KEY POINTS**

**Rheumatic disease women should conceive during a period of inactive disease for optimal pregnancy outcome.**

**Preconception evaluation should include assessment for disease damage, current disease activity, safety of medications for pregnancy, and aPL, anti-Ro/SS-A, and anti-La/SS-B antibodies.**

General principles of pregnancy management for rheumatic disease patients include a structured pre-pregnancy assessment for risk for maternal and obstetric complications, communication of risk and prognosis through counseling, and coordinated rheumatology and obstetric care. Assessment of rheumatic disease patients considering pregnancy should follow the same protocol regardless of the specific diagnosis. Determination of risk should include identification of serious disease-related organ damage that might affect the patient's ability to safely carry a pregnancy, evaluation of current and recent disease activity, review of medication safety, and serologic evaluation for autoantibodies associated with adverse maternal, fetal, or neonatal outcome.

### Severe Disease Damage

Severe manifestations of disease damage may preclude pregnancy. These include severe cardiomyopathy, cardiac valve disease, pulmonary arterial hypertension (PAH), interstitial lung disease, neurologic manifestations, and renal insufficiency. PAH in particular is associated with a high risk of pregnancy-related mortality.[3] The most important predictors of permanent renal disease in pregnant women with chronic kidney disease (CKD) are GFR <40 mL/min/1.73 $m^2$ and proteinuria greater than 1 g/24 hours.[4] The potential complications associated with severe chronic disease manifestations make pregnancy extremely high risk for these patients. If such patients are intent on having a biologic child, it may be appropriate to consider the safety of in vitro fertilization (IVF) with a surrogate.

### Disease Activity

Pregnancy reports in almost every rheumatic disease suggest that active disease increases the risk of adverse events. These patients should hopefully defer pregnancy if possible, use suitable contraception, and be treated aggressively: when disease has been inactive for approximately 6 months, they should be reassessed.[5] Even in RA, disease activity during pregnancy is associated with a small increase in the risk of lower birth weight and pre-term delivery.[6]

### Medication Review

If disease is inactive and there is no indication of severe damage, it is appropriate to assess the patient's present medical regimen. When current medications are contraindicated for use in pregnancy, one option is to taper and discontinue the current medications, allowing an appropriate period of time for high-risk medications to leave the system and for disease to demonstrate stability. The other option is to change to permissible medications and observe for stability on the new regimen.

### Assessment of Autoantibodies

Assessment of autoantibodies helps determine the type and frequency of pregnancy monitoring, the need for potential additional therapy, and to inform both physician and patient regarding risk. Every patient with SLE and patients who have an adverse obstetric history or history of thrombosis should be evaluated for the presence of anti-phospholipid antibodies (aPL). Patients with SLE, RA, undifferentiated connective tissue disease (UCTD), and Sjögren's syndrome should be evaluated for the presence of anti-Ro/SS-A and La/SS-B antibodies.

### Counseling

The full reproductive spectrum from fertility through lactation should be reviewed during the preconception visit. Education should include the risks of pregnancy for the individual patient given her particular clinical profile and is based on damage, disease activity, renal function, aPL, anti-Ro/SS-A and La/SS-B, and medications. Each patient and her partner need to understand the risk to her health, the anticipated pregnancy outcome, and the potential risk to offspring (most commonly pre-term birth or small size for dates). The necessary follow-up and monitoring during pregnancy should be considered, and assure neonatal supportive care if complications develop. Long-term outcomes of children born to mothers with rheumatic disease have received recent attention. Children of both SLE and antiphospholipid syndrome (APS) patients are thought to have a slightly increased risk for developmental disability, thus these children should be monitored accordingly.[7]

## SYSTEMIC LUPUS ERYTHEMATOSUS

**KEY POINTS**

**There is a higher risk of pre-eclampsia, pre-term delivery, fetal loss, and low birth weight infants in SLE patients.**

**SLE can flare up during pregnancy especially in patients with pre-existing renal disease and highly active disease at the time of conception.**

**Anti-malarial medications should be continued during SLE pregnancy; prednisone and prednisolone may be used when necessary, as may azathioprine, cyclosporine, and tacrolimus.**

SLE has its peak incidence in women in their reproductive years, thus co-existing pregnancy in this patient population

is a frequent occurrence. Fertility is generally unimpaired in SLE patients in the absence of severe disease activity or history of cyclophosphamide administration.

## Maternal Outcomes

For many years it was thought that most patients with SLE would worsen during pregnancy, and patients were counseled against becoming pregnant. Recent advances in management and a better understanding of SLE pregnancy has rendered this approach archaic. At present, the majority of women with SLE can anticipate successful pregnancies resulting in a healthy baby. Nonetheless, patients can have flare ups during pregnancy and do experience a higher incidence of pregnancy-related complications. The data on whether SLE significantly worsens during pregnancy or remains stable are conflicting.[8,9] Conflicting results reflect the lack of uniformity in definition of disease flare, differing patient populations, lack of control groups, and varied medication use. Most agree that patients with active disease in the 6 months before conception are at greatest risk for flare during pregnancy. The overall flare rate in patients with active disease in the preconception is estimated at 60%, whereas pregnancy flare rates may be as low as 10% in those with quiescent disease.[10,11]

Women with SLE have a two to fourfold increase in the rate of pregnancy complications. Pre-eclampsia will develop in 25% of women with SLE during pregnancy.[12] Distinguishing a lupus flare from pre-eclampsia is challenging. In general, one expects laboratory and clinical evidence suggesting active disease in a lupus flare, whereas pre-eclampsia is more likely associated with stable disease parameters and an acellular urine despite presence of proteinuria (Table 39-1). Differentiation is important because pre-eclampsia is managed with expectant delivery, whereas lupus flares are managed with medication. In reality, management often includes treatment for both because differentiation may be impossible and disease flare and pre-eclampsia may coexist. In addition to the higher rate of pre-eclampsia, one-third of pregnancies in SLE patients are complicated by pre-term birth, and one-third are delivered by Cesarean section.[13]

## Fetal and Neonatal Outcomes

Fetal loss rate in SLE pregnancies, as manifested by combined miscarriage and still-birth rates, approaches 20%. Although miscarriage rates are not significantly higher than in the general population, the rates of still-birth are significantly increased.[14] Premature delivery, active disease (particularly nephritis), and medication use in SLE pregnancies may contribute to the increased risk of intrauterine growth restriction (IUGR), lower birth weight, and pre-eclampsia.[15,16] Examination of placentas in SLE pregnancies suggests that placental vasculopathy, with or without the presence of aPL, may play an important role.[17] Patients with anti-Ro/SS-A and anti-La/SS-B antibodies are at risk for giving birth to children with neonatal lupus and congenital complete heart block.[18]

## Management

Patients with SLE should be co-managed by a rheumatologist and a maternal-fetal medicine physician. Before pregnancy, patients should have a baseline evaluation including history, physical examination, and laboratory testing as summarized in Table 39-2. Patients should be maintained on antimalarial medication throughout pregnancy because studies suggest benefits for mother and neonate.[19] Patients with active renal disease, central nervous system disease, recent cerebrovascular accident, or PAH should be counseled against pregnancy. In patients with aPL or those who have other risk factors for pre-eclampsia, low-dose aspirin should be prescribed. Those with coexisting APS are managed as outlined below. Patients should have well-controlled disease on low-risk medications for six months prior to attempting conception. Disease flares are managed with nonfluorinated glucocorticoids and, if necessary, the introduction of immunosuppressive agents compatible with pregnancy.

**TABLE 39-1** Differentiating Systemic Lupus Erythematosus Flare during Pregnancy From Pre-eclampsia

| Systemic Lupus Erythematosus Flare | Pre-eclampsia |
|---|---|
| Low white blood cell count | Normal to high white blood cell count |
| Occasional thrombocytopenia | Frequent thrombocytopenia |
| Normal liver function tests | Elevated liver function tests |
| Elevated blood pressure | Elevated blood pressure |
| Proteinuria | Proteinuria |
| Cellular urine with red blood cells and casts | Acellular urine |
| Normal uric acid | Elevated uric acid |
| Low complement levels | Normal to elevated complement levels |
| Increase anti-dsDNA levels | No change in dsDNA levels |

**TABLE 39-2** Pre-pregnancy Work-Up for Systemic Lupus Erythematosus

| |
|---|
| Complete blood count with differential and platelets |
| Complete metabolic panel |
| Urinalysis including microscopic analysis |
| Spot protein/creatinine urine ratio or 24-hour urine collection for protein* |
| Complement levels (C3, C4) |
| Anti-dsDNA level |
| Anti-Ro/SS-A and Anti-La/SS-B antibodies |
| Anti-phospholipid antibodies: Anti-cardiolipin antibodies, anti-beta2 glycoprotein I antibodies, and lupus anti-coagulant |
| Uric acid levels |

*As appropriate.

## MIXED CONNECTIVE TISSUE DISEASE AND UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE

**KEY POINTS**

**Patients with mixed connective tissue disease or undifferentiated connective tissue disease are likely to have uncomplicated pregnancies unless accompanied by PAH or progression to more well-defined systemic rheumatic disease.**

The mixed connective tissue disease (MCTD) has many clinical features in common with SLE. Undifferentiated connective tissue disease (UCTD) is terminology applied to patients who have autoantibodies and clinical findings suggestive of rheumatologic disease but who cannot be classified with a specific disorder. Fertility is not reduced in women with MCTD or UCTD.

### Maternal Outcomes

Limited data are available on pregnancy outcomes in women with these disorders. In a series of ten patients with MCTD, three had flares during pregnancy.[20] Flares included proteinuria, myositis, synovitis, and serositis. There are case reports of PAH appearing in pregnant MCTD patients[21]; thus pre-pregnancy echocardiogram screening for PAH should be considered. In 25 reported UCTD pregnancies, six patients had flares and symptoms meeting criteria for a new SLE diagnosis developed in one patient.[22]

### Fetal and Neonatal Outcomes

There is no increase in fetal loss in women with MCTD[23] although there are rare case reports of skeletal abnormalities in offspring of MCTD mothers.[24] In general, patients with MCTD and UCTD have good pregnancy outcomes, although they should be monitored for disease flares and progression to other systemic rheumatic diseases.

## SJÖGREN'S SYNDROME

**KEY POINTS**

**Mothers who are Anti-Ro/SS-A and anti-La/SS-B antibody-positive are recommended to have serial fetal echocardiograms to assess for development of congenital complete heart block; hydroxychloroquine may have a protective effect against congenital complete heart block.**

Although there are no reports of diminished fertility in patients with Sjögren's syndrome, some women experience dyspareunia because of vaginal dryness related to exocrine dysfunction in their underlying disease.

### Maternal Outcomes

Case reports exist of new onset renal disease and pericarditis in women with Sjögren's syndrome during pregnancy,[25] but no large-scale studies exist that document a pregnancy-associated increase in disease activity.

### Fetal and Neonatal Outcomes

Small series have suggested an increased risk of fetal loss in women with Sjögren's syndrome,[26] but larger studies have not substantiated this finding.[27] Sixty percent of patients with Sjögren's syndrome have anti-Ro/SS-A and anti-La/SS-B antibodies. These antibodies confer risk for the development of congenital complete heart block (CHB) (2%) and neonatal lupus erythematosus manifestations, including reversible thrombocytopenia, transaminitis, and photosensitive rash in their offspring (7% to 16%).[18]

### Management

Antibody-positive women should undergo screening fetal echocardiograms between 18 and 28 weeks of pregnancy to monitor for the development of CHB. Fluorinated glucocorticoids and intravenous immunoglobulin (IVIG) have been used to prevent development of CHB when first or second degree heart block is detected, but controlled studies have failed to demonstrate efficacy. Preliminary data suggest that hydroxychloroquine (HCQ) may reduce CHB risk.[28]

## ANTI-PHOSPHOLIPID ANTIBODY

**KEY POINTS**

**Presence of lupus anticoagulant is the most important risk factor for adverse pregnancy outcome in aPL-positive women.**

**Standard prophylactic therapy for obstetric anti-phospholipid syndrome is a combination of low-dose aspirin and low-dose heparin (low-molecular-weight heparin or unfractionated heparin).**

The presence of anti-phospholipid antibody (aPL) is a risk factor for pregnancy loss and other adverse pregnancy outcomes, especially when present in association with SLE. Clinical criteria for obstetric anti-phospholipid syndrome (OB-APS) include pregnancy loss (three or more consecutive losses at less than 10 weeks of gestation or one or more losses on or after 10 weeks of gestation) or delivery at less than 34 weeks of gestation because of pre-eclampsia, IUGR, or fetal distress. Laboratory criteria include persistent lupus anticoagulant (LAC) or moderate to high titer IgG or IgM isotypes of anti-cardiolipin (aCL) or anti-beta-2-glycoprotein I (aβ2GPI) antibodies.[29] Importantly, other causes of pregnancy loss must be ruled out with appropriate reproductive medicine evaluation. When considering a diagnosis of OB-APS, all three criteria aPL (LAC, aCL, and aβ2GPI) should be tested. The usefulness of alternative (non-criteria) aPL testing is uncertain.

The potential effect of aPL on fertility has been controversial, with the concern that aPL may interfere with implantation, particularly after IVF. However, the Practice Committee of the American Society for Reproductive Medicine has released guidelines based on extensive literature review stating that there is no indication to check aPL as

part of a fertility work-up, or to treat aPL-positive women for the purpose of improving IVF cycle outcome.[30]

### Maternal Outcomes

Maternal complications associated with OB-APS include pregnancy loss, pre-eclampsia, eclampsia, and HELLP syndrome. Anti-phospholipid antibody associated with HELLP syndrome generally occurs early (at 28 to 36 weeks), with hepatic infarction in one-third of cases and frequent progression to other thrombotic complications.[31] Other maternal complications include thrombosis (including catastrophic APS) and severe third-trimester thrombocytopenia.

### Fetal and Neonatal Outcomes

The most frequent neonatal complications are premature birth and IUGR. Premature births are more common in patients who have both APS and SLE. In primary APS pregnancies, risk factors for poor neonatal outcome are similar to predictors for adverse pregnancy outcomes overall, and include LAC, triple antibody positivity (defined as presence of LAC, aCL, and aβ2GPI), and history of vascular thrombosis. Patients with previous pregnancy morbidity alone without history of thrombotic manifestations may have a more favorable neonatal outcome.[32] Transplacental passage of anti-cardiolipin antibody has been documented, but thrombosis in the fetus or neonate is rare. Neonatal APS has been reported in less than 20 infants, and many infants had additional thrombotic risk factors, such as indwelling catheters.[33]

### Management

Effective management of the aPL-positive patient during pregnancy requires accurate assessment of risk for adverse pregnancy outcome. In the prospective multicenter PROMISSE (Predictors of Pregnancy Outcome: Biomarkers in Anti-phospholipid Syndrome and Systemic Lupus Erythematosus) study, LAC was identified as the most important risk factor for adverse pregnancy outcome in aPL-positive women. Multivariate analysis showed relative risk for adverse pregnancy outcome with LAC to be 12.33.[34] Other PROMISSE risk factors included younger age, history of thrombosis, and SLE. Importantly, aCL and aβ2GPI status were not independently associated with adverse pregnancy outcome.[34] Triple aPL positivity has also been suggested as an important risk factor in other cohorts,[35] and presence of low complement levels may also be associated with increased risk.[36]

A 1992 prospective study by Cowchock et al. showed that low-dose heparin plus low-dose aspirin is equal in efficacy to prior therapy with corticosteroids plus low-dose aspirin, with fewer side effects.[37] This combination remains the standard of care, with either low-molecular-weight heparin (LMWH) or unfractionated heparin (UF). Meta-analyses of treatment trials confirm the benefit of combination therapy; however, controversy continues regarding the details of the efficacy of this therapy. One analysis of five studies with 334 primary APS patients suggested success rates of 75% vs. 56% for combination therapy versus low dose aspirin alone[38]; another meta-analysis found combination therapy to be effective for early but not late losses, and found UF, but not LMWH, to improve outcomes.[39] Second-line therapy for treatment failure is usually the addition of IVIG based on case reports, although randomized treatment trials do not support its benefit.[40] Future therapies based on murine models may include complement[41] or tumor necrosis factor (TNF) inhibitors.[42] No strong data currently exist to support the treatment of obstetric patients with asymptomatic aPL antibodies, but low-dose aspirin is often used empirically for patients with high-risk aPL profiles. Low-dose aspirin seems especially reasonable for patients with additional risk factors for pre-eclampsia, including concomitant SLE, hypertension, or renal insufficiency. Patients with previous thrombosis require therapeutic heparin throughout pregnancy, with a change from warfarin either preconception or before six weeks' gestation, to avoid warfarin embryopathy. Heparin dosing usually requires adjustment later in pregnancy because of increased clearance, and LMWH is frequently changed to UF near term because of its shorter half-life. Fetal monitoring with nonstress tests, Doppler studies, or serial ultrasound is routine in the third trimester. Postpartum anticoagulation should be continued for 6 to 12 weeks. Recommendations for long-term thrombosis prophylaxis in patients with OB-APS without a history of thrombosis are lacking, although one large observational cohort study reported an increased risk for subsequent deep vein thrombosis and stroke in these women.[43]

## INFLAMMATORY ARTHRITIS

**KEY POINTS**

- **RA tends to improve during pregnancy.**
- **Active RA during pregnancy can result in lower birth weight infants.**
- **Preconception planning for inflammatory arthritis patients may require medication adjustments: anti-malarials and sulfasalazine can potentially be continued, methotrexate and leflunomide must be discontinued, and recent data suggest TNF inhibitors may be continued if necessary.**

### Rheumatoid Arthritis

Rheumatologists are frequently required to manage RA during pregnancy and, less commonly, to manage pregnancy in women with psoriatic arthritis (PsA) and the spondyloarthropathies (SpA). Smaller family size has been reported in women with RA diagnosed before childbearing.[44,45] Decreased family size is likely multifactorial and may include delays in conception because of disease activity or medication adjustment. Reduced fertility does not seem to be related to ovarian function because anti-Müllerian hormone levels (a marker of ovarian reserve) are normal.[46]

#### Maternal Outcomes

Clinically meaningful remission in RA during pregnancy may be difficult to define, in part because inflammatory markers, including ESR and c-reactive protein (CRP), are elevated in healthy pregnancies and do not provide a good reflection of disease activity. Hench first noted in the 1930s

that many RA patients improve during pregnancy.[47] Although early reports suggest approximately 70% of RA patients enter remission during pregnancy,[48] a 2008 study that used the Disease Activity Scale (DAS) 28 found that only 48% of RA patients improved.[49] Patients who are anti-cyclic citrullinated peptide (CCP) antibody and rheumatoid factor (RF) negative are more likely to remit.[50]

### Fetal and Neonatal Outcomes

RA does not increase the rate of fetal loss during pregnancy.[51] Even after controlling for medication use, women with active RA during pregnancy are found to be at risk for giving birth to small for gestational age infants.[6,52] Minimizing disease activity during pregnancy appears to be important for optimal fetal outcome.

## Psoriatic Arthritis and Ankylosing Spondylitis

Case series suggest that remission rates for PsA are similar to those for RA.[53] Results are more disparate for pregnancy in spondyloarthropathies. One survey of 649 women with ankylosing spondylitis (AS) found that roughly one-third of patients worsened, one-third stayed the same, and one-third improved during pregnancy.[53,54] In another case-controlled study of 35 pregnancies in mothers with AS, 51% of patients reported decreased pain.[55] Cesarean section rates may be as high as 58% in the spondyloarthropathies, with a reported miscarriage rate of 15% and normal rates of term delivery.[53,54]

## Management

Teratogenic medications such as methotrexate and leflunomide must be discontinued before pregnancy; current evidence suggests that other medications such as anti-malarials and sulfasalazine may be continued. Recent evidence suggests that TNF inhibitors may be continued during pregnancy through the second trimester in women with active disease. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids may be used sparingly during pregnancy. Evaluation of cervical spine stability and hip range of motion is suggested before delivery.

# INFLAMMATORY MYOSITIS

**KEY POINTS**

- **Quiescent disease at conception is associated with better pregnancy outcomes for idiopathic inflammatory myopathy patients.**
- **Corticosteroids, intravenous immunoglobulin and azathioprine are useful for idiopathic inflammatory myopathy, flares during pregnancy.**

Idiopathic inflammatory myopathies (IIM) include polymyositis (PM), dermatomyositis (DM), juvenile myositis (JM), and inclusion body myositis (IBM). Because the age of onset is bimodal, either in childhood or older adulthood, it is uncommon for patients to have pregnancies after the diagnosis of adult-onset myositis, and data are limited. One series found myositis developed in only 14% of patients before or during the childbearing years[56]; another recent series reported 8 in 51 patients with pregnancies following diagnosis.[57] There are no data on fertility in patients with IIM.

## Maternal Outcomes

Pregnancies have been reported during both quiescent and active periods of disease, and a number of reports have described new onset disease during or immediately after pregnancy.[56,58] New disease onset during pregnancy is often acute and severe, and rhabdomyolysis and myoglobinuria have been reported.[58] Risk of flare during pregnancy for patients in remission before conception is low.[57] Although disease with onset during pregnancy has a more severe course, maternal outcome is good overall for patients with IIM, and maternal mortality is rare.

## Fetal and Neonatal Outcomes

Neonatal outcomes for patients with pre-existing disease who are in remission at time of conception are best, with no increased risk for fetal loss. Active disease early in the pregnancy has an adverse effect on fetal and neonatal outcomes, whereas flare later in pregnancy does not. Neonatal outcomes are worst for patients with new-onset disease during pregnancy, with only a 38% survival rate.[59,60] In addition to fetal loss, prematurity, and IUGR, other uncommon fetal outcomes have been reported. Two infants with elevated creatine kinase (CK) levels at birth were born to mothers with IIM.[61] Massive perivillous fibrin deposition of the placenta has also been reported, a pathology finding associated with clinical evidence of placental insufficiency and poor fetal outcomes.[56,58,62]

## Management

Patients with IIM are most likely to have successful pregnancies if disease is quiescent before conception. No evidence supports prophylactic corticosteroid treatment, but careful follow-up with prompt treatment of even mild disease flares may improve pregnancy outcome. IVIG has been used successfully with disease onset in the first trimester.[63] Azathioprine may be considered, either alone or in combination with IVIG.

# SYSTEMIC SCLEROSIS

**KEY POINTS**

- **Systemic sclerosis patients with active renal disease, PAH, or significant cardiac compromise should avoid pregnancy because of high risk for poor maternal outcome.**
- **Systemic sclerosis patients with early diffuse disease should defer pregnancy due to a higher risk of scleroderma renal crisis.**

Systemic sclerosis (SSc) is an uncommon disease with a predilection for women during the fifth to sixth decade of life; as a result, there is limited information on this disorder during pregnancy. However, while more women extend

childbearing into their forties, pregnancy issues in SSc become more relevant. Data on whether SSc impacts fertility are conflicting. One retrospective study reports a greater than twofold incidence of infertility before diagnosis,[64] but another study did not find decreased fertility.[65]

### Maternal Outcomes

Raynaud's syndrome in SSc pregnancy generally improves because of increased blood flow to the periphery, whereas gastroesophageal reflux often worsens because of diaphragmatic relaxation. Skin disease does not progress.[66] Patients with early diffuse SSc and active skin thickening may have a higher risk of scleroderma renal crisis during pregnancy.[67] Management of renal crisis during pregnancy is challenging because angiotensin-converting enzyme (ACE)-inhibitors and angiotensin receptor blockers (ARBs) are contraindicated; however, when life-threatening disease occurs, these medications should be used. Scleroderma renal crisis may be difficult to differentiate from pre-eclampsia. Patients with pre-existing PAH are at very high risk for serious pregnancy complications: fluid shifts during pregnancy and especially during delivery may lead to right heart failure and, in some instances, death.[3] These patients should be counseled to avoid pregnancy.

### Fetal Outcomes

Retrospective studies reveal a twofold increase in spontaneous abortion rate in women who are subsequently diagnosed with SSc,[68] and women with SSc have higher rates of preterm delivery. Other reported complications include IUGR and low-birthweight infants.[66]

### Management

Given the potential for renal crisis, SSc patients should be followed by a rheumatologist and a maternal-fetal medicine specialist. Patients with PAH should be counseled about the high risk of maternal morbidity and mortality. Those with pre-existing renal disease should have their disease well-controlled on medications compatible with pregnancy.

## VASCULITIS

**KEY POINTS**

- **Pregnancy outcome in Takayasu's arteritis patients is largely determined by the presence of hypertension and pre-existing vascular damage. Aortic valve disease and the presence of aortic or renal aneurism are contraindications to becoming pregnant.**
- **Patients with medium- and small-vessel vasculitides are likely to have safe and successful pregnancies if conception is during a period of disease remission.**
- **Pregnancy in Behçet's disease has a variable effect on disease activity, but overall, fetal/neonatal outcomes are good.**
- **Medical management for patients with vasculitis during pregnancy may involve corticosteroids, azathioprine, IVIG, and rarely, cyclophosphamide in the third trimester for life-threatening disease.**

There are a limited number of reported cases of pregnancy in systemic vasculitis, resulting from both the older age of onset and male predominance. The available literature suggests active disease at the time of conception or new disease onset during pregnancy carries the most serious prognosis for maternal health and pregnancy outcome. Presence of vasculitis activity and end-organ damage may both affect pregnancy outcome. There are no data on fertility in these patients. In general, women who conceive after the diagnosis of any vasculitis report higher rates of pregnancy loss and pre-term delivery.[69] Flares of vasculitis activity during pregnancy vary from 18% to 50%.[69,70] A prospective series reported rates of miscarriage to be 20%; PROM, 33%; and pre-term delivery, 50%.[70]

### Large Vessel Vasculitis: Takayasu's Arteritis

Takayasu's arteritis is unique among the vasculitides in that it most commonly affects young women. Pregnancy complications are usually the result of vascular damage rather than disease activity, and pregnancy and maternal outcomes are optimized in patients with fewer damaged vessels. Risk of hypertension, pre-eclampsia, and IUGR is high, whereas likelihood of relapse of active vasculitis is low. In a large systematic review of 214 pregnancies in Takayasu's patients, pre-eclampsia occurred in 45% and pre-term delivery occurred in 16% of pregnancies.[71] Although pregnancy did not affect disease activity, pre-existing aortic valve disease or aortic or renal artery aneurysm conferred high risk for maternal mortality.[71] Another case series reported serious maternal complications of congestive heart failure, renal insufficiency, and cerebral hemorrhage. Neonatal effects included low birth weight, premature delivery, and IUGR in as many as 40% of newborns, but favorable outcome overall in 85%.[72]

Risk of complications such as cerebral hemorrhage or infarction in patients with Takayasu's arteritis is greatest at delivery because of fluctuations in regional blood flow. Monitoring of central aortic blood pressure is recommended for patients with severe vascular disease, with cautious use of epidural anesthesia to circumvent blood pressure vacillations. Active disease is unlikely and so most patients will not require immunosuppressive therapy.

### Medium Vessel Vasculitis: Polyarteritis Nodosa

The earliest reports of polyarteritis nodosa (PAN) in pregnancy suggested high rates of maternal mortality. New onset disease during pregnancy mimicked pre-eclampsia, and diagnosis was delayed in most cases.[73] Recent reports of pregnancies in established disease suggest better outcomes during periods of remission.[74] Fetal outcome overall is good. Because the new onset disease is most often in the third trimester or postpartum period, the risk to the pregnancy is that of prematurity and low birth weight rather than fetal loss. Infants with transient cutaneous vasculitis have been reported.[75] Management includes use of corticosteroid and immunosuppressive medications.

### ANCA-Associated Vasculitis

In granulomatosis with polyangiitis (GPA), there is a 25% relapse rate during pregnancy, but a high rate of successful

pregnancy outcome. As with PAN, adverse outcome is more likely in those with active or new manifestation of disease during pregnancy. Because most cases of new diagnoses have been in the third trimester, most of these pregnancies result in live-born births but pre-term deliveries. Quiescent disease at conception yields better outcomes.[76,77] Successful case reports describe the use of IVIG, azathioprine, and plasma exchange. Rarely, critically ill patients have been treated with cyclophosphamide in the second or third trimester with good neonatal outcome.[76] Severe subglottic stenosis may complicate delivery and require temporary tracheotomy to protect the airway.

Eosinophilic granulomatosis with polyangiitis (EGPA) (Churg-Strauss syndrome) case reports suggest maternal morbidity to be lower than in PAN.[78] Microscopic polyangiitis in pregnancy has rarely been reported, including a neonate with (transplacental) myeloperoxidase (MPO) antibodies, pulmonary hemorrhage, and renal disease.[79]

### Behçet's Disease

There are contradictory reports of pregnancy effects on disease activity in patients with Behçet's disease. A recent literature review including 220 pregnancies found improved disease activity in 63% of cases and relapse in 28% of cases.[80] Necrotizing neutrophilic vasculitis in two placentas has been described.[81] Pregnancy outcome overall is good, with a miscarriage rate of 20%.[82] Infants with pustulonecrotic skin lesions have been described,[83] and there is a potential increased risk of thrombosis.

## MEDICATIONS DURING PREGNANCY AND BREASTFEEDING

**KEY POINTS**

- **NSAIDs may be associated with early fetal loss, and their use should be minimized during early pregnancy. NSAIDs should be discontinued after 30 weeks' gestation because of the risk of premature closure of the ductus arteriosus.**
- **Prednisone and prednisolone are the preferred corticosteroids for use during pregnancy because low amounts are transferred across the placenta; fluorinated steroids such as dexamethasone and betamethasone are reserved for use when treatment of the fetus is desired.**
- **Hydroxychloroquine, sulfasalazine, azathioprine, 6-mercaptopurine, cyclosporine, tacrolimus, and IVIG are compatible with pregnancy.**
- **TNF inhibitors can be used when needed to control active inflammatory arthritis. They should be discontinued after the second trimester if possible circumvent immunosuppression in the newborn. Avoid live vaccines for six months in infants exposed to biologics during pregnancy.**

Medication management during pregnancy and lactation is challenging. Potential risks to the developing fetus and newborn must be weighed against the benefits of disease control. Limited information exists regarding drug safety in pregnancy. The current Food and Drug Administration (FDA) labeling system is imperfect because it utilizes a graded system of A, B, C, D, and X, which incorrectly implies an increased risk with ascending letter grade. A soon-to-be-released revision of the FDA labeling system will include three sections for each drug: a risk summary, clinical considerations, and a data section. When assessing any medication, risk must be measured against the reported background congenital anomaly rate of approximately 3%. Although benefits of breastfeeding extend to both the mother and the infant, medication use during the postpartum period can complicate lactation. Drugs are transferred to breast milk by diffusion: nonprotein bound, low-molecular-weight, nonionized, and lipid-soluble medications are most likely to enter breast milk.[84] Below is a discussion on the safety of the more commonly used medications for rheumatologic disorders in pregnancy and lactation (Table 39-3).

Ideally, medications should be adjusted before conception, but inadvertent exposure to teratogenic medications does occur. If this happens, the patient should be provided with information on the potential risks of exposure, and referred to appropriate registries such as Organization of Teratology Information Specialists, or OTIS (http://www.mothertobaby.org/). If available, high-resolution fetal ultrasound should be performed to assess for detectable anomaly.

### Aspirin, Nonsteroidal Anti-inflammatory Medications, and Cyclooxygenase-2 Inhibitors

High dose aspirin and NSAIDs are teratogenic in animals, but congenital anomalies have not been reported in humans.[85] NSAIDs can cause premature closure of the ductus arteriosus in the third trimester and should be discontinued by 30 weeks' gestation. Both NSAIDs and cyclooxygenase-2 (COX-2) inhibitors can potentially interfere with implantation and ovulation, and should be avoided during a conception cycle.[86] There are conflicting data as to whether NSAIDs and COX-2 inhibitors increase risk of spontaneous abortion during the first trimester.[87,88] Although findings are inconclusive, it seems reasonable to minimize the use of these medications during pregnancy. NSAIDs cross into breast milk at very low concentration; thus these medications are thought to be compatible with nursing. Mothers of jaundiced infants should avoid those medications metabolized by the liver, and all NSAIDs should be avoided in lactating mothers whose infants have thrombocytopenia.

### Glucocorticoids

The nonfluorinated glucocorticoids prednisone and prednisolone, which are typically used in the management of rheumatologic diseases, cross the placenta in low concentrations. In contrast, fluorinated glucocorticoids such as betamethasone readily cross the placenta to reach the developing fetus.[89] Recent evidence suggests that there is no increased rate of congenital anomalies after in utero glucocorticoid exposure.[90] Glucocorticoid use throughout pregnancy increases risk of pre-term delivery, small-for-gestational age infants, maternal hypertension, and gestational diabetes. Prednisone and prednisolone cross into breast milk in very low concentrations[91] and can be used in lactating women. When the dose is greater than 20 mg a day, avoiding breastfeeding within 4 hours of drug administration is recommended.

**TABLE 39-3** Risks of Rheumatic Disease Medications during Pregnancy and Lactation

| Drug | Maternal | Fetal | Lactation |
|---|---|---|---|
| ***Minimal Risk*** | | | |
| Hydroxychloroquine | None | None | Compatible |
| Sulfasalazine | Additional folic acid required as absorption is decreased | None | Compatible-one case of bloody diarrhea reported in neonate |
| IVIG | Risk of hepatitis C | Risk of hepatitis C | Compatible |
| Unfractionated heparin | Bleeding | None | Compatible |
| LMW heparin | Bleeding | None | Compatible |
| Aspirin (low dose) | Bleeding | None | Compatible |
| ***Low to Moderate Risk*** | | | |
| NSAIDs | Interferes with ovulation and implantation, possible increase in first trimester pregnancy losses | Safe; discontinue after 30 weeks of gestation because of increased risk of premature closure of the ductus arteriosus | Compatible but avoid NSAIDs with long half-life and enterohepatic circulation |
| Prednisone and Prednisolone | PROM, gestational diabetes, hypertension | Possible<br>No increase in congenital anomalies | Compatible but at dosing greater than 20 mg a day, discard breast milk for 4 hours after dose |
| Azathioprine<br>6-Mercaptopurine<br>Cyclosporine A<br>Tacrolimus | None<br>None<br>Renal Insufficiency<br>None | Transplant and IBD literature endorse the safety of these immunosuppressives during pregnancy. All increase the risk of PROM, SGA, IUGR | Low risk<br>Low risk<br>Low risk<br>Low risk |
| Etanercept<br>Adalimumab<br>Infliximab<br>Golimumab<br>Certolizumab | None<br>None<br>None<br>None<br>None | | TNF inhibitors: Low concentration in breast milk- low risk |
| Colchicine | None | Limited studies in FMF suggest that this medication can be used during pregnancy | |
| ***High Risk*** | | | |
| Methotrexate | | Embryotoxic and Teratogenic | Avoid |
| Leflunomide | | Congenital anomalies reported | Avoid |
| Cyclophosphamide | Maternal infection | Teratogenic | Avoid |
| Mycophenolate Mofetil | | Congenital anomalies reported | Avoid |
| Warfarin | Bleeding | Teratogenic | Avoid |
| ***Unknown Risk*** | | | |
| Rituximab | | | Avoid |
| Abatacept | | | Avoid |
| Tocilizumab | | | Avoid |
| Anakinra | | | Avoid |
| Belimumab | | | Avoid |
| Tofacitinib | | | Avoid |

IBD, Inflammatory bowel disease; IVIG, intravenous immunoglobulin; IUGR, intrauterine growth retardation; FMF, familial Mediterranean fever; LMW, low-molecular-weight; NSAIDS, nonsteroidal anti-inflammatory drugs; PROM, premature rupture of the membranes; SGA, small for gestational age newborns; TNF, tumor necrosis factor.

Modified from Bermas, BL: The medical management of the rheumatology patient during pregnancy in *Contraception and pregnancy in patients with rheumatic disease*. Springer New York, 2014, p. 275.

## Antimalarial Agents

Chorioretinotoxicity has been reported in rodent offspring after in-utero exposure to anti-malarials, and one case report suggests hearing issues in human offspring after chloroquine therapy during pregnancy.[92] However, multiple case series have not demonstrated an increased risk of teratogenicity in human pregnancy.[93] A 2002 survey of North American rheumatologists revealed that 69% prescribed anti-malarials during pregnancy.[94] Importantly, no ocular toxicity was found on examination of 588 neonates exposed to these medications in utero.[95] Anti-malarials cross into breast milk at low concentration, but exposed neonates showed no ocular toxicity.[96] These medications are considered to be compatible with nursing.

## Sulfasalazine

Although sulfasalazine and its metabolites do cross the placenta, large case series have not shown evidence for teratogenicity.[97] Sulfasalazine interferes with folic acid absorption, and thus pregnant women on this medication should take

additional folic acid. Men on this medication should discontinue the drug 3 months before conception to avoid azoospermia. Sulfasalazine appears in breast milk in significant concentrations. A single case of bloody diarrhea in a breast-fed infant has been reported,[98] but this medication is considered compatible with nursing. Because the active metabolite of sulfasalazine can displace bilirubin, women nursing premature infants, infants with hyperbilirubinemia, or infants deficient in glucose-6-phosphate dehydrogenase should avoid this medication.

## Immunosuppressive Agents

Methotrexate is both teratogenic and abortogenic and is contraindicated during pregnancy. In humans, methotrexate exposure during pregnancy particularly between 6 and 8 weeks' gestation can lead to craniofacial and limb malformations as well as significant developmental delays.[99] Although no definitive data suggest that men taking this medication are at increased risk for children with congenital anomalies, it is recommended that men discontinue methotrexate 3 months before conception to allow for a cycle of spermatogenesis. In women, methotrexate should be discontinued three months prior to conception. Limited data suggest that methotrexate is poorly transmitted into breast milk; however, current recommendations are to avoid use in lactating women.

Leflunomide is considered a potent teratogen in rodents.[100] Although recent studies suggest early exposure results in few anomalies in humans,[101] current recommendations are to either stop this medication 2 years before conception or to treat the patient with a cholestyramine wash-out to remove active metabolites in anticipation of pregnancy. There are no data as to whether leflunomide crosses into breast milk in significant concentrations. However, given the long half-life, leflunomide should be avoided in lactating women.

Although azathioprine and 6-mercaptopurine (6-MP) are category D drugs, large transplant registries have followed thousands of treated pregnancies and do not demonstrate an increased rate of congenital anomalies in exposed infants.[102] Minimal levels are found in breast milk[103]; thus use of these medications in nursing mothers of full-term infants is low risk, although testing for thiopurine S-methyltransferase (TPMT) levels in the newborn may be considered. Cyclosporine does not carry an increased risk of congenital anomalies. Although low levels of this medication have been found in breast milk, a single breast-fed infant was found to have a therapeutic level after nursing.[104] Tacrolimus has been used in the management of lupus nephritis, and this medication is considered compatible with pregnancy.[105] Little tacrolimus is transferred into breast milk and thus breastfeeding may be low risk for the neonate.[106] Mycophenolate mofetil, although a cornerstone of lupus nephritis management, has been associated with patterns of congenital anomalies and is contraindicated during pregnancy and breastfeeding.[107] Cyclophosphamide is extremely teratogenic and is contraindicated during pregnancy, although there are rare successful case reports of use during the third trimester for the management of vasculitis.[108] It is contraindicated in nursing women.

## Intravenous Immunoglobulin

There are limited data on the safety of IVIG during pregnancy, but no cases of congenital anomalies have been reported[109]; it is compatible with pregnancy and lactation.

## Tumor Necrosis Factor Inhibitors

Most TNF inhibitors are not actively transported through the placenta until 15 weeks' gestation, when elevated levels in cord blood can be found. A concerning report in 2009 described a clustering of congenital anomalies suggestive of VACTERL syndrome (vertebral anomalies, anal atresia, cardiac defects, tracheoesophageal fistula, esophageal atresia, renal anomalies, and limb dysplasia) in infants exposed to TNF inhibitors in utero.[110] Although subsequent analysis did not support an association,[111] this report informed decision-making for many rheumatologists who avoided prescribing these medications during pregnancy. PEGylated forms (those linked to a polyethylene glycol, or PEG, chain) cross the placenta in very limited amounts and thus may be preferred for use during pregnancy.[112] A 3-month old infant of a mother treated with infliximab throughout pregnancy died of disseminated infection after bacille-Calmette-Guérin (BCG) immunization,[113] thus current recommendations are to discontinue these medications by the third trimester of pregnancy and to avoid immunizations with live vaccines for 6 months in exposed infants. Transfer of TNF inhibitors into breast milk is minimal.[114]

## Other Biologic Agents

Limited information exists on the safety of other biologic medications during pregnancy. One large series of 153 pregnancies in rituximab-exposed fetuses demonstrated that the congenital malformation rate was similar to the background rate.[115] Given that little IgG crosses the placenta before 12 weeks of gestation, it seems unlikely that significant amounts of biologic agents would reach the developing fetus if medication is discontinued before conception. There are varied recommendations by manufacturers as to when these medications should be discontinued prior to pregnancy, with ranges from a few months (belimumab, abatacept) to a year (rituximab).

## Other Medications

Anticoagulation is the mainstay of therapy for the management of APS during pregnancy. Although warfarin is contraindicated during pregnancy because of its teratogenicity, heparin and LMWH are considered compatible with pregnancy and lactation. There is insufficient information on the newer anti-coagulants to assess their safety during pregnancy and lactation. Colchicine is rarely used during pregnancy; however, available data suggest that use during pregnancy does not cause congenital anomalies.[116] ACE inhibition and ARBs are contraindicated during pregnancy and lactation because of the risk of oligohydramnios and renal failure in the newborn; transition to another anti-hypertensive in advance of conception is recommended.

# PREGNANCY-RELATED ISSUES FOR RHEUMATIC DISEASE PATIENTS

**KEY POINTS**

- **Contraceptive options should be discussed with all female patients of reproductive age, with recommendations tailored to the individual patient's medical and social situation.**
- **Combined hormonal contraceptives may be used in stable SLE patients but are contraindicated in patients with positive aPL.**
- **Levonorgestrel IUDs or progesterone subdermal implants are good alternatives for most aPL-positive patients.**

## Contraception

Rheumatic disease patients are strongly advised to use contraception to avoid pregnancy if they have severe disease-related damage, active disease, or are taking teratogenic medications. In practice, however, rheumatic disease patients underutilize effective contraception. In a series of 97 SLE patients at risk for pregnancy, 23% had unprotected sex "most of the time."[117] In another series, 55% of those who used contraceptives were using less effective barrier methods, even those on teratogenic medications.[118] Inconsistent contraceptive use may reflect a lack of counseling by physicians.[119] Efforts at formalizing patient education to improve physician and patient awareness of effective contraception include educational materials, the use of institutional quality indicators, and adherence to FDA recommendations.

### Contraceptive Methods

Currently available contraceptives include barrier methods, hormonal contraceptives, intrauterine devices (IUDs), and subdermal implants. In general, long-acting reversible contraceptives such as IUDs or subdermal implants have the greatest efficacy, followed by other hormonal contraceptives; barrier or natural methods of contraception are least effective.

IUDs generally contain either progesterone (levonorgestrel) or copper. They have a low risk of infection for most patients; however, patients treated with immunosuppressive medications have not been specifically studied. Reassuringly, studies show no increased infection risk in women infected with human immunodeficiency virus.[120]

Hormonal contraceptives may be combined estrogen-progesterone or progesterone-only. Combined hormonal contraceptives include the pill, transdermal patch, and vaginal ring. Serious side effects include a three to five times increase in the risk of venous thromboembolism and a two time increase in stroke risk. Common medications, including warfarin and mycophenolate, may interact with these agents. In the past, concern about estrogen-induced flares has limited use of combined oral contraceptives in patients with SLE; however, prospective controlled studies in women with mild or stable disease activity showed no increased risk of flares with combined oral contraceptive (COC) use.[121,122] Oral contraceptives containing the progestin drospirenone may increase potassium levels and should be used with caution in patients with nephritis or on ACE-inhibitors. While the vaginal ring provides equal or lower estrogen levels than the pill, the patch provides 60% greater estrogen levels and may further increase thrombosis risk. Estrogen-containing contraceptives are not advised for use in aPL-positive patients.

Progesterone-only contraceptives include oral and intramuscular forms, IUDs, and the subdermal etonogestrel implant. Prolonged depot medroxyprogesterone acetate (DMPA) may decrease bone density due to inhibition of ovulation: it is best avoided in corticosteroid-treated patients. Progesterone-only contraceptives represent the best option for aPL-positive patients: the risk for thromboembolism is low and they generally decrease menstrual bleeding, a potential benefit for patients on anticoagulation. Emergency contraception is an option for all rheumatic disease patients and includes the copper IUD, prescription progesterone-receptor modulators, and over-the-counter levonorgestrel. Levonorgestrel is effective, convenient, and not contraindicated in patients with thrombophilia or cardiovascular disease.

Contraceptive choice in patients with rheumatic disease is challenging but important. The progesterone IUD or subdermal implant is preferable for most patients. Ultimately, decisions regarding contraceptive method in patients must take into account not only the risk of the method but also the risk of unplanned pregnancy, the ease of use, and the efficacy of each method. See Table 39-4.

## Fertility and Assisted Reproductive Techniques

**KEY POINTS**

- **Measures to preserve fertility in rheumatology patients include use of leuprolide during treatment with cyclophosphamide and embryo or oocyte cryopreservation.**
- **Risk of lupus flare and thrombosis are concerns for SLE and aPL/APS patients undergoing ovarian induction/in vitro fertilization; careful management generally results in successful outcomes.**

Fertility is generally unimpaired by rheumatic diseases, with important exceptions. Patients treated with cyclophosphamide (CYC) are at risk for gonadal failure, especially with older age and greater cumulative dose.[123] Active disease, high-dose corticosteroid, and chronic renal failure may adversely affect the hypothalamic-pituitary-ovarian axis. Tests of ovarian reserve include follicle-stimulating hormone (FSH), antral follicle count, and anti-Müllerian hormone level.

Prevention of medication-induced infertility is important. Concurrent treatment with a long-acting GnRH analogue (e.g., leuprolide) may decrease risk of premature ovarian failure in CYC-treated patients, although long-term data are limited.[124] Embryo and oocyte cryopreservation are good options to preserve fertility in patients who are stable enough to undergo ovarian hyperstimulation but are not able or ready to pursue pregnancy.

Common assisted reproduction techniques include ovarian induction (OI), with or without IVF, and embryo

**TABLE 39-4** Benefits and Risks of Contraceptive Methods for Rheumatic Disease Patients

| | Copper IUD | LNG IUD | Progestin Pill | DMPA | Progestin Implant | Comb OC | Vaginal Ring | Patch |
|---|---|---|---|---|---|---|---|---|
| **Frequency** | 10 years<br>Insertion by MD | 3-5 years<br>Insertion by MD | Daily oral<br>Take same time each day | Every 3 mo<br>Injection by MD | 3 years<br>Insertion by MD | Daily oral | Monthly | Weekly |
| **Relevant Side Effects** | Increased cramps/ bleeding | Little to no systemic progestin effects<br>Decreased cramps, bleeding | Break-through bleeding | Delayed return to fertility<br>Decreased bone density | Rapid return to fertility<br>No effect on bone density | Prothrombotic effect<br>Frequent medication interactions<br>May increase bone density | | |
| **Rheumatic Disease-Related Concerns** | Uncertain but unlikely increased infection risk in immunosuppressed pts*<br>LNG IUD decreases menstrual bleeding in anticoagulated pts<br>No significant thrombosis or lupus flare risk | | Decreased menstrual bleeding<br>No significant thrombosis or lupus flare risk | Decreased menstrual bleeding<br>No significant thrombosis or lupus flare risk<br>Significant risk of osteoporosis; avoid in RA or steroid-treated patients | Decreased menstrual bleeding<br>No significant thrombosis or lupus flare risk | No increased risk of flare in stable SLE<br>Increased risk thrombosis: Avoid with (+) aPL | Similar estrogen levels to OC<br>Increased risk thrombosis: Avoid with (+) aPL | Higher estrogen levels than OC<br>Increased risk thrombosis: Avoid with (+) aPL |

*Avoid if patient has had multiple sexual partners.

aPL, Anti-phospholipid antibody; DMPA, depot medroxyprogesterone acetate; IUD, intrauterine device; LNG IUD, levonorgestrel intrauterine device; OC, oral contraceptive; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

transfer. IVF cycles require more aggressive hyperstimulation with surgical extraction of oocytes, fertilization, and reimplantation. Although rare, ovarian hyperstimulation syndrome (OHSS) is an important complication that results in capillary leak with pleural effusion and ascites; severe OHSS increases risk for thrombosis and renal compromise, particular issues of concern for rheumatology disease patients. Important risks for rheumatic disease patients relate to the elevated estrogen levels and include lupus flare and thrombosis.[125,126] SLE patients with OI/IVF–induced flare generally have good outcomes. Thrombosis in aPL-positive or APS patients undergoing OI/IVF appears to be rare, although most reported patients have been treated prophylactically with aspirin or LMWH. Data do not support aPL as a cause of failed IVF or infertility, and so anticoagulation is not indicated to improve IVF cycle outcome.[30] Pre-IVF assessment should mimic the pre-pregnancy evaluation for rheumatic disease patients. OI/IVF should be planned for patients with stable inactive disease on medications compatible with pregnancy. Prophylactic anticoagulation should be considered in patients with high-risk aPL profiles, and is mandatory for confirmed APS patients.

## CONCLUSION

Caring for women with rheumatic disease throughout their reproductive lifespans is both challenging and rewarding. All reproductive-aged rheumatic disease patients on potentially teratogenic medications need to be counseled about effective contraceptive methods. In patients who are treated with cyclophosphamide it is important to discuss fertility-sparing approaches before the initiation of therapy. Common principles of reproductive care for rheumatic disease patients, regardless of specific diagnosis, include identifying the limited number of patients with severe disease-related damage who should avoid pregnancy, counseling patients to conceive when disease has been stable and inactive on medications considered low risk for pregnancy, and assessing particular risk factors for outcome such as anti-phospholipid and anti-Ro/SS-A and anti-La/SS-B antibodies. Finally, a plan for medication use in the case of disease flare during pregnancy should be agreed upon by the patient and treating clinician at the onset of pregnancy. With careful planning, most women with rheumatic disorders can anticipate a successful pregnancy with a good outcome.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 40

# History and Physical Examination of the Musculoskeletal System



John M. Davis III • Kevin G. Moder • Gene G. Hunder

**KEY POINTS**

- A detailed and accurate history is crucial to make the correct diagnosis in patients with musculoskeletal diseases.
- The primary symptoms of musculoskeletal disease are pain, joint stiffness, swelling, limitation of motion, weakness, fatigue, and loss of function.
- An understanding of the anatomy, the planes of motion and, particularly, the configuration of the synovial lining, is imperative for proper physical diagnosis of musculoskeletal diseases.
- It is important to record qualitative and quantitative aspects of the joint examination to monitor disease activity in patients with inflammatory arthropathies.
- Early recognition of how patients' psychosocial factors affect their musculoskeletal symptoms and musculoskeletal examination enhances clinical assessment.

## HISTORY IN A PATIENT WITH MUSCULOSKELETAL DISEASE

An accurate and comprehensive history of a patient's musculoskeletal symptoms is crucial to make the correct diagnosis. This history must include a precise understanding of what the patient means by his or her description of symptoms. The physician must obtain a detailed account of symptom onset, location, patterns of progression, and severity, as well as exacerbating and alleviating factors and associated symptoms. The relationship of the symptoms to psychosocial stressors is important and should be determined. The impact of the symptoms on all aspects of the patient's functioning must be assessed to guide therapy.

The effects of current or previous therapy on the course of the illness are helpful in efforts to understand current symptoms. Response to anti-inflammatory or glucocorticoid medications may suggest an inflammatory origin. Such responses are not specific to inflammatory rheumatic diseases, however, and must be considered in light of the entire history and physical examination. The physician must assess compliance with therapies for musculoskeletal diseases. Noncompliance with the recommended treatment must be differentiated from treatment failure as the explanation for the patient's lack of improvement.

While the physician is taking the patient's history, the patient provides verbal and nonverbal clues to the nature of the illness and how the patient has responded to it. Patients with early rheumatoid arthritis (RA) may hold their hands in a flexed posture to minimize intra-articular pressure and pain. Some patients may be overly concerned, whereas others may seem inappropriately indifferent to their symptoms. The physician must appreciate the patient's understanding of the illness and attitudes toward it to begin effective treatment.

### Pain

Pain is the most common symptom that brings a patient with musculoskeletal diseases to the physician. Pain is a subjective hurting sensation or experience that is described in various terms, often of actual or perceived physical damage. Pain is a complex sensation that is difficult to define, qualify, and measure. The patient's pain may be modified by emotional factors and previous experiences.

The character of the pain usually is best defined early in the interview because this can be helpful in categorizing the patient's complaints. Aching in a joint area suggests an arthritic disorder, whereas burning or numbness in an extremity may indicate a neuropathy. Descriptions of pain as "excruciating" or "intolerable" when the patient is otherwise able to function provide a clue that emotional or psychosocial factors are contributing to or amplifying the symptoms.

The physician must elicit the distribution of the patient's pain and determine whether this fits with anatomic structures. Patients describe their pain location in terms of body part names, but frequently the terms are used in a nonanatomic manner. Patients frequently complain of "hip" pain when they are actually referring to pain in the low back, buttock, or thigh. The interviewer must attempt to clarify this complaint by asking the patient to point to the area of pain with one finger. Pain localized in the distribution of a joint or joints likely reflects an articular disorder. Pain may localize to bursae, tendons, ligaments, or nerves, implying disorders of these structures. In contrast to superficial structures, deep structures often give rise to poorly localizing pain. Similarly, pain arising from small, peripheral joints is often more focal than pain arising from proximal, large joints, such as the shoulders and hips. Pain

that is widespread, is vaguely described, and does not respect anatomic distributions generally suggests a chronic pain syndrome, such as fibromyalgia or psychiatric disease.

The severity of the pain should be assessed. A common approach is to ask the patient to describe the level of pain on a numeric scale of intensity from 0 (no pain) to 10 (very severe pain). To monitor disease activity of inflammatory arthritis, measuring pain on a visual analogue scale by having the patient mark the severity of pain during the past week on a 100-mm line can be helpful. Similar scales are used in validated instruments, such as the McGill Pain Questionnaire.

The physician must determine what exacerbates and alleviates the pain. Joint pain present at rest but worse with movement suggests an inflammatory process, whereas pain that occurs primarily with activity and is relieved by rest usually indicates a mechanical disorder such as degenerative arthritis. Timing of pain symptoms during the day and night also provides important information, as discussed in the next section.

## Stiffness

Stiffness is a common complaint among patients with arthritis. What is meant by stiffness varies from patient to patient, however. Some patients may use the term *stiffness* to refer to pain, soreness, weakness, fatigue, or limitation of motion.[1] Rheumatologists generally use the term *stiffness* to describe discomfort and limitation when the patient attempts to move the joints after a period of inactivity. This "gel" phenomenon occurs usually after an hour or more of inactivity. The duration of stiffness related to inactivity varies, with mild stiffness lasting minutes and severe stiffness lasting hours.

Morning stiffness is an early feature of inflammatory arthropathies and is particularly noted in RA and polymyalgia rheumatica, in which morning stiffness may last for several hours. The absence of morning stiffness does not exclude inflammatory arthritis, but its absence is uncommon. A useful question to assess morning stiffness is this: "In the morning, how long does it take for your joints to limber up to as good as they are going to get for the day?" Morning stiffness associated with non-inflammatory joint diseases, such as degenerative arthritis, generally is of short duration (usually <30 minutes) and is less severe than stiffness of inflammatory joint disease. Additionally, the degree of stiffness in non-inflammatory joint diseases is related to the extent of use of the damaged joint: stiffness is worse after excessive use, generally improving within several days to the baseline level. Morning stiffness is not specific for inflammatory arthropathies and may be described by patients with fibromyalgia or chronic idiopathic pain syndromes, neurologic disorders, such as Parkinson's disease (although generally without limbering up), and sleep-related breathing disorders.

## Limitation of Motion

Limitation of motion is a common complaint among patients with articular disorders. This complaint must be differentiated from stiffness, which usually is transient and variable, whereas limitation of motion secondary to joint disease is generally fixed and varies less with time. The interviewer should determine the extent of disability resulting from the restriction in joint motion. The duration of the restriction in joint motion frequently predicts the likelihood of improvement with interventions such as oral and intra-articular glucocorticoids or physical therapy. Determining the rapidity of onset of the limitation of motion may be helpful in the differential diagnosis; abrupt onset of the limitation of motion suggests a structural derangement, such as a tendon rupture or torn knee cartilage, whereas insidious onset of restricted joint motion is more common with inflammatory joint disease.

## Swelling

Joint swelling is an important symptom in patients with rheumatic diseases. The presence of true joint swelling narrows the differential diagnosis in a patient with arthralgia. To determine whether the swelling is related to joint synovitis as opposed to soft tissue conditions, clarifying the anatomic location and distribution of the swelling is key. Diffuse soft tissue swelling can occur because of venous or lymphatic obstruction, soft tissue injury, or obesity. The description of swelling in patients with such conditions usually is ill defined or is not in a distribution of particular joints, bursae, or tendons. Obese patients may interpret normal adipose tissue over the medial aspect of the elbow, the knee, or the lateral aspect of the ankle as joint swelling. In contrast, patients with inflammatory arthritis may describe swelling of joints in a distribution typical of a specific disease—symmetric swelling of the metacarpophalangeal joints and wrists in RA, or swelling of several toes and a knee in psoriatic arthritis.

It is useful to delineate the onset and progression of swelling and the factors that influence it. Swelling of a joint resulting from synovitis or bursitis frequently is associated with discomfort with motion because of tension on the inflamed tissues. If swollen tissues are periarticular, however, no discomfort may be present with joint motion because the inflamed tissues are not stressed. Swelling of a confined structure, such as a synovial cavity or bursa, is most painful when it has developed acutely, whereas a similar degree of swelling that has developed slowly often is much more tolerable.

# WEAKNESS

Weakness is another common complaint that can be associated with myriad different subjective meanings. True weakness is the loss of muscle power. When present, it is demonstrable on physical examination.

The temporal course of weakness is important to the differential diagnosis. Weakness of sudden onset without trauma often indicates a neurologic disorder, such as an acute cerebrovascular event, which generally results in a fixed, nonprogressive deficit. Weakness of insidious onset more often suggests a muscle disease, such as an inflammatory myopathy (e.g., polymyositis). The latter tends to be ongoing and progressive. Weakness that is intermittent suggests a disorder of the neuromuscular junction, such as myasthenia gravis. Patients with this disease may describe muscle fatigue with activity as opposed to true weakness.

The physician should determine the distribution of the patient's weakness. Proximal weakness that is bilateral and symmetric suggests an inflammatory myopathy. In contrast, inclusion body myositis causes an asymmetric and more distal weakness. The presence of a unilateral or isolated deficit generally indicates a neurogenic origin. Distal weakness, in the absence of joint findings, generally indicates neurologic disorders, such as peripheral neuropathy. Patients with peripheral neuropathies also complain of pain and sensory symptoms, such as paresthesias. In contrast, patients with inflammatory myopathy often present with painless weakness.

Inquiring about the patient's family history may provide valuable information. A history of other family members with similar symptoms may increase the likelihood that the patient has a hereditary disorder, such as muscular dystrophy or familial neuropathy.

It also is important to review medication taken recently or currently. Many medications, including corticosteroids and lipid-lowering agents, can cause muscle injury. Less commonly, environmental exposure can lead to symptoms of weakness. Heavy metal poisoning causes a peripheral neuropathy. Dietary exposure also should be investigated, such as eating undercooked pork as a source of trichinosis. Excessive alcohol intake has been associated with neuropathy and myopathy.

Taking a complete review of systems is helpful in evaluating a patient with weakness. Constitutional symptoms, such as weight loss and night sweats, may indicate the presence of a malignancy as the cause of generalized weakness. Rash, arthralgia, or Raynaud's phenomenon may prompt further testing for a connective tissue disease.

### Fatigue

Patients with musculoskeletal disorders frequently complain of fatigue. *Fatigue* can be defined as an inclination to rest even though pain and weakness are not limiting factors. Fatigue after varying degrees of activity that is relieved by rest is normal. Patients with rheumatic diseases experience fatigue even without activity. Fatigue generally improves as the systemic rheumatic disease improves. Malaise frequently occurs with, but is not synonymous with, fatigue. Malaise indicates the lack of well-being that often occurs at the onset of an illness. Fatigue and malaise may occur in the absence of identifiable disease, and psychosocial factors, anxiety, or depression may account for the symptoms.

### Loss of Function

The comprehensive history should include an assessment of the patient's ability to perform activities of daily living, as loss of function is a common manifestation of musculoskeletal disease with serious impact on health and quality of life. The extent of disability may vary from loss of the ability to use one finger joint because of arthritis to complete physical incapacitation resulting from severe inflammatory polyarthritis. Irrespective of the cause, loss of physical function often has a profound impact on patient social activities, exercise routine, work capacity, and even basic self-care. Assessing for the presence and degree of functional disability is important to evaluate the severity of illness and in making treatment recommendations, particularly in RA, in which disability is among the strongest predictors of long-term outcomes and mortality.[2-4]

Functional capacity is assessed first by asking general questions about the patient's ability to perform daily activities, including grooming, dressing, bathing, eating, walking, climbing stairs, opening doors, carrying objects, and so forth. A report of a specific loss of function, such as difficulty opening a milk carton, should be investigated further to clarify why the task is difficult, which will inform the differential diagnosis and guide clinical examination. This information will also yield important information for management, such as opportunities for physical and occupational therapy, use of splints/braces, and so forth. Overall functional capacity may be evaluated with the use of an instrument such as the Health Assessment Questionnaire (see Chapter 33), which is widely used in research and in the clinic to monitor changes in physical function in response to therapy among patients with RA and other rheumatic diseases.

## SYSTEMATIC METHOD OF EXAMINATION

The musculoskeletal examination should be a systematic, thorough assessment of the status of the joints, periarticular soft tissues, tendons, ligaments, bursae, and muscles. Rheumatologists commonly begin by examining the upper extremities followed by the trunk and lower extremities, but many routines may be effective, provided that a systematic, consistent approach is used. Gentle handling of tender and painful joints enhances cooperation by the patient and allows an accurate evaluation of the joints.

The general aim of the examination of the joints is to detect abnormalities in structure and function. Key signs of articular disease include swelling, tenderness, limitation of motion, crepitus, deformity, and instability.

### General Observation

A general examination of the patient should be performed to look for any signs of systemic illness. This should include an examination of the skin, noting signs of pallor (which may suggest anemia), nodules (which may suggest RA or gout), or rashes (which may suggest lupus, vasculitis, or dermatomyositis). The patient should be appropriately undressed for examination. The physician should assess gait by asking the patient to walk in the examining room or down the hallway, because an antalgic gait may be seen in various musculoskeletal disorders of the spine or lower extremities, and various gait disorders may occur in neuromuscular diseases. The ability of the patient to arise and transfer to the examining table should also be evaluated, as this will provide information on pain, proximal muscle strength, and overall physical function. The patient should be assessed for appearance of the muscles, including bulk, tone, and tenderness. Muscle bulk should be compared on one side of the body with the other to look for any asymmetry, hypertrophy, or atrophy. The patient's manner and body language may provide information on his or her mood and anxiety level, which merits consideration in evaluating pain and tenderness.

## Swelling

Swelling around a joint may be caused by intra-articular effusion, synovial proliferation, periarticular subcutaneous tissue inflammation, bursitis, tendinitis, bony enlargement, or extra-articular fat pads. A keen understanding of the anatomic configuration of each joint's synovial membrane is crucial in differentiating soft tissue swelling secondary to a joint effusion from swelling of periarticular tissues. First, the examiner should inspect the joints for visible evidence of swelling, such as loss of normal landmarks or contours. It is frequently helpful to visually compare the same joints on both sides of the body to detect subtle evidence of swelling and to appreciate symmetry.

Second, the examiner should palpate each joint. The normal synovial membrane is too thin to palpate, whereas the thickened synovial membrane in many chronic inflammatory arthritides, such as RA, may have a "doughy" or "boggy" consistency. In some joints, such as the knee, the extent of the synovial cavity can be delineated on physical examination by compressing the fluid into one of the extreme synovial recesses. The edge of the resulting bulge may be palpated more easily. If this palpable edge is within the anatomic confines of the synovial membrane and disappears on release of compression, the distention usually represents synovial effusion; if it persists, it is an indication of a thickened synovial membrane. Reliable differentiation between synovial membrane thickening and effusion is not always possible by physical examination, however. Ultrasonography is used increasingly as an extension of the physical examination, allowing the examiner to differentiate between synovial proliferation and effusion.

## Tenderness

In the musculoskeletal examination, tenderness is unusual discomfort when the physician palpates and puts pressure on articular and periarticular tissues. Localizing the tenderness to palpation may assist the examiner in determining whether the pathology is intra-articular or periarticular in location, such as a fat pad, tendon attachment, ligament, bursa, muscle, or skin. It can be useful to palpate structures that are not involved to assess the importance of tenderness. Finding tender joints in a patient who also has numerous other myofascial tender points is less of a concern for arthritis than finding tender joints in a patient with no extra-articular tenderness.

## Limitation of Motion

Limitation of motion is a common manifestation of articular disease; the examiner must know the normal type and range of motion for each joint. Comparison of the affected joint with an unaffected joint of the opposite extremity is useful to evaluate individual variation. Restricted joint motion may be caused by changes in the joint itself or in periarticular structures. To distinguish these possibilities, it is crucial to compare the passive with the active range of motion. If the passive range of motion is greater than the active range of motion, the restriction may be the result of pain, weakness, or the state of articular or periarticular structures. It also is important to distinguish muscle tension from a true limitation of joint motion, emphasizing the importance of ensuring relaxation of the patient. Pain that occurs with attempts to move a joint passively to the limit of range of motion in one plane is referred to as *stress pain*. Pain in the joint with attempted active or passive range of motion usually indicates an abnormality in the joint.

## Crepitus

Crepitus is a palpable or audible grating or crunching sensation produced by motion. This sensation may or may not be accompanied by discomfort. Crepitus occurs when roughened articular or extra-articular surfaces are rubbed together by active motion or by manual compression. Fine crepitus often is palpable over joints involved by chronic inflammatory arthritis and usually indicates roughening of the opposing cartilage surfaces as a result of erosion or the presence of granulation tissue. Coarse crepitus may be caused by inflammatory or non-inflammatory arthritis. Bone-on-bone crepitus produces a higher-frequency, palpable, audible squeak. Crepitus from within a joint should be differentiated from cracking or popping sounds caused by the slipping of ligaments or tendons over bony surfaces during motion. The latter phenomena are usually less contributory to the diagnosis of joint disease and may be heard over normal joints. In scleroderma, a distinct, coarse, creaking, leathery crepitus may be palpable or audible over tendon sheaths.

## Deformity

Deformity of the joints may manifest as a bony enlargement, articular subluxation, contracture, or ankylosis in nonanatomic positions. Deformed joints usually do not function normally, frequently restrict activities, and may be associated with pain, especially with overuse. Occasionally, a deformed joint may function well but is a cosmetic concern. Joint deformities may be reversible or irreversible. Multiple swan neck deformities of the fingers that can be corrected with manipulation may indicate Jaccoud's arthropathy of lupus. In contrast, hand deformities in RA generally are not correctable.

## Instability

Joint instability is present when the joint has greater than normal movement in any plane. *Subluxation* refers to a joint in which there is partial displacement of the articular surfaces but still some joint surface-to-surface contact. A dislocated joint has lost all cartilage surface-to-surface contact. Instability is best determined by supporting the joint between the examiner's hands and stressing the adjacent bones in directions in which the normal joint does not move. The patient must be relaxed during the examination because muscle tension may stabilize an otherwise unstable joint. A knee with a deficient ligament might appear stable if the patient contracts the quadriceps muscles during evaluation.

## Other Aspects of the Examination

Examinations of the cervical spine and low back are discussed in Chapters 45 and 47.

## RECORDING THE JOINT EXAMINATION

Documentation of the joint examination is important in making decisions about therapy, monitoring the activity of arthritis, and determining the efficacy of interventions. Many different recording methods have been described. Abbreviations for each joint can be used, such as *PIP* for the proximal interphalangeal joints. The S-T-L system has been used historically to record the degree of swelling (S), tenderness (T), and limitation of motion (L) of each joint on the basis of a quantitative estimate of gradation.[5] This method remains useful but is used less commonly today because of increasing reliance on electronic medical records. It is easier to describe joint findings in narrative form, for example, "there is 2+ swelling of the second and third metacarpophalangeal (MCP) joints," where grade 0 indicates no swelling, grade 1 indicates palpable synovial thickening, grade 2 indicates loss of normal joint contours, and grade 3 indicates frank cystic swelling of the metacarpophalangeal joint. An alternative method is to record joint examination findings by using a schematic skeleton or homunculus. When accuracy is necessary, the range of motion of individual joints may be measured by using a goniometer.

Joint counts are standard assessments to monitor the activity of inflammatory arthritides in practice and in clinical trials.[6] For monitoring disease activity of RA, a 28-joint count for tenderness and swelling has been recommended. To assess the tender joint count, the examiner documents which joints the patient indicates are painful on palpation with enough pressure to blanch the nail bed of the examiner's thumb and index fingers. To assess the swollen joint count, the examiner documents which joints have palpable soft tissue swelling or fluctuance, excluding joints affected only by deformity or bony hypertrophy. The 28-joint count[7] includes the shoulders, elbows, wrists, first to fifth MCP joints, first to fifth proximal interphalangeal joints, and knees on both sides of the body. Compared with more extensive joint counts, the 28-joint count has the advantage of being quick and easy to perform; however, it is limited by the fact that the ankles and metatarsophalangeal joints are not included, so active disease in the feet may be underestimated. The 28-joint count is used to calculate the Disease Activity Score 28 (DAS28),[8] which is a validated instrument used to monitor disease activity.

The function of the joints in normal use is not captured by assessments of tenderness, swelling, or range of motion, so other examination techniques are necessary. Other tests are available that attempt to measure joint function by assessing the patient's ability to perform a coordinated task (e.g., shoulder arc of motion, measuring the 50-foot walk time). The results of such functional tests may vary, however. Biologic factors, such as circadian changes in joint size and grip strength among rheumatoid patients observed during a 24-hour interval, contribute to variability.

## INTERPRETING THE JOINT EXAMINATION

The physician must understand the significance of specific joint findings, both their presence and absence, to make appropriate treatment decisions. As with any diagnostic assessment, the accuracy and reliability of the joint examination are important considerations. With regard to accuracy in detecting physical signs of inflammatory synovitis, numerous studies have shown that joint examination is far less sensitive in detecting synovitis or effusions than high-resolution ultrasonography or magnetic resonance imaging (MRI).[9-11] Although it is true that swollen joints are more specific for active synovitis, recent clinical studies have suggested that joint tenderness has similar value compared with swelling in predicting the progression of radiographic joint damage.[12] Demonstrable physical signs of arthritis may be particularly subtle for patients with early disease.[13] Considering that MRI bone marrow edema is a predictor of radiographic damage, it is notable that one study reported that 35% to 57% of joints with bone marrow edema were negative for physical signs of synovitis.[14] Thus the examiner must consider the physical findings in view of the complete history of joint symptoms to make an accurate diagnosis, assess prognosis, and prescribe management. Ultrasound examination can also be useful in clarifying the interpretation of joint pathology and enhancing confidence in clinical decisions about therapies.[15]

The joint examination is also affected by variability. For observations such as joint tenderness or grip strength, interobserver variability usually is greater than intraobserver variability. Considerable intraobserver variability may be noted in observations of the same patient, even during a short interval. Interobserver reliability, in general, is higher for joint-line tenderness than for swelling and is specifically related to the underlying disease, such as higher reliability of the examination for joint swelling in RA than in psoriatic arthritis.[11]

## EXAMINATION OF SPECIFIC JOINTS

### Temporomandibular Joint

The temporomandibular joint is formed by the condyle of the mandible and the fossa of the temporal bone just anterior to the external auditory canal. It is difficult to visualize swelling of this joint. The examiner may palpate the joint by placing a finger just anterior to the external auditory canal and asking the patient to open and close the mouth and to move the mandible from side to side.[16] The presence of synovial thickness or swelling of minimal or moderate degree can be detected most easily if the synovitis is unilateral or asymmetric compared with the other side. To assess vertical movement of the temporomandibular joint, the examiner should ask the patient to open the mouth maximally and then measure the distance between the upper and lower incisor teeth, normally 3 to 6 cm. Lateral movement can be determined by using incisor teeth as landmarks. Audible or palpable crepitus or clicking may be present in patients with and without evidence of severe arthritis.

Many arthritides can affect the temporomandibular joints, including juvenile and adult RA. Micrognathia may develop in children in whom these joints are affected, as a result of arrested bone growth of the mandible. Arthralgias of the temporomandibular joint may develop in patients without inflammatory arthritis, consistent with the temporomandibular joint syndrome (see Chapter 51). This syndrome is thought by some investigators to result from bruxism and is likely to be a form of myofascial pain, similar to fibromyalgia.

## Cricoarytenoid Joints

The paired cricoarytenoid joints are formed by the articulation of the base of the small pyramidal arytenoid cartilage and the upper posterolateral border of the cricoid cartilage. The vocal ligaments (true vocal cords) are attached to the arytenoid cartilages. The cricoarytenoid joints are diarthrodial joints that normally move medially and laterally and rotate during opening and closing of the vocal cords. Examination of these joints is performed by direct or indirect laryngoscopy. Erythema, swelling, and lack of mobility during phonation may result from inflammation of the joints. The cricoarytenoid joints may be involved in RA, trauma, and infection. Involvement in RA is more common than is clinically apparent. Symptoms may include hoarseness or a sense of fullness or discomfort in the throat, which is worse on speaking or swallowing. Severe airway obstruction may occur in rare cases.

## Sternoclavicular, Manubriosternal, and Sternocostal Joints

The medial ends of the clavicles articulate on each side of the sternum at its upper end to form the sternoclavicular joints. The articulations of the first ribs and the sternum (sternocostal joints) are immediately caudal. The articulation of the manubrium and the body of the sternum is at the level of attachment of the second costal cartilage to the sternum. The third through seventh sternocostal joints articulate distally along the lateral borders of the sternum. The sternoclavicular joints are the only articulations in this group that are always diarthrodial; the others are amphiarthroses or synchondroses. The sternoclavicular joints are the only true points of articulation of the shoulder girdle with the trunk. These joints are just beneath the skin; synovitis usually is visible and palpable. These joints have only slight movement, which cannot be accurately measured.

The sternoclavicular joints are commonly involved by ankylosing spondylitis, RA, and degenerative arthritis, although this involvement is often subclinical. The sternoclavicular joint may be the site of septic arthritis, especially in injection drug users. These joints should be examined for tenderness, swelling, and bony abnormalities. Tenderness of the manubriosternal or sternocostal joints is much more frequent than actual swelling. Tenderness of these joints without actual swelling has been termed *costochondritis;* if actual swelling is present, *Tietze's syndrome* may be the term used.

## Acromioclavicular Joint

The acromioclavicular joint is formed by the lateral end of the clavicle and the medial margin of the acromion process of the scapula. Arthritis of the acromioclavicular joint is most commonly attributable to trauma leading to degenerative arthritis. Bony enlargement of this joint is typically observed, but soft tissue swelling is not usually visible or palpable. Tenderness or pain with adduction of the arm across the chest indicates pathology of the acromioclavicular joint. Movement of this joint occurs with shoulder motion but is difficult to measure accurately. The acromioclavicular joint may be involved by RA or spondyloarthropathies, although these often are not severe enough to come to clinical attention.

## Shoulder

See Chapter 46 for a detailed description.

## Elbow

The elbow joint is composed of three bony articulations (Figure 40-1). The principal articulation is the humeroulnar joint, which is a hinge joint. The radiohumeral and proximal radioulnar articulations allow rotation of the forearm.

To examine the elbow joint, the examiner places his or her thumb between the patient's lateral epicondyle and the olecranon process in the lateral para olecranon groove and places one or two fingers in the corresponding groove medial to the olecranon. The examiner relaxes and passively moves the elbow through flexion, extension, and rotation. The examiner should examine the skin around the elbow joint carefully, noting abnormalities such as psoriatic plaques, rheumatoid nodules, or tophi. It is useful to palpate the olecranon bursa carefully to exclude the presence of small nodules or tophi. Limitation of motion and crepitus should

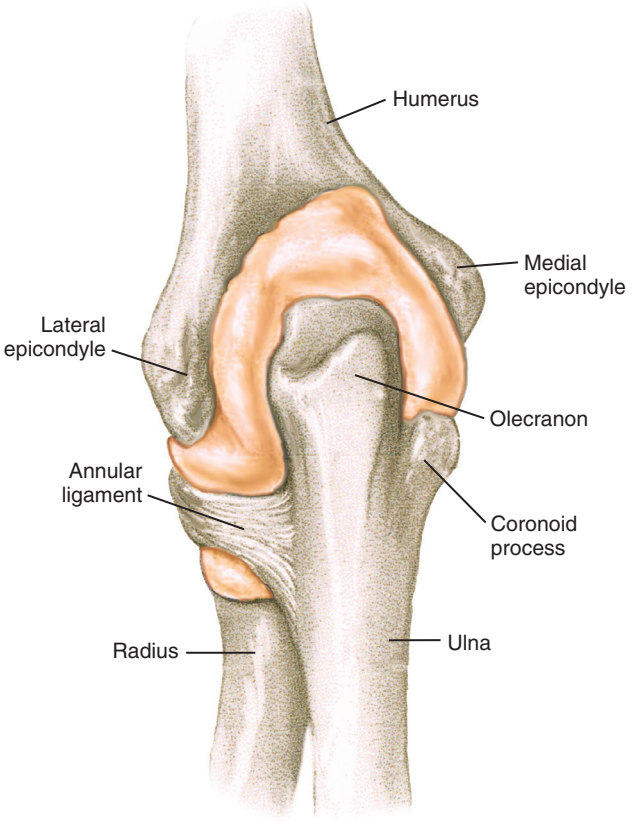



**Figure 40-1** Diagram of the elbow. Posterior aspect of the elbow joint shows radius and ulna in extension and the distribution of the synovial membrane in distention. *(From Polley HF, Hunder GG:* Rheumatologic interviewing and physical examination of the joints*, ed 2, Philadelphia, 1978, WB Saunders. With permission from the Mayo Foundation for Medical Education and Research.)*

be noted. Synovial swelling is most easily palpated because it bulges under the examiner's thumb when the elbow is passively extended. The synovial membrane sometimes can be palpated over the posterior aspect of the joint between the olecranon process and the distal humerus. Synovitis or effusion generally results in limitation of elbow extension.

The olecranon bursa overlies the olecranon process of the ulna. Olecranon bursitis is common after chronic local trauma and in rheumatic diseases, including RA and gout. A septic olecranon bursitis may occur. A patient who has olecranon bursitis usually presents with a swelling over the olecranon process, which is often tender and may be erythematous. Sometimes a large collection of fluid over the area is palpable as a cystic mass, often requiring aspiration and drainage. There is generally no pain with elbow movement.

The medial and lateral epicondyles of the humerus are the sites of attachment of the common flexor and extensor tendons, controlling hand and wrist motion. Tenderness at the epicondyles without swelling or other signs of inflammation may indicate overuse tendinopathy, termed *lateral epicondylitis* (tennis elbow) and *medial epicondylitis* (golfer's elbow). In lateral epicondylitis, discomfort can be elicited by resisted supination of the forearm or resisted extension of the pronated wrist.[17] In medial epicondylitis, discomfort can be elicited by resisted flexion of the supinated wrist.

To assess motor function of the elbow, flexion and extension can be assessed. The principal flexors of the elbow are the biceps brachii (nerve roots C5 and C6), brachialis (C5 and C6), and brachioradialis (C5 and C6) muscles. The principal extensor of the elbow is the triceps brachii muscle (C7 and C8). Occasionally, a patient may rupture the attachment site of one of the heads of the biceps, resulting in visible and palpable muscle swelling on the anterior upper arm.

## Wrist and Carpal Joints

The wrist is a complex joint formed by many articulations between the radius, the ulna, and the carpal bones. The true wrist or radiocarpal articulation is a biaxial ellipsoid joint formed proximally by the distal end of the radius and the triangular fibrocartilage and distally by a row of three carpal bones: scaphoid (navicular), lunate, and triquetrum (triangular). The distal radioulnar joint is a uniaxial pivot joint. The midcarpal joints are formed by the junction of the proximal and distal rows of the carpal bones. The midcarpal and carpometacarpal articular cavities often communicate. The intercarpal joints refer to the articulations between individual carpal bones.

Movements of the wrist include flexion (palmar flexion), extension (dorsiflexion), radial deviation, ulnar deviation, and circumduction. Pronation and supination of the hand and forearm occur primarily at the proximal and distal radioulnar joints. The only carpometacarpal joint that moves to a notable degree is the carpometacarpal joint of the thumb. This joint is saddle-shaped and moves in three planes. Crepitus at this joint is common because it is frequently involved in degenerative arthritis.

The wrist normally can be extended to 70 to 80 degrees and flexed to 80 to 90 degrees. Ulnar and radial deviation should allow 50 degrees (ulnar) and 20 to 30 degrees (radial) of movement. Loss of extension is the most incapacitating functional impairment of wrist motion.

The long flexor tendons of the forearm musculature cross the volar aspect of the wrist and are enclosed in the flexor tendon sheath under the flexor retinaculum (transverse carpal ligament). The flexor retinaculum and the underlying carpal bones form the carpal tunnel. The median nerve passes through the carpal tunnel superficial to the flexor tendons. The extensor tendons of the forearm musculature are enclosed by six synovial lined compartments.

The palmar aponeurosis (fascia) spreads out into the palm from the flexor retinaculum. Dupuytren's contracture, a fibrosing condition, affects the palmar aponeurosis, which becomes thickened and contracted and may draw one or more fingers into flexion at the metacarpophalangeal joint. The fourth finger is frequently affected first.

Swelling of the wrist may be caused by effusion, synovial proliferation, or both, of the tendon sheaths (tenosynovitis), the wrist joint, or a combination thereof. When swelling is attributable to tenosynovitis, swelling is localized to the distribution of a particular tendon sheath or compartment (i.e., ulnar swelling attributable to tenosynovitis of the flexor carpi ulnaris tendon), tends to be more localized, and moves with flexion and extension of the fingers (Figure 40-2). Articular swelling tends to be more diffuse and protrudes anteriorly and posteriorly from under the tendons (Figure 40-3).

Synovitis of the wrist is best detected by palpation of the dorsal aspect of the joint. Accurate localization of the synovial margins is difficult because of structures overlying the volar and dorsal aspects of the wrist. To examine the wrist, the examiner should palpate the joint gently between the thumbs dorsally and the fingers on the volar aspect. Thickening or frank synovial proliferation of the synovium should be noted. When this thickening or proliferation is severe, the range of motion of the wrist joint frequently is limited and associated with stress pain.

A ganglion is a cystic enlargement arising from a joint capsule. Ganglions characteristically occur at the volar or dorsal aspect of the wrist between the tendons.

Subluxation of the ulna may develop as a result of severe chronic inflammatory arthritis. The subluxated ulna appears as a prominence on the dorsomedial wrist. Chronic irritation of the extensor tendons, primarily the fourth and fifth finger extensor tendons, may cause these tendons to rupture.

*Trigger fingers* secondary to stenosing tenosynovitis can be detected by palpating crepitus or nodules along the tendons in the palm while the patient slowly flexes and extends the fingers.[18] The patient usually gives a history of the affected finger catching or locking with movement.

Tenosynovitis of the first extensor compartment, which encloses the abductor pollicis longus and extensor pollicis brevis muscles of the thumb, is known as de Quervain's tenosynovitis. Patients complain of pain at the radial aspect of the wrist. Tenderness may be elicited by palpating near the radial styloid process. The examiner performs the Finkelstein test for de Quervain's tenosynovitis by asking the patient to make a fist with the thumb enclosed in the palm of the hand, then to move the wrist into ulnar deviation. Severe pain over the radial styloid is a positive finding, often indicating stretching of the thumb tendons in a stenosed tendon sheath.

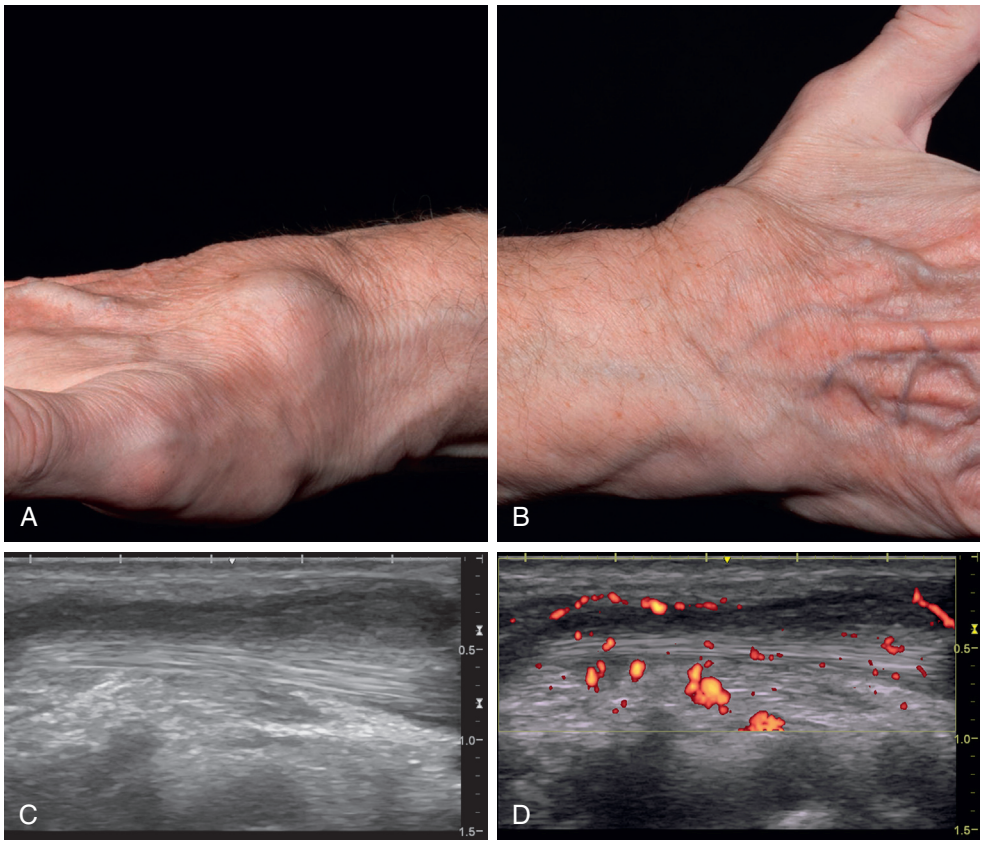



**Figure 40-2** Tenosynovitis of the wrist. **A**, Radial and **B**, dorsal views of the wrist in a patient with rheumatoid arthritis. Note the localized swelling at the dorsoradial aspect of the wrist. Sonographic evaluation of this patient revealed evidence of **C**, hypoechoic thickening of the third dorsal extensor compartment with **D**, hyperemia as detected by power Doppler signal, consistent with active inflammatory tenosynovitis.

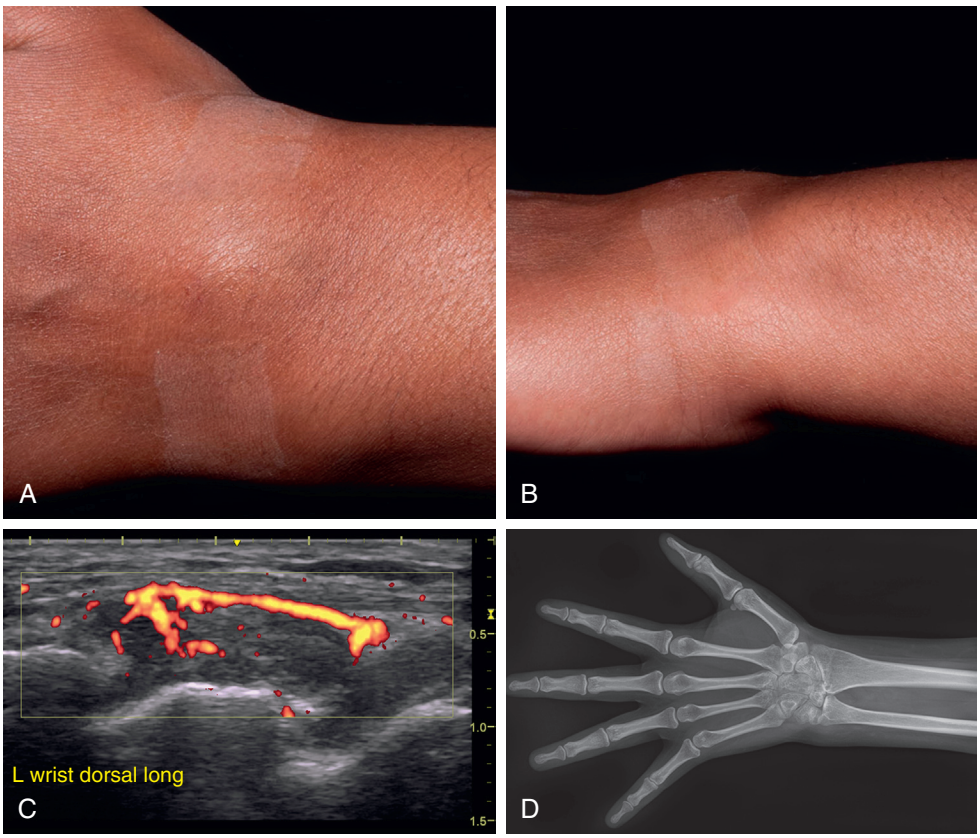



**Figure 40-3** Synovitis of the wrist. **A**, Dorsal and **B,** radial views of the wrist in another patient with rheumatoid arthritis. Note diffuse swelling through the dorsal recess of the wrist. **C**, Sonographic evaluation reveals hypoechoic thickening and power Doppler hyperemia. **D**, Corresponding plain radiograph of the wrist demonstrates carpal erosions and joint space narrowing.

Carpal tunnel syndrome results from pressure on the median nerve in the carpal tunnel. Carpal tunnel syndrome is discussed in detail in Chapter 50.

Muscle function of the wrist may be measured by testing flexion and extension and supination and pronation of the forearm. The principal flexors of the wrist are the flexor carpi radialis (nerve roots C6 and C7) and flexor carpi ulnaris (C8 and T1) muscles. Each of these muscles can be tested separately. This testing can be accomplished if the examiner provides resistance to flexion at the base of the second metacarpal bone in the direction of extension and ulnar deviation in the case of the flexor carpi radialis muscle and resistance at the base of the fifth metacarpal in the direction of extension and radial deviation in the case of the flexor carpi ulnaris muscle. The principal extensors of the wrist are the extensor carpi radialis longus (C6 and C7), extensor carpi radialis brevis (C6 and C7), and extensor carpi ulnaris (C7 and C8) muscles. The radial and ulnar extensor muscles can be tested separately. The principal supinators of the forearm are the biceps brachii (C5 and C6) and supinator (C6) muscles. The principal pronators of the forearm are the pronator teres (C6 and C7) and pronator quadratus (C8 and T1) muscles.

## Metacarpophalangeal and Proximal and Distal Interphalangeal Joints

The metacarpophalangeal joints are hinge joints. Lateral collateral ligaments that are loose in extension tighten in flexion, preventing lateral movement of the digits. The extensor tendons that cross the dorsum of each joint strengthen the articular capsule. When the extensor tendon of the digit reaches the distal end of the metacarpal head, it is joined by fibers of the interossei and lumbricales muscles and expands over the entire dorsum of the metacarpophalangeal joint and onto the dorsum of the adjacent phalanx. This expansion of the extensor mechanism is known as the *extensor hood*.

The proximal and distal interphalangeal joints also are hinge joints. The ligaments of the interphalangeal joints resemble those of the metacarpophalangeal joints. When the fingers are flexed, the bases of the proximal phalanges slide toward the palmar side of the heads of the metacarpal bones. The metacarpal heads form the rounded prominences of the knuckles, with the metacarpal joint spaces situated about 1 cm distal to the apex of the prominences.

To examine the metacarpophalangeal joints, the examiner should palpate the dorsal and volar aspects of each joint in 20 to 30 degrees of flexion (Figure 40-4). The skin on the palmar surface of the hand is thick and covers a fat pad between it and the metacarpophalangeal joint. This makes palpation of the palmar surface of the joint difficult. It is especially helpful in examining the small joints to compare one with another to detect subtle synovitis. Gentle lateral compression with force applied at the base of the second and fifth metacarpophalangeal joints (the *squeeze test*) often elicits pain if synovitis is present.

The proximal and distal interphalangeal joints are best examined by palpating gently over the lateral and medial aspects of the joint, where the flexor and extensor tendons do not interfere with assessment of the synovial membrane. Alternatively, the joint can be compressed anteroposteriorly by the thumb and index finger of one of the examiner's hands, while the other thumb and index finger palpate for synovial distention medially and laterally. The Bunnell test is useful in differentiating synovitis of the proximal interphalangeal joints from tightening of the intrinsic muscles (see Chapter 50).



**Figure 40-4** Palpation of the metacarpophalangeal joints is done with the examiner's thumbs palpating the dorsal aspect of the patient's joint, while the forefingers palpate the volar aspect of the metacarpal head. The joints should be examined while the examiner holds the patient's hand in a relaxed position of partial flexion.

Swelling of the fingers may result from articular or periarticular causes. Synovial swelling usually produces symmetric enlargement of the joint itself, whereas extra-articular swelling may be diffuse and may extend beyond the joint space. Asymmetric enlargement, involving only one side of the digit or joint, is less common and usually indicates an extraarticular process. Diffuse swelling of an entire digit, known by the terms *dactylitis* and *sausage digit*, may result from tenosynovitis and is seen most commonly in the spondyloarthropathies, such as reactive arthritis or psoriatic arthritis. Rheumatoid nodules are firm periarticular swellings that frequently overlie the joints or bony prominences in patients with chronic rheumatoid disease. Chronic swelling with distention of the metacarpophalangeal joints tends to produce stretching and laxity of the articular capsule and ligaments. This laxity, combined with muscle imbalance and other forces, eventually results in the extensor tendons of the digits slipping off the metacarpal heads to the ulnar sides of the joints. The abnormal pull of the displaced tendons is one of the factors that cause ulnar deviation of the fingers in chronic inflammatory arthritis (Figure 40-5).

Swan neck deformity describes a finger with a flexion contracture of the metacarpophalangeal joint, hyperextension of the proximal interphalangeal joint, and flexion of the distal interphalangeal joint. These changes are produced by contraction of the interossei and other muscles that flex the metacarpophalangeal joints and extend the proximal interphalangeal joints. This deformity is characteristic of RA but may be seen in other chronic arthritides (Figure 40-6).

Boutonnière deformity describes a finger with a flexion contracture of the proximal interphalangeal joint associated with hyperextension of the distal interphalangeal joint. The



**Figure 40-5** Destructive rheumatoid arthritis. Chronic synovial pannus formation involves the metacarpophalangeal joints of both hands and both wrist joints. Subluxation and ulnar deviation are present in the right hand metacarpophalangeal joints. Swan neck deformities are present in the right third through fifth and left second through fourth digits.



**Figure 40-6** Swan neck deformity in a patient with psoriatic arthritis. Note hyperextension of the proximal interphalangeal joint and hyperflexion of the distal interphalangeal joint of the second digit. Also note the psoriatic changes of the third and fourth fingernails.

deformity is common in RA and occurs when the central slip of the extensor tendon of the proximal interphalangeal joint becomes detached from the base of the middle phalanx, allowing palmar dislocation of the lateral bands. The dislocated bands cross the fulcrum of the joint and act as flexors instead of extensors of the joint.

Another abnormality is telescoping or shortening of the digits produced by resorption of the ends of the phalanges secondary to destructive arthropathy. This may be seen in the arthritis mutilans form of psoriatic arthritis. Shortening of the fingers is associated with wrinkling of the skin over involved joints and is called *opera-glass hand* or *la main en lorgnette*.

A mallet finger results from avulsion or rupture of the extensor tendon at the level of the distal interphalangeal joint. With this deformity, the patient is unable to extend the distal phalanx, which remains in a flexed position. This deformity frequently results from traumatic injuries.

The Murphy sign is a test for lunate dislocation. The patient is asked to make a fist. The third metacarpal head usually is more prominent than the second and fourth. If the third metacarpal is level with the second and fourth, the finding is positive for lunate dislocation.

Involvement of the distal interphalangeal joints in RA is uncommon. Bony hypertrophy and osteophyte formation are commonly seen, however, at the distal and proximal interphalangeal joints in patients with osteoarthritis. Enlarged, bony, hypertrophic distal interphalangeal joints are called *Heberden nodes*, whereas similar changes at the proximal interphalangeal joints are called *Bouchard nodes*. These usually are easily differentiated from the synovitis of inflammatory arthritis because, on palpation, the enlargement is hard or bony. In addition, signs of inflammation are minimal. Heberden and Bouchard nodes should be easily distinguished from rheumatoid nodules, but patients occasionally confuse these when describing swellings over joints. The examiner should be aware of other causes of joint enlargement or nodules on the hands, including tophaceous gout (Figure 40-7) and, rarely, multicentric reticulohistiocytosis. The first carpometacarpal joint also is often affected in osteoarthritis (Figure 40-8).

The patient's fingernails should be inspected for evidence of clubbing or other abnormalities. Often in patients with psoriatic arthritis, ridging, onycholysis, or nail pitting is present. Occasionally, patients with osteoarthritis develop a groove deformity of the nail on a digit with a Heberden node. (This nail deformity has been called a *Heberden node nail*.) The abnormality is believed to occur secondary to synovial cyst encroachment on the nail bed by the evolving osteoarthritis process. With time, the nail may return to normal.

A crude but sometimes useful assessment of hand function can be made by asking the patient to make a fist. An estimate of the patient's ability to form a full fist can be recorded as a percentage fist, with 100% a complete fist. A fist of 75% indicates that the patient can touch the palm with the fingertips. The ability to oppose fingers, especially the thumb, is crucial to hand function because of the necessity to grasp or at least pinch for objects. If the patient is unable to form a full fist, the ability or inability to pinch or oppose fingers can be demonstrated by asking the patient to pick up a small object.

Strength of the hands can be assessed crudely by asking the patient to grip firmly two or more of the examiner's fingers. More accurate measures of grip strength can be made by using a dynamometer or by having the patient squeeze a partially inflated sphygmomanometer (at 20 mm Hg). It sometimes is useful to test the strength of the fingers separately. The prime movers of flexion of the second through fifth MCP joints are the dorsal and palmar interossei muscles (nerve roots C8 and T1). The lumbricales muscles (C6, C7, and C8) flex the metacarpophalangeal joints when the proximal phalangeal joints are extended. The flexors of the proximal interphalangeal joints are the flexor digitorum superficialis muscles (C7, C8, and T1), and the flexor of the distal interphalangeal joints is the flexor digitorum profundus muscle (C7, C8, and T1).

The prime extensors of the MCP and interphalangeal joints of the second through fifth fingers are the extensor

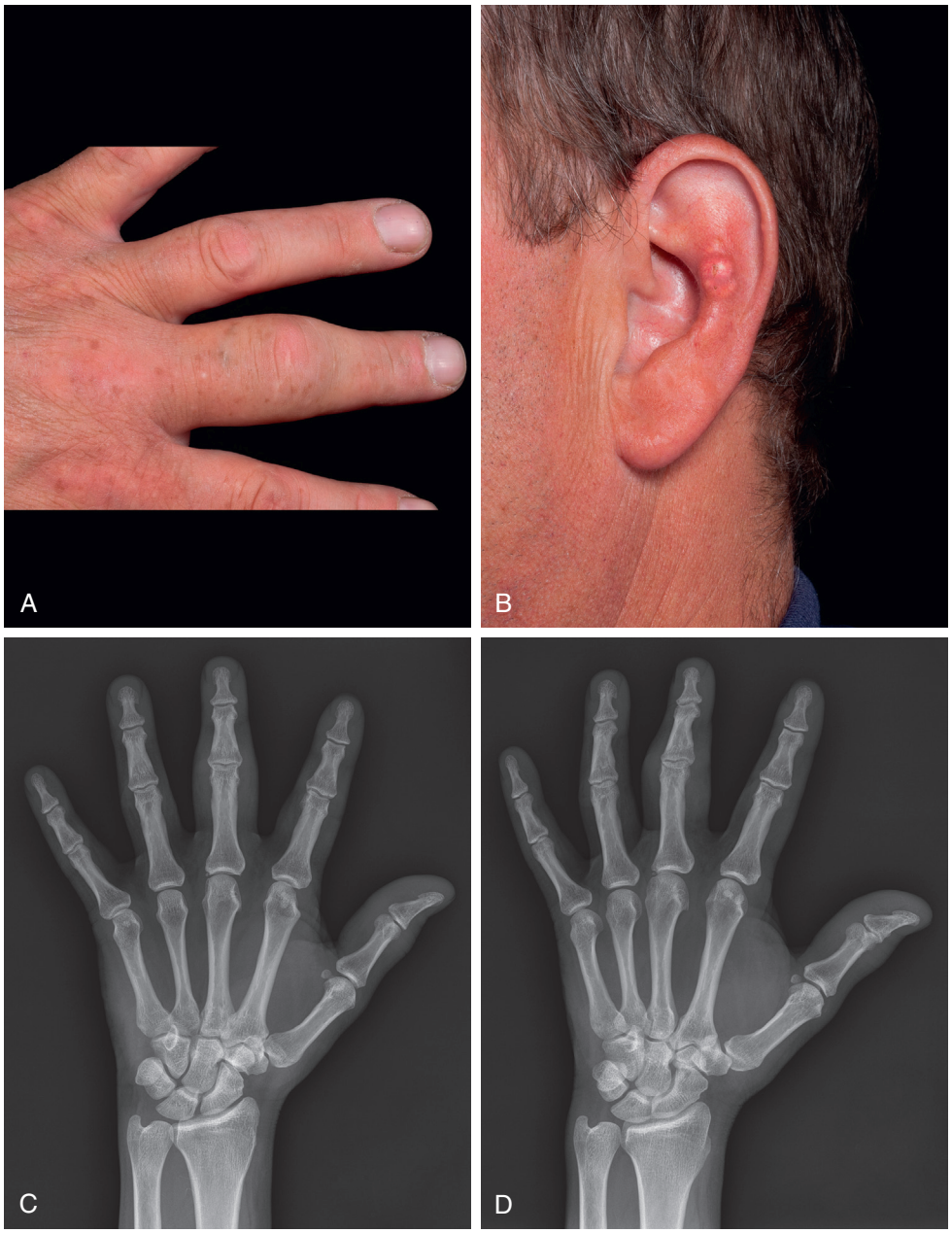



**Figure 40-7** Gout. **A**, Note the swelling and enlargement of the left third proximal interphalangeal joint in a patient with crystal-proven gout. **B**, The same patient was found to have a tophus in the left ear. **C** and **D,** The corresponding posteroanterior and oblique radiographs of the hands demonstrate soft tissue swelling and increased density about the left third proximal interphalangeal joint likely due to tophaceous deposits.

digitorum communis (nerve roots C6, C7, and C8), extensor indicis proprius (C6, C7, and C8), and extensor digiti minimi (C7) muscles. The interossei and lumbricales muscles simultaneously flex the metacarpophalangeal joints and extend the interphalangeal joints. The dorsal interossei (C8 and T1) and abductor digiti minimi (C8) muscles abduct the fingers, whereas the palmar interosseous muscles adduct the fingers.

The thumb is moved by several muscles. The prime flexor of the first metacarpophalangeal joint is the flexor pollicis brevis muscle (nerve roots C6, C7, C8, and T1). The prime flexor of the interphalangeal joint is the flexor pollicis longus muscle (C8 and T1). The metacarpophalangeal joint of the thumb is extended by the extensor pollicis brevis muscle, and the prime extensor of the interphalangeal joint is the extensor pollicis longus muscle (C6, C7, C8, and C9).

The principal abductors of the thumb are the abductor pollicis longus (nerve roots C6 and C7) and the abductor pollicis brevis (C6 and C7) muscles. Motion occurs primarily at the carpometacarpal joint. The principal adductor of the thumb is the adductor pollicis muscle (C8 and T1). Motion occurs primarily at the carpometacarpal joint. The principal movers in opposition of the thumb and fifth fingers are the opponens pollicis (C6 and C7) and opponens digiti minimi (C8 and T1) muscles.

Sensation and nerve injuries in the upper extremity are discussed in Chapters 45 and 50.



**Figure 40-8** Osteoarthritis of the hands. Note the advanced hypertrophic enlargement at the base of both thumbs. Both second distal interphalangeal joints are notable for advanced bony enlargement, and more moderate changes are seen in the other interphalangeal joints.

## Hip

The hip is a spheroidal or ball-and-socket joint formed by the rounded head of the femur and the cup-shaped acetabulum (see Chapter 48). Stability of the joint is ensured by the fibrocartilaginous rim of the glenoid labrum and the dense articular capsule and surrounding ligaments, including the iliofemoral, pubofemoral, and ischiocapsular ligaments that reinforce the capsule. Support also is provided by the powerful muscle groups that surround the hip. The principal hip flexor is the iliopsoas muscle assisted by the sartorius and rectus femoris muscles. Hip adduction is accomplished by the three adductors (longus, brevis, and magnus) plus the gracilis and pectineus muscles. The gluteus medius is the major hip abductor, whereas the gluteus maximus and hamstrings extend the hip. Several clinically important bursae are found around the hip joint. Anteriorly, the iliopsoas bursa lies between the psoas muscle and the joint surface. The trochanteric bursa lies between the gluteus maximus muscle and the posterolateral greater trochanter, and the ischiogluteal bursa overlies the ischial tuberosity.

Examination of the hip should begin by observation of the patient's stance and gait. The patient should stand in front of the examiner so that the anterior iliac spines are visible. Pelvic tilt or obliquity may be present and related to structural scoliosis, anatomic leg-length discrepancy, or hip disease.

Hip contractures may result in abduction or adduction deformities. To compensate for an adduction contracture, the pelvis is tilted upward on the side of the contracture. This allows the legs to be parallel during walking and weight bearing. With a fixed abduction deformity, the pelvis becomes elevated on the normal side during standing or walking. This elevation causes an apparent shortening of the normal leg and forces the patient to stand or walk on the toes of the normal side or to flex the knee on the abnormal leg. Viewed from behind with the legs parallel, the patient with hip disease and an adducted hip contracture may have asymmetric gluteal folds secondary to pelvic tilt, with the diseased side elevated. In this situation, the patient is unable to stand with the foot of the involved leg flat on the floor. In abduction contracture, the findings are reversed; with both legs extended and parallel, the uninvolved side is elevated.

A hip flexion deformity commonly occurs in diseases of the hip. Unilateral flexion of the hip in the standing position reduces weight bearing on the involved side and relaxes the joint capsule, causing less pain. This posture is best noted by observing the patient from the side. A hyperlordotic curve of the lumbar spine compensates for lack of full hip extension.

Gait should be assessed in the patient with possible hip joint disease. With a normal gait, the abductors of the weight-bearing leg contract to hold the pelvis level or to elevate the non–weight-bearing side slightly. Two abnormalities of gait may be commonly observed in patients with hip disease. The most common abnormality seen with a painful hip is the antalgic (limping) gait. With this gait, the individual leans over the diseased hip during the phase of weight bearing on that hip, placing the body weight directly over the joint to avoid painful contraction of the hip abductors. With a Trendelenburg gait, with weight bearing on the affected side, the pelvis drops and the trunk shifts to the normal side. Although the antalgic gait is frequently seen with painful hips, and the Trendelenburg gait is seen in patients with weak hip abductors, these gaits are not specific, and either may occur as a result of hip pain from one of several causes. A mild Trendelenburg gait is seen often in healthy individuals.

The Trendelenburg test assesses the stability of the hip, together with the ability of the hip abductor muscle to stabilize the pelvis on the femur.[19] It is a measure of the gluteus medius hip abductor strength. The patient is asked to stand while bearing weight on only one leg. Normally, the abductors hold the pelvis level or the nonsupported side slightly elevated. If the non–weight-bearing side drops, the test is positive for weakness of the weight-bearing side hip abductors, especially the gluteus medius muscle. This test is nonspecific and may be used in primary neurologic or muscle disorders and in hip diseases that lead to weakness of the hip abductors.

The motion of the hip should be assessed with the patient in the supine position. Range of motion of the hip includes flexion, extension, abduction, adduction, internal and external rotation, and circumduction. The degree of flexion permitted varies with the manner with which it is assessed. When the knee is held flexed at 90 degrees, the hip normally flexes to an angle of 120 degrees between the thigh and the long axis of the body. If the knee is held in extension, the hamstrings limit hip flexion to approximately 90 degrees. The presence of a hip flexion contracture is suggested by the persistence of lumbar lordosis and pelvic tilt, which masks the contracture by allowing the involved leg to remain in contact with the examination table. The Thomas test shows the flexion contracture. With this test, the opposite hip is fully flexed to flatten the lumbar lordosis and fix the pelvis. The patient's involved leg should be extended toward the examination table as far as possible. Flexion contracture of the diseased hip becomes more obvious and can be estimated in degrees from full extension. Measurement for leg-length discrepancy is performed with

the patient in a supine position and the legs fully extended. Each leg is measured from the anterior superior iliac spine to the medial malleolus. A difference of 1 cm or less is unlikely to cause any abnormality of gait and may be considered normal. In addition to true leg-length asymmetries, apparent leg-length discrepancies may result from pelvic tilt or abduction or adduction contractures of the hip.

Abduction is measured with the patient in a supine position and the leg in an extended position, perpendicular to the pelvis. Pelvic stabilization is achieved by the examiner placing an arm across the pelvis with the hand on the opposite anterior iliac spine. With the other hand, the examiner grasps the patient's ankle and abducts the leg until the pelvis begins to move. Abduction to approximately 45 degrees is normal. It is helpful to compare one side with the other because the normal range of motion may vary. Alternatively, the examiner could stand at the foot of the table, grasp both of the patient's ankles, and simultaneously abduct both legs. Abduction is commonly limited in hip joint disease. The examiner assesses adduction by grasping the patient's ankle and raising the leg off the examination table by flexing the hip enough to allow the tested leg to cross over the opposite leg. Normal adduction is approximately 20 to 30 degrees. Hip rotation may be tested with the hip and knee flexed to 90 degrees or with the leg extended. Normal hip external rotation and internal rotation are observed to 45 degrees and 40 degrees, respectively. The difference in rotation between the flexed and extended hip is attributable to increased stabilization of the joint by surrounding ligaments in the extended position. Rotation decreases with extension. To test hip rotation, the examiner grasps the extended leg above the ankle and rotates it externally and internally from the neutral position. Limitation of internal rotation of the hip is a sensitive indicator of hip joint disease.

Extension is tested with the patient in the prone position. Estimating hip extension can be difficult because some of the apparent motion arises from hyperextension of the lumbar spine, pelvis rotation, motion of the buttock soft tissue, and flexion of the opposite hip. The pelvis and the lumbar spine can be partially immobilized by the examiner placing an arm across the posterior iliac crest and the lower lumbar spine. The examiner places the other hand under the thigh with the knee flexed and hyperextends the thigh. Normal extension ranges from 10 to 20 degrees. Limitation of extension often occurs secondary to a hip flexion contracture

Swelling around the hip only rarely can be discerned on examination. The flexion abduction external rotation (FABER) test, also known as the *Patrick* test, is a commonly used screening test for intra-articular hip pathology.[20] To perform this test, the examiner has the patient lie in a supine position with the foot ipsilateral to the test hip, resting on the contralateral knee. The examiner then slowly lowers the patient's test leg toward the examining table, applying gentle pressure to the knee of the test hip and the contralateral anterior superior iliac spine. Normally, the test leg will fall at least parallel to the opposite leg. The FABER test is considered positive when the maneuver reproduces the patient's pain. Although very sensitive for hip joint disease, this test is not specific because a positive test may indicate iliopsoas tightness or sacroiliac joint disease.

The iliotibial band is a part of the fascia lata that extends from the iliac crest, sacrum, and ischium over the greater trochanter to the lateral femoral condyle, tibial condyle, and fibular head, and along the lateral intermuscular system, separating the hamstrings from the vastus lateralis muscle. The tensor fasciae latae muscle may produce an audible snap while it slips over the greater trochanter if the weight-bearing leg moves from hip flexion and adduction to a neutral position, as when climbing stairs. Most commonly observed in young women, the snapping hip usually does not cause severe pain. The Ober test evaluates the iliotibial band for contracture. The patient lies on the side, with the lower leg flexed at the hip and knee. The examiner abducts and extends the upper leg with the knee flexed at 90 degrees. The hips should be slightly extended to allow the iliotibial band to pass over the greater trochanter. The examiner slowly lowers the patient's limb with the muscles relaxed. A positive test result indicative of an iliotibial band contracture occurs if the leg does not fall back to the level of the tabletop.

A common cause of lateral hip pain is trochanteric bursitis. Patients with this condition often complain of pain and tenderness when they attempt to lie on the affected side or climb stairs. The greater trochanter should be palpated for tenderness and compared with the opposite side. In trochanteric bursitis, this area is usually exquisitely tender. The pain of trochanteric bursitis is aggravated by actively resisted abduction of the hip. Aching and tenderness over the buttock area may be secondary to an ischial bursitis. Other causes of lateral and posterior hip (buttock) discomfort include pain at muscle and tendon insertion sites.

Anterior hip and groin pain may be secondary to hip abnormality, most commonly degenerative arthritis. The examiner should take note of decreased range of motion in these patients. Other causes include iliopsoas bursitis, in which swelling and tenderness may be noted in the middle third of the inguinal ligament lateral to the femoral pulse. This pain is aggravated by hip extension and is reduced by flexion. The bursitis may be a localized problem or may represent extension of hip synovitis. It usually is impossible to distinguish between a localized bursitis and an extension of hip synovitis on the basis of the physical examination. If the patient has tenderness in the region of the iliopsoas bursa, but no swelling is palpable, the examiner should consider tendinitis of the iliopsoas muscle. The inguinal region should be palpated for other abnormalities, such as hernias, femoral aneurysms, adenopathy, tumor, and psoas abscess or masses.

Muscle strength testing should include the hip flexors, extensors, abductors, and adductors. The primary hip flexor is the iliopsoas muscle (nerve roots L2 and L3). Flexion may be tested with the patient sitting at the edge of a table. The examiner exerts downward pressure against the thigh proximal to the knee while the patient attempts to flex the hip. The pelvis may be stabilized by the examiner's other hand placed on the ipsilateral iliac crest. Alternatively, with the patient supine and holding the leg in 90 degrees of flexion at the hip, the examiner may attempt to straighten the hip.

Hip extension is tested with the patient lying prone. The primary hip extensor is the gluteus maximus muscle (L5 and S1). With the patient's knee flexed to remove hamstring action, the patient is instructed to extend the hip and thigh

off the surface of the table while the examiner places a forearm across the posterior iliac crest to stabilize the pelvis and applies downward pressure to prevent the lateral trunk muscles from elevating the pelvis and leg off the table.

Abduction may be tested with the patient prone or supine. The patient should abduct the thigh and leg against resistance from the examiner applied at the midthigh level.

The primary adductor is the adductor longus muscle (nerve roots L3 and L4). The examiner holds the upper leg proximal to the knee in slight abduction, while the patient resists and attempts to adduct the leg. Testing for abduction and adduction also may be done in the two legs simultaneously. The patient lies supine with the legs fully extended and the hips moderately abducted. To test abduction, the patient actively pushes out against the examiner's resistance against the lateral malleoli. Adduction is tested by movement against resistance at the medial malleoli.

## Knee

The knee is a compound condylar joint with three articulations: the patellofemoral and the lateral and medial tibiofemoral condyles with their fibrocartilaginous menisci. The knee is stabilized by its articular capsule, the patellar ligament, medial and lateral collateral ligaments, and anterior and posterior cruciate ligaments. The collateral ligaments provide medial and lateral stability, whereas the cruciatus provide anteroposterior and rotatory stability. Normal knee motion is a combination of flexion or extension and rotation. With flexion, the tibia internally rotates, and with extension, it externally rotates on the femur. The surrounding synovial membrane is the largest of the body's joints; it extends 6 cm proximal to the joint as the suprapatellar pouch beneath the quadriceps femoris muscle. Several important bursae are found around the knee, including the superficial prepatellar bursa, the superficial and deep infrapatellar bursae, the pes anserine bursa distal to the medial tibial plateau, and the posterior medial semimembranous and posterolateral gastrocnemius bursae. Knee extension is primarily mediated by the quadriceps femoris muscle; knee flexion is mediated by the hamstrings. The biceps femoris muscle externally rotates the lower leg on the femur, whereas the popliteus and semitendinous muscles mediate internal rotation.

In taking the history of a patient with knee complaints, the examiner should ask the patient about symptoms of knee locking, catching, or giving way. *Locking* is the sudden loss of ability to extend the knee; it usually is painful and may be associated with an audible noise, such as a click or pop. It often implies extensive intra-articular abnormality, including loose bodies or cartilaginous tears. *Catching* refers to a subjective sensation of the patient that the knee might lock; the patient may experience a momentary interruption in the smooth range of motion of the joint but is able to continue with normal motion after this brief hesitation. Catching usually implies less abnormality than true locking and may occur in various pathologic conditions. True giveway indicates that the knee actually buckles and gives out in certain positions or with certain activities. It is important to elicit details of the patient history to verify this common subjective complaint. Patients often experience a sensation that the knee will give out when it actually does not. Other patients say their knees are "giving out" to describe severe pain that necessitates stopping an activity. True give-way implies severe intra-articular abnormality, such as an unstable joint from ligamentous injury or incompetence.

Examination of the knees should always include observation of the patient while standing and walking (see Chapter 48). Deviation of the knees, including genu varum (lateral deviation of the knee joint with medial deviation of the lower leg), genu valgum (medial deviation of the knee with lateral deviation of the lower leg), and genu recurvatum (hyperextension deformity of the knee), is most easily evaluated with the patient standing. The patient also should be observed ambulating for evidence of gait abnormalities.

Inspection should be done with the patient standing and supine. It is essential to compare side to side, noting any asymmetry that may be caused by swelling or muscle atrophy. Suprapatellar swelling with fullness of the distal anterior thigh that obliterates the normal depressed contours along the sides of the patella usually indicates knee joint effusion or synovitis. Localized swelling over the surface of the patella is generally secondary to prepatellar bursitis (Figure 40-9). Patellar alignment should be noted, including

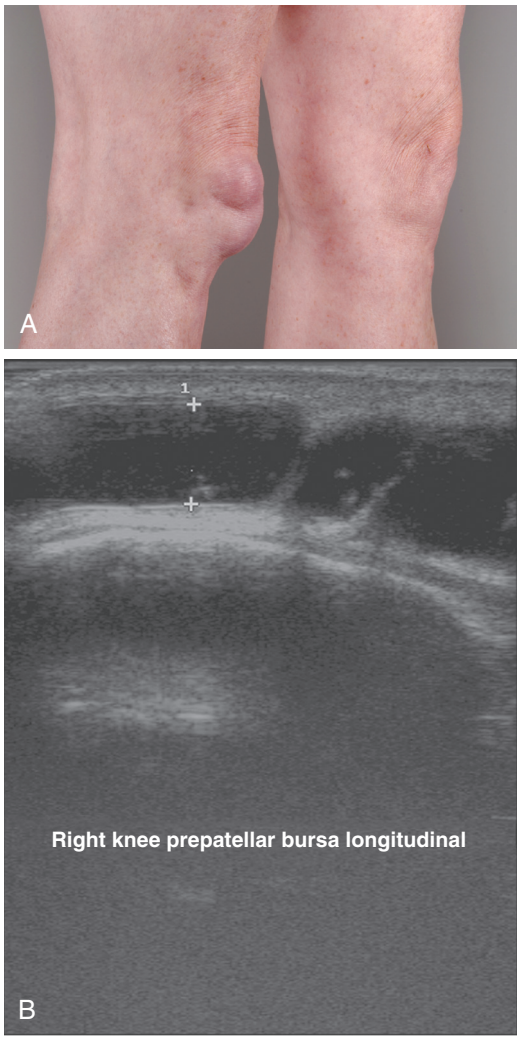



**Figure 40-9** Prepatellar bursitis. **A,** Note the cystic swelling overlying the right patella, with the appearance of two separate compartments of bursal swelling. **B,** High-resolution ultrasound image of the same patient showing collection of anechoic fluid with an internal septation.

high-riding or laterally displaced patellae. The examiner also should inspect the knee from behind to identify popliteal swelling caused by a popliteal or Baker cyst, most commonly caused by medial semimembranous bursal swelling. If the calves appear asymmetric, calf circumference should be measured and compared bilaterally. Popliteal cysts may rupture and dissect down into the calf muscles, resulting in enlargement and palpable fullness. Edema may be present if the cyst causes secondary venous or lymphatic obstruction. Acute rupture and dissection of a popliteal cyst can mimic thrombophlebitis, with local pain, heat, redness, and swelling. This is probably a more common cause of unilateral calf swelling in patients with RA than is deep venous thrombosis. The two conditions may be difficult to distinguish on physical examination alone.

Quadriceps femoris muscle atrophy usually develops in chronic arthritis of the knee. Atrophy of the vastus medialis muscle is the earliest change and may be appreciated by comparing the two thighs for medial asymmetry and circumference. Measurement of the thigh circumference should be performed at 15 cm above the knee to avoid spurious results due to suprapatellar effusions.

Palpation of the knee should be performed with the joint relaxed. This usually is best accomplished with the patient supine and the knees fully extended and not touching. Palpation should begin over the anterior thigh approximately 10 cm above the patella. To identify the superior margin of the suprapatellar pouch, which is an extension of the knee joint cavity, the examiner should palpate the anterior thigh, moving distally toward the knee. Swelling, thickening, nodules, loose bodies, tenderness, and warmth should be noted. A thickened synovial membrane has a boggy, doughy consistency, which differs from the surrounding soft tissue and muscle. It usually is palpated earlier over the medial aspect of the suprapatellar pouch and the medial tibiofemoral joint. To enhance detection of knee fluid, any fluid in the suprapatellar pouch is compressed with the palm of the hand placed just proximal to the patella. The synovial fluid forced into the inferior distal articular cavity is palpated with the opposite thumb and index finger laterally and medially to the patella. If the examiner alternates compression and release of the suprapatellar pouch, the synovial thickening can be differentiated from a synovial effusion. An effusion intermittently distends the joint capsule under the thumb and index finger of the opposite hand, whereas synovial thickening does not.

The examiner should not compress the suprapatellar pouch too firmly or push the tissues distally because the patella or normal soft tissue, including the fat pads, fills the palpated space and could be misinterpreted as synovitis or joint swelling. With a large effusion, the patella can be balloted by pushing it posteriorly against the femur with the right forefinger, while maintaining suprapatellar compression with the left hand.

At the other extreme, effusions of 4 to 8 mL can be detected by eliciting the bulge sign. This test is performed with the patient's knee extended and relaxed. The examiner strokes or compresses the medial aspect of the knee proximally and laterally with the palm of a hand to move fluid from the area. The lateral aspect of the knee is tapped or stroked, and a fluid wave or bulge appears medially (Figure 40-10). A so-called *spontaneous bulge sign* occurs if, on compression along the medial side of the joint space, fluid reaccumulates with no pressure or compression along the lateral side of the joint.

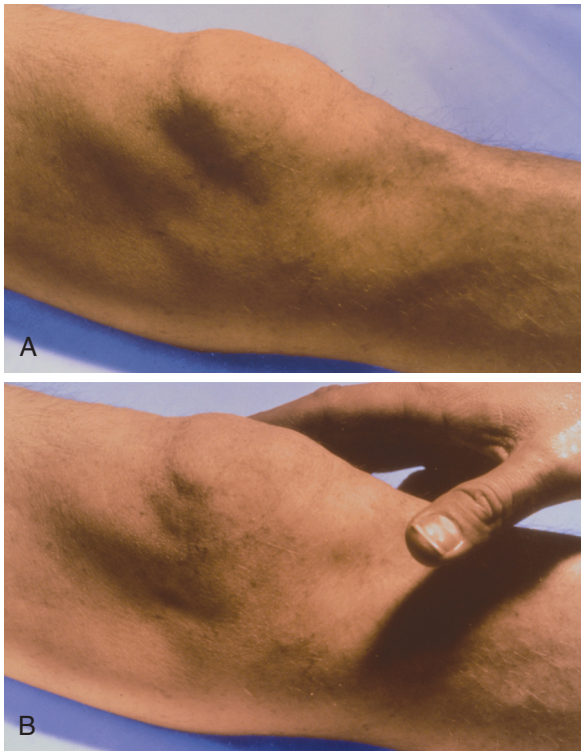



**Figure 40-10** Demonstration of the bulge sign for a small synovial knee effusion. The medial aspect of the knee has been stroked to move the synovial fluid from this area *(shaded depressed area in **A**)*. **B** shows a bulge in the previously depressed area after the lateral aspect of the knee has been tapped.

The medial and lateral tibiofemoral joint margins are palpated for tenderness and bony lipping or exostosis, as can be seen in degenerative joint disease. Joint margins can be palpated easily with the hip flexed to 45 degrees, the knee flexed to 90 degrees, and the foot resting on the examining table. Tenderness localized over the medial or lateral joint margins may represent articular cartilage disease, medial or lateral meniscal abnormality, or medial or lateral collateral ligament injury. Other causes of tenderness include pathologic conditions in the underlying bony structures.

Bursitis is another cause of localized tenderness around the knee; the two most common sites are the pes anserine and the prepatellar bursae. Exquisite local tenderness usually can be elicited if bursitis is present. Mild swelling also may be appreciated. Occasionally, the prepatellar bursa can become quite swollen. It is important not to interpret this swelling mistakenly as knee joint synovitis. The two can be differentiated because the bursal margins can be outlined by palpation; other features of true joint effusion, such as the bulge sign, are absent.

Patellofemoral malalignment is another common cause of knee pain. It is more common in female patients because of the wider Q angle caused by the broader female pelvis. The Q angle is the angle formed between the quadriceps and the patellar tendon. Patients with patellofemoral disease may complain of stiffness in the knee after a period of

flexion (the moviegoer sign) or may have particular difficulty with stair climbing. Some patients may experience a sensation of catching as the patella moves over the distal femur. Patellar palpation is best performed with the knee extended and relaxed. The patella is compressed and moved so that its entire articular surface comes into contact with the underlying femur. Slight crepitation may be observed in many normally functioning knees. Pain with crepitation may suggest patellofemoral degenerative arthritis or chondromalacia patellae.

Retropatellar pain occurring with active knee flexion and extension and secondary to patellofemoral disease may be differentiated from tibiofemoral articular pain. To test this, the examiner should attempt to lift the patella away from the knee, while passively moving the knee through the range of motion. Painless motion during this maneuver indicates that the patellofemoral joint is the likely source of the pain. In addition, the "patellar grind" test is useful in patients with extensive patellofemoral abnormality. In this test, the examiner compresses the patella distally away from the femoral condyles, while instructing the patient to contract the quadriceps isometrically. Sudden patellar pain and quadriceps relaxation indicate a positive test result. This test has frequent false-positive results, however.

Patellar stability should be assessed. The Fairbanks apprehension test is done with the patient supine, the quadriceps relaxed, and the knee in 30 degrees of flexion. The examiner slowly pushes the patella laterally. A sudden contraction of the quadriceps and a distressed reaction from the patient constitute a positive apprehension test result. A patient who has had previous patellar dislocations usually has a positive apprehension test result. The patella also can be examined for subluxation while the knee is moved through a range of motion from full flexion to extension.

The plica syndrome occasionally causes symptoms that suggest patellofemoral disease. Plicae are bands of synovial tissue, most often located on the medial side of the knee. If present, a tender band-like structure may be palpated parallel to the medial border of the patella. During flexion and extension, palpable or audible snapping may be heard, and the patient may experience symptoms of catching. Many plicae are asymptomatic, however, and they are common, so they may be considered a normal variant.

The normal knee range of motion should be from full extension (0 degrees) to full flexion of 120 to 150 degrees. Some normal individuals may be able to hyperextend to 15 degrees. Loss of full extension that is generally reversible frequently occurs with a knee joint effusion, synovitis, or both. However, permanent loss of extension caused by flexion contracture is a common finding that accompanies chronic arthritis of the knee. In advanced arthritis, such as in some cases of RA, posterior subluxation of the tibia on the femur may be observed.

Testing for ligamentous and meniscal derangements are covered in Chapter 48.

Muscle strength testing includes testing flexion supplied by the hamstrings (i.e., the biceps femoris, semitendinosus, and semimembranosus) (nerve roots L5 to S3) and extension supplied by the quadriceps femoris (L2, L3, and L4). The hamstrings are best tested with the patient prone and attempting to move the knee from 90 degrees to maximal flexion. The ankle should be kept in neutral position or dorsiflexed to remove gastrocnemius action. With the leg externally rotated, the biceps femoris, which inserts on the fibula and lateral tibia, is primarily tested, whereas flexion with internal rotation tests the semitendinosus and semimembranosus muscles, which insert on the medial side of the tibia. Extension is tested with the patient sitting upright with the knee fully extended. The examiner stabilizes the thigh with downward pressure just proximal to the knee and places downward pressure at the ankle to test the knee extensors.

## Ankle

The true ankle is a hinged joint, and movement is limited to plantar flexion and dorsiflexion. It is formed by the distal ends of the tibia and fibula and the proximal aspect of the body of the talus. Inversion and eversion occur at the subtalar joint (see Chapter 49). The tibia forms the weight-bearing portion of the ankle joint, whereas the fibula articulates on the side of the tibia. The malleoli of the tibia and fibula extend downward beyond the weight-bearing part of the joint and articulate with the sides of the talus. The malleoli provide medial and lateral stability by enveloping the talus in a mortise-like fashion. The articular capsule of the ankle is lax on the anterior and posterior aspects of the joint, allowing extension and flexion, but it is tightly bound bilaterally by ligaments. The synovial membrane of the ankle on the inside of the capsule usually does not communicate with any other joints, bursae, or tendon sheaths.

The medial and lateral ligaments surrounding the ankle contribute to medial and lateral stability of the joint. The deltoid ligament, the only ligament on the medial side of the ankle, is a triangle-shaped fibrous band that resists eversion of the foot. It may be torn in eversion sprains of the ankle. The lateral ligaments of the foot consist of three distinct bands forming the posterior talofibular, the calcaneofibular, and the anterior talofibular ligaments. These ligaments may be injured in inversion sprains of the ankle.

All tendons crossing the ankle joint lie superficial to the articular capsule and are enclosed in synovial sheaths for part of their course across the ankle. On the anterior aspect of the ankle, the tendons and the synovial tendon sheaths of the tibialis anterior, extensor digitorum longus, peroneus tertius, and extensor hallucis longus muscles overlie the articular capsule and synovial membrane. On the medial side of the ankle, posterior and inferior to the medial malleolus, lie the flexor tendons and tendon sheaths of the tibialis posterior, flexor digitorum longus, and flexor hallucis longus muscles (Figure 40-11). All three of these muscles plantar flex and supinate the foot. The tendon of the flexor hallucis longus is located more posteriorly than the other flexor tendons and lies beneath the Achilles tendon for part of its course. The calcaneus tendon (Achilles tendon), the common tendon of the gastrocnemius and soleus muscles, inserts into the posterior surface of the calcaneus, where it is subject to external trauma, various inflammatory reactions, and irritations from bone spurs beneath it. On the lateral aspect of the ankle, posterior and inferior to the lateral malleolus, a synovial sheath encloses the tendons of the peroneus longus and peroneus brevis. These muscles extend the ankle (plantar flex) and evert (pronate) the foot.

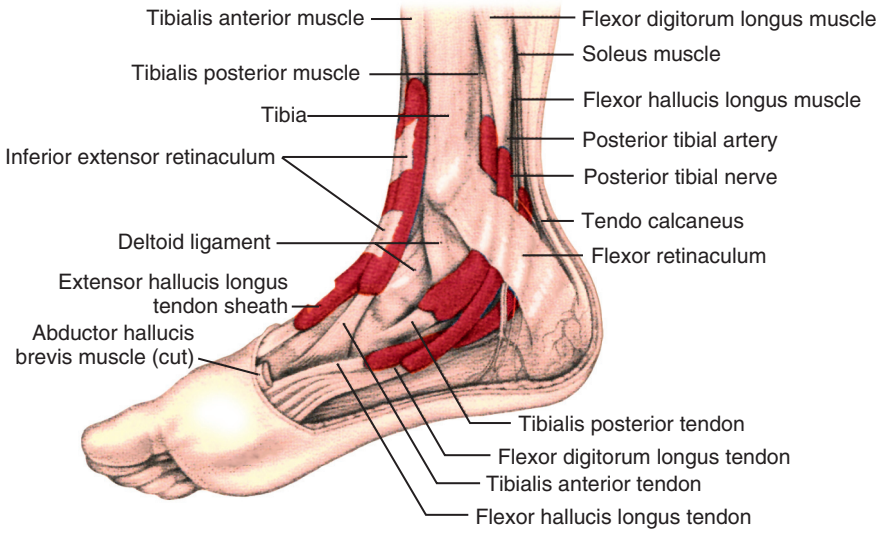



**Figure 40-11** Diagram of the ankle. Medial aspect of the ankle shows the relationship between tendons, ligaments, artery, and nerve. *(From Polley HF, Hunder GG:* Rheumatologic interviewing and physical examination of the joints, *ed 2, Philadelphia, 1978, WB Saunders. With permission from the Mayo Foundation for Medical Education and Research.)*

Each of the tendons adjacent to the ankle may be involved separately in traumatic or disease processes.

Three sets of fibrous bands or retinacula hold down the tendons that cross the ankle in their passage to the foot. The extensor retinaculum consists of a superior part (transverse crural ligament) in the anterior and inferior portions of the leg and an inferior part in the proximal portion of the dorsum of the foot. The flexor retinaculum is a thickened fibrous band on the medial side of the ankle. On the lateral side of the ankle, the peroneal retinaculum forms a superior and an inferior fibrous band. These bands bind down tendons of the peroneus longus and peroneus brevis muscles as they cross the lateral aspect of the ankle.

Synovial swelling of the ankle joint is most likely to cause fullness over the anterior or anterolateral aspect of the joint because the capsule is more lax in this area. Mild swelling of the joint may not be apparent on inspection because of the many structures crossing the joint superficially. Efforts should be made to differentiate superficial linear swelling localized to the distribution of the tendon sheaths from more diffuse fullness and swelling attributable to involvement of the ankle joint. Swelling of the heels may be observed from behind the standing patient and may be caused by enthesitis of the Achilles tendon insertion, which can occur in spondyloarthropathies (Figure 40-12).



**Figure 40-12** Achilles tendinitis in a patient with reactive arthritis. Note swelling of the left Achilles tendon insertion caused by active enthesitis and milder swelling of the right Achilles tendon insertion. *(With permission from the Mayo Foundation for Medical Education and Research, 2010.)*

It is difficult to observe synovitis of the intertarsal joints. Intertarsal joint synovitis may produce an erythematous puffiness or fullness over the dorsum of the foot.

From the normal position of rest in which there is a right angle between the leg and the foot, labeled 0 degrees, the ankle normally allows approximately 20 degrees of dorsiflexion and approximately 45 degrees of plantar flexion. Inversion and eversion of the foot occur mainly at the subtalar and other intertarsal joints. From the normal position of the foot, the subtalar joint normally permits approximately 20 degrees of eversion and 30 degrees of inversion. To test the subtalar joint, the examiner grasps the calcaneus with a hand and attempts to invert and evert it, holding the ankle motionless.

A general assessment of muscular strength of the ankle can be obtained by asking the patient to walk on toes and on heels. If the patient can walk satisfactorily on the toes and on the heels, the muscle strength of the flexors and extensors of the ankle can be considered normal. If this cannot be accomplished, it is desirable to test the muscles individually.

The principal flexors of the ankle are the gastrocnemius (nerve roots S1 and S2) and the soleus (S1 and S2) muscles. The principal extensor (dorsiflexors) of the ankle is the tibialis anterior muscle (L4, L5, and S1). The tibialis

posterior muscle (L5 and S1) is the principal inverter. To test the tibialis posterior muscle, the foot should be in plantar flexion. The examiner applies graded resistance on the medial border of the forefoot while the patient attempts to invert the foot. The principal everter of the foot are the peroneus longus (L4, L5, and S1) and peroneus brevis (L4, L5, and S1) muscles.

## Foot

See Chapter 49 for a detailed description.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 41

# Acute Monoarthritis



Laura McGregor • Max Field

| KEY POINTS |
| --- |
| Inflammatory arthritis causes morning stiffness and pain with restricted joint movement. |
| Pain that worsens after movement usually indicates mechanical joint pain. |
| Soft tissue disorders usually produce pain in a periarticular distribution. |
| Acute monoarthritis presentations (i.e., those that develop suddenly) are usually related to trauma. |
| Monoarthritis presentations (i.e., those that develop during a 1 to 2 day period) are usually inflammation- or infection-related. |
| Chronic monoarthritis presentation indicates pre-existing joint disease, but causes of acute monoarthritis need to be excluded. |
| Synovial fluid analysis is the most valuable laboratory test in acute monoarthritis. |

The sudden onset of monoarthritis in a synovial joint is a significant clinical event. The most serious cause is joint infection because of associated mortality (approximately 15%) and probability of impaired long-term function. However, the most common of the differential diagnoses (Table 41-1) is trauma or inflammation. Each case of monoarthritis requires immediate investigation and treatment to limit pain and prevent joint destruction. The British Society of Rheumatology and British Orthopaedic Association developed guidelines (Figure 41-1) to assist physicians in the diagnosis of the acutely swollen joint[1] based on history, examination, radiologic, and blood and synovial investigations.

Monoarthritis can also be a new presentation of more chronic forms of arthritis, and monoarticular pain can result from non-inflammatory arthropathies, periarticular problems, and bone and soft tissue disorders. These are discussed in the context of a formal differential diagnosis.

## PATIENT ASSESSMENT

### History

Physicians should inquire about the patient's age, occupation, and social, drug, travel, and sexual history. Pain and joint stiffness, its diurnal variation, and aggravating and relieving factors should be sought, and a history of swelling should be established. Specific features of relevance include trauma, joint locking, and the presence of systemic symptoms (fevers, sweats, rigors, and weight loss). Inquiring about ocular, oral, respiratory, gastrointestinal, or skin symptoms can facilitate the diagnosis if the monoarthritis is a presentation of a chronic arthropathy, related inflammatory disease, postinfectious phenomenon, or connective tissue disease. Table 41-2 summarizes symptoms and signs that characterize patients with inflammatory or non-inflammatory joint disease and soft tissue disorders[2] to help initial evaluation.

## EXAMINATION

The Regional Examination of the Musculoskeletal System (REMS) can be used to identify monoarticular abnormalities.[3] Physicians should look, feel, and move joints to assess function—comparing the affected joint and normal side, and to look for evidence of more widespread disease. In any acutely swollen joint, examination should include looking for local signs of inflammation (pain, erythema, swelling, heat, and loss of function). Any patient with pre-existing arthritis should be assessed for chronic changes to joint structure and disability. Local synovial swelling and/or effusion, joint instability, limited movement, and deformity of any single joint necessitate detailed investigation. Local monoarticular tenderness without swelling could indicate enthesitis, tendinitis, bursitis, or bone disease. A complete examination should look specifically for ocular signs, skin rashes, ulcers, and nodules.

## INVESTIGATIONS

### Blood

Inflammatory, septic, and crystal arthritides cause elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and white cell count (WCC), often associated with anemia. Systemic disease involvement is assessed by renal, liver, muscle, or bone biochemical screening and protein electrophoresis. Raised uric acid levels suggest a diagnosis of gout. In acute hemarthrosis, a platelet count, international normalized ratio, and clotting studies are warranted. Blood cultures are mandatory in patients with suspected septic arthritis and should *precede* antibiotic prescription. Viral screening (IgG and IgM antibodies), antistreptolysin-O test (ASOT), and Lyme serology can be diagnostic in relevant situations.

Rheumatoid factor and anti-nuclear antibodies (ANAs) can suggest rheumatoid arthritis (RA) or systemic lupus

**TABLE 41-1** Causes of Acute Monoarticular Joint or Periarticular Pain*

| |
|---|
| Common Acute Monoarthritis |
| Septic arthritis (nongonococcal, gonococcal) |
| Crystal arthritis (gout, pseudogout) |
| Reactive arthritides |
| Lyme disease |
| Plant thorn synovitis |
| Other infections (mycobacterial, viral, soft tissue) |
| Trauma or Internal Derangement |
| Loose bodies |
| Stress fractures |
| Ischemic necrosis |
| Hemarthrosis |
| Existing Inflammatory Polyarthritis |
| Psoriatic arthritis |
| Enteropathic arthritis |
| Rheumatoid arthritis/palindromic rheumatism |
| Juvenile inflammatory arthritides |
| Non-Inflammatory Joint Disease |
| Osteoarthritis |
| Charcot's joints |
| Storage diseases (hemochromatosis, ochronosis) |
| Synovial Disease |
| Pigmented villonodular synovitis |
| Lipoma arborescens |
| Synovial osteochondromatosis |
| Reflex sympathetic dystrophy |
| Sarcoidosis |
| Amyloid |
| Existing Systemic Disease |
| Systemic lupus erythematosus |
| Vasculitides (anti-neutrophil cytoplasmic antibody positive and negative) |
| Henoch-Schönlein purpura |
| Behçet's disease |
| Bacterial endocarditis |
| Familial Mediterranean fever |
| Relapsing polychondritis |
| Bone Disease |
| Paget's disease |
| Osteomyelitis (Brodie's abscess) |
| Osteogenic/osteoid tumors |
| Metastatic disease |
| Pulmonary hypertrophic osteoarthropathy |
| Soft Tissue Lesions |

*This table shows the causes of inflammation in any one joint (monoarthritis) and pain around the joint that presents without inflammation (monoarthropathy).

erythematosus (SLE), although autoantibodies can be present in infectious diseases, other autoimmune diseases, and in normal individuals. Similar limitations apply to results for anti-neutrophil cytoplasmic antibodies (ANCAs) and angiotensin-converting enzyme (ACE), so positive results should be interpreted in the clinical context. Anti-citrullinated protein antibody (ACPA) antibodies are sensitive for RA and are useful in early presentation. Other relevant blood tests include thyroid function, ferritin, vitamin D levels, and human leukocyte antigen (HLA) genes.

### Urine

The urinary tract can be a source of Gram-negative bacteria in septic arthritis in the elderly. Significant proteinuria and/or hematuria and red cell casts indicate renal damage in SLE, vasculitis, or subacute bacterial endocarditis.

### Imaging Studies

A range of imaging studies assist in diagnosis of acute monoarthritis.

- Plain radiographs identify soft tissue swelling, calcium in periarticular tissues, fractures, local bone disease, and loose bodies, as well as destructive changes in long-standing arthritides.
- Computed tomography (CT). CT scanning better identifies fractures, bone diseases, and intra-abdominal and chest pathology. It is useful when magnetic resonance imaging (MRI) is contraindicated. In acute arthritis, CT scan can show osteomyelitis in addition to acute inflammation.
- Musculoskeletal ultrasound. Ultrasound is increasingly reliable for use in rheumatology practice, as long as practitioners are appropriately trained.[4] Ultrasound is quick, efficient, inexpensive, and equally effective versus MRI and clinical examination in detecting synovitis[5] and soft tissue damage in acute shoulder injury.[6] In acute monoarthritis ultrasound can identify loculated synovial fluid for aspiration and/or injection.[7] Power Doppler views can demonstrate increased blood flow in active synovitis.[8] Ultrasound is recommended for detection of synovitis by the European League Against Rheumatism (EULAR) in early arthritis.[9]
- MRI. Although it is the best technique for soft tissue imaging, MRI is time consuming and costly. MRI can diagnose internal ligament damage and tendon enthesitis and is most effective in identifying avascular necrosis of bone. MRI is also useful when identifying the extent of inflammation in acute monoarthritis and subclinical joint involvement. EULAR also suggests MRI for diagnosis of acute early arthritis.[9]
- Arthrography. Imaging internal joint structure after injection of radiopaque solutions in association with CT scanning is useful for hip cartilage tears and in situations when MRI is not feasible.
- Radionuclide scans. Bone scanning with technetium-labeled methylene diphosphonate identifies osteoid osteomas, bone sarcomas, bony metastases, osteomyelitis, and stress fractures not seen on plain radiographs. Bone scintigraphy is helpful when excluding bone and joint disorders in patients with chronic pain syndromes. Although not specific in acute arthritides, bone scans show differences in the pattern of joint involvement between inflammatory conditions and osteoarthritis (OA). Labeled white cell scans can identify areas of infection, especially when the source of infection is uncertain in patients with septic arthritis.

## SYNOVIAL FLUID

The most useful test in acute monoarthritis is examination of synovial fluid, which should be analyzed for color and cloudiness (Table 41-3). Microscopy should be used to characterize the predominant cells, Gram stain to detect bacteria, and polarized light analysis to identify uric acid or

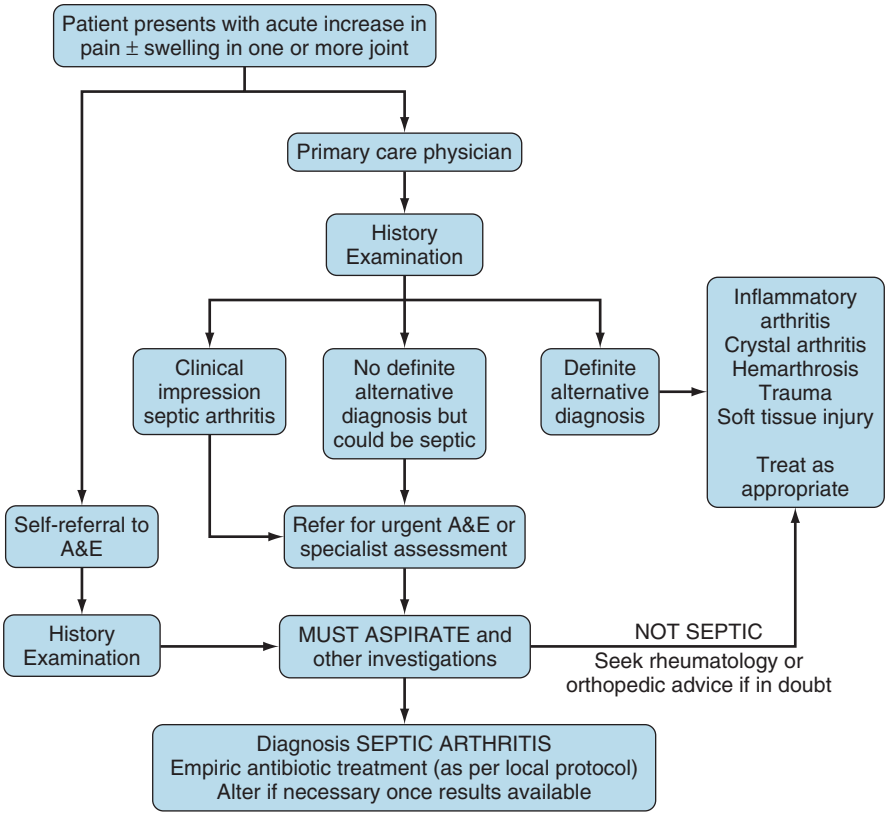



**Figure 41-1** Algorithm for assessing patients with acute monoarthritis. A&E, Accident and emergency department (or emergency room).

**TABLE 41-2** Symptoms and Signs About Which Patients Complain When They Have Inflammatory Joint Pain, Non-inflammatory Joint Pain, and Soft Tissue Problems*

| Feature | Inflammatory | Non-inflammatory | Soft Tissue Injury |
|---|---|---|---|
| **Symptoms** | | | |
| Morning stiffness | Usually >30 min | Local usually <30 min | Localized and brief |
| Constitutional symptoms | Present (fever, malaise) | None | None |
| Time of major discomfort | After prolonged inactivity | After prolonged use | During and after use |
| Locking/instability | Unlikely in acute joint disease | Suggests internal joint derangement | Unusual unless tendon damage/tear present |
| **Signs** | | | |
| Swelling | Common | Can be bone | Unusual |
| Tenderness | Diffuse over joint space | Mild over joint line | Localized periarticular |
| Inflammation | Common | Unusual | Over tendon/bursa |
| Instability | Uncommon | Occasional | Uncommon |
| Multisystem disease | More common | No | Unusual |

*Modified from 1996 American College of Rheumatology guidelines for evaluation of adults with acute musculoskeletal symptoms.
From Schmerling R, Fuchs H: Guidelines for the initial evaluation of the adult patient with acute musculoskeletal symptoms. *Arthritis Rheum* 39:1–8, 1996.

calcium pyrophosphate dihydrate (CPPD) crystals.[1] Identification of bacteria following synovial fluid culture can provide results even when a Gram stain is negative. *Neisseria* organisms are fastidious with low yields from culture, but PCR can detect *Neisseria*-specific DNA.

Many centers measure synovial fluid WCC, which may differentiate inflammatory and non-inflammatory presentations but crucially fails to differentiate between inflammation and infection. Low synovial fluid glucose and high lactate[10] and procalcitonin[11] are found in septic arthritis, but evidence that these can distinguish infectious from inflammatory arthritis in a clinically meaningful way is limited. Bacterial identification in synovial fluid from patients with septic arthritis can be inhibited by prior antibiotic use, and hence a careful drug history should be elicited.[12] CPPD crystals are difficult to detect, so in both cases a clinical diagnosis should not be excluded if symptoms, signs, and investigations are suggestive.

**TABLE 41-3** Synovial Fluid Characteristics in Clinical Situations, with Imaging and Investigation Techniques Best Used to Identify the Cause

| Diagnosis | Cells | Microorganisms | Appearance | Imaging Modality | Comments |
|---|---|---|---|---|---|
| Bacterial arthritis | Neutrophils, 10,000-100,000 | Gram stain usually positive | Turbid/pus | Aspiration to dryness; may need ultrasound | Systemic symptoms, Gram stain, blood and synovial fluid culture |
| Gonococcal arthritis | Neutrophils, 10,000-100,000 | Gram stain usually positive | Turbid/pus | Aspiration to dryness; may need ultrasound | Systemic symptoms, Gram stain, blood and synovial fluid culture |
| Crystal arthritis | Neutrophils, 10,000-100,000 | — | Turbid/pus | XR, CPPD | Presence of appropriate crystals<br>Acute serum urate unreliable |
| Tuberculous arthritis | Mononuclear 5000-50,000 | Acid-fast stain often negative | Turbid/pus | | At-risk population; Ziehl-Neelsen stain biopsy may be necessary |
| Inflammatory monoarthropathies | Neutrophils 5000-50,000 | — | Slightly turbid | Ultrasound/MRI for early synovitis and erosions | Serum autoantibodies such as RF, ACPA, ANA |
| Osteoarthritis | Mononuclear 0-2000 | — | Clear | XR changes | Usually non-inflammatory<br>CPPD may be present |
| Internal derangement | Red blood cells | — | Clear/turbid | MRI | Arthroscopy may be necessary |
| Trauma | Red blood cells | — | Clear/turbid | XR | Tc bone scan may aid diagnosis if radiograph normal |
| Ischemic necrosis | | — | | MRI in early disease | XR abnormal only in advanced cases |
| **Uncommon Causes**<br>Sarcoidosis | Mononuclear, 5000-20,000 | — | | CXR | |
| PVNS | Red blood cells | — | Turbid | Ultrasound and MRI | Synovial biopsy essential |
| Charcot's | Mononuclear, 0-2000 | — | | XR | CPPD may be present |
| Lyme disease | Neutrophils, 0-5000 | — | Clear/turbid | | SF eosinophilia may be found<br>Serology for *Borrelia* |
| Amyloid | Mononuclear 2000-10,000 | — | Turbid | | Synovial biopsy for Congo red stain |

ACPA, Anti-citrullinated protein antibody; ANA, anti-nuclear antibody; CPPD, calcium pyrophosphate dehydrate deposition; CXR, chest radiograph; MRI, magnetic resonance imaging; PVNS, pigmented villonodular synovitis; RF, rheumatoid factor; SF, synovial fluid; Tc, technetium; XR, radiograph.

## SYNOVIAL OR BONE BIOPSY

Arthroscopic synovial biopsy is rarely necessary, but in tuberculosis, sarcoidosis, amyloid, pigmented villonodular synovitis, lipoma arborescens, and foreign body synovitis it is often required for diagnosis.[13] Proteomic and genomic assays begin to show differences in the synovium from chronic arthritides, so synovial biopsy use may increase in the future. Bone biopsy may be needed to identify tumors and relevant factors in nonresolving osteomyelitis.

## SPECIFIC DIAGNOSES

### Acute Monoarthritis

#### Septic Arthritis (Bacterial)

The presence of bacteria in synovial fluid is a medical emergency as the mortality rate is between 7% and 15%.[14,15] Large limb joints are most frequently involved,[16] usually associated with underlying OA or inflammatory arthropathies, especially RA.[14] Patients are particularly at risk after joint surgery, including arthroplasty and intra-articular injection, as are patients with distant infections.[17] Patients with underlying disease affecting the immune response or those taking drugs that impair immune function are also at risk,[18] as are the elderly and those living in deprived socioeconomic circumstances.[14]

History often elicits an acute, painful, swollen monoarthritis, but more than 10% of patients with native joint infections are seen with polyarticular infection. An identifiable distant site of infection may be found at presentation, but fever occurs in more than 50% of cases and sweats/rigors in approximately 30%.[15] Blood cultures may identify bacteria in patients in whom synovial fluid culture is negative. When patients are first seen, ESR and CRP are almost invariably raised, but approximately 35% of all septic arthritis patients do not have a raised WCC. When infection is in the context of underlying RA, this figure rises to 50%. Impaired renal and liver function when the patient is first examined can predict a poorer outcome.[14] Plain radiographs can show soft tissue swelling, but are invariably normal at first examination. Ultrasound can localize synovitis and fluid collection to target aspiration, but MRI is required to demonstrate osteomyelitis.

Synovial fluid is usually cloudy with a high WCC, and a Gram stain should be performed.[1] High levels of synovial WCC are often noted but fail to differentiate between septic arthritis and inflammatory arthritis. Studies assessing the use of other serum and synovial fluid markers, such as procalcitonin, interleukin (IL)-6, and tumor necrosis

factor (TNF) have been performed with varying success.[19] Synovial fluid culture is mandatory but detects the organism in only approximately 50% of cases. Thus local protocols for antibiotic use should be instigated before culture results are available. Organisms detected most commonly include staphylococci (*Staphylococcus aureus* and *Staphylococcus epidermidis*), streptococci, and Gram-negative bacteria, with an increasing prevalence of methicillin-resistant *S. aureus*.[12,20] Prompt intervention can reduce mortality[10,14]; hence the joint must be aspirated to dryness, but this can usually be done without recourse to surgical intervention.[21]

Interestingly, results from history, examination, routine investigations, and outcome are identical when comparing patients with bacteria-proven septic arthritis with patients in whom a clinical diagnosis of septic arthritis is made but in whom bacteria cannot be isolated.[12] Hence normal investigations at first examination should not delay treatment in the presence of a strong clinical suspicion.

Infection with *Neisseria gonorrhoeae* should be suspected in sexually active patients who are seen with migratory arthralgias, tenosynovitis, skin rashes, and vesicles. Untreated *Neisseria* infection can also lead to destructive arthritis. Most patients will be febrile with a raised acute phase response and blood WCC, but as with other bacterial causes of septic arthritis, these may be normal at presentation.[22] Investigations should include swabs from the urethra, cervix, pharynx, and rectum, inoculated immediately on Thayer-Martin medium. In contrast with other bacterial infections, PCR has been used on synovial fluid to improve positive results.[23]

## Crystal Arthritis

See Chapters 95, Clinical Features and Treatment of Gout, and 96, Calcium Crystal Disease.

### Gout

Podagra is the classic monoarthritis of the first metatarsophalangeal joint. Other lower limb joints can be affected,[24] as can small joints of the hands, elbows, and periarticular structures.[25] Patients tend to be obese males, aged 40 to 50 years with hypertension, and consumers of excess alcohol.[26] However, post-menopausal females with low estrogen and who take loop diuretics for hypertension are also predisposed to gout. Tophi are indicative of the diagnosis.

Fever is present in 34% of patients, especially in polyarticular presentations.[27] In acute settings, blood WCC, ESR, and CRP are raised, and to this extent gout mimics septic arthritis. Serum uric acid may be raised, but levels are low in 33% during acute attacks.[28] Renal and liver function should be assessed. Negatively birefringent needle-shaped uric acid crystals are present in synovial fluid or tophi aspirates and confirm the diagnosis.[29] Synovial fluid should be examined for bacteria to exclude concomitant septic arthritis.

Routine radiographs frequently show no bony abnormalities but may identify erosions after repeated or prolonged attacks. Ultrasound and dual-energy CT (DECT) are superior imaging techniques compared with conventional radiographs.[30] A double-contour sign overlying cartilage is suggestive of gout on ultrasound. Cortical erosions, however, may be more clearly identified on MRI.[31]

### Acute Calcium Pyrophosphate Crystal Arthritis

Formally known as pseudogout, acute calcium pyrophosphate (CPP) crystal arthritis is the third commonest inflammatory arthritis.[32] It is rare in patients younger than 50 years, and the initial symptom is usually monarticular inflammation, commonest at the knees and wrists, often with concomitant OA.[32] Acute attacks often occur following a trigger such as infection, trauma, or surgery.[33] It can also occur in relation to specific diseases, such as hemochromatosis and primary hyperparathyroidism. Calcinosis of cartilage and periarticular tissues can be seen on radiographs and ultrasound, with ultrasound the more sensitive modality.[32] Synovial fluid microscopy demonstrates rhomboid-shaped crystals at ×400 magnification, and this remains the diagnostic gold standard.[34] Culture should be undertaken to exclude coexistent septic arthritis. Hemarthrosis necessitates review for an occult fracture. Repeat imaging (e.g., using MRI) may be necessary to formally exclude bone injury if clinical suspicion exists.

### Acute Calcific Periarthritis

Calcium hydroxyapatite deposition in periarticular tissues is predominately seen at the shoulder but can occur throughout the body. Underlying inflammatory arthritides, metabolic disease, and repetitive injury can be associated. Hypercalcemia necessitates that hyperparathyroidism should be excluded. Many patients are asymptomatic, although flares can be mistaken for commoner crystal arthritides.[35] Calcium crystals can be identified on routine radiographs, ultrasound, or MRI.[36]

### Calcium Phosphate Crystal Arthritis

Intra-articular deposits of basic calcium phosphate (BCP) are rare but present in older female patients with OA, with a destructive shoulder arthropathy usually on the dominant side (Milwaukee shoulder) as an acute on chronic monoarthritis.[37] The effusion is not inflammatory, but synovial fluid can be viscous and blood stained and may contain calcium aggregates and cartilage fragments. Plain radiographs show upward shoulder dislocation. CT is superior to a radiograph in identifying calcification. Even rarer clinical manifestations include tenosynovitis, peripheral nerve and spinal cord compression, and pseudotumoral deposition.[38]

### Cholesterol Crystal Arthritis

Cholesterol crystals have been reported in synovial fluid, albeit rarely, and often in association with inflammatory arthropathies. Whether these large rhomboid-shaped crystals are truly a separate cause of synovial inflammation remains a subject for speculation.

## Reactive Arthritis

Reactive arthritis characteristically presents as a large joint monoarthritis, a flitting asymmetric oligoarthritis, or

enthesitis with a history of preceding throat, urogenital, or gastrointestinal tract infection.[39] Uveitis, which may precede reactive arthritis, requires urgent review. Patients can also be seen with coexistent circinate balanitis, sterile urethritis, and keratoderma blennorrhagica. Urethral cultures should be performed. The finding of sterile inflammatory synovial fluid without crystals should exclude other acute arthritides. The acute phase response is usually high, and conventional radiologic investigations and ultrasound can identify synovial swelling and effusions. MRI is particularly useful for identifying enthesitis and extra-articular soft tissue disease.[36] Reactive arthritis is within the spondyloarthropathy spectrum, and as many as 30% of patients can have acute inflammatory back pain.[40] The high radiation exposure and insensitivity associated with standard sacroiliac joint radiographs has led to the increased use of MRI to assess for active sacroiliitis. Autoantibody tests are usually negative. The link with HLA-B27 may be suggestive but is of limited diagnostic use, although it may predict perpetuation to a chronic arthritis.[40] Antibodies against *Chlamydia*, *Shigella*, *Salmonella*, and *Campylobacter* can be found but are often negative.[41]

## LYME DISEASE

Patients with Lyme disease live in, or travel through, recognized at-risk geographic areas and are seen with expanding erythematous rashes (typically erythema migrans) after a "tick bite" resulting from *Borrelia* infection, usually *Borrelia burgdorferi*. The rash can be absent in as many as 30% of patients.[42] Large joint monoarthritis develops a few weeks after initial infection,[43] associated with a high ESR and CRP. Specific IgG antibodies, detected 4 weeks after infection, are diagnostic. Low levels of rheumatoid factor and ANAs can be detected. Synovial fluid contains polymorphs, and *Borrelia* organisms may be cultured, but PCR of synovial fluid for *Borrelia* DNA represents a superior test.[44]

### Plant Thorn Synovitis

Foreign bodies, including plant thorns, cause inflammation in intra-articular and tendon synovial tissue in hands or feet, but history of penetrating injury may be absent. Ultrasound, CT, and MRI are helpful[45] in localizing foreign bodies, but synovial biopsy can make the diagnosis. Synovial fluid can be sparse, and although usually sterile, may be infected with *Enterobacter agglomerans*,[46] a Gram-negative bacillus commonly found in soil.

#### Other Organisms That Produce Monoarthritis

Monoarthritis resulting from tuberculosis, including atypical forms that are most common,[47] should be considered in at-risk populations and people with a relevant social history. Synovial fluid contains mononuclear cells, but synovial biopsy may be required to identify organisms.

Polyarthralgia after viral infection is common. Rubella, herpes, and hepatitis B and C infections are reported to appear as monoarthritis. Arthropathies occur during infection with, and treatment for, human immunodeficiency virus. Other features in the patient's initial history and examination findings can be diagnostically useful. Arthritis, tenosynovitis, and bone lesions are reported following fungal and parasitic infections, but most infectious lesions are extensions of underlying disease. A reactive arthritis (Poncet's disease) can occur after tuberculosis infection elsewhere and may also follow viral and fungal infections.

## TRAUMA AND INTERNAL DERANGEMENT

Trauma, either acute or after repeated injury, is the most common cause of acute monoarticular pain, especially in the knee and the ankle. In the knee, torn menisci or loose bodies in the synovial fluid "wedge" between articulating surfaces and lead to sudden and painful locking and weakness when walking, which the patient describes as "giving way." Patient history is diagnostic, and an examination that uses the REMS can elicit locking by using McMurray's test. Examination for other ligament damage is mandatory, with use of tests for cruciate or collateral knee ligament stability. Careful attention to inversion and eversion stability of the ankle is also essential for monoarticular pain in that area. Plain radiographs may demonstrate abnormal architecture, dislocation, or loose bodies, but MRI is diagnostically superior and will usually establish the cause of a trauma-related diagnosis. Arthrography may be required to assess the hip for damage, particularly to establish tears in the acetabular labrum.

Stress fractures can cause monoarticular or periarticular pain on weight bearing and occur after repeated minor trauma (e.g., March metatarsal fracture) but may occur secondary to underlying local or systemic bone disease, particularly in sedentary individuals and those on prolonged bisphosphonate therapy.[48] These fractures can be missed on standard radiographs; thus CT, MRI, or bone scintigraphy are helpful in the context of persistent localizing regional articular pain.

Osteonecrosis commonly affects the hip in children (Legg-Calvé-Perthes) but can involve metatarsal heads (Freiberg), humerus, or lunate.[49] Osteonecrosis after neck or femur fracture results from impaired vascular supply and can occur in patients with connective tissues diseases, particularly when receiving high-dose glucocorticoids. Other causes of osteonecrosis include decompression sickness, hemoglobinopathies, and patients with hyperlipidemia, hyperuricemia, or high alcohol consumption. History is of monoarticular pain, and examination can be normal, but early MRI is diagnostic when plain radiographs are often normal.[50]

The presence of acute hemarthrosis suggests trauma, inherited/acquired clotting abnormalities, hemangiomas, or pigmented villonodular synovitis (PVNS). In the context of recurrent blood-stained effusions that are resistant to local interventions such as glucocorticoid injection, MRI is the investigation of choice before proceeding to synovial biopsy, which is essential for pathologic confirmation of PVNS.[50]

## POLYARTHRITIS PRESENTING AS ACUTE MONOARTHRITIS

Monoarthritis, digital dactylitis, or enthesitis may develop in patients with a personal or family history of psoriasis.[51] Radiographs may show proliferative new bone formation

along the metacarpal shaft or periarticular erosions in long-standing disease. Ultrasound and MRI can differentiate inflammation at tendon insertions from synovitis[52] and can localize synovial fluid that, if aspirated, is usually aseptic and inflammatory in character.

As many as 25% of inflammatory bowel disease patients are seen with a seronegative lower limb large joint acute monoarthritis,[53] often during exacerbation of bowel symptoms. Synovial biopsy from Crohn's disease patients may show granulomas. In cases of Whipple's disease, 60% of patients are seen with large migratory joint monoarthritis or oligoarthritis.[54] Blood tests confirm a high acute phase response with leukopenia, whereas synovial fluid usually shows a high WBC. The diagnosis is based on histologic analysis of jejunal or synovial biopsy and molecular biologic tests for *Tropheryma whippelii*.[54] There are reports of short-lived peripheral joint oligoarthritis in approximately 25% of patients with celiac disease.[55] A seronegative erosive arthritis with inflammatory synovial fluid and lymphocytic infiltrate on biopsy may occur after jejunoileal surgery for obesity,[56] but to date arthropathies are not a significant problem after less invasive gastric banding surgery.[57]

RA is reported to develop within 5 years in as many as 20% of patients who are seen at early arthritis clinics with acute knee synovitis (even for short periods as in palindromic rheumatism). Patients with a positive rheumatoid factor, ACPA, and a high acute phase response should have synovitis confirmed by MRI and/or ultrasound.[58] By achieving the American College of Rheumatology (ACR)/EULAR 2010 diagnostic criteria for RA,[59] many such patients merit earlier therapeutic intervention.

In adolescent boys, a monoarthritis may be the harbinger of spondyloarthropathy. Features of inflammatory back pain, duration of pain for more than 3 months, and presence of HLA-B27 should raise this suspicion and prompt urgent assessment for peripheral and axial inflammatory disease. Most juvenile inflammatory arthritis (JIA) patients with monoarthritis close to the 16-year age cutoff have lower limb arthritis, commonly knee or ankle involvement, and blood and radiologic investigations are similar to those with adult-onset disorders. Uveitis with inflammatory arthritis suggests the ANA-positive variety and requires regular ophthalmologic investigation. Systemic adult-onset JIA is characterized by polyarthritis, a transient salmon-pink rash, fever, and serositis. A high serum ferritin and acute phase response is found, and abnormal liver function is common. Synovial fluid shows a high neutrophil count.[60]

## MONOARTHROPATHIES ARISING FROM OTHER JOINT DISEASES

Osteoarthritis is seen as a chronic non-inflammatory polyarthropathy with a classic joint distribution. A history of intra-articular fracture or recurrent occupational-related injury (carpet fitter's knee) or previous arthrotomy can lead to a more localized OA. In younger patients with hip OA, slipped epiphysis, congenital dislocation, or avascular necrosis may antedate disease. Blood investigations will usually be normal, and provided there are no additional pathologies, synovial fluid shows a non-inflammatory level WCC.[61] Plain radiographs usually confirm the diagnosis in established disease, but ultrasound and MRI can clarify the extent of inflammation. In the presence of more inflammatory symptoms, it is important to exclude crystal deposition and/or infection,[14] but inflammatory OA may develop and require additional therapy.[61]

Neuropathic arthropathy (Charcot's joint) should be suspected in patients in whom there is a monoarthritis in the distal lower limb with severe OA on plain radiograph in association with demonstrable peripheral neuropathy. The reduced syphilis incidence means that diabetes mellitus is the commonest cause of peripheral neuropathy in the Western world.[62]

Accumulation of iron leads to hemochromatosis in synovial tissue with a secondary arthropathy. Abnormal liver function and iron saturation tests are found in association with sequence variations in the *HFE* gene, which regulates iron transport, and in more chronic cases osteophytes occur in second and third fingers. The arthropathy associated with alkaptonuria affects the spine and large joint degenerative arthritis on radiographs. Discoloration of ear cartilage and sclera is diagnostic.

## SYNOVIAL CAUSES OF NON-INFLAMMATORY MONOARTICULAR PAIN

Patients with primary or secondary tumors in periarticular tissues can be seen with single-joint pain and are usually diagnosed following routine radiographs and/or MRI.[36] Lipoma arborescens is a benign tumor, often presenting with knee swelling, in which synovium is replaced by mature fat cells. This tumor is more widely recognized with the increased use of MRI, which shows villous proliferation and characteristic features similar to subcutaneous fat.[63]

Patients with synovial osteochondromatosis are first seen with symptoms of pain and locking in the large joints, predominantly hips and knees. Synovial fluid is pale in color with few cells. Plain radiographs show calcification in synovial tissues, and synovial histology after biopsy shows the formation of osteocartilaginous bodies in the synovial membrane.[64]

Complex regional pain syndrome type I (reflex sympathetic dystrophy or algodystrophy) usually affects upper limb extremities, often following a triggering event. Pain is diffuse with a burning quality, is worsened after minor stimuli, and local weakness limits function. The affected limb is swollen and warmer than the normal side. Bone scintigraphy shows increased blood flow, and MRI patchy osteoporosis. Synovial fluid contains high protein levels but a low cell count and synovial biopsy shows hypervascularity and hyperplasia of arteriole walls with only limited cellular infiltrate or proliferation.[65]

## MONOARTICULAR ARTHRITIS IN SYSTEMIC DISEASES

Systemic vasculitides, including giant cell arteritis, ANCA-associated vasculitis, Henoch-Schönlein purpura, Behçet's disease, and connective tissue diseases, such as Sjögren's syndrome and SLE, may present with arthralgia and occasionally oligoarticular joint pain and synovitis. Relevant symptoms and signs and appropriate investigations should make the diagnosis. Sudden onset of new monoarticular

symptoms, particularly in those with SLE, when taking high-dose steroids, should raise the possibility of infection, trauma with tendon damage[66] or avascular necrosis. MRI is the investigation of choice.[67]

Sarcoidosis represents approximately 4% of cases in early synovitis clinics being linked with ankle arthritis, erythema nodosum, and bilateral hilar lymphadenopathy.[68] Patients are seronegative for rheumatoid factor and have a high acute phase response, but reported hypercalcemia is not common at presentation. ACE levels may be raised in patients with active widespread sarcoidosis but are often negative in acute joint disease.[69] Synovial fluid contains lymphocytes, and biopsy shows noncaseating granulomas.[70] Chronic arthritis is uncommon, but bone sarcoidosis may present as dactylitis with plain radiographs showing bone cysts in the phalanges.[36]

Many different fibrillar proteins located in the synovium can be associated with joint pain. Amyloid deposition is diagnosed by green birefringence after staining with Congo red on organ biopsy.[71] Amyloidosis can be primary (AL) with deposition of light chains in patients with myeloma or secondary (AA) amyloid, often associated with chronic infectious or inflammatory diseases or, rarely, of familial etiology. More than 40% of AL patients are reported to have joint related symptoms that include arthropathy, including some with synovial amyloid deposition.[72]

In hereditary periodic fever syndromes, patients, often with a Mediterranean connection, may have acute short-lived episodes of monoarthritis with serositis and high fevers. The acute phase response is high, as might be expected from the neutrophil synovial infiltrate. *HFE* gene associations now exist across a range of these disorders.[73]

Relapsing polychondritis is an episodic disorder in middle-aged Caucasians that destroys synovial joint cartilage and mimics acute monoarthritis. Ear pinna, nose, larynx, bronchi, and sclera can be affected, as can the aortic valves and aortic root. Synovial fluid is non-inflammatory, but the acute phase response is raised, often with a leukocytosis. Biopsy shows an active cellular infiltrate into cartilage, and antibodies can be detected against a variety of cartilage constituents. Repeated episodes lead to local damage and deformity.[74]

## REGIONAL DIFFERENTIAL DIAGNOSIS OF MUSCULOSKELETAL PAIN

Injuries to soft tissues can present with pain close to local joints and are often misconstrued as "arthritis." Table 41-4 shows a list of the most frequent causes of soft tissue injury. These conditions are not usually associated with active joint synovitis. Blood investigations are virtually always normal, as are plain radiographs unless there is local soft tissue swelling, calcification, or underlying joint damage. Ultrasound is the quickest method of imaging to demonstrate local injury to tendons, entheses, or bursae.[75] MRI is also reliable but costly to perform.[36]

## MONOARTICULAR PAIN RESULTING FROM PERIARTICULAR DISORDERS

Local skin infections, especially over superficial joints, can mimic the pain of acute synovitis. Deep intra-abdominal infections can cause pressure on femoral and sciatic nerves presenting with hip, knee, or sacroiliac joint pain.[76] The limited signs can lead to delay in diagnosis, with consequent increasing systemic toxicity. Investigations will be similar to those of septic arthritis, but ultrasound, CT scanning, MRI, and nuclear medicine scans, now including PET,[77] can localize unusual sites of infection. Neuropathic pain can mimic that from acute arthropathies. Pain from carpal tunnel syndrome can radiate down the arm to the fingers but equally can produce symptoms in the upper arm. Ultrasound and MRI can be used to identify pressure on the median nerve, but nerve conduction studies are the investigation of choice before injection or surgery are performed. Pressure on the lumbar (and cervical) nerve roots can cause pain that radiates into the affected limb and can localize into single joints. MRI of the spine is the investigation of choice to examine for disc protrusion or other local pathology. Blood investigations are usually normal.

Patients with chronic pain syndromes have poorly localized widespread pain often with headaches, irritable bowel syndrome, fatigue, and multiple pressure point tenderness. Symptoms can start from localized pain in a single area, which may be close to joints and hence mimic monoarthritis. Multiple trigger points are found on examination, but serum, radiologic investigations, including MRI, bone scintigraphy, and ultrasound, are normal.

**TABLE 41-4** Common Soft Tissue Disorders That Arise after Local Injury by Region

| |
|---|
| **Upper Limb** |
| Shoulder Region |
| Rotator cuff tendinitis |
| Rotator cuff tears |
| Subacromial bursitis |
| Frozen shoulder |
| Acromioclavicular pain |
| Referred pain |
| Elbow |
| Medial and lateral epicondylitis |
| Olecranon bursitis |
| Wrist and Hand |
| De Quervain's tenosynovitis |
| Trigger finger/thumb |
| **Lower Limb** |
| Hip Region |
| Iliopsoas bursitis |
| Ischiogluteal bursitis |
| Trochanteric bursitis |
| Adductor tendinitis |
| Referred pain |
| Knee |
| Prepatellar bursitis |
| Patellar tendinitis |
| Anserine bursitis |
| Ankle |
| Achilles tendonitis |
| Calcaneal and retrocalcaneal bursitis |
| Foot |
| Plantar fasciitis |
| Longitudinal arch problems |
| Hallux valgus/rigidus |
| Metatarsalgia |
| Morton's neuroma |

Bone pain may present as referred monoarticular pain from an undiagnosed fracture, undiagnosed metastatic bone disease, osteoid osteoma, Paget's disease, or osteomyelitis. Acute bone pain may be indicative of osteosarcoma when associated with a limp in adolescents. Imaging investigations may require technetium bone scans or MRI if standard radiography is not helpful.

## CONCLUSION

Monoarticular pain results from infectious, inflammatory, and non-inflammatory causes. The use of acute referral clinics can bring together apposite clinical expertise, appropriate blood and synovial fluid investigations, and imaging modalities to enable efficient diagnosis of patients' problems. Consequently, more effective treatments are used earlier in disease management, and some improved outcomes for patients with inflammatory joint diseases are being seen.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 42

# Evaluation and Differential Diagnosis of Polyarthritis



Ronald F. van Vollenhoven

**KEY POINTS**

- The differential diagnosis of polyarthritis is extensive.
- Diagnosing polyarthritis depends on an accurate medical history, a thorough physical examination, and appropriately chosen laboratory and imaging investigations.
- Laboratory testing and imaging, when used correctly, can lead to rapid diagnosis and diagnostic certainty.
- An early (presumptive) diagnosis and appropriate management will contribute to better outcomes.

> **"For the physician, it is essential to recognize what diseases are and whence they come; which are long and which are short; which are in the process of changing into others; which are major and which are minor."**
>
> —Hippocrates

Diagnosing patients with musculoskeletal symptoms remains one of the rheumatologist's core responsibilities. Although in the general field of medicine significant advances have been made in the use of sophisticated laboratory analyses and imaging modalities, in rheumatology, making correct diagnoses remains as much an art as a science today as in the days of Hippocrates. Moreover, for patients with polyarthritis, a purposeful and expeditious diagnostic workup has gained in importance as the benefits of early intervention have been identified for some specific diagnoses, most notably rheumatoid arthritis (RA).

In this chapter I will review the current state of the art of diagnosing polyarthritis in adults (the evaluation of children with joint symptoms is covered in Chapters 106, 107, and 108), with special reference to the proper use of laboratory testing and imaging but without detracting from the central role of a detailed history and a thorough physical examination.

## DIFFERENTIAL DIAGNOSIS OF POLYARTHRITIS

The differential diagnosis of polyarthritis, which is generally defined as arthritis occurring in four or more joints, is large; however, information that is readily available at the initial presentation will in many cases narrow the diagnosis to a limited number of possibilities. Thus, when in early spring polyarthritis of recent onset is seen in a young woman and is associated with febrile symptoms and a maculopapular rash, a viral diagnosis is very likely, and parvovirus B19 infection is a good guess. In contrast, if a middle-aged man being treated with hemodialysis wakes up one night with four hot, red, and swollen joints in his feet, it does not take an expert diagnostician to suspect gout. Nonetheless, surprises do occur, and it remains important to consider the entire spectrum of possible diagnoses when first evaluating the patient. Table 42-1 lists a comprehensive differential diagnosis of polyarthritis. The most important disease categories to be considered are outlined in the next sections.

### Viral Infections

Many different viral infections can cause a transient and self-limiting polyarthritis. One may suspect that the relatively common occurrence of self-limiting polyarthritis without further explanation usually represents viral infection, even if it is not so diagnosed. Viral arthritis is usually a symmetric polyarthritis of the small joints in the hands and feet and may therefore trigger a workup for early RA. The following viral infections are important to consider in the evaluation of polyarthritis:

- Parvovirus B19, which occurs seasonally and often appears in teenagers or young adults, is sometimes is severe enough to trigger suspicions of RA. The discovery that this infection can be associated with transient positivity for rheumatoid factor (RF) led to speculation that parvovirus might be the cause of RA.[1] Later studies clearly ruled out that possibility.[2,3] The course of parvovirus arthritis is self-limiting, but treatment may be needed for several days or weeks.[4]
- Although rubella virus infection has become uncommon as a result of vaccination, it may nonetheless be encountered from time to time in young adults, and diagnosis is, of course, extremely important when pregnancy is an issue. Rubella virus infection is self-limiting and usually mild.[5]
- Hepatitis virus infection: Each of the hepatitis viruses can cause polyarthritis as the first and sometimes only clinical manifestation.[6-8] The fact that RF can be positive in persons with viral hepatitis may lead even experienced clinicians astray.[9]
- HIV infection may cause polyarthritis, which may be the first manifestation of HIV.[10] Because early diagnosis and treatment is very important, this disease must always be considered. In contrast to most types of viral arthritis, HIV-related polyarthritis can be severe.[11]

**TABLE 42-1** The Differential Diagnosis of Polyarthritis

| Mechanism of Disease | Types of Disease |
|---|---|
| *Infections* | |
| Viral | Parvovirus B19<br>Rubella virus<br>Hepatitis A, B, C<br>Human immunodeficiency virus<br>Alphaviruses, including Chikungunya infection |
| Bacterial | Multiple infected joints in the presence of overwhelming sepsis<br>Initial phase of gonorrhea and chlamydia<br>Early phase of Lyme arthritis |
| *Diseases triggered by infection but presumed autoimmune* | |
| Reactive arthritis | After urogenital infections (*Chlamydia* and *Ureaplasma*); after gastrointestinal infections (*Yersinia, Shigella, Campylobacter,* and *Salmonella*) |
| Acute rheumatic fever | After infection with group A streptococcus |
| *Autoimmune diseases* | |
| Primary arthritides | Rheumatoid arthritis<br>Psoriatic arthritis<br>Spondyloarthropathies<br>Juvenile inflammatory arthritis |
| Transient and recurring polyarthritides | Palindromic rheumatism<br>Recurrent symmetric seronegative synovitis with pitting edema (RS3PE syndrome) |
| Systemic autoimmune disease | Systemic lupus erythematosus<br>Mixed connective tissue disease<br>Primary Sjögren's syndrome<br>Progressive systemic sclerosis and limited scleroderma<br>Behçet's disease<br>Sarcoidosis<br>Vasculitis |
| *Autoinflammatory diseases* | Adult-onset Still's disease<br>Familial Mediterranean fever and other cryopyrin-associated fever syndromes<br>Various genetic autoinflammatory conditions usually manifested first in childhood |
| *Degenerative diseases*<br>Osteoarthritis<br>Hypertrophic osteoarthropathy<br>Osteonecrosis | Includes "erosive inflammatory osteoarthritis" |
| *Metabolic diseases* | |
| Thyroid diseases | Hypothyroidism |
| Hemochromatosis | Hyperthyroidism (Graves' disease; early phase of Hashimoto's disease) |
| Hemoglobinopathies | Sickle cell anemia |
| Hemochromatosis | Thalassemiae |
| *Crystal diseases* | Gout<br>Pseudogout |
| *Deposition diseases* | Glycogen storage diseases; amyloid deposition in primary amyloidosis; mucopolysaccharidoses; light- and heavy-chain deposition diseases; others |
| *Drug-Induced diseases*<br>Generalized vasculitic drug reactions, serum sickness | |

- Alphavirus infections, including Chikungunya arthritis: In tropical countries, several alphaviruses (i.e., viruses belonging to the family of togaviruses and the class of arboviruses that are transmitted by mosquitos) are not uncommonly encountered. These infections with fanciful names such as Chikungunya[12] ("bending up" in the Akonde language), Sindbis,[13] and O'nyong-nyong[14] (meaning "severe joint pain" in the East African Acholi language) are not always self-limiting and may sometimes be destructive. Ross River virus arthritis in Australia is also caused by an alphavirus.[15] Until recently these types of viral arthritis were rarely encountered in practice outside tropical regions, but a remarkable change in the epidemiology of Chikungunya virus arthritis is taking place. As recently as 2013, this virus gained a foothold in the Caribbean region, where more than a million persons are since estimated to have been infected.[16] Frequent travel between that region and North America is now making Chikungunya infection in general, and Chikungunya arthritis more specifically, a diagnosis to be reckoned with in daily practice in the United States and Canada. Moreover, evidence indicates that the virus is adapting to mosquito strains that are ubiquitous in the United States, so the infection may become endemic in the United States as well. Chikungunya infection almost invariably causes moderate or severe myalgias and arthralgias that frequently persist weeks or months after other signs of the acute infection have subsided.[17,18]

Frank arthritis may also be seen, usually in a small joint pattern that is difficult to differentiate from seronegative RA.[19]

## Bacterial Infections

Typically, bacterial infection causes a septic monoarthritis. In rare cases, multiple joints can be seeded simultaneously, but the clinical setting will usually clarify this from the outset. Some bacterial infections behave atypically, such as gonorrhea, which can cause polyarthritis in the small joints in a relatively early phase[20]; this occurrence is believed to be due to the dissemination of immune complexes into the joints rather than to direct infection. Similarly, Lyme disease, which was initially identified as a new form of juvenile inflammatory arthritis, may cause polyarthritis in an early phase of the infection.[21] In this disease, both direct infection of the joints by *Borrelia burgdorferi* and an autoimmune reaction triggered by that organism may play a role; this possibility is discussed in more detail in Chapter 110.

Several other bacterial infections may also lead to rheumatic syndromes through mechanisms other than direct infection of the joints. A classic finding in rheumatic fever after infection with group A streptococcus is a migratory aseptic arthritis of the large joints (discussed further in Chapter 115), and various gram-negative infections of the gastrointestinal system (e.g., *Shigella* and *Campylobacter*) and genitourinary tract (e.g., *Chlamydia* and *Ureaplasma*) may trigger reactive arthritis.

## Malignancy-Associated Polyarthritis

Although direct invasion of the joint by tumor cells or metastases is very rare, polyarthritis can be a paraneoplastic phenomenon.[22] Relatively little is known about the epidemiology or pathophysiology of this entity. Diagnostic clues may be the rapid onset of a fulminant polyarthritis and associated symptoms, such as weight loss and diffuse pain; in the absence of an obvious clue, these cases can be diagnostically very challenging.[23]

## Crystal-Associated Polyarthritis

Most cases of crystal deposition disease progress from recurrent monoarticular episodes to oligoarticular and then polyarticular phases, especially when left untreated. The initial locations of the gout attack are usually the feet, ankles, and knees, but the later polyarticular manifestations may also involve the upper extremities. Thus gout in the polyarticular phase can sometimes mimic RA, and this may be particularly important to consider if patients are partially treated with nonsteroidal anti-inflammatory drugs (NSAIDs). The first presentation of gout is hardly ever polyarticular, but in pseudogout, caused by the deposition of calcium pyrophosphate dihydrate crystals, this presentation may sometimes occur. Pseudogout occurs mostly in elderly persons and affects the knees and ankles, but also the joints of the toes, and even the joints of the wrists and hands; thus it can mimic many other diseases. The deposition of hydroxyapatite crystals can give rise to an inflammation of the shoulder ("Milwaukee shoulder").

## Degenerative Arthritis

Although osteoarthritis (OA) is considered a degenerative disease by many persons, some inflammation is often detected in the affected joint. Less commonly, overt clinical inflammation is present, and the moniker "erosive-inflammatory OA," although not very well defined, is used.[24,25] The presentation in this case may be a true polyarthritis, although the experienced clinician will have little difficulty recognizing this entity, based on the distribution (predominantly the distal and proximal interphalangeal joints and frequently the first carpometacarpal joint) and typical bony hypertrophy around the affected joints.

## Metabolic Diseases

Metabolic diseases such as hemochromatosis may cause progressive degenerative and inflammatory changes in multiple joints,[26] and a polyarticular presentation may on occasion be encountered in primary amyloidosis[27] and in other deposition diseases as well.[28-30] Both hypothyroidism and hyperthyroidism may be associated with a range of musculoskeletal symptoms[31,32]; monoarticular or oligoarticular arthritis of the large joints with large effusions is most typical of hypothyroidism,[33] and muscular symptoms are most typical of hyperthyroidism,[34] but frank polyarthritis occasionally may be seen in both conditions.

## Autoimmune Diseases

Autoimmune inflammation of the joints is the most important concern for the rheumatologist when evaluating a patient with new-onset polyarthritis. In addition to diseases in which polyarthritis is the dominant manifestation, such as RA and psoriatic arthritis (PsA), consideration must be given to the spondyloarthropathies (SpAs) and to the systemic inflammatory diseases for which polyarthritis may be the first presenting manifestation, including systemic lupus erythematosus (SLE), systemic vasculitis, Sjögren's syndrome, progressive systemic sclerosis, Behçet's disease, sarcoidosis, and others.

Transient syndromes in which prominent polyarthritis is self-limiting but recurrent include palindromic rheumatism[35] and the syndrome of recurrent symmetric seronegative synovitis with peripheral edema (RS3PE syndrome).[36]

Autoinflammatory diseases with high fever, skin rashes, lymphadenopathy, and polyarthritis are more commonly diagnosed in the pediatric population but can on occasion become manifest first in adults. Important considerations in the patient with polyarthritis and fever are adult-onset Still's disease and cryopyrin-associated periodic syndromes including familial Mediterranean fever.

## Drug-Induced Arthritis and Serum Sickness

Classic descriptions of a drug-induced vasculitis or "serum sickness" include polyarthritis, which may variably be inflammatory and oligoarticular or polyarticular.[37] The clinical setting is usually sufficient to make the diagnosis, and the course is self-limiting.

## APPROACH TO THE PATIENT WITH POLYARTHRITIS

### History

Obtaining a thorough history remains a sine qua non for diagnosis, even in this age of high-tech investigations. The "seven dimensions" of the history of present illness as encountered in documentation guidelines—location, quality, quantity, duration, context, modifying factors, and associations—all provide clues to the diagnosis. The location of the symptoms makes it clear whether we are dealing with a monoarticular, oligoarticular, or polyarticular disease, but patients may sometimes focus on just one particularly bothersome joint even though additional joints are inflamed. The quality of the symptoms may provide some information; for example, pain associated with persistent morning stiffness is a notable feature of inflammation, whereas the gel phenomenon—a more short-lived stiffness when first starting to move—is typical of OA. Obviously, it is important to ascertain the severity of the symptoms. Asking the patient about the duration of symptoms, how they first started, how they fluctuate during the day, and what (s)he has done to find relief will come quite naturally. It may be less intuitive, but at least as important, to ask specifically about other symptoms, including fever and weight loss, gastrointestinal symptoms, skin rashes and mucosal lesions, ophthalmologic issues, and urogenital symptoms. The probable cause of the polyarthritis frequently becomes obvious after associated features that the patient often does not believe are important have been elicited by the clinician. Likewise, one must investigate risk factors for infectious causes of polyarthritis, such as possible exposures to infectious organisms, risk of sexually acquired infections, and recent travel to tropical countries or to areas where Lyme disease is endemic. Routinely inquiring about these types of exposures minimizes the risk of missing an important clue. Most practitioners now use a standardized form to collect this information in advance of the patient encounter.

### Physical Examination

Rheumatologists take pride in their ability to detect inflammation by simple clinical means, but they must hone this skill continuously. An inflamed joint is typically swollen, and upon palpation the swelling is soft or doughy rather than hard (as in the osteoarthritic joint). This synovial swelling may be quite obvious in some cases but is harder to establish in others. Even experienced joint assessors will not always agree where to draw the line between swollen and not swollen. In one study, six rheumatologists achieved complete agreement in only about 70% of cases,[38] and other studies have also highlighted the lack of complete agreement between experienced specialists. Fortunately, one such study also showed that specific training improves consistency.[39] Because it may not be in the patient's interest to define joints as swollen in borderline cases, I generally teach my fellows to be restrained in assessing joint swelling. One study suggested that the "squeeze test"—that is, pain on lateral compression of the metacarpophalangeal (MCP) or metatarsophalangeal joints—was more specific than other tests for arthritis, but a recent study found a low sensitivity for this test.[40,40a]

In addition to swelling ("tumor") and tenderness ("dolor"), inflamed joints may exhibit other classic signs of inflammation. Redness ("rubor") is frequently seen over joints that are affected by highly acute inflammation, such as in septic arthritis or gout, but redness is seen only rarely in RA and other autoimmune inflammations. Warmth ("calor") is more confidently assessed in larger than in smaller joints but may be a useful clue, especially in the knees, where the normal temperature gradient—with the knee being slightly cooler than the proximal musculature—may be reversed. Finally, impaired function is an almost universal finding in inflamed joints, and here it is important to distinguish between true limitations in range of motion (contractures) and limitations solely caused by pain.

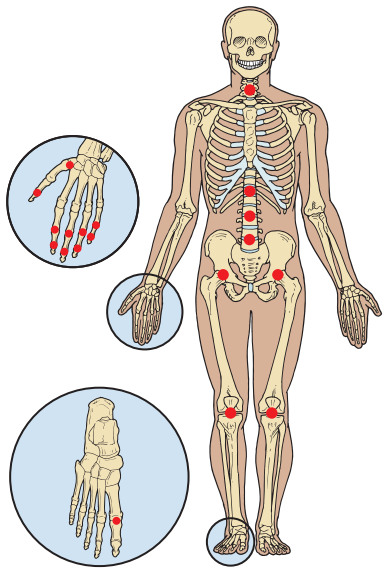
The proper technique for examining joint swelling has been a matter of some discussion. The European League against Rheumatism (EULAR) has published a set of recommendations for examining the joints, along with instructional images; this handbook may serve as a good starting point for learning joint examination or for achieving standardization across different sites. Inflamed large joints often have palpable effusions, and in the knee the signs of "fluid wave" and "ballottement" can be elicited in the case of moderate or large effusions. In the initial workup of the patient with polyarthritis, a detailed examination and documentation of the individual inflamed joints, including those in the feet, will be necessary to document the range of movement (predictably decreased in the inflamed joint) or early anatomic changes. Moreover, when first evaluating a patient, a full joint examination must be performed, whereas for follow-up purposes, a more limited but standardized joint examination is recommended, the most practical one being the 28-joint count, where the MCP and proximal interphalangeal (PIP) joints of both hands, the wrist, elbows, shoulders, and knees are examined.[41] It is good practice to document such a standardized joint examination in the medical record for most visits. The more extensive 44-joint count and the even more comprehensive systematic joint count based on 68 joints are rarely used in practice for long-term follow-up of patients with arthritis.

Perhaps the two most useful pieces of information gained from the physical examination of the affected joints are the nature of the swelling (assuming swelling is present) and the pattern of joint involvement. Synovial inflammation is palpated as a soft, "doughy" swelling around the joint line, obstructing the possibility of feeling the two apposed bony edges. In contrast, hypertrophy emanating from these bony edges, such as occurs in OA, is palpated as a hard structure. Patterns of joint involvement that are easily recognized and usually point at the correct diagnosis are the symmetric involvement of wrists, MCP and PIP joints of the hands, and the corresponding joints in the feet, suggesting RA; the asymmetric involvement of predominantly the lower extremity larger joints in reactive arthritis and SpA; and involvement of multiple distal interphalangeal (DIP) joints in OA but also in some patients with PsA and prominent nail changes. The typical pattern of joint involvement in various conditions is summarized in Table 42-2. The important distinction between RA and OA is further illustrated in Figure 42-1.

**TABLE 42-2** The Typical Distribution of Joint Involvement in Some Commonly Encountered Forms of Polyarthritis

| | Rheumatoid Arthritis | Osteoarthritis | Psoriatic Arthritis | Gout/Pseudogout |
|---|---|---|---|---|
| Large weight-bearing joints | Knees and ankles, usually symmetric; hips only rarely | Hips, knees, ankles | Knees and ankles, usually asymmetric | Knees and ankles; wrists in pseudogout |
| Small joints | MCP and PIP joints in the hands; MTP joints in the feet | DIP, PIP, and first CMC joints in the hands; first MTP joint in the feet | Frequently DIP joints along with nail involvement; other small joints | Small joints of the feet; MCP joints in pseudogout |
| Spine | Cervical spine | Cervical spine and LS spine | LS spine and SI joints | No |

CMC, Carpometacarpal; DIP, distal interphalangeal; LS, lumbo-sacral; MCP, metacarpophalangeal; MTP, metatarsophalangeal; PIP, proximal interphalangeal; SI, sacroiliac.



**Osteoarthritis**

| | |
|---|---|
| Age of onset | Most commonly occurs in individuals older than 50 years |
| Speed of onset | Slow; over years |
| Systemic symptoms | Lack of systemic symptoms |
| Joint symptoms | Joints painful without swelling or with hard, bone swelling<br>may affect joints symmetrically or asymmetrically;<br>affects small joints, large weight-bearing joints such as hips and knees, and/or the spine |
| Stiffness | Short-lived morning stiffness; "gel phenomenon";<br>stiffness when starting movement after inactivity |
| Radiologic findings | Joint-space narrowing, subchondral sclerosis, cystic changes, osteophytes |
| Lab findings | Essentially normal findings; RF, anti-CCP negative |

**Rheumatoid arthritis**

| | |
|---|---|
| Age of onset | Usual age of onset is 40-60 years<br>May begin any time in life |
| Speed of onset | May be sudden or gradual over weeks to months |
| Systemic symptoms | Frequent fatigue, low-grade fever, anorexia, muscle/joint aches, stiffness<br>Extra-articular manifestations; rheumatoid nodules, Sjögren's syndrome |
| Joint symptoms | Joints are painful, swollen and stiff;<br>affects joints symmetrically;<br>primarily affects small joints, but also affects large ones<br>Joints are swollen, warm, tender, and painful |
| Stiffness | Morning stiffness > 45 minutes;<br>may recur after rest |
| Radiologic findings | Bony erosions, soft tissue swelling, angular deformities, periarticular osteopenia, joint-space narrowing |
| Lab findings | Elevated ESR and CRP; normocytic anemia; mild leukocytosis; thrombocytosis; positive RF and/or anti-CCP |

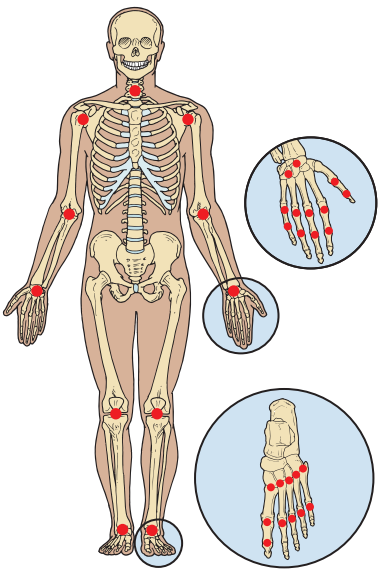


**Figure 42-1** The typical distribution of joint involvement in rheumatoid arthritis and osteoarthritis. Note that hips can be involved in late rheumatoid arthritis as well. CCP, Cyclic citrullinated peptide; CRP, C-reactive protein; DIP, distal interphalangeal; ESR, erythrocyte sedimentation rate; MCP, metacarpophalangeal; PIP, proximal interphalangeal; RF, rheumatoid factor. *(From* http://www.zoomout-ph.com/2012/02/rheumatoid-arthritis-concept-map.html*.)*

The spine must be examined even if no symptoms are reported to detect early signs of axial involvement in SpA.

Deep palpation of the large muscle groups around the shoulders and hips sometimes reveals exquisite tenderness, which, together with the patient's age of 50 years or older and a high erythrocyte sedimentation rate (ESR), might suggest a diagnosis of polymyalgia rheumatica (PMR), in which case some peripheral joint arthritis also may be present.

Furthermore, a general examination should be performed with special attention to the skin and integuments (psoriasis

and nail pitting in PsA), mucous membranes (ulcers in reactive arthritis, Behçet's disease, or SLE), lymph glands, salivary glands, thyroid, heart, lungs, eyes, and other organs based on specific suspicion.

## Laboratory Investigation

After a comprehensive history and physical examination, a laboratory workup will most likely be needed. Although some tests can be ordered "routinely," clearly many tests should be ordered on the basis of the possible differential diagnosis at this stage, at which time most often the possibilities already have been narrowed down considerably.

Inflammation can be reflected by several routine measures, including leukocytosis and neutrophilia, normocytic anemia ("of chronic disease"), and thrombocytosis. The latter in particular may be a strong indicator of systemic inflammation of significant duration and severity. The ESR is typically elevated in patients with inflammation, although it lacks specificity or sensitivity for any single diagnosis, and the same applies to the C-reactive protein (CRP). A normal ESR or CRP argues against infectious causes for the polyarthritis but does not preclude a rheumatologic diagnosis, whereas extremely high ESR or CRP values should trigger suspicion of a more serious disease underlying the polyarthritis.

Serologic testing is one of the rheumatologist's most treasured domains. The demonstration of rheumatoid factors (IgM antibodies binding to IgG; RF) in the 1940s was one of the first indications that RA was an autoimmune disease[42,43]—a concept considered inadmissible for many decades. RFs are indeed commonly seen in RA but are not more than 60% to 70% sensitive, and in the setting of an undiagnosed polyarthritis, the test must be interpreted with caution because several viral infections can cause polyarthritis with a positive RF, including parvovirus and hepatitis B and C. Moreover, RF can be positive in a wide range of rheumatologic diseases—not only RA but also Sjögren's syndrome, scleroderma, SLE, and vasculitis, for example. Nonetheless, in an early undifferentiated arthritis clinic, the presence of RF conferred a considerably increased risk of the development of persistent disease and radiologic damage.[44-47] In other words, although RF is useful in the diagnostic process, it must not be relied upon blindly; however, once the diagnosis of RA is established, the presence of RF suggests a worse prognosis.

More recently, it has become standard to test for antibodies against citrullinated proteins using an assay based on cyclic citrullinated peptides (CCPs). The test is therefore often called the anti-CCP test, but the antibodies that are detected should more properly be called anti-citrullinated protein antibodies (ACPA) because the CCP is a laboratory construct that does not exist in nature. Regardless, anti-CCP or ACPA are about equally sensitive for RA as RF but are much more specific.[48,49] Combined testing for RF and ACPA is the current standard for evaluating a patient with polyarthritis when RA is a possible diagnosis, and the specificity of the combined positivity was indeed 100% in one study,[49] although of course the sensitivity was reduced.

Other serologic tests can be useful in the evaluation of the patient with polyarthritis but must be interpreted with even greater caution. Anti-nuclear antibodies (ANAs) are positive in many autoimmune diseases, as well as in various infections, malignancies, drug-induced conditions, and so on. When ANAs are tested by immunofluorescence on human cell lines, they can be helpful in that a negative test provides a strong argument against a diagnosis of SLE, whereas a positive test will allow further testing for more specific autoantibodies (but does not prove that the patient has any particular disease). ANA by enzyme-linked immunosorbent assay (ELISA) methods may have more false-negative results. Antibodies against extractable nuclear antigens (ENAs) can point in the direction of various systemic inflammatory diseases, whereas anti-Ro/La (SS-A/SS-B) will point to Sjögren's syndrome, and anti-neutrophil cytoplasmic antibodies (ANCA) will point to systemic vasculitis.

Testing for specific infectious causes of polyarthritis can and should also be performed on the basis of clinical suspicion at this stage. Testing for hepatitis virus, HIV, and various other viruses can be considered, but it would be excessive to test for all viruses in all patients. The same can be said for serologic testing for Lyme disease or for enteric or urogenital pathogens.

### Genetic Testing

The HLA-B27 gene marker is found in approximately 3% to 8% of the population, but it is found in more than 90% of patients with ankylosing spondylitis and in 50% to 80% of patients with other SpAs. It has been argued that the HLA-B27 test is not useful because of the prevalence of this gene marker in healthy persons, but this argument does not do justice to the subtle "probabilistic" process of diagnosis; in the right clinical setting, a positive HLA-B27 test can raise the diagnostic likelihood from modest to high. Moreover, the HLA-B27 test only needs to be performed once, making it a rather cost-effective way to obtain an additional diagnostic clue.

Other genetic tests have not yet become useful in rheumatology practice. Determining whether the patient has the "shared epitope" (a constellation of HLA-DR genes that confer an elevated risk for RA) is not yet part of standard care. A genetic test exists for hereditary hemochromatosis, that is, the *HFE* gene; this test can be performed in the right clinical setting.

### Synovial Fluid Analysis

If at all possible, synovial fluid should be obtained and analyzed in the initial workup of polyarthritis. The fluid from an inflamed but aseptic joint typically is yellow and turbid and contains 5000 to 50,000 white cells/$mm^3$, mostly neutrophils. Higher leukocyte counts suggest bacterial infection but may also occur in crystal diseases, whereas lower counts would be expected in degenerative diseases. Crystal arthritis can be definitively diagnosed by demonstrating intra-cellular crystals in the joint aspirate.

## Imaging and Additional Diagnostic Procedures

Although conventional radiography is not often the key to making a diagnosis in the patient with polyarthritis, ordering plain radiographs of some of the affected joints may

nonetheless be useful. Such radiographs may reveal unexpected findings that provides diagnostic clues; in addition, radiographs may provide an indication of the severity of inflammation (juxta-articular osteopenia) and form a baseline for future evaluation, and in some cases, they may establish the diagnosis, such as when typical erosions are found and the diagnosis of RA can be made. Typical "clues" on plain radiographs are summarized in Table 42-3.

Magnetic resonance imaging (MRI) has become an important diagnostic modality in musculoskeletal diseases and has largely replaced computerized tomography, conventional tomography, and scintigraphy in this setting. MRI combines several very favorable attributes: It is noninvasive and carries minimal risk; it offers unparalleled structural detail in soft tissues; and it can be adapted to demonstrate inflammation, such as by T2 weighting, fluid-attenuated inversion recovery (FLAIR) sequencing, and the use of contrast media. The disadvantages of MRI are that it is quite time consuming for the patient and may be associated with physical and psychological discomfort (e.g., lying still in an uncomfortable position and claustrophobia); the images it provides are not as good for bone as for other tissues; only a relatively limited region can be imaged at one time; and both acquisition and pass-through costs are very high. Some of these disadvantages are lessened for low-magnetic-strength "office" MRI.[50] With use of a device that can be operated easily in a general practice setting, images of reasonable quality can be obtained and at a lower cost. However, the region that can be imaged is small, and image acquisition time is significantly longer. Based on all these considerations, when initially evaluating a patient with polyarthritis, the use of MRI will only rarely be indicated.

Use of musculoskeletal ultrasound examination (MSUS) has been increasing rapidly in the past several years. MSUS combines several attractive features: It is easily accommodated in the practice setting and even adds to the patient-physician interaction, images of good quality can be obtained and evaluated in real time based on the clinical situation, inflammation is readily detected with use of Doppler technology, and the costs are surmountable. Inflammation is easily identified using MSUS, as demonstrated in Figure 42-2. Distinct disadvantages of MSUS are that it requires an experienced physician or physician assistant to perform the procedure, it remains rather more subjective compared with MRI, and soft tissue structures can be "hidden" from view by overlying bone. Nonetheless, MSUS has increasingly become a valuable tool in rheumatology practice. Sensitivity and specificity have been shown to be similar to MRI.[51-53] Moreover, when assessing patients with polyarthritis, MSUS has proven useful to increase the diagnostic certainty on the part of the rheumatologist, both in terms of establishing that polyarthritis is truly present[54] in cases where there may be doubt and in establishing a more definitive diagnosis.[55]

Fluorescence optical imaging (FOI; "Rheumascan") is approved in Europe for evaluating arthritis in the hands. The technique is based on the intravenous injection of a fluorochrome, after which images of the hands are taken in rapid succession with a specially adapted camera. FOI has been used at some European centers, and several recent studies have demonstrated that it has similar sensitivity and

**TABLE 42-3** Clues to Diagnosis on Plain Film Radiographs

| Finding | Disease Suggested |
|---|---|
| Juxta-articular osteopenia | Early RA |
| Joint-space narrowing | RA, PsA, or OA |
| Subchondral sclerosis | OA |
| Eburnation | OA |
| Entheseal calcifications | PsA, SpA |
| Bony erosions | RA, gout |
| Osteophytes | OA |
| Chondrocalcinosis | Pseudogout |

OA, Osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthropathy.

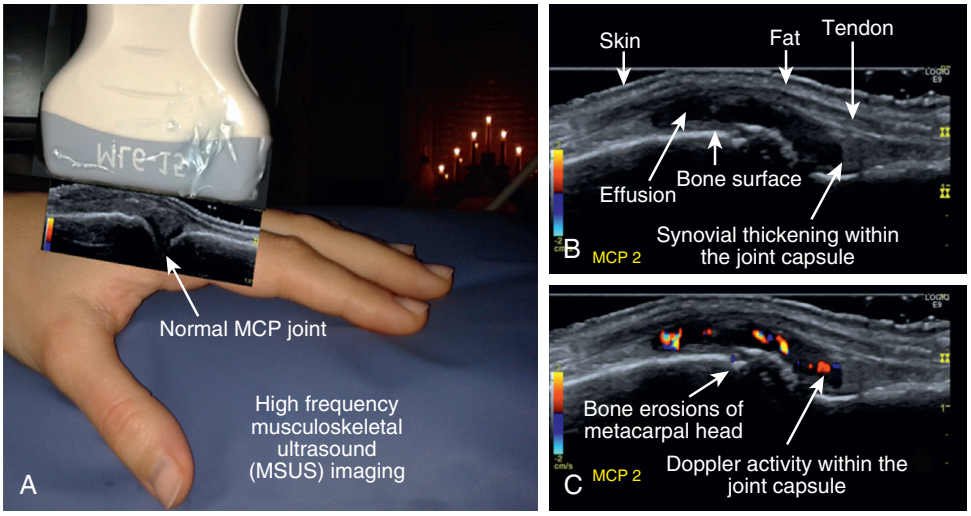



**Figure 42-2** Musculoskeletal ultrasound examination can complement the clinical examination. **A,** A normal metacarpophalangeal (MCP) joint. **B,** Synovial thickening in "gray scale" mode and an effusion. **C,** A distinct Doppler signal in the synovium, indicating inflammation. *(Original photographs courtesy Mr. Yogan Kisten, the Karolinska Institute.)*

specificity to MSUS.[56-59] However, its use is limited to the hands, and the injection can in rare instances cause allergic reactions. The device is not yet approved in the United States.

## FORMAL CRITERIA AND THEIR ROLE IN CLINICAL DIAGNOSIS

"Classification criteria" have been developed for many rheumatologic diseases, perhaps most notably for RA. These criteria were developed originally to achieve uniformity between clinicians in different health care settings, regions, or countries, and primarily so for epidemiologic or other research purposes. They were explicitly not developed as diagnostic criteria. Nonetheless, the very existence of these criteria has led to changes in the way RA and other rheumatologic diseases are perceived, and many clinicians do, in fact, rely on the classification criteria for making clinical diagnoses. The most recent classification criteria for RA, which were developed internationally under the auspices of the American College of Rheumatology (ACR) and the EULAR, were quite significantly different from previous versions[60] (Table 42-4). First, it was recognized that if a radiograph showed incontrovertible evidence for RA, then the diagnosis could be made and no other evidence was needed. Unfortunately, the criteria did not make it entirely clear exactly how certain the radiographic evidence would have to be, and most radiologists agree that there can be considerable variations in the interpretations of radiographic findings.

When radiographic evidence is lacking, the classification of RA is based on a system of points, where the number and nature of the inflamed joints, combined with several other characteristics, determines whether the patient "has" or "does not have" RA. It remains critically important to emphasize that these criteria were benchmarked on the opinions of expert clinicians and were shown to have sensitivity and specificity in the 90% range, indicating that in 1 in 10 patients, an experienced clinician will disagree with the criteria. This is not to belittle the importance of these criteria but to underscore that their use should not replace the clinical judgment of a competent specialist.

Similar considerations apply to other rheumatologic diagnoses, with the added caveat that classification criteria for diseases such as PsA,[61] ankylosing spondylitis,[62] and gout[63] usually achieve sensitivity and specificity lower than that for RA.

**TABLE 42-4** The 2010 American College of Rheumatology/European League against Rheumatism Classification Criteria for Rheumatoid Arthritis*

| Criteria | Score |
|---|---|
| Joint involvement | |
| 2-10 large joints | 1 |
| 1-3 small joints (with or without involvement of large joints) | 2 |
| 4-10 small joints (with or without involvement of large joints) | 3 |
| >10 joints (with at least 1 small joint) | 5 |
| Serology (at least 1 test result is needed for classification) | |
| Negative RF and negative ACPA | 0 |
| Low-positive RF or low-positive ACPA | 2 |
| High-positive RF or high-positive ACPA | 3 |
| Acute-phase reactants | |
| Normal CRP and normal ESR | 0 |
| Abnormal CRP or abnormal ESR | 1 |
| Duration of symptoms | |
| <6 wk | 0 |
| ≥6 wk | 1 |

*Target population (patients who can be evaluated using these criteria): those who have *at least one joint with definite clinical synovitis* (swelling), with the synovitis not better explained by another disease.

- A score of 6 or greater is needed for classification of a patient as having definite rheumatoid arthritis.
- If incontrovertible radiographic evidence of rheumatoid arthritis exists, the diagnosis can be made even if the criteria provided are not fulfilled.
- If a patient has previously fulfilled the criteria for rheumatoid arthritis, the diagnosis is maintained even if the criteria are not fulfilled on current re-examination.

ACPA, Anti-citrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

## PRELIMINARY DIAGNOSES, WORKING DIAGNOSES, PRESUMPTIVE TREATMENTS, AND REASSESSMENTS: A LONGITUDINAL VIEW

Even after applying all possible diagnostic acumen and each and every available test, the diagnosis may not be entirely clear. All clinicians are familiar with the fact that medical diagnostics remains, to some extent, a probabilistic venture. Nonetheless, there comes a point where a preliminary diagnosis must be made, the patient must be informed, and a course of treatment must be chosen. This situation poses some challenges in terms of patient-physician communication. Understandably, clinicians may not want to appear uncertain when speaking with their patients, but nonetheless, it may be better to be frank and say something to the effect that "I think it is likely that you have X, but we cannot be certain. There are no further tests right now to be done, and I propose that we start treatment with Y. We will reassess in a few weeks' or months' time."

Most important, a time point for reassessment must be chosen. This point could be a few weeks or several months into the future, depending on the situation, but by explicitly marking this point, the patient will have greater acceptance if a preliminary diagnosis must be revised.

## DIFFERENTIAL DIAGNOSIS OF POLYARTHRITIS: FUTURE PERSPECTIVES

Modern rheumatologic diagnostics, applied in the setting of a clinic with experienced specialists, will achieve a final diagnosis in 60% to 90% of patients presenting with new-onset polyarthritis.[64,65] It does seem likely that further advances can be made in diagnosing specific rheumatologic disorders earlier, especially RA; the interest in this possibility is based not only on the desire to achieve an expeditious diagnosis but also on the potential for very early intervention, taking advantage of a presumed "window of opportunity" and thereby achieving better long-term results for the patient.[66] However, if this earlier diagnosis is to be achieved,

it may well have to done in the pre-clinical phase, before the patient actually has polyarthritis.

In summary, for the patient with polyarthritis, a diagnosis often can be made rapidly and confidently. Additional refinements in tools for diagnosing polyarthritis will most likely be forthcoming, and when combined with better access to medical specialty care for persons with serious musculoskeletal symptoms, excellent results will increasingly be achieved.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 43

# Skin and Rheumatic Diseases



Lela A. Lee • Victoria P. Werth

**KEY POINTS**

- The skin is often affected in rheumatic diseases, and many patients present with skin findings.
- Accurate diagnosis of cutaneous lesions requires knowledge of the differential diagnosis, understanding of when and when not to obtain additional studies (e.g., biopsy), and ability to interpret the results in the context of the clinical presentation.
- Skin biopsy findings of inflammatory conditions are often not definitively diagnostic.

## DIAGNOSIS OF SKIN LESIONS ASSOCIATED WITH RHEUMATIC DISEASES

The skin is a highly visible organ that is frequently affected in rheumatic diseases, and the presence of skin lesions may be helpful diagnostically. Certain caveats are worth noting before discussing the specifics. First, a major pitfall for the nondermatologist in evaluating skin lesions is incomplete knowledge of the entities in the differential diagnosis. For example, malar erythema occurs frequently in patients with systemic lupus erythematosus (SLE), but the differential diagnosis for malar erythema is rather extensive and includes conditions that are much more prevalent than lupus (e.g., rosacea), as well as conditions that are far less prevalent (e.g., Rothmund-Thomson syndrome). Patients frequently have more than one skin condition, which often makes diagnosis more challenging.

Another caveat for the nondermatologist concerns skin biopsy. The ability to determine when a skin biopsy might be diagnostically useful in many cases requires a great deal of specialized knowledge, as does the interpretation of pathology reports. It is often the case with inflammatory skin conditions that the microscopic findings are actually less diagnostically specific than the clinical examination. Unfortunately, it is common for a pathology report to list a diagnosis with no context to help the clinician understand how definitive the findings were. For example, a skin biopsy report may list psoriasis as the final diagnosis, but, depending on the specific case, the unstated additional possibilities may include nummular dermatitis, atopic dermatitis, seborrheic dermatitis, lichen simplex chronicus, dermatophytosis, or drug eruption. Particularly with regard to inflammatory skin conditions, a working knowledge of both dermatopathology and dermatology and placement of the histologic findings in the context of the clinical presentation may be necessary for the clinician to arrive at the correct diagnosis.

The above considerations notwithstanding, it is useful for the physician caring for patients with rheumatic diseases to be well versed in their cutaneous manifestations. In this chapter, we provide an overview of these manifestations, as well as a perspective on diagnosis and differential diagnosis. Therapy is discussed briefly in conditions in which treatment may be specifically directed toward the skin lesions. Etiology and pathogenesis of these diseases are covered elsewhere in this text.

## PSORIASIS

**KEY POINTS**

- The major phenotypes of psoriasis are chronic plaque, guttate, localized pustular, generalized pustular, and erythroderma. Chronic plaque and guttate are the most common phenotypes.
- Guttate psoriasis may occur a few weeks after a streptococcal infection.
- Nail changes are common but are not specific for psoriasis.
- Systemic corticosteroids should be avoided in psoriasis, if possible, because severe flaring may occur upon tapering.
- Skin findings of reactive arthritis include circinate balanitis, keratoderma blenorrhagica, oral mucosal erosions, and psoriasiform plaques.
- Skin biopsy cannot distinguish between psoriasis and reactive arthritis.

Psoriasis is one of the most common inflammatory skin diseases, affecting approximately 2% of the general population. There is a wide range of severity of skin lesions, from a few relatively asymptomatic plaques to extensive, disabling disease. The onset may be at any time during life. Once present, it may exhibit exacerbations and remissions, but it does not tend to resolve permanently. In general, onset in childhood portends more severe disease.

Skin lesions of psoriasis characteristically are sharply demarcated plaques with silvery scale and underlying erythema, although there may be a paucity of scale if the lesions have been partially treated or if they occur in

intertriginous areas. When the scale is removed, pinpoint bleeding may be observed (Auspitz sign). Lesions may occur in areas of trauma (Koebner phenomenon) such as in surgical scars. In some cases lesions contain small pustules.

General phenotypes of psoriasis are chronic plaque, guttate, localized pustular, generalized pustular, and erythroderma.[1] Chronic plaque and guttate psoriasis are the most common, and generalized pustular psoriasis and erythroderma are typically the most disabling and even life threatening. Chronic plaque psoriasis lesions are often relatively large in diameter and occur preferentially on elbows, knees, scalp, genitalia, lower back, and the gluteal cleft, although they may occur in many other locations. It is quite common for only one area of skin, such as the scalp, to be affected. Guttate lesions are relatively small in diameter and usually quite numerous, distributed preferentially on the trunk and proximal extremities (Figure 43-1). Guttate psoriasis occurs relatively commonly in children and young adults, often manifesting a few weeks after a streptococcal infection.

Nail changes are common, occurring in approximately half of patients, and are often mistaken for fungal infection. Findings include pitting, onycholysis ("oil spots"), dystrophy of nails, and loss of the nail plate. These changes are not specific for psoriasis. Notably, pitting may occur as a result of trauma. Nail changes are more frequent in patients with arthritis of the distal interphalangeal joints.[2]




**Figure 43-1** **A**, Guttate psoriasis resembles "drops" of discrete scaly papules with erythema, often on the trunk. **B,** Plaque psoriasis with thick micaceous scale on the thighs. (***A**, Courtesy Dr. Nicole Rogers, Tulane University School of Medicine, New Orleans; **B**, courtesy Dr. Abby Van Voorhees, University of Pennsylvania).*

Arthritis occurs more often in patients with severe cutaneous disease, but cutaneous disease need not be present at all. Remissions and exacerbations of arthritis do not correlate well with remissions and exacerbations of skin disease. The presence of psoriatic skin lesions may be helpful in supporting a diagnosis of psoriatic arthritis, although many patients with psoriasis have joint disease unrelated to psoriasis.

The physician usually makes the diagnosis of psoriatic skin disease on clinical grounds alone, largely on the basis of the morphology and distribution of lesions. The differential diagnosis may be extensive and includes in selected cases nummular eczema, seborrheic dermatitis, candidiasis (in intertriginous areas), pityriasis rubra pilaris, Bowen's disease or Paget's disease (for isolated plaques), drug eruption, pityriasis rosea, pityriasis lichenoides, dermatophytosis, lichen planus, secondary syphilis, parapsoriasis, cutaneous lupus (especially subacute cutaneous lupus erythematosus [SCLE]), and dermatomyositis. In cases in which the diagnosis is not clear cut, biopsy may be helpful. The histologic findings may range from virtually diagnostic for psoriasis to merely consistent with but not diagnostic. Histologically, psoriasis cannot generally be distinguished from the skin lesions seen in reactive arthritis.

Common topical therapies include corticosteroids, tar, anthralin, calcipotriene, and tazarotene.[3] Phototherapy with sunlight, narrow-band ultraviolet B (UVB), or psoralen ultraviolet A (PUVA)-range is a mainstay of therapy for many patients. Common systemic therapies include methotrexate, acitretin, and biologic agents targeting tumor necrosis factor (TNF) or interleukin (IL)-12/23. Cyclosporine may be useful to attain relatively rapid control of severe psoriasis, but it is less often used as a long-term therapy. Although topical corticosteroids are an acceptable treatment for many patients, systemic corticosteroids are avoided for the treatment of cutaneous disease, in particular because of the observation of severe flaring of psoriasis following withdrawal of systemic corticosteroids.

## REACTIVE ARTHRITIS

Circinate balanitis is the most common of the characteristic mucocutaneous lesions of reactive arthritis.[4] Small erythematous papules and pustules coalesce to form serpiginous erosive or crusted plaques on the glans penis. In uncircumcised men, the appearance is more often that of erosion rather than crust because the moisture and trauma minimize the formation of crust. In circumcised men, crusting may be more obvious than erosion.

Lesions that are initially similar to the small erythematous papules and pustules of the genital region may develop on the palms and, particularly, the soles of the patient. With time, these lesions, termed *keratoderma blenorrhagica*, tend to become markedly hyperkeratotic (Figure 43-2). They may coalesce into large plaques or generalized hyperkeratosis involving the entire plantar surface, or they may remain discrete, erythematous, hyperkeratotic papules a few millimeters in diameter.



**Figure 43-2** Reactive arthritis with keratoderma blennorrhagica of the feet.

Erythematous, scaly plaques indistinguishable from psoriasis may appear elsewhere on the skin, including the scalp, elbows, and knees. When lesions occur around the nails, it is common for there to be hyperkeratosis underneath the nails. Pitting is not typical of reactive arthritis, but thickening, ridging, or shedding of the nail plate may occur. Erosions of the oral mucosa are relatively common on the tongue, buccal mucosa, and palate.

The diagnosis of the cutaneous lesions is usually made on a clinical basis. Skin biopsy may be helpful in excluding many entities in the differential diagnosis, but in general, it cannot exclude psoriasis. Unfortunately, the major condition in the differential diagnosis of the skin lesions is usually psoriasis. One somewhat distinguishing histologic feature is that the older lesions of keratoderma blenorrhagica may have a considerably thickened stratum corneum, corresponding to the markedly hyperkeratotic papules seen grossly.

For the genital lesions, conditions to consider in the differential diagnosis may include candidiasis, psoriasis, dermatitis, Bowen's disease, Paget's disease, squamous cell carcinoma, Zoon's balanitis, erosive lichen planus, lichen sclerosus (balanitis xerotica obliterans), aphthosis, fixed drug eruption, and certain infectious diseases. The differential diagnosis for lesions on the soles and palms may include psoriasis, hereditary or acquired hyperkeratosis of the palms and soles, pustular eruption of the palms and soles, pompholyx, scabies, and dermatophytosis. The differential diagnosis for oral lesions may include geographic tongue, lichen planus, candidiasis, aphthae, and autoimmune bullous diseases.

The approach to treatment of skin lesions is similar to that for psoriasis, particularly in cases in which the lesions are persistent. Choice of topical therapies may be somewhat limited because of the sites involved. The oral mucosa is a difficult site to deliver medication topically, and the genital area may develop irritant reactions to certain topical medications. Often, topical corticosteroids are preferred for both areas because of low potential for irritation and the availability of topical preparations designed for these sites. In the genital area, suprainfection with *Candida* may occur, and concurrent therapy with a topical or systemic anticandidal medication may be necessary on occasion.

## RHEUMATOID ARTHRITIS

**KEY POINTS**

- **Skin findings associated with RA are characteristically granulomatous or neutrophilic.**
- **The major granulomatous cutaneous finding is the rheumatoid nodule. The major neutrophilic conditions are vasculitis, Sweet's syndrome, and pyoderma gangrenosum.**
- **Sweet's syndrome and pyoderma gangrenosum are part of a group of noninfectious inflammatory conditions called *neutrophilic dermatoses*. These conditions may be associated with internal diseases, including rheumatic diseases.**
- **Interstitial granulomatous dermatitis (IGD) and palisaded neutrophilic and granulomatous dermatitis (PNGD) are rare conditions that may be associated with RA or other rheumatic diseases. It is not clear whether IGD and PNGD are distinct entities or variants of the same condition.**
- **The cutaneous eruptions of juvenile idiopathic arthritis and adult-onset Still's may be similar to viral exanthems or drug eruptions, but they are distinctive in that they clear completely between episodes.**

The major skin manifestations associated with rheumatoid arthritis (RA) generally fall under granulomatous lesions, exemplified by the rheumatoid nodule, and neutrophilic lesions, exemplified by vasculitis and pyoderma gangrenosum.

Rheumatoid nodules are the most common cutaneous manifestations of RA.[5] They occur more often in seropositive patients and correlate somewhat with higher rheumatoid factor titers, more severe arthritis, and increased risk for vasculitis. Nodules are usually relatively deep, firm, and painless and tend to develop over areas of pressure and trauma, such as the extensor forearms, fingers, olecranon processes, ischial tuberosities, sacrum, knees, heels, and posterior scalp (Figure 43-3). In patients who wear glasses, nodules may develop under the bridge or nosepieces. In most cases, rheumatoid nodules are in the subcutaneous tissue and/or deep dermis, but occasionally they may occur more deeply or more superficially.

Clinically, depending on the presentation, numerous entities may be in the differential diagnosis, including infections, inflammatory disorders, and benign tumors. If needed, biopsy of a nodule may be quite helpful in establishing the diagnosis. Rheumatoid nodules exhibit a distinctive histologic finding called necrobiosis, a fibrinoid degeneration of the connective tissue, surrounded by palisaded histiocytes. Necrobiosis is also a characteristic feature of granuloma annulare and necrobiosis lipoidica diabeticorum. Although necrobiosis lipoidica diabeticorum is easily distinguished from rheumatoid nodule clinically, the subcutaneous variant of granuloma annulare may be difficult to distinguish, both clinically and histologically.



**Figure 43-3** Rheumatoid nodule over the extensor tendon of the distal interphalangeal joint.

The term *rheumatoid nodulosis* has been used to describe an entity characterized by subcutaneous rheumatoid nodules, cystic bone lesions, rheumatoid factor positivity, and arthralgias in patients with little or no evidence of systemic manifestations of RA or erosive joint disease.[6] Older males are preferentially affected.

The development of nodules in RA patients undergoing treatment with methotrexate has been noted by several observers and termed *accelerated rheumatoid nodulosis*.[7] The nodules are newly appearing and occur preferentially on the hands. There are also case reports of the phenomenon in RA patients treated with etanercept.

The other major type of cutaneous lesion associated with RA is neutrophil predominant. Rheumatoid vasculitis occurs more frequently in patients who are seropositive and have rheumatoid nodules, and it often occurs relatively late in the course of the disease.[8] Vessels of any size may be affected. In the skin, vasculitis may appear as purpuric papules and macules, nodules, ulcerations, or infarcts. Bywaters lesions are periungual or digital pulp purpuric papules that represent a small vessel vasculitis but are not necessarily associated with vasculitic lesions elsewhere.

The differential diagnosis of purpuric or petechial lesions may include stasis dermatitis, Schamberg's purpura, platelet dysfunction, petechial drug eruptions, viral exanthems, emboli, thromboses, and sludging. Of these, Schamberg's purpura, a relatively common condition unassociated with systemic disease, is probably the most frequently confused with small vessel vasculitis. Skin biopsy may be helpful in establishing the diagnosis of vasculitis, particularly if an early lesion is sampled, although rheumatoid vasculitis cannot be distinguished histologically from many other causes of small vessel vasculitis. Immunofluorescent examination of an early lesion may be helpful in ruling out immunoglobulin (Ig)A-predominant vasculitis. The differential diagnosis of ulcers and infarcts is extensive. Biopsy is often unrewarding because nonspecific changes present in established lesions may make interpretation difficult, but on occasion biopsy of ulcers or infarcts may result in a definitive diagnosis of vasculitis.

The neutrophilic dermatoses are a group of diseases inflammatory rather than infectious in origin, typified by pyoderma gangrenosum and Sweet's syndrome. These conditions have been associated with a variety of extracutaneous diseases, including RA. The classic pyoderma gangrenosum lesion is a rapidly appearing, large, destructive ulcer in which the border is undermined. The classic lesion of Sweet's syndrome is an erythematous, edematous plaque with a surface often described as mammillated, pseudovesicular, or microvesicular. Clinical appearances intermediate between these two have been described. For pyoderma gangrenosum, the differential diagnosis is usually that of conditions causing leg ulcer, and the diagnosis is mainly clinical, with biopsy primarily serving to exclude some of the other entities under consideration. For Sweet's syndrome, the differential diagnosis may include infections, halogenoderma, and other neutrophilic dermatoses. Biopsy often provides helpful supporting evidence. The mainstay of therapy for acute lesions of both conditions is systemic corticosteroids. For more persistent lesions, a variety of options may be considered, cyclosporine and infliximab being two of the more common. Colchicine or potassium iodide may be first-line therapies in patients with infections or contraindications to corticosteroids.

The term *rheumatoid neutrophilic dermatitis* has been given to describe chronic, erythematous, urticarial-like plaques that occur primarily on the distal arms.[9] Clinically and histologically, rheumatoid neutrophilic dermatitis is similar to Sweet's syndrome and may be a variant of it.

Palisaded neutrophilic and granulomatous dermatitis (PNGD) of connective tissue disease is an unusual condition or set of conditions for which consistent terminology is still evolving. As the name implies, the major bases for diagnosis of this entity are the histologic appearance and the occurrence in a patient with connective tissue disease, often RA.[10] The clinical appearance ranges from erythematous or flesh-colored papules that appear primarily on fingers and elbows to erythematous or flesh-colored linear cords on the trunk. Some authors classify the latter as interstitial granulomatous dermatitis with cutaneous cords or interstitial granulomatous dermatitis with arthritis (IGDA). Treatment of PNGD and IGDA can be challenging. PNGD may respond to dapsone or sulfapyridine. IGDA can be treated with anti-malarials or immunosuppressives, but evidence is based on case reports and small case series. Patients can progress to a severe deforming arthritis. In some cases, granuloma annulare and rheumatoid nodule may be in the differential diagnosis.

## JUVENILE RHEUMATOID ARTHRITIS/STILL'S DISEASE

The majority of patients with classic Still's disease manifest an exanthematous eruption coincident with daily fever spikes.[11] The lesions are evanescent, usually nonpruritic, erythematous macules occurring over the trunk, extremities, and face. The differential diagnosis includes viral exanthem, drug eruption, familial periodic fever syndromes, and rheumatic fever. It is not unusual for exanthems of any type to be more prominent during fevers, but it is not expected that viral exanthems and drug eruptions will clear completely between fever spikes. However, it should be noted that the eruption of erythema infectiosum (fifth disease) due to parvovirus B19 may resolve completely but reappear when the skin temperature rises, as with warm baths or

exercise. Adult-onset Still's disease is also typified by an evanescent erythematous, sometimes salmon-colored, eruption over the trunk and extremities, associated with high fever. Skin biopsy may be nonspecific. Some patients with adult-onset Still's disease have more persistent, pruritic lesions. The chronic lesions have been described as hyperpigmented plaques with a linear and rippled morphology. These lesions may exhibit a distinctive histology consisting of dyskeratotic keratinocytes in the upper epidermis, along with increased dermal mucin.[12]

Subcutaneous nodules may develop in both juvenile-onset and adult-onset Still's disease. The lesions tend to occur at the same sites of the body as do rheumatoid nodules in RA, but histologically they appear similar to nodules of rheumatic fever.

## LUPUS ERYTHEMATOSUS

| KEY POINTS |
|---|
| Patients with lupus erythematosus can have a wide variety of lupus-specific and lupus-nonspecific skin lesions. |
| Nonspecific skin lesions are more frequently seen in patients who have systemic lupus erythematosus or overlap syndromes. |
| Acute cutaneous lupus erythematosus is associated with a high risk for significant systemic disease. |
| Patients who present with other lupus-specific skin lesions have a low risk for moderate or severe systemic disease. |
| Subacute cutaneous lupus erythematosus is associated with antibodies to Ro/SSA and may sometimes be drug induced. |
| Neonatal lupus is associated with maternal IgG autoantibodies to Ro/SSA. Clinical findings may include cutaneous lesions, cardiac disease (usually complete heart block), hepatobiliary disease, or hematologic cytopenias. |
| The major skin findings in patients with Sjögren's syndrome consist of dryness and sequelae of dryness of the skin and mucous membranes. Vasculitis is also a relatively common finding. |
| Some patients with Sjögren's syndrome have subacute cutaneous lupus lesions or an annular erythematous eruption. |

The skin is involved at some time in the course of disease in the majority of patients with lupus erythematosus (LE), and skin lesions may be important in establishing the diagnosis. Some skin lesions are highly likely to be associated with "systemic" (i.e., extracutaneous) disease, whereas others may or may not be associated with extracutaneous disease. The phenomenon of lupus skin lesions occurring in the absence of systemic disease has previously been termed *discoid lupus* by some. However, dermatologists use this term to denote a specific type of skin lesion, regardless of presence or absence of systemic disease. It is the latter meaning of discoid lupus to which we refer in this chapter.

### Lupus-Specific Skin Lesions

James Gilliam classified cutaneous lesions as specific or nonspecific for lupus, with discoid lupus lesions an example of the former and palpable purpura an example of the latter.[13] Although this division of lesions is useful, sometimes a lupus-specific lesion occurs in a patient who has a primary autoimmune disease other than LE. For example, SCLE lesions may occur in patients whose primary condition is Sjögren's syndrome, and discoid lesions may be seen in a variety of conditions, such as mixed connective tissue disease. Many of the lupus-specific skin lesions can occur in patients who have no evidence of extracutaneous disease.

The characteristic morphologies of the various lupus-specific skin lesions are largely a function of the depth and intensity of the inflammatory infiltrate, presence or absence of epidermal basal cell damage, involvement of hair follicles, abundance of dermal mucin, and tendency to scar. In practice, there may be some overlap of these features, and there may be more than one type of lesion present in a given patient, which makes classification difficult. Because therapy for most of the lupus-specific lesions is similar, it is not always important to distinguish among the various types of lesions. However, it can be useful to identify conditions that are more likely to scar, to target more aggressive therapy, and to identify conditions that are highly likely or highly unlikely to be associated with systemic disease.

Acute cutaneous lupus (ACLE) lesions are typified by malar erythema, the classic butterfly rash (Figure 43-4). The inflammation tends to be superficial, with little propensity to scar. Precipitation or exacerbation of lesions by sun exposure is common, and lesions tend to be distributed on the sun-exposed face, neck, extensor arms, and dorsal hands, where the skin over the knuckles is relatively spared. Often the lesions are quite transient, but they may be persistent. When the face is severely affected, facial edema may be prominent. Oral lesions are often present concurrently. Acute eruptions with considerable focal basal cell damage can result in erythematous papules with dusky centers that clinically mimic erythema multiforme. The major importance of recognition of ACLE is its strong association with systemic disease. The differential diagnosis of malar rash may include several conditions. In some cases, the facial rash of ACLE may be difficult to distinguish from rosacea. Seborrheic dermatitis, atopic dermatitis, and photosensitive

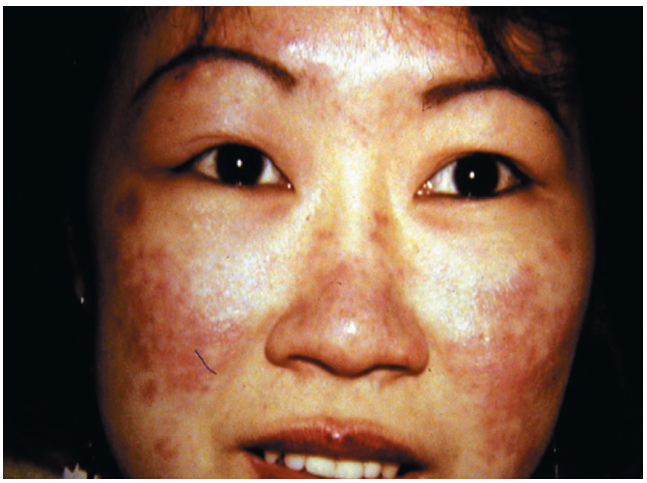


**Figure 43-4** Acute malar rash in a butterfly distribution in systemic lupus erythematosus.

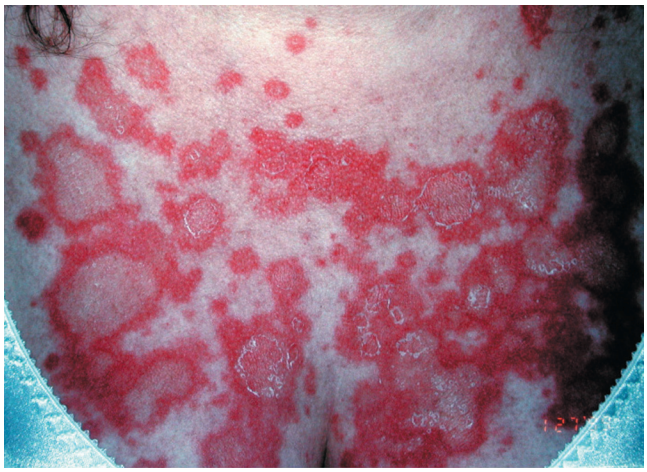


**Figure 43-5** Subacute cutaneous lupus erythematosus: annular-polycyclic type.

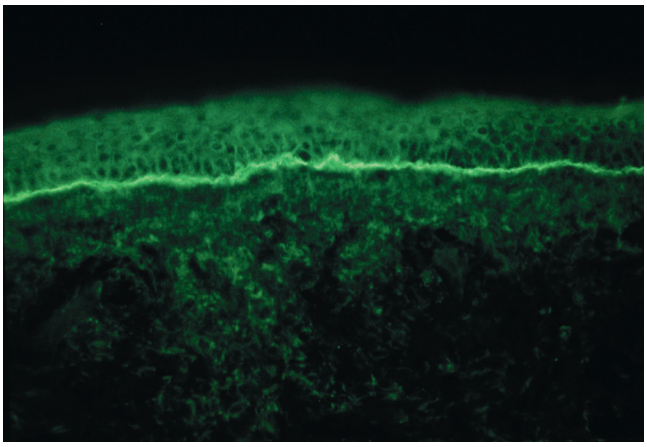


**Figure 43-6** Direct immunofluorescence (lupus band test) demonstrating IgG deposits at the dermal-epidermal junction.

eruptions, such as polymorphous light eruption and drug-induced photosensitivity, may also be considered. Dermatomyositis (DM) may cause a photosensitive facial erythema with edema similar to ACLE, although the erythema tends to be more violaceous. Persistent lesions on the neck and arms may be indistinguishable from SCLE. Discoid lupus lesions occasionally appear in a butterfly distribution, where they can result in disfiguring scarring. Skin biopsy is usually not performed on malar erythema because of its transient character, the scar resulting from biopsy, and the availability of other means of establishing the diagnosis of SLE. If a biopsy is done, it should be noted that DM and SCLE cannot be distinguished from ACLE by histology, and, also, that skin biopsy findings are sometimes nonspecific.

SCLE is a photosensitive eruption usually associated with anti-Ro/SSA autoantibodies.[14] Lesional morphology is of two main types: annular erythematous plaques and scaly erythematous psoriatic plaques. Lesions are distributed over sun-exposed skin of the arms, upper trunk, neck, and sides of the face (Figure 43-5). Inexplicably, the midfacial area is usually uninvolved. Fair-skinned individuals are preferentially affected. Lesions may resolve with hypopigmentation or even depigmentation, but they rarely scar. Several drugs, particularly hydrochlorothiazide and terbinafine, have been reported to induce SCLE.[15] The risk for systemic disease is not fully known, but perhaps 15% of patients with SCLE have or will develop significant systemic disease, often SLE, Sjögren's syndrome, or an overlap of the two disorders. Depending on the morphology of the lesions and the clinical presentation, the differential diagnosis may include psoriasis, tinea, polymorphous light eruption, reactive erythema, and erythema multiforme. Skin biopsy for routine histology is often helpful in establishing the diagnosis. The characteristic finding of skin biopsy for immunofluorescence is a particulate deposition of IgG in the epidermis (Figure 43-6), both in lesions and uninvolved skin.[16] This pattern can be reproduced in animal models by infusing anti-Ro, and thus immunofluorescence results provide information that duplicates serologic testing for anti-Ro.[17] The particulate epidermal pattern seen in normal skin does not indicate an increased risk for SLE. However, increased risk is indicated by the finding of granular deposits of IgG at the dermal-epidermal junction of normal skin (the nonlesional lupus band test). It should be noted that many immunofluorescence laboratories do not routinely report particulate epidermal staining.



**Figure 43-7** Discoid lupus erythematosus of the scalp, with scarring alopecia and central hypopigmentation. *(Courtesy Dr. Nicole Rogers, Tulane University School of Medicine, New Orleans.)*

Discoid lupus erythematosus (DLE) lesions are the most common of the persistent lupus-specific skin lesions. Population-based studies indicate that cutaneous lupus, to include DLE and SCLE, has an incidence similar to that of SLE.[18,19] Active DLE lesions are erythematous papules and plaques that, when palpated, feel indurated because of the substantial numbers of inflammatory cells that infiltrate the dermis. Involvement of hair follicles may be grossly evident as follicular plugs and scarring alopecia. Dyspigmentation is common, often with hypopigmentation or even depigmentation in the center and hyperpigmentation at the periphery (Figure 43-7). Visible scale is common and occasionally is pronounced in a clinical variant called *hypertrophic DLE*. In established lesions, scarring may be disfiguring. Lesions tend to occur on the scalp, ears, and face but may be widespread and occasionally involve mucosal surfaces. It is unusual to have lesions below the neck in the absence of lesions above

the neck. Sun exposure may exacerbate DLE in some cases, but the presence of lesions in sun-protected areas of the scalp and ears and the frequent absence of a history of photosensitivity indicates that sun exposure is probably not a trigger in every instance. There are case reports of squamous cell carcinoma developing in patients with established DLE lesions. In a patient who presents with DLE lesions only, the risk for development of moderate to severe systemic symptoms is probably approximately 5% to 10%, although mild systemic symptoms such as arthralgias are relatively common. As many as 15% to 20% may meet the criteria for SLE, but many do so solely on the basis of mucocutaneous findings.[20] The differential diagnosis of DLE lesions is often that of conditions that exhibit intense lymphocytic or granulomatous infiltrates such as sarcoid, Jessner's lymphocytic infiltrate, granuloma faciale, polymorphous light eruption, lymphocytoma cutis, and lymphoma cutis. In the scalp, lichen planopilaris and other scarring alopecias may be considered. Skin biopsy for routine histology often establishes the diagnosis definitively. In more difficult cases, biopsy for immunofluorescence may provide additional supporting diagnostic information. Lesions are expected to have granular deposits of Igs at the dermal-epidermal junction. Unless there is concomitant systemic disease, normal skin is expected not to have Ig deposits.

Tumid lupus (TLE) skin lesions are similar to DLE lesions in that they are erythematous indurated papules and plaques with a substantial lymphocytic infiltrate. Unlike DLE, though, the lesions do not exhibit epidermal abnormalities, follicular involvement, or scarring. Considerable mucin is present in the dermis, which gives the lesions a somewhat boggy look and feel. In some reports, lesions are most common on the face and may be reproduced by phototesting.[21] The risk for SLE appears to be low, and Ig deposits are not generally present in skin biopsies. Jessner's lymphocytic infiltrate and other lymphocytic and granulomatous infiltrative conditions (see earlier) are in the differential diagnosis. Skin biopsy for routine histology is valuable in establishing the diagnosis, with the exception of reliably distinguishing TLE from Jessner's lymphocytic infiltrate. Some have argued that Jessner's lymphocytic infiltrate and TLE are one and the same, and it might reasonably be argued that what is called TLE is not appropriately classed as a form of chronic cutaneous LE but rather as an independent entity. However, the presence of TLE lesions in some patients with lupus is evidence to the contrary.

Lupus panniculitis (LEP) lesions have inflammation in the subcutaneous tissue, resulting in deep indurated plaques that become disfiguring, depressed areas (Figure 43-8). Usual sites of involvement are the face, scalp, upper trunk, breasts, upper arms, buttocks, and thighs. The risk for SLE is not known precisely, but clearly some patients with LEP have or will develop SLE. The differential diagnosis is that of the panniculitides, but the distribution exhibited in LEP is unusual for most other conditions that cause panniculitis. The correlation of clinical with histologic findings usually serves to establish the diagnosis.

Some unusual variants of cutaneous lupus are chilblain lupus (red or dusky plaques on colder areas of skin, such as fingers, toes, nose, elbows, knees, and lower legs), cutaneous lupus/lichen planus overlap, and a bullous eruption associated with autoantibodies to type VII collagen or other basement membrane zone proteins. Not all bullae related to lupus are due to autoantibodies to basement membrane proteins, however. It is not unusual for bullae to develop in the patient simply from intensive destruction of the basal cell layer in ACLE, SCLE, or, rarely, DLE.



**Figure 43-8** Lupus profundus (panniculitis) with extensive atrophy.

Treatment of the lupus-specific lesions is relatively similar for most of the subtypes, with some exceptions and modifications.[22] Sun protection is critical for lesions that are initiated or exacerbated by sun exposure. Many or most patients underestimate the amount of sunscreen needed to apply, the potential damage of the seemingly minimal exposure one has in the course of day-to-day activities, and the value of protective clothing. Tobacco use appears to be an exacerbating factor and can prevent response to therapy.[23] Topical therapy is often used to avoid side effects of systemic medications or to provide adjunctive therapy, although topical agents are unlikely to be beneficial if the disease process is deep, as in panniculitis. Topical or intralesional corticosteroids are the most often used local therapy, but there are some reports of benefit from topical calcineurin inhibitors and topical retinoids. The first-line systemic medication for cutaneous lupus is antimalarial therapy, most often hydroxychloroquine. For skin disease not responsive to hydroxychloroquine, changing to chloroquine or adding quinacrine has been helpful in some patients. For antimalarial-resistant skin disease, a wide variety of medications have been used, but there is no clear second choice when patients have not responded to anti-malarials . When evaluating response to therapy, it is important for the clinician to distinguish active disease, as manifested by erythema or development of new lesions, from "damage," as exemplified by scarring or dyspigmentation. Although dapsone is arguably not helpful in most types of cutaneous lupus, it may be helpful in neutrophil-predominant bullous eruptions.[24] Measures to keep the skin warm may be useful for chilblains lupus.

## Nonspecific Cutaneous Lesions

A wide variety of lupus nonspecific skin lesions has been reported. Many of these, such as vasculitic lesions, are cutaneous clues to the possibility of extracutaneous disease.

Livedo reticularis is noteworthy in this regard. The netlike erythema of livedo reticularis is a vascular phenomenon caused by lowered oxygenation at the periphery of the area supplied by a particular vessel. This can simply be due to vasoconstriction, such as occurs in a cold environment, and thus can be a benign finding. If livedo is more prominent than usual, not corrected by warming, and persistent, it can indicate lowered flow caused by pathology such as vasculitis, atherosclerotic disease, or sludging. In lupus, livedo reticularis may be a sign of the presence of anti-phospholipid antibodies.[25]

Other lupus nonspecific skin lesions include Raynaud's phenomenon, palmar erythema, periungual telangiectasia, alopecia, erythromelalgia, papulonodular mucinosis, and anetoderma. Sclerodactyly, calcinosis, and rheumatoid nodules have been reported but may be more likely in overlap syndromes than in SLE.

## NEONATAL LUPUS SYNDROME

Neonatal lupus erythematosus (NLE) is associated with maternal IgG autoantibodies to Ro/SSA and La/SSB.[26] Affected children may have cutaneous lesions, cardiac disease (notably complete heart block and/or cardiomyopathy), hepatobiliary disease, or hematologic cytopenias. Most children have only one or two features of the disease. Similar to the anti-Ro/SSA-associated SCLE of adults, the skin lesions are often photosensitive, have relatively superficial inflammatory infiltrates, and do not tend to scar. The lesions usually appear in infants at a few weeks of age but have been noted at birth in several cases. The natural history of the skin disease is that the lesions last for weeks or months and resolve spontaneously, usually leaving no residuum. In a few cases, persistent telangiectasias have been noted. Individual lesions appear as erythematous annular papules or plaques. Lesions are usually more numerous and more intensely inflamed on the face and scalp, but may additionally occur on the patient's trunk and extremities. Confluent periorbital erythema, which gives the appearance of an erythematous mask, is common and diagnostically helpful. Even though the skin disease resolves and most children without extracutaneous involvement remain otherwise healthy, there is a possibility that children who have had NLE are at increased risk for autoimmune disease later in childhood.[27]

Differential diagnosis of the skin lesions may include reactive erythema, drug eruption, erythema multiforme, and urticaria. Annular NLE lesions usually have little or no scale, unlike the annular lesions of tinea. In areas where there is intense destruction of the basal cell layer, lesions may be crusted and look similar to bullous impetigo. Treatment of skin lesions consists largely of sun protection and mild topical steroids.

The pathogenesis of lupus is covered elsewhere, but it is noteworthy that SCLE-like, anti-Ro/SSA–associated skin lesions may occur in neonates, but other lupus-specific skin lesions do not appear to be maternally transmissible.

## SJÖGREN'S SYNDROME

The most common mucocutaneous findings of Sjögren's syndrome are related to glandular dysfunction. Lacrimal gland dysfunction causes dryness and irritation of the eyes and can lead to keratitis and corneal ulceration. Salivary gland dysfunction causes dry mouth and may result in angular cheilitis and numerous dental caries. Vaginal xerosis may cause burning and dyspareunia. The skin may be dry, cracked, and pruritic. Mildly dry mucous membranes and even severely dry skin may be present in a substantial percentage of healthy individuals who live in dry climates, thus the findings should be interpreted in the context of the setting. In Japanese patients with Sjögren's syndrome, an annular erythema has been described that is somewhat reminiscent of annular SCLE or annular lesions of NLE, although more indurated.[28]

Vasculitis is a relatively common finding. In one series of 558 patients with primary Sjögren's syndrome, 52 had vasculitis, typically involving the small vessels. In most cases, lesions were purpuric, but in some, urticarial vasculitis was the clinical presentation. Patients with cutaneous evidence of vasculitis generally had more severe systemic disease.[29]

## DERMATOMYOSITIS

**KEY POINTS**

- **Patients with DM may present with only skin or skin and lung involvement in the absence of muscle disease.**
- **Patients with DM often have erythema on the upper eyelids, over joints on the hands, on the elbows, and in the malar area that extends to involve the nasolabial fold.**
- **Skin biopsies are important to rule out other diseases that can mimic DM, such as psoriasis, eczema, or multicentric reticulohistiocytosis. Clinical-pathologic correlation is critical because skin biopsies of cutaneous lupus and DM can be identical.**
- **Patients with involvement of the sides of the fingers are more likely to have interstitial lung disease. All patients with DM should have screening pulmonary function tests.**

The current American College for Rheumatitis (ACR) criteria for DM do not recognize the existence of amyopathic dermatomyositis (ADM). This omission has led to a problem in diagnosing patients with predominantly skin involvement with DM. Revised classification criteria[30] are currently addressing this problem. ADM, or dermatomyositis sine myositis, refers to classic cutaneous manifestations of DM without evidence of inflammatory myopathy. ADM has also been defined as biopsy-proven cutaneous findings of classic DM that occur for 6 months or longer without any clinical evidence of proximal muscle weakness, serum muscle enzyme abnormalities, or abnormal muscle testing. This latter definition excludes any patient treated with systemic immunosuppressive therapy for 2 or more consecutive months during the first 6 months of cutaneous manifestations of DM because therapy could suppress clinically significant myositis. It also excludes any patient using drugs that are associated with DM-like skin changes (e.g., hydroxyurea).[31]

The most common cutaneous manifestations of active DM include Gottron's papules (Figure 43-9) and Gottron's sign, which are pathognomonic of DM. Other characteristic



**Figure 43-9** Gottron's papules over the interphalangeal joints in dermatomyositis.



**Figure 43-10** Heliotrope eruption of dermatomyositis with characteristic edema.

findings of DM include heliotrope rash of the periorbital and upper eye area (Figure 43-10), V- or shawl-shaped macular erythema over the chest and back, cuticular overgrowth, and periungual telangiectasias. Patients with active disease can have widespread erythema over the trunk and extremities, with accentuation of the extensor arms and legs, as well as lateral thighs. Erythema and scale of the scalp can result in extensive alopecia. Hyperkeratosis of the palmar and lateral surfaces of the fingers, called *mechanic's hands*, can be associated with anti–Jo-1 autoantibodies and interstitial lung disease. Rarely, patients can have a panniculitis. Vasculopathy, with livedo reticularis and ulceration, can occur. Itching can result in excoriations and lichenification. Damage lesions include postinflammatory hyperpigmentation, poikiloderma, calcinosis, lipoatrophy, and depressed scars.[32] Poikiloderma is a descriptive term for a pattern of finely mottled white areas and brown pigmentation, telangiectasia, and atrophy.

Skin biopsy from a patient with cutaneous DM is identical to that seen with cutaneous lupus erythematosus. A diagnosis of DM is made by clinical-pathologic correlation and does not need to include muscle disease. In the absence of clinical muscle findings, the workup for DM should include muscle enzyme testing, pulmonary function testing, chest radiograph, electrocardiogram, and evaluation for underlying malignancy. Patients with ADM have the same incidence of pulmonary involvement as classic DM, and approximately 25% of patients have evidence of pulmonary fibrosis on high-resolution CT.[33] There is an increased risk for malignancy in adult patients with DM, including ADM. The most frequent malignancies are lung, ovarian, pancreatic, stomach, colorectal, and non-Hodgkin's lymphoma.[34] The increased risk of malignancy occurs for at least 5 years after the diagnosis of DM, and thus patients should receive routine cancer screening during that time.[35] Patients with DM frequently experience a delay in obtaining a diagnosis, and the presence of photosensitivity, malar rash, oral ulcers, and a positive anti-nuclear antibody (ANA) test means that they frequently are misdiagnosed as having SLE, having met the current criteria for the disease.

The patients with both skin and muscle disease frequently experience resolution of their muscle disease after aggressive treatment with glucocorticoids, with or without immunosuppressives. Patients are sometimes treated with intravenous immunoglobulin (IVIG), cyclosporine, or tacrolimus. Patients with AMD or residual skin disease after treatment often benefit from hydroxychloroquine. Patients who do not improve with a single antimalarial can benefit from the addition of quinacrine or a switch from hydroxychloroquine to chloroquine.[36] Immunosuppressives such as methotrexate, azathioprine, or mycophenolate mofetil can be of additive benefit for patients with resistant skin disease. IVIG can be of benefit for skin findings of DM, and studies related to the role of biologics in treatment are ongoing.[37]

## SCLERODERMA AND OTHER SCLEROSING CONDITIONS

**KEY POINTS**

- **Scleroderma can occur as localized or systemic disease.**
- **The localized form of the disease occurs as localized or generalized morphea, linear scleroderma, or facial hemiatrophy, otherwise known as *Parry-Romberg syndrome*.**
- **Linear forms of the disease occur more often in children, whereas morphea and scleroderma are more common in adults.**
- **Eosinophilic fasciitis involves the fascia below the fat and can be associated with eosinophils in the tissue and is more responsive to glucocorticoids than localized or systemic scleroderma.**
- **Some fibrosing conditions, including scleromyxedema and POEMS, are associated with monoclonal gammopathies.**
- **Nephrogenic systemic fibrosis is related to gadolinium exposure in patients with decreased renal function. The histology is indistinguishable from scleromyxedema.**

### Morphea

Scleroderma can occur as localized or systemic disease. The localized form of the disease occurs as localized or generalized morphea, linear scleroderma, or facial hemiatrophy,

otherwise known as *Parry-Romberg syndrome*. Linear scleroderma can occur over the forehead, in a variant called *en coup de sabre* (Figure 43-11). Morphea is seen more in adults, with increased incidence with advancing age, whereas linear scleroderma occurs more frequently in children and adolescents.[38] Although remissions are reported to occur in 3 to 5 years, ongoing clinical activity or reactivation is not unusual.

Localized scleroderma patients typically lack sclerodactyly, Raynaud's phenomenon, or internal organ involvement. The level of involvement in localized scleroderma can be in the dermis (morphea), fat (subcutaneous morphea), fat and fascia (morphea profundus), and fascia (eosinophilic fasciitis). Morphea typically has round and/or oval, irregular plaques that are initially dull red/violaceous, smooth, and indurated. They frequently progress to chalky, white atrophic lesions, although some patients have residual hyperpigmentation overlying the lesions. Morphea can have different presentations, including an overlap with lichen sclerosus et atrophicus, in which there are flat-topped papules that coalesce to form a white plaque, sometimes combined with a deeper morphea lesion. Some lesions are small and oval, known as *guttate morphea*. Occasionally, patients with localized scleroderma overlap with other autoimmune diseases such as SLE. Many mimics of morphea exist, including radiation-induced morphea, injection-induced morphea-like lesions, morphea-like Lyme disease (seen in Europe), eosinophilia-myalgia syndrome, toxic oil syndrome, and, more recently, nephrogenic systemic fibrosis (NSF).

Linear scleroderma is frequently located on the lower limbs, upper limbs, frontal head area, and anterior trunk. It is frequently unilateral and can result in joint deformity, joint contractures, and limb atrophy. Some cases are associated with seizures or other focal neurologic symptoms. Parry-Romberg syndrome can occur in the first or second decade of life and leads to unilateral facial atrophy in 95%. Half of patients with linear scleroderma start with en coup de sabre and progress to have soft tissue involvement in the upper face. Patients can have seizures, headaches, visual changes, and atrophy of the salivary glands and hemiatrophy of the tongue on the same side as the facial atrophy. Any reparative surgical treatment should be timed to occur no sooner than 1 year after cessation of the ongoing atrophic process. ANAs are positive 46% of the time in cases of localized scleroderma. A positive ANA correlates with disease severity.[39] A peripheral eosinophilia and hypergammaglobulinemia can be seen.

Treatment of localized scleroderma includes protection from trauma and cold, anti-malarials (hydroxychloroquine alone, hydroxychloroquine and quinacrine together, or chloroquine with or without quinacrine), low-dose prednisone for eosinophilic fasciitis, topical calcipotriene, methotrexate, and mycophenolate mofetil. Phototherapy with narrow-band UVB, UVA1, PUVA, and topical photodynamic therapy has also been used.[40]



**Figure 43-11** Linear scleroderma of the forehead. *(Courtesy Dr. Victoria Werth, University of Pennsylvania Department of Dermatology and Philadelphia Veterans Administration Medical Center, Philadelphia.)*

## Systemic Scleroderma

Patients with early limited cutaneous scleroderma have Raynaud's phenomenon for many years, minimal constitutional symptoms, puffy fingers, limited skin thickening, and anticentromere antibody. Patients with early diffuse cutaneous scleroderma frequently have delayed Raynaud's, acute onset, many constitutional symptoms, arthralgias, tendon friction rubs, swollen puffy hands, and early diffuse skin thickening. They may have anti–Scl-70 antibody, as well as anti–RNA polymerase III. The major and minor criteria for diffuse cutaneous scleroderma include many skin findings. Major criteria include proximal scleroderma. Minor criteria include sclerodactyly (Figure 43-12), digital pitting and scars of the fingertips, loss of substance of the finger pad, and bibasilar pulmonary fibrosis.[41] New criteria to identify patients with early disease have been proposed.[42] Ischemia and skin changes in systemic scleroderma can result in skin ulcers. Therapies used in the cutaneous treatment of systemic scleroderma include D-penicillamine, methotrexate,



**Figure 43-12** Sclerodactyly with flexion contractures.

cyclophosphamide, mycophenolate mofetil, photopheresis, and hematopoietic stem cell transplant.[43,44] Treatment of Raynaud's phenomenon is covered elsewhere. There are reports of bosentan, an endothelin receptor antagonist, and phosphodiesterase inhibitors, such as sildenafil, being helpful in the treatment of skin ulcers.[45,46] Patients who have pruritus may benefit from systemic antihistamines.

## Eosinophilic Fasciitis

Eosinophilic fasciitis (EF) involves inflammation of the fascia overlying muscle and results in swelling of the extremities, followed by fibrosis and contractures. The digits are typically spared. There is often a rapid onset of disease activity, particularly following physical exertion. A contaminant of L-tryptophan was associated with an EF-like disease in the early 1990s.[47] Approximately 30% of EF patients have morphea concurrently.

Diagnosis is normally based on a deep, usually excisional biopsy of the skin that includes fascia. There are inflammatory cells among collagen bundles, thickening of collagen, sclerosis of the dermis and fat/fascia, and absent sweat glands and hair. MRI of active EF demonstrates thickening of the fascia overlying the muscle, hyperintense signal within the fascia on fluid-sensitive sequences, and fascial enhancement after IV contrast.[48]

## POEMS Syndrome

POEMS syndrome includes polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes. To be diagnosed with POEMS, a patient must have polyneuropathy and a monoclonal plasmaproliferative disorder, along with one other minor criterion that includes sclerotic bone lesions, Castleman's disease, organomegaly, edema, endocrinopathy, papilledema, or skin changes. Other associated findings may include ascites, pleural effusions, thrombocytosis, fingernail clubbing, and white nails. The sensorimotor polyneuropathy has both demyelinating and axonal features and is slowly progressive and debilitating in most cases. Organomegaly may be hepatosplenomegaly or lymph node enlargement. Endocrinopathies include diabetes, impotence, gynecomastia, and hypothyroidism. The monoclonal (M) protein abnormality consists of IgA or IgG heavy chains with λ light chains. The skin changes may consist of hyperpigmentation, hypertrichosis, hyperhidrosis, skin thickening, telangiectasia, and glomeruloid hemangiomas.[49] Treatment with corticosteroids, low-dose alkylators, lenalidomide, and high-dose melphalan and autologous peripheral blood stem cell transplantation has been reported to be successful.[50,51]

## Scleromyxedema

Scleromyxedema is a rare disorder frequently characterized by dysproteinemia and widespread skin changes. The paraprotein stimulates fibroblast production of mucin, suggesting a causal role and a rationale for lowering it. Patients have waxy papules on the face, neck, upper trunk, forearms, hands, and thighs that become confluent and occur in association with underlying sclerosis, which can lead to joint contractures, sclerodactyly, and carpal tunnel syndrome. Common extracutaneous manifestations include upper gastrointestinal dysmotility, muscle weakness, joint contractures, and neurologic symptoms, such as seizures, encephalopathy, coma, and obstructive or restrictive pulmonary disease. Ninety percent of patients have a monoclonal gammopathy, most frequently IgGλ, but occasionally IgGκ.[52,53]

Skin biopsy shows mucin deposition and a proliferation of fibroblasts in the upper dermis. Treatment has included prednisone, IVIG, PUVA, systemic retinoids, thalidomide, lenalidomide, interferon (IFN) alfa-2a, plasmapheresis, photopheresis, low-dose melphalan, and high-dose dexamethasone.[52] Chemotherapeutic agents such as melphalan, cyclosporine, cladribine, cyclophosphamide, methotrexate, and chlorambucil have been used. Successful remission after autologous stem cell transplantation has been reported.[54]

## Nephrogenic Systemic Fibrosis

NSF is a relatively new illness that occurs in patients with renal disease; most of the patients have undergone dialysis for renal failure.[55] NSF presents as either a morphea-like disease or a more diffuse acral sclerosis. Morphea-like presentations include ill-defined indurated plaques, with islands of sparing and finger-like projections that involve lower more than upper extremities. More diffuse confluent acral sclerosis, sometimes with truncal involvement, can occur. There are often yellow plaques on the conjunctiva. Patients can experience pain, severe itching, joint contractures, fibrosis and calcification of the skin, subcutaneous tissue, fascia, muscle, myocardium, lungs, renal tubules, and testes. Patients typically do not have Raynaud's syndrome. Skin biopsy is identical to that seen with scleromyxedema, with stellate fibroblasts, glycosaminoglycans, and thickening of collagen.[56] Transforming growth factor (TGF)-β is increased in lesional skin.[57] There is no proven effective therapy, and prognosis depends on the extent, rapidity of skin involvement, and severity of the systemic disease.[57] Treatments that have been reported to potentially help include plasmapheresis, IVIG, immunosuppressives, glucocorticoids, IFN-α, thalidomide, PUVA, UVA1, photopheresis, and imatinib mesylate.[58] Recent studies suggest a strong association of NSF with gadolinium exposure, with some cases of delayed onset after exposure.[59]

# PRIMARY VASCULITIS INVOLVING THE SKIN

**KEY POINTS**

- **In cutaneous diseases, the term *vasculitis* is typically reserved for lesions that histologically show damage to vessel walls and a neutrophilic or granulomatous infiltrate.**
- **The major determinant of the clinical appearance is the size of the vessel affected.**
- **Small vessel vasculitis unassociated with rheumatic diseases is often short lived and limited to the skin.**
- **A characteristic lesion of cutaneous small vessel vasculitis is palpable purpura in dependent areas of the body. It is common for the majority of lesions to be nonpalpable.**

**Systemic corticosteroids are not routinely indicated for skin-limited disease.**

**Urticarial vasculitis lesions are morphologically similar to urticaria but more long lasting. Hypocomplementemic urticarial vasculitis is associated with a higher risk of significant internal disease.**

**Two major forms of small vessel vasculitis in children are Henoch-Schönlein purpura, which is characteristically associated with internal involvement, and acute hemorrhagic edema of infancy, a dramatic-appearing but benign and self-limited condition.**

**Skin biopsy of granulomatosis with polyangiitis and of eosinophilic granulomatosis with polyangiitis often shows small vessel vasculitis rather than true granulomatous vasculitis.**

**A variant of PAN, termed *benign cutaneous PAN* ,or *primarily cutaneous PAN* is characterized by tender subcutaneous nodules and livedo reticularis.**



**Figure 43-13** Leukocytoclastic vasculitis demonstrating nonblanching, purpuric macules.

In cutaneous diseases, *vasculitis* is a term typically reserved for lesions characterized by damage to vessel walls and a neutrophilic or granulomatous infiltrate. Classification, pathogenesis, diagnostic evaluation, and therapy are covered elsewhere in the text. In this chapter, the focus is on the skin findings and differential diagnosis. The primary determinant of the appearance is the size of the vessel affected.

## Cutaneous Small Vessel Vasculitis and Its Variants

Cutaneous small vessel vasculitis is a rather common condition with characteristic histologic findings of fibrinoid necrosis of vessel walls, a neutrophil-predominant infiltrate, and leukocytoclasis (i.e., fragmented nuclei resulting from degeneration of neutrophils).[60] In the skin, the vessels involved are in the dermis, and damage to these vessels results in lesions of a characteristic size. The lesions are both erythematous and purpuric and are distinctly palpable if there are sufficient numbers of neutrophils in the lesion. On physical examination, it may be helpful for the physician to palpate several lesions because it is common for the majority of the lesions to be nonpalpable. The usual diameter of the purpuric papules is approximately 0.3 to 0.6 cm, although smaller and larger lesions may be observed (Figure 43-13). Discrete lesions have a round shape. The center may look dusky, pustular, or ulcerated, or it may appear as a hemorrhagic vesicle. Larger ulcerations may occur when lesions coalesce. Particularly in larger lesions, the devitalized tissue may be a focus for secondary bacterial infection. Lesions tend to occur in dependent areas. Thus for ambulatory patients, lesions are most numerous on the lower legs. Koebnerization, the appearance of lesions along lines of trauma, such as scratches, is sometimes observed.

As mentioned under the discussion of rheumatoid vasculitis, the differential diagnosis of purpuric or petechial lesions may include stasis dermatitis, pigmented purpuric dermatosis, platelet dysfunction or deficiency, petechial drug eruptions, viral exanthems, emboli (cholesterol, septic), thromboses, and sludging. Skin biopsy is often helpful in establishing the diagnosis of small vessel vasculitis, especially if an early lesion is sampled. Immunofluorescence of an early lesion may establish whether the vasculitis is IgA predominant.

Small vessel vasculitis unassociated with connective tissue disease and not IgA predominant is sometimes called *hypersensitivity vasculitis*. It is often apparently confined to the skin and therefore has a good prognosis. In some cases, infection or drugs may be implicated, but often no definitive initiating event is discovered. Therapy is directed first toward treating the underlying cause, if a cause is found. If there is no significant extracutaneous disease, treatment is often symptomatic. Leg elevation, compression stockings, and reduction of activity may be helpful. Nonsteroidal anti-inflammatory drugs (NSAIDs) or antihistamines are sometimes used. Systemic corticosteroids are not routinely indicated for skin-limited disease. For patients with persistent disease confined to the skin, colchicine and dapsone have each been used with some success.[61]

A common variant of small vessel vasculitis is Henoch-Schönlein purpura (HS purpura). HS purpura occurs commonly in children and is often associated with extracutaneous findings of gastrointestinal or renal involvement. The typical lesion of IgA-predominant small vessel vasculitis is an erythematous or urticarial macule or papule that evolves rapidly into palpable purpura. It has been noted that IgA-predominant vasculitis in particular may display superficial plaques of palpable purpura or a retiform configuration of lesions.[62] The most reliable means of establishing that the vasculitis is IgA predominant is biopsy for immunofluorescence. IgA-predominant vasculitis is not unusual in adults. Compared with the presentation in children, there is a lower association with preceding upper respiratory infection and higher association with medication use.

Mixed cryoglobulinemia can present as a small vessel vasculitis. Additional skin findings that may occur include livedo reticularis, urticarial papules, cold urticaria, Raynaud's syndrome, acrocyanosis, leg ulcers, and digital ulceration or gangrene. Hepatitis C is a frequent association. Patients with type I monoclonal cryoglobulinemia may more often have purpura caused by cryogelling rather than a true vasculitis.

A distinct subset of patients with small vessel vasculitis has lesions that are primarily urticarial rather than purpuric.

The main diagnosis in the clinical differential is urticaria. Individual lesions of urticaria tend to be short lived, usually less than 24 hours, whereas individual lesions of urticarial vasculitis tend to last several days. Additional skin findings may include angioedema, livedo reticularis, nodules, and bullae. In some lesions, foci of purpura may be observed. Urticarial vasculitis has been classified into two groups: normocomplementemic and hypocomplementemic. Extracutaneous disease is more likely to occur in the hypocomplementemic group, and some of these patients have underlying SLE. It has been proposed that there is a distinctive subset of the hypocomplementemic group characterized by IgG antibodies to C1q, angioedema, ocular inflammation, arthritis, obstructive pulmonary disease, and renal disease. The pulmonary disease tends to be severe and life threatening. This subset has been termed *hypocomplementemic urticarial vasculitis syndrome*.[63,64]

Erythema elevatum diutinum is an unusual form of small vessel vasculitis that is characterized by erythematous or violaceous papules, plaques, and nodules over the dorsal hands, ears, knees, heel, and buttocks. In the clinical differential diagnosis are Sweet's syndrome, multicentric reticulohistiocytosis, sarcoidosis, and lymphoma, among others. With time, fibrosis often occurs. Established lesions may be disfiguring and may have an appearance somewhat reminiscent of keloids. Although significant extracutaneous involvement is not expected and many patients are otherwise well, erythema elevatum diutinum has been reported in association with various autoimmune, infectious, and hematologic conditions, including streptococcal infection, paraproteinemia, inflammatory bowel disease, RA, SLE, and HIV. For active skin lesions, dapsone is the usual treatment.[65] Intralesional corticosteroids are sometimes used for fibrotic lesions.

Acute hemorrhagic edema of childhood is an uncommon but generally benign and self-limited form of vasculitis usually occurring in children younger than 2 years, commonly preceded by an upper respiratory infection or medication.[66] The clinical appearance may be dramatic, with large purpuric plaques on the face, ears, and extremities. On the basis of the appearance of the skin lesions, meningococcemia is sometimes suspected but the child with acute hemorrhagic edema appears relatively healthy. Generally there is no extracutaneous involvement. Treatment is symptomatic.

## Granulomatous Vasculitides

Skin lesions are occasionally the presenting feature of granulomatosis with polyangiitis.[67] The most common type of lesion is palpable purpura, with or without necrosis. Many other types of lesions have been noted, including papules, ecchymoses, hemorrhagic bullae, necrotic papules, subcutaneous nodules, and ulcers. Ulcerative lesions may be similar in appearance to pyoderma gangrenosum, although lacking an undermined border. Oral ulcers are relatively common but nonspecific in appearance. A more specific oral finding is hypertrophic gingival inflammation with petechiae.

Skin biopsy is sometimes helpful diagnostically, but unfortunately, biopsies are often nonspecific or show small vessel vasculitis. True granulomatous vasculitis is not often observed in skin specimens. Extravascular granulomatous inflammation is sometimes noted, and may be more likely to occur in nonpurpuric papules or nodules than in palpable purpura.

Differential diagnosis of the skin lesions includes other small vessel vasculitides, particularly when cutaneous lesions consist of palpable purpura and the biopsy finding is leukocytoclastic vasculitis. If granulomatous inflammation is observed, the differential diagnosis may include eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), polyarteritis nodosa, microscopic polyangiitis, RA, SLE, infection, lymphoproliferative disorders, chronic active hepatitis, erythema nodosum, granuloma annulare, and inflammatory bowel disease.

Patients with eosinophilic granulomatosis with polyangiitis characteristically present with respiratory symptoms, but skin lesions are common during the vasculitic phase of the disease.[68] Hemorrhagic lesions ranging from petechiae to palpable purpura to ecchymoses, cutaneous nodules with or without ulceration, subcutaneous nodules, and nonspecific erythematous eruptions are most common. As with granulomatosis with polyangiitis, hemorrhagic lesions tend to show small vessel vasculitis on biopsy, and nodules are more likely to demonstrate granulomas. The clinical and histologic differential diagnoses often include polyarteritis nodosa, granulomatosis with polyangiitis, and microscopic polyangiitis. As alluded to earlier, the histologic finding of Churg-Strauss granuloma is not specific for eosinophilic granulomatosis with polyangiitis, and may be seen in several entities. Large numbers of eosinophils in the biopsy may be diagnostically helpful but are not definitive. Hypereosinophilic syndrome may share clinical and laboratory features with eosinophilic granulomatosis with polyangiitis, but vasculitis is not characteristic.

## Polyarteritis Nodosa and Related Conditions

Classic polyarteritis nodosa (PAN) and microscopic polyangiitis have historically been classified as PAN but appear to be distinct conditions distinguishable in part by the size of vessels affected. Classic PAN involves medium-sized vessels, whereas microscopic polyangiitis primarily involves vessels ranging in size from capillaries to arterioles.

Cutaneous findings of classic PAN represent damage downstream of the affected vessel and consist of ulceration, digital gangrene, ecchymoses from vessel rupture, livedo reticularis, and subcutaneous nodules that may follow the course of arteries. Because the affected vessel is proximal to the skin, skin biopsy is likely to show nonspecific findings.

The skin findings of microscopic polyangiitis reflect the smaller size of vessels affected. Petechiae, palpable purpura, purpuric plaques, erythematous nodules, and ulcerations may be observed. There have been some reports of livedo reticularis in association with microscopic polyangiitis The cutaneous pathology is that of a leukocytoclastic vasculitis, but, in contrast to many of the small vessel vasculitides previously discussed, may involve small arterioles. The differential diagnosis is mainly that of other small vessel vasculitides. Size of vessels affected, anti-neutrophilic cytoplasmic antibody (ANCA) positivity, paucity or absence of antibody deposits in vessels, and spectrum of extracutaneous

involvement aid in distinguishing microscopic polyangiitis from other vasculitides.

There is a variant of PAN termed *benign cutaneous PAN* or, perhaps more accurately, *primarily cutaneous PAN*. In this condition, arterioles in the subcutaneous fat and lower dermis are affected, and the presentation is often that of tender subcutaneous nodules and livedo reticularis. Associations with Crohn's disease, hepatitis B, and hepatitis C have been reported. Clinically, panniculitides such as erythema nodosum and erythema induratum may be considered, although livedo reticularis is not expected. A biopsy for diagnosis should include subcutaneous fat. Even after biopsy, microscopic polyangiitis may be difficult to exclude. Although relatively little information is available concerning ANCA in cutaneous PAN, a negative ANCA is more consistent with cutaneous PAN than with microscopic polyangiitis. Although the outcome is benign, the course is often chronic and relapsing. Therapy is typically conservative and may consist of intralesional corticosteroids, NSAIDs, low-dose methotrexate, dapsone, or, occasionally, systemic corticosteroids.

Kawasaki's disease is considered a polyarteritis nodosa variant because of the involvement of coronary arteries. Skin findings constitute several of the criteria for diagnosis. None of the findings is specific for Kawasaki's disease, but the constellation of findings establishes the diagnosis. Kawasaki's disease usually affects young children but has been reported in adults. Criteria for diagnosis are otherwise unexplained fever for at least 5 days and four of the following five findings: (1) bilateral nonexudative conjunctivitis; (2) injected pharynx, strawberry tongue, or injected or fissured lips; (3) erythema of palms or soles, hand and foot edema, and, in the convalescent phase, desquamation; (4) erythematous, polymorphous, generalized skin eruption; and (5) cervical lymphadenopathy. In addition, erythema of the perineal region is common and transverse lines across the fingernail beds have been noted in a few cases.

### Large Vessel Vasculitis

Skin findings of temporal arteritis (giant cell arteritis) consist mainly of palpable temporal arteries, skin tenderness in the area, and scalp nodules or ulcerations. Skin lesions in patients with Takayasu's arteritis may include Raynaud's phenomenon, livedo reticularis, ulcerated nodules, subcutaneous nodules, and pyoderma gangrenosum–like ulcers. Skin biopsy is generally not performed in these conditions.

## INFECTIONS

**KEY POINTS**

**Infections frequently occur on the skin and can mimic autoimmune skin diseases.**

**Skin biopsies can help confirm some types of skin infections, and some require a skin biopsy for culture to determine the organism and drug sensitivity.**

Patients with many infectious diseases present with both skin and rheumatologic findings.[69] This section will highlight a few examples.

### Lyme Borreliosis

*Borrelia burgdorferi*, the causative agent of Lyme disease in North America, is associated with erythema migrans (EM). In Europe the related genospecies *Borrelia afzelii* is associated with both EM and acrodermatitis chronica atrophicans (ACA), and several European studies have found compelling evidence for *B. afzelii* infection in patients with morphea. There has been no similar association of *Borrelia* with morphea in the United States.[70] Hematogenous dissemination from the initial skin site is believed to cause secondary skin lesions and extracutaneous manifestations, and only certain subtypes of *B. burgdorferi* are associated with dissemination.

EM is the first manifestation of Lyme disease in 60% to 80% of people and occurs at the site of the tick bite.[71] At the time of the skin lesion, which occurs within a few days to a month after the bite, the spirochetes enter the circulation and disseminate. The skin findings may be associated with fever, chills, fatigue, headache, neck stiffness, myalgias, arthralgias, conjunctivitis, erythematous throat, and regional or generalized lymphadenopathy. The lesions of EM begin as red macules that become papular and then expand into an erythematous, annular plaque (Figure 43-14). Two forms of EM exist. In one, there is an expanding red plaque with varying intensities of redness within the plaque. In the second, there is a target, with a central red plaque surrounded by normal-appearing skin, which in turn is surrounded by another band of erythema. They can enlarge rapidly, and multiple lesions resulting from hematogenous spread are seen 17% of the time. While the lesion enlarges, the central erythema can fade. The central portion of the lesion may be edematous, vesicular, urticarial, or crusted. Triangular and elongated oval lesions have been described, but circular lesions are most frequent. The most common locations are the inguina, axillae, abdomen, and behind the knees. EM lesions are usually asymptomatic but can be pruritic or painful. Untreated EM lesions resolve in a median of 28 days, with a range from 1 day to 14 months. Resolution is within a few days after treatment with antibiotics such as doxycycline or penicillin.



**Figure 43-14** Lyme disease with characteristic erythematous, annular plaques. *(Courtesy Dr. Joshua Levin, University of Pennsylvania Department of Dermatology, Philadelphia.)*

ACA is associated with late-stage Lyme disease. It occurs mainly in women between the ages of 40 and 70 years. The lesions begin on an extremity, usually the lower leg or foot as a bluish-red edematous plaque. Fibrous bands may develop, especially on the ulnar and tibial regions, and fibrous nodules may form near joints. Regional lymphadenopathy is often present. Over many years, the skin becomes atrophic.[72] *B. burgdorferi* has been isolated from the skin of patients with ACA.[73]

### Parvovirus

The skin findings of patients seen with parvovirus B19 include an erythematous "slapped cheeks" appearance; a lacy, reticulated proximal extremity rash; a febrile petechial eruption; and papular-purpuric gloves and socks syndrome (PPGSS). The infection is self-limited and generally resolves spontaneously within 1 to 2 weeks. Laboratory findings may include mild or severe leukopenia, transient neutropenia or relative neutrophilia, eosinophilia, and mild thrombocytopenia. Adults who are infected often contract the virus from infected children and commonly present with systemic disease, including arthropathy and a flu-like illness, and with additional skin lesions of periflexural pattern and purpura.[74]

### Atypical Infections: *Mycobacterium marinum*

Many types of mycobacteria, atypical mycobacteria, and deep fungal infections can affect skin and joints. *Mycobacterium marinum* is an example and can be acquired through exposure to fresh water, salt water, fish tanks, swimming pools, fish or aquatic exposures, timber cuts, or splinters. The incubation period is usually about 3 weeks, although much longer periods are possible. The disease often occurs after inoculation into abrasions or after penetrating injuries to the fingers and hands. This is an indolent disease, with nodules or ulcerated plaques, occasionally with extension to deep tissue. Common areas of involvement are the fingers, dorsum of the hands, and knees. The lesions can be localized or sporotrichoid (25%), with dissemination 2% of the time.

## PANNICULITIS

**KEY POINTS**

**There are many causes and types of panniculitis. Some are associated with systemic conditions and require a careful history and physical examination, along with a skin biopsy, to determine the cause.**

**Diseases such as amyloidosis and sarcoidosis can involve the skin and can be diagnosed with the help of a skin biopsy. A diagnosis of these diseases in the skin should prompt a search for systemic involvement. Treatment is determined by the cause of the disease and the organs involved.**

Panniculitis refers to a group of diseases that manifest as inflammation or alterations in the subcutaneous fat. The complexity of etiologies for even one form of panniculitis, such as erythema nodosum, the relative rarity of most forms of panniculitis, and the number of different panniculitides has slowed progress in elucidating pathogenesis. The etiologies for many panniculitides are still poorly understood.

Panniculitis may be primary without an identifiable cause, or secondary. Common secondary causes of panniculitis include infection, trauma, pancreatic disease, immunodeficiency states, malignancies, and connective tissue disease. Erythema nodosum remains the most common form of panniculitis, and although there is a long list of diseases and medications with which it is associated, it is frequently not associated with an identifiable underlying condition. Some underlying conditions associated with erythema nodosum include inflammatory bowel disease; sarcoidosis; malignancies such as leukemia and lymphoma; infections (bacteria, *Yersinia*, rickettsiae, chlamydial, spirochetal, and protozoal disease); pregnancy; drugs (sulfonamides and contraceptives); and autoimmune diseases such as Behçet's disease, Sjögren's syndrome, reactive arthritis, and SLE.[75]

Although the understanding of lobular panniculitis has expanded, cases that were lumped into the wastebasket diagnosis of "Weber-Christian" disease are now recognized to be clearly definable and separate entities, such as lupus panniculitis, cytophagic histiocytic panniculitis, subcutaneous panniculitis-like T cell lymphoma, $\alpha_1$-anti-trypsin deficiency, factitial panniculitis, traumatic panniculitis, calciphylaxis, and drug-induced.[76-78] Infections are recognized as a trigger of panniculitis, as exemplified by erythema nodosum caused by streptococcal infection; hepatitis B or C associated with polyarteritis nodosa; infectious panniculitides, often in immunocompromised hosts; and most recently, some cases of erythema induratum/nodular vasculitis associated with *Mycobacterium tuberculosis*. In addition, atypical infections can themselves cause lesions that resemble panniculitis.

An understanding of the heterogeneity of lymphomas that involve the fat is still evolving, but advances in differentiating various histologic and clinical outcomes are occurring.[79] Some patients thought to have lupus panniculitis on the basis of cytopenias and laboratory tests are actually diagnosed with subcutaneous lymphoma after careful review of their pathology.[79,80]

Patients with panniculitis frequently have erythematous tender nodules, and the clinical presentation frequently is not specific enough to allow for determination of the exact subtype of panniculitis without a biopsy. Patients with panniculitis can have associated symptoms such as low-grade fevers, fatigue, arthralgias, and myalgias.

An adequate skin biopsy, often involving an elliptical excision, is essential to properly diagnose the various entities that fall into the category of panniculitis (Table 43-1). Panniculitis is typically classified into four main subgroups: septal, lobular, mixed panniculitis, and panniculitis with vasculitis, and the exact nature of the cellular infiltrate also contributes to a proper diagnosis. There is no question that these overall categorizations help to narrow the differential in any given case, but at times, there are overlapping features or reaction patterns that do not allow for a specific diagnosis. Clinical-pathologic correlation is important, as emphasized by a published review that has an expanded and useful classification of panniculitis.[81]

Anecdotes exist about the efficacy of combination antimalarials, such as hydroxychloroquine and quinacrine, in

**TABLE 43-1** Classification of Panniculitis

| |
|---|
| **I. Without Prominent Vasculitis** |
| A. Septal inflammation |
| 1. Lymphocytic and mixed: erythema nodosum and variants |
| 2. Granulomatous: palisaded granulomatous diseases, sarcoidosis, subcutaneous infection: tuberculosis, syphilis |
| 3. Sclerotic: scleroderma, eosinophilic fasciitis, lipodermatosclerosis, toxins |
| B. Lobular inflammation |
| 1. Neutrophilic: infection, ruptured folliculitis and cysts, pancreatic fat necrosis |
| 2. Lymphocytic: lupus panniculitis, poststeroid panniculitis, lymphoma/leukemia |
| 3. Macrophagic: histiocytic cytophagic panniculitis |
| 4. Granulomatous: erythema induratum/nodular vasculitis, palisaded granulomatous diseases, sarcoidosis, Crohn's disease |
| 5. Mixed inflammation with many foam cells: $\alpha_1$-anti-trypsin deficiency, Weber-Christian disease, traumatic fat necrosis |
| 6. Eosinophilic: eosinophilic panniculitis, arthropod bites, parasites |
| 7. Enzymatic fat necrosis: pancreatic enzyme panniculitis |
| 8. Crystal deposits: sclerema neonatorum, subcutaneous fat necrosis of the newborn, gout, oxalosis |
| 9. Embryonic fat pattern: lipoatrophy, lipodystrophy |
| **II. With Prominent Vasculitis (Septal or Lobular)** |
| A. Neutrophilic: leukocytoclastic vasculitis, subcutaneous polyarteritis nodosa, thrombophlebitis, ENL |
| B. Lymphocytic: nodular vasculitis, perniosis, angiocentric lymphomas |
| C. Granulomatous: nodular vasculitis/erythema induratum, ENL, granulomatosis with polyangiitis, Churg-Strauss allergic granulomatosis |
| **III. Mixed Patterns** |

ENL, Erythema nodosum leprosum.

treating subcutaneous sarcoid, but no studies exist that support definitive recommendations. Reports on the use of newer therapies, such as mycophenolate mofetil and thalidomide to treat inflammatory causes of panniculitis, such as nodular panniculitis and erythema nodosum, are already in the literature, and indicate that these drugs will likely evolve to be useful for these conditions.[82,83] The effectiveness of these and more established drugs, such as NSAIDs, anti-malarials, and methotrexate, need to be studied, and outcomes will hopefully be more systematically evaluated.

## RELAPSING POLYCHONDRITIS

The diagnosis of relapsing polychondritis (RP) is based on the typical clinical manifestations, with auricular findings seen in 90% of patients. Nasal and respiratory tract chondritis can occur, along with nonerosive inflammatory arthritis, cardiac valvular insufficiency, vasculitis, eye, and audiovestibular involvement. The estimated prevalence of 3.5 per million makes controlled trials nearly impossible. The etiology is unknown, but the pathogenesis appears to be mediated by an immune reaction to type II collagen. Clinical skin manifestations include inflammation of the ear, with sparing of the earlobe. Diagnosis includes the presence of a positive serum antibody test to type II collagen and a wedge biopsy that shows cartilage necrosis and perichondral inflammation with lymphocytes and histiocytes. Involvement of other cartilage areas, including the upper airway, should be assessed. Glucocorticoids are the therapeutic choice for reducing the inflammatory process in patients with RP. For patients with sustained disease, many immunosuppressive drugs have been used as steroid-sparing agents. There have been reports of response to TNF inhibitors, rituximab, tocilizumab, and abatacept in patients otherwise refractory to therapy.[84]

## INFILTRATIVE DISEASES

### Amyloid

Type AL amyloidosis (primary amyloidosis) is rare, with an incidence of less than 1 per 100,000 individuals. Skin lesions may occur in as many as 40% of these patients. Skin lesions can be an early sign of the disease and may include purpura, petechiae, and ecchymoses caused by infiltration of blood vessels by amyloid. Other skin findings include alopecia, plaques, and nodules, often found on flexor surfaces, the face, or the buccal mucosa. Bullae and nail dystrophy are occasionally seen. Diagnosis is confirmed by biopsy of lesional or nonlesional skin, along with urine and serum for immunoelectrophoresis to confirm the presence of a circulating monoclonal protein. Skin biopsy shows Congo-red positive, homogeneous, hyaline, fibrillary deposits. Treatment includes autologous stem cell transplantation, with approximately 50% of patients achieving prolonged remission with such therapy. Other effective therapies include the combination of melphalan with high-dose dexamethasone, thalidomide, and recent stem cell transplant in conjunction with melphalan.[85,86] The prognosis depends on the stage at the time of diagnosis, which emphasizes the importance of recognizing the disease.

### Sarcoidosis

Cutaneous involvement occurs in 20% to 25% of sarcoidosis cases and is most likely to be seen early in the disease. Cutaneous lesions can be classified as nonspecific, typically erythema nodosum, and specific or granulomatous. Erythema nodosum occurs frequently as part of Lofgren's syndrome, with bilateral hilar lymphadenopathy and acute iridocyclitis. This variant has a good prognosis and resolves in 80% of patients within 2 years. The skin lesions of sarcoidosis generally have no prognostic significance or correlation with disease activity. Skin involvement has no effect on the course of the disease, and the number of skin lesions does not correlate with systemic disease. Skin plaques tend to be more persistent and commonly associated with chronic forms of the disease. Lupus pernio (Figure 43-15), with violaceous plaques on the nose, ears, cheeks, lips, and fingers, is often seen in long-standing sarcoidosis and is associated with upper airway involvement and pulmonary fibrosis.[87] Other forms of cutaneous sarcoidosis include papules, follicular papules, subcutaneous nodules, ulcerative lesions, alopecia, and ichthyosis. Cutaneous sarcoid can arise in scars. Because of the many types of presentation, diagnosis can be challenging, and a skin biopsy is necessary to confirm the clinical suspicion. Mimickers of papules include xanthelasma, rosacea, trichoepithelioma, syphilis, LE, and granuloma annulare. Plaques can resemble lupus vulgaris, necrobiosis lipoidica, morphea, leprosy, *Leishmania*, or lupus erythematosus. Nodules can



**Figure 43-15** Sarcoidosis with "apple-jelly" plaques on the face and lupus pernio, or nasal rim lesions.

resemble lymphoma or other types of panniculitis. Treatment of cutaneous sarcoidosis depends on the degree of systemic involvement. Clearly, patients who need prednisone for systemic disease often experience improvement of their cutaneous sarcoid. Patients with isolated skin disease or systemic disease that does not require aggressive therapy can benefit from topical or intralesional corticosteroids, topical tacrolimus, minocycline, hydroxychloroquine, combination anti-malarials with hydroxychloroquine and quinacrine, or chloroquine. If anti-malarials are not adequate, then methotrexate or oral retinoids may be used. There have been case reports and small case series of successful therapy with thalidomide and TNF inhibitors, as well as laser remodeling of lupus pernio. There is some concern that TNF inhibitors and IFN-α therapy may induce sarcoidosis.

## MISCELLANEOUS SKIN DISEASES AND ARTHRITIS

**KEY POINTS**

**There are a number of autoinflammatory diseases, including Behçet's disease, familial Mediterranean fever, and cryopyrin-associated periodic syndromes that involve mutations of the inflammasome and can be treated with inhibitors of upregulated cytokines.**

### Behçet's Disease

Behçet's disease is most prevalent in the Middle East and the Far East. Human leukocyte antigen (HLA)-B5 and HLA-B51 genes are important in the pathogenesis of the disease. The criteria for Behçet's disease (BD) include recurrent oral and genital ulcers, eye lesions (uveitis or retinal vasculitis), characteristic skin lesions, and a positive pathergy test.[88] The pathergy test involves use of a sterile needle to prick the forearm. The results are positive when the puncture causes an aseptic erythematous nodule or pustule that is more than 2 mm in diameter at 24 to 48 hours. A diagnosis is made if patients have recurrent oral ulceration plus at least two of the other findings without other clinical explanations. Skin lesions include erythema nodosum, pseudofolliculitis, or papulopustular lesions or acneiform nodules in postadolescents. Oral ulcers are painful and occur on the gingiva, tongue, and buccal and labial mucosa. Genital ulcers, usually larger and deeper than oral ulcers, are typically on the scrotum and penis in men and the vulva in women. Venous involvement, including superficial thrombophlebitis and deep venous thrombosis, can occur. On skin biopsy, small vessel vasculitis is common. Ulcer treatment includes topical corticosteroids, colchicine, and thalidomide, and there are some reports of biologics, including inhibitors of IL-1, IL-6, IL-12/23, and TNF, being helpful.[89] Systemic corticosteroids are prescribed for unresponsive erythema nodosum.

### Familial Mediterranean Fever

Familial Mediterranean fever (FMF) is an autosomal recessive disease that tends to affect certain ethnic groups, including Sephardic Jews, Arabs, Armenians, and Turks. There is a mutation on the short arm of chromosome 16, and the mutant protein pyrin likely plays an inhibitory role in the control of inflammation.[90,91] It is characterized by recurrent, self-limited attacks of peritonitis, pleuritis, and synovitis. Erysipelas-like erythema (ELE) is the pathognomonic skin manifestation. This is characterized by tender erythematous, well-demarcated plaques, usually located on the lower legs.[92] They may be triggered by physical effort and subside spontaneously within 48 to 72 hours of bed rest. Fever and leukocytosis may accompany this condition. Other associated skin findings include Henoch-Schönlein purpura; nonspecific purpura; erythema of the face, trunk, or palm; angioneurotic edema; Raynaud's phenomenon; pyoderma; and subcutaneous nodules. Secondary generalized amyloidosis may lead to chronic renal failure and death if not recognized. Skin biopsy shows edema of the superficial dermis and sparse perivascular infiltrate composed of a few lymphocytes, neutrophils, and nuclear dust, without vasculitis. Direct immunofluorescence shows deposits of C3 in the wall of small superficial vessels. Early treatment with colchicine, which prevents or diminishes the frequency and severity of the inflammatory episodes, can be beneficial. Refractory patients may benefit from IL-1 blockade.

### Multicentric Reticulohistiocytosis

Multicentric reticulohistiocytosis (MRH) is a rare condition of unknown etiology that most frequently occurs in Caucasian women in their fifth and sixth decades. There is destructive symmetric arthritis, with arthritis mutilans developing in approximately 45% of cases, associated with cutaneous papulonodular lesions. Skin findings include cutaneous red-to-brown papules or nodules, typically on the face, dorsum of the fingers, and over the proximal and distal interphalangeal joints, but they can be in a more generalized distribution. A rarer presentation includes photodistributed

erythema, often with targeting over joints that masquerades as DM.[93] Diagnosis is made by skin biopsy, which shows infiltration of histiocytes and multinucleated giant cells. These changes can be seen in a variety of tissues, including the heart, lungs, skeletal muscle, and gastrointestinal tract. The differential diagnosis of skin disease includes other infiltrative processes, such as sarcoidosis and even leprosy. Treatment recommendations, based on mostly small case reports, include glucocorticoids and methotrexate, cyclophosphamide, and finally chlorambucil if the condition is unresponsive.[94] Cyclosporine, TNF inhibitors, and bisphosphonates have also been used with reported benefit.[95] In approximately one-third of patients, MRH may precede or follow an underlying malignancy. Reported associated malignancies include breast, cervix, colon, stomach, lung, larynx, ovary, lymphoma, leukemia, sarcoma, melanoma, mesothelioma, and metastatic cancer of unknown primary.

## Cryopyrin-Associated Periodic Syndromes

Cryopyrin-associated periodic syndromes (CAPS) are inherited autoinflammatory conditions characterized by recurrent bouts of systemic inflammation related to inappropriate activation of the innate immune system. The syndrome encompasses a continuum of three diseases. The mildest is familial cold autoinflammatory syndrome (FCAS). Patients with FCAS present with recurrent, cold-induced episodes of fever, urticaria-like rash, arthralgia, and conjunctivitis. Patients with Muckle-Wells syndrome (MWS) have an intermediate phenotype. In MWS, the disease tends to be chronic, with fever, rash, and arthritis or arthralgia; sensorineural hearing loss; and AA amyloidosis in adulthood. The most severe (neonatal-onset multisystem inflammatory disease [NOMID]), also referred to as *chronic infantile neurologic cutaneous articular* [CINCA] syndrome). In CINCA/NOMID, fever is rare. Patients present with an early onset, intermittent, urticaria-like rash, and neurosensory involvement. Hypertrophic arthropathy with contractures and bone deformity can occur in severely affected patients with CINCA/NOMID. CAPS are caused by dominantly inherited, or de novo gain-of-function mutations within the *NLRP3* gene. Somatic mutations have been recently described in CINCA/NOMID germline-mutation–negative patients. *NLRP3* encodes cryopyrin, a cytosolic protein complex that controls activation of caspase-1, which then activates IL-1β. Mutations in *NLRP3* are associated with overactivation of the inflammasome and thus overexpression of IL-1β. Inhibition of IL-1β can dramatically improve all the clinical manifestations related to this type of inflammation.[96] Isolated reports also suggest that TNF inhibitors and thalidomide can be beneficial therapeutically in these patients.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 44

# The Eye and Rheumatic Diseases



James T. Rosenbaum

**KEY POINTS**

- Symptoms of uveitis vary widely based on the location of the inflammation within the eye and the suddenness of onset.
- Ankylosing spondylitis is the systemic rheumatic disease most often associated with uveitis in North America and Europe.
- During a lifetime, acute anterior uveitis develops in about 40% of patients with ankylosing spondylitis.
- The uveitis associated with HLA-B27 tends to be unilateral, recurrent, and sudden in onset. Recurrences sometimes affect the opposite eye.
- Sarcoidosis frequently manifests as uveitis.
- Most patients with retinal vasculitis do not have systemic vasculitis.
- Many patients with scleritis have a systemic disease, such as rheumatoid arthritis.
- Anti-neutrophilic cytoplasmic antibody testing helps identify a subset of patients with severe scleritis.
- Granulomatosis with polyangiitis is the rheumatic disease that most frequently involves the orbit.
- Anterior ischemic optic neuropathy is the most common ocular manifestation of giant cell arteritis.
- Most patients with visual loss resulting from optic nerve ischemia do not have arteritis.

Virtually all of the systemic inflammatory diseases that require rheumatologic care tend to affect the eye or its surrounding structures. Table 44-1 presents the prototypic ocular manifestations of rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, spondyloarthropathies, vasculitides including granulomatosis with polyangiitis and giant cell arteritis (also known as temporal arteritis), scleroderma, Behçet's disease, relapsing polychondritis, and dermatomyositis. Each of these diseases is addressed elsewhere in this text. This chapter focuses on specific ocular structures—the uvea, cornea, orbit, and optic nerve—and illustrates how inflammation of each might relate to an autoimmune or inflammatory process.

## OCULAR ANATOMY AND PHYSIOLOGY

A diagram of the eye is shown in Figure 44-1. The eye is a tiny but elegantly complex structure. The anterior segment of the eye includes the cornea, which is avascular and transparent when healthy. The lens also is an avascular structure. The anterior chamber is filled with aqueous humor, which has homology to cerebrospinal fluid. When the blood-aqueous barrier is intact, the aqueous humor contains no leukocytes and very little protein. The blood-aqueous barrier, which resembles the blood-synovial barrier, is disrupted in anterior uveitis. In this case, a routine, noninvasive biomicroscopic or slit lamp examination would reveal leukocytes and increased protein in the anterior chamber. An ophthalmologist has the opportunity to observe two universal hallmarks of inflammation noninvasively.

The term *uvea* derives from the Latin word for "grape." The anterior uvea includes the iris and the ciliary body. The aqueous humor is synthesized by the ciliary body. The posterior portion of the uvea is the choroid, which is a highly vascular tissue just posterior to the retina. Any portion of the uveal tract could become inflamed, and adjacent tissue also is frequently inflamed. Anatomic subsets of uveitis include anterior uveitis, which consists of iritis or iridocyclitis (ciliary body inflammation); intermediate uveitis, in which leukocytes are present within the vitreous humor; and posterior uveitis, in which the choroid and the retina are inflamed. *Panuveitis* occurs when all portions of the uveal tract are inflamed. An attempt has been made to standardize the nomenclature used to describe uveitis by the Standardization of Uveitis Nomenclature Working Group,[1] although ambiguities persist because not all ophthalmologists follow these definitions as yet.

Signs and symptoms of uveitis depend on the portion of the uveal tract that is affected. An anterior uveitis, especially if it begins suddenly, is associated with redness, pain, and photophobia. Visual loss varies and often is due to macular edema if present (Figures 44-2 and 44-3). An intermediate uveitis usually causes floaters as a result of leukocytes that enter the visual axis, although most floaters are due to aging or other changes within the vitreous humor. Posterior uveitis by itself does not usually produce pain or redness. Visual loss depends on the location and extent of the inflammatory process.

The outer tunic of the eye is known as the *sclera*. At the front of the eye, the sclera meets the cornea at a tissue known as the *limbus*. The most interior layer of the eye is an extension of the brain that responds to visual signals, the *retina*. The eye shares some common features with joints, including the presence of hyaluronic acid primarily in the vitreous humor and the presence of type II collagen, although ocular inflammation is not a reported accompaniment of collagen-induced arthritis. Aggrecan is a proteoglycan present both in the eye and in joints. An autoimmune response to aggrecan in BALB/c mice can produce both arthritis and uveitis.[2]

**TABLE 44-1** Most Characteristic Ocular Findings of Selected Rheumatic Diseases

| Disease | Most Characteristic Ocular Findings |
|---|---|
| Rheumatoid arthritis | Sicca<br>Scleritis |
| Systemic lupus erythematosus | Sicca<br>Cotton-wool spots |
| Sjögren's syndrome | Sicca |
| Spondyloarthritis | Acute anterior uveitis |
| Granulomatosis with polyangiitis | Scleritis<br>Orbital inflammation |
| Giant cell arteritis | Anterior ischemic optic neuropathy |
| Scleroderma | Sicca |
| Behçet's disease | Uveitis, retinal arteritis |
| Relapsing polychondritis | Scleritis, episcleritis, uveitis |
| Dermatomyositis | Heliotrope eyelids |

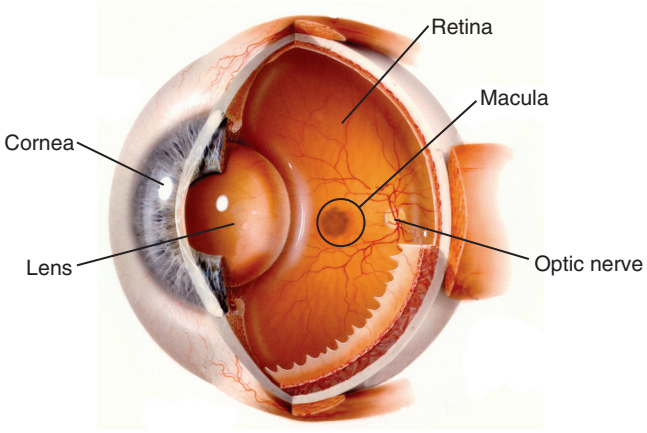



**Figure 44-1** Diagram of the eye.

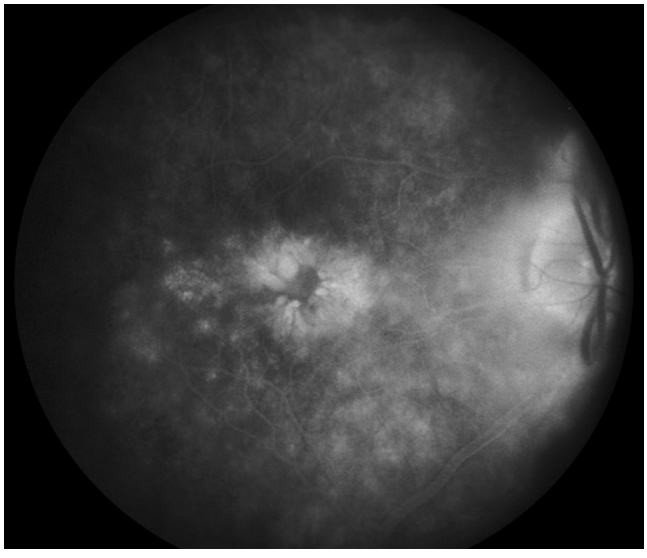


**Figure 44-2** A fluorescein angiogram. The normal macula is avascular and does not stain with fluorescein dye. This patient has macular edema, as indicated by the donut-shaped pattern of dye in the center of the photo. The optic nerve is at the 3-o'clock position in the photo. Macular edema can complicate uveitis, even anterior uveitis.

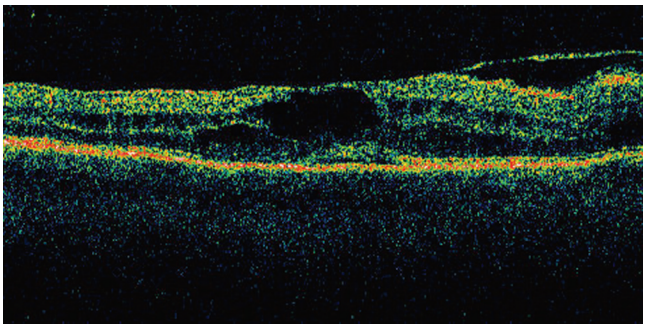


**Figure 44-3** Optical coherence tomography produces precise imaging of the retinal structure. The ovoid black hole in the center of the image is due to macular edema, a major cause of visual loss in patients with uveitis.

Because uveitis and arthritis co-exist in multiple diseases, it is logical to seek the mechanism for this co-existence. It is likely that the mechanism for one disease, such as ankylosing spondylitis, might differ from the mechanism that explains the co-existence of eye and joint disease in Blau syndrome or in juvenile idiopathic arthritis (JIA). In a disease such as reactive arthritis, bacterial products might be trapped in synovium, where they become the target of an immune response. A similar pathogenesis in the iris is difficult to prove because of a relative inability to obtain a biopsy specimen of uveal tissue while it is inflamed, but many microbial products are inflammatory within the eye.[3] Because the eye and joints share specific antigens such as aggrecan, an autoimmune response could also account for the simultaneous inflammation of uvea and joints.

## OCULAR IMMUNE RESPONSE

The eye generally is regarded as an immune privileged site.[4] From a teleologic perspective, many scientists believe that the eye has developed mechanisms to avoid becoming inflamed because of the consequences that inflammation has for visual acuity. Similar to the brain, the internal portion of the eye has no lymphatics, although the conjunctiva on the ocular surface has lymphatic drainage. Portions of the eye—the cornea and the lens—are avascular. The aqueous humor contains several factors that are known to be immunosuppressive, including transforming growth factor-β and α-melanocyte–stimulating hormone. Several tissues within the eye express ligands that promote apoptosis, including tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) and Fas ligand. If a soluble antigen is injected into the anterior chamber, a cellular immune response is suppressed. This phenomenon is known as anterior chamber–associated immune deviation (ACAID). These factors are important to consider in the effort to understand why the eye sometimes is targeted as part of an immune or inflammatory disease.

## UVEITIS

Rheumatologists may be consulted to identify a systemic disease in a patient with uveitis, and a rheumatologist often is asked to assist in the management of immunosuppression in select patients with uveitis. In some referral practices for patients with uveitis, 40% of patients might have an

associated systemic illness. Table 44-2 lists the differential diagnoses of uveitis. The immunologic diseases most likely to be associated with uveitis are listed in Table 44-3.

The most common systemic illness associated with uveitis in most North American practices is ankylosing spondylitis. From an epidemiologic perspective, anterior uveitis is more common than posterior or intermediate uveitis.[5]

About 50% of persons with anterior uveitis are positive for human leukocyte antigen (HLA)-B27.[6] The uveitis associated with HLA-B27 is almost always unilateral, recurrent, of relatively short duration (<3 months per attack), resolves completely between attacks, and is associated with reduced intraocular pressure (in contrast to herpes simplex, which can cause recurrent anterior uveitis associated with increased intraocular pressure).[7] Hypopyon or pus in the anterior chamber sometimes is present in patients with HLA-B27–associated uveitis (Figure 44-4). Recurrent episodes can affect the contralateral eye, but simultaneous bilateral involvement is rare. Many studies have tried to address the question of how frequently a patient with HLA-B27–associated anterior uveitis has an associated spondyloarthropathy. A wide range of answers have been suggested, and the percentage depends on the definition of spondyloarthropathy, but one reasonable estimate is that 80% of HLA-B27$^+$ patients with acute anterior, unilateral uveitis have associated spondyloarthropathy.[6]

**TABLE 44-2** Differential Diagnosis of Uveitis

| |
|---|
| Infections—toxoplasmosis, syphilis, herpes simplex, herpes zoster, and cytomegalovirus |
| Systemic, immune-mediated diseases |
| Masquerade syndromes, such as lymphoma |
| Syndromes confined to the eye, such as pars planitis, birdshot chorioretinopathy, and serpiginous choroiditis |

**TABLE 44-3** Immune-Mediated Diseases Most Often Associated with Uveitis

| |
|---|
| Ankylosing spondylitis |
| Behçet's disease |
| Drug/hypersensitivity reactions |
| Familial granulomatous synovitis |
| Inflammatory bowel disease |
| Interstitial nephritis |
| Juvenile idiopathic arthritis |
| Multiple sclerosis |
| Neonatal-onset multisystem inflammatory disease |
| Psoriatic arthritis |
| Reactive arthritis |
| Sarcoidosis |
| Sweet's syndrome |
| Systemic lupus erythematosus |
| Vasculitis, especially Cogan's syndrome and Kawasaki's disease |
| Vogt-Koyanagi-Harada syndrome |

The uveitis associated with reactive arthritis is indistinguishable from the uveitis associated with ankylosing spondylitis. In either of these entities, acute anterior uveitis develops in about 40% of patients during a lifetime. Although conjunctivitis is part of the classic triad of reactive arthritis (in association with arthritis and nongonococcal urethritis), conjunctivitis is uncommon. A genome-wide screen for susceptibility genes for acute anterior uveitis identified loci that predispose to ankylosing spondylitis and loci that seem to be unassociated with susceptibility to ankylosing spondylitis.[8]

Uveitis develops in approximately 5% of persons with inflammatory bowel disease and 7% of persons with psoriatic arthritis. Although some of these persons have disease that is unilateral, anterior, and recurrent, many have disease that is bilateral, chronic in duration, and posterior to the lens.[9,10] About half of all patients with Crohn's disease or psoriatic arthritis and uveitis are positive for HLA-B27.

Sarcoidosis is the second most common systemic disease associated with uveitis, at least in North America, and in some geographic areas it might be more common than spondyloarthritis. Sarcoidosis is promiscuous within the eye, meaning that it can affect a wide range of structures, including the orbit, lacrimal gland, anterior uvea, vitreous humor, choroid, retina, or optic nerve. Ocular inflammation with sarcoidosis frequently is termed *granulomatous* because large collections of cells deposit on the back of the cornea (Figure 44-5). A retinal vasculitis can be a prominent feature of sarcoidosis, even though systemic vasculitis is not a typical feature of the disease; this phenomenon results in part from the manner in which vasculitis is diagnosed in the retina.

Histologic evidence of vessel wall destruction is rarely obtained because of the morbidity associated with a retinal biopsy. Instead, retinal vasculitis is diagnosed on the basis of perivascular sheathing along a vessel as seen on funduscopic examination (Figure 44-6), intraretinal hemorrhages that must be secondary to vascular injury, and fluorescein angiography indicating increased vascular permeability.[11] Most rheumatologists find the term *retinal vasculitis* misleading because the classic systemic vasculitides, such as polyarteritis nodosa and granulomatosis with polyangiitis, are rarely associated with retinal vasculitis.[12]

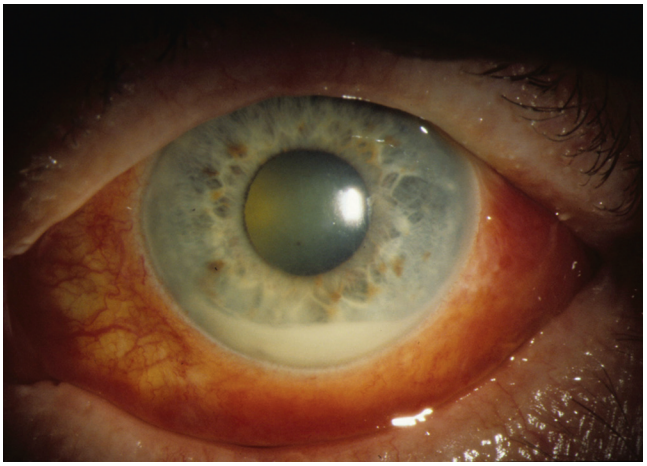


**Figure 44-4** The creamy material at the bottom of the pupil is an accumulation of leukocytes, also known as a hypopyon.

Initially, sarcoidosis frequently manifests as an ocular problem.[13] An ocular symptom is the initial manifestation almost as frequently as a pulmonary symptom. Sarcoidosis frequently involves the conjunctiva, which is an accessible tissue for biopsy confirmation of the diagnosis. In most series of patients with uveitis, about 30% of patients have uveitis that defies placement within a diagnostic category.[14] Many of these patients may have sarcoidosis that is difficult to find outside the eye. The sensitivity and specificity of studies such as a serum angiotensin-converting enzyme level or gallium scan for sarcoid that is primarily ocular are unknown. I consider obtaining a chest computed tomography (CT) scan to look for symmetric hilar adenopathy in any patient who has uveitis of unknown origin.[15] The therapeutic implications of the result of the scan must be balanced against the cost and potential harm from radiation exposure.

JIA comprises several different diseases. Patients with juvenile ankylosing spondylitis resemble their adult counterparts in that a sudden-onset, unilateral anterior uveitis can develop. The subset of JIA that is most classically associated with uveitis tends to be female, with onset of arthritis between the ages of 2 and 8 years.[16] The joint disease is pauciarticular, and most patients are positive for antinuclear antibodies. The uveitis tends to have an insidious onset, such that pain and redness are almost always absent. Joint disease can be minimal as well, and some patients are not diagnosed until a visual screening examination is performed when starting school. The eye disease usually is bilateral and very persistent, although remissions have been well described. Band keratopathy, which is the deposition of calcium superficially in the cornea, is a well-known and frequent complication of this form of uveitis (Figure 44-7). Patients also may experience glaucoma and posterior *synechiae*, a term that describes adhesions of the iris to the lens.

Other forms of uveitis associated with joint disease include Behçet's disease, relapsing polychondritis, and vasculitis such as Cogan's syndrome and Kawasaki's disease. In Behçet's disease, uveitis is often the symptom that "drives" the therapy—that is, it is often the manifestation that most often requires systemic immunotherapy.[17] Eye inflammation usually is bilateral and recurrent. In contrast to the recurrences typical of ankylosing spondylitis, recurrences of uveitis with Behçet's disease, usually do not have complete resolution between attacks. A hallmark of Behçet's disease–associated uveitis is a retinal vasculitis. Retinal arteries are especially prone to be affected. The visual prognosis with Behçet's disease can be grim, and blindness is a frequent concomitant of untreated ocular disease.

Relapsing polychondritis can have an impact on almost any portion of the eye, including the episclera, sclera, and uveal tract.[18] Ocular inflammation is common.

Cogan's syndrome is classically defined as sensorineural hearing loss with corneal disease, especially interstitial keratitis. This definition usually is broadened to include any ocular inflammatory process, such as uveitis or scleritis. Although uveitis can occur with polyarteritis or granulomatosis can occur with polyangiitis, scleral disease is typical. In contrast, anterior uveitis in association with conjunctivitis is present in most patients with Kawasaki's disease.

Uveitis and arthritis occasionally can result from an infection such as Whipple's disease or Lyme disease. Uveal involvement with Lyme disease has been described but is extremely rare.

Some autoinflammatory diseases are associated with uveitis. Autoinflammatory diseases are characterized by

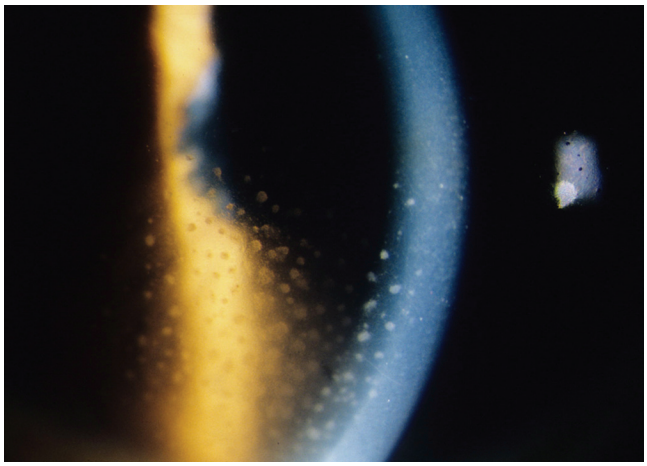


**Figure 44-5** Keratic precipitates. The white dots result from concretions of cells depositing against the corneal endothelium. These keratic precipitates are large and usually are described as granulomatous even though a granuloma is not present histologically.

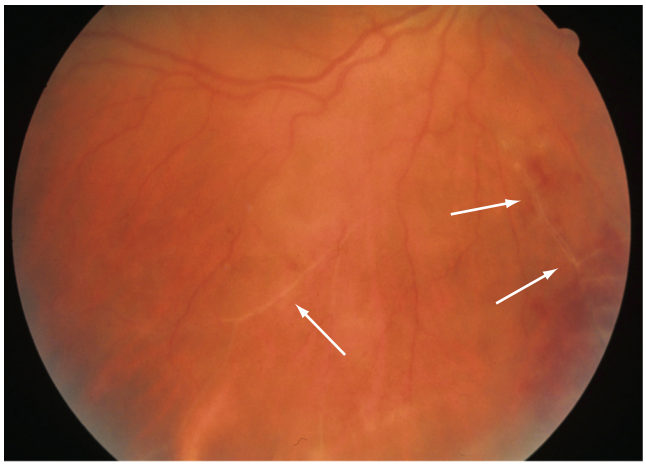


**Figure 44-6** Retinal vasculitis. *Arrows* indicate areas of vascular sheathing or occlusion.

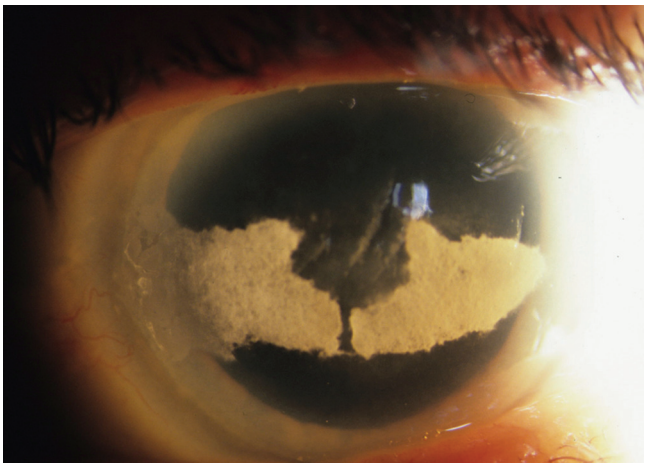


**Figure 44-7** Band keratopathy is illustrated by calcific patches stretching across the cornea.

widespread inflammation in the absence of detectable autoantibodies. Many autoinflammatory syndromes respond dramatically to inhibition of interleukin (IL)-1. Blau syndrome, which also is known as *familial granulomatous synovitis*, results from a single base change in the nucleotide-binding domain of the *NOD2* gene, which used to be known as *CARD15* or *NLRC2*.[19] Polymorphisms elsewhere in this same gene predispose to Crohn's disease. Blau syndrome is characterized by childhood onset of uveitis, arthritis, and dermatitis. Inflammation in additional organ systems also has been described. The disease is autosomal dominant. The histopathology of affected skin or joint can show noncaseating granuloma, as in sarcoidosis. Lung involvement has not been described in Blau syndrome, however. Gene sequencing has shown that many patients thought to have so-called early-onset sarcoidosis actually have new mutations in the *NOD2* gene.[20]

Neonatal-onset multisystem inflammatory disease (NOMID), which also is known as *chronic infantile neurologic cutaneous articular syndrome* (CINCA), is an autosomal dominant autoinflammatory syndrome. Ocular involvement in NOMID is more variable than in Blau syndrome. Characteristic findings include papilledema and uveitis.[21]

Treatment of uveitis depends on multiple factors, such as severity, location within the eye, patient preference, and the specific diagnosis (e.g., Behçet's disease might be especially responsive to infliximab[22] or interferon alfa[23]). For noninfectious causes of uveitis that involve the anterior portion of the eye, treatment usually begins with topical corticosteroids and often dilating drops to prevent posterior synechiae and to relieve spasm of the ciliary muscle. Periocular or intraocular corticosteroid injections, usually with triamcinolone, are given for inflammation posterior to the lens that is not responding to topical medication. Local corticosteroids can increase intraocular pressure, induce cataracts, interfere with the response to infection, and delay wound healing. A long-lasting corticosteroid implant containing fluocinolone has been approved by the U.S. Food and Drug Administration.[24] A cataract develops in all patients who elect to receive this type of therapy if the lens has not already been surgically removed, and glaucoma develops in most patients, with many persons requiring surgery to control the intraocular pressure. Intravitreal corticosteroid therapy can also be given in the form of dexamethasone in a slow-release formulation or as triamcinolone.

Systemic immunosuppressive therapy generally is reserved for patients with active, noninfectious causes of inflammation. For systemic immunosuppression to be indicated, the inflammation usually is bilateral and severe enough to interfere with activities of daily living. A variety of immunomodulatory medications have been tried to treat intraocular inflammation,[25] including azathioprine, chlorambucil, cyclophosphamide, cyclosporine, daclizumab, infliximab, methotrexate, mycophenolate mofetil, and tacrolimus. The optimal choice depends on many factors, not the least of which is the empiric result of any therapeutic approach. Maintaining a treatment for uveitis usually requires some efficacy in association with good tolerability. In this regard, at least one study found methotrexate to be superior to other anti-metabolites.[26] Because of the range of diseases being treated and the range of clinical response, room for all of these options exist in the therapeutic armamentarium of a uveitis clinic.

The optimal therapy for uveitis presumably depends on the underlying cause of the uveitis. The approach to uveitis in association with JIA should differ from the approach to uveitis resulting from Vogt-Koyanagi-Harada syndrome. However, the relative rarity of uveitis is such that few studies have been performed on specific forms of uveitis. For JIA-associated uveitis, many clinicians follow the algorithm of using topical therapy with prednisolone acetate initially and adding methotrexate if the inflammation is not controlled by drops used up to three times per day. If methotrexate does not help gain control of the inflammation, a monoclonal antibody to TNF is generally added to the regimen.

Biologic therapies increasingly are being used to treat patients with uveitis when the disease has proved refractory to other forms of immunomodulatory therapy. Infliximab is especially useful in the treatment of Behçet's disease.[22] One prospective study that assessed infliximab for a variety of forms of uveitis concluded that the drug was often effective but that it was also surprisingly toxic.[27] Consequently, the role of biologic therapies in most specific forms of uveitis is inadequately studied.[28]

In one review, a group of experts summarized the role of biologics in the therapy of various forms of uveitis.[29] A variety of investigational approaches for uveitis were being investigated at the time, including a monoclonal antibody to IL-17, a monoclonal antibody to IL-1β, and the intravitreal injection of rapamycin.

## SCLERITIS AND CORNEAL MELT

Many diseases cause a red eye, including conjunctivitis, keratitis, iritis, acute closed-angle glaucoma, episcleritis, and scleritis. Conjunctivitis is compared with episcleritis and scleritis in Table 44-4. A rheumatologist should refer patients with red eyes to an ophthalmologist if the problem is associated with pain, photophobia, and a change in visual acuity. Patients with persistent redness should also be referred.

**TABLE 44-4** Some Causes of Red Eyes: A Comparison

| | Conjunctivitis | Episcleritis | Scleritis |
|---|---|---|---|
| Discomfort | Scratchy | Scratchy | Pain |
| Redness | Diffuse, includes palpebral conjunctivae of lids | Diffuse or a sector, spares palpebral conjunctivae | Diffuse or a sector, spares palpebral conjunctivae |
| Causes | Allergy, viral, chemical irritant | Usually idiopathic but presumed immune response | Usually idiopathic but presumed immune response; can be an infection |
| Relation to systemic disease | Rare | Rare | Common (approximately 40%) |

Scleritis often is divided into five categories: diffuse anterior, nodular, necrotizing, scleromalacia perforans, and posterior (Figure 44-8). Each of the first three categories results in a red, painful eye. Pain is more variable in scleromalacia perforans, in which a nodule pathologically similar to a rheumatoid nodule forms in the sclera (Figure 44-9). Pain also varies with posterior scleritis, and because the sclera extends back to the optic nerve, posterior scleritis can occur in a localized fashion that does not lead to a red eye. Because of the risk of perforation, the sclera is not normally biopsied, but biopsy studies have shown that scleritis is often a granulomatous inflammation of scleral tissue.[30]

Patients with scleritis can experience complications within the eye, including uveitis, glaucoma, optic nerve edema, and retinal or choroidal distortion. A corneal melt or peripheral thinning of the cornea sometimes develops in persons with severe scleritis and represents a potentially blinding complication of the disease (Figure 44-10).

About 50% of patients with scleritis have an associated systemic illness.[31] The most common of such illnesses are limited granulomatosis with polyangiitis and rheumatoid arthritis. Generally, the associated rheumatoid arthritis is long standing and seropositive. Patients may have associated nodules, vasculitis, or pleuropericarditis. They have a shortened life expectancy compared with other patients with rheumatoid arthritis.[32] It is unusual for scleritis to be an initial manifestation of rheumatoid arthritis.

Granulomatosis with polyangiitis is commonly associated with scleritis. In contrast to rheumatoid arthritis, scleritis can be the initial manifestation of granulomatosis with polyangiitis. Obtaining anti-neutrophilic cytoplasmic antibody serology for any patient who presents with scleritis without an obvious systemic disease association is appropriate. Other systemic associations with scleritis include inflammatory bowel disease, relapsing polychondritis, other vasculitides such as giant cell arteritis, and ankylosing spondylitis. Infections are a rare but possible cause of scleritis. Tophaceous gout also has been reported as an unusual cause of scleritis.

Scleritis tends to be a painful and persistent disease that often lasts for years. In contrast, episcleritis involves more superficial tissue and is usually transient. Episcleritis may be a feature of rheumatoid arthritis, although many patients with episcleritis may not have any associated systemic illness. Complications within the eye, such as glaucoma or uveitis, are absent. Mild discomfort, rather than frank pain, is the usual presenting symptom. In contrast to scleritis, patients with episcleritis have vessels that constrict completely after 2.5% phenylephrine is placed on the surface of the eye.

Some patients with scleritis, especially those who do not have an associated systemic illness, are treated adequately with an oral nonsteroidal anti-inflammatory drug. Some experts treat scleritis with locally injected corticosteroids, but this approach should be avoided if the sclera is thin (a sign of necrotizing disease). In addition, corticosteroids have the theoretical risk of promoting thinning. The usual option for patients who do not respond to nonsteroidal anti-inflammatory drugs is oral prednisone. Some patients can be effectively treated with low doses of prednisone, but many patients require the addition of an antimetabolite as a steroid-sparing drug. Scleritis usually responds to treatment of the underlying disease if an associated disease is present. Thus control of rheumatoid arthritis or



**Figure 44-8** A scleral nodule. Active scleritis is present superior to the limbus. In this patient, scleritis has taken on a nodular configuration.

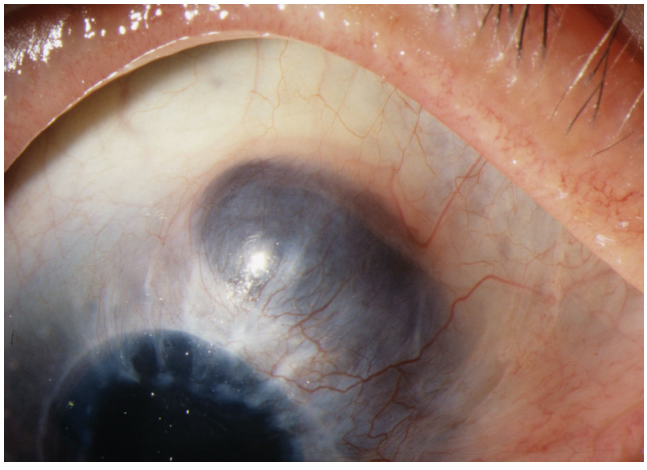


**Figure 44-9** Scleromalacia has resulted in ulceration of the sclera and a bluish appearance.

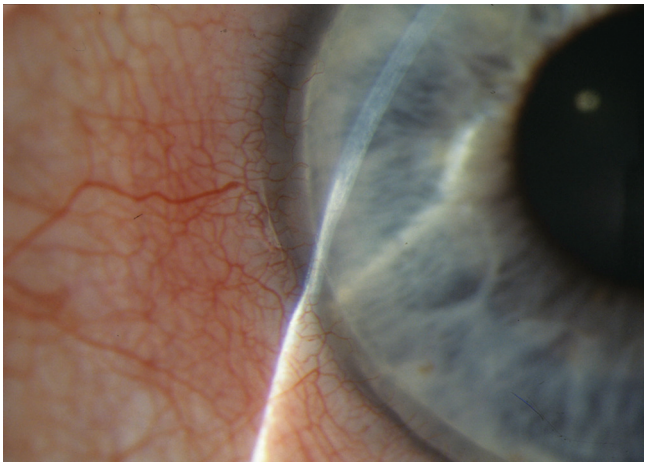


**Figure 44-10** Corneal melt. The white light of the slit lamp beam narrows over the peripheral cornea, where the tissue is thin.

inflammatory bowel disease usually results in control of associated scleritis. Rituximab is reportedly effective for the majority of patients with scleritis who fail to respond to antimetabolite therapy.[33]

## ORBITAL DISEASE

Graves' disease, the most common orbital inflammatory disease, generally results in an orbital myositis that can be identified on imaging such as CT, ultrasound, or MRI. From a rheumatologic perspective, granulomatosis with polyangiitis is the disease that most commonly affects the orbit. The inflammation can be extremely painful and may result in blindness. Orbital inflammation sometimes is more recalcitrant to therapy than other aspects of granulomatosis with polyangiitis. A small series suggested that rituximab may be efficacious in patients with granulomatosis with polyangiitis, including those with orbital involvement.[34]

Orbital pseudotumor, or nonspecific orbital inflammatory disease, is a diagnosis of exclusion that is made on the basis of objective orbital swelling as documented by imaging and a biopsy showing an inflammatory process that cannot be ascribed to another process, such as Graves' disease. A biopsy specimen of the orbit is not always obtained, but it can be useful in ruling out lymphoma or a metastatic malignancy as the cause of the proptosis. Methotrexate is a therapeutic option to treat nonspecific orbital inflammation.[35] Other systemic diseases that commonly affect the orbit include sarcoidosis. Rituximab has been used successfully to treat orbital inflammation that is refractory to antimetabolite therapy.[36]

## OPTIC NEURITIS

Optic nerve disease can result from many insults, including toxins (some of which are medications), vascular insufficiency (such as the insufficiency that occurs as a result of atherosclerotic disease or giant cell arteritis), and immunologic attack. The immune-mediated disease that most commonly affects the optic nerve is multiple sclerosis. This demyelinating condition generally starts suddenly in one eye with pain, an afferent pupillary defect, loss of color vision, and visual field loss typical of optic nerve disease. Initially the optic nerve may show papilledema, or it may appear normal if the inflammation is retrobulbar. Over several weeks, the affected nerve usually becomes pale.

Demyelinating disease affecting the optic nerve generally is not treated by a rheumatologist, but on rare occasions, patients with optic nerve disease have inflammation that might require long-term immunosuppression. These patients carry a diagnosis labeled variously as autoimmune optic neuropathy or, sometimes, steroid-sensitive optic neuropathy. This diagnosis is clinically distinct from optic neuritis associated with multiple sclerosis in that MRI of the head should not indicate a demyelinating process, the disease is often bilateral, the kinetics of the inflammation is different from that of multiple sclerosis, and the disease usually responds to oral corticosteroids. In most centers, a neuro-ophthalmologist would be involved in establishing this diagnosis. Systemic lupus erythematosus and sarcoidosis may affect the optic nerve in this way, but many patients with this diagnosis do not have an associated systemic illness. Therapy with an alkylating agent or an antimetabolite can be beneficial for many patients with this entity.[37]

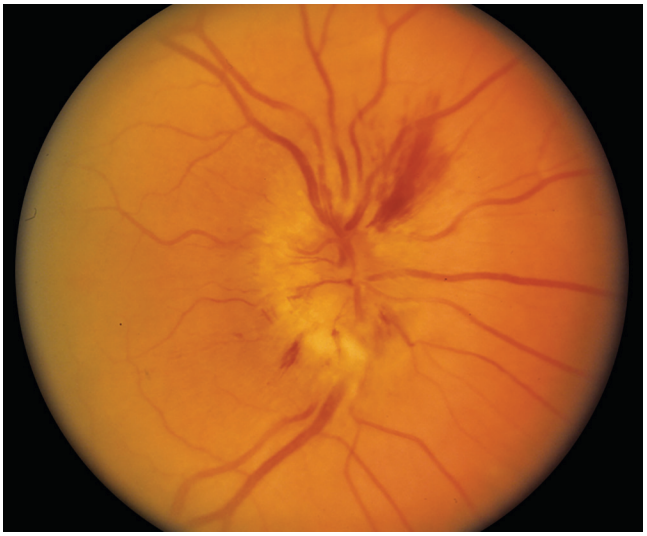


**Figure 44-11** Anterior ischemic optic neuropathy resulting from giant cell arteritis. The optic nerve is swollen, and surrounding hemorrhages are evident.

Sudden blindness is arguably the most feared consequence of giant cell arteritis. This disease is characterized by granulomatous inflammation of multiple vessels above the waist. These vessels frequently include the temporal artery and the posterior ciliary arteries. Inflammation in these latter vessels leads to anterior ischemic optic neuropathy (AION), which is ischemia of the optic nerve that manifests as sudden visual loss (Figure 44-11). Giant cell arteritis also can affect the central retinal artery, which may result in blindness. In this condition, the funduscopic appearance of the eye shows markedly reduced arteriolar flow and a cherry-red spot in the macula. Giant cell arteritis can cause diplopia by affecting circulation to extraocular muscles.

The visual loss associated with giant cell arteritis is frequently labeled arteritic AION to distinguish it from the more common nonarteritic AION, which usually is attributable to small vessel atherosclerosis. Patients with arteritic AION typically are older than 50 years and have an erythrocyte sedimentation rate greater than 50 mm/hr. Many patients with arteritic AION have associated symptoms of polymyalgia rheumatica, jaw claudication, scalp tenderness, or temporal artery tenderness. The biopsy specimen of the temporal artery shows vasculitis in about 80% of patients with giant cell arteritis if an adequate length of vessel is sampled. Twenty percent of patients with giant cell arteritis might have a biopsy specimen of the artery that is negative because the disease has spared this vessel or has affected it in a sufficiently patchy distribution that the biopsy specimen did not reveal the disease. Patients with nonarteritic AION tend to have small optic nerve cups.

## MEDICATION-RELATED OCULAR TOXICITY

A variety of medications have the potential to cause uveitis. These medications include rifabutin,[38] intravenously administered bisphosphonates,[39] moxifloxacin,[40] TNF inhibitors,[41] and ipilumumab.[42]

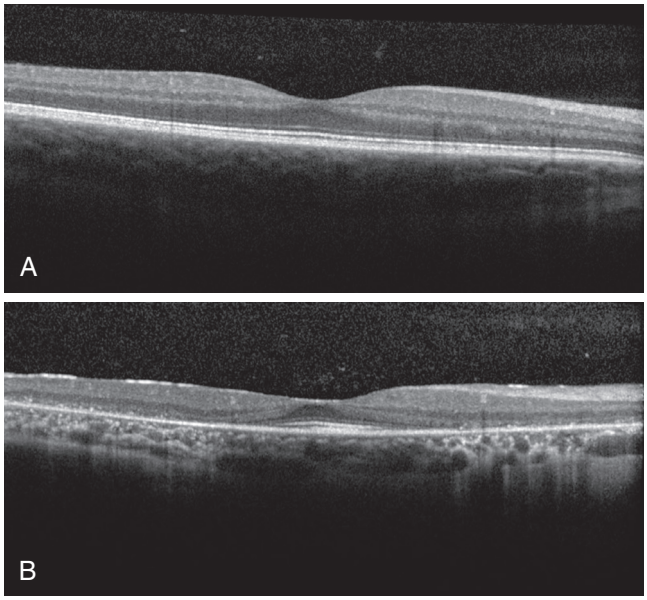



**Figure 44-12** Optical coherence tomography (OCT) demonstrating characteristic changes from antimalarial toxicity. **A,** An OCT of a normal retina. The "valley" at the center of the image is the macula. **B,** Characteristic changes from hydroxychloroquine toxicity. The thinning of the outer nuclear layer in the fovea results in a configuration sometimes called the "flying saucer sign." *(Photograph courtesy Mark Pennesi.)*

Rheumatologists need to be especially aware of the potential for antimalarial agents to cause retinal toxicity. The widespread use of optical coherence tomography (OCT) has resulted in the ability to image retinal layers accurately, quickly, and noninvasively. Although hydroxychloroquine was once considered to be a rare cause of retinal toxicity, OCT studies now show that retinal toxicity is roughly 7.5% among persons who take hydroxychloroquine (see Figure 44-12).[43] The likelihood of toxicity is related to both dosage and duration of therapy.[43] The American Academy of Ophthalmology has revised its recommendations to monitor for antimalarial toxicity. After an initial examination, routine examinations can be deferred until usage reaches 5 years. In addition to a dilated examination and a visual field examination, either OCT, fundus autofluorescence, or multifocal electroretinography, should be used to monitor for toxicity.[44]

## CONCLUSION

In some ways, from a rheumatologist's perspective, the eye is a microcosm of the body. Its complex structures frequently reflect inflammation elsewhere. Treatment of many forms of ocular inflammation requires collaboration between a rheumatologist and an ophthalmologist.

The references for this chapter can also be found on ExpertConsult.com.

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PART 6 DIFFERENTIAL DIAGNOSIS OF REGIONAL AND DIFFUSE MUSCULOSKELETAL PAIN

CHAPTER 45



# Neck Pain

Joseph S. Cheng • Raul Vasquez-Castellanos • Cyrus Wong

**KEY POINTS**

- Neck pain is a ubiquitous condition associated with enormous medical and legal costs.
- Physicians need to differentiate causes of neck pain that can be managed conservatively from causes that require more aggressive treatments.
- Knowledge of the anatomy helps with the diagnosis and the differentiation of symptoms from a musculoskeletal, neurogenic, or vascular cause.
- The history and clinical examination help focus the differential diagnosis and identify the origin of the neck pain based on the anatomy and physiology.
- Indicated imaging studies, neurophysiologic procedures, and laboratory studies aid in diagnosis and in determining a treatment plan for the patient's symptoms.
- In the absence of spinal instability, neurologic deficit, infectious process, or neoplastic process, the patient may benefit from conservative treatment with recovery expected in time.

## EPIDEMIOLOGY

Pain is an evolutionary protective mechanism to prevent further tissue damage. Neck pain is a ubiquitous condition with a lifetime prevalence of 67% to 71%.[1] The annual total cost for neck and low back pain corresponds to 1% of the gross national product in Sweden, with direct health-service costs representing only a small fraction of this percentage.[2-4] The medical and legal expenses associated with neck pain can be enormous, such as in whiplash injuries, which can result in $29 billion in costs annually in the United States alone.[5]

Neck pain may originate from various anatomic structures, including paraspinal soft tissues, the intervertebral joints and disk, compression of the spinal cord or nerve roots, and referred visceral pain. The cause of neck pain has a wide differential diagnosis, which can include trauma, degenerative changes, infection, autoimmune disorders such as rheumatoid arthritis (RA) and ankylosing spondylitis, and lifestyle modifiable factors like smoking. Several studies have identified smoking as the cause of myofascial pain syndromes, including neck pain.[6-11]

The perception and resultant reporting of this pain varies significantly based on cultural and social circumstance. Honeyman and Jacobs[12] noted that Australian Aborigines significantly underreport pain and are rarely disabled by pain. Social circumstances also play an important role in an individual's ability to cope with and overcome neck pain. Studies have demonstrated worse outcomes after diskectomy for patients with a workers compensation claim or litigation surrounding their condition.[13] These studies indicate nonorganic contributions to neck pain for secondary gain. Fortunately, most episodes of acute neck pain resolve with the "tincture of time" and patient education.

Physicians need to be able to differentiate causes of neck pain that can be managed with a conservative approach from those that require more aggressive treatments. An understanding of the anatomy and physiology and their association with the pathogenesis of neck pain provides the basis for obtaining a thorough history, physical examination, and ancillary data with the ultimate goal of effective treatment.

## ANATOMY

The cervical spine consists of seven vertebrae (C1 through C7; Figure 45-1). The bony anatomy of the atlas (C1) and axis (C2) are unique, whereas C3 to C7 demonstrate fairly consistent anatomy.[1] The atlas has no vertebral body; its lateral masses articulate with the skull's occipital condyles, forming the atlanto-occipital joints, which are supported by the anterior and posterior occipital membranes.[14] The atlanto-occipital joint is responsible for approximately 50% of total flexion and extension in the neck, with clear functional implications when this motion is lost. The axis (C2) has the dens or an odontoid peg that projects upward and anterior to articulate with the posterior aspect of the anterior arch of the atlas. The principal stabilizer of the odontoid to the anterior arch of the atlas is the transverse ligament, with the alar and apical ligaments acting as secondary stabilizers. C2 is a true synovial joint and thus is susceptible to inflammatory processes such as RA. There is no intervertebral disk between the atlanto-occipital joint and the atlanto-axial joint, and without the conferred

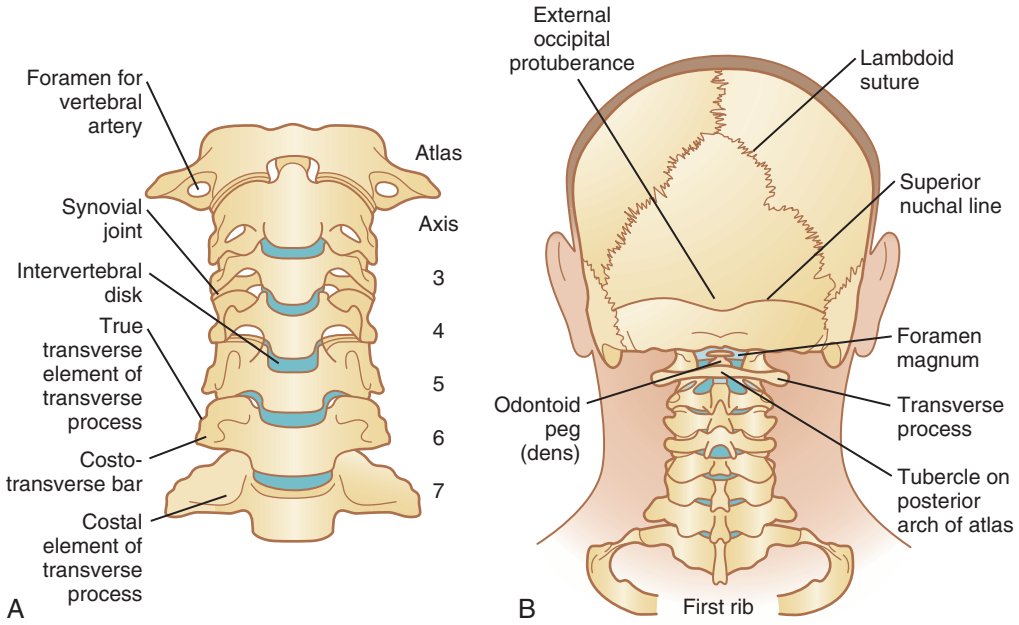



**Figure 45-1** Cervical spine anatomy. **A,** Anterior view. **B,** Posterior view.

stability from a disk, these joints are often affected by destructive inflammatory arthritides, which may result in instability.[15] The atlanto-axial articulation also provides approximately 50% of the rotatory motion of the neck.

The subaxial cervical spine consists of the C3 through C7 vertebrae, which all demonstrate fairly similar anatomy. Each vertebra consists of a body, two interconnecting pedicles, two lateral masses, two transverse processes, two lamina, and spinous processes. The transverse and spinous processes project outward, providing attachment for ligaments and muscles and creating a moment arm to facilitate motion. The spinous processes of C3 through C6 are bifid, whereas the C7 spinous process usually is not bifid. The C7 spinous process is large and the most prominent and easily palpable spinous process below C2.

Five articulations are found between each vertebra from C2 through C7, including the intervertebral disk, two uncovertebral joints, and two facet (zygoapophyseal) joints. The facet joints are true apophyseal joints with hyaline cartilage articulations, intervening menisci, synovial lining, and a joint capsule. This composition makes them susceptible to degenerative changes and systemic arthritides. The cartilage and synovial lining are aneural, whereas the joint capsule is highly innervated by the dorsal primary ramus. The facet joints are angled approximately 45 degrees from the transverse plane, articulating in concert with the uncovertebral joints and ligaments.

The intervertebral disks increase in size from C2 downward, giving the cervical spine its characteristic lordotic shape. Each disk consists of an outer annulus fibrosus and an inner nucleus pulposus, as well as a cephalad and caudal end plate. The annulus fibrosus consists of type I collagen, which gives form to the disk and provides tensile strength. The annulus fibrosus is innervated by the sinuvertebral nerve, formed by branches of the ventral nerve root and the sympathetic plexus.[16] The nucleus pulposus consists of type II collagen and proteoglycans, which interact with water to resist compressive stress. The pressure within the disk is highest with flexion, which may explain why individuals with a disk herniation find this position most uncomfortable.[17] Disk degeneration with aging includes loss of water content with resultant loss of height, annular tears, and myxomatous changes, increasing the risk of disk herniation. This process typically occurs in the posterolateral aspect of the disk, medial to the uncovertebral joints, where the posterior longitudinal ligament is not present and the annulus fibrosus is at its weakest.

The spinal column is supported by an interplay of ligaments and muscles (Figure 45-2). The anterior longitudinal ligament and posterior longitudinal ligament course along the anterior and posterior aspect of the vertebral bodies, with the anterior longitudinal ligament resisting hyperextension and the posterior longitudinal ligament resisting hyperflexion. The ligamentum flavum joins the laminae of adjacent vertebra and with degeneration may buckle and thicken, resulting in spinal canal stenosis and the potential for cord impingement. In a similar manner, the interspinous ligament joins the spinous process of adjacent vertebrae. The supraspinous ligament originates as the nuchal ligament at the occiput and extends caudally as an aponeurosis until it attaches to the tip of the spinous processes of C7 and then continues to the lumbar region. Fourteen paired anterior, lateral, and posterior muscles help orchestrate the complex movements of the neck.

A brief review of the spinal cord and nerve roots is beneficial and assists in performing a thorough evaluation. In general, the spinal cord is divided into the posterior column, the lateral column, and the anterior column. The posterior column mediates proprioceptive, vibratory, and tactile sensation; the lateral column is a conduit for motor fibers, along with pain and temperature sensation from the contralateral side of the body; and the anterior column conveys crude touch sensation. Humans have a total of eight cervical nerve roots as the dorsal and ventral roots converge to form

the spinal nerve within the vertebral foramen. Cervical nerve roots enter the intervertebral foramina by passing over the top of the corresponding pedicle, except the C8 cervical nerve, which lies between C7 and T1. Therefore, a C5-C6 posterolateral disk herniation will affect the C6 nerve root. The nerve root occupies approximately one-third of the foramen (Figure 45-3). The space available for the nerve root is decreased with neck extension and degenerative changes and increased with neck flexion.

The anterior spinal artery arises from the vertebral arteries and supplies the majority of the spinal cord, excluding the posterior columns. The posterior columns receive their blood supply from the two posterior spinal arteries, which originate from either the inferior cerebellar artery or the vertebral arteries. The vertebral arteries arise from the subclavian arteries and course cephalad through the C6 transverse foramen, passing anterior to the emerging cervical nerve root at each level. They pass behind the lateral mass of C1 and enter the foramen magnum. Diseases such as arterial dissection can be associated with severe neck pain and impairment of blood flow through the vertebral artery and can result in posterior circulation signs that include nystagmus, vertigo, drop attacks, dysarthria, and visual impairment. These symptoms are often associated with head position, and if a critical reduction of blood flow occurs, it can result in a cerebellar infarction.

The cervical spine is the most mobile segment of the spine; it has approximately 90 degrees of motion in flexion and extension, with three-fourths of this mobility due to extension (Table 45-1). The maximal range of motion in the sagittal plane within the subaxial spine is at the C5-C6

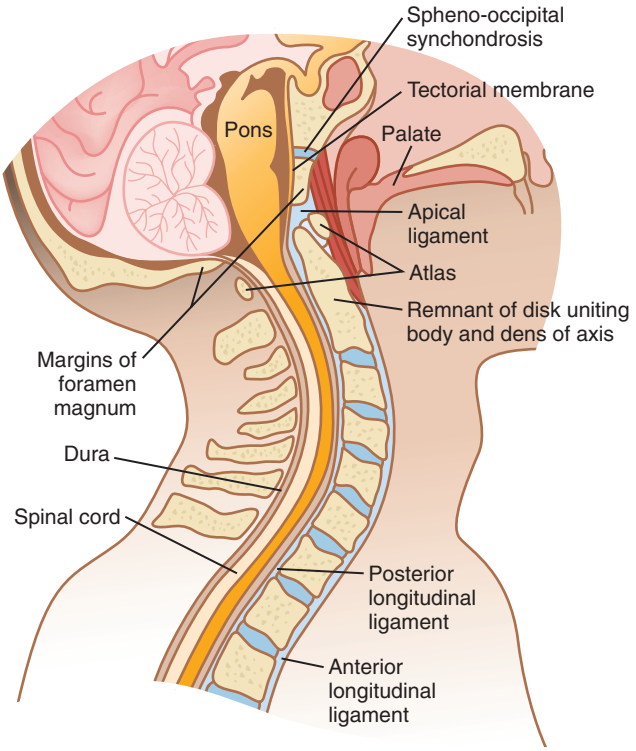



**Figure 45-2** Cervical spine anatomy: sagittal view.

**TABLE 45-1** Age and Normal Cervical Movement

| Age (yr) | Flexion-Extension (degrees) | Lateral Rotation (degrees) | Lateral Flexion (degrees) |
|---|---|---|---|
| <30 | 90 | 90 | 45 |
| 31-50 | 70 | 90 | 45 |
| >50 | 60 | 90 | 30 |

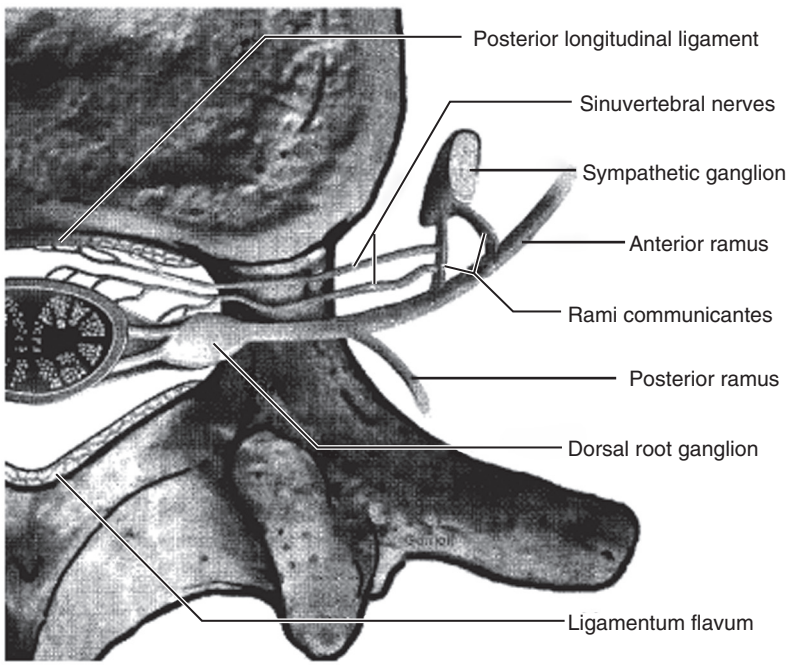



**Figure 45-3** Cervical spine anatomy. *(From Levin KH, editor:* Neck and back pain: continuum 7 [No. 1]. *Philadelphia, 2002, Lippincott Williams & Wilkins, p 9.)*

level, making it a common site of disk degeneration. Rotation encompasses approximately 80 to 90 degrees of motion, with 50% of this motion occurring at the atlanto-axial joint. Much like extension, rotation also reduces the cross-sectional area of the spinal canal. The cervical spine demonstrates 30 degrees of lateral mobility in each direction, which typically occurs with some degree of rotation as a result of the orientation of the facet joints.

## AXIAL NECK PAIN

Axial neck pain may originate from any tissue that receives innervation, including the zygoapophyseal joints, cervical disks, vertebral periosteum, posterior neck muscles, cervical dura mater, occipito-atlanto-axial joints, and the vertebral artery. The cause of neck pain may include degenerative, traumatic, malignant, infectious, and systemic inflammatory processes, as well as smoking. The most direct supporting data available link zygoapophyseal joints and cervical disks to the origin of axial neck pain. Provocative injections into the facet joint in asymptomatic volunteers invoke a reproducible pattern of occipital or axial neck pain.[18] This pattern of pain can be accurately diagnosed and treated, for at least a short period, with anesthetic injections targeted at the joint capsule itself or by blocking its respective dorsal primary ramus.[19-21] Degenerative arthritis within the upper cervical spine can manifest as suboccipital headaches, termed "cervicogenic headaches," which are thought to result from irritation of the greater occipital nerve. Typically arthritis within the atlanto-occipital joints is made worse with provocative neck flexion and extension, whereas atlanto-axial arthritis is made worse with rotation. This phenomenon is supported by a study that found that this pattern of pain was reproduced when asymptomatic volunteers underwent injections at the atlanto-occipital and atlanto-axial joints.[22] Relief of suboccipital pain can be obtained by fluoroscopically guided injection of corticosteroid into the diseased joint, or fusion of these joints in recalcitrant cases.

The cervical disk is a more controversial source of axial neck pain, with pain occurring as a result of insult of the highly innervated annulus fibrosis. This idea is based on provocative diskography, whereby a diseased disk is fluoroscopically injected to a given pressure. A positive test occurs when there is reproduction of pain in reliable patterns. To be a true positive or a "concordant study," an adjacent normal disk should not produce pain when injected. Using this method, several studies have implicated cervical disks as a source of axial neck pain.[16,23,24] Even with use of careful technique, a false-positive finding can occur, and it is not unusual to have a "nonconcordant study" with multiple disks eliciting a pain response despite being normal.[16] Interventions directed at treating the disk for isolated axial neck pain have been found to be unpredictable, despite the fact that the cervical disk likely contributes to axial neck pain.

Myofascial pain due to irritation of the muscles in the neck region can contribute to axial neck pain. Patients with chronic myofascial pain have been shown to have a lower level of high-energy phosphates in the involved muscle tissue.[25] Myofascial pain can be the original source of neck pain or, more commonly, a manifestation of postural adaptations, compensatory overuse of normal tissue that remains after the injured structure heals, and lifestyle habits like smoking. A more generalized form of myofascial pain is fibromyalgia, a widespread disorder defined by diffuse pain that affects all four quadrants of the body, with at least 11 of 18 pressure points being positive. Patients have associated symptoms of fatigue, cognitive difficulties, irritable bowel syndrome, and a nondermatomal pattern of dysesthesias, weakness, and parasthesias.[26]

Systemic inflammatory arthropathies causing neck pain typically demonstrate the classic pattern of morning stiffness, polyarticular involvement, rigidity, and associated cutaneous manifestations. RA often involves the cervical spine, initially causing stiffness and later causing pain and potentially leading to instability. After the hands and feet, the cervical spine is the most common site of disease involvement in RA.[27] The upper cervical spine is most commonly involved (occiput to C2), followed by the subaxial cervical spine (C3 to C7). The likelihood of developing cervical spine disease can be predicted by the amount of rheumatoid changes seen in the hands and feet. Basilar invagination is one such manifestation, whereby the C1 lateral masses erode, allowing the odontoid peg to settle into the foramen magnum and place pressure on the brain stem with the potential for instantaneous death. The atlanto-axial joint can also demonstrate instability with the potential for neurologic injury. Because of these potential catastrophic complications, dynamic radiographs of the cervical spine should be obtained prior to any procedure requiring intubation. The seronegative spondyloarthropathies that can manifest with neck pain include ankylosing spondylitis, psoriatic arthritis, and reactive arthritis. Psoriatic arthritis will manifest with skin lesions before the development of arthritis in 70% of patients, and reactive arthritis rarely involves the cervical spine.

Ankylosing spondylitis often affects the entire axial skeleton with limitation of lumbar motion and chest expansion and later involvement of the cervical spine. In progressive patterns the cervical spine will take on a kyphotic deformity, and as the spine fuses it biomechanically becomes similar to a long bone. As such, minor trauma with neck pain should be taken very seriously in these patients. Even in the face of negative plain radiographs, these patients should undergo an extensive workup with a CT scan, observe strict spine precautions with appropriate neutral alignment according to their baseline spinal curvature, and undergo frequent neurologic evaluations to assess for development of an epidural hematoma.

Infection and neoplasms can cause axial neck pain through bone destruction, with irritation of vertebral body periosteal nerves and altered biomechanics on the facet joints and cervical disks. The onus is on the clinician to identify these patients at the initial visit because a delay in diagnosis can have catastrophic consequences. Red flags for axial neck pain that require further workup at the initial presentation include elderly patients or patients with a history of malignancy, immunocompromised patients, fevers, chills, unexplained weight loss, fatigue, nighttime awakening, recent antecedent bacteremia, and severe nonmechanical neck pain.[26]

Patients who have undergone previous cervical spine surgery should be evaluated for the presence of a pseudoarthrosis or iatrogenic instability. A pseudoarthrosis is failure

of an attempted arthrodesis or fracture to fully heal with bridging bone. These patients describe a "honeymoon" period in which they did well for 3 to 6 months after surgery but then experience worsening axial neck and interscapular pain with associated headaches. Pseudoarthrosis can be diagnosed on plain radiographs with evidence of hardware loosening or movement on dynamic images. A CT scan with coronal and sagittal reconstructions should be obtained to more definitively diagnose a pseudoarthrosis. Once a pseudoarthrosis has been diagnosed, a surgical consult should be obtained, a bone metabolic workup should be undertaken, and the patient should be counseled on smoking cessation, given the deleterious effect of nicotine and carbon monoxide on bone healing. An additional cause of neck pain after surgery is iatrogenic instability, whereby the surgery itself created pathologic motion. This condition requires a surgical consultation to determine if stabilization is warranted.

## RADICULOPATHY AND MYELOPATHY

The clinician must determine if there is evidence of nerve root compression, termed *radiculopathy*, versus a spinal cord compression, termed *myelopathy*. Cervical spondylosis and changes within the disk may cause loss of height with posterior bulging of the disk into the spinal canal and foramen. As the disk collapses, the posterior soft tissue structures, including the ligamentum flavum and facet joint capsule, buckle inward, further compromising the spinal canal and neural foramen. Pressure that was once dispersed throughout the disk is then transferred to the facet joints and uncinate processes, resulting in the development of bone overgrowth or osteophytes, which cause extrinsic pressure on the nerve root or spinal cord.

Radiculopathy is a problem with the peripheral nervous system that affects the exiting nerve root. With radiculopathy, mechanical distortion of the nerve occurs, leading to increased vascular permeability, resulting in chronic edema and eventually fibrosis. This condition leads to hypersensitivity of the nerve root with an inflammatory response mediated by chemicals released from the cell bodies of sensory neurons and the cervical disk.[28] Compression of the dorsal root ganglion is believed to be especially important in producing radicular pain.[29] Clinically, this compression presents with pain in a dermatomal distribution, which is easily identifiable for roots C5-T1. However, dermatomes for the higher cervical nerve roots, including C3 and C4, are along the posterior scapula and should not be confused with isolated axial neck pain.[30] Minor symptoms that are tolerable may be treated with conservative care, but persistent compression on a nerve root can lead to sensory loss and weakness. Disabling deficits should be treated operatively given that prolonged nerve compression can result in irreversible changes. In patients without a neurologic deficit, it is reasonable to expect a good outcome with conservative care.[31]

Myelopathy is a problem with the central nervous system and is a clinical presentation of long tract signs from compression of the spinal cord. Factors that contribute to the development of myelopathy include a congenitally narrow spinal canal, dynamic cord compression, dynamic thickening of the spinal cord, and vascular changes. The anterior-posterior diameter in the subaxial spine for a normal adult measures 17 to 18 mm, with diameters of less than 13 mm considered congenitally stenotic. The cord measures 10 mm. The shape of the spinal cord deformity has a high association with development of myelopathy because patients with a banana-shaped cord on axial views had evidence of myelopathy 98% of the time.[32] Ono and associates[33] described a ratio whereby the anterior-posterior diameter of the spinal cord is divided by the transverse diameter of the cord. Patients with a ratio of less than 0.40 tended to have severe neurologic deficits.[33] Certain patients may have dynamic cord compression with signs and symptoms of myelopathy only during neck flexion and extension. The space available for the cord is decreased during neck extension because of infolding of the ligamentum flavum and overlapping of the lamina. Also during extension, the spinal cord shortens as the distance from the skull base to the cervical spine is lengthened, effectively increasing cord diameter and making it more prone to compression by the posterior structures. In flexion, the cord lengthens and drapes over anterior degenerated disks and osteophytes.[34]

Myelopathy can be exacerbated by altered biomechanics from degenerated segments. For example, when a given level stiffens, the level above can become hypermobile.[35] A certain subset of patients can experience myelopathy in the absence of mechanical compression, which has been attributed to ischemic insult.[36] Despite the prevalent theory of ischemia, the precise molecular mechanisms that contribute to the development of myelopathy are not completely known. Some studies have debated the role of ischemia, proposing a complex cascade of bimolecular events that leads to the disease.[37] Patients with mild cases of myelopathy that do not affect activities of daily living can be closely monitored.[38] Patients with more severe deficits or progressive deficits tend to deteriorate over time with conservative care, and it is recommended that these patients undergo surgery to decompress the spinal cord.[39]

### Infection/Neoplasm

Outside the degenerative cascade, infection and neoplasm can be the source of axial neck pain or neurologic deficit. In terms of infection, several entities are often seen: diskitis, osteomyelitis, or epidural abscess. Risk factors for infections of the spine include immunosuppression, intravenous drug use, and alcohol abuse, among others. Infection can also spread after lumbar puncture or epidural anesthesia.

The most common symptoms of spinal infection include excruciating pain and tenderness, accompanied by constitutional symptoms, including fever, night sweats, and lethargy. Disease progression may eventually lead to neurologic deficits such as motor deficits, sensory loss, and bowel/bladder dysfunction. Neurologic deficits are more common with epidural abscesses and are less commonly seen with osteomyelitis and diskitis. Whether CT or MRI scans are performed, contrast is needed in the setting of infection. MRI best evaluates epidural abscesses, but if no deficit is seen, CT may help with evaluating the bony destruction and mechanical instability.

The most common organisms seen in spinal infections include *Staphylococcus aureus*, streptococcus, *Escherichia coli*, *Pseudomonas aeruginosa* (especially in intravenous drug

abusers), and *Haemophilus influenza* (pediatric diskitis). In 29% to 50% of the cases an organism is not identified, especially when antibiotics have been started.[40]

Diskitis and osteomyelitis often can be treated nonsurgically. Long-term treatment with antibiotics in combination with bracing often can treat the underlying infection and avoid progressive deformity.[40] The antibiotics used most often include vancomycin, rifampin, and a third-generation cephalosporin for 4 to 6 weeks with or without an oral agent. These patients are watched closely for progression of disease—that is, worsening pain or neurologic deficit—and also are monitored with serial inflammatory markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Because of the nature of the antibiotics and the prolonged treatment time, kidney function must be monitored as well. Refractory infections and/or suspected instability cases should be referred for a surgical consultation.

Because of their proximity to the spinal cord and high chance for rapid neurologic deterioration, epidural abscesses have a lower threshold for surgical treatment, and a surgical consultation should always be considered.[40-43] Nonsurgical management is indicated in the neurologically intact patient with a small abscess or in patients too unstable for surgery.[40] In the setting of a progressive deficit or large abscess, the main goals of surgery are to decompress the neural elements and disrupt the abscess capsule, which helps with antibiotic penetration and infection control.

Neoplasms represent another source of neck pain. Similar to infection, neoplastic processes cause axial neck pain through bone destruction and irritation of periosteal nerves and altered biomechanics of facet joints and the anterior column, leading to progressive cervical kyphosis. The spine is the most frequent site of bone metastases, and spinal metastases will develop in 5% to 10% of patients with cancer.[44-47] Common tumors that metastasize to the spine are lung, breast, and prostate. A smaller percentage of cervical spine tumors are primary spinal tumors, including ependymomas, astrocytomas, schwannomas, neurofibromas, or meningiomas. Spinal cord tumors are often classified based on location: extradural, intradural-extramedullary, or intramedullary. MRI with contrast is the imaging modality of choice to visualize the tumor. Consultation with a spine surgeon should be requested in all neoplasm cases.

Treatment of spinal tumors is based on tumor disease, neural compression, and the presence of instability. The main driver of treatment in terms of tumor biology is its radiosensitivity. Radiosensitive tumors can be emergently treated with radiation even in the setting of compression of neural elements. The most common radiosensitive tumors include lymphoma and multiple myeloma. Stereotactic radiation is becoming increasingly popular as a method of maximizing radiation to the surrounding tumor without damaging the sensitive spinal elements.[48-51] For metastatic spinal tumors causing spinal cord compression, the standard of treatment is surgical decompression followed by radiation.[52]

## CLINICAL FEATURES

In terms of functional anatomic pathways, neck pain is mediated via somatic or autonomic pathways.[53] Somatic pain, the most common, is perceived in dermatomes, myotomes, or sclerotomes. Pain originating in the autonomic pathway, or sympathetic nervous system, may fall into somatic segmental distributions, vascular supply distributions, peripheral nerve distributions, or nonconforming patterns. Because pain mediation pathways may have significant overlap, additional clinical information regarding characteristics of the neck pain along with diagnostic studies will complement the determination of the pain origin.

### Patient History

Neck pain is the most common symptom of cervical spine disease, and correctly characterizing it helps to identify conditions requiring immediate treatment. Important characteristics to note include onset, distribution, frequency, duration, quality, aggravating factors, and the presence of neurologic symptoms. In general, pain that is present only intermittently may be indicative of instability or motion, whereas constant and increasing pain is a source of concern for a mass effect. The new onset of generalized neck pain that is of relatively short duration is likely related to benign disease, including muscle strain, whereas a longer duration of symptoms indicates significant or progressive disease. A well-localized quality of pain indicates specific nerve root irritation, whereas poorly defined pain may derive from irritation of deep connective tissue structures such as muscle, joint, bone, or disk. Aggravating and relieving factors may help elucidate biomechanical changes in the cervical spine that are contributing to the symptomatology.

Localized axial neck pain is commonly reported as originating posteriorly with extension into the shoulder or occiput. Localized pain of myofascial origin may worsen with neck flexion, whereas diskogenic neck pain will worsen with neck extension or rotation. Referred pain to the occiput usually indicates pathologic changes in the upper cervical spine and may radiate down the neck and to the ear. Shoulder girdle pain develops as a result of postural adaptations from initial neck pain symptoms. It is not uncommon for pain to be referred from the shoulder, heart, lungs, viscera, or temporomandibular joint to the neck region because of overlapping nerve distribution. Symptoms may arise as a result of irritation or activation of receptors directly, as with articular pain, pseudoarticular pain, vascular pain, cervicogenic headaches, pseudoangina pectoris, eye and ear symptoms, and throat symptoms.

Articular symptoms arise from innervation to the facet and uncovertebral joints, causing local pain and stiffness. Patients often state that their symptoms are made worse with inactivity and describe feelings of clicking, grating, or "sand" in the neck. Pseudoarticular pain may be felt in the shoulder and elbow, with the true disease originating from the neck. Vascular symptoms result from compression of the vertebral artery by osteophytes or a protruding disk. Symptoms may intensify with neck movement or certain postures. Tenosynovitis and tendonitis may involve the rotator cuff and tendons about the elbow, wrist, or hand. Stenosis or fibrosis of tendon sheaths or palmar fascia may be present, along with trigger points over the affected joints, giving a false impression of local disease.[54]

**TABLE 45-2** Cervical Nerve Root Segments and Corresponding Clinical Signs

| Nerve Root | Symptom | Correlate |
|---|---|---|
| C3<br>C4 | Suboccipital pain with extension to the back of the ear<br>Pain from the caudal aspect of the neck to the superior aspect of shoulder | If C3, C4, and C5 are all involved, paradoxical breathing might occur |
| C5 | Numbness over the shoulder and down the lateral aspect of the arm to the mid portion; the deltoid muscle may be weak and the biceps reflex may be affected | |
| C6 | Radiating pain and numbness down the lateral aspect of the arm and forearm to the thumb and index finger ("six shooter"); weakness in wrist extension, elbow flexion, and supination; diminished brachioradialis and biceps reflex | Sensory component can mimic carpal tunnel syndrome |
| C7 | Numbness and pain down the posterior aspect of the arm and forearm to the long finger; weakness in the triceps, wrist flexion, and finger extensors | Most frequent; posterior interosseous nerve entrapment can mimic motor component, but no sensory deficits are present |
| C8<br>T1 | Pain and numbness down the medial aspect of the arm and forearm into the 4th and 5th digits; weakness in the flexor digitorum profundus to IF and LF and FPL | AIN entrapment can mimic motor component of C8 or T1; however, sensory changes and involvement of thenar muscles will not be present; ulnar nerve entrapment will spare short thenar muscles with the exception of ADP; does not involve FPL or FDP to IF and LF |

ADP, Adductor pollicis; AIN, anterior interosseous nerve; FDP, flexor digitorum profundus; FPL, flexor pollicis longus; IF, index finger; LF, long finger.

## Localization of Pain Generators

Pain may be somatic or autonomic and is not always felt in precise anatomic zones. Overlapping sensory supplies may be present, as well as radiation in spinal segments by recruitment within the spinal column, causing difficulty in localization. Somatic pain is caused by cervical nerve root irritation. It is the most common type of pain, and diabetics are more susceptible to this nerve root irritation. Neurologic deficits correspond with the offending disk level in 80% of patients.[55]

Neuralgic and myalgic pain describes symptoms resulting from compression of different areas of the nerve root. Neuralgic pain originates from irritation of the dorsal sensory root and has a "lightning" or "electric" sensation that tends to be dermatomal and associated with numbness and paresthesias. The pain tends to present more proximally, with paresthesias more distally. Myalgic pain occurs with irritation of the ventral motor root. This pain is described as a deep, boring, and unpleasant sensation that tends to be poorly localized because of its referral to sclerotomal areas. These sensations conform to the areas of muscles that are innervated by the compressed nerve root. Autonomic-mediated symptoms result in dizziness, blurring of vision, tinnitus, retroocular facial pain, and jaw pain.

It is important to determine if the axial neck pain is isolated or if there is associated radiating pain, weakness, changes in sensation, or alterations in proprioception. The compression of a nerve root often can be localized by identifying the distribution of pain, paresthesia, or weakness as it follows segmental distribution of that respective nerve root (Table 45-2). Sensory loss may be described by the patient in precise terms with a description of numbness, or alternatively the patient may have vague symptoms of swelling or bogginess to the skin. If the face, head, or tongue is involved, the upper three nerve roots of the cervical plexus may be affected. Numbness of the neck, shoulder, arm, forearm, or fingers indicates involvement of C5-T1. Weakness, as with sensory changes, occurs in a graded fashion depending on the amount of compression on the nerve root. This weakness will present clinically with an obvious functional deficit or more subtle findings, made obvious only after repetitive testing. The clinician must be attuned to these subtle complaints of weakness, which may be described by the patient as a feeling of heaviness of the limbs, early fatigue, or insufficient power in the grip. If obvious atrophy is present in a muscle, then more than one nerve root is affected because of the evolutionary benefit of multiple levels of innervation for a given muscle. Alterations in proprioception are due to compression of the dorsal column of the spinal cord and will be described by the patient as symptoms of clumsiness, with reports of tripping or dropping objects. This presentation raises concern for the more ominous condition of myelopathy, which is secondary to spinal cord compression.

Cervical spinal disease classically causes isolated axial neck pain or a radicular pain that radiates to the shoulder or down the upper extremity (Table 45-3). However, less commonly it can be the cause of headache, pseudoangina pectoris, and otolaryngologic sensations. Cervicogenic occipital headaches can be compounded by adaptive changes in the posterior occipital muscles and often spread

**TABLE 45-3** Cervical Nerve Root Pain Referral Pathways

| Location of Pain | Source |
|---|---|
| Upper posterolateral cervical region | C0-C1, C1-C2, C2-C3 |
| Occipital region | C2-C3, C3 |
| Upper posterior cervical region | C2-C3, C3-C4, C3 |
| Middle posterior cervical region | C3-C4, C4-C5, C4 |
| Lower posterior cervical region | C4-C5, C5-C6, C4, C5 |
| Suprascapular region | C4-C5, C5-C6, C4 |
| Superior angle of scapula | C6-C7, C6, C7 |
| Midscapular region | C7-T1, C7 |

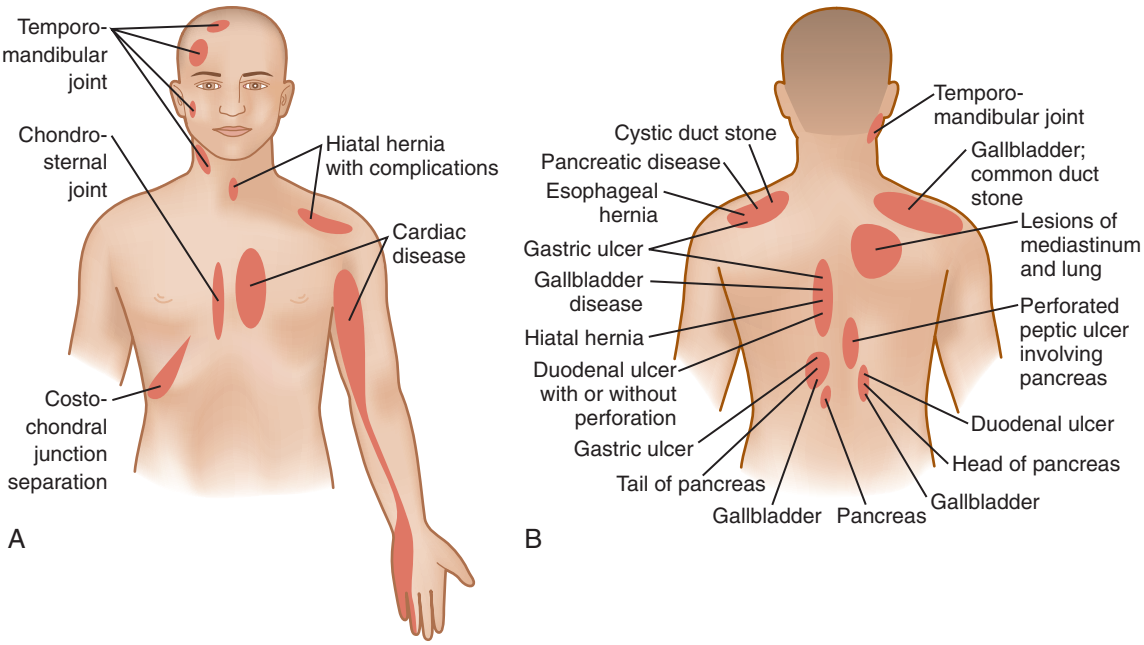



**Figure 45-4** Patterns of referred pain. **A,** Anterior distribution. **B,** Posterior distribution.

to the eye region, manifesting as a dull rather than a pulsating pain. These headaches are unique in that they are aggravated by neck movements. They typically have migraine-like symptoms, including phonophobia or photophobia.

Pseudoangina pectoris has been reported as a result of cervical spinal disease and can be confused with angina pectoris or breast pain in women (Figure 45-4). In the presence of a C6-C7 lesion, neuralgic or myalgic pain may be present along with tenderness in the precordium or scapular region. Differentiation of heart disease from symptoms associated with C6-C7 dysfunction is made on the basis of muscle weakness, fasciculations, sensory changes, or reflex changes. Differentiation of these two diseases may be difficult when true angina and pseudoangina coexist in the same patient.[56]

Cervical spinal disease may manifest in the form of eye, ear, and throat symptoms (Figure 45-4). Eye and ear symptoms may arise from irritation of the plexuses surrounding the vertebral and internal carotid arteries. Eye symptoms can present with blurring of vision relieved by changing neck position, increased tearing, orbital and retro-orbital pain, and descriptions of eyes being "pulled backward" or "pushed forward." Altered equilibrium with associated gait disturbances may result from irritation of surrounding sympathetic plexus or vertebral insufficiency. Hearing can be affected with tinnitus and altered auditory acuity. Throat symptoms, including dysphagia, may be related to anterior vertebral osteophytes that cause direct compression, as well as cranial nerve and sympathetic nerve communications.

Symptoms of dyspnea and cardiac arrhythmia and drop attacks may have a cervical spinal origin. Dyspnea can be related to a deficit in C3-C5 innervation of the diaphragm. Cardiac palpitations and tachycardia resulting from cervical spine disease can be differentiated from other causes by the fact that these symptoms are associated with unusual positions or hyperextension of the neck. This presentation is caused by irritation of C4 innervation of the diaphragm and pericardium or irritation of the cardiac sympathetic nerve supply. Drop attacks suggest posterior circulation insufficiency, resulting in an abrupt loss of proprioception without the loss of consciousness.

Myelopathy, or spinal cord compression, initially presents with subtle complaints of hand clumsiness or difficulty with balance. Patients will report worsening handwriting in the past few months or difficulty buttoning shirts. Patients also may have nausea and emesis caused by equilibrium dysfunction. Paresthesias and dysesthesias may be present, often involving bilateral upper extremities and not following a dermatomal distribution. This presentation is often mistaken for peripheral neuropathy or carpal tunnel syndrome but should be considered when bilateral extremity symptoms are present. As the disease progresses over time, more advanced manifestations occur, including most commonly weakness in the triceps, hand intrinsic, and hip flexors. Late manifestations include spasticity, as well as bowel and bladder dysfunction.

Finally, neck pain may present concomitant with systemic disease with varied symptoms that require further investigation. Examples include inflammatory arthritides, infection or tumor, multiple sclerosis, subacute combined degeneration, or syrinx. Inflammatory arthritides often present with morning stiffness, polyarticular involvement, or cutaneous manifestations. Fever, weight loss, or night pain points to an infectious or neoplastic cause. A Pancoast's tumor is a neoplastic process of the apical portion of the lung that can cause a mass effect on the caudal cervical nerve roots. This tumor should always be considered in a person with radicular symptoms and a history of smoking, with workup including a chest radiograph. Idiopathic brachial plexus neuritis, formerly known as Parsonage-Turner syndrome, is caused by viral infection of the brachial plexus that presents with severe arm pain involving multiple nerve

roots. The classic presentation is weakness and variable deficits after the acute phase of severe pain resolves. Subacute combined degeneration from $B_{12}$ deficiency is a consideration when greater sensory deficit is present in the lower extremities.

## Clinical Examination

A careful clinical examination provides additional focus to the differential diagnosis. The clinical examination begins by broadly observing the patient's gait, as well as his or her head and neck posture. Results of further palpation, range of motion testing, and a neurologic examination for motor signs, reflexes, sensory signs, autonomic signs, and articular signs are assessed (Table 45-4).

Careful palpation with knowledge of key anatomic bony and soft tissue landmarks in the cervical spine may localize pain to a particular cervical level and location. Anteriorly or anterolaterally, the transverse process of C1 is palpated between the angle of the jaw and the styloid process. C3 is identified by palpation of the hyoid bone. C4-C5 is at the level of the thyroid cartilage, and C6 is at the level of the cricoid ring and the carotid tubercle. In the process of examining the neck, the sagittal balance with retention or loss of normal cervical lordosis also should be noted. Posteriorly and posterolaterally, the occiput, inion, superior nuchal line, mastoid processes, and spinous processes of C2 and C7-T1 are palpable.

Soft tissues about the anterior and posterior triangles of the neck, occipital region, and posterior paraspinal muscles

**TABLE 45-4** Nerves and Tests of Principal Muscles

| Nerve | Nerve Roots | Muscle | Test |
|---|---|---|---|
| Accessory | Spinal | Trapezius | Elevation of shoulders<br>Abduction of the scapula |
| | Spinal | Sternocleidomastoid | Tilting of the head to the same side with rotation to the opposite side |
| Brachial plexus | | Pectoralis major | |
| | C5, C6 | Clavicular part | Adduction of the arm |
| | C7, C8, T1 | Sternocostal part | Adduction, forward depression of the arm |
| | C5, C6, C7 | Serratus anterior | Fixation of the scapula during forward thrusting of the arm |
| | C4, C5 | Rhomboid | Elevation and fixation of the scapula |
| | C4, C5, C6 | Supraspinatus | Abduction of arm initiated |
| | (C4), C5, C6 | Infraspinatus | External rotation of the arm |
| | C6, C7, C8 | Latissimus dorsi | Adduction of horizontal, externally rotated arm, coughing |
| Axillary | C5, C6 | Deltoid | Lateral and forward elevation of the arm to the horizontal position |
| Musculocutaneous | C5, C6 | Biceps<br>Brachialis | Flexion of the supinated forearm |
| Radial | C6, C7, C8 | Triceps | Extension of the forearm |
| | C5, C6 | Brachioradialis | Flexion of the semiprone forearm |
| | C6, C7 | Extensor carpi radialis longus | Extension of the wrist to the radial side |
| Posterior interosseous | C5, C6 | Supinator | Supination of the extended forearm |
| | C7, C8 | Extensor digitorum | Extension of the proximal phalanges |
| | C7, C8 | Extensor carpi ulnaris | Extension of the wrist to the ulnar side |
| | C7, C8 | Extensor indicis | Extension of the proximal phalanx of the index finger |
| | C7, C8 | Abductor pollicis longus | Abduction of the first metacarpal in plane at a right angle to the palm |
| | C7, C8 | Extensor pollicis longus | Extension of the first interphalangeal joint |
| | C7, C8 | Extensor pollicis brevis | Extension of the first metacarpophalangeal joint |
| Median | C6, C7 | Pronator teres | Pronation of the extended forearm |
| | C6, C7 | Flexor carpi radialis | Flexion of the wrist to the radial side |
| | C7, C8, T1 | Flexor digitorum superficialis | Flexion of the middle phalanges |
| | C8, T1 | Flexor digitorum profundus (lateral part) | Flexion of the terminal phalanges, index, and middle fingers |
| | C8, T1 | Flexor pollicis longus (anterior interosseous nerve) | Flexion of the distal phalanx, thumb |
| | C8, T1 | Abductor pollicis brevis | Abduction of the first metacarpal in plane at right angle to the palm |
| | C8, T1 | Flexor pollicis brevis | Flexion of the proximal phalanx, thumb |
| | C8, T1 | Opponens pollicis | Opposition of the thumb against the fifth finger |
| | C8, T1 | First and second lumbricals | Extension of the middle phalanges while the proximal phalanges are fixed in extension |
| Ulnar | C7, C8 | Flexor carpi ulnaris | Observation of tendons during testing of abductor digiti minimi |
| | C8, T1 | Flexor digitorum profundus (medial part) | Flexion of distal phalanges of the ring and little fingers |
| | C8, T1 | Hypothenar muscles | Abduction and opposition of the little finger |
| | C8, T1 | Third and fourth lumbricals | Extension of the middle phalanges while the proximal phalanges are fixed in extension |
| | C8, T1 | Adductor pollicis | Adduction of the thumb against the palmar surface of the index finger |
| | C8, T1 | Flexor pollicis brevis | Flexion of the proximal phalanx, thumb |
| | C8, T1 | Interossei | Abduction and adduction of the fingers |

are examined. The sternocleidomastoid muscle is involved with whiplash injuries, whereby abrupt hyperextension of the neck occurs. The muscle may be tender to palpation, or the patient may be splinting the neck with the head turned away from the injured muscle. This posturing of the neck is termed *torticollis*, and the clinician should remember that the head is turned away from the side of the involved sternocleidomastoid. Flexion injuries may traumatize the trapezius muscle. Midline cervical tenderness is more of a concern for ligament injury, whereas paraspinal muscle tenderness is typically a more benign process.[57] The greater occipital nerves are located lateral to the inion and may be involved in traumatic inflammation associated with flexion or extension injuries resulting in suboccipital headaches. Skin markings or visible trauma should be noted at this time.

Range of motion examination may reveal pain or limitations in flexion-extension, lateral bending, and rotation. Flexion limitation may be assessed by placing fingers between the patient's chin and sternum at maximum flexion, with 50% the motion occurring at the occiput-C1 joint and the remaining 50% distributed over C2-C7. If the patient is unable to place the chin on the chest, the interval should be measured. One finger width shows a limitation of 10 degrees, whereas three fingers width indicates a 30- degree limitation in flexion. Upon extension the distance between the base of the occiput and the spinous process of T1 should be measured. Lateral flexion should allow the ear to touch the shoulder, with motion being shared across all cervical vertebrae. Upon rotation, the chin should touch the shoulder, with 50% of rotation occurring at C1-C2 and the remaining 50% distributed in the subaxial spine between C3-C7. A natural decrease in range of motion occurs with age, even in healthy individuals.[58]

Range of motion tests the ligaments, capsules, and fascia, and range of motion will be reduced in the presence of cervical spinal muscular spasm or pain. Patients with degenerative changes of the cervical spine will have back pain with decreased range of motion of the cervical spine without resistance. The most common findings due to changes in the cervical spine articulations are (in order): restriction of movement with or without pain, pain upon movement, and local tenderness. Lateral flexion is the earliest and most impaired movement in degenerative diseases, with rotation first impaired in RA because of involvement of the odontoid peg. A uniformly stiff neck may be caused by diffuse idiopathic skeletal hyperostosis, which is present in a quarter of elderly patients but also may be due to ankylosing spondylosis or recent trauma to the neck.[59] If articular signs are found, the examiner must evaluate the entire vertebral column and peripheral joints for evidence of further arthritis and search for extra-articular manifestations.

Motion against resistance testing is performed after active and passive range of motion is established. Muscle groups tested include the flexors and extensors of the neck. In testing flexor muscles, a hand is placed between the forehead and chest. The primary flexor is the sternocleidomastoid muscle, with secondary flexors being the three scalene muscles and small pre-vertebral muscles. Extensor muscles are tested by placing a hand on the shoulder and head for resistance. Primary extensors include the paravertebral extensor mass, splenius, semispinalis capitis, and trapezius. Secondary flexors include the small intrinsic muscles of the neck. Rotators are examined by placing a hand on the shoulder and chin for resistance. The sternocleidomastoid muscle and the intrinsic muscles of the neck provide rotational force. Motion against resistance testing should include an active maximum effort strength testing to the extremes of flexion, extension, and rotation to assess muscle strength. Causes of decreased range of motion of the cervical spine include joint locking and bony ankylosis from degenerative changes or arthritides, fibrous contractures, muscle spasm, and splinting over painful joints, as well as nerve root or spinal cord compression or irritation. Decreased range of motion in the presence of pain or weakness warrants further investigation.

Sensation for light touch, pinprick, temperature, and proprioception should be performed. These tests are admittedly subjective, and therefore both extremities should be compared to assess differences in sensation. Comparing an unaffected area such as the face to the area of decreased sensation can also be helpful. Pinprick can be performed using a sterile needle, and temperature using an alcohol pad can be used to assess functioning of the spinothalamic tract that traverses the anterolateral aspect of the spinal cord. Light touch and proprioception assess functioning of the posterior spinal column.

Dermatomes are anatomically distributed, as noted in Figure 45-5. The lower extremities demonstrate a unique dermatomal map that correlates with embryologic development whereby the limb starts in a supinated position and with longitudinal growth pronates. Perineal sensation and rectal tone are important to examine because an abnormality may indicate compression of the spinal cord or cauda equina, requiring immediate surgical intervention. Isolating the level of disease can at times be challenging. Nerve roots with proximal compression are more susceptible to distal compression. When the nerve is compressed both proximally and distally it is called a "double crush" phenomenon. The cervical spine should always be considered as the potential etiology in patients who present with symptoms of carpal or cubital tunnel syndrome and peripheral neuropathy. Ancillary imaging and nerve conduction studies can help elucidate the cause.

After palpation, range of motion testing, and assessment of sensation, muscle strength testing is continued for localization of any positive findings. Lower motor neuron disease is indicated by weakness, hypotonia, and fasciculations. Upper motor neuron disease is indicated by spasticity. Motor function should be graded using the standard 0-5 nomenclature: grade 0, no function; 1, a trace of function; 2, full range of joint motion with gravity eliminated; 3, antigravity function; 4, function against slight resistance; and 5, normal strength against resistance (Table 45-5). If weakness is

**TABLE 45-5** Strength Grading in Motor Examination

| | |
|---|---|
| 0 | No function with total paralysis |
| 1 | Trace movement with palpable or visible contraction |
| 2 | Full range of joint motion with gravity eliminated |
| 3 | Active movement against gravity |
| 4 | Active movement against slight resistance |
| 5 | Normal strength |

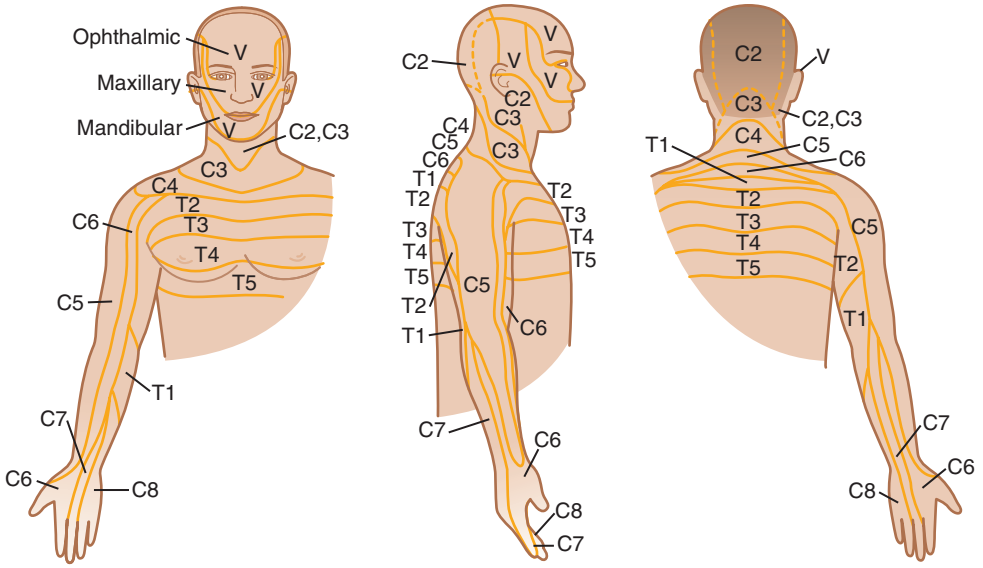



**Figure 45-5** Dermatome distributions. Dermatome distribution of nerve fibers from C1 through T5, carrying senses of pain, heat, cold, vibration, and touch to the head, neck, arm, hand, and thoracic area. The sclerotomes and myotomes are similar but show some overlap. Pain arising from structures deep to the deep fascia (myotome and sclerotome) does not precisely follow the dermatome distribution.

present, a more focused examination should be performed to look at other muscles innervated by that same nerve root.

Deep tendon stretch reflexes should be performed and graded from 0 to 3, as follows: 0, no response; 1, hyporeflexive; 2, normal; and 3, hyperreflexive. C5 is tested by striking the biceps tendon; C6, the brachioradialis; C7, the triceps; L4, the patellar tendon; and S1, the Achilles tendon. To facilitate reflex testing, it may be helpful to use muscle loading or Jendrassik's maneuver (performed by having the patient flex both sets of fingers into a hook-like form, interlocking the hands, and pulling apart). This technique creates a diversion to help relax the patient and better assess lower extremity reflexes. If difficulty with reflex testing persists, one must ensure that no peripheral neuropathy is present. In addition to deep tendon reflex testing, the abdominal reflex, Babinski test, and bulbocavernosus test should also be assessed.

Provocative tests that can be helpful in confirming compressive extradural monoradiculopathy include Spurling's test, the arm abduction test, and the axial compression and traction test. All of these tests are meant to change the diameter of the neural foramen, thus increasing or decreasing the symptoms, respectively. Spurling's test is performed by having the patient extend his or her neck and rotating toward the side of pain. The test is positive if the radicular pain is made worse in this position and indicates foraminal stenosis with potential compression of a nerve root. The arm abduction sign is positive if the patient's pain is relieved by placing the hand on the affected side, on top of the head.[60] The axial compression test is performed by pressing on top of the patient's head with the neck in the neutral position, with a positive result if the radicular symptoms are exacerbated by this maneuver and relieved by placing traction on the head and opening up the foramina.

Provocative tests that are helpful in diagnosing myelopathy include the presence of Hoffmann's sign, the finger escape sign, the abnormal grip-release test, and Lhermitte's sign. A positive (pathologic) Hoffmann's sign is performed by holding the middle finger extended and suddenly extending the distal interphalangeal joint, resulting in flexion of the index finger and thumb. A positive finger escape sign occurs when a person cannot hold all of the fingers in an adducted and extended position without the ulnar two digits falling into flexion and abduction over time. The grip-release test reveals an inability to rapidly open and close a fist because of weakness and spasticity of the hand. Lhermitte's sign evaluates changes in the spinal cord itself and occurs when the patient's neck is forcefully flexed, resulting in electric-like shocks that travel down the arms and legs. This reaction indicates changes in the white matter of the spinal cord and may be a result of cervical myelopathy or multiple sclerosis.

## DIAGNOSTIC EVALUATION

After completing the history and clinical examination, performing indicated imaging studies, neurophysiologic procedures, and laboratory studies may aid in completing the differential diagnosis and treatment plan for the patient's neck pain. Cervical radiographs often show degenerative changes in asymptomatic people in their sixties.[61] In the absence of trauma, constitutional symptoms, or worsening neurologic deficit, 4 to 6 weeks of conservative care is indicated prior to obtaining radiographs.[62] Dynamic radiographs should also be used for screening patients with RA prior to endotracheal intubation, given the risk of cervical instability. One study demonstrated that 61% of patients with RA had evidence of instability defined by at least 3 mm of atlanto-axial subluxation on pre-operative screening radiographs.[63]

In the presence of significant degenerative changes and end plate osteophytes, CT myelography can be helpful in further characterizing the bony involvement. CT myelography should be thought of as a complimentary test to MRI.[64]

It should only be used as the primary test to evaluate neural involvement when MRI is contraindicated because MRI is superior for evaluating spinal cord changes such as syringomyelia, myelomalacia, or neoplasm.[65] MRI scanning is indicated for progressive neurologic deficit, disabling weakness, or long tract signs and is recommended for patients with persistent cervical radiculopathy after 6 weeks of conservative care.[62] The addition of gadolinium contrast enhancement is helpful in evaluating infection and neoplasm and differentiating scarring from recurrent disk herniation in patients who have undergone previous spinal surgery. MRI results must be correlated with physical examination findings given that asymptomatic volunteers have been found to have abnormal cervical spine MRI findings.[53] Increased signal on the T2 sequence can be representative of a spectrum of disease from edema to myelomalacia and syrinx formation. Therefore the presence of increased signal within the spinal cord warrants a surgical consultation and operative intervention if the examination and history correlate, or close follow-up at a minimum. If the MRI findings do not correlate with the history and physical examination findings, further studies may be required, such as CT myelography. CT myelography is superior at detecting bony foraminal stenosis. Nuclear bone scanning techniques, including the single photon emission computerized tomography scans, have been used to identify and characterize acuity in occult fractures, periosteal injury, and post-traumatic osteoarthritis in the absence of positive radiograph findings.[66]

Neurophysiologic procedures are indicated when the clinical examination and imaging studies fail to correlate or when there is conflicting information. Electromyography (EMG), nerve conduction studies, and somatosensory evoked responses help differentiate cervical spine disorders from peripheral nerve entrapment syndromes and help in differentiating intrinsic joint disease from a radiculopathy. These tests are complementary to plain radiographs and an MRI scan or CT myelogram.

Neck pain is typically a result of mechanical causes, and laboratory studies generally are not helpful in its diagnosis. However, laboratory studies can be critical in ruling out infection, neoplasm, and systemic arthritides. ESR indirectly measures the acute-phase response with a high sensitivity but low specificity. Patients younger than 50 years should have an ESR less than 20 mm/hour, with the accepted normal range increasing as the patient ages. Values greater than 100 are seen with infection and neoplasm, whereas less dramatic elevations are seen with RA and after surgery.[67] CRP is an acute-phase reactant synthesized by the liver, with elevations peaking by day 2 of the inciting event and returning to normal within 3 to 7 days of removing the insult.[68] The complete blood cell count with differential and a spinal tap can also be helpful if meningitis is a concern.

## DIFFERENTIAL DIAGNOSIS AND TREATMENT

Using the combination of history, physical examination, and diagnostic studies is detrimental to establish the differential diagnosis of benign axial neck pain, radiculopathy, myelopathy, infection, neoplasm, systemic arthritides, and referred pain. Most axial neck pain is self-limiting and will resolve with appropriate conservative care.[69] Axial neck pain with associated radiculopathy also has a fairly benign course, with 75% of patients having only one recurrence or mild symptoms at 19-year follow-up with conservative treatment.[70] Patients with isolated neck pain and a negative radiographic and laboratory workup are best treated with a multimodal approach. During the acute phase, patients can be treated with a soft collar to reduce inflammation, but the collar should not be worn for more than 2 weeks to avoid deconditioning. Multimodal treatments have been found to be the most effective at treating axial neck pain, including proprioceptive training, exercises with resisted strengthening, muscle relaxants, and NSAIDs.[71-76] Evidence is inconclusive as to the effectiveness of radiofrequency denervation of facet joints, acupuncture, transcutaneous electrical nerve stimulation unit, iontophoresis, EMG biofeedback, or local injections for treatment of axial neck pain.[77-80]

Cervical traction may be prescribed, with a typical regiment of 3.5 to 4.5 kg for 15- to 20-minute sessions with the device at 20 to 25 degrees of flexion. However, this treatment has only shown short-term relief of radicular symptoms.[81] Fluoroscopically guided interlaminar and transforaminal epidural steroids have been shown to be effective at treating lumbar radiculopathy, but not in the cervical spine.[82] Cervical injections carry a higher risk, with complications in up to 16% of patients, including neurologic deficits.[83,84] Given these increased risks with epidural steroid injections in the cervical spine, a more conservative approach should be taken in prescribing this treatment modality. Atlanto-axial facet joint osteoarthritis may be treated successfully with a facet block and NSAIDs. If conservative therapy with NSAIDs fails, a fusion may be indicated.

In addition to degenerative changes to the cervical spine, trauma, and acute disk herniation, more insidious causes of neck pain exist, such as schwannomas, Pancoast's tumor, brachial plexus neuritis, and complex regional pain syndrome. Schwannomas, if intradural, may involve a sensory nerve root, causing dermatomal pain along with a myelopathy or radiculopathy from compression. Pancoast's tumor involving the lung apex may cause caudal cervical nerve root and sympathetic changes in addition to nerve root or brachial plexus compression. Brachial plexus neuritis (Parsonage-Turner syndrome), which is of viral origin, causes severe arm pain followed by weakness and then pain resolution followed by a return of arm strength. This condition may progress to complex regional pain syndrome in a small number of cases associated with diffuse burning pain along with autonomic changes, including discoloration of the skin.

Follow-up and vigilance are in order because a progressive neurologic deficit, segmental instability, or persistent radicular symptoms for at least 6 weeks may be indications for surgical intervention. In a prospective randomized study comparing surgery, physical therapy, or cervical collar use for long-standing cervical radiculopathy, no difference between the three groups was found at 12 months.[85] Cervical myelopathy with very mild deficits can be followed closely; however, the natural course of the illness is long periods of stability with episodes of deterioration. Definitive indications for surgery include the presence of myelopathy for 6 months or longer, progression of signs or symptoms, difficulty walking, or change in bowel or bladder function.

**TABLE 45-6** Rheumatologic Disorders Causing Neck Pain

| |
|---|
| Rheumatoid arthritis |
| • Without disease of the C1-C2 joint |
| • With structural cervical abnormalities |
| • C1-C2 subluxation |
| • C1-C2 facet involvement |
| Spondyloarthropathies |
| • Ankylosing spondylitis |
| • Reactive arthritis |
| • Psoriatic arthritis |
| • Enteropathic arthritis |
| Polymyalgia rheumatica |
| Osteoarthritis |
| Fibromyalgia |
| Nonspecific musculoskeletal pain |
| Miscellaneous spondyloarthropathies |
| • Whipple's disease |
| • Behçet's disease |
| • Paget's disease |
| • Acromegaly |
| • Ossification of the posterior longitudinal ligament |
| • Diffuse idiopathic skeletal hyperostosis |

Surgery is directed at decompressing the spinal cord and preventing further deterioration rather than improving neurologic deficits.

Systemic arthritides, infection, and tumors can affect the cervical spine with variable neurologic and constitutional symptoms. RA typically causes atlanto-axial subluxation, atlanto-axial impaction, and subaxial subluxation (Table 45-6). Surgical stabilization is indicated with progressive neurologic deficit, persistent axial neck pain with radiographic evidence of instability, canal diameter of less than or equal to 14 mm (posterior atlanto-dens interval), and odontoid migration of greater than or equal to 5 mm above McGregor's line. In the setting of atlanto-axial subluxation or subaxial subluxation, the involved levels are fused posteriorly and atlanto-axial impaction should be treated with occipitocervical fusion.[15] Patients with rheumatoid disease who have evidence of instability demonstrate radiographic progression over time, but this progression correlates poorly with neurologic outcome.[86] Regardless, these patients require close follow-up given that once myelopathy develops, most patients die within 1 year.[87]

Ankylosing spondylitis commonly affects the cervical spine, resulting in a kyphotic deformity over time with altered biomechanics, which has implications in the setting of trauma. The kyphotic deformity can have significant functional implications as the person's gaze moves toward the floor, making interaction with the surrounding world difficult. Corrective osteotomies are available but carry the risk of neurologic deficit and intra-operative bleeding. However, earlier diagnosis and treatment with tumor necrosis factor blocking agents will help make cervical deformity a thing of the past.[88]

Infection and neoplastic processes both can cause destruction with mechanical or nonmechanical neck pain, constitutional symptoms, and variable neurologic deficit. The goal is similar with eradication of the infection or tumor, decompression if a neurologic deficit exists, and stabilization of the spinal column.

In summary, a careful history, physical examination, and ancillary studies will help one arrive at a fairly narrow differential diagnosis. In the absence of spinal instability, neurologic deficit, or an infectious or neoplastic process, the patient may benefit from conservative treatment with expectant recovery using "tincture of time."



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 46

# Shoulder Pain



Scott David Martin • Shivam Upadhyaya • Thomas S. Thornhill

**KEY POINTS**

- **Comprehension of functional anatomy allows diagnosis of most causes of shoulder pain upon clinical examination.**
- **History and clinical examination aided by ancillary tests will usually guide application of the most appropriate treatment for shoulder pain.**
- **The differential diagnosis of shoulder pain includes not only common local disorders (e.g., of tendons and adjacent structures) but also should include consideration of causes arising from distant anatomic sites via referred pain-mediated pathways.**
- **A variety of specific diagnostic tests can greatly aid in the diagnosis of shoulder pain.**
- **Most causes of shoulder pain can be treated with a structured physical therapy program. Successful treatment programs can benefit potential surgical candidates, including those who fail to respond to conservative treatment.**
- **Systemic arthropathies occasionally present with shoulder disease and often involve the shoulder over time. Early assessment in such patients is essential.**

Shoulder pain, which is one of the most common musculoskeletal maladies, may arise from diverse causes. Accurate diagnosis of shoulder pain is made difficult by the unique anatomy and position of the shoulder, which serves as a link between the upper extremity and the thorax. One of the most complex and mobile joints of the body, the shoulder is traversed by muscle, tendon, and bone and is surrounded by major neurovascular structures, all of which may serve as potential sources of local and referred pain.

Determining the source of shoulder pain is essential in order to recommend the proper method of treatment. The examining physician must be able to differentiate the occurrence of shoulder pain caused by intrinsic or local factors, extrinsic or remote factors, or a combination of the two. Intrinsic factors originate from the shoulder girdle and include glenohumeral and periarticular disorders, whereas extrinsic factors occur outside of the shoulder girdle with secondary referral of pain to the shoulder (Table 46-1). An example of an extrinsic factor is left shoulder pain as the initial presentation of coronary artery disease. Hepatic, gallbladder, and splenic disease also may manifest initially as shoulder pain.

Accurate evaluation, diagnosis, and treatment require a thorough understanding of shoulder anatomy, including pain referral patterns. A complete and systematic physical examination is crucial for an accurate diagnosis. During the initial evaluation, care must be taken to discern all possible causes of shoulder pain. The final diagnosis may require repeated office examinations and correlation of diagnostic tests with symptoms, along with response to selective injections. Improvements in diagnostic tests, such as MRI, computed tomography (CT)-arthrography, ultrasonography, and electromyography (EMG), have facilitated early diagnosis of shoulder pain and have provided a better understanding of shoulder disease. This chapter provides practical guidelines for the diagnosis and treatment of painful shoulder disorders that may be encountered in a rheumatology or general practice. A detailed analysis of shoulder problems and information on the treatment of major trauma are beyond the scope of this chapter and have been addressed by other authors.

## ANATOMY AND FUNCTION

Because of the shoulder's complexity, clinicians treating shoulder pain must have an understanding of the structural and functional anatomy of this joint. The shoulder is the most mobile joint of the body, although this mobility is gained at the sacrifice of stability. Only 25% of the humeral head surface has contact with the glenoid at any time. The labrum increases the contact area of the articular surface and confers stability to the joint.[1] Lesions of the labrum may result from instability, and the type of lesion may indicate the type of instability. Labral tears also may be a source of pain from internal derangement of the shoulder.[2] Joint stability is provided by a thin capsule and by the glenohumeral ligaments, which are thickenings of the capsule anteriorly, posteriorly, and inferiorly.[1] Anterior stability is predominantly conferred by the anterior band of the inferior glenohumeral ligament.

The rotator cuff, which provides dynamic stability of the joint, is composed of four musculotendinous units: the supraspinatus, infraspinatus, and teres minor posteriorly, and the subscapularis anteriorly. The shoulder consists of three joints: the acromioclavicular (AC), sternoclavicular, and glenohumeral joints, and two gliding planes—the scapulothoracic and subacromial surfaces.

Figure 46-1 shows the musculoskeletal and topographic localization of pain associated with common shoulder disorders. Figure 46-2 shows the relationship of the three posterior rotator cuff muscles coursing anteriorly underneath the acromion to insert on the greater tuberosity. The subscapularis, the only anterior rotator cuff muscle, inserts on the lesser tuberosity. By understanding the relationship

**TABLE 46-1** Common Causes of Shoulder Pain

| |
|---|
| **Intrinsic Causes** |
| Periarticular Disorders |
| Rotator cuff tendinitis or impingement syndrome |
| Calcific tendinitis |
| Rotator cuff tear |
| Bicipital tendinitis |
| Acromioclavicular arthritis |
| Glenohumeral Disorders |
| Inflammatory arthritis |
| Osteoarthritis |
| Osteonecrosis |
| Cuff arthropathy |
| Septic arthritis |
| Glenoid labral tears |
| Adhesive capsulitis |
| Glenohumeral instability |
| **Extrinsic Causes** |
| Regional Disorders |
| Cervical radiculopathy |
| Brachial neuritis |
| Nerve entrapment syndromes |
| Sternoclavicular arthritis |
| Reflex sympathetic dystrophy |
| Fibrositis |
| Neoplasms |
| Miscellaneous |
| • Gallbladder disease |
| • Splenic trauma |
| • Subphrenic abscess |
| • Myocardial infarction |
| • Thyroid disease |
| • Diabetes mellitus |
| Renal osteodystrophy |

between the rotator cuff and the subacromial region, bounded inferiorly by the humeral head and superiorly by the undersurface of the acromion, the clinician can visualize the problems of impingement syndrome and administer an injection into this space with accuracy. Knowledge of the route of the tendon of the long head of the biceps through the bicipital groove and onto the superior aspect of the glenoid helps in understanding bicipital tendinitis. Before attempting to diagnose and treat shoulder pain, the clinician should review in detail one of the many sources describing the structural and functional relationships of the shoulder girdle.[3,4]

# DIAGNOSIS

## Clinical Evaluation of the Shoulder

Accurate diagnosis and successful treatment of a shoulder disorder begin with a thorough history and physical examination. Most of the information needed to make a correct diagnosis can be elicited with basic clinical skills, rather than by relying on expensive and highly technologic investigative aids. Diagnostic tests should be used only to confirm an established diagnosis or to assist in cases with a challenging presentation.

### History

When establishing a diagnosis, it is important to consider the patient's age and chief reason for presenting. The differential diagnosis of shoulder pain in a 70-year-old sedentary person is entirely different from that in a 20-year-old athlete. Did the pain occur slowly over time, or suddenly after a particular event? Gradual onset of pain over the anterolateral or deltoid region that is increased with forward elevation of the shoulder, along with nocturnal pain, suggests impingement with rotator cuff tendinopathy. The presence of significant weakness with pain upon engaging in overhead actions suggests impingement with a rotator cuff tear. Pain and weakness also may be noted upon reaching behind the back with the shoulder in extension and external rotation, as when reaching into the back seat of a car. Initiating factors relative to the onset of symptoms should be elicited, and any history of shoulder pain or trauma should be carefully documented.

Pain intensity, character, location, and periodicity and aggravating or alleviating factors should be assessed. Pain should be graded on a visual analog scale of 0 to 10, with 0 indicating no pain and 10 indicating the worst pain the patient has ever experienced. Another indication of the severity of pain is disruption of sleep; the patient should be asked if the pain prevents sleep or awakens the patient and if he or she can lie on the affected shoulder. The patient also should be asked if the pain is sharp or dull. Sharp, burning pain over the top of the shoulder indicates a neurogenic origin, whereas a dull, aching pain over the lateral deltoid suggests rotator cuff disease with impingement. The location or distribution of the pain should be identified: Is it local around the shoulder girdle, or does the pain radiate down the arm? Is concomitant sensory loss or weakness present? Periodicity of the pain as constant or intermittent should be determined, along with factors that aggravate or alleviate the pain. Pain caused by rotator cuff tendinopathy usually is exacerbated by repetitive activities that involve the elbow away from the side of the body.

Any history of neck pain should be considered, along with a history of radicular pain. Radicular-type pain frequently extends below the elbow and is associated with sensory loss and weakness. Pain located in the paracervical region may indicate a cervical origin, or it can be localized to the trapezius. Trapezial pain often is associated with shoulder pain and results from the patient trying to favor the shoulder. Assuming a military brace position may produce fatigue, spasm, and trigger points of the trapezius.

Any pertinent medical history, such as a history of malignancy, should be considered. Neurologic, visceral, and vascular disease can produce referred pain to the shoulder and should always be considered, especially in a patient with painless range of motion.

### Physical Examination

Proper physical examination of the shoulder includes close inspection of the shoulder girdle from the front and back. The evaluation is started by standing behind the patient, who has both shoulders exposed. The normal shoulder is always inspected and compared with the injured shoulder. Examination can be performed with the patient in the sitting or standing position. Contour and symmetry are observed and compared between shoulders, and any atrophy or asymmetry in shoulder position or level is assessed.

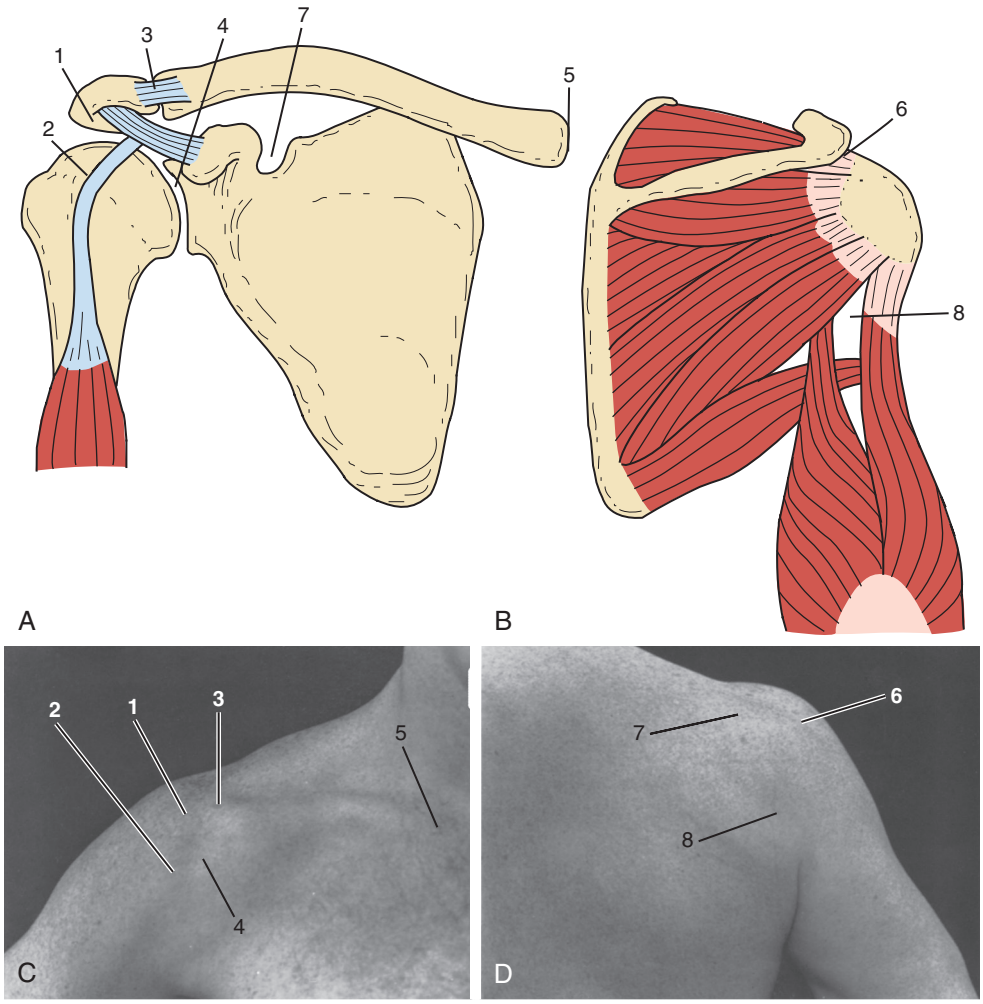



**Figure 46-1** Musculoskeletal (**A** and **B**) and topographic (**C** and **D**) areas localizing pain and tenderness associated with specific shoulder problems. *1,* Subacromial space (rotator cuff tendinitis/impingement syndrome, calcific tendinitis, and rotator cuff tear). *2,* Bicipital groove (bicipital tendinitis, biceps tendon subluxation, and tear). *3,* Acromioclavicular joint. *4,* Anterior glenohumeral joint (glenohumeral arthritis, osteonecrosis, glenoid labrum tears, and adhesive capsulitis). *5,* Sternoclavicular joint. *6,* Posterior edge of acromion (rotator cuff tendinitis, calcific tendinitis, and rotator cuff tear). *7,* Suprascapular notch (suprascapular nerve entrapment). *8,* Quadrilateral space (axillary nerve entrapment). These areas of pain and tenderness frequently overlap.

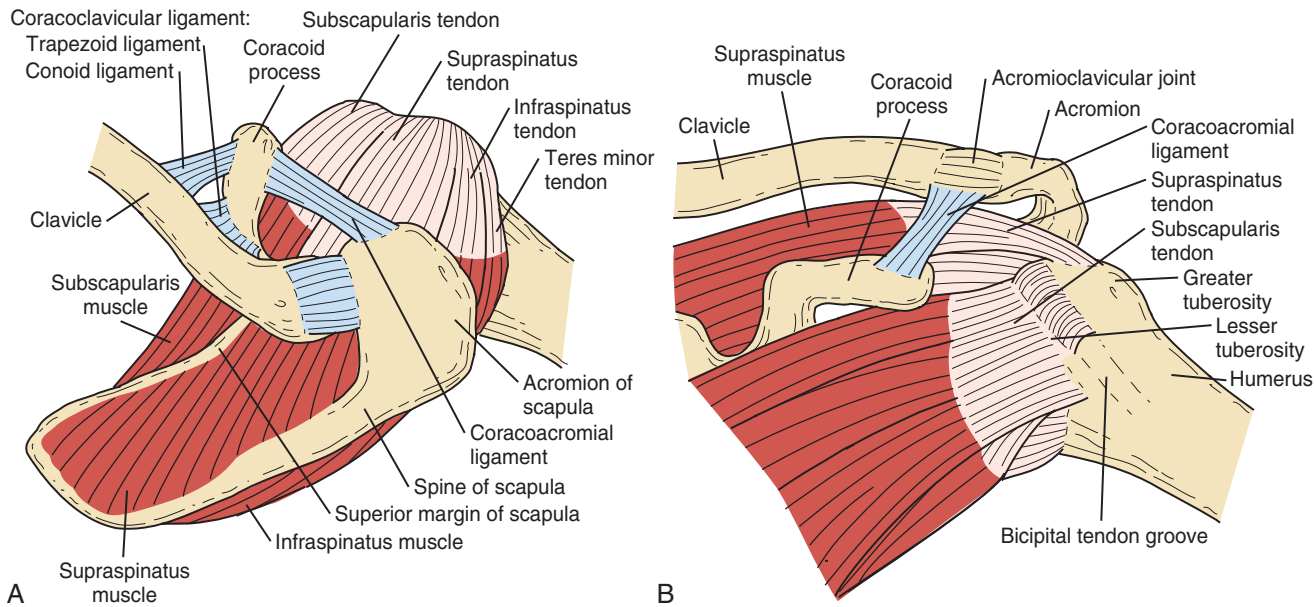



**Figure 46-2** **A,** Superior view of the rotator cuff musculature as it courses anteriorly underneath the coracoacromial arch to insert on the greater tuberosity. **B,** Anterior view of the shoulder reveals the subscapularis, which is the only anterior rotator cuff muscle inserting on the lesser tuberosity. It internally rotates the humerus and provides dynamic anterior stability to the shoulder. *(From* the Ciba Collection of Medical Illustrations, *Volume 8, Part I. Netter Illustration from* www.netterimages.com. *Copyright Elsevier Inc. All rights reserved.)*

Spinatus muscle atrophy may result from disuse, chronic cuff tear, or suprascapular or brachial neuropathy.[5] If scapular winging is evident, the patient should be asked to perform a wall push-up, which accentuates winging.

Range of motion should be carefully recorded, along with notation of any absence of rhythmic shoulder motion or excessive scapulothoracic motion that may compensate for the lack of glenohumeral motion. Internal rotation of the shoulder is checked by having the patient reach behind his or her back with the thumb while the examiner notices the vertebral level. Loss of internal rotation is seen early with shoulder pain and usually indicates some tightness of the posterior shoulder capsule. The biceps tendon is palpated, along with the coracoid, lesser, and greater tuberosities and the posterior cuff, and any tenderness is gauged (Figure 46-3A). Tenderness upon palpation of the long head of the biceps is frequently associated with rotator cuff tendinopathy and tenderness of the greater tuberosity. Any spasm or tenderness of the trapezius or levator scapulae may be associated with rotator cuff or cervical spine disease. Cervical range of motion is evaluated, and the paracervical muscles are palpated. Paracervical tenderness and limited range of motion of the neck may indicate cervical spondylosis or neurogenic disease. A Spurling test is performed by flexing the neck laterally while applying axial compression to the skull. Pain that radiates to the ipsilateral shoulder is considered a positive test result and indicates radiculopathy.

To elicit the impingement sign, the shoulder is elevated passively in forward flexion while the scapula is depressed with the opposite hand, forcing the greater tuberosity against the anterior acromion and producing pain in cases of impingement (Figure 46-3B).[6] This maneuver also may be painful in the presence of conditions such as adhesive capsulitis, glenohumeral and AC arthritis, glenohumeral instability, and calcific tendinitis. A dynamic impingement test, known as the circumduction-adduction shoulder maneuver (also called the Clancy test), is 95% sensitive and 95% specific for diagnosing rotator cuff tendinopathy,

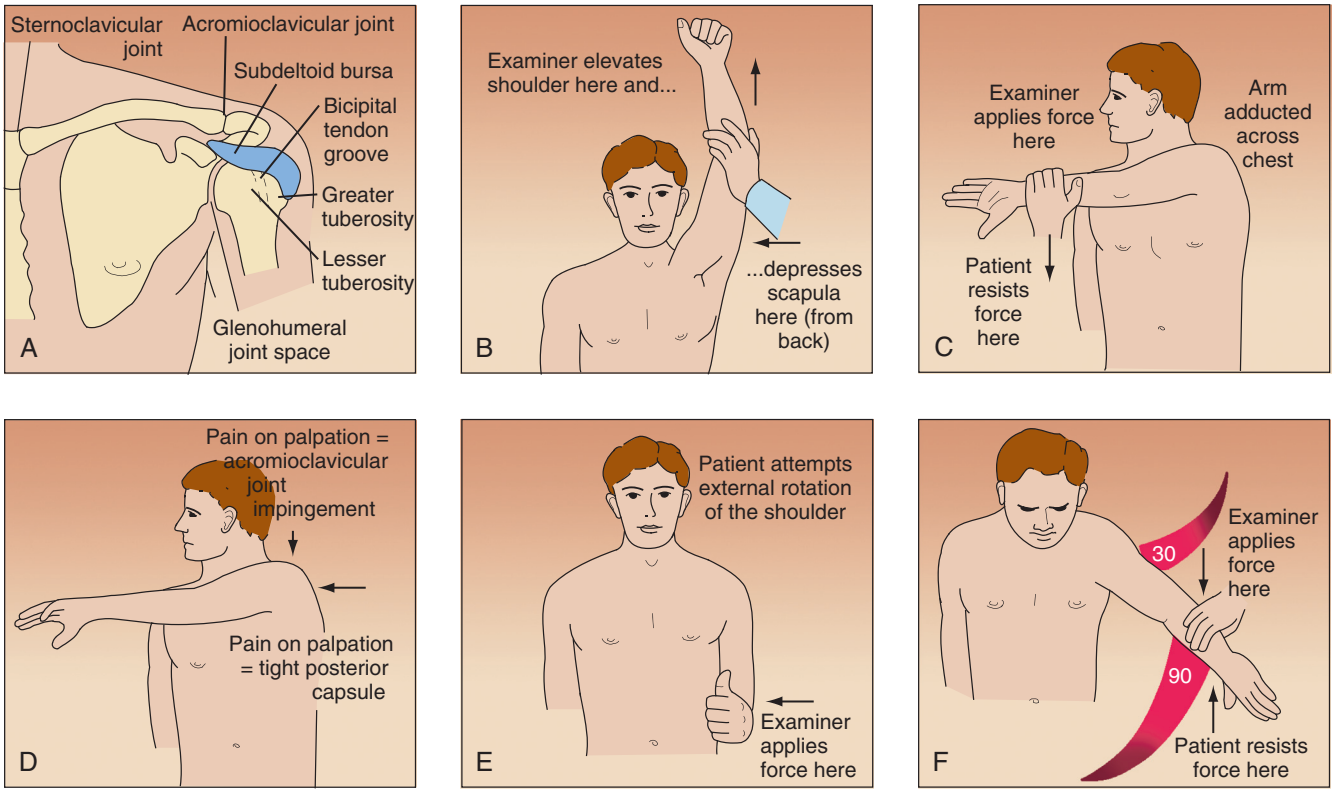



**Figure 46-3** **A,** Tenderness upon palpation of trigger points may help localize the site of disease. Tenderness upon palpation of the long head of the biceps and greater tuberosity suggests impingement with possible cuff tendinopathy. **B,** To elicit the impingement sign, the shoulder is elevated in forward flexion while the scapula is depressed with the opposite hand, forcing the greater tuberosity and the rotator cuff against the anterior acromion and producing pain when impingement exists. Relief of pain after injection of local anesthetics (i.e., the impingement test) provides additional evidence of subacromial disease. **C,** The Clancy test is performed with the patient standing and with the head turned toward the contralateral shoulder. The affected shoulder is circumducted and adducted across the body to shoulder level, keeping the elbow in extension with the arm internally rotated with the thumb pointed toward the floor. In this position, the patient is asked to resist maximally as a uniform downward force is applied to the extended arm by the examiner. Production of pain or weakness localized to the anterior lateral portion of the shoulder is considered a positive test result. **D,** The test is performed by forward flexion of the arm at 90 degrees and subsequent cross-chest adduction of the arm. Pain localized to the acromioclavicular joint is considered a positive test result. **E,** The test is performed with the patient's elbow flexed at 90 degrees and held at the patient's side by the examiner. The patient is asked to attempt external rotation of the shoulder from a neutral position (0 degrees of adduction) as the examiner applies resistance to the forearm. Strength is compared with that of the contralateral arm. **F,** Abduction strength testing is performed with the patient's shoulder in 30 degrees of forward flexion and 90 degrees of abduction and with the thumb pointed toward the floor. The patient is asked to resist as the examiner exerts a downward force on the abducted arm. Strength is compared with the contralateral shoulder. *(From Martin TL, Martin SD: Rotator cuff tendinopathy.* Hosp Med *12:23–31, 1998.)*

including partial tears.[7] The test is performed with the patient in the standing position, with the head turned to the contralateral shoulder. The affected shoulder is circumducted and adducted across the body to shoulder level while the elbow is kept in extension, the shoulder is in internal rotation, and the thumb is pointing toward the floor (Figure 46-3C). In this position, the patient is instructed to resist maximally as a uniform downward force is applied to the extended arm by the examiner. The test result is considered positive if pain or weakness is elicited during the maneuver, with pain localized to the anterolateral aspect of the shoulder. A strong positive correlation of pain and weakness is noted with a complete cuff tear.[7]

The sternoclavicular and AC joints should be observed for prominences and palpated for stability and tenderness. Many patients with impingement have tenderness upon direct downward palpation of the AC joint as a result of impingement on the cuff from undersurface osteophytes of the distal clavicle.[3,5]

AC joint tenderness also may result from primary AC joint arthrosis and should be differentiated by physical examination, including the cross-chest adduction test and O'Brien's test.[8] Radiographic evidence of AC joint arthrosis is common in patients older than 40 years, but this condition usually is not painful.[9]

The cross-chest adduction test or the horizontal adduction test is performed by forward flexing the shoulder 90 degrees with subsequent cross-chest adduction of the arm (Figure 46-3D). Pain localized to the AC joint is considered a positive test result. If pain occurs posteriorly over the shoulder, a tight posterior capsule with impingement is suspected. O'Brien's test is performed by forward flexing the arm 90 degrees and adducting the arm 10 degrees out of the sagittal plane of the body. The first part of the test is performed with the hand maximally pronated with the thumb pointed down. In this position, the patient is asked to resist as the examiner applies a downward force on the arm. If the test elicits pain, the patient is asked if the pain is on top of the shoulder or deep inside. Pain localized to the top of the shoulder indicates AC joint pain, and pain deep inside the shoulder indicates a superior labrum anterior posterior (SLAP) lesion. In the second part of the test, the patient is asked to supinate the hand maximally, while the examiner applies a downward force to the arm. If the patient notices significantly less pain, the test result is positive for a SLAP lesion. If the pain is unchanged and is located on top of the shoulder, the test result is positive for AC joint disease.[8]

If the cause of AC joint tenderness is still in question, a lidocaine injection should be administered. The clinician should carefully avoid injecting the lidocaine into the subacromial space by advancing the needle too far inferiorly through the AC joint, which can lead to a false interpretation. Painful degenerative changes of the AC joint may exist concomitantly with subacromial impingement and should be evaluated thoroughly when surgical treatment (i.e., distal clavicle excision) is being considered.[10]

In patients with pain out of proportion to objective findings, other causes of shoulder pain should be sought, including calcific tendinitis, infection, reflex sympathetic dystrophy, and fracture. Patients with significant wasting of the supraspinatus and infraspinatus muscles and posterior shoulder pain, especially younger patients, may have suprascapular neuropathy or brachial neuropathy (Parsonage-Turner syndrome).[5,11]

Patients with chronic cuff disease frequently have variable disuse atrophy of the supraspinatus and infraspinatus fossae. In cases of chronic massive cuff tears, atrophy and weakness can be severe. Strength testing of external rotation should be performed with the elbow at the side and supported by the examiner; the patient is asked to attempt external rotation of the shoulder from a neutral position (0 degrees of adduction) while the examiner applies resistance (Figure 46-3E).[12] Weakness in this position may suggest a tear of the infraspinatus tendon. Abduction strength testing against resistance is performed with the shoulder in 30 degrees of forward flexion and 90 degrees of abduction and with the thumb pointed toward the floor (Figure 46-3F).[13,14] Weakness in this position may suggest a tear of the supraspinatus tendon. A lift-off test should be performed with the shoulder in internal rotation; the patient is asked to try to hold his or her hand away from the back. Inability to do so indicates a subscapularis tear.

If impingement is suspected after a thorough physical examination, an impingement test should be performed with injection of 5 mL of a local anesthetic into the subacromial space.[15,16] Before the test is performed, the patient is asked to grade the pain during the impingement signs on a visual analog scale of 0 to 10, with 0 equal to no pain and 10 equal to the most severe pain the patient has ever experienced. The injection may be performed anteriorly, laterally, or posteriorly, depending on the physician's preference. Ten minutes after injection of a local anesthetic into the subacromial space, the patient should be re-examined and asked to grade the pain again on the same visual analog scale. A 50% or greater reduction in pain is thought to be a positive test result for impingement; otherwise, an alternative cause of shoulder pain should be sought, or inadequate placement of the anesthetic should be suspected. If the AC joint is thought to be contributing to the shoulder pain, 1 to 2 mL of a local anesthetic should be injected into the joint, and the shoulder should be re-examined. When subacromial impingement and the AC joint are thought to be contributing to shoulder pain, serial injections during separate office visits may be required to evaluate the shoulder while minimizing discomfort to the patient.[9]

In cases of suspected bicipital tendinitis, Speed's test is performed by having the patient flex the shoulder and extend the elbow while a downward force is applied to the arm. The production of pain over the long head of the biceps is a positive test result and suggests bicipital tendinitis.

Upper extremity strength testing should be performed and compared with the contralateral side so that any atrophy is detected. Grip strength is checked, and the hands are examined carefully for evidence of intrinsic atrophy. The biceps (C5), triceps (C7), and brachioradialis (C6) reflexes are checked for symmetry and briskness.

Light touch sensory testing should be conducted, and the dermatomal distribution of any deficits that may suggest that cervical radiculopathy should be identified. The cervical, supraclavicular, axillary, and epitrochlear regions should be palpated for enlarged lymph nodes, which may suggest malignancy.

## Imaging

### Radiographic Assessment

For nontraumatic painful shoulder evaluation, standard radiographic profiles are used. An impingement series should be obtained, which includes anteroposterior views with a 30-degree caudal tilt (Rockwood view), an outlet view (scapular Y with a 10- to 15-degree caudal tilt), and an axillary view. Internal and external rotational views may be obtained if calcific tendinitis or instability is suspected. The Rockwood view can reveal any osteophytes off the anterior acromion and AC joint.[17] In cases of traumatic injury, a trauma series is obtained that includes a true anteroposterior view, a scapular Y view, and an axillary view. The axillary view is useful in assessing posterior or anterior subluxation of the humeral head. Additional views, such as the West Point view, which evaluates the glenoid for evidence of a bony Bankart lesion, or the Styker notch view, which assesses the humeral head for a Hill-Sachs lesion, may be obtained to assist in the evaluation if the diagnosis of instability is in doubt. Secondary impingement-type rotator cuff tendinitis may be caused by increased anterior translation with subluxation of the humeral head. In such cases, an axillary view or fluoroscopy can help show the subluxation.[18,19]

When AC joint disease is suspected, a 10-degree, cephalic tilt view of the AC joint at 50% penetrance, as described by Zanca,[20] should be obtained (Figure 46-4). Stress views of the AC joint may be obtained by strapping 5- to 10-lb weights to the patient's forearms and determining AC separation. Comparing the coracoclavicular distance of both shoulders may be helpful. When clinically indicated, cervical spine radiographs should be obtained to exclude cervical spondylosis as a cause of shoulder pain.

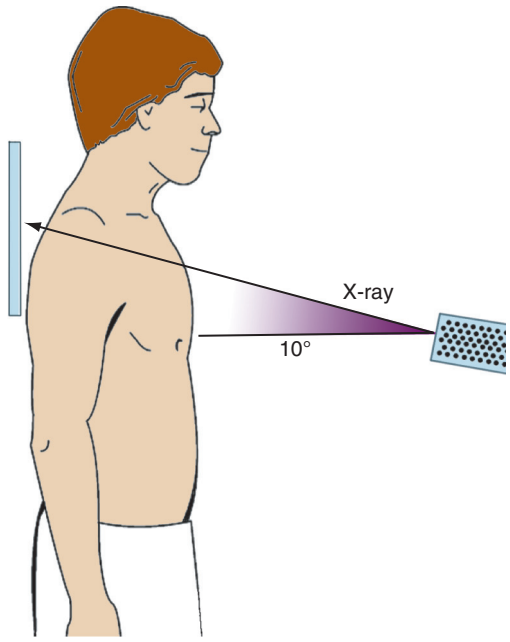



**Figure 46-4** The Zanca view of the acromioclavicular joint is obtained with a 10-degree cephalic tilt and 50% penetrance. *(From Rockwood CA Jr, Young DC: Disorders of the acromioclavicular joint. In Rockwood CA Jr, Matsen TA III, editors:* The shoulder. *Philadelphia, 1985, WB Saunders, pp 413–476.)*

### Scintigraphy

Technetium 99m (Tc 99m) methyl diphosphonate (MDP) or gallium may be of diagnostic help in evaluating skeletal lesions in the area of the shoulder joint. Bone scans generally are not helpful in the diagnosis of non-neoplastic or noninfectious shoulder disease.

Scintigraphy may have a role in identifying patients with complete rotator cuff tears that evolved to cuff-tear arthropathy. This distinction is important because patients with complete rotator cuff tears may do well, whereas patients who experience progressive changes of cuff-tear arthropathy have progressive arthritis, pain, and significant functional impairment. Synovitis or calcium pyrophosphate deposition disease may be an important factor in the pathogenesis of cuff-tear arthropathy. In such cases, scintigraphy may show the increased blood flow and blood pooling associated with chronic synovitis.

### Arthrography

Double-contrast arthrotomography (DCAT) can be used to evaluate problems of the rotator cuff, glenoid labrum, biceps tendon, and shoulder capsule.[21-24] Figure 46-5 shows normal DCAT of the shoulder. Rotator cuff tears can be shown by single-contrast or double-contrast studies. Proponents of double-contrast arthrography believe that the extent of the tear, the preferred surgical approach, and the quality of the rotator cuff tissue are best determined by double-contrast studies.[21-26] Arthrography without MRI or CT can be misleading and may result in underestimation of the extent of a rotator cuff tear. Multidetector CT can enhance the accuracy of diagnosing labral and rotator cuff tears, especially in patients for whom MRI is not possible (Figure 46-6).

Tears of the glenoid labrum without shoulder dislocation are sources of anterior shoulder pain in athletes.[2] Glenoid

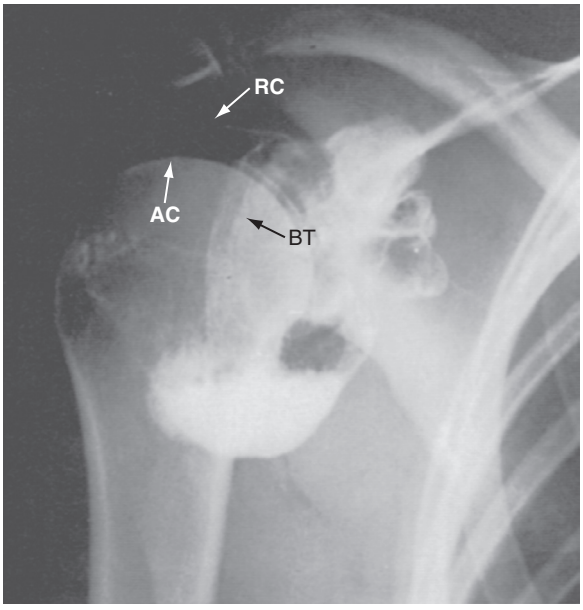



**Figure 46-5** Normal double-contrast arthrography shows the inferior edge of the rotator cuff (RC) as it courses through the subacromial space to the greater tuberosity, the tendon of the long head of the biceps (BT), and the articular cartilage of the humeral head (AC).

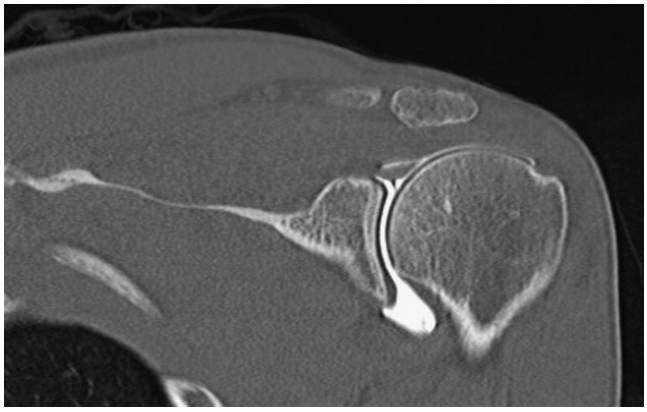


**Figure 46-6** Multidetector computed tomography reveals a superior labral tear of the shoulder.



**Figure 46-7** Double-contrast arthrotomography shows a tear of the anterior-inferior portion of the glenoid labrum *(arrow)*.

labrum tears (Figure 46-7), with or without associated glenohumeral subluxation, frequently can be identified by DCAT.[24,25] Kneisl and colleagues[27] described 55 patients who underwent DCAT followed by diagnostic shoulder arthroscopy. DCAT predicted the arthroscopic findings in 76% of anterior labrum studies and 96% of posterior labrum studies. This test was 100% sensitive and 94% specific in diagnosing complete rotator cuff tears. Partial rotator cuff tears identified at arthroscopy were missed in 83% of patients who underwent DCAT. Investigators believed that DCAT was more effective in diagnosing intra-articular and cuff disease in cases of instability than when pain alone was the presenting diagnosis.[27]

Shoulder arthrography can confirm a diagnosis of adhesive capsulitis by showing a contracted capsule with an obliterated axillary recess (Figure 46-8). The use of subacromial bursography has been beneficial in visualizing the outer surface of the rotator cuff and the subacromial space in cases of impingement.[28,29] Fukuda and associates[30] reported

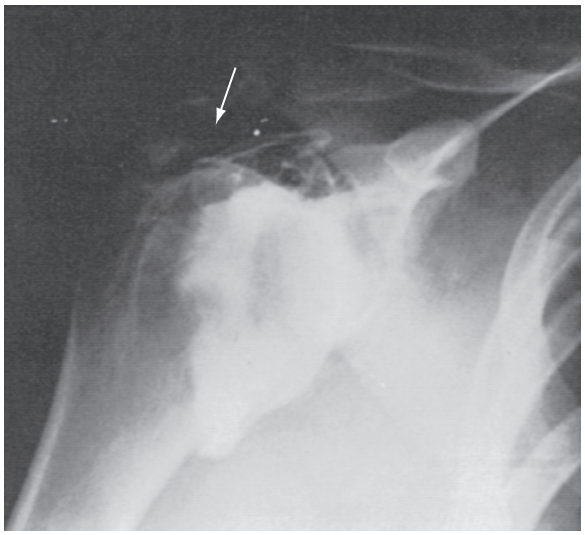


**Figure 46-8** Double-contrast arthrography of a patient with calcific tendinitis *(arrow)* and adhesive capsulitis. Notice the contracted capsule with diminution of the synovial space and obliteration of the axillary recess.

a small series of younger patients (average age, 41.8 years) who underwent subacromial bursography after a negative glenohumeral arthrographic result. These patients showed pooling of contrast medium on the bursal side of a tear, which was confirmed at the time of surgery. Subacromial bursography is not routinely used diagnostically, and in our opinion, it is of little value in planning surgical procedures.

## Computed Tomography

CT is helpful in evaluating the musculoskeletal system, and CT combined with contrast arthrography (CT-arthrography) has become a major diagnostic tool for the evaluation of glenoid labrum tears, loose bodies, and chondral lesions (Figure 46-9). Rafii and co-workers[31] reported using CT-arthrography in an evaluation of shoulder derangement and found 95% accuracy of CT-arthrography for investigating lesions of the labrum and articular surface.[34] More recently, multidetector CT-arthrography scans have been used to evaluate partial cuff tears (Figure 46-10A), cystic lesions (Figure 46-10B), and calcific tendinopathy (Figure 46-10C).

## Ultrasonography

Technologic improvements in ultrasound equipment have led to improved ultrasound study of the rotor cuff. The technique is noninvasive and rapid and involves no radiation exposure.[27-29,32] The cuff is examined in the horizontal and transverse planes with the arm in different positions to allow visualization of various areas of the cuff. These techniques generally provide visualization of the distal cuff, where most rotator cuff tears are located. Figure 46-11 shows normal and abnormal ultrasound images of the rotator cuff in longitudinal and transverse planes.

Several studies report high sensitivity and specificity for the diagnosis of a rotator cuff tear by ultrasound.[29-32] The

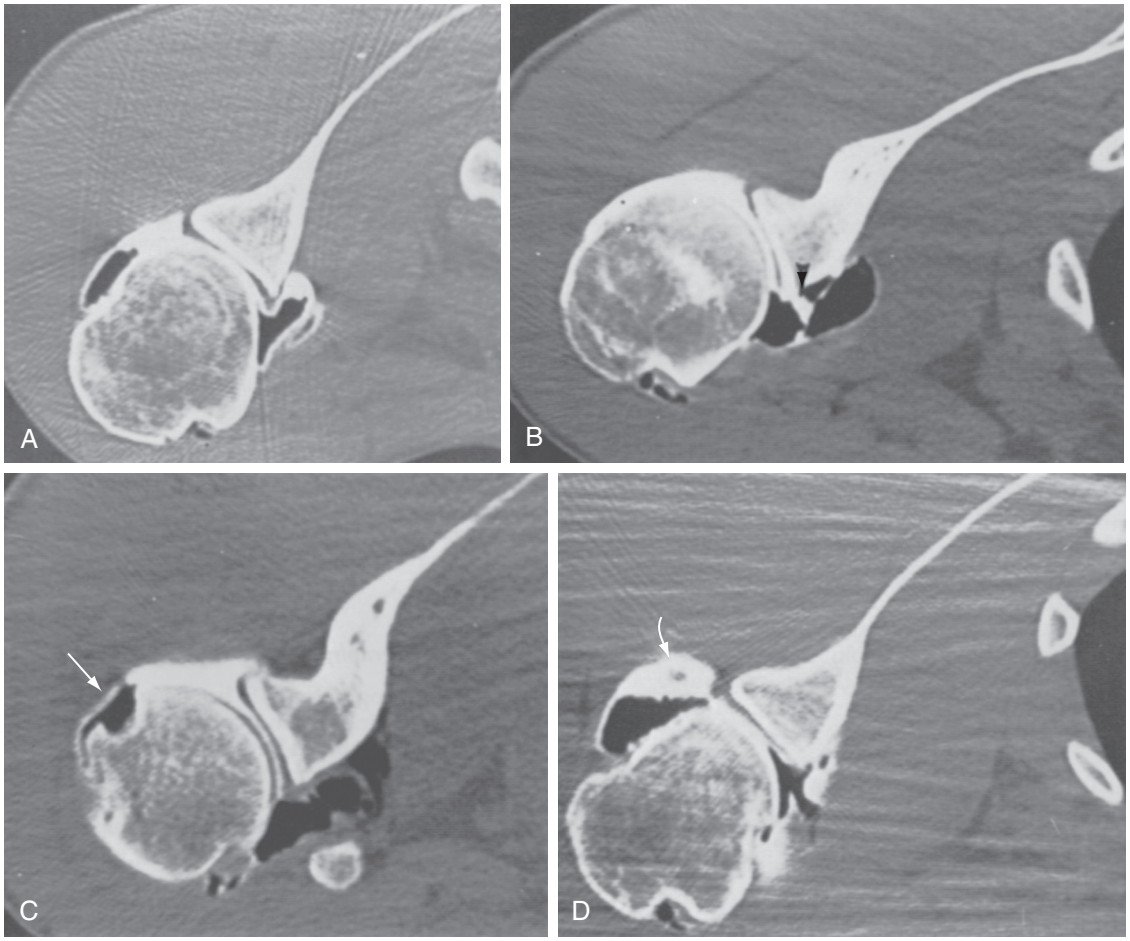



**Figure 46-9** Computed tomography–arthrography of the shoulder. **A,** Normal findings. **B,** A tear of the anterior glenoid labrum. **C,** A large defect of the articular surface of the posterior portion of the humeral head (Hill-Sachs lesion) *(arrow)*. **D,** A loose body in the posterior recess *(arrow)*.

specificity and sensitivity of the procedure are reported to be greater than 90% as determined by arthrographic and surgical correlations.[31,32] This technique has also been used for the post-operative evaluation of a rotator cuff repair and for evaluation of abnormalities of the biceps tendon.[33-37]

Gardelin and Perin[38] reported ultrasound to be 96% sensitive in determining rotator cuff and biceps tendon disease. Mack and associates[33] found ultrasound to be valuable in evaluating post-operative patients with recurrent shoulder symptoms. In a prospective study, Hodler and colleagues[36] compared ultrasound with MRI and arthrography in evaluating rotator cuff lesions in 24 shoulders. Ultrasound identified 14 of 15 torn cuffs, MRI identified 10 of 15, and arthrography identified 15 of 15.[36] Ultrasound identified 7 of 9 intact rotator cuffs, whereas MRI was accurate in 8 of 9 intact cuffs.[36] Vestring and colleagues[39] found ultrasound to be as accurate as MRI in the diagnosis of humeral head defects and joint effusions but inferior to MRI in the diagnosis of labrum lesions, rotator cuff lesions, subacromial spurs, and synovial inflammatory disease. In the hands of an experienced sonographer, ultrasound may be the most cost-effective test for the initial evaluation of a rotator cuff injury, but most surgeons require CT-arthrography or MRI confirmation before beginning surgical exploration.[33,36,38-40]

### Magnetic Resonance Imaging

MRI has been used to diagnose partial-thickness and full-thickness rotator cuff tears, biceps tendon tears, impingement of the rotator cuff, synovitis, articular cartilage damage, and labral disease associated with glenohumeral instability.[41-43] In rheumatoid arthritis (RA), MRI is reported to be more sensitive than plain radiographs in determining soft tissue abnormalities and osseous abnormalities of the glenoid and humeral head.[44]

One of the most valuable diagnostic uses of MRI is in rotator cuff disease. Morrison and Offstein[45] studied 100 patients with chronic subacromial impingement syndrome using arthrography and MRI. MRI was 100% sensitive but only 88% specific in confirming arthrography-proven rotator cuff tears. Nelson and associates[46] studied 21 patients with shoulder pain and found MRI to be more accurate than CT-arthrography or ultrasound in identifying partial-thickness cuff tears. These investigators also reported MRI to be as accurate as CT-arthrography in the diagnosis of abnormalities of the glenoid labrum.[46]

Characteristic MRI findings in persons with rotator cuff tears include a hypointense gap within the supraspinatus muscle tendon complex on T1-weighted films, absence of a

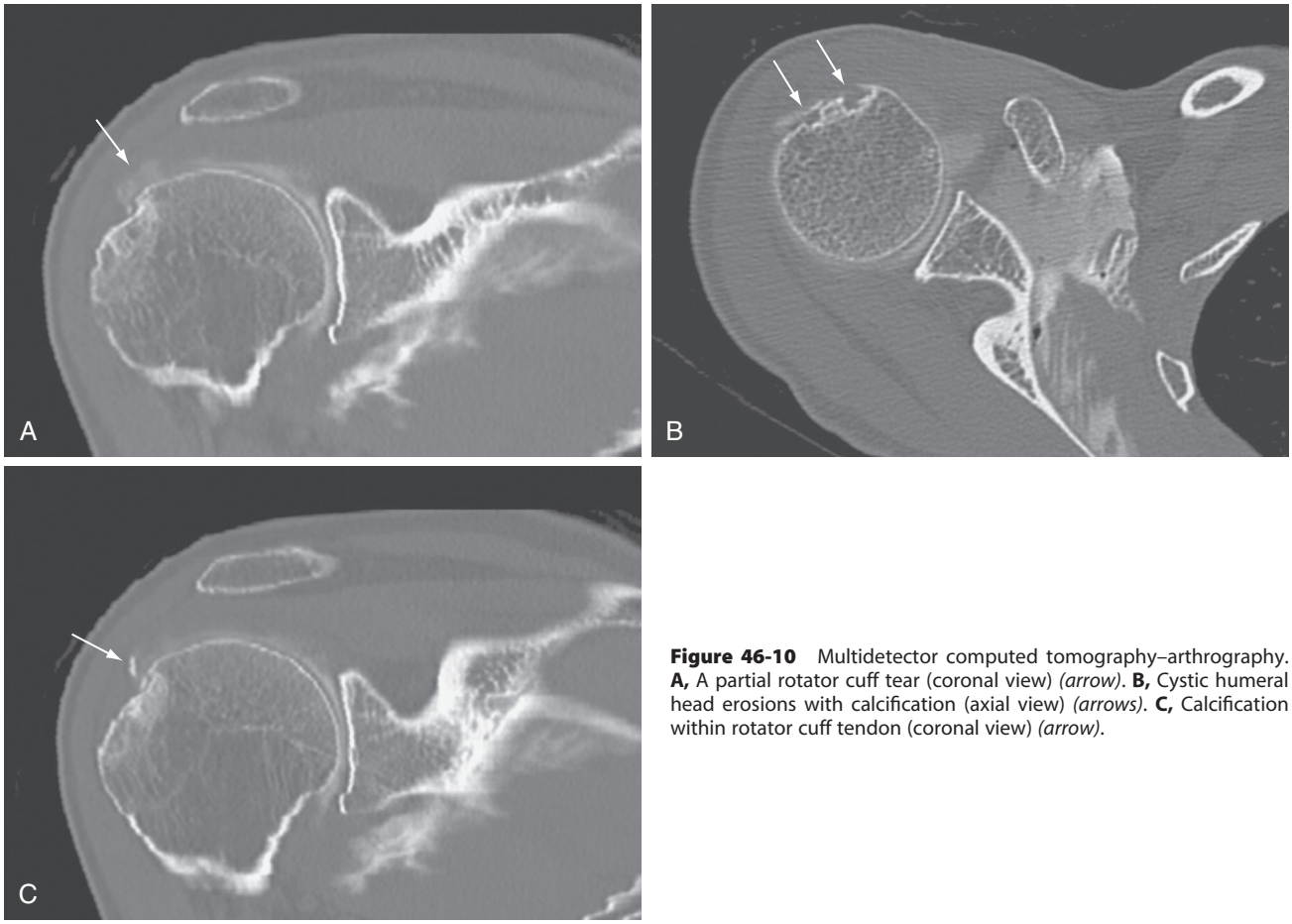



**Figure 46-10** Multidetector computed tomography–arthrography. **A,** A partial rotator cuff tear (coronal view) *(arrow)*. **B,** Cystic humeral head erosions with calcification (axial view) *(arrows)*. **C,** Calcification within rotator cuff tendon (coronal view) *(arrow)*.

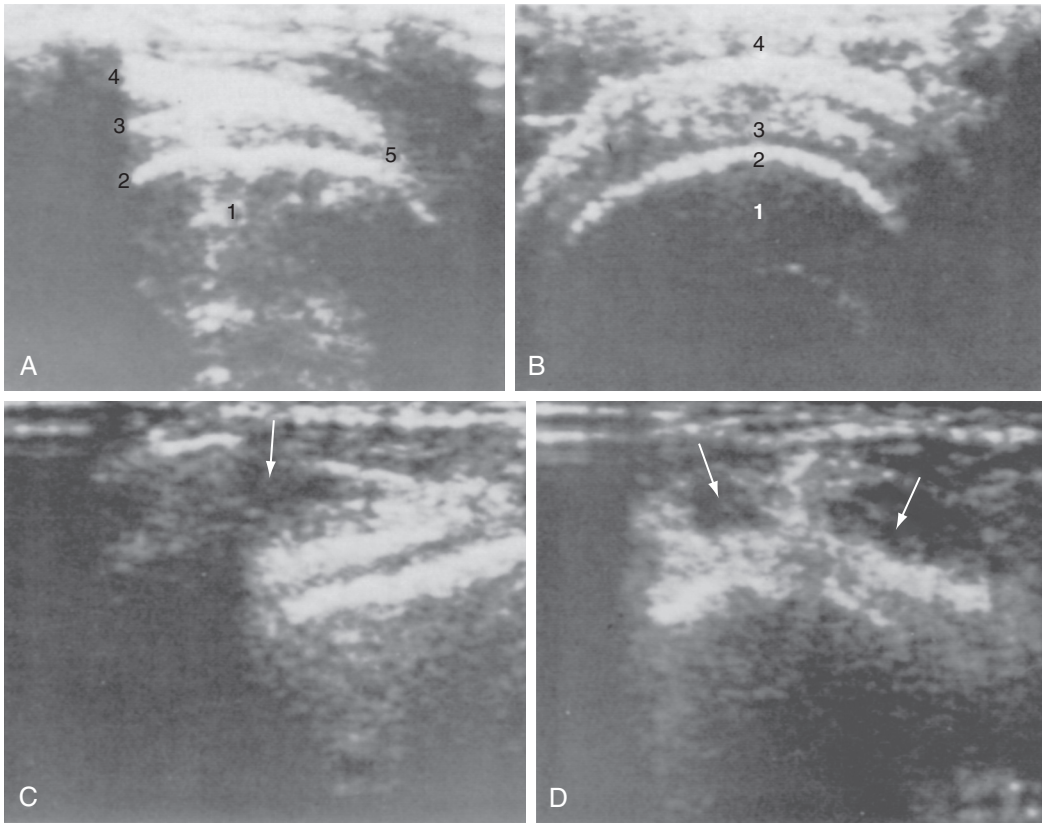



**Figure 46-11** **A,** A normal longitudinal view of the rotator cuff by ultrasound shows the humeral head *(1),* the superior articular surface *(2),* the rotator cuff *(3),* the deltoid tendon *(4),* and tapering of the cuff to its insertion on the greater tuberosity *(5).* **B,** A transverse view of a normal intact rotator cuff covering the humeral head. **C,** A rotator cuff tear showing a hypoechoic area *(arrow)* on a longitudinal view. **D,** A rotator cuff tear showing a hypoechoic area *(arrows)* on a transverse view.

demonstrable supraspinatus tendon with narrowing of the subacromial space, and an increased signal within the supraspinatus tendon on T2-weighted images.[47] Seeger and colleagues,[48] who reported the results of 170 MRI studies, found that T1-weighted images were highly sensitive for identifying abnormalities within the supraspinatus tendon but that T2-weighted images were required to differentiate tendinitis from a small supraspinatus tendon tear. However, large full-thickness tears could be identified on T1- and T2-weighted images. Figure 46-12 depicts common shoulder disease as seen by MRI. Compared with scintigraphy, MRI is almost as sensitive and more specific in the diagnosis of osteonecrosis and neoplastic lesions in the shoulder area.

## Arthroscopy

The use of arthroscopy for the diagnosis of shoulder disease increased in the 1980s, in part because of its accuracy, which was far greater than that of clinical examination and better than the accuracy of other diagnostic modalities of the time. With technologic advances in fiberoptics, video output, and arthroscopic instrumentation, the use of arthroscopy to diagnose and treat shoulder problems exponentially increased to include procedures previously used only for open techniques.[49]

Compared with DCAT, arthroscopy is more accurate in the diagnosis of intra-articular lesions associated with a

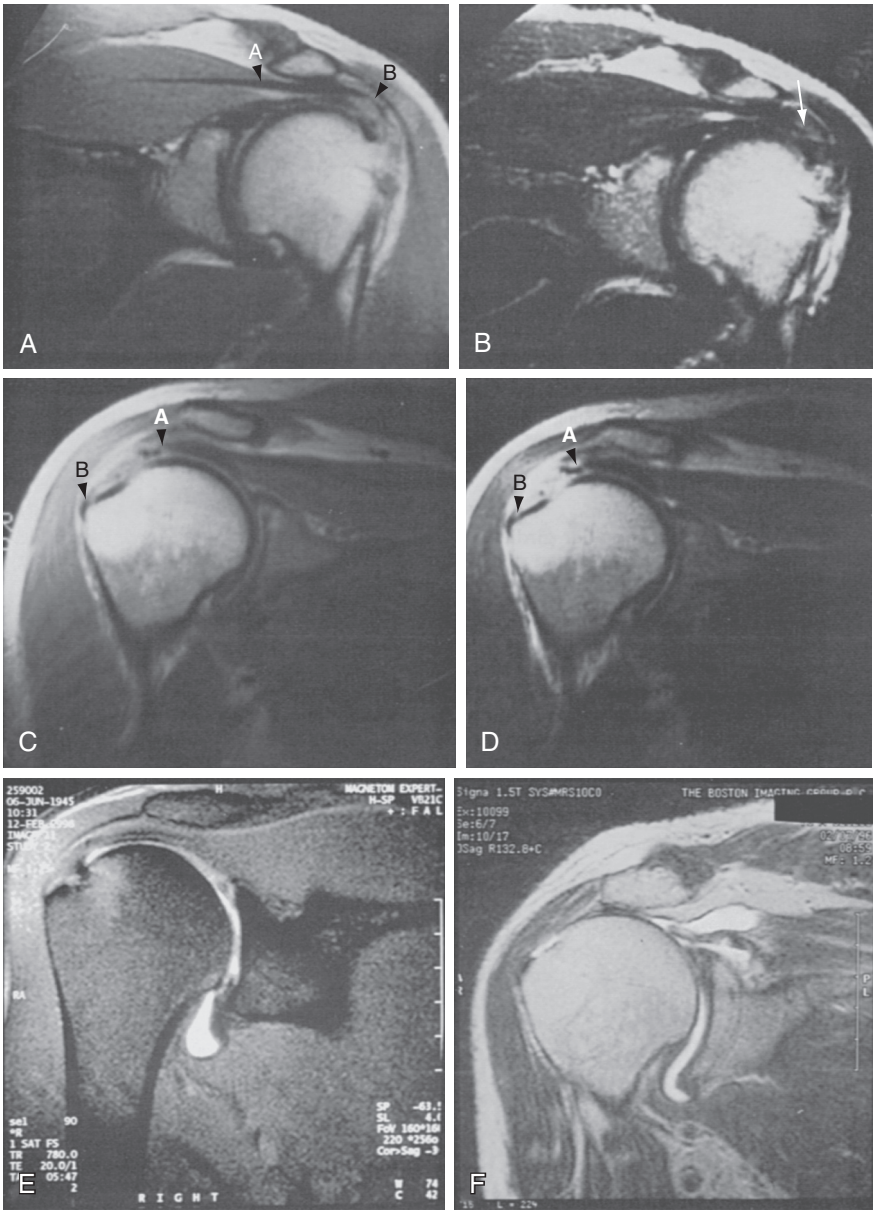



**Figure 46-12** **A,** An MRI proton density–weighted coronal view shows the supraspinatus tendon as a black band *(A)* that has an increased signal as it nears insertion on the greater tuberosity *(B)*. **B,** A similar view with a T2-weighted image shows increased signal as gray *(arrow),* indicating a partial-thickness tear or tendinitis. **C,** MRI proton density–weighted coronal view shows abrupt end of supraspinatus tendon as it courses right to left *(A)*. From A to B is an area of increased signal followed by a short portion of tendon *(B)* inserting at the greater tuberosity. **D,** A similar view on a T2-weighted image shows increased signal as white (fluid density), indicating fluid in the gap of a complete rotator cuff tear. **E,** MR arthrography shows a normal rotator cuff. **F,** MR arthrography shows a chronic cuff tear with retraction.

painful shoulder.[27] An additional benefit is that arthroscopy can be used to diagnose and treat shoulder problems of the glenohumeral joint and the subacromial region. With increased accuracy of MRI-arthrography in detecting partial cuff tears and labral lesions, diagnostic shoulder arthroscopy has become less common in the absence of clear indications and specific treatment plans. In combination with a detailed history and physical examination, and along with examination under the effects of anesthesia, shoulder arthroscopy has been helpful in the diagnosis of chronic instability patterns of the glenohumeral joint.[49-52]

The indications and usefulness of shoulder arthroscopy in the treatment of common pathologic conditions have continued to increase as the technology improves and as understanding of the pathophysiology of shoulder problems grows. Shoulder arthroscopy has been used routinely to confirm and treat SLAP lesions, labral tears, partial cuff tears, refractory adhesive capsulitis, partial biceps tendon tears, and multidirectional instability. Other conditions that are routinely treated arthroscopically include rotator cuff tears, glenohumeral instability, AC joint disease, loose bodies, sepsis, osteochondritis dissecans, synovitis, chondral lesions, subacromial impingement, and calcific tendinitis.[2,10,49,52]

## Electromyography and Nerve Conduction Velocity Studies

EMG and nerve conduction velocity studies can help differentiate shoulder pain from pain of neurogenic origin. They also may be beneficial in determining the localization of neurogenic pain to a particular cervical root, the brachial plexus, or a peripheral nerve.[53,54]

## Injection

Injection of local anesthetics and glucocorticoids is a useful technique for the diagnosis and treatment of shoulder pain.[55] The physician must have a thorough understanding of the anatomy of the shoulder girdle and a presumptive diagnosis to direct the injection properly. Injection of referred pain areas may be misleading. In a patient with lateral arm pain resulting from deltoid bursal involvement from calcific tendinitis of the supraspinatus tendon, the injection should be directed to the subacromial space, rather than the area of referred pain in the deltoid muscle. It is often better to use a posterior or lateral subacromial approach when performing an injection for rotator cuff tendinitis in a patient with anterior impingement symptoms because it is easier to enter the subacromial region posteriorly or laterally, and this approach is less traumatic for contracted anterior structures.

The instillation of rapidly acting local anesthetics can be beneficial in determining the source of shoulder pain. Obliteration of pain by injection of a local anesthetic along the bicipital groove can confirm a diagnosis of bicipital tendinitis. The use of local anesthetics is less helpful when the subacromial space is injected because of its extensive communication with the remainder of the shoulder girdle, but relief of symptoms after such an injection can exclude pain from conditions such as cervical radiculopathy or entrapment neuropathy.

**TABLE 46-2** Relative Costs of Shoulder Diagnostic Procedures in 2014

| Procedure | Initial Fee (USD) | Technical Fee (USD) | Interpretation Fee (USD) |
|---|---|---|---|
| **Medicare B Fee Schedule** | | | |
| Initial office visit (30 min) | 108.18 | | |
| Plain radiography (3 views) | | 91.36 | 29.36 |
| Arthrography | | 340.49 | 72.78 |
| MRI | | 492.63 | 86.33 |
| Computed tomography | | 287.65 | 61.79 |
| **Institutional Charges** | | | |
| Initial office visit (30 min) | 394.00 | | |
| Plain radiography (3 views) | | 436.00 | 35.00 |
| Arthrography | | 625.00 | 302.00 |
| MRI | | 4785.00 | 350.00 |
| Computed tomography | | 2184.00 | 181.00 |

USD, U.S. dollars.

## Potential Diagnostic Tests

Table 46-2 lists reimbursement and charges for various shoulder diagnostic tests based on 2014 Medicare fee schedules and 2014 charges at a single institution. The choice of a specific test depends on its sensitivity, specificity, and cost-benefit analysis. History and physical examination are the most important factors in establishing diagnosis of the painful shoulder. Plain radiographs (three views) should be the first radiographic tests performed. Although they are not as sensitive as the more sophisticated tests, plain radiographs can identify arthritic change, calcific tendinitis, established osteonecrosis, and most neoplasms.

If intra-articular disease (e.g., a labrum tear, capsular tear, loose body, or chondral defect) is suspected, MRI-arthrography is preferable to CT-arthrography. In diagnosing acute rotator cuff tears in a younger patient, ultrasound is the most cost-effective test to confirm a clinical suspicion. In cases of impingement syndrome, MRI is sensitive, but it is difficult to differentiate tendinitis, partial tears, and small complete tears without MRI-arthrography. Orthopedic surgeons prefer use of MRI-arthrography for verification of labral tears or partial rotator cuff tears. In the case of a suspected full-thickness rotator cuff tear, MRI is preferred to determine the size of the tear, the amount of muscle atrophy and tendon retraction, and the quality of remaining tissue for repair.

# INTRINSIC FACTORS CAUSING SHOULDER PAIN

## Periarticular Disorders

### Shoulder Impingement and Rotator Cuff Tendinopathy

One of the most common nontraumatic causes of shoulder pain is impingement with rotator cuff tendinopathy. In

1972, Neer[6] described his results of 100 anatomic shoulder dissections and coined the term *impingement syndrome*. Impingement may be defined as the encroachment of the acromion, coracoacromial ligament, coracoid process, or AC joint on the rotator cuff as it passes beneath them during glenohumeral motion. The function of the posterior rotator cuff is to abduct and externally rotate the humerus. The cuff with the biceps tendon serves as a humeral head depressor to maintain the head centered within the glenoid fossa as the cuff and to use the deltoid to elevate the arm.[56-58]

Controversy continues, however, as to the exact cause of impingement—that is, whether it is a primary, intrinsic, degenerative event within the tendon with superior migration of the head on arm elevation and secondary impingement on the acromion, or purely mechanical attrition of the tendon with primary impingement against the acromion. The mechanical impingement of the rotator cuff may be influenced by variations in the shape and slope of the acromion.[59,60] The supraspinatus outlet may become narrowed from proliferative spur formation of the acromion or degenerative changes in the AC joint. These changes, along with intrinsic degenerative changes of the rotator cuff, may lead to a rotator cuff tear, but the exact pathogenesis remains controversial. Many studies have found a strong correlation between degenerative hypertrophic spur formation, with its resulting narrowing of the supraspinatus outlet, and the presence of full-thickness cuff tears,[6,16,61-68] but clinical studies have failed to confirm whether hypertrophic changes in the coracoacromial arch are caused by the cuff lesions or whether these changes themselves cause the lesions.

Neer[6] developed a staging system for the description of impingement lesions of the shoulder. A stage I lesion involves edema and hemorrhage of the rotator cuff and typically is found in persons younger than 25 years who are active in athletics that involve repetitious overhead activities. The condition usually responds to conservative treatment that includes rest, anti-inflammatory medication, and physical therapy. Stage II lesions usually occur in persons in their 30s or 40s and represent the biologic response of fibrosis and thickening of the tendon after repeated episodes of mechanical impingement over time. Lesions are treated conservatively, as in stage I, but attacks may recur. If symptoms persist despite adequate conservative management for longer than 6 to 12 months, surgical intervention is warranted. Stage III lesions involve rotator cuff tears, biceps tendon rupture, and bone changes, and they rarely occur before age 40 years. Patients may present with pain, weakness, or supraspinatus atrophy, depending on the chronicity of the tear. Surgical treatment depends on the patient's age, loss of function, weakness, and pain.

Patients usually present to the clinician with a report of pain that has failed to resolve after a variable period. Pain can be sudden and incapacitating in cases of traumatic cuff tears, or more commonly it may manifest as a dull ache in cases of chronic impingement. Pain usually is located over the anterior and lateral aspects of the shoulder and may radiate into the lateral deltoid. It may worsen after sleeping on the affected extremity and is exacerbated by overhead activity. Tenderness on palpation may be elicited over the greater tuberosity and the long head of the biceps within the bicipital groove, indicating an associated biceps tendinitis. In cases with concomitant degenerative changes in the AC joint, tenderness may be noted on palpation over the AC joint, as an offending osteophyte impinges on the rotator cuff beneath.

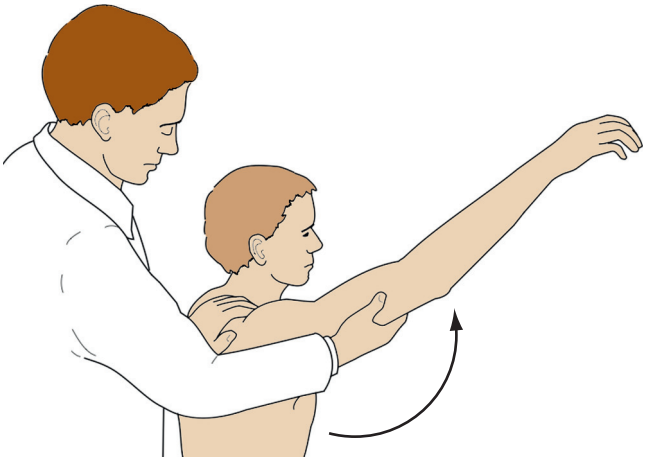


**Figure 46-13** The impingement sign is elicited by forced forward elevation of the arm. Pain results as the greater tuberosity impinges on the acromion. The examiner's hand prevents scapular rotation. This maneuver may be positive in other periarticular disorders. *(From Neer CS II: Impingement lesions.* Clin Orthop Relat Res *173:70, 1983.)*

The impingement sign as described by Neer[6] (Figure 46-13) is useful in the diagnosis of rotator cuff tendinopathy. The patient often describes a catch as the arm is brought into the overhead position. The patient may be observed to raise the arm by abduction and external rotation to clear the greater tuberosity of the acromion, bypassing the painful area. A typical painful arc usually occurs between 70 and 110 degrees of abduction. Neer[6] also described an impingement test that involves injection of lidocaine into the subacromial bursa. Relief of pain is a positive impingement test result and usually indicates rotator cuff origin of the shoulder pain.

Radiographs in the early stages of cuff tendinopathy may be normal or may reveal a hooked acromion. As the disease progresses, sclerosis, cyst formation, and sclerosis of the anterior third of the acromion and the greater tuberosity may be observed. An anterior acromial traction spur may appear on the undersurface of the acromion lateral to the AC joint and represents contracture of the coracoacromial ligament. Late radiographic findings include narrowing of the acromiohumeral gap, superior subluxation of the humeral head in relation to the glenoid, and erosive changes in the anterior acromion.[71] Arthrography, MRI, and ultrasound may be helpful in diagnosing a full-thickness tear of the rotator cuff in association with stage III disease. In some cases of chronic large rotator cuff tears, proximal migration of the humeral head leads to a pattern of degenerative arthritis termed *cuff-tear arthropathy*.

The choice of treatment and, frequently, its result are functions of the stage of the impingement and the response to pain. In stage I disease, in which little mechanical impingement occurs, most patients respond to rest. It is important to avoid immobilizing the shoulder for any period because contraction of the shoulder capsule and periarticular structures can produce an adhesive capsulitis. After a

period of rest, a progressive program of stretching and strengthening exercises generally restores the shoulder to normal function. Use of aspirin and other NSAIDs may shorten the symptomatic period. Modalities such as ultrasound, neuroprobe, and transcutaneous electrical nerve stimulation generally are not helpful. Patients with stage I or II disease may have a dramatic response to local injection of glucocorticosteroids and local anesthetic agents. For stage II disease in which fibrosis and thickening occur anteriorly, it is frequently better to perform the injection with a posterior approach. We prefer a combination of 3 mL of 1% lidocaine, 3 mL of 0.5% bupivacaine, and 20 mg of triamcinolone. This injection combines a short-acting anesthetic to help confirm the diagnosis, a longer-acting anesthetic for analgesic purposes, and a steroid preparation in a depot form.

An integrated program of occupational and physical therapy often precludes the need for surgery in patients with stage II disease. Job modification for persons with impingement syndrome caused by overuse may alleviate symptoms. Businesses are becoming increasingly aware of the cost savings associated with proper job ergonomics.[69,70]

The initial rehabilitation in stage II impingement consists of cessation of repetitive overhead activity. Use of ice, NSAIDs, and local injections also may be beneficial. Initial physical therapy includes passive, active-assisted, and active range of motion combined with stretching and mobilization exercises to prevent contracture. As pain and inflammation subside, isometric or isotonic exercises are used to strengthen the rotator cuff musculature. Isokinetic training at variable speeds and in variable positions is instituted before the patient is allowed to return to full activity. For patients with a job-related injury, it is crucial to review and modify job mechanics to prevent recurrent episodes that can cause further disability and may precipitate the need for surgery.[69]

Neer[16] suggested that a patient with refractory stage II disease may respond to division of the coracoacromial ligament and bursectomy of the subacromial bursa. Open anterior acromioplasty as described by Neer has become accepted as the procedure of choice for stage II and III impingement lesions, with many investigators reporting high success rates in treating impingement syndrome and rotator cuff tears.[71-74] Reported results show good and excellent relief of symptoms in 71% to 87% of patients treated with the open surgical procedure.[75-78]

In 1985, Ellman[50] described the technique of arthroscopic subacromial decompression. His initial results[51] and the results of others are comparable with those of open surgical techniques.[52,79] Arthroscopic subacromial decompression has become a widely accepted treatment for refractory stage II and III impingement lesions. The procedure can be performed as outpatient surgery, and because no deltoid is detached, as with the open technique, the procedure facilitates rehabilitation and increases overall recovery rates.

### Calcific Tendinitis

Calcific tendinitis is a painful condition around the rotator cuff that is associated with deposition of calcium salts, primarily hydroxyapatite.[80-82] The cause of calcific tendinitis is unknown. The commonly accepted cause is degeneration of the tendon, which leads to calcification through a dystrophic process.[82] A common clinicopathologic correlation is seen in three distinct phases of the disease process: the precalcific or formative phase, which can be relatively painless; the calcific phase, which tends to be quiescent and may last months to years; and the resorptive or postcalcific phase, which tends to be painful, as calcium crystals are resorbed.[80] Although calcific tendinitis is more common in the right shoulder, at least a 6% incidence of bilaterality has been reported. Patients with bilateral shoulder involvement often have the syndrome of calcific periarthritis, in which calcium hydroxyapatite crystals are found at multiple sites.[83] Patients usually present with impingement-type pain in the affected shoulder during overhead activity. The pain may seem to be out of proportion to any objective physical findings. The patient may describe difficulty sleeping on the shoulder and trouble falling asleep. Symptoms may last a few weeks or a few months.

The incidence of calcific tendinitis among asymptomatic persons varies from 2.7% to 20% in the literature. Most calcification occurs in the supraspinatus tendon, and 57% to 76.7% of patients are women. The average age of patients is 40 to 50 years.[80,84]

Codman[85] pointed out the localization of calcification within the tendon of the supraspinatus. He provided a detailed description of the symptoms and the natural history of this condition. In describing the phases of pain, spasm, limitation of motion, and atrophy, he noted the lack of correlation between symptoms and the size of the calcific deposit. According to Codman,[85] the natural history includes degeneration of the supraspinatus tendon, calcification, and eventual rupture into the subacromial bursa. During the latter phase, pain and decreased motion can lead to adhesive capsulitis (see Figure 46-8).

Several factors may affect localization of calcium within the supraspinatus. Many patients have an early stage of impingement that compresses the supraspinatus tendon on the anterior portion of the acromion.[6,16] This long-standing impingement may lead to local degeneration of tendon fibers. In patients without impingement, localization of calcium within the supraspinatus may be related to the blood supply of the rotator cuff, which normally is derived from an anastomotic network of vessels from the greater tuberosity or from the bellies of the short rotator muscles.[81] The watershed of these sources is just medial to the tendinous attachment of the supraspinatus.[86] Rathburn and Macnab[87] referred to this watershed as the critical zone and pointed out that during abduction this area was rendered ischemic.

Treatment of calcific tendinitis depends on the clinical presentation and the presence of associated impingement. Patients can have an acute inflammatory reaction that may resemble gout. The acute inflammation can be treated with a local glucocorticoid injection, NSAIDs, or both. Ultrasound may be beneficial. If impingement is associated, treatment depends on the stage at presentation. The radiographic appearance of the calcification can direct and perhaps predict the response to therapy. In the resorptive state, deposits appear floccular, suggesting that the process is in the phase of repair and that a conservative program is indicated.

Patients with discrete calcification and perhaps associated adhesive capsulitis (see Figure 46-8) may be at a stable

phase, in which calcium produces a mechanical block and is unlikely to be resorbed. For these patients, mechanical removal of calcific deposits and correction of associated pathologic lesions may be necessary.[88-90] Percutaneous disruption of calcified areas may be performed using a needle directed by fluoroscopy. This technique allows lavage and injection, but it does not treat associated impingement. Subacromial arthroscopy allows mechanical débridement of calcific deposits under direct visualization. This technique can be combined with arthroscopic removal of the inflamed bursa and decompression of associated impingement. Improved results have been noted with complete removal of calcific deposits.[91] In many cases of refractory calcific tendinitis associated with impingement, open or arthroscopic acromioplasty, subacromial bursectomy, and decompression are indicated.

### Rotator Cuff Tear

**Pathophysiology.** Spontaneous tear of the rotator cuff in an otherwise healthy person is rare.[16] It can occur in patients with RA or systemic lupus erythematosus as part of the pathologic process with invasion from underlying pannus. Metabolic conditions such as renal osteodystrophy and agents such as glucocorticoids occasionally are associated with cuff tears. Most patients report a traumatic episode, such as falling on an outstretched arm or lifting a heavy object. The usual presenting symptoms are pain and weakness of abduction and external rotation. Crepitus and even a palpable defect may be associated with the tear. Long-standing tears generally are associated with atrophy of the supraspinatus and infraspinatus muscles. It may be difficult to differentiate painful tendinitis from a partial-thickness or a small full-thickness cuff tear.

The exact cause of cuff tendinopathy continues to be controversial.[86,90,92,93] Most likely, the pathophysiology involves a combination of factors, including decreased vascularity and cellularity of the tendon, along with changes in the collagen fibers of the tendon that occur with aging.

Loss of motion with subsequent capsular tightness, particularly in the posterior capsule, may lead to cephalad migration of the humeral head, with subsequent impingement of the cuff under the coracoacromial arch.[94] Rehabilitation exercises focus on regaining a normal range of motion. To achieve full, painless motion, the normal relationship of glenohumeral to scapulothoracic motion must be achieved.[13,14,95]

**Diagnosis—History.** Patients with nontraumatic tears of the rotator cuff report symptoms of chronic impingement. Loss of motion and a feeling of stiffness are often noted with extremes of motion, along with difficulty during activities of daily living, such as combing one's hair, hooking a bra strap, putting on a shirt or coat, and reaching into the back pocket. In chronic cases of cuff tendinopathy, loss of motion usually occurs. Limitation of internal rotation caused by posterior capsular contracture occurs initially and is often associated with posterior shoulder pain with adduction of the ipsilateral shoulder. Further shoulder impingement occurs with forward flexion because of superior migration of the humeral head against the anterior inferior acromion. This upward translation is analogous to the action of a yo-yo climbing on a string.[94,96] Over time, loss of forward flexion, abduction, and external rotation occurs with passive and active motion of the shoulder.

**Diagnosis—Imaging.** In acute cases, a history of trauma, such as a fall onto the affected shoulder, may be reported. In cases involving an anterior shoulder dislocation with subsequent profound weakness of the rotator cuff, a large cuff tear or a greater tuberosity avulsion should be suspected, in addition to axillary nerve palsy. In younger patients, traumatic failure of the cuff under tensile overload may result in cuff failure caused by forced adduction of the affected shoulder or active abduction against resistance, and this outcome may occur with traumatic dislocation. Repetitive tensile overload also can result in partial rotator cuff tears in an athlete whose sport entails repetitive overhead actions.

Plain radiographs are used in the initial evaluation of impingement-type shoulder pain with cuff tendinopathy. An impingement series should be ordered, including an anteroposterior radiograph with a 30-degree cephalic tilt (the Rockwood view), which can reveal osteophytes of the anterior os acromion and AC joint; a scapular Y view with a 10-degree cephalic tilt (the supraspinatus outlet view), which can evaluate the type of acromion and reveal anterior and AC osteophytes; and an axillary view, which can evaluate the acromion for possible os acromiale. Calcific deposits within the rotator cuff tendon can be viewed best with rotational anteroposterior radiographs. Cuff arthropathy should be suspected if the acromial-humeral distance is less than 7 mm, or with the presence of cyst formation within the greater tuberosity, humeral head osteopenia, sclerosis around the greater tuberosity, or humeral head collapse. In advanced stages of cuff arthropathy, complete loss of glenohumeral joint space may be seen with superior migration and abutment of the humeral head against the undersurface of the acromion.[56]

In the past, shoulder arthrography was considered the "gold standard" for diagnosing full-thickness and partial-thickness rotator cuff tears, with greater than 90% sensitivity and specificity.[30,97] Currently, arthrography with CT or MRI is routinely used to diagnose rotator cuff disease, including full-thickness and partial-thickness tears.

Ultrasonography has been accurate in the diagnosis of full-thickness rotator cuff tears.[36,98-101] Ultrasonography offers the advantages of being inexpensive and noninvasive, but disadvantages include unproven effectiveness in determining subacromial impingement, capsular and labral abnormalities, and partial cuff tears. The procedure and its results are technician dependent. Ultrasonography may have a useful role in determining the post-operative integrity of the cuff repair.[35]

MRI has been invaluable in evaluating rotator cuff tears. The sensitivity and specificity of MRI for diagnosing full-thickness cuff tears are 100% and 95%.[102] With the use of gadolinium or saline solution, partial tears that are otherwise difficult to detect with conventional imaging can be detected.

Diagnosing cuff tears with MRI usually is based on discontinuity of the tendon on T1-weighted images and consistency with fluid signal on T2-weighted images. Ancillary findings include fluid in the subacromial space on T2-weighted images, loss of the subacromial fat plane on T1-weighted images, and proliferative spur formation of

the acromion or AC joint. Large, chronic cuff tears also may be associated with cephalad migration of the humeral head and fatty atrophy of the supraspinatus muscle. Periarticular soft tissues, including the capsulolabral complex and the biceps tendon, as well as the rotator cuff, can be thoroughly examined. The degree of tear and tendon retraction and evidence of muscle atrophy can be evaluated, all of which are crucial in pre-operative planning for possible cuff repair.

**Treatment—Nonsurgical.** Codman and Akerson[61] recommended early operative repair for acute full-thickness rotator cuff tears and reported the first documented repair in 1911. McLaughlin[63] recommended early repair in cases of grossly displaced tuberosity fractures or massive tears. Several other clinical studies have supported the concept that a full-thickness tear does not preclude good shoulder function. DePalma[103] reported that 90% of patients with rotator cuff tears responded to conservative measures, such as rest, analgesics, anti-inflammatory agents, and physiotherapy.

The reported percentage of patients responding to nonsurgical treatment in the literature varies from 33% to 90%.[4,15,104] Conservative treatment includes pain control with NSAIDs, ultrasound, application of heat before shoulder stretching and exercise, and application of ice after overhead activity. Deep massage therapy is used to reduce trigger point tenderness within the trapezius, levator scapulae, and periscapular muscles. Patients taking anti-inflammatory medications on a long-term basis are monitored periodically for evidence of gastrointestinal bleeding and for hepatic or renal toxicity. Opiate-based drugs are used only in the acute setting, such as after a fall, or in the perioperative period.

Steroid and local anesthetic injections are used when the patient has significant pain that prohibits rehabilitation. Injections may be repeated once every 3 months if needed; injection into the cuff tendon is to be avoided. If the patient fails to improve after 3 months of conservative treatment or does not continue to improve after three sequential injections, surgical options should be discussed.

The mainstay of conservative therapy is exercise. Rehabilitation stresses pain relief with exercises aimed at restoring shoulder motion and strengthening remaining cuff muscles, deltoid muscles, and scapular stabilizers. Therapy can be divided into three phases. The goals of the initial phase of therapy are to relieve pain and restore shoulder motion. Motion therapy includes pendulum exercises, passive motion with use of a wand with assistance of the uninvolved shoulder, an overhead pulley system, and posterior capsular stretching. The arc of motion is gradually increased and is guided by the patient's discomfort to avoid painful impingement arcs.

The second phase of therapy is entered after the patient has return of motion and little discomfort with overhead activity. Emphasis is placed on strengthening the remaining rotator cuff musculature and deltoid and periscapular muscles. Strengthening with elastic surgical tubing provides variable degrees of resistance, depending on the size of the tubing. Initial strengthening is performed out of the impingement arc (with 70 to 120 degrees of shoulder flexion). The goal of this phase is to strengthen the shoulder to prevent dynamic proximal humeral migration with impingement during active shoulder elevation.[56,59] Normal shoulder kinematics rely on combined and synchronous glenohumeral flexion and scapular rotation.[57,90] In addition to strengthening the cuff and deltoid, the scapular rotators, including the trapezius and the serratus anterior muscles, are emphasized.[105]

After the patient has successfully completed phase two of the rehabilitation program with minimal symptoms and good shoulder function, the final phase is entered. Phase three is characterized by a gradual return to normal overhead activities, including work and sporting activities. This part of the rehabilitation program should be tailored to the individual patient's needs and the demands placed on the shoulder.

**Treatment—Surgical.** A Cochrane review of the effectiveness of surgery for rotator cuff disease failed to reach any firm conclusions about the effectiveness or safety of rotator cuff surgery.[106]

Severity and duration of pain are the primary indications for surgical intervention in a rotator cuff tear. Other factors important in surgical decision making include shoulder dominance, activity level, physiologic age, acuteness of the tear, degree of the tear, loss of function, the amount of tendon retraction, and fatty atrophy of the remaining cuff musculature.

A systematic review of indications for rotator cuff surgery found that earlier surgical intervention may be needed for patients with cuff tears who have weakness and significant functional disability. In addition, older chronologic age did not portend a worse outcome; however, pending workman's compensation claims negatively affected treatment results.[107]

**Acute Tears.** Acute tears of the rotator cuff can be treated with conservative measures of periscapular and cuff strengthening along with capsular stretching to restore motion. Early surgical intervention should be considered in a young patient, especially an athlete whose sport entails repetitive overhead actions. Conservative shoulder rehabilitation should be maintained for 3 to 6 months before a decision is made regarding surgery for an older sedentary patient, in whom functional results without surgery may be acceptable. Many older patients may function well with chronic cuff tears, but they may become debilitated if an acute tear is superimposed on chronic changes. Surgical intervention may be required in these cases to return the patient to baseline function by repairing the acute tear and attempting to repair the chronic tear if possible.

**Chronic Tears.** For elderly patients whose pain and weakness do not create a functional problem, a conservative program is preferable for chronic tears. Pain that is unresponsive to conservative management is the main indication for surgery in an older patient with a chronic rotator cuff tear. In these cases, surgery should be considered on an individual basis after at least 3 months of conservative treatment, including subacromial steroid injection. If the cuff tear is massive and irreparable, débridement and subacromial decompression may provide good pain relief without extensive surgery and prolonged immobilization.[51,79,108-112] In a younger patient with a chronic tear and weakness, surgery to repair the cuff may be indicated to improve strength and prevent further extension of the tear.[110] In cases of rotator cuff arthropathy with glenohumeral joint degeneration, a reverse total shoulder replacement may be indicated. This



**Figure 46-14** Reverse total shoulder replacement in a 72-year-old man who had severe cuff arthropathy.

type of total shoulder replacement reverses the normal relationship between scapular and humeral components, moving the center of rotation medially and distally to increase the lever arm length of the deltoid muscle. The deltoid compensates for the deficient rotator cuff, allowing as near-normal function as possible (Figure 46-14). A recent publication also suggested reverse total shoulder arthroplasty for the treatment of irreparable rotator cuff tear with disability and no glenohumeral arthritis.[113]

**Economic Aspect of Rotator Cuff Repair.** With the recent shift of health care management toward reducing costs and efficient treatment, rotator cuff repairs have become an area of consideration. Evaluation fees, including visit and imaging fees, have gradually increased in recent years, generating a larger burden on both the health care system and the economy at large. A common aspect of health care costs that is not taken into consideration is the opportunity cost faced by patients when they choose to seek and follow medical advice. To fully understand the scope of the financial impact of rotator cuff repair, indirect costs such as travel costs to and from medical facilities, missed wages, decrease in labor and productivity, and disability support must be added into the equation. Indirect costs, combined with the increased costs reflected in Table 46-2, can show the real fiscal significance.

In subsequent debates it has been argued whether operative treatment is superior to nonoperative treatment, with both sides utilizing hidden costs as the deciding factor. Mather et al.[114] published a study showcasing this exact quandary of operative versus nonoperative treatment in cost reduction and societal benefit. The investigators utilized a decision-based, semirandom optimization framework, the Markov decision process, in comparing the repair of full-thickness tears to nonoperative management after an assumed failure of a course of nonoperative therapy. Patients in the model were reimbursed by the standards of average Medicare payouts at the time and several indirect costs, such as those previously mentioned, were used as measuring points. The results showed a significant cost efficacy at any age with repair, with a substantial impact on patients younger than 40 years because of their increased earning potential and financial activity.[114] Although the savings margin was increased for all ages, it was much more reduced in persons older than 70 years. The total savings calculated was approximately $3.44 billion, and the authors commented that although surgery is not indicated for all cases, it assisted with the global costs of rotator cuff tear management.

A more recent level I study conducted in Norway included 103 patients randomized into nonoperative or operative management for both full- and partial-thickness tears not exceeding 3 cm.[115] Although this study did not focus on the economic aspect of care, the results favored operative management and by doing so indicated certain financial benefits. The two major areas in which operative treatment was shown to excel and have a meaningful influence were in quality of life as measured by several outcome measures and rate of retearing and persistent tears during a 5-year period. The assumption can be made that with increased quality of life and decreased symptomatic retearing, patients most likely stayed on disability for shorter periods and fewer resources were allocated to symptom assessment and treatment.

Although these studies highlight certain aspects of the economics of rotator cuff repair, several limitations must be considered, such as sample size, assumptions made to develop the logic model, and financial motivation. At present there is still a dearth of analysis with regard to the fiscal environment surrounding health care costs for persons undergoing management of symptomatic rotator cuff tears.

## Bicipital Tendinitis and Rupture

The long head of the biceps passes through the bicipital groove, crosses over the head of the humeral, and inserts on the superior rim of the glenoid (see Figure 46-1A).[116] The biceps tendon aids in flexion of the forearm, supination of the pronated forearm if the elbow is flexed, and forward elevation of the shoulder.[4] Bicipital tendinitis, subluxation or dislocation of the biceps tendon within the bicipital groove, and rupture of the long head of the biceps generally are associated with anterior shoulder pain.

Bicipital tendinitis is sometimes an associated feature of a rotator cuff tear. The rotator cuff tear compromises centering of the humeral head on the glenoid. This compromise results in increased mechanical loading of the long head of the biceps, which initiates a hypertrophic tendinitis.[117]

Dislocation of the long head of the biceps usually is combined with a lesion of the subscapularis tendon.[9] Isolated rupture of the long head of the biceps tendon is rare when the rotator cuff is intact. Rupture of the long head of the biceps is common, however, when a co-existing rotator cuff tear is present.[118] The effects of rotator cuff tear and concomitant biceps tendon rupture on strength can be substantial.[9]

Early phases of bicipital tendinitis are associated with hypervascularity, edema of the tendon, and tenosynovitis.[119]

Persistence of this process leads to adhesions between the tendon and its sheath, along with impairment of the normal gliding mechanism in the groove. Stretching of the adhesions may be associated with chronic bicipital tendinitis.[120] The diagnosis of bicipital tendinitis is based on localization of tenderness. It is often confused with impingement symptoms and is frequently seen with an impingement syndrome.[21] Isolated bicipital tendinitis can be differentiated by the fact that the tender area migrates with the bicipital groove as the arm is abducted and externally rotated. Many eponyms are associated with tests to identify bicipital tendinitis.[4] Yergason's supination sign refers to pain in the bicipital groove when the examiner resists supination of the pronated forearm with the elbow at 90 degrees. Ludington's sign refers to pain in the bicipital groove when the patient interlocks the fingers on top of the head and actively abducts the arms.

Biceps tendon rupture can occur in some patients who report no history of shoulder pain. Patients often report an acute onset of pain and ecchymosis around the anterior shoulder and sagging of the biceps muscle belly. In these cases, a concomitant rotator cuff injury should be excluded by clinical examination. More often, the biceps tendon rupture is preceded by painful shoulder symptoms that often improve or disappear after the rupture.[120,121]

Treatment generally is conservative and consists of rest, analgesics, NSAIDs, and local injection of glucocorticoids. The use of ultrasound and a neuroprobe is more beneficial in this condition than in isolated rotator cuff tendinitis. Patients with refractory bicipital tendinitis and recurrent symptoms of subluxation are treated by arthroscopic biceps tenodesis or open tenodesis—that is, opening the bicipital groove and resecting the proximal portion of the tendon with tenodesis of the distal portion into the groove or beneath the pectoralis tendon.

## Acromioclavicular Disorders

The AC joint is a common source of shoulder pain. Acute causes of AC joint pain are often related to direct trauma of the affected shoulder that may result in a distal clavicle injury with an intra-articular chondral fracture, or in AC joint instability from ligamentous disruption.[122]

Post-traumatic distal clavicle osteolysis, with resorption of the distal clavicle, may ensue 4 weeks after a shoulder injury, leading to AC joint pain.[123,124] Osteolysis may be caused by microfracture of the subchondral bone and subsequent attempts at repair.[125] Other authors believe the cause to be an autonomic nerve dysfunction affecting the blood supply to the clavicle. The increased blood supply leads to resorption of bone from the distal clavicle.[123,126] More commonly, chronic osteolysis results from repetitive microtrauma to the AC joint from activities such as weight lifting, gymnastics, and swimming.[125,127,128]

The underlying pathophysiology is believed to be an inflammatory process caused by stress fracture of the subchondral bone with hyperemic resorption of the distal clavicle.[125,129] Other causes of osteolysis include rheumatoid arthrosis, hyperparathyroidism, and sarcoidosis, which should be considered in the differential diagnosis, especially in bilateral cases.[123,124] Patients with atraumatic osteolysis of the distal clavicle should be forewarned that bilateral involvement may occur; an incidence of 70% was reported for one study with long-term follow-up.[130] Other chronic causes of AC pain include idiopathic, intra-articular disk disease, post-traumatic degenerative arthrosis from joint incongruity, primary degenerative arthrosis, and rheumatoid arthrosis.

Evaluation should always include a detailed history, physical examination, and radiographic evaluation. A history of trauma to the AC joint from a direct fall or blow to the ipsilateral shoulder may be reported. Less commonly, the AC joint may have been injured indirectly, as during a fall on the outstretched arm with forces transmitted through the arm to the AC joint.[122,131] Patients with osteolysis of the distal clavicle sometimes report a history of acute trauma, although the more common cause is repetitive microtrauma to the AC joint caused by activities such as weight lifting or gymnastics.[123,124,127,128]

Patients frequently report having pain over the AC joint when adducting the ipsilateral shoulder, such as during a golf swing or when buckling a seat belt. Often, pain occurs when the patient sleeps on the affected shoulder. Athletes may experience AC joint pain when bench pressing and when performing push-ups and dips.[130,132,133] Pain and weakness of the affected shoulder also may be experienced with forward flexion and adduction of the arm.[123]

On physical examination, a visible step-off may be observed between the medial acromion and the distal clavicle, indicating a probable AC separation. Pain usually can be elicited on direct palpation of the AC joint and is made worse by a cross-arm adduction maneuver. This test is performed by internally rotating the arm, which is maximally adducted across the chest, and is considered positive if pain is produced in the AC joint (see Figure 46-3D). Pain also may be elicited by moving the arm from a horizontally abducted position to the extended position and upon maximal internal rotation of the shoulder.[132,134] These tests cause rotation and compression of the AC joint and are sensitive but less specific. They also may be positive with other disorders of the shoulder, such as posterior capsular stiffness.[135]

Frequently, AC joint pain co-exists with subacromial impingement and rotator cuff disease. In these cases, impingement signs are positive, and rotator cuff weakness may be present. Otherwise, no muscle weakness should be detectable on manual resistance testing, and no evidence of muscle atrophy should be found.[130,135,136] The AC joint and the subacromial space may require injection on separate occasions to determine the true source of the symptoms. Some physicians have noticed an association of AC joint symptoms with shoulder instability.[130] Glenohumeral motion can vary, depending on chronicity and isolation of the problem to the AC joint. In isolated cases, some loss of internal rotation of the affected shoulder may be caused by pain.

Radiographs should include anteroposterior views of the shoulder in the scapular plane in neutral, internal, and external rotation; a transcapular Y view; an axillary view; and a 15-degree cephalic tilt view of the AC joint at 50% penetrance, as described by Zanca (see Figure 46-4).[20] Stress views may be obtained by strapping 5- to 10-lb weights to the forearms and determining AC separation. Comparing the coracoclavicular distance of both shoulders also may be

helpful. When clinically indicated, cervical spine radiographs should be obtained to exclude cervical spondylosis.

Radiographic evaluation may reveal AC joint arthrosis with microcystic changes in the subchondral bone, sclerosis, osteophytic lipping, and joint space narrowing.[137] In cases of osteolysis, radiographs may reveal loss of subchondral bone detail with microcystic appearances in the subchondral region of the distal clavicle and osteopenia of the lateral one-third of the clavicle.[124,125,127,128] In late stages of osteolysis, resorption of the distal end of the clavicle results in marked widening of the AC joint and sometimes complete resorption of the distal clavicle. AC separation may be evident with widening of the coracoclavicular distance and post-traumatic ossification of the coracoclavicular ligaments.

AC symptoms do not always correlate with the radiographic appearance of the joint. DePalma[138] found AC joint degeneration to be an age-related process, with symptoms not always correlating with radiographic findings of AC joint arthrosis.[20] AC joint pain may occur despite normal findings of radiographs.[139]

A Tc 99m phosphate bone scan may assist in the diagnosis, revealing increased uptake in the distal clavicle and the medial acromion.[125] In cases of atraumatic osteolysis of the distal clavicle, increased uptake may be isolated to the distal clavicle, but in approximately 50% of cases, scintigraphic activity of the adjacent medial acromion is increased.[130] The bone scan may reveal pathologic changes in the AC joint when plain radiographs appear normal.

In selected cases, MRI can be valuable in determining a diagnosis and evaluating the glenohumeral and subacromial regions for co-existing disease (Figure 46-15). AC joint involvement may reveal increased fluid with synovitis, soft tissue enlargement, and periarticular ossifications with encroachment on underlying bursal and cuff tissue.

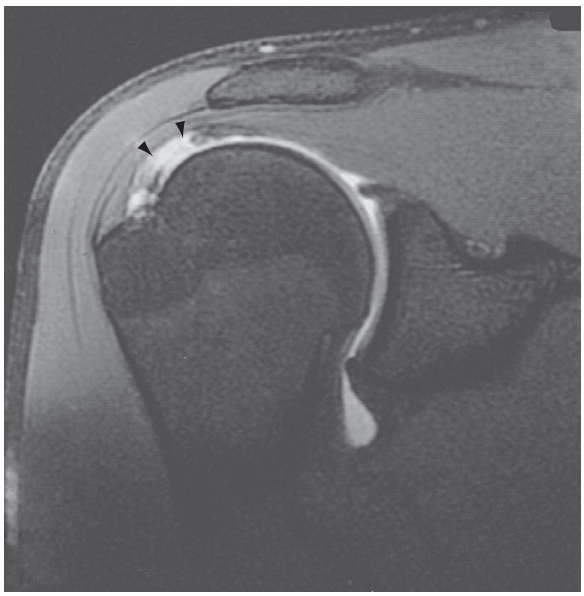


**Figure 46-15** Sagittal section MRI of the shoulder in a 32-year-old weight lifter with shoulder pain. A fat-suppressed proton density fast spin-echo image shows a bursal-side high-grade partial cuff tear *(arrowheads)*.

Patients with AC joint pain usually respond well to nonoperative treatment; however, complete relief of symptoms may require an extended period. Conservative therapy includes application of heat, use of NSAIDs, steroid injections, shoulder rehabilitation, and avoidance of painful positions and activities. Steroid injections are repeated at 3-month intervals if painful conditions persist.

Open resection of the distal clavicle for chronic AC joint pain was initially reported by Gurd[140] and by Mumford,[141] both with good results. Since that time, other surgeons have reported similar good results with open resection; however, significant morbidity, such as disruption of the deltotrapezial fascia and anterior deltoid rupture, can occur.[124,124,137,139,142] Arthroscopic resection of the distal clavicle has been described with results similar to those of open resection.[10,129,134,136,143-146]

## Glenohumeral Disorders

The various arthritides that affect the shoulder joint are discussed in detail in other chapters. They are presented here to address aspects that are unique to the glenohumeral joint. The usual presentation of intra-articular disorders consists of pain with motion and symptoms of internal derangement, such as locking and clicking. Pain is generalized throughout the shoulder girdle and sometimes is referred to the neck, back, and upper arm. The usual response to pain includes decreased glenohumeral motion and substitution with increased scapulothoracic mobility. Patients with adequate elbow and scapulothoracic motion require little glenohumeral motion for activities of daily living, and patients with glenohumeral arthrodesis can achieve adequate function.[147,148] The response to pain consists of diminution of motion and secondary soft tissue contractures with muscle atrophy. With increasing weakness and involvement of adjacent joints, pain, limitation of motion, and weakness can cause a substantial functional deficit.

## Inflammatory Arthritis

Although the most common inflammatory arthritis involving the shoulder joint is RA, other systemic disorders such as systemic lupus erythematosus, psoriatic arthritis, ankylosing spondylitis, reactive arthritis, and scleroderma may cause glenohumeral degeneration. Motion is limited by splinting of the joint with secondary soft tissue contractures or by primary soft tissue involvement with scarring or rupture. Plain radiographs confirm glenohumeral involvement (Figure 46-16A). Narrowing of the glenohumeral joint space may occur, with erosion and cyst formation and without significant sclerosis or osteophytes. As the disease progresses, superior and posterior erosion of the glenoid with proximal subluxation of the humeral head may occur. Eventually, secondary degenerative changes and even osteonecrosis of the humeral head may occur.

Treatment is initially conservative and is directed toward controlling pain, inducing a systemic remission, and maintaining joint motion through physical therapy. The use of intra-articular glucocorticoids may be beneficial in controlling local synovitis. In RA, the involvement of periarticular structures with subacromial bursitis and rupture of the rotator cuff magnifies the functional deficit. When synovial

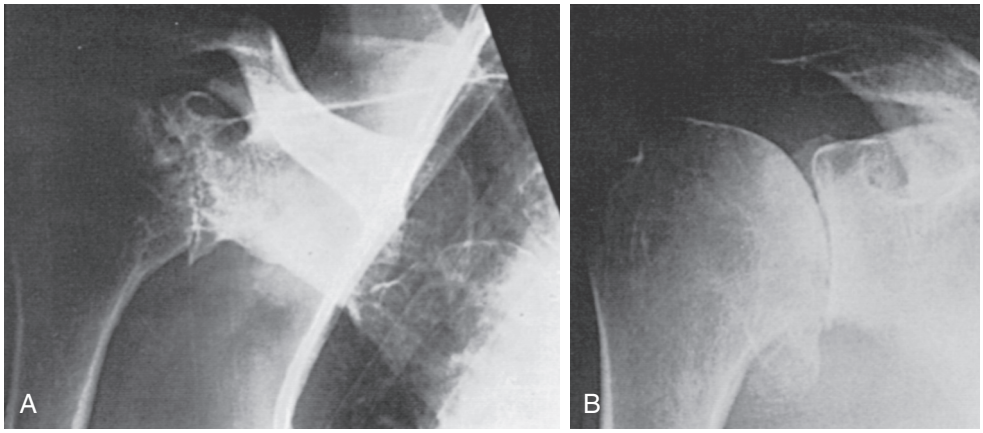



**Figure 46-16** Plain radiographs. **A,** Rheumatoid arthritis with loss of joint space, cyst formation, glenohumeral erosion, and early proximal subluxation of the humerus, indicating a rotator cuff tear. **B,** Osteoarthritis with narrowing of the glenohumeral joint space, sclerosis, and osteophyte formation. Notice the preservation of the subacromial space, suggesting an intact rotator cuff.

cartilage interactions produce significant symptoms and radiographic changes that cannot be controlled by conventional therapy, glenohumeral resurfacing should be considered.

When following up on a patient with RA who has shoulder involvement, the rheumatologist should assess range of motion carefully and obtain radiographs periodically. Patients with progressive loss of motion or radiographic destruction should be referred for evaluation for possible surgical treatment. The treatment of choice is an unconstrained total shoulder arthroplasty.[149,150] Total shoulder arthroplasty is best performed in patients with RA before end-stage bony erosion and soft tissue contractions have occurred.[151,152] Acute inflammatory arthritis of the glenohumeral joint may be associated with gout, pseudogout, hydroxyapatite deposition of renal osteodystrophy, and recurrent hemophilic hemarthrosis.

## Osteoarthritis

Osteoarthritis of the glenohumeral joint is less common than that in the hip, its counterpart in the lower extremity; this condition is caused by non–weight-bearing characteristics of the shoulder joint and the distribution of forces throughout the shoulder girdle. Osteoarthritis is divided into conditions associated with high unit loading of articular cartilage and conditions in which an intrinsic abnormality within the cartilage causes abnormal wear at normal loads. Because the shoulder is normally a non–weight-bearing joint and is not usually susceptible to repeated high loading, the presence of osteoarthritis of the glenohumeral joint should alert the physician to consider other factors. Has the patient engaged in unusual activities, such as boxing, heavy construction, or long-term use of a pneumatic hammer? Has some disorder, such as epiphyseal dysplasia, created joint incongruity with high unit loading of the articular cartilage? Is this a neuropathic process caused by diabetes, syringomyelia, or leprosy? Have associated hemochromatosis, hemophilia, or gout altered the ability of articular cartilage to withstand normal loading? Is unrecognized chronic dislocation responsible?

Pain is the usual presentation, but generally it is not as acute or it may be associated with the spasm seen in inflammatory conditions. Plain radiographs show narrowing of the glenohumeral joint, osteophyte formation, sclerosis, and some cyst formation (Figure 46-16B). Because the rotator cuff usually is intact, less bone erosion of the glenoid and proximal subluxation of the humerus is noted. Patients with osteoarthritis of the glenohumeral joint frequently do well with functional adjustments and conservative therapy. Analgesics and NSAIDs may provide symptomatic relief. The use of glucocorticoid injections is less beneficial, unless evidence of synovitis is observed. Patients with severe involvement who fail to respond to conservative therapy are best treated with shoulder arthroplasty.

## Osteonecrosis

Osteonecrosis of the shoulder refers to necrosis of the humeral head seen in association with a variety of conditions. Symptoms are due to synovitis and joint incongruity resulting from resorption, repair, and remodeling.

The most common cause of osteonecrosis of the shoulder is avascularity resulting from a fracture through the anatomic neck of the humerus.[153] Fracture through this area disrupts intramedullary and capsular blood supplies to the humeral head.[154] Another common cause of osteonecrosis of the shoulder is steroid therapy provided in conjunction with organ transplantation, systemic lupus erythematosus, or asthma. Other conditions associated with osteonecrosis of the humeral head include hemoglobinopathies, pancreatitis, and hyperbarism.

Early diagnosis is difficult because the presence of symptoms is often delayed. Bone scans may be helpful in early cases, before radiographic changes are evident. MRI is highly sensitive and is more specific than scintigraphy. Plain radiographs show progressive phases of necrosis and repair. In early stages, the films may be normal or may show osteopenia or bone sclerosis. A crescent sign representing subchondral fracture or demarcation of the necrotic segment appears during the reparative process. Patients who fail to undergo remodeling show collapse of the humeral head with

secondary degenerative changes. A considerable discrepancy is often noted between symptoms and radiographic involvement. Patients with extensive bone changes may be asymptomatic. Treatment should be directed by the patient's symptoms rather than by the radiographs and is similar to that provided for osteoarthritis. Arthroscopy occasionally is helpful by removing loose chondral fragments and débriding chondral incongruities.[155] Patients with severe symptoms that cannot be controlled by conservative means are best treated with unconstrained shoulder arthroplasty, hemiarthroplasty, or resurfacing arthroplasty.[149]

## Cuff-Tear Arthropathy

In 1873, Adams described the pathologic changes that characterize RA of the shoulder and a condition that since that time has been referred to as "Milwaukee shoulder" or cuff-tear arthropathy.[156] McCarty called the condition Milwaukee shoulder and reported that factors predisposing to this syndrome included deposition of calcium pyrophosphate dihydrate crystals, direct trauma, chronic joint overuse, chronic renal failure, and denervation.[157,159] Patients with Milwaukee shoulder have elevated levels of synovial fluid 5-nucleotidase activity and elevated levels of synovial fluid inorganic pyrophosphate and nucleotide pyrophosphohydrolase activity.[158]

Neer and colleagues[159] reported a similar condition in which untreated massive tears of the rotator cuff with proximal migration of the humeral head are associated with erosion of the humeral head. Erosion of the humeral head differs from that seen in other arthritides and is presumed to be caused by a combination of mechanical and nutritional factors acting on the superior glenohumeral cartilage.

Patients with cuff-tear arthropathy present a difficult therapeutic problem because bone erosion and disruption of the cuff jeopardize the functional result from an unconstrained prosthesis.[151] Hemiarthroplasty or a reverse total shoulder arthroplasty may be indicated.[160,161] The major challenge in treating cuff-tear arthropathy is to determine which patients with massive rotator cuff tears will proceed to the syndrome of cuff-tear arthropathy. Patients with massive rotator cuff tears in whom localized calcium pyrophosphate disease develops may be predisposed to further proximal migration and further joint destruction. This situation poses a dilemma for the treating physician. Many patients with massive rotator cuff tears remain stable and require little or no treatment. Occasionally, symptomatic patients can be treated by arthroscopic débridement of the cuff tear. In a recent study, patients who had massive rotator cuff tears without arthritis did well when treated with reverse total shoulder arthroplasty.[113] It is crucial to define the patient who will proceed to the syndrome of cuff-tear arthropathy. If crystal deposition disease predisposes patients to proximal migration and joint destruction, joint aspiration with crystal analysis and scintigraphy to determine synovial reaction may be helpful diagnostic tools.

Hamada and co-workers[162] followed up 22 patients with massive rotator cuff tears who were treated conservatively. Radiographic findings included narrowing of the acromiohumeral interval and degenerative changes in the humeral head, tuberosities, acromion, AC joint, and glenohumeral joint. Five of seven patients followed up for longer than 8 years progressed to cuff-tear arthropathy. Investigators concluded that progressive radiographic changes were associated with repetitive use of the arm in elevation, rupture of the long head of the biceps, impingement of the humeral head against the acromion, and weakness of external rotation.[162]

## Septic Arthritis

Septic arthritis can masquerade as any of the conditions classified as periarticular or glenohumeral disorders. Sepsis must be included in any differential diagnosis of shoulder pain because early recognition and prompt treatment are necessary to achieve a good functional result. The diagnosis is confirmed by joint aspiration with synovial fluid analysis and culture. Cultures should include aerobic, anaerobic, mycobacterial, and fungal studies.

## Labral Tears

The glenoid labrum increases the depth of the glenoid and serves as an anchor for the attachment of the glenohumeral ligaments. Historically, labral tears have been difficult to diagnose. Findings on physical examination can be confused with impingement and rotator cuff tendinopathy and bicipital tendinitis. Diagnosis can be confirmed with MRI-arthrography, CT-arthrography, and DCAT.[24] Arthroscopy has greatly increased our knowledge of the glenoid labrum in normal and pathologic situations and has aided clinicians in the diagnosis and treatment of labral lesions.

Labral tears can be divided into tears associated with symptoms of internal derangement and tears associated with anterior or posterior instability. A soft tissue Bankart lesion is associated with a tear of the anterior band of the inferior glenohumeral ligament and with anterior instability. Isolated labral tears that do not involve detachment of the ligaments can cause internal derangement and may have an arthroscopic appearance similar to that of a meniscal tear of the knee.

Andrews and associates[2] first described lesions of the anterior superior labrum in athletes whose sport entails throwing; these lesions were often associated with biceps tendon tears (10%), which may result from traction of the biceps tendon. Snyder and co-workers[163] introduced the term *SLAP lesion* in 1990 to describe an injury involving the long head of the biceps tendon and the superior portion of the glenoid labrum.

The long head of the biceps tendon originates at the supraglenoid tubercle and the glenoid labrum in the superior-most portion of the glenoid. The major portion of the tendon blends with the posterior superior aspect of the labrum. The most common mechanism of a SLAP injury is a fall onto an outstretched arm with the shoulder in abduction and slight forward flexion.[163] The lesion also can result from acute traction on the arm and from an abduction and external rotation mechanism.[164,165]

Patients usually report pain with overhead activities and a frequent catching or popping sensation in the shoulder. The most reliable diagnostic test is O'Brien's test. The test is performed against resistance with the arm in forward flexion and with the elbow extended and the forearm pronated. In the second part of the test, the arm is supinated.

Less pain during the latter part of the test suggests a SLAP lesion.[163] The most accurate diagnostic test is MRI-arthrography with gadolinium.[166] Treatment for symptomatic SLAP lesions is surgical.

## Adhesive Capsulitis

Adhesive capsulitis, or frozen shoulder syndrome (FSS), is a condition characterized by limited motion of the shoulder joint with pain at the extremes of motion. It was first described by Putman[167] in 1882 and later by Codman.[85] The initial presentation is pain, which is generalized and is referred to the upper arm, back, and neck. As pain increases, loss of joint motion ensues. The process generally is self-limiting and in most cases resolves spontaneously within 10 months, unless an underlying problem is present.

The exact cause of FSS is unknown.[90,168] It is frequently associated with conditions such as diabetes mellitus, parkinsonism, thyroid disorders, and cardiovascular disease. When one of these conditions exists, a history of some mild trauma that initiated the frozen shoulder is often reported. Major skeletal trauma and soft tissue injury may co-exist with FSS. It also may be seen with a variety of other conditions, including apical lung tumor, pulmonary tuberculosis, and cervical radiculopathy, as well as after myocardial infarction.[169-171] In one review of FSS, 3 of 140 patients with this syndrome had local primary invasive neoplasms.[172] Another study described three patients with adhesive capsulitis who subsequently were found to have a neoplastic lesion of the midshaft of the humerus.[173] In a high-risk patient with an underlying disorder, even minor surgery or trauma in a remote location, such as the hand, can precipitate FSS.

The pathophysiology involves a diffuse inflammatory synovitis with subsequent adherence of the capsule and loss of the normal axillary pouch and joint volume, which leads to significant loss of motion. Capsular contracture is thought to result from adhesion of the capsular surfaces or fibroblastic proliferation in response to cytokine production.[168,174,175] The condition is common in women in their 40s and 50s. Typically, the patient relays a history of diffuse, dull aching around the shoulder, with weakness and loss of motion occurring over a few months.

Usually, three distinct clinical stages of the syndrome can be identified. Stage one is the painful inflammatory or freezing phase. During this stage, pain is severe, is exacerbated by any attempts at movement, and usually lasts a few weeks or months. The patient usually feels most comfortable with the arm at the side in an adducted and internally rotated position. Phase two, the adhesive or stiffening phase, generally lasts 4 to 12 months. Pain is usually minimal during this phase, although periscapular symptoms may develop from compensatory motion to achieve elevation of the arm. The third phase of the syndrome is the resolution or thawing phase, which may last 5 to 26 months. During this time, pain eases and motion slowly improves, although some patients may improve dramatically over a short period.[176]

In the early stages, any attempts at motion may produce severe pain and associated weakness. The syndrome usually is associated with a prolonged period of immobilization.[177] Pain at night is common, along with an inability to sleep on the associated shoulder; these findings are similar to those of impingement syndrome.

In patients with a history of minimal or no trauma and FSS, a metabolic cause should be excluded. A complete blood cell count, erythrocyte sedimentation rate, serum chemistry, and thyroid function tests are performed as a screening panel. Further testing is performed if results suggest that the patient may have a systemic illness. Plain radiographs should include true anteroposterior, axillary, and scapular Y views of the shoulder. In patients with no underlying detectable illness and a negative workup, a Tc 99m pertechnetate scan may show increased uptake in FSS, but more important, it is used to exclude occult lesions or metastasis.[178]

A literature review reveals a multitude of treatment options, along with significant deficits on accurate reporting of disease staging with response to treatment.[179]

Treatment of FSS is mainly conservative and consists of intra-articular injections, application of heat, gentle stretching, NSAIDs, and modalities such as transcutaneous electrical nerve stimulation. The disease usually is self-limited, and after the painful phase it is not severely disabling. Communication between the physician and the patient, together with a thorough explanation of the condition, is essential, because resolution of the syndrome occurs slowly. Closed manipulation and surgery (open and arthroscopic) are reserved for patients whose condition is recalcitrant to conservative measures or for whom the diagnosis is in question. Paramount in the prevention of FSS is avoiding overimmobilization with a minor shoulder injury, in addition to careful identification of patients at risk for FSS.

Fareed and Gallivan[180] reported good results with hydraulic distention of the glenohumeral joint using local anesthetic agents. Rizk and associates[181] conducted a prospective, randomized study to assess the effects of steroid or local anesthetic injection in 48 patients with FSS. No significant difference in outcome was noted between persons who received intrabursal or intra-articular injection. Steroid with lidocaine offered no advantage compared with lidocaine alone in restoring shoulder motion. However, transient pain relief occurred in two-thirds of patients treated with steroids.[181]

General anesthesia occasionally is indicated for closed manipulation. Hill and Bogumill[182] reported the results of manipulation of 17 frozen shoulders in 15 patients who did not respond to physical therapy. On average, 78% of persons who were working before their shoulder problems returned to work 2.6 months after manipulation. Investigators concluded that manipulation allowed patients to return to a normal lifestyle and to work sooner than the reported natural history of the condition.[182] Surgical intervention for adhesive capsulitis should be limited to treatment of an underlying problem, such as calcific tendinitis or an impingement syndrome.

## Glenohumeral Instability

Glenohumeral instability is a pathologic condition that manifests as pain associated with excessive translation of the humeral head on the glenoid during shoulder motion. Instability can range from excessive laxity with episodes of subluxation to frank dislocation of the joint. Traumatic dislocation of the glenohumeral joint reveals characteristic clinical and radiographic findings that are beyond the scope

of this chapter and have been reviewed in detail elsewhere.[183] The most common type of instability is anterior, although posterior and multidirectional laxity of the shoulder is increasingly recognized as a cause of shoulder pain. Anterior dislocation usually occurs with the arm in an abducted and externally rotated position, and the diagnosis is usually obvious. Posterior dislocation is frequently associated with convulsive disorders or unusual trauma with the arm in a forward flexed and internally rotated position. The diagnosis is often missed and should always be suspected in the patient who is unable to rotate the arm externally after trauma.

Recurrent subluxation without dislocation may be difficult to diagnose and may mistakenly be identified as impingement with chronic cuff tendinitis. An athlete whose sport entails overhead actions may experience repetitive stresses to the shoulder, causing microtrauma to the static stabilizers. Jobe and colleagues[18] described a syndrome of shoulder pain in athletes who engage in overhead activities or throwing that manifests as impingement but is caused by anterior subluxation of the joint, with the humeral head impinging on the anterior aspect of the coracoacromial arch. Fu and co-workers[184] underscored this distinction by dividing the causes of rotator cuff tendinitis into primary impingement of the tendon on the coracoacromial arch and anterior subluxation with secondary impingement in young athletes performing overhead movements. Walch and colleagues[185] described intra-articular impingement between the undersurface of the rotator cuff (supraspinatus and infraspinatus) and the posterior superior glenoid rim and labrum. This "internal impingement" usually is observed in athletes who engage in overhead activities and have subtle anterior glenohumeral instability, and it results in tendinitis or partial tears of the rotator cuff (see Figure 46-16).

The diagnosis of glenohumeral instability with subluxation in one or multiple directions is made with the combination of a detailed history and physical examination and the use of adjuncts, such as arthrography, CT, MRI, and arthroscopy with examination under the effects of anesthesia. The syndrome of multidirectional instability has been recognized in patients with symptomatic inferior instability, in addition to anterior or posterior instability. Approximately 50% of affected patients have evidence of generalized laxity. Frequently, the syndrome occurs in young athletic patients who are loose jointed, in particular in the dominant arm of pitchers, racket sports players, and swimmers. In these types of athletes, repetitive microtrauma may cause stretching of the shoulder, resulting in a large capsular pouch without labral detachment. A traumatic event may damage the shoulder, resulting in the syndrome of multidirectional instability and a Bankart lesion.[186]

The most common manifestation in these patients is pain, which often is mistakenly considered to be rotator cuff tendinitis. The patient may relate a history of minor trauma causing acute pain and a "dead arm" syndrome lasting minutes or hours. Other associated symptoms include a sense of instability, weakness, and radicular symptoms suggestive of neuropathy. Few or no positive physical findings may be associated with chronic subluxation or multidirectional instability. The patient may have signs of generalized ligamentous laxity, and pain may be reproduced by subluxating the glenohumeral joint in multiple directions. One particularly helpful sign of inferior laxity is the sulcus sign, which refers to the subacromial indentation that occurs when longitudinal traction is applied to the humerus with the arm at the side. This sign occurs with inferior translation of the humeral head. Because this syndrome frequently occurs in athletes with highly developed musculature around the shoulder girdle, physical findings of subluxation may be difficult to reproduce in the office setting.

Plain radiographs are generally normal, although some inferior subluxation may be shown on stress radiographs obtained with the use of weights. Special radiographs, as discussed previously, may show a Bankart lesion (i.e., avulsion of the anterior inferior glenoid rim) or a Hill-Sachs lesion (i.e., osteochondral defect of the posterior humeral head) with subluxation of the humeral head in front of the anterior glenoid rim. CT-arthrography or MRI-arthrography may show increased capsular volume, a labral detachment, or a Hill-Sachs lesion (see Figure 46-9). When surgery is indicated, examination under the effects of anesthesia and shoulder arthroscopy may assist in diagnosing the primary direction of instability in the syndrome of multidirectional instability. In selected patients with traumatic anterior dislocation who have no history of multidirectional instability, arthroscopic stabilization may be performed with stabilization of the capsulolabral complex.

Treatment of patients with chronic subluxation or the syndrome of multidirectional instability is first directed toward prolonged rehabilitation. Activities that stress the shoulder and produce symptoms are avoided. Strengthening exercises of the shoulder girdle may control symptoms, dynamically stabilizing the glenohumeral joint, and may obviate the need for surgical intervention. If a conservative treatment program fails, surgery is performed on the side associated with the greatest clinical instability. Stabilization is directed toward tightening of the capsular structures to stabilize the glenohumeral joint.[186,187]

## EXTRINSIC OR REGIONAL FACTORS CAUSING SHOULDER PAIN

Because the shoulder girdle connects the thorax with the upper extremity, and because major neurovascular structures pass in proximity to the joint, shoulder pain is a hallmark of many nonarticular conditions.

### Cervical Radiculopathy

Cervical disease may manifest with associated shoulder pain. The area of referred pain has a dermatomal pattern, consistent with the distribution of dermatomal nerve roots. Isolation of the pain usually defines the exact location of associated cervical disease. Pain can be differentiated from shoulder pain on the basis of history, physical examination, EMG, cervical radiographs, and myelography or MRI when indicated. Because conditions causing cervical neck pain and conditions causing shoulder pain, such as calcific tendinitis and cervical radiculopathy, may co-exist, it often is difficult to distinguish which lesion is responsible for the symptoms. These conditions often can be differentiated by injection of local anesthetics to block certain components of the pain.

The thoracic outlet is an interval created by the anterior and middle scalene muscles and the first rib through which the brachial plexus and vessels pass to the arm. In thoracic outlet syndrome, compression of these nerves and vessels often manifests as vague shoulder pain with numbness of the ipsilateral fourth and fifth digits. Cervical rib or hypertrophy of the scalene muscles can be related to the onset of pain.[188-190] The occurrence of pain also has been related to scapular ptosis, poor posture, and clavicular fracture with malunion or copious formation of callus.

## Brachial Neuritis

In the 1940s, Spillane[191] and Parsonage and Turner[192,193] described a painful condition of the shoulder associated with limitation of motion. As pain subsided and motion improved, muscle weakness and atrophy became apparent. The deltoid, supraspinatus, infraspinatus, biceps, and triceps are the most frequently involved muscles,[194] although diaphragmatic paralysis also has been reported.[193,195] The cause is unclear, but the clustering of cases suggests a viral or postviral syndrome.[192,193] Occasionally, an associated influenza-like syndrome or previous vaccination has been reported.[194]

Hershman and colleagues[196] described acute brachial neuropathy in athletes. Findings that suggest an acute brachial neuropathy include acute onset of pain without trauma; persistent, severe pain that continues despite rest; and patchy neurologic signs. The diagnosis is confirmed by EMG and nerve conduction studies.[196] The prognosis for recovery is excellent, although full recovery may take 2 to 3 years. Tsairis and associates[197] reported 80% recovery within 2 years and more than 90% recovery by the end of 3 years.

## Nerve Entrapment Syndromes

Peripheral compression neuropathies of the upper extremities may produce referral pain to the shoulder. Distant compression neuropathies, such as carpal tunnel (median nerve) and cubital tunnel (ulnar nerve) syndromes, may manifest with concomitant and separate shoulder impingement with rotator cuff disease. Associated numbness and paresthesias with mapping of the dermatomal distribution and with peripheral neuropathy often direct the examiner to the appropriate diagnosis. Patients often give a history of dropping objects and a feeling of clumsiness with the affected hand. A Tinel sign may be elicited over the region of entrapment at the elbow or wrist. Provocative maneuvers such as Phalen's test may be positive and usually indicate median nerve compression at the wrist. Diminished vibratory sensation is an early finding in the disease and is easily reproducible,[198,199] whereas decreased two-point discrimination and intrinsic atrophy are late findings of peripheral compression neuropathy.[198]

The diagnosis usually can be made by clinical examination with exclusion of other possible causes. EMG and nerve conduction velocity tests may reveal slowed conduction and latency at appropriate compression points to aid in diagnosis. Spinal accessory nerve injury with subsequent denervation of the trapezius may cause weakness and pain in the shoulder consistent with impingement. The injury can occur from traction injury to the neck or a direct blow or pressure to the base of the neck. Iatrogenic nerve injury may result from surgical procedures on the neck such as lymph node biopsy.[200] The injury produces weakness in shoulder abduction with associated pain that radiates from the neck into the trapezius and shoulder. Subsequent atrophy of the trapezius may lead to dyssymmetry and ptosis of the involved shoulder, with narrowing of the supraspinatus outlet and secondary impingement with shoulder pain. Definitive diagnosis can be made by EMG examination.

Early treatment is conservative. If return of function is not evident at 6 months, surgical exploration of the nerve with possible tendon transfers may be indicated.[201]

Injury to the long thoracic nerve (cervical fifth, sixth, and seventh roots) can lead to scapular winging. The resultant scapular dysrhythmia and weakness can lead to a painful shoulder that may mimic rotator cuff disease.[200] Patients also report pain and discomfort with active forward flexion of the shoulder. Patients who remain symptomatic after conservative treatment may require surgery for scapulothoracic fusion or tendon transfer with use of the pectoralis major or minor to stabilize the scapula.[202,203]

In quadrilateral space syndrome, the axillary nerve is compressed by fibrous bands in the quadrilateral space.[200,204,205] This syndrome typically occurs when the arm is held in abduction and external rotation, with subsequent tightening of fibrous bands across the nerve.[206] It is most commonly seen in the dominant shoulder of young athletic persons such as pitchers, tennis players, and swimmers who function with an excessive amount of overhead activity. Pain may occur throughout the shoulder girdle and may radiate down the arm in a nondermatomal pattern. Findings of neurologic and EMG testing may be normal. Diagnosis often is made by an arteriogram of the subclavian artery. A positive arteriogram reveals compression of the posterior humeral circumflex artery as it traverses the quadrangular space when the arm is in the abducted and externally rotated position. Surgical intervention may be required to release the fibrotic bands or the tendon of the teres minor if the patient does not respond to conservative treatment.[200,207]

Suprascapular nerve entrapment syndrome can be caused by a traction lesion resulting from repetitive overhead activities, a compression lesion, or both, involving the nerve; it is caused by tethering of the nerve at the suprascapular notch by the suprascapular ligament or at the spinoglenoid notch by the transverse ligament. Suprascapular nerve entrapment syndrome also can result from direct compression of a space-occupying lesion, such as a ganglion or a lipoma. Rengachary and co-workers[208] described variations in size and shape of the suprascapular notch that may predispose the nerve to entrapment. Several authors have noted an association of suprascapular neuropathy with massive rotator cuff tears, presumably resulting from a traction injury to the nerve.[209,210]

The resulting suprascapular neuropathy produces pain in the posterolateral aspect of the shoulder that may radiate into the ipsilateral extremity, shoulder, or side of the neck. Although this condition is uncommon, it can have a prolonged and disabling course when it is not diagnosed. Because the suprascapular nerve has no cutaneous innervation, no numbness, tingling, or paresthesias are associated

with this condition. Weakness is usually noted in abduction and external rotation, and significant atrophy is often observed at diagnosis. The pain frequently is described as a deep burning or aching that can be well localized and often can be elicited by palpation over the region of the suprascapular notch. Any activity that brings the scapula forward, such as reaching across the chest, may aggravate the pain.[211] The location of pain and other symptoms can mimic more common entities, such as impingement, rotator cuff disease, cervical disk disease, brachial neuropathy, biceps tendinitis, thoracic outlet syndrome, AC disease, and instability of the shoulder.[212]

Fritz and colleagues[213] reported the efficacy of MRI in the diagnosis of suprascapular nerve entrapment as a result of space-occupying lesions. Definitive diagnosis is made with EMG and nerve conduction studies. EMG changes usually reveal spontaneous activity in the muscle at rest and fibrillations indicating motor atrophy and denervation. Nerve conduction studies may reveal slowing across the site of entrapment. As with axillary nerve entrapment, the syndrome is often associated with young, athletic persons who engage in excessive overhead activity.[214] It also has been associated with trauma.[212,215,216]

Lack of consensus continues regarding the optimal treatment of suprascapular neuropathy.[1,212,214,217-219] Post and Grinblat[217] reported good to excellent results with surgical treatment in 25 of 26 cases. No difference in residual atrophy and in strength deficits has been shown, however, for operative and nonoperative treatments. Ferretti and co-workers[214] evaluated 96 top-level volleyball players from the 1985 European Championships and found that 12 had isolated suprascapular neuropathy with atrophy of the infraspinatus of the dominant shoulder. All players were unaware of any impairment, however, and played without limitations. After a space-occupying lesion has been excluded, a 6-month trial of conservative treatment may be indicated for some persons. If the entrapment does not improve or if symptoms worsen with conservative treatment, surgical decompression for pain relief is warranted; however, resolution of atrophy and strength gains can vary.[212]

## Sternoclavicular Arthritis

Occasionally, traumatic, nontraumatic, or infectious conditions can cause pain in the area of the sternoclavicular joint (see Figure 46-1). The most common problem involves ligamentous injury and painful subluxation or dislocation, which can be diagnosed by palpable instability and crepitus over the sternoclavicular joint. Radiographic sternoclavicular views may show dislocation.[220]

Inflammatory arthritis of the sternoclavicular joint has been associated with RA, psoriatic arthritis, ankylosing spondylitis, and septic arthritis. The association of palmoplantar pustulosis with sternoclavicular arthritis has been reported.[221] Seven of 15 patients who underwent biopsy for this condition had cultures positive for *Propionibacterium acnes*, suggesting an infectious origin of the condition.[221]

Two other conditions involving the sternoclavicular joint are Tietze's syndrome, a painful, nonsuppurative swelling of the joint and adjacent sternochondral junctions, and Friedrich's syndrome, a painful osteonecrosis of the sternal end of the clavicle.[4] Condensing osteitis of the clavicle is a rare benign idiopathic lesion of the medial one-third of the clavicle. This condition, which is better described as aseptic enlarging osteosclerosis of the clavicle, is most commonly seen in middle-aged women and manifests as a tender swelling over the medial one-third of the clavicle.[222]

## Reflex Sympathetic Dystrophy

Since its original description by Mitchell[223] in 1864, reflex sympathetic dystrophy (RSD) has remained a poorly understood and frequently overlooked condition. Its cause is unknown but may be related to sympathetic overflow or short-circuiting of impulses through the sympathetic system. Any clinician who deals with painful disorders must be familiar with the diagnosis and treatment of this condition. Bonica's[224] excellent review covers the clinical presentation, various stages of the disease, and the importance of early intervention to ensure a successful outcome.

RSD has been called causalgia, shoulder-hand syndrome, and Sudeck's atrophy, which has caused some confusion. It is generally associated with minor trauma and is to be differentiated from causalgia, which involves trauma to major nerve roots.[223] RSD is divided into three phases, which are important in determining appropriate treatment.[224] Phase one is characterized by sympathetic overflow with diffuse swelling, pain, increased vascularity, and radiographic evidence of demineralization. If left untreated for 3 to 6 months, the condition may progress to phase two, which is characterized by atrophy. The extremity may now be cold and shiny, with atrophy of the skin and muscles. Phase three refers to progression of trophic changes, with irreversible flexion contracture and a pale, cold, painful extremity. It has been speculated that phase one is related to peripheral short-circuiting of nerve impulses, phase two represents short-circuiting through the internuncial pool in the spinal cord, and phase three is controlled by higher thalamic centers.[224,225]

Steinbrocker[226] reported that recovery is possible as long as vasomotor activity with swelling and hyperemia is evident. After the trophic phase two or three is established, the prognosis for recovery is poor. Prompt recognition of the syndrome is important because early intervention to control pain is mandatory. Careful supervision and reassurance are crucial because many of these patients are emotionally labile as a result of the pain or an underlying problem. The syndrome may be reversed to a remarkable degree by a sympathetic block. Patients who receive transient relief from sympathetic blockade may be helped by surgical sympathectomy.

## Neoplasms

Primary and metastatic neoplasms may cause shoulder pain by direct invasion of the musculoskeletal system or by compression with referred pain.[2,227] Primary tumors are more likely to occur in younger persons. More common lesions have a typical distribution, such as the predilection of a chondroblastoma for the proximal humeral epiphysis or an osteogenic sarcoma for the metaphysis.[228] The differential diagnosis of spontaneous onset of shoulder pain in older persons should include metastatic lesions and myeloma.

Neoplasms are best identified by plain radiographs, MRI, Tc 99m MDP scintigraphy, and CT.

Neoplasms also may involve the shoulder region through metastases to the region. An associated history of carcinomas should alert the examiner to the possibility of a bone tumor, especially in patients who have had malignancies with a predilection for metastasis to bone (e.g., thyroid, renal, lung, prostate, and breast malignancies). Pain often is present at rest and is exacerbated at night. Atypical pain distribution that is not relieved by injection without specific dermatomal distribution should alert the examiner to other underlying possibilities. Plain radiographs should be evaluated thoroughly for any cortical destruction and for lytic lesions.

Pancoast syndrome or apical lung tumor may manifest as shoulder pain or cervical radiculitis caused by invasion of the brachial plexus or invasion of C8 or T1 roots.[229-231] With invasion of the cervical sympathetic chain, the patient also may experience Homer's syndrome.

## Miscellaneous Conditions

With increasing numbers of patients undergoing long-term maintenance hemodialysis, a shoulder pain syndrome known as dialysis shoulder arthropathy has been described. This syndrome consists of shoulder pain, weakness, loss of motion, and functional limitation. The cause and pathogenesis of this syndrome are unclear, although rotator cuff disease, pathologic fracture, bursitis, and local amyloid deposition have been implicated as causative factors.[232] Surgical or necropsy data are insufficient to confirm a specific diagnosis. Patients generally respond poorly to local measures of injection, application of heat, and NSAIDs, but their condition may improve with correction of underlying metabolic disorders, such as osteomalacia and secondary hyperparathyroidism.

In patients older than 50 years who have bilateral shoulder pain and stiffness, polymyalgia rheumatica should be considered as a diagnosis. This condition is twice as common among females and is almost exclusively found in white persons.[233] Most patients have sedimentation rates greater than 40 mm/hr and respond to low doses of corticosteroids.[234]


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 47



# Low Back Pain

Rajiv Dixit

## KEY POINTS

- Low back pain (LBP) affects as many as 80% of individuals, and degenerative changes of the lumbar spine is the most common cause.
- More than 90% of these patients are mostly pain free within 8 weeks.
- The initial evaluation should focus on identification of the few patients with neurologic involvement, fracture, or possible systemic disease (infection, malignancy, or spondyloarthritis) because they may need urgent or specific intervention.
- Psychosocial and other factors that predict risk of chronic disabling LBP should be assessed.
- Imaging is rarely indicated in the absence of significant neurologic involvement, trauma, or suspicion of systemic disease.
- Imaging abnormalities, often the result of age-related degenerative changes, should be carefully interpreted because they are frequently present in asymptomatic individuals.
- A precise pathoanatomic diagnosis with identification of the pain generator cannot be made in up to 85% of patients.
- Persistent LBP should be treated with an individually tailored program that includes analgesia, core strengthening, stretching, aerobic conditioning, loss of excess weight, and patient education.
- Intensive interdisciplinary rehabilitation with an emphasis on cognitive-behavioral therapy should be strongly considered if conservative measures fail.
- There is no evidence for the effectiveness of epidural corticosteroid injections in patients without radiculopathy secondary to disk herniation.
- A large number of injection techniques, physical therapy modalities, and nonsurgical interventional therapies lack evidence of efficacy.
- The major indication for back surgery is presence of a serious or progressive neurologic deficit.
- In the absence of neurologic deficits, back surgery, especially spinal fusion for degenerative changes, has not been shown to be more effective than conservative care.

## EPIDEMIOLOGY

Low back pain (LBP) is one of the most common conditions encountered in clinical medicine. It affects the area between the lower rib cage and gluteal folds.

An estimated 65% to 80%[1] of the population will experience LBP during their lifetime. LBP is the most prevalent chronic pain syndrome and the leading cause of limitation of activity in patients younger than 45 years. It is also the second most frequent reason for a visit to the physician's office and the third most common reason for surgery.[2] The prevalence of LBP increases with age until between 60 and 65 years, then gradually declines.[3] LBP is more common in women.

The natural history of back pain, especially the duration and chronicity, is somewhat controversial. Regardless, studies show that pain and function improve substantially in most patients within 1 month,[4] and more than 90% are better at 8 weeks.[5] These patients, however, remain susceptible to future relapses that also tend to be brief. Chronic LBP develops in the remaining 7% to 10%, and these individuals are largely responsible for the high costs associated with LBP. Expenditures on spinal conditions in the United States have significantly increased in recent years, despite little change in the health status among people who experience these conditions.[6] The general consensus is that approximately $90 billion is spent on the diagnosis and management of LBP, and an additional $10 to $20 billion is attributed to economic loses in productivity each year.[6-8]

A number of risk factors have been associated with LBP, including heredity, psychosocial factors, heavy lifting, obesity, pregnancy, weaker trunk strength, and cigarette smoking.[9] Persistence of disabling LBP has been associated with the presence of maladaptive pain coping behavior, nonorganic signs, functional impairment, poor general health status, and psychiatric comorbidities.[10]

## ANATOMY

The lumbar spine is composed of five vertebrae. Each vertebra consists of a body anteriorly and a neural arch that encloses the spinal canal posteriorly (Figure 47-1). Their cartilaginous end plates cover the superior and inferior surfaces of the vertebral body.

Adjacent vertebrae are united by an intervertebral disk. The outer circumference of the disk is made up of concentric layers of dense, tough fibrous tissue, the annulus fibrosus.

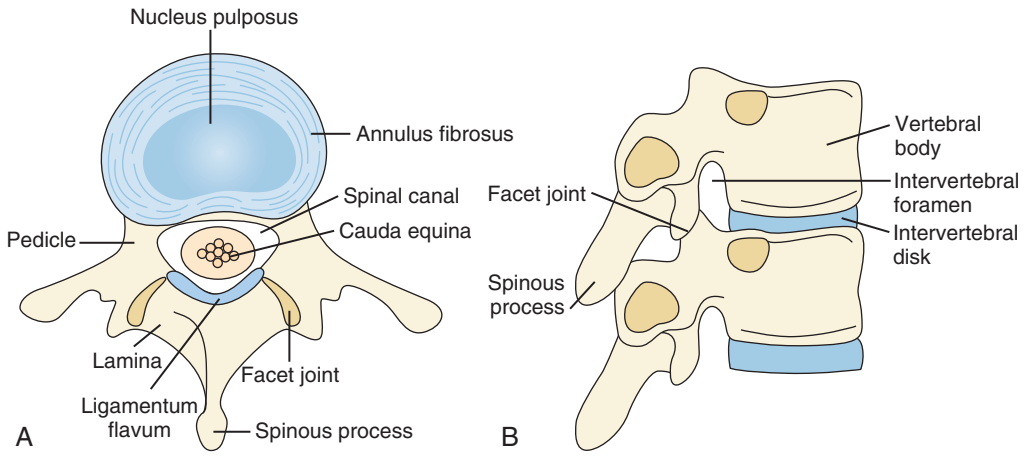



**Figure 47-1** Anatomy of the lumbar spine. **A,** Cross-sectional view through a lumbar vertebra. **B,** Lateral view of the lumbar spine.

The annulus encloses a shock-absorbing gelatinous nucleus pulposus. In addition to the anteriorly placed diskovertebral joint (formed by the intervertebral disk and adjacent vertebrae), at each level of the lumbar spine, there are two posterolaterally placed synovial facet (apophyseal) joints. These are formed by articulation of the superior and inferior articular processes of adjacent vertebrae.

The vertebral column is further stabilized by ligaments and paraspinal muscles (erector spinae, trunk, and abdominal muscles). The anterior and posterior longitudinal ligaments run the length of the spinal column. They anchor the anterior and posterior vertebral body surfaces and intervertebral disks. The ligamentum flavum interconnects the laminae, whereas the interspinous and supraspinous ligaments interconnect the spinous processes. The intertransverse ligaments interconnect the transverse processes.

The sacroiliac joints join the spinal column to the pelvis. The anterior and inferior part of the joint is lined with synovium, whereas the posterior and superior part is fibrous. There is little or no movement at the sacroiliac joint.

The spinal canal in the lumbar region contains the cauda equina (the bundle of lumbar and sacral nerve roots that occupy the vertebral canal below the cord), blood vessels, and fat. Because the spinal cord ends at the L1 level, cord compression is generally not a feature of lumbar pathology. At each level a pair of nerve roots leaves the spinal canal and exits through the intervertebral foramina.

## CLINICAL EVALUATION

LBP has a large number of causes, and the spectrum of clinical presentation is broad. Many individuals will have self-limited episodes of acute LBP that resolve without specific treatment, whereas others may be seen with recurrent or chronic LBP. A thorough history is the most important part of the clinical evaluation of these patients. Imaging is often unnecessary.

### History

The major focus in the initial evaluation of a patient with LBP is to identify the small fraction[11] (<5%) of patients who may have neural compression, fracture, or underlying systemic disease (infection, malignancy, or spondyloarthritis) as the cause of back pain. These patients require early diagnostic testing (mostly imaging) and may require specific treatment (e.g., antibiotics for vertebral osteomyelitis) or urgent treatment (e.g., surgical decompression in a patient with major or progressive neural compression). As such, clues to the presence of the previously mentioned conditions[12-14] (Table 47-1), often referred to as "red flags," should be carefully sought. The prevalence of serious spine disorders is low and the sensitivity and specificity of most red flags is modest. As a result, recent studies have highlighted the limited predictive value of most red flags, and suggested that performing imaging with the presence of any red flag would result in unnecessarily high rates of imaging. It has therefore been suggested that use of imaging should be guided by the full clinical picture and observations during

**TABLE 47-1** Red Flags for Potentially Serious Underlying Causes of Low Back Pain

| |
|---|
| **Spinal Fracture** |
| Significant trauma |
| Prolonged glucocorticoid use |
| Age >50 yr |
| **Infection or Cancer** |
| History of cancer |
| Unexplained weight loss |
| Immunosuppression |
| Injection drug use |
| Nocturnal pain |
| Age >50 yr |
| **Cauda Equina Syndrome** |
| Urinary retention |
| Overflow incontinence |
| Fecal incontinence |
| Bilateral or progressive motor deficit |
| Saddle anesthesia |
| **Spondyloarthritis** |
| Severe morning stiffness |
| Pain improves with exercise, not rest |
| Pain during second half of night |
| Alternating buttock pain |
| Age <40 yr |

time, rather than by the uncritical use of individual red flags. The presence of multiple red flags generates higher predictive values.[15] It is also important to look for any social or psychological distress, such as job dissatisfaction, pursuit of disability compensation, and depression, that may amplify or prolong the pain.[14]

Mechanical LBP is due to an anatomic or functional abnormality in the spine that is not associated with inflammatory or neoplastic disease. It typically increases with physical activity and upright posture and tends to be relieved by rest and recumbency. More than 95% of LBP is mechanical,[11] and degenerative change in the lumbar spine is the most common cause of mechanical LBP.[12] Severe and acute mechanical LBP in a post-menopausal woman would suggest a vertebral compression fracture secondary to osteoporosis. Nocturnal pain suggests the possibility of underlying infection or neoplasm as the cause of LBP.

Inflammatory LBP,[16] as seen in the spondyloarthritides, is more common in men younger than 40 years. It is associated with marked morning stiffness that usually lasts for more than 30 minutes. The pain frequently improves with exercise but not with rest. Pain is often worse during the second half of the night, and some patients complain of alternating buttock pain.

It is important to ask the patient if the back pain radiates into the lower extremities, suggesting pseudoclaudication (neurogenic claudication) secondary to spinal stenosis or sciatica (usually secondary to a herniated disk or spinal stenosis). Young adults are more likely to be seen with the clinical syndrome of disk herniation, and elderly patients are more likely to be seen with the clinical features of spinal stenosis. Sciatica results from nerve root compression and produces pain in a dermatomal (radicular) distribution, usually to the level of the foot or ankle. The pain is often lancinating, shooting, and sharp in quality. It is frequently accompanied by numbness and tingling and may be accompanied by sensory and motor deficits. Sciatica resulting from disk herniation typically increases with cough, sneezing, or the Valsalva maneuver. Sciatica should be differentiated from non-neurogenic sclerotomal pain. This pain can arise from pathology within the disk, facet joint, or lumbar paraspinal muscles and ligaments. Similar to sciatica, sclerotomal pain is often referred into the lower extremities, but unlike sciatica, sclerotomal pain is nondermatomal in distribution, it is usually dull in quality, and the pain usually does not radiate below the knee or have associated paresthesias. Most radiant pain is sclerotomal.[12] Bowel or bladder dysfunction should suggest the possibility of the cauda equina syndrome.

## Physical Examination

A physical examination usually does not lead to a specific diagnosis. Nevertheless, a general physical examination including a careful neurologic examination may help identify those few but critically important cases of LBP that are secondary to a systemic disease or have clinically significant neurologic involvement (see Table 47-1).

Inspection may reveal the presence of scoliosis. This can be either structural or functional. A structural scoliosis is associated with structural changes of the vertebral column and sometimes the rib cage as well. In adults structural scoliosis is usually secondary to degenerative changes, although some adults may have a history of adolescent idiopathic scoliosis. With forward flexion, structural scoliosis persists. In contrast, functional scoliosis, which usually results from paravertebral muscle spasm or leg length discrepancy, usually disappears. A tuft of hair in the lumbar spine region may indicate a congenital structural abnormality such as spina bifida occulta.

Palpation can detect paravertebral muscle spasm. This often leads to loss of the normal lumbar lordosis. Point tenderness on percussion over the spine has sensitivity but not specificity for vertebral osteomyelitis. A palpable step-off between adjacent spinous processes suggests spondylolisthesis.

Limited spinal motion (flexion, extension, lateral bending, and rotation) is not associated with any specific diagnosis because LBP from any cause may limit motion. Range-of-motion measurements, however, can help in monitoring treatment.[9] Chest expansion of less than 2.5 cm has specificity but not sensitivity for ankylosing spondylitis.[16]

The hip joints should be examined for any decrease in range of motion because hip arthritis, which normally causes groin pain, may occasionally refer pain to the back. Trochanteric bursitis with tenderness over the greater trochanter of the femur can be confused with LBP. The presence of more widespread tender points, especially in a female patient, suggests the possibility that LBP may be secondary to fibromyalgia.

A complete neurologic examination should be performed because neurologic abnormalities in the upper extremities, such as hyper-reflexia, may indicate a more proximal etiology of a patient's lower extremity complaints. In patients with a history of LBP that radiates into the lower extremities (sciatica, pseudoclaudication, or referred sclerotomal pain), a straight leg–raising test (Figure 47-2) should be performed. With the patient lying on his or her back, the examiner places the heel in the palm of his or her hand and progressively raises the patient's leg with the knee fully extended. This movement places tension on the sciatic nerve (that originates from L4, L5, S1, S2, and S3) and thereby stretches the nerve roots (especially L5, S1, and S2). If any of these nerve roots is already irritated, such as by impingement from a herniated disk, further tension on the nerve root by straight leg raising will result in radicular pain that extends below the knee. The test is positive if radicular pain is produced when the leg is raised less than 70 degrees. Dorsiflexion of the ankle further stretches the sciatic nerve and increases the sensitivity of the test. Pain

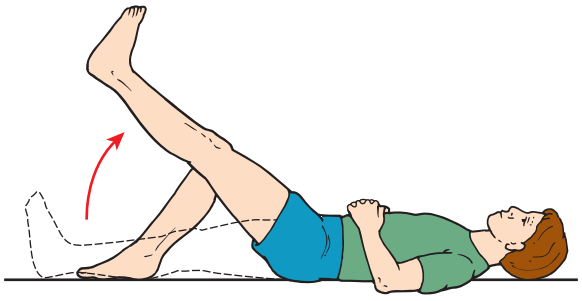


**Figure 47-2** Straight leg–raising test. A very sensitive test for nerve root impingement at the L4-5 or L5-S1 level.

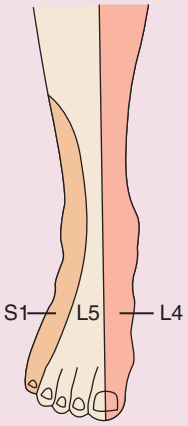
| Lower extremity dermatome | Disk | Nerve root | Motor loss | Sensory loss | Reflex loss |
|---|---|---|---|---|---|
|   | L3-4 | L4 | Dorsiflexion of foot | Medial foot | Knee |
| | L4-5 | L5 | Dorsiflexion of great toe | Dorsal foot | None |
| | L5-S1 | S1 | Plantarflexion of foot | Lateral foot | Ankle |

**Figure 47-3** Neurologic features of lumbosacral radiculopathy.

experienced in the posterior thigh or knee during straight leg–raising is generally from hamstring tightness and does not represent a positive test. The straight leg–raising test is sensitive but not specific for clinically significant disk herniation at the L4-5 or L5-S1 level (the sites of 95% of clinically meaningful disk herniations). False-negative tests are more frequently seen with herniation above the L4-5 level. The straight leg–raising test is usually negative in patients with spinal stenosis. The crossed straight leg–raising test (with sciatica reproduced when the opposite leg is raised) is highly specific but insensitive for a clinically significant disk herniation.[9,14,17,18]

The neurologic evaluation (Figure 47-3) of the lower extremities in a patient with sciatica can identify the specific nerve root involved. The evaluation should include motor testing with focus on dorsiflexion of the foot (L4), great toe dorsiflexion (L5), and foot plantar flexion (S1); determination of knee (L4) and ankle (S1) deep tendon reflexes; and tests for dermatomal sensory loss. The inability to toe walk (mostly S1) and heel walk (mostly L5) indicate muscle weakness. Muscle atrophy can be detected by circumferential measurements of the calf and thigh at the same level bilaterally.[9]

Patients involved with litigation or with psychological distress occasionally exaggerate their symptoms. They may display nonorganic signs where the objective findings do not match the subjective complaints, such as with nonanatomic motor or sensory loss. A number of tests to detect this have been described by Waddell and co-workers.[19] The most reproducible tests are the presence of superficial tenderness, over-reaction during the examination, and observation of a discrepancy in the straight leg–raising test done in the seated and supine positions.

## Diagnostic Tests

### Imaging

There is concern about overuse of lumbar spine imaging, especially in the United States where imaging capacity is high. Indiscriminate spine imaging leads to a low yield of clinically useful findings, a high yield of misleading findings, radiation exposure, and costs.[15] The major function of diagnostic testing, especially imaging, is the early identification of pathology in those few patients who have evidence of a major or progressive neurologic deficit, and those in whom an underlying systemic disease or vertebral fracture is suspected (see Table 47-1). Otherwise, imaging is not required unless significant symptoms persist beyond 6 to 8 weeks. This approach avoids unnecessary early testing because more than 90% of the patients will have largely recovered by 8 weeks.[9,11] Furthermore, neither MRI nor plain radiographs obtained early in the course of LBP evaluation improves clinical outcome, predicts recovery course, or reduces the overall cost of care.[2,20]

A significant problem with all imaging studies is that many of the anatomic abnormalities identified in patients with LBP are also commonly present in asymptomatic individuals and are frequently unrelated to the back pain.[12] Often these abnormalities result from age-related degenerative changes, which begin to appear even in early adulthood and are among the earliest degenerative changes in the body.[21] Although clinically challenging and sometimes impossible, one should refrain from making causal inferences based solely on imaging abnormalities in the absence of corresponding clinical findings because this may lead to unnecessary, invasive, and costly interventions.[22,23]

Given the weak association between imaging abnormalities and symptoms, it is not surprising that in as many as 85% of patients a precise pathoanatomic diagnosis with identification of the pain generator cannot be made.[14] Patients should understand that the reason for imaging is to rule out serious conditions and that common degenerative findings are expected. Ill-considered attempts to make a diagnosis on the basis of imaging studies may falsely reinforce the suspicion of serious disease (leading to "fear avoidance" behavior), magnify the importance of nonspecific findings, and label patients with spurious diagnoses.

Plain radiographs and MRI are the major modalities used in the evaluation of patients with LBP. In patients with

persistent LBP of greater than 6 to 8 weeks' duration despite standard therapies, radiography may be a reasonable first option if there are no symptoms suggesting radiculopathy or spinal stenosis.[24] Standing anteroposterior and lateral views are usually adequate. Oblique views substantially increase radiation exposure and add little new diagnostic information. Gonadal radiation in a woman from a two-view radiograph of the lumbar spine is equivalent to radiation exposure from a chest radiograph taken daily for more than 1 year.[24]

Abnormalities on radiography, such as single-disk degeneration, facet joint degeneration, Schmorl's nodes (protrusion of the nucleus pulposus into the spongiosa of a vertebra), spondylolysis, mild spondylolisthesis, transitional vertebrae (the "lumbarization" of S1 or "sacralization" of L5), spina bifida occulta, and mild scoliosis, are equally prevalent in individuals with and without LBP.[11,12,25]

MRI without contrast is generally the best initial test for patients with LBP who require advanced imaging. It is the preferred modality for the detection of spinal infection and cancers, herniated disks, and spinal stenosis.[11] MRI testing for LBP should largely be limited to patients in whom there is a suspicion of systemic disease (such as infection or malignancy), for the pre-operative evaluation of patients who are surgical candidates on clinical grounds[14,24] (e.g., the presence of a significant or progressive neurologic deficit), or for those patients with radiculopathy who are candidates for epidural corticosteroids.[24] Disk abnormalities are commonly noted on MRI studies but often have little or no relationship with the patient's symptoms. A disk bulge is a symmetric, circumferential extension of disk material beyond the interspace. A disk herniation is a focal or asymmetric extension. Herniations are subdivided into protrusions and extrusions. Protrusions are broad-based, whereas extrusions have a "neck" so that the base is narrower than the extruded material. Bulges (52%) and protrusions (27%) are common in asymptomatic adults, but extrusions are rare.[11] MRI with the intravenous contrast agent gadolinium may be useful for the evaluation of patients with prior back surgery (with no hardware present) to help in the differentiation of scar tissue from recurrent disk herniation.

MRI is generally preferred versus CT scanning in the evaluation of patients with LBP. However, when bone anatomy is critical, CT is superior. Unlike MRI, CT can safely be done in patients with a ferromagnetic implant, although imaging artifacts may make interpretation difficult. CT myelography is therefore sometimes preferred in patients with surgically placed spinal hardware.

Bone scanning is used primarily to detect infection, bony metastases, or occult fractures and to differentiate them from degenerative changes. Bone scans have limited specificity due to poor spatial resolution, and thus abnormal findings often require further confirmatory imaging such as MRI.

### Electrodiagnostic Studies

Electrodiagnostic studies can be helpful in the evaluation of some patients with lumbosacral radiculopathy. The main procedures are electromyography and nerve conduction studies. These studies can confirm nerve root compression and define the distribution and severity of involvement. Whereas studies such as MRI can only provide anatomic information, electrodiagnostic studies provide physiologic information that may support or refute the findings on imaging. Electrodiagnostic testing is therefore mostly considered in patients with persistent disabling symptoms of radiculopathy in which there is discordance between the clinical presentation and findings on imaging. Electromyography and nerve conduction studies can also be helpful in differentiating the limb pain of peroneal nerve palsy or lumbosacral plexopathy from that of L5 radiculopathy. These studies are also useful to evaluate possible factitious weakness. Electrodiagnosis is unnecessary in a patient with an obvious radiculopathy. It should be noted that electromyographic changes depend on the development of muscle denervation following nerve injury and may not be detected for 2 to 3 weeks after the injury. In contrast, nerve conduction tests become abnormal immediately after nerve damage. Another limitation is that electromyographic abnormalities may persist for more than a year following decompressive surgery.[26]

### Laboratory Studies

Laboratory studies are used mostly in identifying patients with systemic causes of LBP. A patient with normal blood cell counts, erythrocyte sedimentation rate, and radiographs of the lumbar spine is unlikely to have underlying infection or malignancy as the cause of LBP.[27]

## DIFFERENTIAL DIAGNOSIS

LBP usually originates from pathology within the lumbar spine or associated muscles and ligaments (Table 47-2). Rarely, pain is referred to the back from visceral disease. In the vast majority of patients with LBP, the pain is

**TABLE 47-2** Causes of Low Back Pain

| |
|---|
| **Mechanical** |
| Lumbar spondylosis* |
| Disk herniation* |
| Spondylolisthesis* |
| Spinal stenosis* |
| Fractures (mostly osteoporotic) |
| Nonspecific (idiopathic) |
| **Neoplastic** |
| Primary |
| Metastatic |
| **Inflammatory** |
| Spondyloarthritis |
| **Infectious** |
| Vertebral osteomyelitis |
| Epidural abscess |
| Septic diskitis |
| Herpes zoster |
| **Metabolic** |
| Osteoporotic compression fractures |
| Paget's disease |
| **Referred Pain to Spine** |
| From major viscera, retroperitoneal structures, urogenital system, aorta, or hip |

*Related to degenerative changes.

mechanical.[14] Degenerative change in the lumbar spine is the largest contributor to the mechanical causes of LBP[11] (see Table 47-2) and indeed the most commonly identified cause of back pain.

## Lumbar Spondylosis

The current common usage of the term *lumbar spondylosis* incorporates degenerative changes in both the anteriorly placed diskovertebral joints and the posterolaterally placed facet joints.[9] These degenerative or osteoarthritic changes are seen radiographically as disk or joint-space narrowing, subchondral sclerosis, and osteophytosis (Figure 47-4).

Imaging evidence of lumbar spondylosis is common in the general population, increases with age, and may be unrelated to back symptoms. Radiographic abnormalities such as single-disk degeneration, facet joint degeneration, Schmorl's nodes, mild spondylolisthesis, and mild scoliosis are equally prevalent in persons with and without back pain.[28] The situation is further complicated by the observation that patients with severe mechanical LBP may have minimal radiographic changes, and conversely patients with advanced changes may be asymptomatic.

The clinical spectrum of mechanical LBP is wide. Patients may present with acute LBP (with recurrent attacks in some), whereas chronic LBP (often with periods of acute exacerbation) may develop in others. Somatic referral may lead to sclerotomal pain that radiates into the buttocks and lower extremities. Lumbar spondylosis predisposes patients to intervertebral disk herniation, spondylolisthesis, and spinal stenosis. Overall, degenerative changes of the lumbar spine are the most common cause of back pathology and pain.

In some patients with facet joint osteoarthritis the pain may radiate into the buttock and posterior thigh, be alleviated with forward flexion, and be exacerbated by bending ipsilateral to the involved joint (facet syndrome).

The terms internal disk disruption and diskogenic low back pain are used interchangeably and remain controversial diagnoses[17] when diagnosed by provocative diskography. Following contrast injection into several disks in sequence, the radiographic appearance and induced pain at each level are assessed. If injection into a disk reproduces a patient's usual LBP, the test is considered positive. Advocates of this technique interpret a positive diskogram as defining the particular disk as the primary pain generator, and spinal fusion or disk arthroplasty is frequently recommended.[2] However, injection into a disk can simulate the quality and location of pain known not to originate from that disk.[29] Furthermore, diskographic abnormalities and induced pain are frequently seen in asymptomatic persons and, more importantly, the diskogenic pain attributed to disk disruption frequently improves spontaneously.[11,14] Finally, long-term follow-up of patients who have undergone diskography has led to a concern that the procedure itself may lead to accelerated disk degeneration and disk herniation.[30] Therefore the clinical importance and appropriate management of this condition when diagnosed by diskography remains unclear.

Modic changes are signal changes in the vertebral endplate and adjacent bone marrow that are commonly reported following lumbar MRI. Modic type I changes are secondary

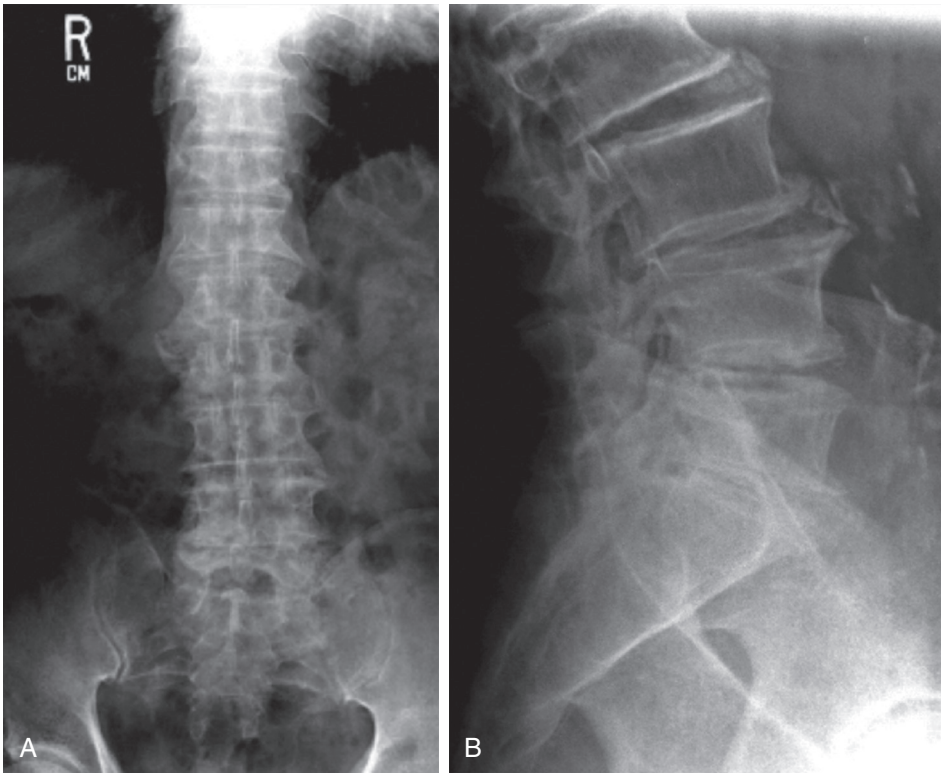



**Figure 47-4** Lumbar spondylosis. Anteroposterior **(A)** and lateral **(B)** radiographs of the lumbar spine show the cardinal features of disk-space narrowing, marginal osteophytes, and end-plate sclerosis. *(Courtesy Dr. John Crues, University of California, San Diego.)*

to marrow edema, Modic type II changes represent fatty replacement of marrow, and Modic type III changes reflect subchondral bone sclerosis.[31,32] The prevalence of these changes, which may progress or regress over time, increases with age and appears to be associated with degenerative disk changes.[33] Modic changes are present in 20% to 40% of LBP patients but may be seen in as many as 10% of asymptomatic adults.[31,32] The clinical usefulness with regard to selecting treatment options of these signal changes are unclear.[34]

Focal high signal in the posterior annulus fibrosus as seen on T2-weighted MRI images, sometimes referred to as a *high-intensity zone*, is believed to represent tears in the annulus fibrosus and to correlate with positive findings on provocative diskography.[11] The high prevalence of high-intensity zones in asymptomatic individuals limits its clinical value.[35]

Spinal instability is seen in some patients with lumbar spondylosis. It is identified by demonstrating abnormal vertebral motion (anteroposterior displacement or excessive angular change of adjacent vertebrae) on lateral radiographs in flexion and extension. However, such spinal motion may be seen in asymptomatic persons and its natural history and relationship to the causation of LBP is unclear. Thus the diagnosis of spinal instability (in the absence of fractures or spondylolisthesis) as a cause of LBP and its treatment by spinal fusion remains controversial.

## Disk Herniation

Intervertebral disk herniation occurs when the nucleus pulposus in a degenerated disk prolapses and pushes out the weakened annulus, usually posterolaterally. Imaging evidence of disk herniation has a high prevalence in the general population. One study found MRI evidence of disk herniation in 27% of asymptomatic individuals.[36] Occasionally, however, the herniated disk can cause nerve root impingement that leads to lumbosacral radiculopathy (Figures 47-5 and 47-6). A herniated intervertebral disk is the most common cause of sciatica in young adults.[11]

The lumbosacral spine is susceptible to disk herniation because of its mobility. Seventy-five percent of flexion and extension occurs at the lumbosacral joint (L5-S1), and 20% occurs at L4-5[37] (with more torsion at the L4-5 level). Probably related to this, 90% to 95% of clinically significant compressive radiculopathies occur at these two levels.[14]

The frequency of disk herniation increases with age. The peak frequency of herniation at the L5-S1 and L4-L5 levels is between the ages of 44 and 50 years, with a progressive decline in frequency thereafter.[38]

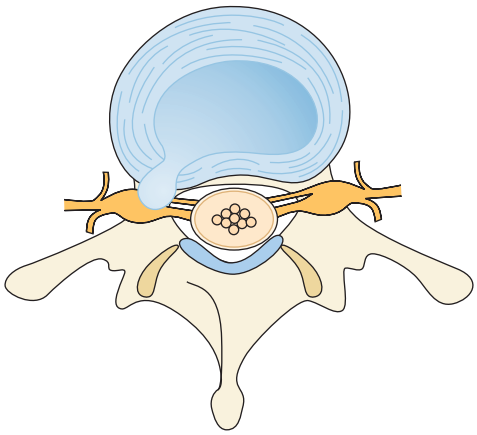


**Figure 47-5** Schematic drawing showing posterolateral disk herniation resulting in nerve root impingement.

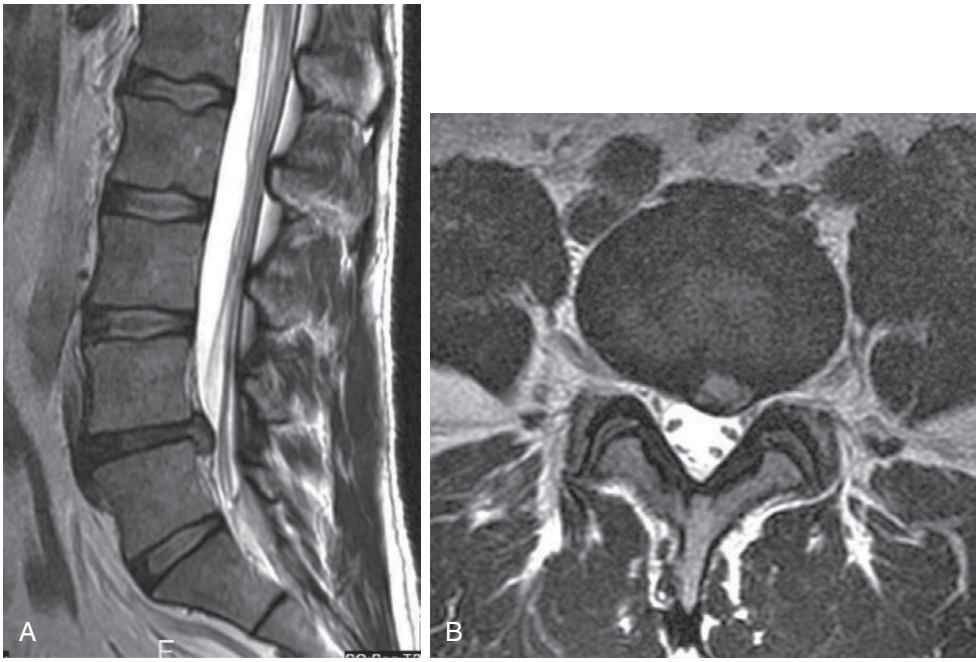



**Figure 47-6** Lumbar disk extrusion. **A,** The sagittal T2-weighted magnetic resonance image shows an extruded disk at the L4-5 level. **B,** The axial image through the L4-5 level shows disk extrusion to the left side of the neural canal and compressing the exiting L5 nerve root against the left lamina. *(Courtesy Dr. John Crues, University of California, San Diego.)*

The genesis of sciatica is felt to have both a mechanical (disk material impinging on a nerve root) and biologic component. Inflammation, vascular invasion, immune responses, and an array of cytokines have been implicated.

The clinical features of disk herniation resulting in lumbosacral radiculopathy have already been discussed (see history, physical examination, and Figure 47-3). It should be noted that immediate imaging is unnecessary in patients without a clinically significant neurologic deficit and no red flags to suggest an underlying systemic pathology (see Table 47-1). L1 radiculopathy is rare and patients are seen with symptoms of pain, paresthesias, and sensory loss in the inguinal region.[39] L2, L3, and L4 radiculopathies are uncommon and more likely to be seen in older patients with lumbar spinal stenosis.

The natural history of disk herniation is favorable, with progressive improvement expected in most patients. Sequential MRI studies reveal that the herniated portion of the disk regresses with time, and there is partial or complete resolution in two-thirds of cases after 6 months.[14,40] Only approximately 10% of patients have sufficient pain after 6 weeks of conservative care, and for this group, decompressive surgery is considered.[14] Even a sequestered fragment (piece of herniated material that breaks off and is free in the epidural space) tends to be reabsorbed with time.[41]

Rarely, a large midline disk herniation, usually L4-5,[12] compresses the cauda equina, resulting in cauda equina syndrome. Patients usually are seen with LBP, bilateral radicular pain, and bilateral motor deficits with leg weakness. Physical examination findings are often asymmetric. Sensory loss in the perineum (saddle anesthesia) is common, and urinary retention with overflow incontinence is frequently present.[14] Fecal incontinence may also occur. The spectrum of signs and symptoms are presented in Table 47-3. Other causes of cauda equina syndrome include neoplasia, epidural abscess, hematoma, and rarely lumbar spinal stenosis. Cauda equina syndrome is a surgical emergency because neurologic results are affected by the time to decompression.[9] Whenever possible, the cauda equina syndrome should be recognized before established incontinence because, once urinary retention has occurred, the prognosis is worse.[42]

## SPONDYLOLISTHESIS

Spondylolisthesis is the anterior displacement of a vertebra on the one beneath it. There are two major types: isthmic and degenerative.

Isthmic spondylolisthesis (Figure 47-7), most commonly seen at the L5-S1 level, is caused by bilateral spondylolysis. Spondylolysis is a defect in the pars interarticularis that is most commonly seen at L5. It is typically a fatigue fracture acquired early in life that is more commonly seen in boys. Based on CT imaging the prevalence of spondylolysis in adults is 11.5%, nearly twice the prevalence of plain radiograph–based studies.[43] Spondylolysis progresses to spondylolisthesis in approximately 15% of patients.[44]

Degenerative spondylolisthesis develops in some patients with severe degenerative changes with subluxation at the facet joints allowing anterior or posterior movement of one

**TABLE 47-3** Spectrum of Signs and Symptoms of Cauda Equina Syndrome

| |
|---|
| Altered/reduced urinary sensation |
| Loss of desire to void or poor stream |
| Sexual dysfunction |
| Saddle area anesthesia |
| Bilateral (or unilateral) sciatica |
| Motor weakness of lower extremities |
| Urinary and/or fecal incontinence |
| Loss of rectal tone |
| Absent voluntary anal contraction |

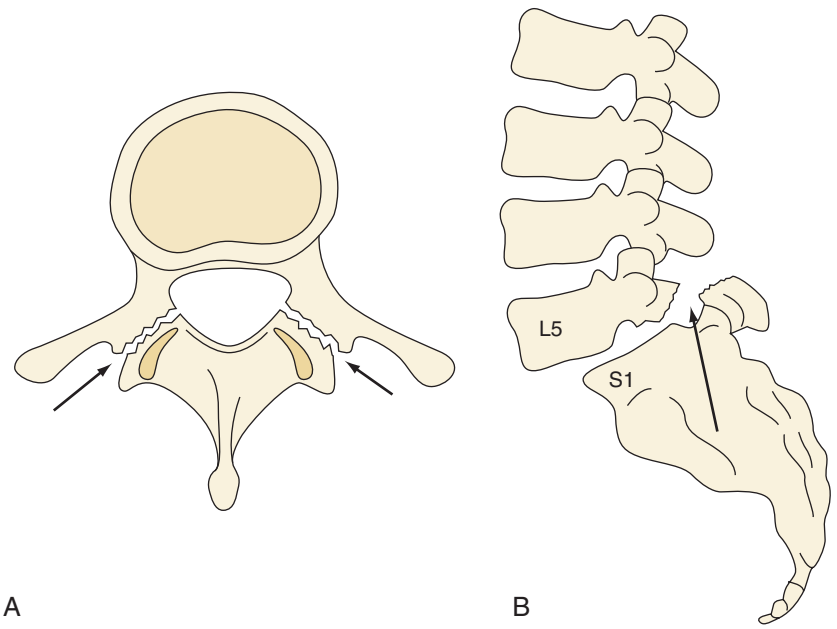



**Figure 47-7** **A,** Spondylolysis with bilateral defects in the pars interarticularis *(arrows)*. **B,** Spondylolysis of the L5 vertebra *(arrow)* resulting in isthmic spondylolisthesis at L5-S1.

vertebra over another. It is usually seen in an older age group (typically older than 60 years), is more common in women, most frequently involves the L4-5 level, and rarely exceeds 30% of vertebral width.[12]

Most patients, especially those with a minor degree of spondylolisthesis, are asymptomatic. Some may complain of an aching mechanical LBP. Neurologic complications may occur in some with greater degrees of spondylolisthesis. Nerve root impingement is more likely to be seen in patients with isthmic spondylolisthesis (especially L5 nerve root), whereas in degenerative spondylolisthesis the more likely clinical presentation is of spinal stenosis. Rarely, extreme slippage results in cauda equina syndrome. In view of its potential dynamic nature, spondylolisthesis may be missed if standing radiographs are not obtained.

## SPINAL STENOSIS

Lumbar spinal stenosis is defined as a narrowing of the central spinal canal, its lateral recesses, and neural foramina that may result in a compression of lumbosacral nerve roots. Spinal stenosis can occur at one or multiple levels, and the narrowing may be asymmetric. It is important to recognize that 20% to 30% of asymptomatic adults older than 60 years have imaging evidence of spinal stenosis.[45] The prevalence of symptomatic lumbar spinal stenosis is not established. It is, however, the most frequent indication for spinal surgery in patients older than 65 years.

Congenital idiopathic spinal stenosis (Table 47-4) is not uncommon and results from congenitally short pedicles. These patients tend to become symptomatic early (in the third to fifth decade of life) when superimposed mild degenerative changes that would normally be tolerated result in sufficient further narrowing of the spinal canal to cause symptoms.[45]

Degenerative changes are the cause of spinal stenosis in the vast majority of cases. The intervertebral disk loses height while it degenerates. This results in a bulging or buckling of the now redundant and often hypertrophied ligamentum flavum into the posterior part of the canal. Any herniation of the degenerated disk narrows the anterior part of the canal, whereas hypertrophied facets and osteophytes may compress nerve roots in the lateral recess or intervertebral foramen (Figures 47-8 and 47-9). Any degree of spondylolisthesis will further exacerbate spinal canal narrowing.

The hallmark of spinal stenosis is neurogenic claudication (pseudoclaudication). The symptoms of neurogenic claudication are usually bilateral but often asymmetric. The patient's primary complaint is of pain in the buttocks, thighs, and legs. The buttock pain is sometimes described as a burning sensation. The pain may be accompanied by paresthesias. Neurogenic claudication is induced by standing erect or walking and relieved by sitting or flexing forward. This forward flexion increases the spinal canal dimensions, and may lead to the patient adopting a simian stance. It is therefore not surprising that these patients often feel relief by stooping forward while holding onto a shopping cart (the "shopping cart sign"), and may exhibit surprising endurance while pedaling a stationary bicycle. Symptoms of neurogenic claudication probably represent intermittent mechanical and ischemic disruption of lumbosacral nerve root function.[46] The patients also often have a sense of weakness in the lower extremities. Unsteadiness of gait, related to compression of proprioceptive fibers, is a frequent complaint. The finding of a wide-based gait in a patient with LBP has more than 90% specificity for lumbar spinal stenosis.[47] Intermittent priapism with walking has been described.[48] Factors that favor a diagnosis of neurogenic claudication versus vascular claudication include preservation of pedal pulses, provocation of symptoms by standing erect just as readily as by walking, relief of symptoms with flexion of the spine, and location of maximal discomfort in the thighs rather than the calves.

The physical examination of a patient with lumbar spinal stenosis is often unimpressive.[9] Severe neurologic deficits are uncommon. Lumbar range of motion may be normal or reduced, and the result of straight leg–raising is usually negative. An abnormal Romberg test is seen in some patients. Deep tendon reflexes and vibration sense may be reduced. Mild diffuse weakness in the lower extremities may be present in others. The significance of these findings is

**TABLE 47-4** Causes of Lumbar Spinal Stenosis

| |
|---|
| **Congenital** |
| Idiopathic |
| Achondroplastic |
| **Acquired** |
| **Degenerative** |
| Hypertrophy of facet joints |
| Hypertrophy of ligamentum flavum |
| Disk herniation |
| Spondylolisthesis |
| Scoliosis |
| **Iatrogenic** |
| Postlaminectomy |
| Postsurgical fusion |
| **Miscellaneous** |
| Paget's disease |
| Fluorosis |
| Diffuse idiopathic skeletal hyperostosis |
| Ankylosing spondylitis |

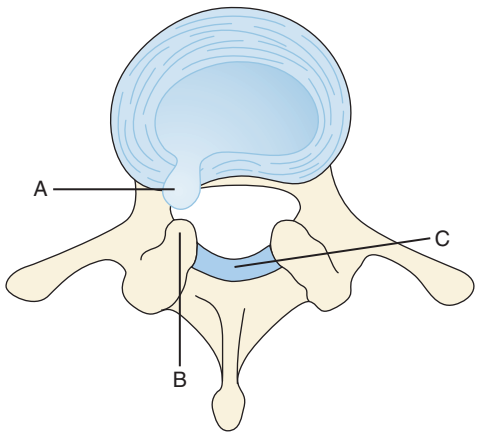



**Figure 47-8** Spinal stenosis secondary to a combination of disk herniation *(A),* facet joint hypertrophy *(B),* and hypertrophy of the ligamentum flavum *(C).*

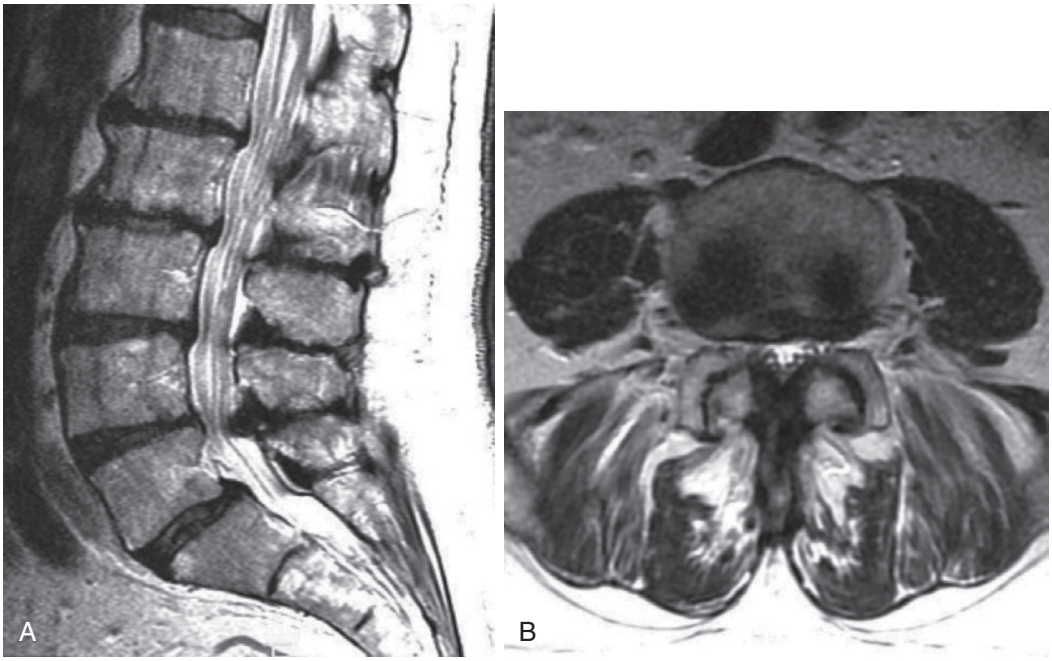



**Figure 47-9** Degenerative spinal stenosis. **A,** The sagittal T2-weighted magnetic resonance image shows decreased anteroposterior diameter of the neural canal at the L4-5 level due to redundancy of the ligamentum flavum. **B,** The axial image through the L4-5 disk shows decreased cross-sectional area of the thecal sac from hypertrophic changes of the facet joints posterolateral to the thecal sac. *(Courtesy Dr. John Crues, University of California, San Diego.)*

often difficult to determine in elderly patients. However, in a few patients with spinal stenosis, a fixed nerve root injury may occur, resulting in a lumbosacral radiculopathy or, rarely, a cauda equina syndrome.

The diagnosis of lumbar spinal stenosis is most often suspected when a history of neurogenic claudication is elicited. The diagnosis is best confirmed by MRI.

Spinal stenosis is generally an indolent condition in which the symptoms evolve gradually and the natural history is benign. In a study of patients with lumbar spinal stenosis followed up for 49 months without surgical intervention, symptoms remained unchanged in 70%, improved in 15%, and worsened in 15%.[49] As such, prophylactic surgical intervention is not warranted.[45]

## DIFFUSE IDIOPATHIC SKELETAL HYPEROSTOSIS

Diffuse idiopathic skeletal hyperostosis (DISH) is characterized by calcification and ossification of paraspinous ligaments and the entheses.[50] It is a non-inflammatory condition of unknown etiology that is not associated with human leukocyte antigen (HLA)-B27 positivity.

DISH has been associated with obesity, diabetes mellitus, and acromegaly.[51] It is rarely diagnosed before 30 years, is more commonly seen in men, and the prevalence rises with age.[52]

The thoracic spine is most commonly involved, although the cervical and lumbar regions may also be affected. Ossification of the anterior longitudinal ligament is best seen on a lateral radiograph of the thoracic spine. The ossification, together with bridging enthesophytes in the spine, give the appearance of flowing wax on the anterior and right lateral aspects of the spine. Involvement of the left lateral aspect in patients with situs inversus has led to speculation that the descending aorta plays a role in the location of the calcification. Intervertebral disk spaces and facet joints are preserved (unless there is co-existing lumbar spondylosis) and the sacroiliac joints appear normal. This helps to differentiate DISH from spondylosis and the spondyloarthritides. Almost any extraspinal osseous or articular site may be affected.[53] Irregular new bone formation ("whiskering") is often best seen at the iliac crests, ischial tuberosities, and femoral trochanters. Ossification of tendons and ligaments at sites of attachment (such as the patella, olecranon process, and calcaneus) and periarticular osteophytes (such as the lateral acetabulum and inferior portion of the sacroiliac joint on pelvic radiographs) may also be seen. Severe ligamentous calcification may be seen in the sacrotuberous and iliolumbar ligaments and heterotopic bone formation following hip replacement in patients with DISH has been described.[54]

DISH may be entirely asymptomatic. The most common complaint encountered is of pain and stiffness involving the spine, often the thoracic region. Usually there is only a moderate limitation of spinal motion. Extensive ossification of the anterior longitudinal ligament, together with large anterior enthesophytes, may occasionally compress the esophagus and cause dysphagia.[50] Ossification of the posterior longitudinal ligament is almost exclusively seen in the cervical spine and may occur either as a discrete disorder or as part of DISH. This can rarely lead to cervical myelopathy. Pain and tenderness may be present at the entheses, and these patients may have findings of lateral or medial humeral epicondylitis, Achilles tendinitis, or plantar fasciitis.

If treatment of DISH is necessary at all, it is symptomatic. Most patients respond to acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), and judicious use of glucocorticoid injections for painful enthesopathy.

## Nonspecific Low Back Pain

This condition is also referred to as *idiopathic LBP.* As mentioned earlier, a precise pathoanatomic diagnosis, with identification of the pain generator, cannot be made in as many as 85% of the patients. This is largely because of the nonspecific nature of the symptoms in patients with LBP, and the weak association of these symptoms with findings on imaging. Thus terms such as lumbago, strain, and sprain have come into use. Strain and sprain have never been histologically characterized. Therefore nonspecific LBP is a more accurate label for these patients who have a mostly self-limited syndrome of acute mechanical LBP. The severity of pain can vary from mild to severe, and whereas sometimes the back pain develops immediately after a traumatic event such as lifting a heavy object or a twisting injury, other patients may simply wake up with LBP. Most patients are better within 1 to 4 weeks,[4] but remain susceptible to similar future episodes. Chronic nonspecific LBP develops in less than 10% of patients.

## Neoplasm

Neoplasms are an uncommon, but nevertheless important, cause of LBP. In a primary care setting, neoplasia accounts for less than 1% of cases of LBP.[14]

In a large prospective study of patients in a walk-in clinic, a history of cancer, unexplained weight loss, failure to improve after 1 month of conservative therapy, and age older than 50 years were each associated with a higher likelihood for cancer.[55] By far the most important predictor for the likelihood of underlying cancer as the cause of LBP was a prior history of cancer.

The typical patient with LBP secondary to spinal malignancy presents with a persistent and progressive pain that is not alleviated by rest and is often worse at night. In some patients a spinal mass can result in a lumbosacral radiculopathy or cauda equina syndrome. Acute LBP may be the presentation in a patient with a pathologic compression fracture. Rarely, leptomeningeal carcinomatosis (in patients with breast cancer, lung cancer, lymphoma, or leukemia) may present with a lumbosacral polyradiculopathy.[56]

Most cases result from involvement of the spine by metastatic carcinoma[5] (especially prostate, lung, breast, thyroid, or kidney) or multiple myeloma. Metastatic vertebral lesions, more commonly seen in the thoracic spine, account for 39% of bony metastases in patients with primary neoplasms.[57] Spinal cord tumors, primary vertebral tumors, and retroperitoneal tumors may in rare cases be the cause of LBP.[14]

Osteoid osteoma, a benign tumor of bone, typically presents with LBP in the second or third decade of life. The pain is often accompanied by a functional scoliosis secondary to paravertebral spasm. Patients may be seen with pain even before the osteoid osteoma is visible radiographically. Osteoid osteomas predominantly involve the posterior elements of the spine, usually the neural arch. A sclerotic lesion measuring less than 1.5 cm with a lucent nidus is pathognomonic.[58] A bone scan, CT scan, or MRI should be ordered if an osteoid osteoma is suspected but not detected on radiography. Symptoms can often be controlled by NSAIDs, presumably because the nidus produces high levels of prostaglandins. Surgical resection is an option for intolerable pain. Osteoid osteomas spontaneously resolve during the course of several years.[59]

Plain radiographs are less sensitive than other imaging tests in detecting neoplastic lesions because approximately 50% of trabecular bone must be lost before a lytic lesion is visible.[11] Metastatic lesions may be lytic (radiolucent), blastic (radiodense), or mixed. The majority of metastases are osteolytic. Vertebral bodies are primarily involved because of their rich blood supply associated with red marrow, and unlike infections, the disk space is usually spared. It should be noted that a purely lytic lesion such as multiple myeloma will not be detected by a bone scan. MRI offers the greatest sensitivity and specificity in the evaluation of spinal tumors and is generally the modality of choice.

Radiation therapy is usually helpful in controlling the pain related to skeletal metastases. Decompressive surgery is often required if the spinal mass results in a nerve root compression syndrome.

## Infection

Vertebral osteomyelitis (spinal osteomyelitis, spondylodiskitis) may be acute (usually pyogenic) or chronic (pyogenic, fungal, or granulomatous). Acute vertebral osteomyelitis evolves during a period of a few days or weeks and is the major focus of this discussion.

Vertebral osteomyelitis usually results from hematogenous seeding, direct inoculation at the time of spinal surgery, or contiguous spread from an infection in the adjacent soft tissue. The lumbar spine is the most common site of vertebral osteomyelitis, followed by the thoracic and cervical spine.[60] *Staphylococcus aureus* is the most common microorganism, followed by *Escherichia coli*. Coagulase-negative staphylococci and *Propionibacterium acnes* are almost always the cause of exogenous osteomyelitis after spinal surgery, particularly if internal fixation devices are used.[60]

A source of infection is detected in approximately half the cases, with endocarditis diagnosed in as many as a third of cases of vertebral osteomyelitis.[60] Other common sites for the primary focus of infection are the urinary tract, skin, soft tissue, a site of vascular access, bursitis, or septic arthritis.[61] Most patients with hematogenous pyogenic vertebral osteomyelitis have underlying medical disorders, such as diabetes, coronary artery disease, immunosuppressive disorders, malignancy, and renal failure.[60,61] Intravenous drug abuse is also a risk factor for vertebral osteomyelitis.

Vertebral osteomyelitis may be complicated by an epidural or paravertebral abscess. This may result in neurologic complication.

Back pain is the initial symptom in most patients. The pain tends to be persistent, present at rest, exacerbated by activity, and at times well localized. Point tenderness on percussion over the spine has sensitivity but not specificity for vertebral osteomyelitis. Fever is present in only approximately half of the patients,[60] in part because most patients

are using analgesic medications. Because most cases of vertebral osteomyelitis result from hematogenous seeding, the dominant manifestations initially may be of the primary infection. An epidural abscess may result in a radiculopathy or cauda equina syndrome.

Leukocytosis is seen in only approximately two-thirds of the patients. However, almost all the patients have increases in the erythrocyte sedimentation rate and C-reactive protein, with the latter best correlating with clinical response to therapy.[61] If blood cultures are negative in a patient suspected of having vertebral osteomyelitis, a bone biopsy (CT-guided or open) with appropriate culture studies and histopathologic analysis is indicated.

Plain radiography is usually the initial imaging study. Radiographic changes, however, occur relatively late and are nonspecific. Typically there is loss of disk height and loss of cortical definition followed by bony lysis of adjacent vertebral bodies. MRI is the most sensitive and specific imaging technique to detect spinal infections. The classic finding of pyogenic osteomyelitis is involvement of two vertebral bodies with their intervening disk.[11] In a patient with neurologic impairment, MRI should be done early to rule out an epidural abscess. Whenever possible, antimicrobial therapy should be directed against an identified susceptible pathogen. There are no data from randomized, controlled trials to guide decisions about specific antimicrobial regimens or the duration of therapy.[61] Intravenous therapy of at least 4 to 6 weeks, and possibly additional oral antibiotic therapy, is usually recommended. Surgery may be necessary to drain an abscess, although CT-guided catheter drainage may be sufficient in some cases. Surgical débridement is always required when infection is associated with a spinal implant with removal of the implant whenever possible.[61]

Tuberculosis and nontubercular granulomatous infections (blastomycosis, cryptococcosis, actinomycosis, coccidioidomycosis, and brucellosis) of the spine should be considered in the appropriate clinical and geographic setting.

Lumbar nerve roots are commonly involved in patients with herpes zoster. In most cases a single unilateral dermatome is involved. Pain is often severe and may precede the appearance of a maculopapular rash that evolves into vesicles and pustules.

Pyogenic sacroiliitis is rare. Patients, often children or young adults, present with back pain that may radiate into the buttock or posterior thighs. The sacroiliac joints are usually tender to palpation. MRI is the most sensitive imaging modality. Immediate CT-guided needle biopsy and culture, followed by appropriate antibiotic therapy, will usually produce an excellent outcome.[62]

## Inflammation

The spondyloarthritides cause inflammatory LBP (see Table 47-1) and are discussed in detail elsewhere (see Chapters 74 to 78).

## Metabolic Disease

The major consideration in this category is the occurrence of acute mechanical LBP secondary to a vertebral compression fracture in a patient with osteoporosis (see Chapter 101). Most patients are post-menopausal women.

Paget's disease of bone (see Chapter 101) is most often detected in an asymptomatic patient by the incidental finding of either an elevated alkaline phosphatase or characteristic radiographic abnormality. The spine is the second most commonly affected site after the pelvis. Within the spine, the L4 and L5 vertebrae are most commonly involved.[63] Paget's disease of the spine may involve single or multiple levels. The vertebral body is almost always involved together with a variable portion of the neural arch. Radiographically, Paget's disease is seen as areas of enlargement of the bone with thickened, coarsened trabeculae. Usually a mixed picture of sclerotic and lytic Paget's disease is encountered. The vertebrae may enlarge, weaken, and fracture. LBP may occur because of the pagetic process itself (with periosteal stretching and vascular engorgement), microfractures, overt fractures, secondary osteoarthritis of the facet joints, spondylolysis with or without spondylolisthesis, or sarcomatous transformation (rare).[63] Neurologic complications secondary to Paget's disease of the lumbar spine include sciatica secondary to nerve root impingement, spinal stenosis, and, rarely, cauda equina syndrome.

## Visceral Pathology

Disease in organs that share segmental innervation with the spine can cause pain to be referred to the spine. In general, pelvic diseases refer pain to the sacral area, lower abdominal diseases to the lumbar area, and upper abdominal diseases to the lower thoracic spine area. Local signs of disease, such as tenderness to palpation, paravertebral muscle spasm, and increased pain on spinal motion, are absent.

Vascular, gastrointestinal, urogenital, or retroperitoneal pathology may on occasion cause LBP. A partial list of causes includes an expanding aortic aneurysm, pyelonephritis, ureteral obstruction due to renal stones, chronic prostatitis, endometriosis, ovarian cysts, inflammatory bowel disorders, colonic neoplasms, and retroperitoneal hemorrhage (usually in a patient taking anti-coagulants).

Most abdominal aortic aneurysms are asymptomatic but may become painful as they expand. Aneurysmal pain is usually a harbinger of rupture. Rarely, the aneurysm may develop leakage. This produces severe pain with abdominal tenderness. Most patients with aortic dissection present with a sudden onset of severe "tearing" pain in the chest or upper back. Pain originating from a hollow viscus such as the ureter or colon is often colicky.

## Miscellaneous

LBP may be part of the clinical spectrum in innumerable conditions. It would not be practical or useful to discuss these entities here. Considered next are some of the more important or controversial causes of LBP.

The piriformis syndrome is thought to be an entrapment neuropathy of the sciatic nerve related to anatomic variations in the muscle-nerve relationship or to overuse. The piriformis is a narrow muscle that originates from the anterior part of the sacrum and inserts into the greater trochanter. It is an external rotator of the hip. There is, however, debate about the existence of the piriformis syndrome as a

discrete entity because of the lack of objective, validated, and standardized tests. The diagnosis is clinical. Patients complain of pain and paresthesias in the gluteal region that radiate down the leg to the foot. Unlike sciatica from lumbosacral nerve root compression, the pain is not restricted to a specific dermatome. The straight leg–raising test is usually negative. There may be tenderness over the sciatic notch. Physical examination maneuvers for the diagnosis of piriformis syndrome are based on the notion that stretching the irritated piriformis muscle may provoke sciatic nerve compression. This can be done by internally rotating the hip (Freiburg's sign) or by flexion, adduction, and internal rotation (FAIR maneuver) of the hip. Physical therapy that focuses on stretching the piriformis muscle and NSAIDs are generally the treatments offered. In refractory patients, local anesthetic, corticosteroid, and botulinum toxin injections have been used.

Sacroiliac joint dysfunction is a controversial diagnosis. It is a term used to describe pain in the sacroiliac region related to abnormal sacroiliac joint movement or alignment. However, tests of pelvic symmetry or sacroiliac joint movement have low intertester reliability, and fluoroscopically guided sacroiliac joint injections have been unreliable in diagnosis and treatment.[64,65] Radiographic degenerative changes of the sacroiliac joint are often noted in the evaluation of patients with LBP. Whether these changes are the primary cause of the back pain remains unresolved.[66]

Lumbosacral transitional vertebrae include sacralization of the lowest lumbar vertebral body (assimilation of L5 to the sacrum) and lumbarization of the uppermost sacral segment (assimilation of S1 to lumbar spine). These common variants can be seen in 15% to 35% of the general population.[67] The association of these variants with LBP remains controversial.

A "back mouse" is a mobile subcutaneous fibro-fatty nodule in the lumbosacral area. The nodule may be tender. Although there are case reports of patients with LBP and "back mice,"[68] the association with LBP remains unproven.

Epidural lipomatosis may be seen in obese patients, but it is more commonly seen as a rare side effect of long-term use of corticosteroids. There is an increase in epidural adipose tissue that causes a narrowing of the spinal canal. This is usually an incidental finding, although it may lead to compression of neural structures.

LBP during pregnancy is common. The pain usually starts between the fifth and seventh months of pregnancy.[69] The etiology of LBP in pregnancy is unclear. Biomechanical, hormonal, and vascular factors have been implicated. Most women have resolution of their pain postpartum.

Fibromyalgia (see Chapter 52) and polymyalgia rheumatica (see Chapter 88) are two frequently encountered rheumatologic conditions in which LBP may be a prominent part of the clinical syndrome.

## TREATMENT

Specific treatment is available only for the small fraction of patients with LBP who have either evidence of clinically significant neural compression or an underlying systemic disease (cancer, infection, visceral disease, and spondyloarthritis). In the vast majority of patients with LBP, either the precise pathoanatomic cause (i.e., the pain generator) cannot be determined or, when the cause is determined, no specific treatment is available. These patients are managed with a conservative program centered on analgesia, education, and physical therapy. The goal of treatment is relief of pain and restoration of function. Surgery is rarely necessary (Figure 47-10).

One should be wary of the proliferation of unproven medical, surgical, and alternative therapies. Most have not been rigorously tested in well-designed randomized controlled trials. Uncontrolled studies can produce a misleading impression of efficacy because of fluctuating symptoms and the largely favorable natural history of LBP in most patients.

For management purposes, patients with LBP are considered to have either acute LBP (duration <3 months), chronic LBP (duration >3 months), or a nerve root compression syndrome.

### Acute Low Back Pain

The typical patient seeks medical attention for sudden onset of severe mechanical LBP. Examination usually reveals paravertebral muscle spasm, which often results in loss of the normally present lumbar lordosis and a severe decrease in range of motion secondary to pain. The prognosis for acute LBP is excellent. Indeed, only approximately a third of these patients seek medical care and more than 90% recover within 8 weeks or less.[70]

Patients with acute LBP are advised to stay active and continue ordinary daily activities within the limits permitted by pain. This leads to more rapid recovery than bed rest.[71] Bed rest of more than 1 or 2 days is discouraged.

Pharmacologic therapy is used for symptomatic relief and does not affect recovery time. Unfortunately, no medication has consistently been shown to result in large average benefits on pain, and evidence of beneficial effects on function is even more limited.[8] In spite of limited efficacy,[72] acetaminophen and NSAIDs are first-line options for analgesia. Short-term use of opioids is reasonable in patients with severe disabling LBP or in those at high risk of complications because of NSAIDs. For patients with acute LBP, short-acting opioids are generally recommended. Muscle relaxants are moderately effective for short-term symptomatic relief but have a high prevalence of adverse effects, including drowsiness and dizziness.[8] It is unclear whether these medications truly relax muscles or if their effects are related to sedation or other nonspecific effects. Benzodiazepines have similar efficacy to muscle relaxants for short-term pain relief but are associated with risks for abuse, addiction, and tolerance.[24]

Back exercises are not helpful in the acute phase, and a physical therapy referral is usually unnecessary in the first month. Later, an individually tailored program that focuses on core strengthening, stretching exercises, aerobic conditioning, functional restoration, loss of excess weight, and education is recommended to prevent recurrences.[9,14] The purpose of back exercises is to stabilize the spine by strengthening trunk muscles. Flexion exercises strengthen the abdominal muscles, and extension exercises strengthen the paraspinal muscles. Numerous exercise programs have been developed and appear to be equally effective.

Patient education, including the use of education booklets, is strongly recommended.[24] The information provided

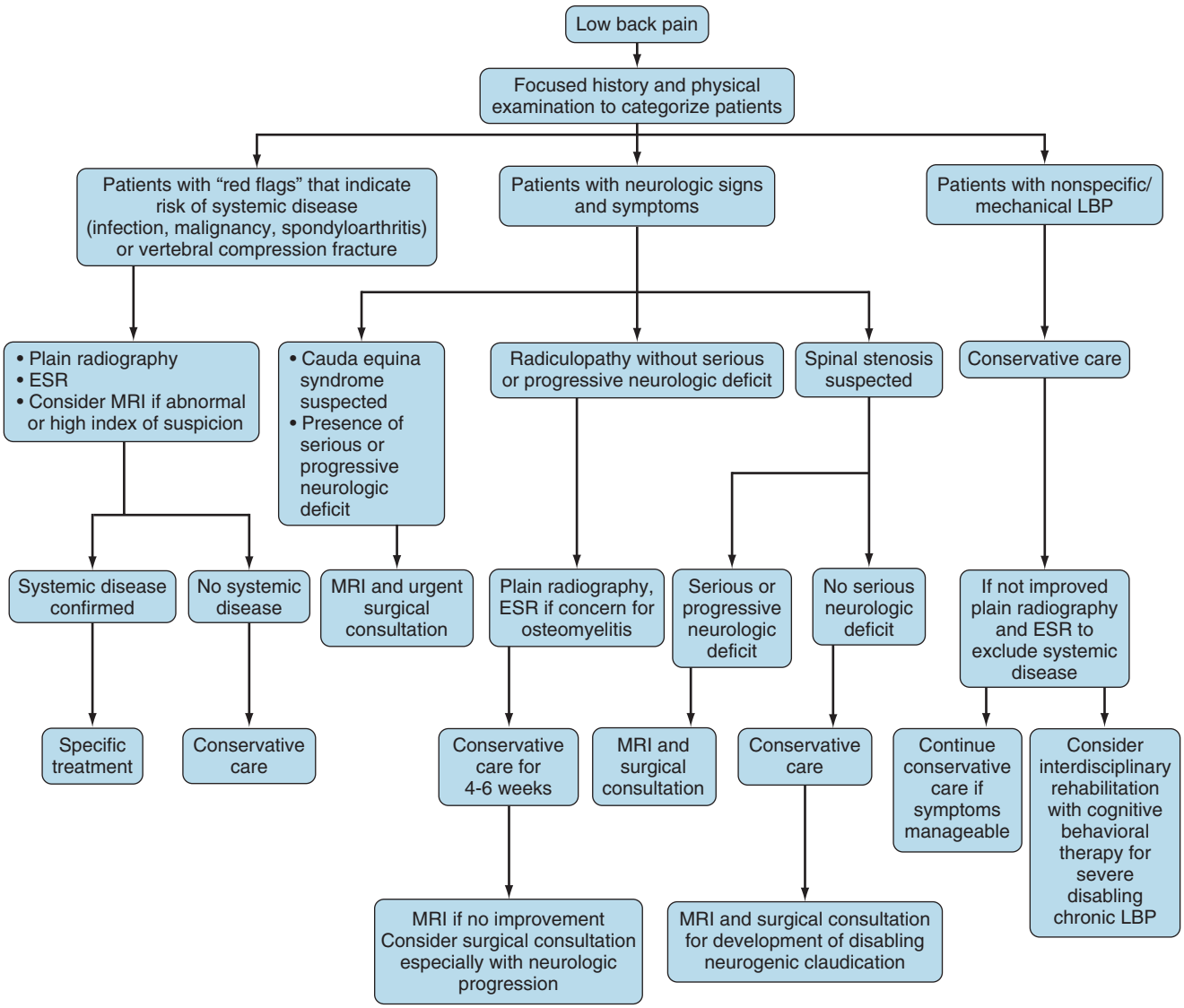



**Figure 47-10** Algorithm for the differential diagnosis and treatment of low back pain. ESR, Erythrocyte sedimentation rate; LBP, low back pain; MRI, magnetic resonance imaging.

should include causes of LBP, basic anatomy, favorable natural history, minimal value of diagnostic testing, importance of remaining active, effective self-care options, and coping techniques.

Spinal manipulation is provided mainly by chiropractors and osteopaths. It may involve low-velocity mobilization or manipulation with a high-velocity thrust that stretches spinal structures beyond the normal range and is frequently accompanied by a cracking or popping sound. For acute LBP, current evidence suggests that manipulative therapy is no more effective than conventional medical therapy.[24] There is no evidence that ongoing manipulation reduces the risk of recurrence of LBP.[73] There is insufficient evidence regarding the efficacy of massage and acupuncture in the treatment of acute LBP.[24]

Application of heat by heating pads or blankets is a reasonable self-care option for short-term relief of acute LBP. However, insufficient evidence exists to recommend application of cold packs or the use of corsets and braces.[24] Traction provides no significant benefit for LBP patients with or without sciatica.[74]

Injection therapy is used mostly in subacute (>6 weeks) and chronic LBP. Epidural corticosteroid injections have gained remarkable, but unjustified, popularity. The rationale for their use is that the genesis of radicular pain, when a herniated disk impinges on a nerve root, is at least partly related to locally induced inflammation. There is evidence of a small treatment benefit compared with placebo injection for short-term relief of leg pain in patients with radiculopathy resulting from a herniated nucleus pulposus.[75,76] However, epidural corticosteroid injections offer no significant functional benefit, nor do they reduce the need for surgery. It is important to note that there is no convincing evidence for the effectiveness of epidural corticosteroid injections in LBP patients without radiculopathy, in patients with spinal stenosis and neurogenic claudication,[77] or for

failed back surgery syndrome. Nonetheless, most of the use of epidural steroid injections occurs in these situations of questionable benefit.[78] The U.S. Food and Drug Administration (FDA) has not approved the use of epidural corticosteroid injections. In April 2014 the FDA issued a drug safety communication requiring label changes to warn of rare but serious neurologic problems (including loss of vision, stroke, and death) after epidural corticosteroid injections. This safety issue is unrelated to the contamination of compounded corticosteroid injections products used for epidural injections that was reported in 2012.

A variety of other injection therapies with glucocorticoids or anesthetic agents, often in combination, are used in individuals with LBP with or without radicular pain and other symptoms in the leg. These include injection of trigger points, ligaments, sacroiliac joints, facet joints, and intradiskal steroid injections. There is no convincing evidence of the efficacy of these interventions.[79,80] Medial branch block for presumed facet joint pain and nerve root blocks for therapeutic or diagnostic purposes are also not recommended.[80] Unfortunately, these invasive and expensive procedures are commonly used in interventional pain clinics.

A number of physical therapy modalities are currently used in the treatment of patients with subacute and chronic LBP. These include transcutaneous electrical nerve stimulation (TENS), percutaneous electrical nerve stimulation, interferential therapy, low-level laser therapy, shortwave diathermy, and ultrasound. There is insufficient evidence of efficacy to recommend their use.

Vertebral compression fractures secondary to osteoporosis are common. There is resolution of pain with fracture healing within a few weeks in most patients. Vertebroplasty and balloon kyphoplasty are two increasingly popular, invasive, and expensive procedures that are used to treat persistent pain associated with these fractures. Both procedures involve the percutaneous placement of needles into the vertebral body through or lateral to the pedicles, as well as the injection of bone cement to stabilize the fracture. Kyphoplasty differs from vertebroplasty in that the cement is injected into a void in the vertebral body created by inflation of a balloon. Several early studies had suggested a positive treatment effect for vertebroplasty.[81] However, two blinded, randomized, placebo-controlled trials of vertebroplasty for painful osteoporotic spinal fractures found no beneficial effect of vertebroplasty compared with a sham procedure.[82,83] A subset analysis of data from these trials confirmed that the lack of benefit from vertebroplasty extended to patients with pain of recent onset (<6 weeks) and to those with severe pain.[84] Therefore, on the basis of current evidence, the routine use of vertebroplasty or indeed kyphoplasty for relief of pain from osteoporotic compression fractures cannot be justified.[85]

## Chronic Low Back Pain

The clinical spectrum in patients with chronic LBP is wide. Some complain of severe, unrelenting pain, but most have a nagging mechanical LBP that may radiate into the buttocks and upper thighs. Patients with chronic LBP may experience periods of acute exacerbation. These exacerbations are managed according to the principles discussed earlier. A significant number of patients with chronic LBP remain functional and continue working, but overall, the results of treatment are unsatisfactory, and complete relief of pain is unrealistic for most. There is evidence that in a subset of patients with chronic LBP a pattern of augmented central nervous system (CNS) pain processing (similar to fibromyalgia) is present suggesting a component of "centralized pain."[86] These patients are more likely to have poor treatment outcomes. Patients with chronic LBP are largely responsible for the high costs associated with LBP. It is therefore incumbent on physicians who treat these patients to judiciously use proven therapies.

For most patients, first-line medication options are acetaminophen or NSAIDs. They may provide some degree of analgesia, but the evidence for their long-term efficacy is not compelling. Opioid analgesics or tramadol are an option when used judiciously in patients with severe disabling pain. Because of substantial risks, including aberrant drug-related behaviors with long-term use in patients vulnerable to abuse or addiction, potential benefits and harms of opioid analgesics should be carefully weighed before starting therapy.[24,87] The co-prescribing of opioids and benzodiazepines should be avoided. There is no evidence that long-acting, around-the-clock dosing is more effective than short-acting or as-needed dosing, and continuous exposure to opioids could induce tolerance and lead to dose escalations.[8] Muscle relaxants are not recommended for long-term use in patients with chronic stable LBP. Anti-depressants that inhibit norepinephrine uptake are thought to have pain-modulating properties independent of their effects on depression. As such, low-dose tricyclic anti-depressants are an option for chronic LBP, although the treatment effect is small and adverse side effects are common.[8] There is no evidence of efficacy of selective serotonin reuptake inhibitors for LBP. Depression is, however, common in patients with chronic LBP and should be treated appropriately. Duloxetine, a serotonin-norepinephrine reuptake inhibitor, may have marginal efficacy in patients with chronic LBP.[88,89] There is insufficient evidence to recommend antiepileptic medications, such as gabapentin and topiramate, for pain relief in patients with LBP with or without radiculopathy.[8]

An individually tailored physical therapy program and patient education, as discussed in the earlier section on the treatment of acute LBP, are particularly important aspects in the management of a patient with chronic LBP. The use of physical therapy modalities and injection techniques (as discussed earlier) is not recommended for patients with chronic LBP. Lumbar supports and traction are ineffective. For most patients with LBP a medium-firm mattress or a back-conforming mattress (waterbed or foam) may be superior to a firm mattress.[90,91]

A number of physical treatments have been used to treat chronic LBP. Spinal manipulation has been shown to be superior to sham manipulation, but it is no more effective than conservative medical therapy.[92] A recent meta-analysis suggested that osteopathic manipulative treatment improves pain and functional status in acute and chronic nonspecific LBP. However, the studies included had small sample sizes, different comparison groups in different studies, and lack of long-term measurement.[93] There is less evidence for the efficacy of massage and acupuncture.[92] Trial data, including randomized studies,[94,95] suggest that a yoga program can be effective in reducing pain and improving function in some

patients with chronic or recurrent LBP, with benefits lasting at least several months.

There has been a proliferation of nonsurgical interventional therapies for back pain. Various injection therapies (such as injections of trigger points, facet joints, and nerve root blocks) as discussed under the treatment of acute LBP, lack convincing evidence of efficacy and as such are not recommended in the treatment of patients with chronic LBP.

Radiofrequency denervation has most commonly been used for the treatment of presumed facet joint pain by targeting the medial branch of the primary dorsal ramus. It involves fluoroscopic placement of an electrode near the nerve and application of heat by using a radiofrequency current to coagulate the nerve. There is a lack of convincing evidence about the effectiveness of this invasive procedure.[79] Intradiskal electrothermal therapy (IDET) and percutaneous intradiskal radiofrequency thermocoagulation (PIRFT) involve placement of an electrode into the intervertebral disk of patients with presumed diskogenic pain and using electric or radiofrequency current to provide heat to thermocoagulate and shrink intradiskal tissue and destroy nerves. Current evidence does not support the use of IDET or PIRFT.[79,96] Prolotherapy (also referred to as *sclerotherapy*) involves repeated injections of an irritant sclerosing agent into ligaments and tendinous attachments. It is based on the hypothesis that back pain in some patients stems from weakened ligaments and repeated injections of a sclerosing agent will strengthen the ligaments and reduce pain. On the basis of trial data, a guideline from the American Pain Society recommends against prolotherapy for chronic LBP.[79]

Spinal cord stimulation is a procedure involving the placement of electrodes, percutaneously or by laminotomy, in the epidural space adjacent to the area of the spine presumed to be the source of pain and applying an electric current in order to achieve sympatholytic and other neuromodulatory effects.[79] Power for the spinal cord stimulator is supplied by an implanted battery. Spinal cord stimulation is associated with a greater likelihood for pain relief compared with reoperation or conventional medical management in patients with failed back surgery syndrome with persistent radiculopathy.[79] At present there is no good evidence for the use of spinal cord stimulation for chronic LBP not related to the failed back surgery or failed back surgery syndrome without radiculopathy. Approximately a third of the patients involved in studies have experienced a complication following spinal cord stimulation implantation, including electrode migration, infection, wound breakdown, and lead- and generator pocket–related complications.[79]

Intraspinal drug infusion systems, with use of a subcutaneously implanted pump with attached catheter, have been used in some patients with chronic intractable LBP for the intrathecal delivery of analgesics, usually morphine. Adequate evidence to support this intervention is not available.

Chronic LBP is a complex condition that involves biologic, psychological, and environmental factors. For patients with persistent and disabling nonradicular LBP despite recommended noninterdisciplinary therapies, the clinician should strongly consider intensive interdisciplinary rehabilitation with an emphasis on cognitive-behavioral therapy.[80] Interdisciplinary rehabilitation (also called *multidisciplinary therapy*) is an intervention that combines and coordinates physical, vocational, and behavioral components and is provided by multiple health professionals with different clinical backgrounds. Cognitive-behavioral therapy is a psychotherapeutic intervention that involves working with cognitions to change emotions, thoughts, and behaviors. There is strong evidence of improved function and moderate evidence of pain improvement with intensive interdisciplinary rehabilitation programs.[29] Functional restoration (also called *work hardening*) is an intervention that involves simulated or actual work in a supervised environment to enhance job performance skills and improve strength, endurance, flexibility, and cardiovascular fitness in injured workers. When combined with a cognitive-behavioral component, functional restoration is more effective than standard care alone to reduce time lost from work.[92]

As previously discussed, the precise identification of the pain generator in an LBP patient with degenerative changes involving the lumbar spine and no radicular pain is usually not possible in contradistinction to the patient with radicular symptoms. It is therefore not surprising that, as a general rule, the results of back surgery are disappointing when the goal is relief of back pain rather than relief of radicular symptoms resulting from neurologic compression. As such, the role of surgical treatment for chronic disabling LBP without neurologic involvement in patients with degenerative disease remains controversial. The most common surgery performed is spinal fusion. In spite of the unclear efficacy, rates of spinal fusion surgery for this indication are rapidly increasing. Interbody fusion is achieved from either a posterior or an anterior approach or both combined for a circumferential fusion. All fusion techniques involve placement of a bone graft between the vertebrae. Instrumentation refers to the use of hardware, such as screws, plates, or cages, that serve as an internal splint while the bone graft heals. Bone morphogenetic proteins are sometimes used to speed fusion. The rationale for fusion is based on its successful use at painful peripheral joints.

The current evidence is that for nonradicular back pain with degenerative changes, fusion is no more effective than intensive interdisciplinary rehabilitation but is associated with small to moderate benefits compared with standard nonsurgical care.[97] Furthermore, the majority of patients who undergo surgery do not experience an optimal outcome defined as no pain, discontinuation or occasional pain medication use, and return of high-level function.[80]

Lumbar disk replacement with a prosthetic disk is a newer alternative to fusion. Disk replacement is approved in the United States for patients with disease limited to one disk between L3-S1 and no spondylolisthesis or neurologic deficit. Approval was based on data showing efficacy equal to that of spinal fusion. This may be faint praise, given the controversy regarding the efficacy of spinal fusion for lumbar disk disease. No data support the hypothetical advantage that, unlike spinal fusion, prosthetic disks will protect adjacent levels from further degeneration by preserving motion. At present there is insufficient evidence regarding the long-term benefits and risk of disk replacement to support its recommendation.

Most patients with LBP, including those with neurogenic signs and symptoms, do not require surgical treatment.

## Spine Patient Outcomes Research Trial

The precise role of surgery in the care of patients with LBP and neurogenic signs and symptoms is often unclear and controversial. This prompted the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) to fund three large parallel randomized[98-101] Spine Patient Outcomes Research Trial (SPORT) studies in an effort to assess the role of surgery in patients with lumbar disk herniation, lumbar degenerative spondylolisthesis with spinal stenosis, or lumbar spinal stenosis. Of note, in each of these milestone studies, all the patients had radicular leg pain with associated neurologic signs or neurogenic claudication. Patients with serious or progressive neurologic deficits require urgent surgical decompression and, as such, were excluded from all the SPORT studies. Each study included a randomly assigned cohort and an observational cohort. Patients in the observational cohort declined to be randomly assigned in favor of designating their own treatment, but agreed to undergo follow-up according to the same protocol. The primary study outcomes were measures of pain, physical function, and disability during a 2-year period. All three studies were compromised by high rates of crossover (as much as 50%) between the assigned treatment, surgical or nonsurgical, in both cohorts. This has caused concern over the validity of the conclusions.

The first study[98,99] looked at surgical (discektomy) versus nonoperative treatment (physical therapy, education, NSAIDs if tolerated) in patients with persistent symptoms despite nonoperative treatment for at least 6 weeks. Both treatment groups improved substantially; the intent-to-treat analysis showed no significant difference in the randomly assigned cohort.[98] Greater improvement with surgery was reported in the observational cohort.[99] However, nonrandomly assigned comparisons of self-reported outcomes are subject to potential confounding and must be interpreted cautiously.

In the second study, in patients with lumbar degenerative spondylolisthesis and spinal stenosis, with persistent symptoms for at least 12 weeks, the intent-to-treat analyses for the randomly assigned cohort showed no significant differences between the surgical (decompressive laminectory with or without fusion) and usual nonsurgical treatment. The nonrandomly assigned "as treated" comparison that combined both cohorts showed greater improvement in the surgical group. "As treated" analyses are significantly confounded and should be interpreted with caution.[102]

In the final study[101] for patients with lumbar spinal stenosis without spondylolisthesis, with persistent symptoms for at least 12 weeks, both the intent-to-treat and "as treated" analyses showed a significant advantage favoring surgery (posterior decompressive laminectomy).

SPORT data also revealed that, in general, spinal surgery improves leg pain more than LBP, and people with diabetes tend to experience less relief from spinal surgery.[103,104]

## Disk Herniation

Patients with a herniated disk with radicular pain secondary to nerve root compression should be treated nonsurgically, as described in the section on acute LBP unless they have a serious or progressive neurologic deficit. Only approximately 10% of patients have sufficient pain after 6 weeks of conservative care that surgery is considered.[14] A decision to continue with nonsurgical therapy beyond 6 weeks in these patients does not increase the risk for paralysis or cauda equina syndrome.[80] Surgery in these patients is associated with moderate short-term (through 6 to 12 weeks) benefits compared with nonsurgical therapy, although differences in outcome diminish with time and are generally no longer present after 1 to 2 years.[74,80]

Open diskectomy or microdiskectomy is the usual surgery performed on patients with serious or progressive neurologic deficit or electively on patients with persistent disabling pain secondary to radiculopathy (Table 47-5). Open diskectomy generally involves a laminectomy, whereas microdiskectomy, by using a smaller incision and an operating microscope, involves a hemilaminectomy to remove the disk fragment compressing the nerve root. There are no clear differences in the outcome between open diskectomy and microdiskectomy. There is insufficient evidence to evaluate the efficacy of sequestrectomy, or various laser-assisted, endoscopic, percutaneous, and other minimally invasive methods.[97,105]

Epidural corticosteroid injections may offer a small treatment benefit for short-term relief of radicular pain but do not offer significant functional benefit and do not reduce the need for surgery.[75]

Anti–tumor necrosis factor (TNF) therapy is under investigation in patients with lumbar radiculopathy.

There is biologic plausibility for its potential efficacy in sciatica because TNF is an important mediator of nerve root inflammation, central sensitization, and neuropathic pain.[106] However, there have been conflicting results in small randomized control trials looking at patients with radiculopathy. The addition of a short course of subcutaneous adalimumab to the treatment regimen of patients with acute sciatica resulted in a small decrease in leg pain and fewer surgical procedures.[107] However, another trial found no difference between intravenous infliximab and a saline infusion.[108] In a three-group trial, leg pain appeared to be reduced more with epidural steroids than with epidural etanercept or saline.[109] The results of the etanercept group were no better than the saline group. Yet a trial that used the transforaminal approach to deliver epidural etanercept concluded that etanercept reduced both leg and back pain compared with placebo.[106]

**TABLE 47-5** Indications for Surgical Referral

| |
|---|
| **Disk Herniation** |
| Cauda equina syndrome (emergency) |
| Serious neurologic deficit |
| Progressive neurologic deficit |
| Longer than 6 weeks of disabling radiculopathy (elective) |
| **Spinal Stenosis** |
| Serious neurologic deficit |
| Progressive neurologic deficit |
| Persistent and disabling pseudoclaudication (elective) |
| **Spondylolisthesis** |
| Serious or progressive neurologic deficit |

### Spinal Stenosis

It is critical to understand the natural history of degenerative lumbar spinal stenosis before making treatment decisions. The symptoms of spinal stenosis remain stable for years in most patients and may improve in some. Dramatic improvement is uncommon. Even when symptoms progress, there is little likelihood of rapid deterioration of neurologic function. Therefore conservative nonoperative treatment is a rational choice for most patients.

There is a paucity of good data to guide the conservative management of lumbar spinal stenosis. Physical therapy is the mainstay of management, but evidence for the efficacy of specific standardized regimens is not available. Most regimens include core strengthening, stretching, aerobic conditioning, loss of excess weight, and patient education. Exercises that involve lumbar flexion, such as bicycling, are better tolerated. Strengthening of abdominal muscles may be helpful by promoting lumbar flexion and reducing lumbar lordosis. Lumbar corsets that maintain slight flexion may provide symptomatic relief. They should only be used for a limited number of hours a day to avoid atrophy of paraspinal muscles.

Acetaminophen, NSAIDs, and mild narcotic analgesics are used for symptomatic relief of pain.

Lumbar epidural corticosteroid injections are used on the assumption that symptoms may result from inflammation at the interface between the nerve root and compressing tissues.[45] To assess this potential for efficacy, a double-blind placebo controlled, randomized trial[77] was carried out in patients with lumbar spinal stenosis and associated moderate to severe leg pain. There was no significant difference between patients assigned to epidural injections of glucocorticoids plus lidocaine and those assigned to lidocaine alone with regards to the co-primary outcomes of functional disability or pain intensity. Based on this well-designed study, the routine use of epidural corticosteroids in patients with lumbar spinal stenosis cannot be recommended.

Surgery is indicated for the few patients with lumbar spinal stenosis who have a serious or progressive neurologic deficit. However, most surgery for lumbar spinal stenosis is elective. The indication for elective surgery is to relieve persistent and disabling symptoms of neurogenic claudication that have not responded to conservative care. In patients without fixed neurologic deficits, delayed surgery produces similar benefits to surgery selected as the initial treatment.[45,110] The surgical goal is to decompress the central spinal canal and the neural foramina to eliminate pressure on the nerve roots. This is accomplished by laminectomy, partial facetectomy of hypertrophied facet joints, and excision of the hypertrophied ligamentum flavum and any protruding disk material. Laminectomy with lumbar fusion should generally be reserved for patients who have spinal stenosis with spondylolisthesis. Unfortunately, there is an alarming increase in spinal fusion surgery with routine use of complex fusion techniques in the absence of evidence of greater efficacy. The techniques include instrumentation, bone graft augmentation with bone cement and human bone morphogenetic proteins, and combined anterior and posterior fusion (often at multiple levels). These techniques are associated with increased perioperative mortality, major complications, rehospitalization, and cost in the absence of evidence of greater efficacy.[111-113]

Overall, for patients with spinal stenosis, with or without spondylolisthesis, who have disabling symptoms of neurogenic claudication despite conservative care, there is some evidence to support the effectiveness of decompressive laminectomy in reducing pain and improving function through 1 to 2 years.[45,80,97,101] Beyond this time frame, the benefits appear to diminish.

A less invasive alternative to decompressive laminectomy is the implantation of a titanium interspinous spacer at one or two vertebral levels. This spacer distracts adjacent spinous processes and thereby imposes lumbar flexion, which in turn potentially increases the spinal canal dimensions. There is preliminary evidence of efficacy in patients with one- or two-level spinal stenosis, without spondylolisthesis, and with a history of relief of neurogenic claudication with flexion.[97] There are no trials comparing the interspinous spacer with decompressive surgery.

### Spondylolisthesis

The vast majority of patients with spondylolisthesis and chronic LBP are treated conservatively. Rarely, a patient may need decompression surgery with fusion if a serious or progressive neurologic deficit develops from nerve root impingement or disabling pseudoclaudication secondary to spinal stenosis develops in the patient. A randomized trial involving patients with isthmic spondylolisthesis and disabling isolated LBP or sciatica for at least a year suggested fusion surgery produced better results than nonsurgical care,[114] although the differences in outcome narrowed during a 5-year follow-up period.[115]

## OUTCOME

The natural history of most patients with acute LBP is favorable. There is substantial improvement in pain and function within a month in the majority of patients,[4] and more than 90% are better at 8 weeks.[5] Only approximately a third of patients with acute LBP seek medical care. Presumably, the rest improve on their own. Relapses, that also tend to be brief, are common and may affect as many as 40% of patients within 6 months.

Improvement is also the norm for patients with sciatica secondary to a herniated disk.[116] A third of these patients are significantly better in 2 weeks, and 75% improve after 3 months.[11] Only approximately 10% of these patients ultimately undergo surgery.

The symptoms of spinal stenosis tend to remain stable in 70% of patients, improve in 15%, and worsen in 15%.[49]

The 7% to 10% of patients in whom chronic pain develops are largely responsible for the high costs associated with LBP and remain a major challenge. Factors that predict persistence of chronic disabling LBP include maladaptive pain coping behaviors, presence of nonorganic signs, functional impairment, poor general health status, psychiatric comorbidities, job dissatisfaction, disputed compensation claims, and a high level of "fear avoidance" (an exaggerated fear of pain leading to avoidance of beneficial activities).[29,117]

## CONCLUSION

Of the general population, 80% will experience LBP. Of these patients, 90% will be largely pain free within 8 weeks. Degenerative change in the lumbar spine is the most commonly identified cause of LBP. The initial evaluation of LBP should focus on identifying the few patients with significant neurologic involvement, fracture, or systemic disease (infection, malignancy, or spondyloarthritis) because they may need urgent or specific intervention. Early imaging is rarely indicated in the absence of trauma, significant neurologic involvement, or suspicion of systemic disease. Imaging abnormalities, often the result of age-related degenerative changes, should be carefully interpreted because they are frequently present in asymptomatic individuals. A precise pathoanatomic diagnosis with identification of the pain generator cannot be made in 85% of patients. Regardless, most patients with the largely self-limited syndrome of acute LBP will only require reassurance, education, and simple analgesics. The management of patients with chronic LBP remains a challenge and complete relief of pain is an unrealistic goal for most. Patients with chronic LBP benefit from an initial program of core strengthening, stretching, aerobic conditioning, loss of excess weight, and education. Intensive interdisciplinary rehabilitation with an emphasis on cognitive-behavioral therapy should be strongly considered if conservative measures fail. Epidural corticosteroids have a small treatment benefit, and their use should be restricted to patients with radiculopathy because of disk herniation. A large number of injection techniques, physical therapy modalities, and nonsurgical interventional therapies lack evidence of efficacy. Decompressive surgery is indicated for serious or progressive neurologic deficit and is rarely needed.

Rates of back surgery (including spinal fusion) in the United States are the highest in the world and continue to rise rapidly.[97] This increase continues in spite of the fact that the role of surgery in the management of patients without serious or progressive neurologic deficit remains controversial. Randomized trials incorporating a sham operation may be the only way to resolve the controversy. Such trials may be justifiable because genuine clinical equipoise exists among clinicians about the merits of the intervention, the surgery is often not performed for a life-threatening condition, the primary outcomes are subjective, and the rate of complications is high.[102,113]

There continues to be a proliferation and increasing utilization of expensive but unproven interventional and alternative therapies. Whenever possible, these need to be subjected to randomized, placebo-controlled trials. This is the only truly valid means to assess the efficacy of interventions for subjective outcomes such as pain. Once the efficacy has been established, these treatments should be further subjected to "comparative effectiveness research" to determine how these effective interventions compare with each other.[118]

A promising new approach for the management of LBP in primary care involves stratified care according to the estimated risk of poor prognosis by using a validated and simple screening method, Patients are allocated into three different risk-defined groups (low, medium, and high) and treated according to three treatment pathways matched to the risk groups. This resulted in better clinical and economic outcomes than did nonstratified conventional care.[119] This strategy should be further explored and confirmed in different settings.

Evidence-based clinical practice guidelines regarding indications for tests or treatment at specific steps in the care of patients with LBP exist in many countries. They are intended to provide a cost-effective road map for rational and efficient care. The implementation of these guidelines, however, remains a challenge. Avenues that will facilitate this implementation should be explored.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 48

# Hip and Knee Pain



James I. Huddleston III • Stuart Goodman

**KEY POINTS**

- The clinician should be able to narrow the differential diagnosis down to two to three diagnoses after the history and physical examination.
- Imaging studies should be used to confirm the diagnosis.
- The initial imaging study ordered should be conventional radiographs.
- Many of the vital structures in the knee can be palpated easily or examined with provocative tests.
- A knee effusion is often associated with internal derangement.
- The clinician should suspect a torn meniscus if a patient has an effusion, joint line tenderness, and pain with hyperextension and hyperflexion.
- Patients with osteoarthritis often report stiffness and pain with activity.
- Inflammatory arthritis should be considered when a patient continues to experience pain despite resting the joint.
- Groin pain with internal rotation of the hip is considered to be due to hip disease until proven otherwise.
- Concurrent hip and lumbosacral disease is common.

Musculoskeletal pain affects one-third to one-half of the general population.[1,2] A substantial increase in the burden of musculoskeletal disease is expected in the next decade as the "Baby Boomers" reach middle age and beyond. This increased burden is exemplified by the increasing prevalence of hip and knee replacement operations, which rose by 16.2% to 884,400 procedures annually in the United States between 2002 and 2004.[3] Furthermore, the prevalence of total knee and total hip arthroplasty procedures is expected to double by 2016 and 2026, respectively.[4] The hip and knee joints are two of the most commonly affected sites of musculoskeletal pain, with the prevalence of hip pain ranging from 8% to 30% in people 60 years and older[5,6] and the prevalence of knee pain ranging from 20% to 52% in individuals 55 years and older. In general, women experience more musculoskeletal pain than do men.[7] Geographic and ethnic variations in the rates of both hip and knee pain also exist. For example, there tends to be significantly less hip and knee pain with decreasing latitude, as well as significantly less hip pain and osteoarthritis in China than in the United States.[8-15]

When evaluating reports of knee or hip pain, knowledge of the anatomy of these joints is necessary to formulate a differential diagnosis. Given the thin soft tissue envelope around the knee and the fact that knee pain is rarely referred, the pain generators around the knee often can be elucidated with a complete history and thorough physical examination. Diagnosis of hip pain may be more challenging because the joint is deeper and the region is not infrequently the site of referred pain from the spine. An understanding of the basic biomechanics of these joints is also important in formulating a differential diagnosis because certain activities are likely to cause specific injuries.

This chapter will focus on the important aspects of the history, physical examination, and imaging modalities involved in evaluating patients with reports of knee and hip pain. An appropriate, thorough workup of these patients will allow the clinician to formulate an accurate differential diagnosis in an efficient manner.

## KNEE PAIN

### History

A detailed history is perhaps the most important step in accurately diagnosing the cause of knee pain. Knee complaints generally fall into two broad categories: pain or instability. Pain may arise from injury to the articular surfaces (e.g., osteoarthritis, inflammatory arthritis, osteochondral defects, and osteochondritis desiccans), torn menisci, quadriceps and patella tendon tears, bursitis, nerve damage, fractures, neoplasia, or infection. Referred pain from the hip or spine is less common. Instability is usually episodic and stems from injuries to the quadriceps-patellar extensor mechanism, collateral ligaments, or cruciate ligaments. It is important to distinguish true instability from the common complaint of "giving way" because the latter is usually due to a robust pain response rather than specific structural disease.

People in certain age groups tend to experience similar injuries. Ligament injuries, acute meniscus tears, and patellofemoral problems are frequently encountered in patients younger than 40 years. In contrast, degenerative conditions such as osteoarthritis and degenerative meniscal lesions tend to occur more frequently in older people.

The location and character of the pain are particularly important when evaluating knee pain because many of the structures vital to proper knee function are subcutaneous and can be palpated easily. We prefer to conceptualize the knee as three separate compartments—medial, lateral, and patellofemoral. Each compartment should be examined separately. The patient should be able to point to the exact

area where the pain is most severe. The time of onset of the pain should be determined. Osteoarthritis and inflammatory arthritis tend to have an insidious onset, whereas injuries to menisci and ligaments are usually associated with a traumatic event. Knowing the details of a traumatic event will be helpful. For example, a twisting injury, especially one sustained with a flexed knee, suggests a meniscus tear, whereas a noncontact knee injury associated with change of direction is more likely to produce a tear of the anterior cruciate ligament (ACL). Pain from degenerative arthritis tends to be associated with stiffness, is generally worse with ongoing activity during the day, and is exacerbated by activities such as exercise, stair climbing, getting up from a chair, and getting in and out of a car.

The presence or absence of knee swelling is an important part of the history because knee effusions (i.e., fluid in the knee joint) usually accompany internal derangement. An effusion also may be present with synovitis, osteoarthritis, inflammatory arthritis, fractures, infection, and neoplasms. Distinguishing between soft tissue swelling around the knee, synovial thickening, and a true knee effusion is critical (described in the following text). The timing or onset of the swelling is also important for determining the diagnosis. An acute cruciate or collateral ligament injury or osteochondral fracture usually presents with an acute hemarthrosis (occurring within an hour), whereas an effusion associated with arthritis tends to be more insidious in nature.

Reports of "locking" are common. In a younger patient, locking may be due to a displaced meniscal tear. In older patients who have degenerative arthritis, locking is often due to loose bodies. It is important to distinguish between true locking and diminished range of motion due to pain (so-called pseudolocking) because this distinction will determine which imaging studies are most appropriate.

Timing of the pain with activity is also important for making the correct diagnosis. Meniscus tears and ligament injuries leading to instability will be particularly troublesome with activities such as walking on uneven surfaces and stairs, movements requiring knee flexion, and pivoting. Osteoarthritis tends to be exacerbated by all load-bearing activities and relieved by rest.

The clinician also should explore the patient's exercise tolerance and ability to perform activities of daily living. These details may give insight into the severity of the injury and also will guide treatment. Important details include the use of ambulatory assist devices (e.g., a cane, crutches, walker, brace, or wheelchair), walking tolerance, and capability for other exercises (e.g., physical therapy).

A history of any previous treatments rendered also should be recorded. A patient's response to physical therapy, analgesics, nonsteroidal anti-inflammatory drugs, nutritional supplements (such as glucosamine and chondroitin), intra-articular injections of corticosteroids or hyaluronic acid derivatives, and any operative treatments will lend further insight into the accurate diagnosis and have implications for treatment once the diagnosis has been confirmed.

After obtaining a detailed history, the clinician should be able to formulate a differential diagnosis with a short list of potential conditions. This information should then allow the physician to concentrate on specific aspects of a focused physical examination that will lead to confirmation of the diagnosis.



**Figure 48-1** Assessment of coronal alignment.

## Physical Examination

### General

After a brief overall assessment of the patient, the physical examination should begin with observation of the patient's lower extremity coronal alignment and leg lengths. We prefer to have patients stand with their legs slightly apart while they face the examiner (Figure 48-1). A goniometer is then used to measure the varus/valgus alignment of the knees. Evaluation of leg lengths should be performed with step blocks of known sizes. The total height of the blocks needed to make the iliac crests level with the floor is equivalent to the leg length discrepancy (Figure 48-2).

Gait is examined next. Although a comprehensive discussion of gait analysis is beyond the scope of this chapter, all clinicians should routinely make a few basics observations when evaluating patients with a knee problem. Antalgic gaits (i.e., a shortened stance phase) and thrusts are commonly seen. Any disorder that causes lower extremity pain may cause an antalgic gait. Seen in the stance phase of gait, thrusts may be due to a progressive angular deformity as a result of degenerative changes or chronic ligamentous instability. Medial thrusts result from medial collateral ligament and/or posteromedial capsular laxity. Lateral thrusts arise from lateral collateral ligament or posterolateral corner laxity (Figure 48-3). Patients also may thrust into recurvatum (the so-called back-knee deformity) as a result of posterior capsular laxity or quadriceps weakness.

The patient should then transfer to the examination table for evaluation in a comfortable supine position. The examination should proceed with inspection and palpation prior to performing any provocative maneuvers. A pillow should be placed under the knee if full extension is not possible because of pain (e.g., as a result of fractures, displaced meniscus tears, or a large effusion). If the patient has no known pre-existing disease, the contralateral knee can serve as an adequate control. The lower extremity should



**Figure 48-2** The total height of the blocks needed to make the iliac crests level is equal to the length discrepancy.

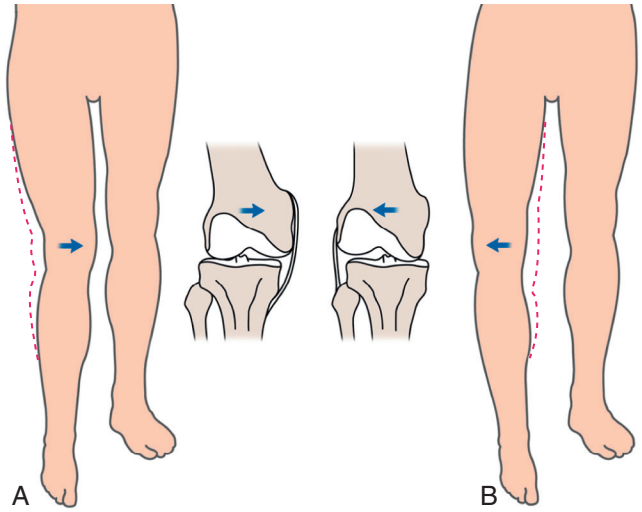



**Figure 48-3** The femur shifts medially during a medial thrust **(A)** and laterally during a lateral thrust **(B)**.

be inspected for any skin lesions, areas of ecchymosis, or surgical scars. Quadriceps atrophy should be noted, and a tape measure should be used to record thigh circumference. It is good practice to measure the thigh circumference at the same distance from the patella or joint line in each knee. The presence of an effusion, which will be seen as fullness or swelling in the suprapatellar pouch, should be noted. The effusion should be confirmed by ballottement of the patella (Figure 48-4). Small effusions will require "milking" of the fluid upward into the suprapatellar pouch, which will allow for quantification of the amount of fluid (Figure 48-5). The active and passive range of motion of both knees should be recorded with a goniometer.

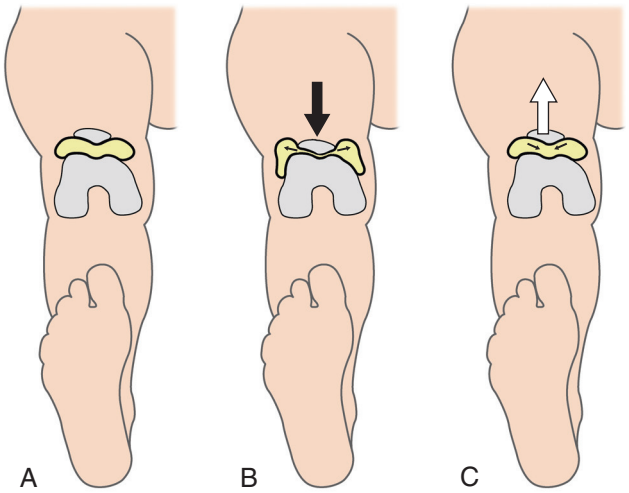



**Figure 48-4** Large effusions can be detected by "balloting" the patella with the knee in extension.



**Figure 48-5** Small effusions can be appreciated by the "milking" of fluid into the suprapatellar pouch.

The examiner should then proceed with palpation of all structures of the knee. It is important to perform this palpation in a systematic manner to ensure completeness. Palpation should be gentle but firm enough to detect subtle disease. Structures to be palpated include the quadriceps tendon, the patella (superior and inferior poles), the pes anserinus bursa, the medial (Figure 48-6A) and lateral (Figure 48-6B) joint lines, the origins and insertions of the collateral ligaments, the tibial tubercle, and the popliteal fossa. Fullness in the posterior knee may be indicative of a Baker's cyst.

## Ligaments

Injuries to the collateral or cruciate ligaments may lead to knee instability. It is important to mention that for each translational and rotational motion of the knee, both primary and secondary restraints exist. When a primary restraint is disrupted, motion will be limited by the secondary restraint. If a secondary restraint is injured and the

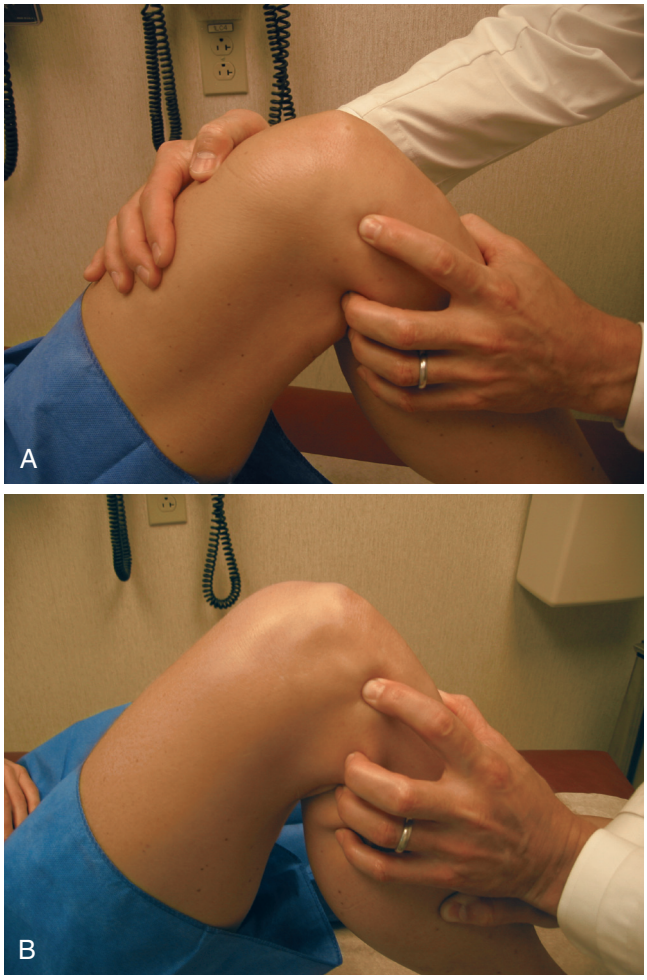



**Figure 48-6** Palpation of the medial **(A)** and lateral **(B)** joint lines.



**Figure 48-7** The anterior drawer test is performed by subluxating the tibia anteriorly with the knee in 90 degrees of flexion. The amount of anterior translation (mm) is noted. The endpoint is characterized as "soft" or "hard."



**Figure 48-8** The Lachman test is performed by applying an anterior force on the tibia while stabilizing the femur with the knee in 30 degrees of flexion.

primary restraint remains intact, then motion will not be abnormal. For example, the ACL is the primary restraint to anterior translation of the tibia, and the medial meniscus is the secondary restraint. ACL disruption will lead to a significant increase in anterior tibial translation. This translation will be increased if the patient underwent a prior medial menisectomy.[16]

The collateral ligaments can be examined with stress applied in the coronal plane. They should be examined in full extension, as well as in 30 degrees of flexion to remove the influence of the cruciate ligaments and the capsular restraints. With the patient in a supine position, a varus force is applied across the knee to test the lateral collateral ligament and a valgus force is applied across the knee to evaluate the medial collateral ligament.

The ACL is one of the most frequently injured structures in the knee. ACL insufficiency is also common in patients with advanced osteoarthritis. Common mechanisms of injury include a direct blow to the lateral side of the knee (the "clipping" injury in football causing the triad of medial collateral ligament, ACL, and medial meniscus injuries[17]), as well as noncontact injuries that occur during cutting, pivoting, and jumping.[18] Patients often report an audible "pop" accompanied by the acute onset of knee swelling. Multiple tests have been described to evaluate the ACL. The most sensitive tests for diagnosis of an ACL injury include the anterior drawer, Lachman,[19] and pivot-shift tests.[20,21] All three tests are performed with the patient in the supine position. The anterior drawer test is performed with the knee flexed to 90 degrees. The examiner places his or her hands on the posterior surface of the proximal tibia and subluxates the tibia anteriorly (Figure 48-7). Any gross movement of the tibia that is different from the contralateral side is considered abnormal. The Lachman test is performed with the knee in 30 degrees of flexion (to remove the contribution of secondary restraints). The examiner applies an anterior force on the tibia while stabilizing the femur with his or her contralateral hand. Any increase in anterior tibial translation relative to the contralateral side is considered abnormal (Figure 48-8). The pivot-shift test is performed with the knee in extension. The examiner holds the tibia in slight internal rotation and applies a

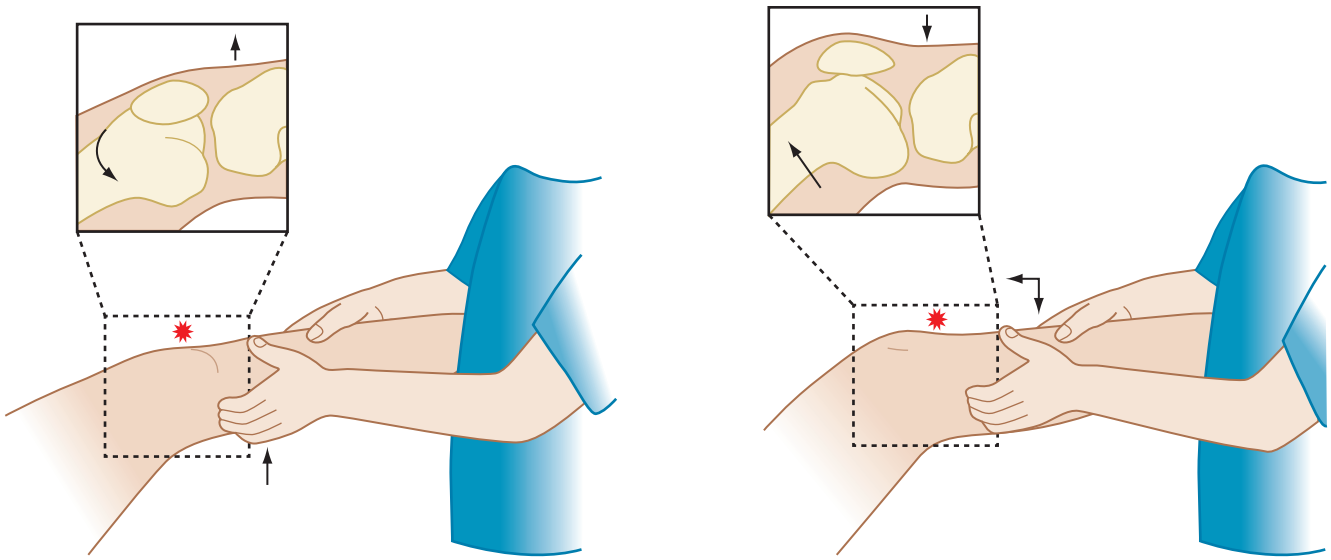



**Figure 48-9** The pivot shift test is positive if the tibia reduces with a "clunk" or a "glide" at 20 to 40 degrees of flexion.

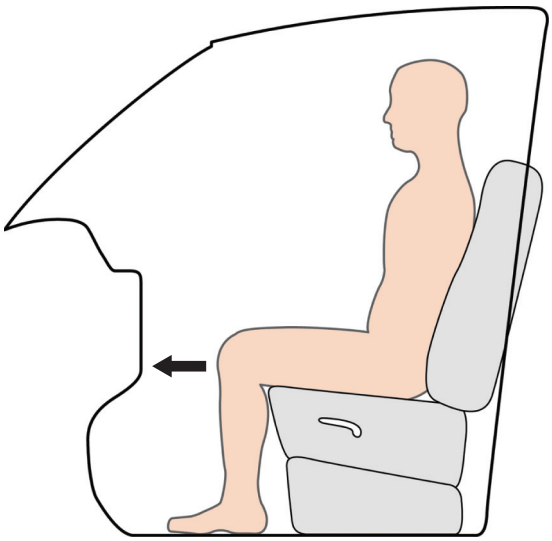


**Figure 48-10** An injury to the posterior cruciate ligament can occur when the tibia strikes the dashboard, causing the tibia to subluxate posteriorly on the femur.



**Figure 48-11** The posterior drawer test is performed by subluxating the tibia posteriorly with the knee in 90 degrees of flexion. The amount of posterior translation (mm) is noted. The endpoint is characterized as "soft" or "hard."

valgus stress while the knee is slowly flexed. This combination of forces should cause the tibia to subluxate anteriorly if the anterior cruciate ligament is injured. The test is positive if the tibia reduces with a "clunk" or a "glide" at 20 to 40 degrees of flexion (Figure 48-9).

The posterior cruciate ligament (PCL) is the strongest ligament in the knee,[22,23] and thus injuries to the PCL are usually a result of significant knee trauma. The "dashboard" injury is a common mechanism for PCL injury and occurs during a motor vehicle accident when the flexed knee strikes the dashboard (Figure 48-10). The PCL can be evaluated with the posterior drawer, posterior sag, and quadriceps active tests. All tests are performed with the patient in the supine position. The posterior drawer test is performed with the knee in 90 degrees of flexion. The examiner applies a posteriorly directed force to the tibia. Placement of one's thumb tips at the anterior joint line permits quantification of any abnormal translation (Figure 48-11). The posterior sag test is positive when the tibia subluxates posteriorly with the knee at 90 degrees of flexion. Loss of the medial tibial step-off at the joint line should alert the examiner to a PCL injury (Figure 48-12).[22] This test is usually positive in the chronic setting or after inducement of anesthesia in the acute setting. The quadriceps active test is performed with the knee in 60 degrees of flexion. The patient is asked to extend the knee while keeping his or her foot on the examination table. Reduction of the tibia will be seen in a positive test.[24]

Injuries to the PCL are often accompanied by injuries to the posterolateral corner, a complex structure that functions as both a static and a dynamic stabilizer of the knee.[23] It is composed of the lateral collateral ligament, the popliteofibular ligament, the popliteomeniscal attachment, the arcuate ligament, and the popliteus tendon and muscle.[25] Injuries to the posterolateral corner and/or the PCL can be examined with the "dial test" (Figure 48-13). The posterolateral corner structures restrain external rotation at 30 degrees of flexion, whereas the PCL restrains external rotation at 90 degrees of flexion. An increase of external rotation at 90 degrees of flexion without an increase in external rotation at 30 degrees of flexion suggests an isolated PCL injury. An increase of external rotation at 30 degrees of flexion without an increase at 90 degrees of flexion suggests an isolated injury to the posterolateral corner. Increased external rotation at both 30 and 90 degrees of flexion suggests combined PCL and posterolateral corner injuries.

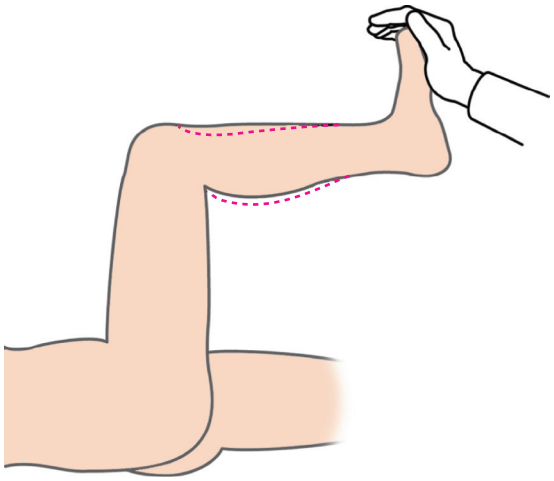


**Figure 48-12** The posterior sag test is positive when the tibia subluxates posteriorly with the knee at 90 degrees of flexion.

## Menisci

Traumatic and degenerative meniscal injuries are among the most common knee injuries. The menisci are considered the "shock-absorbing" cartilages of the knee and also provide rotational and translational restraint. The medial meniscus tends to be more bean-shaped and is both larger and less mobile than the lateral meniscus. The lateral meniscus tends to be more C-shaped. These anatomic differences have implications for the different injuries patterns seen in these two structures.

Meniscal tears usually occur with rotation of the flexed knee as it moves into extension. Tears of the medial meniscus are more common than tears of the lateral meniscus, likely because of the relative lack of mobility of the medial meniscus.[26] Patients frequently report "locking" and "clicking" or of something not being right with the knee, which usually results from displacement of the torn meniscus during motion. Common physical findings include pain with hyperflexion and with hyperextension, joint line tenderness, and an effusion. Many provocative tests have been described to diagnose meniscal tears. The McMurray[27] and Apley compression[28] tests are frequently performed, although they lack sensitivity and specificity. The flexion McMurray test is performed with the patient supine and the hip and knee flexed to 90 degrees. A compressive and rotational force is applied to the knee as it is moved from a flexed to an extended position. The test is positive if the patient reports pain (Figure 48-14). The Apley compression test is performed with the patient prone and the knee flexed to 90 degrees. When the test is positive, the patient will report pain with rotation of the tibia. An arthroscopic photograph in Figure 48-15 shows a tear in the posterior horn of the medial meniscus.

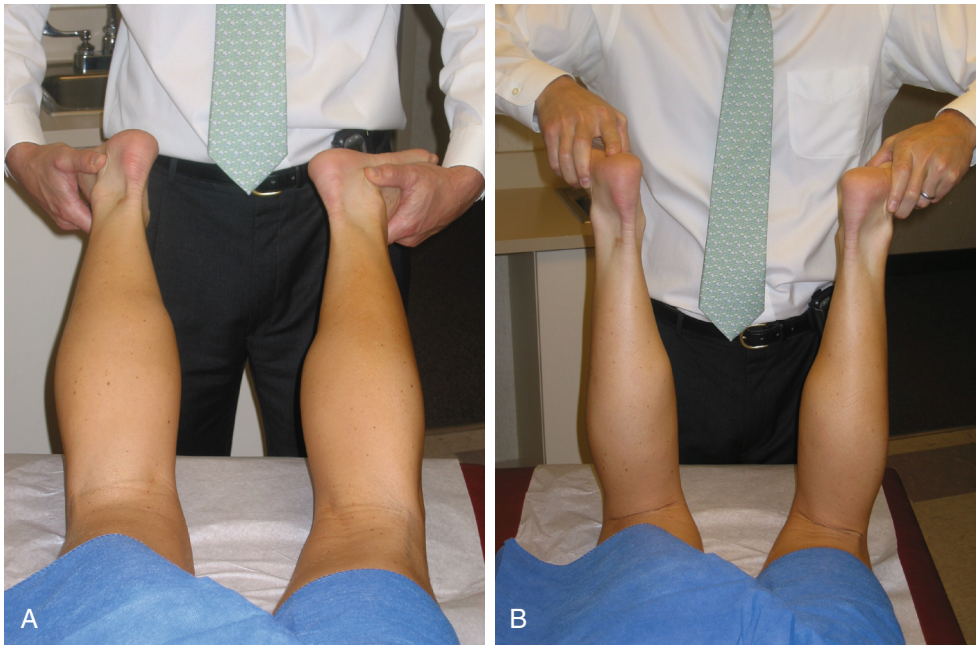



**Figure 48-13** The degree of tibial external rotation is measured in the "dial" test.

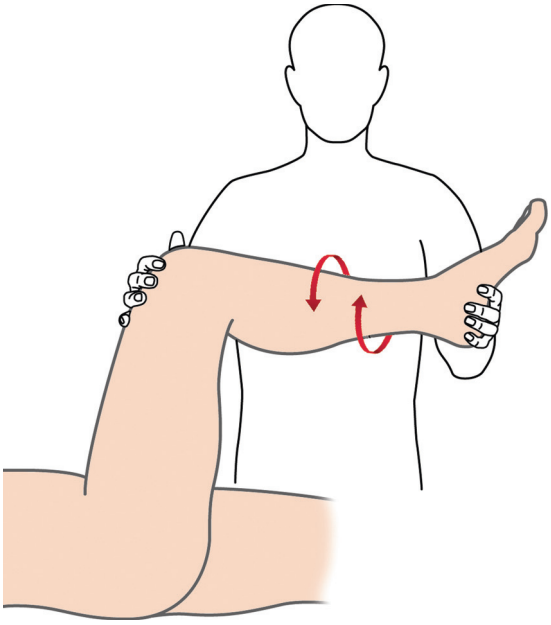


**Figure 48-14** A positive flexion McMurray test may indicate a torn meniscus.

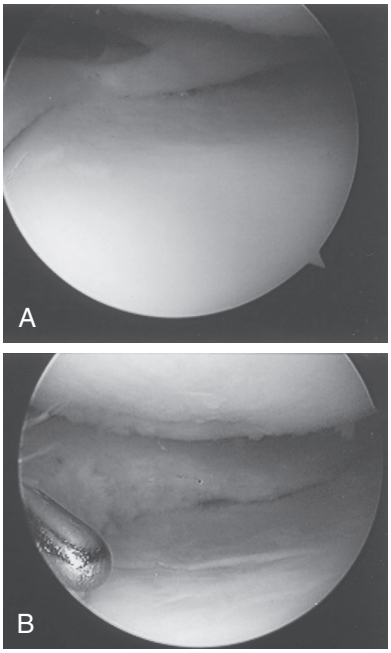



**Figure 48-15** An arthroscopic photograph of a tear in the posterior horn of the medial meniscus before **(A)** and after **(B)** debridement.

## Quadriceps Tendon

Injuries to the quadriceps tendon are most common in the sixth and seventh decades of life. Patients with systemic lupus erythematosus, renal failure, endocrinopathies, diabetes, and various other systemic inflammatory and metabolic diseases tend to be at a higher risk for these injuries. The occurrence of quadriceps tendon rupture after total knee arthroplasty is a rare complication (with an incidence of 0.1%), but when it occurs it is devastating.[29] Patients usually present with intense anterior knee pain after experiencing an eccentric quadriceps contraction during a fall or twisting

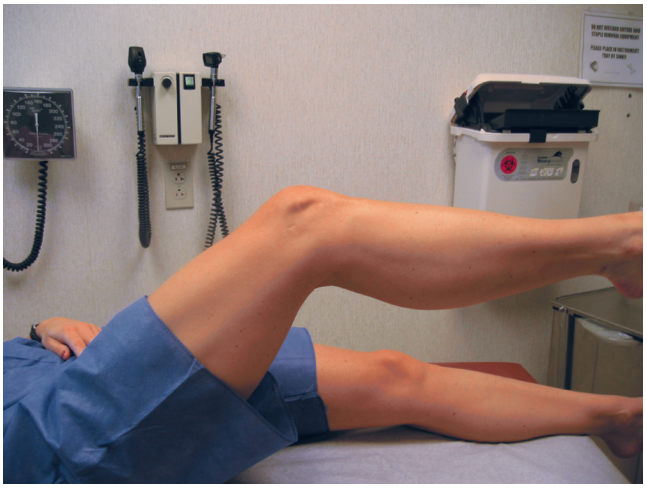


**Figure 48-16** An extensor lag due to a complete tear in the quadriceps tendon.

injury. Physical examination reveals a palpable defect in the tendon, an effusion resulting from hemarthrosis, and hypermobility of the patella. Patients usually will not be able to fully extend their knee (Figure 48-16).

## Patella Tendon

Problems with the infrapatellar tendon include tendonitis and rupture. Tendonitis is usually an overuse injury and is often associated with jumping, changes in activity level, and eccentric contractions during falls. Patients exhibit tenderness at their tibial tubercle or at the inferior pole of their patella. Rupture of the patella tendon usually occurs in patients younger than 40 years and is associated with chronic patella tendonitis. Patients usually present with anterior knee pain and the inability to extend their knee.

## Patellofemoral Pain

Persons who report having anterior knee pain are commonly seen by orthopedic surgeons. Anterior knee pain is more common in women and accounts for up to 25% of all sports-related knee injuries.[30] A variety of factors contribute to the biomechanics of the patellofemoral joint, including overuse, the depth of the trochlea, the shape of the patella, quadriceps strength, the line of pull of the quadriceps relative to the patella tendon (the Q angle), the length of the patella tendon, the shape of the femoral condyles, and the articular cartilage. Abnormalities of any of these factors may contribute to this pain syndrome, and successful treatment is possible only with correct identification of any contributing factors.

Physical examination of the patellofemoral joint begins with an analysis of coronal alignment of the knee because any valgus deformity may contribute to lateral subluxation. The height of the patella relative to the tibial tubercle should be noted (patella alta or baja). The J sign is present when the patella slides laterally at terminal extension, indicating excessive pull of the vastus lateralis. The vastus medialis obliquus is the primary stabilizer against lateral pull by the vastus lateralis. With the knee extended and the quadriceps relaxed, the examiner should make note of any

patellar tilt. Any crepitus, either audible or palpable, should be noted as well. Crepitus is common in persons with osteoarthritis. A Q angle greater than 15 degrees in women and greater than 8 to 10 degrees in men is considered abnormal.[30] Patellar mobility should be assessed using a quadrant system for passive mediolateral displacement of the patella relative to the trochlear groove. The normal patella should not be displaced medially or laterally beyond the second quadrant. Any abnormality in mobility may stem from changes in the tightness of the retinaculum. The apprehension test is performed by attempting to subluxate the patella with the knee in extension. The test is positive when it elicits pain and an unwillingness to allow the examiner to move the patella laterally (Figure 48-17).



**Figure 48-17** The apprehension test is positive when subluxation of the patella causes pain.

At the conclusion of the history and physical examination, the astute clinician should have formulated a short list of possible diagnoses. With this list in mind, the appropriate imaging studies can now be obtained. The goal of the initial imaging studies should be to confirm the diagnosis with the most appropriate and least-expensive study. Advanced imaging studies should not replace a thorough history and physical examination.

## Imaging

### Conventional Radiographs

Conventional radiographs are usually the first study obtained after knee injury and should be read in a systematic fashion. Soft tissues should be evaluated before examining the bony structures. Findings should be described in terms of radiolucent and radiopaque lines. Only after the findings have been described should the interpretation phase begin. Bypassing the description and proceeding directly to interpretation is a natural tendency, but if this tendency is followed, it is likely that certain findings will be missed or dismissed prematurely.

The basic radiographic evaluation of the knee consists of standing anteroposterior (AP) weight-bearing, lateral, and Merchant's views. The AP view permits evaluation of coronal alignment and the height of the tibiofemoral joint spaces. The normal coronal alignment of the knee should be 5 to 7 degrees of anatomic (tibiofemoral) valgus. The lateral tibiofemoral joint space should be wider than the medial tibiofemoral joint space in a normal knee. The presence of marginal osteophytes, joint space narrowing, subchondral sclerosis, and cystic change will be seen in the presence of osteoarthritis (Figure 48-18). Periarticular

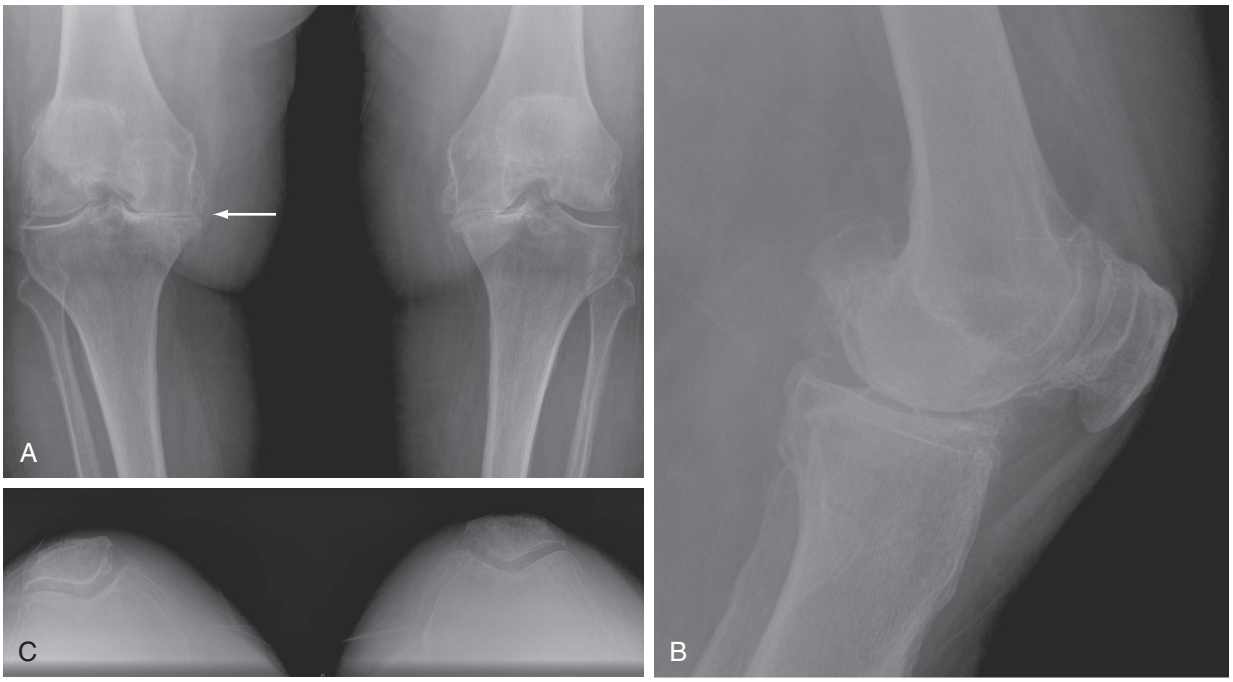



**Figure 48-18** Standing anteroposterior **(A)**, lateral **(B)**, and Merchant's **(C)** views of an osteoarthritic knee.

osteopenia, concentric joint space narrowing, and a paucity of osteophytes are commonly seen in persons with inflammatory arthritis (Figure 48-19). The lateral radiograph allows for evaluation of an effusion, patella tendon length, and the quadriceps tendon. The Merchant's view, which is taken tangential to the patellofemoral joint,[31] permits detection of patellofemoral arthritis and malalignment.

Additional views include a posteroanterior (PA) standing view with the knees flexed approximately 45 degrees, the tunnel or intercondylar notch view, and the 36-inch AP standing view of bilateral lower extremities. The flexed PA standing view is taken with the radiographic beam directed 10 degrees caudad from anterior to posterior, which permits evaluation of the posterior femoral condyles for joint space narrowing.[32] The tunnel view is obtained with the knee flexed and the radiographic beam directed inferiorly at an angle perpendicular to the tibial plateau. It is useful in detecting posterior tibiofemoral joint space narrowing, tibial spine fractures, loose bodies, and osteochondral lesions on the medial aspect of the femoral condyles. The 36-inch standing view is used for determining the mechanical axis of the lower extremity, as well for evaluating any deformity that may be present. The normal mechanical axis is a straight line joining the center of the hip, knee, and ankle joints. Surgeons use it for pre-operative planning and post-operative evaluation in total knee arthroplasty, as well for the planning of distal femoral and proximal tibia osteotomies in arthritis surgery.

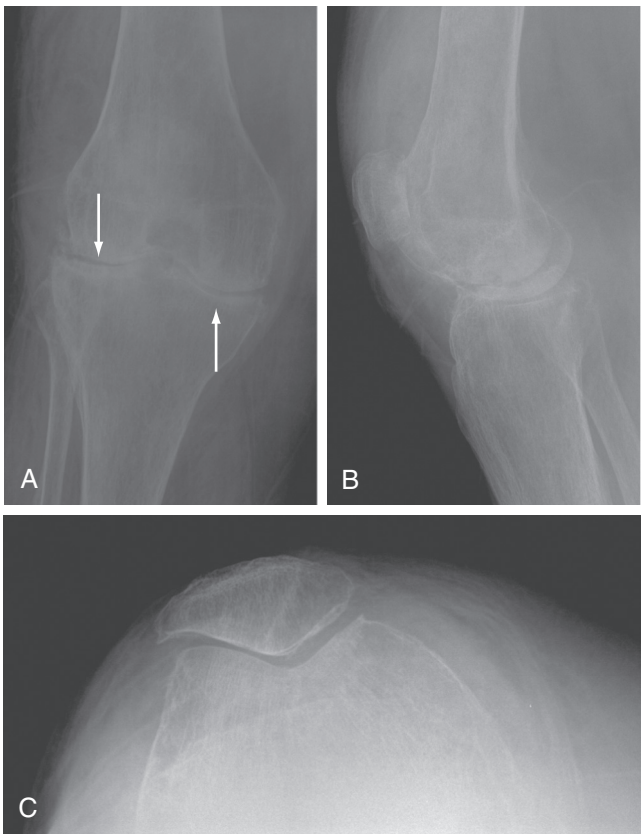



**Figure 48-19** Standing anteroposterior **(A)**, lateral **(B)**, and Merchant's **(C)** views of the knee in a patient with rheumatoid arthritis.

## Computed Tomography

CT has largely been replaced by MRI in evaluation of routine knee problems. CT is now used primarily for detection of bony tumors and in the trauma setting for detection of subtle fractures that are not easily visualized with conventional radiographs, as well as for a more thorough evaluation of intra-articular fractures. In cases of distal femoral or proximal tibia fractures, CT is used to help the surgeon plan operative treatment. CT is also used to assess axial alignment of the femoral and tibial components in cases of the painful total knee arthroplasty.[33,34]

## Ultrasound

The use of ultrasound has become more common in the diagnosis and treatment of knee disorders. Some practitioners use ultrasound routinely in both hospital and office settings to assist in arthrocentesis of the knee. Ultrasound is an attractive imaging modality because of its low cost, real-time capabilities, and portability. The ability to perform provocative maneuvers during sonography is particularly appealing. Ultrasound can easily and reliably detect joint effusions and popliteal cysts (Figure 48-20), as well as quadriceps and patella tendon disruptions. It has been reported that ultrasound can detect a 1-mm increase in joint fluid.[35]

## Nuclear Scintigraphy

Nuclear scintigraphy is very sensitive but not specific and is used to detect areas of increased osseous remodeling. It requires clinical correlation and should be used in conjunction with other imaging modalities. Technetium phosphate compounds are injected intravenously. Approximately 50% of the tracer is excreted by the kidneys, and the remainder is taken up in areas of increased osseous turnover. Imaging of the skeleton is typically performed 2 to 3 hours after injection because this delay allows for maximum contrast between the soft tissues and the skeletal structures while still providing for an adequate photon count.[36]

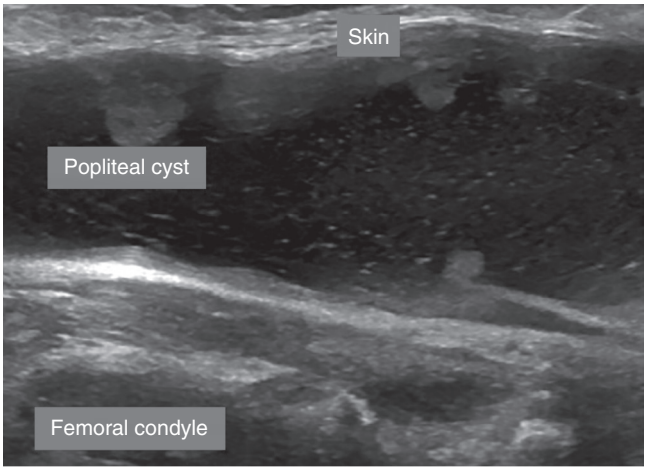



**Figure 48-20** This ultrasound image shows a large popliteal cyst in a patient with symptomatic knee osteoarthritis that was treated with aspiration and injection of corticosteroid.

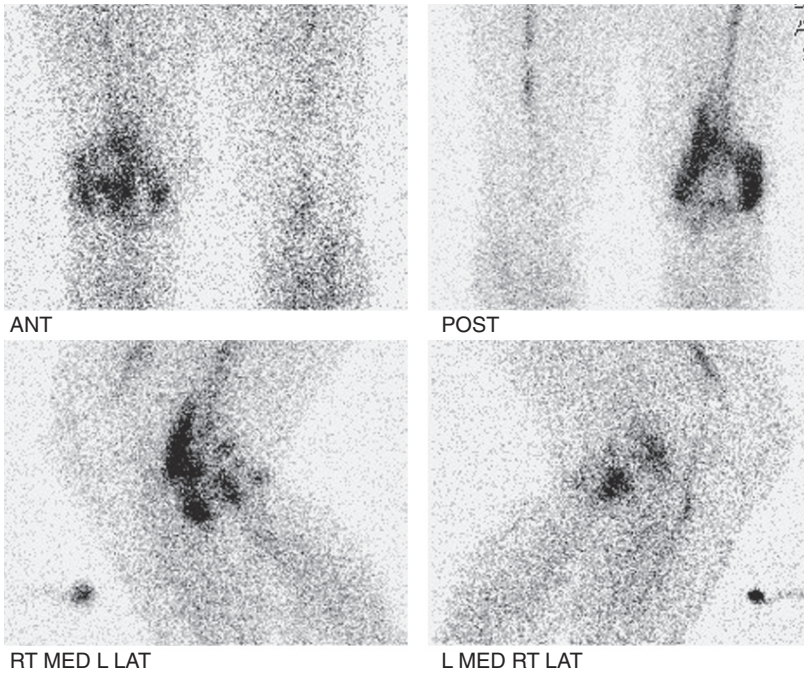



**Figure 48-21** A bone scan reveals increased uptake of radiotracer around the distal femur in this patient with an infected total knee arthroplasty and septic loosening of his femoral component. ANT, Anterior; L MED RT LAT, left medial right lateral; POST, posterior; RT MED L LAT, right medial left lateral.

Three-phase bone scanning can yield additional information. The three phases include an angiographic pool, followed by blood pool and bone imaging. The angiographic phases allows for detection of regional hyperemia. This technique has been reported to have greater specificity and can be used in cases of suspected osteomyelitis, osteonecrosis, stress fracture, and implant loosening.[36] It has been reported that increased radionuclide uptake can be seen for up to 12 to 18 months after total knee arthroplasty. Asymmetric uptake in one area around the prosthesis should raise the question of loosening or periprosthetic fracture (Figure 48-21).[37,38] The addition of labeled leukocytes to the technetium-99m sulfur colloid yields an 80% sensitivity and 100% specificity for diagnosing infection.[39]

### Magnetic Resonance Imaging

MRI has supplanted many imaging modalities because of its direct multiplanar capabilities and superior soft tissue contrast. Although conventional radiographs remain the gold standard for defining osseous structures, MRI provides excellent visualization of articular cartilage, the cruciate ligaments, the collateral ligaments, the patella tendon, the quadriceps tendon, and the menisci (Figure 48-22). It is also highly sensitive for detecting bone marrow edema (contusion), stress fractures, and mass lesions. Use of the "two-slice touch" rule has improved the sensitivity and specificity of MRI in accurately diagnosing meniscal tears. This rule classifies a meniscus as torn if two or more MR images show abnormal findings and as possibly torn if only one MR image shows an abnormal finding. Using fast spin-echo imaging, the sensitivity and specificity for diagnosing medial and lateral meniscal tears was 95% and 85%, and 77% and 89%, respectively, translating to a positive predictive value of 91% to 94% for medial meniscus tears and 83% to 96% for lateral meniscus tears.[40]

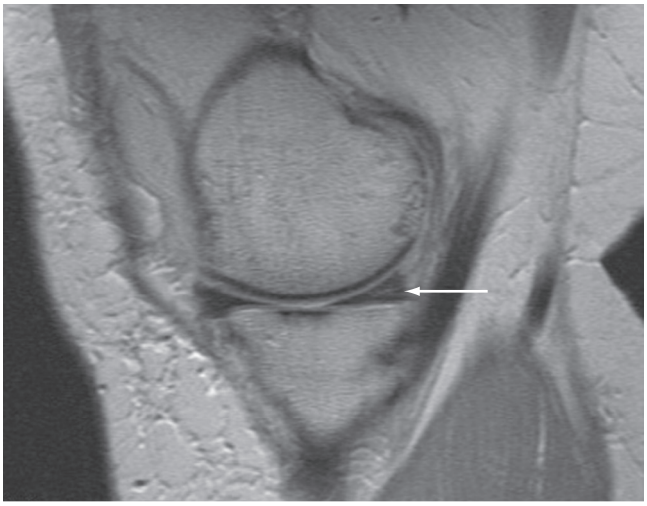


**Figure 48-22** This sagittal MRI shows linear signal change extending to the meniscal surface consistent with a tear *(arrow)* in the posterior horn of the medial meniscus.

## Common Disorders in the Differential Diagnosis of Knee Pain

### General

Though many diseases exist that may involve the knee, a limited number of them are encountered frequently. In

evaluating knee pain, the clinician should be familiar with osteoarthritis, rheumatoid arthritis, inflammatory arthritis associated with the seronegative spondyloarthropathies, tears of the menisci, ligaments, and tendons, osteochondritis desiccans, osteochondral fractures, fractures, referred pain from the hip (such as with slipped capital femoral epiphysis in adolescents), vascular claudication, neurogenic claudication, complex regional pain syndrome, sarcoma, metastases, and infection.

### Bursitis

The prepatellar bursa lies between the retinaculum and the subcutaneous fat and runs from the patella to the tibial tubercle. The bursa may become inflamed and fill with fluid when exposed to a direct blow or repetitive microtrauma (e.g., kneeling). Patients with prepatellar bursitis present with anterior knee pain upon flexion and a fluctuant mass over the anterior knee. If the area becomes warm, tender to palpation, and erythematous, septic bursitis should be ruled out with aspiration. The pes anserinus bursa, located over the insertions of the sartorius, gracilis, and semitendinosus muscles on the proximal medial tibia, also can be a source of knee pain if it is inflamed.

### Neoplasia

Tumors in the area of the knee are often diagnosed after trauma prompts medical evaluation. Pain at night, pain at rest, and constitutional symptoms should alert the clinician to consider the appropriate workup. Some of the benign tumors seen in the area of the knee include enchondroma, pigmented villonodular synovitis, osteochondromatosis, and giant cell tumor. Malignant tumors seen in the area of the knee include but are not limited to metastases, osteosarcoma, Ewing's sarcoma, chondrosarcoma, and malignant fibrous histiocytoma.

### Popliteal Cysts

A popliteal cyst, originally called Baker's cyst, is a synovial fluid-filled mass located in the popliteal fossa. The most common synovial popliteal cyst is considered to be a distension of the bursa located beneath the medial head of the gastrocnemius muscle. Usually, in an adult patient, an underlying intra-articular disorder (osteoarthritis) is present. In children, the cyst can be isolated and the knee joint can be normal. Patients usually present with episodic posterior knee pain.[41] The diagnosis is made by ultrasonography or MRI. Treatment options include benign neglect, aspiration, surgical excision, or removal of the underlying disease process (arthritis) with knee arthroplasty.

## HIP PAIN

### History

Obtaining an accurate history is an important initial step in formulating a differential diagnosis for patients who present with hip pain. In general, more conditions should be considered in the differential diagnosis for hip pain than for knee pain, because the hip is a common site for referred pain from lumbosacral and intrapelvic disease. A detailed, comprehensive history will direct the clinician to a focused physical examination.

Most patients who present with hip disease report having pain. It is important to define the exact location of the pain because "hip" pain may refer to discomfort in the groin, lateral thigh, or buttock. Pain in the groin or medial thigh region is usually due to hip disease and is believed to arise from irritation of the capsule and/or synovial lining.[42] Pain generated in the lumbosacral spine may be referred to the buttocks and/or lateral thigh.[43] Lateral thigh pain may stem from so-called "trochanteric bursitis" (usually abductor tendonitis) as well. Activities or positions that aggravate and relieve the pain should be explored. The severity, frequency, and patterns of radiation of the pain also should be evaluated. It is not uncommon for knee pain to be generated from the hip joint. Metastatic and primary tumors that occur in the pelvic and proximal thigh regions should always be included in the differential diagnosis. Intrapelvic disease from the prostate, seminal vesicles, hernias, ovaries, gastrointestinal system, and vasculature also should be considered.[44,45]

Knowledge of the patient's general level of functioning is important, because this information will lend insight into the severity of disease and may influence treatment. Patients with hip disease may have difficulty trimming their toenails, donning shoes and socks, and using stairs. Walking tolerance and use of assist devices also should be recorded. The Harris Hip Score and Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index are two rating scales that are widely used to assess function in this patient population.[46,47]

The patient should be asked about any hip problems that he or she encountered in childhood. Diseases such as developmental dysplasia, slipped capital femoral epiphysis, Legg-Calvé-Perthes disease, polio, and trauma may lead to osteoarthrosis later in life.[48-50] The patient should be asked about any treatment rendered for these diseases as well.

Osteoarthritis and inflammatory arthritis are two common causes of hip pain. In general, pain from osteoarthritis is exacerbated by activity and relieved by rest. Mild arthritis of the hip may not become symptomatic until a certain activity level is reached. Stiffness (usually from synovitis) is also commonly reported with both degenerative and inflammatory arthritis. When the hip pain continues despite a trial of rest, an underlying inflammatory or infectious process should be considered.

Any previous treatments for hip pain should be discussed. The patient's response to nonsteroidal anti-inflammatory medications, nutritional supplements (such as chondroitin and glucosamine), physical therapy, corticosteroid injections, local anesthetic injections, hyaluronic acid injections, ultrasound, and operative interventions should be recorded. Lastly, a more general medical history should be explored. The physician should be aware of alcoholism, neuromuscular disorders, smoking history, and general support systems.

### Physical Examination

The physical examination of the patient with hip pain begins as the clinician observes the patient for the first time.

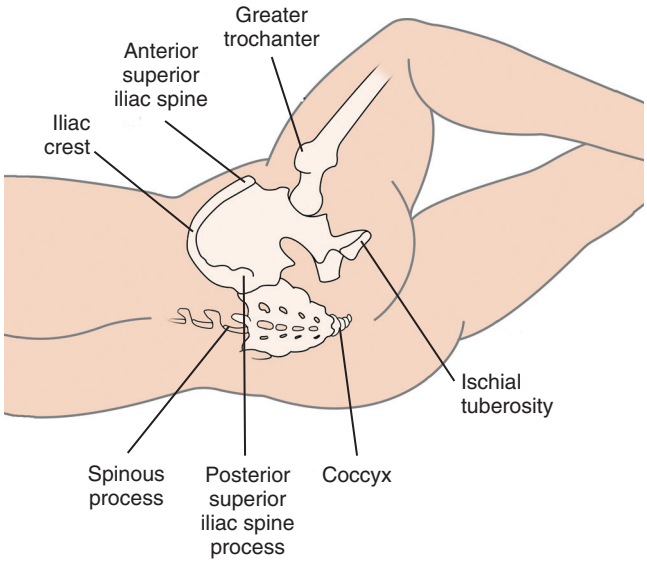



**Figure 48-23** The bony landmarks on the pelvis that can be palpated during physical examination.

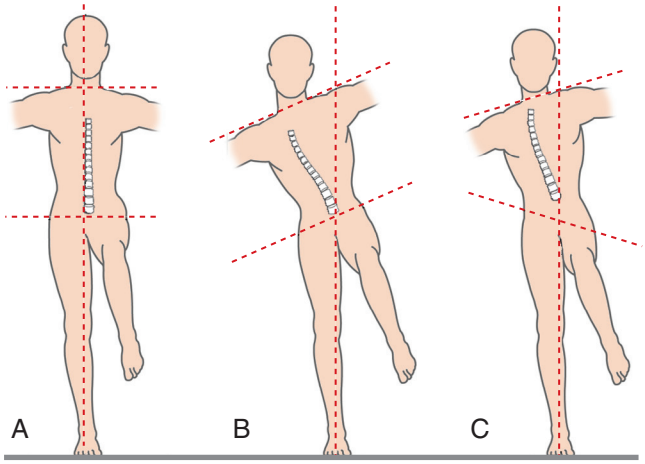



**Figure 48-24** Physical examination of abductor function: **A,** Normal single-legged stance. **B,** Positive Trendelenburg lurch and negative Trendelenburg's sign. **C,** Positive Trendelenburg lurch with pelvic obliquity and leaning over the involved hip to shift the body's center of gravity.

Ease of rising from a chair, postures, and walking speed all provide insight into the extent of a patient's disability. A general evaluation of the patient's spine, lower extremity alignment, and leg lengths is performed next. With the examiner behind the patient, the spine is examined for coronal and sagittal balance. The patient is asked to touch his or her toes. A rib hump indicates the presence of scoliosis. Any gross deformity of the spine will alert the examiner to the potential of a pelvic obliquity and resultant leg length discrepancy.

The overall coronal alignment of the lower extremities is evaluated next. If a leg length discrepancy is detected, blocks can be used, as discussed previously, to determine the amount of apparent inequality. If the leg length discrepancy is due to a fixed pelvic obliquity from lumbosacral disease, blocks may not be able to level the pelvis. Previous surgical scars in the area of the hip are noted. Palpation of the bony landmarks (iliac crest, anterior superior iliac spine, posterior superior iliac spine, ischial tuberosity, coccyx, spinous processes, and greater trochanter) should be performed (Figure 48-23). The femoral neck is located approximately three fingerbreadths below the anterior superior iliac spine.

A basic evaluation of gait should be performed. Although gait analysis is a complex science, all clinicians should feel comfortable evaluating patients for common abnormalities. The patient with hip pain may present with an antalgic gait. The severity of the limp should be classified as mild, moderate, or severe. Mild limps can only be detected by trained observers. Moderate limps will be noticed by the patient. A severe limp will be readily apparent and have a significant impact on the speed of ambulation. Common causes of a limp include pain and abductor (gluteus medius and gluteus minimus) weakness; differentiating between these two causes of limp is an important part of the physical examination.

The patient with abductor dysfunction will likely have an abductor or Trendelenburg lurch.[51] With a Trendelenburg lurch, the patient compensates for abductor dysfunction by leaning over the involved hip to shift the body's center of gravity in that direction (Figure 48-24). If the patient has a Trendelenburg lurch, we proceed to evaluate for Trendelenburg's sign. A positive Trendelenburg's sign occurs when the pelvis tilts toward the unsupported side during one-legged stance. This test is best performed with the examiner behind the patient. Causes of abductor weakness are numerous and may include a contracted or shortened gluteus medius, coxa vara, fracture, dysplasia, neurologic conditions (e.g., superior gluteal nerve injury, radiculopathy, poliomyelitis, myelomeningocele, and spinal cord lesions), and slipped capital femoral epiphysis.

The patient is then asked to lay supine on the examination table. The range of motion of both hips should be evaluated by recording flexion, extension, adduction, abduction, internal rotation in extension, and external rotation in extension. Hip extension is best evaluated with the patient in the prone position. Normal range of motion values include 100 to 135 degrees for flexion (the knee should be flexed to relax the hamstrings), 15 to 30 degrees for extension, 0 to 30 degrees for adduction, 0 to 40 degrees for abduction, 0 to 40 degrees for internal rotation, and 0 to 60 degrees for external rotation. Motion is often limited in cases of deformity (such as limited internal rotation in slipped capital femoral epiphysis) and advanced osteoarthritis. Internal rotation and abduction are usually the first motions to be limited in osteoarthritis. Motion will be painful in patients with synovitis as well. Areas that are painful should be palpated.

A series of special tests can be performed to evaluate for subtle muscle contractures and limitation of motion. The presence of a hip flexion contracture is common in patients with moderate to severe hip disease and can be quantified with the Thomas test (Figure 48-25).[52] This test is performed by having the patient bring his or her thighs to the chest while in the supine position. This maneuver allows for flattening of the spine, and the hip to be evaluated is allowed to extend to neutral. If the patient is unable to reach neutral, the amount of flexion contracture is recorded. The Ober test measures tightness of the iliotibial band. The patient lies on the unaffected side and the examiner helps

the patient abduct the hip with the hip extended and the knee flexed to 90 degrees. The leg is slowly released from abduction to neutral, and the hip will remain abducted if contracture of the iliotibial band occurs. Ely's test will detect a tight rectus femoris. The knee is passively flexed with the patient in the prone position. If the rectus femoris is tight, the ipsilateral hip will spontaneously flex. If the rectus femoris is normal, the hip will remain flush with the examination table.

Patients occasionally report a "snapping" sensation in their hip. Although it may be difficult for the clinician to reproduce snapping, patients may be able to demonstrate it by flexing and internally rotating their hip. Extra-articular causes of hip snapping include a thickened iliotibial band snapping over the greater trochanter, the iliopsoas tendon gliding over the iliopectineal eminence, the long head of the biceps tendon rubbing on the ischial tuberosity, and the iliofemoral ligament rubbing on the femoral head. Intra-articular causes of snapping hip syndrome include loose bodies and large labral tears.



**Figure 48-25** In the Thomas test, a hip flexion contracture is measured by flexing the contralateral hip to eliminate compensatory lumbar lordosis. The ipsilateral hip is then allowed to extend with gravity. The angle between the examination table and the thigh is the degree of flexion contracture.

In addition to using blocks with the patient standing, leg lengths can be measured while the patient is in the supine position (Figure 48-26). The *apparent* leg length is the distance from the umbilicus to the medial malleolus. The *true* leg length is measured from the anterior superior iliac spine to the medial malleolus. Pelvic obliquity and abduction/adduction of the hip will create an apparent leg-length discrepancy.

Sacroiliac disease should be included in the differential diagnosis of hip pain. Although multiple provocative tests have been described to elicit sacroiliac disease, the FABER test (also known as Patrick's test) can help distinguish between hip and sacroiliac joint disease. With the patient supine, the clinician has the patient place his/her hip in the flexion, abduction, and externally rotated position. The clinician then presses the flexed knee and the contralateral anterior superior iliac spine toward the floor. Pain in the buttocks suggests sacroiliac joint disease, whereas pain in the groin points to hip disease. If the sacroiliac joint is implicated, it is recommended that multiple other provocative tests be performed. It has been shown that by using a combination of the distraction, thigh thrust, compression, sacral thrust, Gaenslen's, and FABER tests, sacroiliac joint disease is the likely pain generator when three or more of the tests are positive.[53,54]

The acetabular labrum is drawing attention as a previously underappreciated cause of hip pain. Clinical presentation of a labral tear of the acetabulum may be variable, and the diagnosis is often delayed. Patients usually see multiple providers before the diagnosis is confirmed. In a series of 66 patients with arthroscopically confirmed tears of the acetabular labrum, 92% reported groin pain, 91% had activity-related pain, 71% reported pain at night, 86% described the pain as moderate to severe, and 95% had a positive impingement sign. The authors recommended that a diagnosis of acetabular labral tear be suspected in young, active patients reporting groin pain with or without trauma.[55] The positive

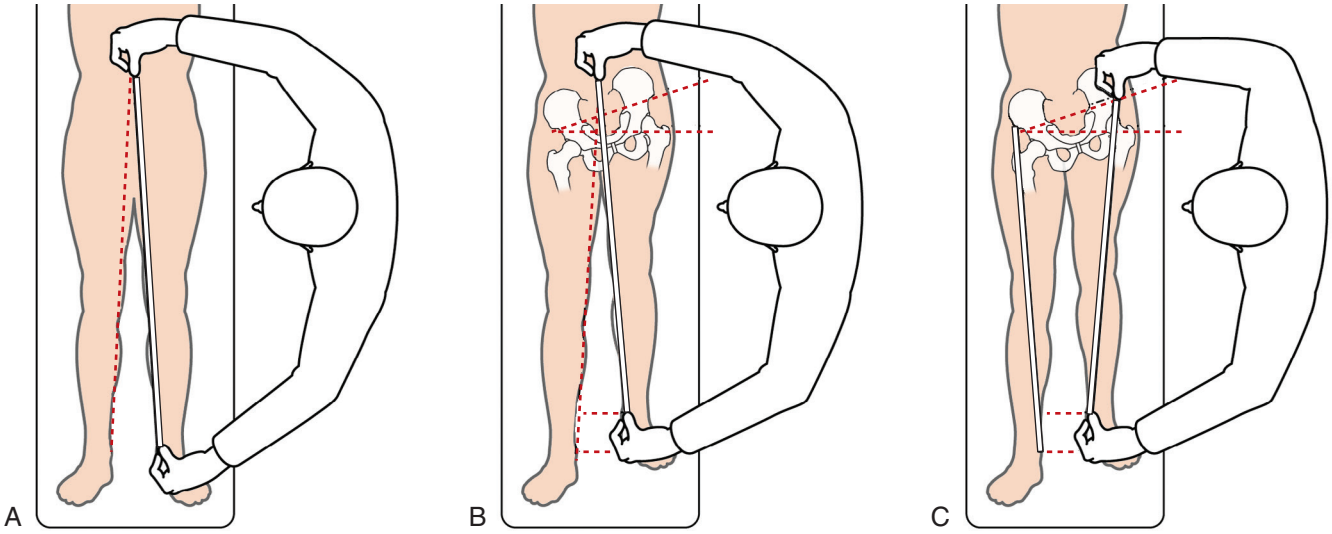



**Figure 48-26** Measurement of leg lengths. **A,** The apparent leg length is the distance from the umbilicus to the medial malleolus. **B,** Pelvic obliquity causing an apparent leg-length discrepancy. **C,** The true leg length is the distance from the anterior superior iliac spine to the medial malleolus.

impingement test helps confirm the diagnosis of a labral tear. The test is positive if the patient experiences groin pain with the hip flexed, adducted, and internally rotated. The positive predictive value of this test has been shown to range from 0.91 to 1.00 in six different studies.[56-61]

A thorough evaluation of the neurovascular system should be completed after the musculoskeletal portion of the physical examination for the hip or knee is completed. This evaluation should include palpation or Doppler evaluation of the femoral, popliteal, dorsalis pedis, and posterior tibial arteries, as indicated. Strength testing with resisted isometric movements for each muscle in the lower extremity is performed, with 5 being normal strength, 4 being full motion against gravity and against some resistance, 3 being fair motion against gravity, 2 being movement only with gravity eliminated, 1 being evidence of muscle contraction but no joint motion, and 0 being no evidence of contractility. Sensation in the lower extremity should be evaluated by assessing for light touch and/or appreciation of pin prick in a dermatomal distribution. Patellar and ankle reflexes should be tested. Lastly, the examiner should test for any abnormal clonus and Babinski reflexes as indicated.

## Imaging

### Conventional Radiographs

Plain radiographs remain the primary diagnostic imaging tool for the evaluation of hip disease. All other imaging modalities should be viewed as complementary to conventional radiographs. Our standard screening series include a low AP pelvis (Figure 48-27), an AP hip (Figure 48-28), a frog-lateral view, and a cross-table lateral view. The frog-lateral view provides a lateral of the proximal femur and is useful for detecting femoral head collapse (as seen in osteonecrosis; Figure 48-29). Numerous other special radiographs of the hip may be obtained, some of which include Judet 45-degree oblique views and the false profile view. Judet views allow easier visualization of the anterior (obturator oblique) and posterior (iliac oblique) columns. The false profile view allows evaluation of the anterior bony coverage of the femoral head in cases of acetabular dysplasia. Developmental dysplasia of the hip is common, and we do not recommend the routine use of any special views prior to referral to an orthopedic surgeon (Figure 48-30).

### Computed Tomography

CT is used for assessment of acetabular fractures, acetabular nonunion, femoral head fractures, subtle femoral neck fractures, neoplasia, and bone stock in the revision total hip arthroplasty setting. Because of its limited soft-tissue




**Figure 48-28** An anteroposterior hip demonstrating the characteristic concentric joint space narrowing, paucity of osteophytes, and periarticular osteopenia seen in rheumatoid arthritis.

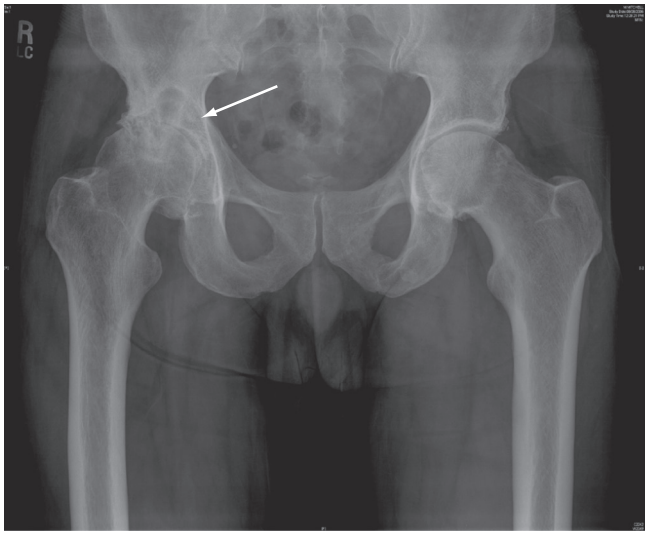



**Figure 48-27** An anteroposterior pelvis demonstrates the characteristic joint space narrowing, cystic changes, and osteophytes seen in osteoarthritis.



**Figure 48-29** A frog-lateral radiograph demonstrating femoral head collapse from osteonecrosis.

contrast, CT has largely been replaced by MRI for detailed evaluation of the soft tissues around the hip.

### Nuclear Scintigraphy

The role of bone scanning in the evaluation of hip disease is similar to its role in the assessment of knee pain. It should always be used in conjunction with other imaging modalities because of its limited specificity (Figure 48-31).



**Figure 48-30** An anteroposterior hip radiograph demonstrates osteoarthrosis from developmental dysplasia. The up-sloping lateral edge of the acetabulum is characteristic for developmental dysplasia of the hip.

### Magnetic Resonance Imaging

MRI provides unprecedented detail of the soft tissues around the hip joint. MRI is now commonly used for diagnosis of osteonecrosis, labral disease, neoplasia, effusion, synovitis, loose bodies, tendonitis, transient osteoporosis of the hip, occult femoral neck fractures, bone edema, gluteus medius tendon avulsions, and nerve injury. MR arthrography of the hip joint is useful for identifying gluteus medius tendon avulsion after total hip arthroplasty (Figure 48-32) and for detecting labral tears. One study showed a 92% sensitivity for the detection of labral tears using MR arthrography.[62] Delayed gadolinium-enhanced MRI of cartilage, a technique designed to measure early arthritis in the hip joint, is now being used clinically in the management of hip dysplasia.[63] Despite the tremendous diagnostic capabilities of MRI, its ability to detect bony disease is limited. As such, conventional radiographs remain the imaging modality of choice for the screening of hip disease.

### Ultrasound

The use of ultrasound for the diagnosis and treatment of hip disorders is common. Some practitioners use ultrasound routinely in the office setting. Common hip conditions that are often managed with ultrasound-guided injections and/or aspirations include osteoarthritis, septic arthritis (Figure 48-33), iliopsoas tendonitis, adverse local tissue reaction associated with metal-on-metal articulating total hip arthroplasties, and trochanteric bursitis.

### Hip Arthrography

Hip arthrography is useful for detecting avulsions of the gluteus medius tendon from the greater trochanter and for

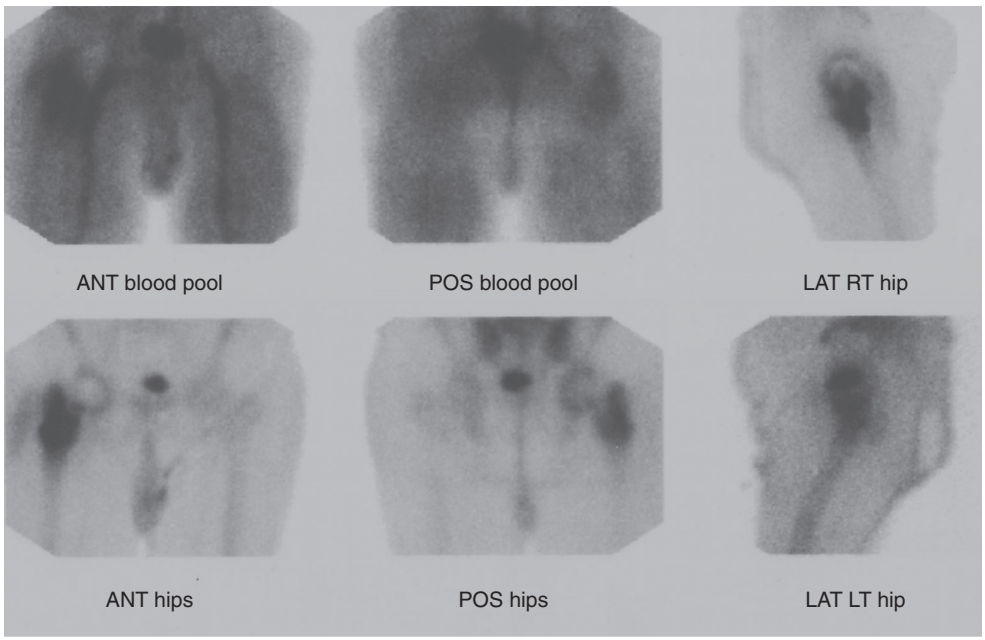



**Figure 48-31** This bone scan shows increased radiotracer uptake at the proximal femur. The patient presented with activity-related thigh pain 1-year after undergoing primary cementless total hip arthroplasty. History, physical examination, and conventional radiographs suggested failure of osseointegration. At the time of surgery, the femoral component was found to be grossly loose. ANT, Anterior; LAT, lateral; LT, left; POS, posterior; RT, right.

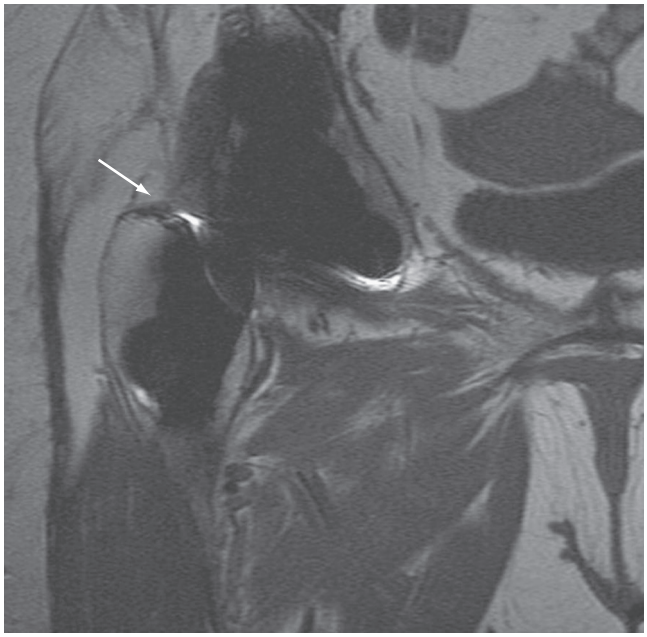


**Figure 48-32** This short tau inversion recovery coronal MRI shows a complete avulsion of the gluteus medius tendon from its insertion on the greater trochanter. Note the signal change along the lateral aspect of the greater trochanter, consistent with accumulation of intra-articular gadolinium at the site where the gluteus medius tendon should be.

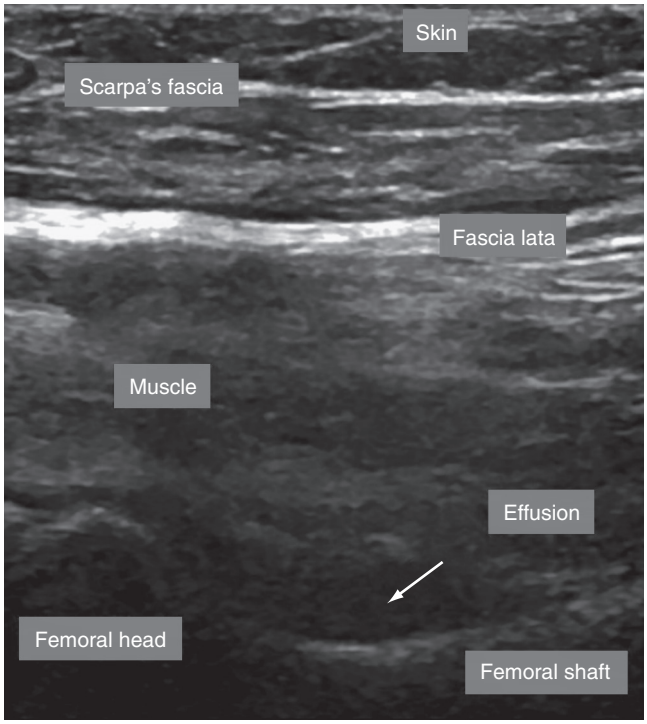



**Figure 48-33** This ultrasound image depicts a small hip effusion in a 9-year-old girl who was evaluated for toxic synovitis versus septic arthritis. Ultrasound images from the asymptomatic, contralateral hip showed comparable amounts of fluid, arguing against septic arthritis.

differentiating intra-articular hip disease from lumbosacral disease. In one study, intra-articular anesthetic injection was 90% accurate in predicting intra-articular disease as confirmed by hip arthroscopy.[64] Anesthetic arthrogram of the hip has shown a 95% positive predictive value and a 67% negative predictive value for pain relief after total hip arthroplasty in patients with concurrent hip and lumbar osteoarthritis.[65]

## Common Disorders in the Differential Diagnosis of Hip Pain

Common causes of hip pain are numerous, and a detailed discussion of these causes is beyond the scope of this chapter. The differential diagnosis of hip pain should include osteoarthrosis (most frequently from developmental dysplasia, Legg-Calvé-Perthes disease, or slipped capital femoral epiphysis), inflammatory arthritis, osteonecrosis, fractures (acetabulum, femoral head, femoral neck, intertrochanteric, or subtrochanteric), trochanteric bursitis, femoroacetabular impingement, tears of the acetabular labrum, transient osteoporosis of the proximal femur, infection, snapping hip syndrome, osteitis pubis, neoplasia (osteosarcoma, chondrosarcoma, pigmented villonodular synovitis, osteochondromatosis, malignant fibrous histiocytoma, or metastases), inguinal hernia, or referred pain (lumbosacral spine, sacroiliac joint, prostate, seminal vesicles, uterus, ovaries, and lower gastrointestinal tract). This list can be efficiently narrowed down by taking a detailed history, performing a comprehensive examination of the musculoskeletal and neurovascular systems, and obtaining the appropriate imaging studies.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 49



# Foot and Ankle Pain

Mark D. Price • Christopher P. Chiodo

**KEY POINTS**

- The differential diagnosis for foot and ankle pain is vast. Localizing symptoms by anatomic region helps narrow this differential.
- On physical examination, most structures in the foot and ankle are immediately subcutaneous and readily palpable.
- Beyond medications, useful nonoperative treatments include bracing, shoe wear modification, orthoses, and physical therapy.
- Most surgical procedures in foot and ankle surgery fall into one of the following categories: arthrodesis, arthroplasty, corrective osteotomy, ostectomy, tendon débridement and transfer, and synovectomy. Patient compliance and soft tissue integrity are important factors when considering surgery.
- Advances in medical management now make joint-sparing procedures possible in many patients with inflammatory arthritis who previously would have required arthrodesis.

Foot and ankle pain are independent risk factors for locomotor instability, impaired balance, and increased risk for falling, as well as compromised functional activities of daily living.[1-5] Foot and ankle pain affect approximately one in five middle-aged to older individuals. Interference with daily activities occurs in one-half to one-third of affected individuals but is rarely disabling outside the context of rheumatoid arthritis. Foot and ankle pain are significantly more common in women, a finding that has been attributed to gender-specific footwear.

## ETIOLOGY

The differential diagnosis of foot and ankle pain is vast and includes disorders of tendons, ligaments, muscle, bone, joints, periarticular structures, nerves, and vessels, as well as referred pain (Table 49-1).

One of the most common cause of pain of the foot and ankle is osteoarthritis (OA). Although OA is the most prevalent joint disease, its pathophysiology remains poorly understood. Ankle and foot OA results from damage and loss of the articular cartilage, which can cause inflammation, stiffness, pain, swelling, deformity, and limitation of function, such as walking or standing. Osteophyte formation can also lead to mechanical impingement. In the foot, OA most commonly occurs in the big toe (hallux), the midfoot, and ankle. In the early stages, pain may occur only at the beginning and at the end of an activity, but while the condition progresses it can become constant and occur even while the individual is at rest.

The ankle is a complex joint that is subject to enormous force during daily activities and in sports, especially running. It is also the joint most commonly injured in the human body and is subject to sprains, fractures, and chondral injuries. This combination of factors predisposes the ankle joint to degenerative changes, although the risk is lower than in other weight-bearing joints, such as the hip and knee. The ankle also rarely develops arthritic changes without an identifiable cause. The most common cause of ankle OA is trauma, including both fracture and ligamentous injury with subsequent chronic instability. Other causes of OA are abnormal mechanics (flat feet and high-arched feet) and, less commonly, systemic diseases such as hemochromatosis.

Foot and ankle pain is the presenting complaint in approximately 15% to 20% of newly diagnosed rheumatoid arthritis (RA) patients.[6] Further, of those patients already diagnosed with RA, the prevalence of foot and ankle involvement has been estimated to be greater than 90%.[7]

The clinician should begin the evaluation of the rheumatoid foot and ankle with a thorough history and physical examination. The location, timing, and duration of symptoms can help to establish a specific diagnosis and guide the subsequent course of treatment. Radiographs and advanced imaging modalities provide useful adjuncts to the evaluation of specific foot and ankle pathologies.

The treatment of the rheumatoid foot and ankle is aimed at both alleviating pain and preserving function (i.e., maintaining the ambulatory status of the patient). Initial nonoperative treatment includes medical management, physical therapy, shoe wear modification, orthotics, and bracing. These measures provide substantial relief for many patients. For recalcitrant symptoms, surgical intervention may be necessary. Most surgical procedures fall into one of the following general categories: arthrodesis (joint fusion), arthroplasty (joint replacement), corrective osteotomy, ostectomy, and synovectomy (joint or tendon).

## FUNCTIONAL ANATOMY AND BIOMECHANICS

The ankle, or tibiotalar joint, is composed of the articulation between the foot (talus) and the lower leg (distal tibia and fibula). Its primary motion is plantar flexion and dorsiflexion in the sagittal plane. In addition, the articulation

**TABLE 49-1** Differential Diagnosis of Foot and Ankle Pain

| |
|---|
| **Tendon, Ligament, and Muscle** |
| Ankle or foot ligament tear or attenuation |
| Chronic joint instability secondary to ligament compromise |
| Joint impingement caused by ligamentous tear and/or hypertrophy |
| Sinus tarsi pathology |
| Achilles tendon pathology |
| Achilles tendon rupture |
| Plantar fasciitis |
| Posterior tibial tendon dysfunction (usually with adult acquired flatfoot) |
| Flexor hallucis longus tenosynovitis and stenosis |
| Tibialis anterior tendon tear |
| Peroneus tendon pathology |
| **Bone** |
| Acute fractures of the ankle or foot |
| Stress fractures |
| Freiberg's infraction (osteonecrosis of the second metatarsal head) |
| Osteonecrosis of talus and navicular |
| Sesamoid bone pathology |
| Metatarsal overload (metatarsalgia) |
| **Joint** |
| Osteoarthritis |
| Gout |
| Rheumatoid arthritis |
| Other inflammatory arthritides |
| Charcot neuroarthropathy |
| Osteochondral lesion of the talus |
| Joint impingement (bone, synovitis, or ligamentous hypertrophy) |
| Hammer toe, claw toe, and mallet toe deformities |
| Hallux rigidus |
| **Nerve Compression** |
| Tarsal tunnel syndrome |
| Anterior tarsal tunnel syndrome (involvement of deep peroneal nerve under the superficial fascia of the ankle) |
| Morton's neuroma |
| Vessels |
| Vasculitis |
| Atherosclerosis |
| Compartment syndrome |
| Neuropathic and Referred Pain |
| Neuropathy |
| Spinal pathology |
| Complex regional pain syndrome |

Courtesy of and modified from Dr. George Raj, No Surgical Spine and Joint Clinic, PS, Bellingham, Wash.

between the distal tibia and fibula allows a lesser amount of internal and external rotation to occur in the axial, or transverse, plane.

The foot may be loosely divided into three anatomic regions: forefoot, midfoot, and hindfoot. The forefoot consists of the toes and metatarsal bones, along with the metatarsophalangeal (MTP) and interphalangeal (IP) joints. The tarsometatarsal (TMT) joints connect the forefoot to the midfoot, which comprises the three cuneiform bones, the navicular, and the cuboid. Finally, the hindfoot, located below the ankle, consists of the talus and calcaneus. The joints of the hindfoot include the talocalcaneal (subtalar), talonavicular, and calcaneocuboid articulations.

Forefoot and midfoot motion is primarily plantarflexion and dorsiflexion in the sagittal plane, with some secondary pronation and supination in the coronal plane and abduction/adduction in the axial plane. Motion in the hindfoot is primarily composed of inversion/eversion in the coronal plane, with secondary internal/external rotation in the axial plane and plantarflexion/dorsiflexion in the sagittal plane.

Knowledge of these anatomic divisions is important because radiographs often demonstrate polyarticular disease in patients with RA. An intimate understanding of the local anatomy is an essential resource for the physician to establish an accurate diagnosis and formulate an appropriate treatment plan.

## DIAGNOSTIC EVALUATION

### Physical Examination

The physician should begin a thorough physical examination of the foot and ankle with gait analysis, even if by simply observing the patient enter the examination room. Normal human gait is divided into two phases. The stance phase is the weight-bearing portion of the gait cycle and comprises roughly 60% of normal walking. This phase begins with heel-strike and then extends through foot-flat to toe-off motion. Meanwhile, the swing phase of gait extends from toe-off to heel-strike and comprises the remaining 40% of the gait cycle.

Patients with an "antalgic" gait pattern will have a shortened stance phase on the side of the affected limb, while they attempt to more quickly transfer their weight to the nonpainful limb. In addition to an antalgic gait, foot and ankle pain often results in the avoidance of ground contact with the painful part of the foot. A further problem noted in stance phase is dynamic collapse of the medial longitudinal arch, most apparent at foot-flat and toe-off.

During the swing phase of gait, a "steppage" gait may be noted. This type of gait is characterized by excessive hip and knee flexion, to allow the foot to clear the ground in the setting of a footdrop. In patients with RA, it may be caused by attritional rupture of the anterior tibialis tendon, which is the main dorsiflexor of the ankle.

After gait analysis, the physician inspects the foot and ankle, both with the patient in the sitting and standing position. The location of swelling is usually well correlated with the joint(s) involved (e.g., the ankle vs. the talocalcaneal joint). Deformity should also be noted. Commonly seen deformities in patients with RA include hallux valgus, or bunion (Figure 49-1); hammertoes; and flatfoot deformity (characterized by hindfoot valgus/forefoot abduction). Callosities develop over regions of increased pressure and are associated with deformity and fat pad atrophy. Rheumatoid nodules can appear anywhere on the foot but are often found in areas of repetitive trauma (i.e., at the site of irritation from a tight shoe counter). Similarly, ulcerations may appear in areas of increased pressure or repeated injury. Finally, wear patterns on shoes should also be noted. As Hoppenfeld observed[8]: "A deformed foot can deform any good shoe; in fact, in many cases the shoe is a literal showcase for certain disorders."

After inspection of the foot and ankle, the physician performs range-of-motion analysis. Passive range of motion of the ankle is normally between 10 and 20 degrees of dorsiflexion and 40 and 50 degrees of plantarflexion. Normal hindfoot inversion and eversion are approximately 20 and 10 degrees, respectively. The first MTP joint should have



**Figure 49-1** Clinical photograph of hallux valgus deformity.

approximately 45 degrees of "plantarflexion" (flexion) and 70 to 90 degrees of "dorsiflexion" (extension). Deviations from these norms should be noted as part of the standard workup.

Next, the physician thoroughly palpates the foot and ankle. The dorsum of the foot and ankle has little overlying musculature. As such, many of the bones and tendons are immediately subcutaneous and a great deal of information can be gained from palpating these structures. It is helpful to palpate the foot and ankle by anatomic location (i.e., forefoot, midfoot, hindfoot, and anterior and posterior ankle).

In the forefoot, the first metatarsal head and MTP joint can be palpated at the base of the hallux, at the medial aspect of the "ball" of the foot. Proceeding laterally, the physician can then sequentially palpate the lesser metatarsal heads and MTP joints. In patients with RA, such palpation often reveals tenderness, synovitis, and bursal swelling. In the second and third MTP joints, sagittal plane instability often results from attenuation of the plantar joint capsule. This condition can be appreciated by gently translating the second and third toes dorsally.

In the hindfoot, the calcaneus is readily palpable, and its various parts can be palpated individually. A stress fracture should always be considered in patients with RA. Further, tenderness over the posterior aspect of the bone may indicate Achilles tendon pathology, while pain over the medial tubercle (palpable on the medial plantar surface) may indicate plantar fasciitis. Tenderness over the "sinus tarsi" of the hindfoot (located laterally, just anterior and distal to the tip of the fibula) usually indicates talocalcaneal joint pathology. Finally, posteromedial tenderness may be secondary to tenosynovitis, posterior tibial tendinosis, and tarsal tunnel syndrome (usually secondary to adjacent tenosynovitis).

In the ankle joint proper, tenderness over the anterior joint line usually correlates with ankle joint pathology including arthritis, synovitis, impingement, and osteochondral defect (OCD).

A more detailed description of these conditions and their correlation with anatomic location is provided later in the chapter and in Table 49-2.

**TABLE 49-2** Anatomic Basis of Common Causes of Foot and Ankle Pain

| Location | Pathology/Dysfunction |
|---|---|
| Forefoot | Arthritis/synovitis<br>Hallux valgus<br>Hammertoes, claw toes, and mallet toes<br>Morton's neuroma<br>Metatarsophalangeal arthritis/synovitis/instability<br>Osteonecrosis of the second metatarsal head (Freiberg's) |
| Midfoot | Arthritis/synovitis, stress fracture, osteonecrosis (navicular) |
| Hindfoot | Arthritis/synovitis<br>Hindfoot valgus deformity<br>Stress fracture<br>Plantar fasciitis |
| Anterior ankle | Arthritis, synovitis, impingement, osteochondral defect |
| Central ankle | Osteochondral lesion<br>Stress fracture |
| Posterior ankle | Achilles tendinitis/tendinosis<br>Retrocalcaneal bursitis<br>Achilles rupture<br>Stress fracture |
| Posterolateral ankle | Peroneal tendinitis, tear, instability |
| Posteromedial ankle | Posterior tibial tendinitis/dysfunction<br>Flexor hallucis longus/flexor digitorum longus tendinitis<br>Tarsal tunnel syndrome |

## IMAGING

Despite the abundant availability of advanced imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT), radiographs remain the imaging mainstay in the evaluation of foot and ankle pain. Weight-bearing images should be obtained whenever possible because joint space narrowing and deformity may not be apparent in nonweight-bearing images. Standard images consist of weight-bearing anteroposterior, lateral, and oblique views of the foot and anteroposterior, mortise, and lateral views of the ankle. Further radiographic findings of RA include periarticular erosions and osteopenia.

MRI provides reliable imaging of soft tissue structures and can be a useful tool in the evaluation of the rheumatoid foot and ankle. Early in the course of RA, MRI allows one to look for signs of the disease such as synovitis, tenosynovitis, periarticular edema, and bursitis.[9] Later, MRI is useful in assessing disease progression and extent of joint involvement, as well as in distinguishing between tendon rupture and tendinitis/tendinopathy (Figure 49-2).

CT scan[10] and nuclear scintigraphy[11] are also used in the evaluation of foot and ankle pain. For example, either method can be quite helpful in post-operative evaluation in fusion surgery. Ultrasound is gaining utility as a method to

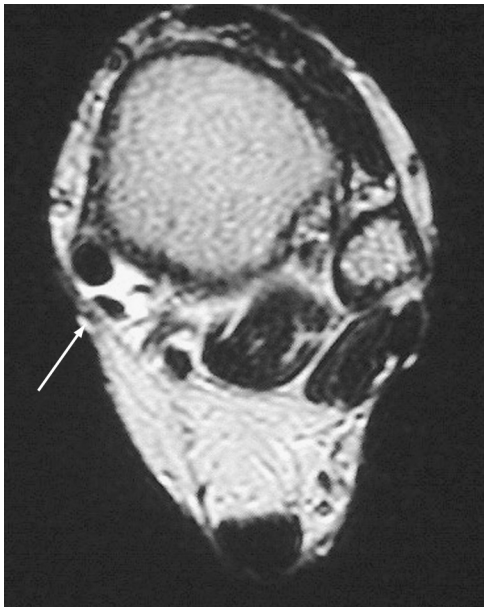


**Figure 49-2** Axial magnetic resonance image of ankle demonstrating posterior tibial tendon degeneration and synovitis *(arrow)*.

evaluate joint inflammation as well as tendon integrity. There is no radiation involved, and the procedure is now frequently performed in the office setting. However, the results are sometimes operator and technique dependent with minimization of artifacts being of utmost concern. In addition, ultrasound may be difficult to use in pre-operative planning because important landmarks are often not included.[12]

Anesthetic arthrograms are an extremely useful adjunct in diagnosing foot and ankle pain in patients with RA. Given the complex and crowded geometry of the foot and the propensity of RA to affect multiple joints and tendons, it is often difficult to determine whether the pain is articular and, if so, which joint is symptomatic. With an anesthetic arthrogram, a mixture of steroid, anesthetic, and contrast material is injected under fluoroscopic guidance into a suspect joint. This allows the clinician to more precisely determine whether or not the injected joint is a significant pain generator. Again, this is especially helpful in the foot and ankle, where multiple joints are in close proximity and may be simultaneously diseased.[13]

## DIFFERENTIAL DIAGNOSIS OF ANKLE PAIN

From a diagnostic standpoint, it is useful for the clinician to conceptualize ankle pain on the basis of anatomic location. This approach applies to patients with virtually any form of ankle or foot pain, and RA will be used to illustrate how to formulate a differential diagnosis and treatment plan while advances in medical management temper disease and "allow" patients to develop other, non-inflammatory disorders.

### Anterior Ankle Pain

Anterior ankle pain, in patients both with and without RA, is most often the result of intra-articular pathology. Anteriorly, the ankle joint is not shielded by the malleoli and is immediately subcutaneous. Further, the anterior extensor tendons are typically not prone to the development of tendinitis and tendinosis.

In early RA, synovitis can cause anterior joint line pain, swelling, and tenderness. Clinically, this results in symptoms of "impingement." Specifically, patients will note pain with ankle dorsiflexion, such as when they walk up stairs or an incline. On physical examination, there may be anterior tenderness and/or pain with terminal passive dorsiflexion. Anterior osteophyte formation may produce even more pronounced impingement symptoms, although such osteophytes are more commonly seen with OA and longstanding ankle instability.

### Central Joint Pain

Two other causes of central ankle pain, stress fracture and OCD, should be considered. Stress fractures are commonly seen in patients with periarticular and generalized osteopenia. An OCD is a focal defect in the articular cartilage and subchondral bone. These lesions are encountered more commonly in patients without inflammatory arthritis. In the setting of RA, their presence may represent an early manifestation of RA or a separate pathologic process.

### Posterior Joint Pain

Posterior ankle pain usually originates from the Achilles tendon, its insertion onto the calcaneal tuberosity, and two associated bursae in this region. The Achilles tendon is the largest tendon in the body but lacks a true synovial lining. As such, isolated Achilles tendinitis is uncommon. In most instances, Achilles pain results from degenerative tendinosis, with or without an overlying tendinitis. Although associated spur formation is common, it is important to remember that Achilles spurs are a manifestation of a disease process. As such, surgeries directed at spur excision also frequently entail tendon débridement and reconstruction, as well as tendon transfer.

The Achilles tendon is associated with two bursae. The "retrocalcaneal" bursa is a larger structure that lies deep to the Achilles. Inflammation of this structure often accompanies Achilles tendinitis/tendinosis. It may also be irritated by an enlarged posterior superior calcaneal tuberosity, sometimes referred to as "Haglund's" deformity. Less commonly, a more superficial, immediately subcutaneous bursa can develop with irritation from ill-fitting shoes with a tight counter ("pump bump").

### Medial and Lateral Ankle Pain

As with anterior, central, and posterior ankle pain, the origin of medial or lateral ankle pain is also anatomically based.

On the medial side, pain directly over the medial malleolus should alert the clinician to the possibility of a stress fracture. Pain anterior to the medial malleolus is usually articular in nature. Pain posterior to the medial malleolus is often caused by inflammation and/or degeneration of the posteromedial flexor tendons. These include the posterior tibial tendon and the flexor hallucis longus and flexor

digitorum longus tendons. The posterior tibial tendon is the largest and strongest of the posteromedial flexor tendons. Its primary function is to invert the hindfoot and thus support the medial longitudinal arch of the foot. Long-standing synovitis and dysfunction of this tendon may ultimately lead to collapse of the arch and the development of an acquired flatfoot deformity.

On the lateral side of the ankle, pain directly over the lateral malleolus may be caused by a stress fracture. This is especially relevant in the setting of hindfoot valgus and a flatfoot, which will increase fibular loading. Similar to the medial side, pain anterior to the lateral malleolus is usually articular in nature. Finally, pain posterior to this lateral malleolus is usually indicative of peroneal tendon pathology. In patients with RA, the peroneal tendons may be affected by tenosynovitis, longitudinal "split" tears, and chronic tendon instability. With the latter, the tendons sublux over the posterolateral edge of the fibula, causing pain as well as attritional tearing.

## DIFFERENTIAL DIAGNOSIS OF FOOT PAIN

Typically, the forefoot is the most common site of involvement early in the course of diseases such as RA but also can occur in patients with gout and OA.[14] The pathogenesis of forefoot pain and deformity in the rheumatoid forefoot is inflammation and progressive synovitis that eventually leads to a capsular distention at the MTP joints and attenuation of the plantar plates.[15] Eventually it progresses to loss of collateral ligament stability and, finally, destruction of the articular cartilage and bone (Figure 49-3A and B). Clinically, this typically manifests as hallux valgus and dorsal subluxation or dislocation of the lesser toe MTP joints with a hallux valgus deformity, and presents in the patient as metatarsalgia.

In the lesser MTP joints, such loss of stability leads to progressive deformity secondary to ligamentous attenuation combined with the standard forces on the forefoot. Muscle imbalance and dorsiflexion forces during the toe-off phase of gait cause progressive subluxation and even dorsal dislocation of the MTP joints. With these changes, the metatarsal head is prone to forming keratotic skin lesions that can ultimately ulcerate. Muscle imbalance can also lead to the development of painful hammertoe and claw toe deformities that can exert a plantar-directed force that further exacerbates symptomatic metatarsalgia. Lesser MTP joint subluxation has been reported to be as high as 70%, with a concomitant incidence of pressure sores in approximately 30% of those patients.

In the hallux, RA can cause both articular erosions and loss of capsular integrity that often results in the development of a hallux valgus deformity, or bunion (see Figure 49-1). The progression of this deformity may be further accelerated by loss of support from the adjacent lesser MTP joints. The incidence of hallux valgus deformity in patients with RA has been estimated to be as high as 70%.

The midfoot is a less common site of involvement in the rheumatoid foot. Radiographically there can be erosions; however, the prevalence of symptoms is often quite low. The most frequent site of involvement is the first tarsometatarsal (TMT) joint. The symptoms seen here, though, may not be from rheumatoid synovitis per se, as seen in the forefoot. Rather, pain may also be due to hindfoot and hallux valgus deformities that lead to increased stresses across the TMT joint. Eventually this increased stress can lead to dorsiflexion of the first TMT joint and resultant lesser toe TMT joint abduction and dorsiflexion, thereby leading to pain in the dorsomedial midfoot. In addition, progressive biomechanical changes can lead to OA of the midfoot TMT joints, causing discomfort and pain with weight bearing.

The three joints of the hindfoot (talonavicular, talocalcaneal, and calcaneocuboid) are commonly affected by RA. Although these joints are affected at different rates, the overall prevalence of hindfoot involvement in patients with RA is between 21% and 29%. The talonavicular joint is most often affected, followed by the talocalcaneal and then calcaneocuboid joints. Further, the hindfoot becomes more symptomatic and involved the longer the duration of RA. The incidence of hindfoot deformity in patients diagnosed with RA for less than 5 years has been estimated to be 8% and increases to 25% in patients with RA for longer than 5 years.[16] Clinically, patients with talocalcaneal or calcaneocuboid involvement will complain of lateral hindfoot pain. Meanwhile, arthritis and synovitis of the talonavicular joint are manifested by dorsal or medial pain.

The deformity most often seen in patients with hindfoot RA is an acquired flatfoot deformity, characterized by heel valgus and forefoot abduction. This usually results from articular deformity and instability, but may also be caused by tenosynovitis and tendinosis of the posterior tibial tendon, the main supporter of the longitudinal arch of the foot.

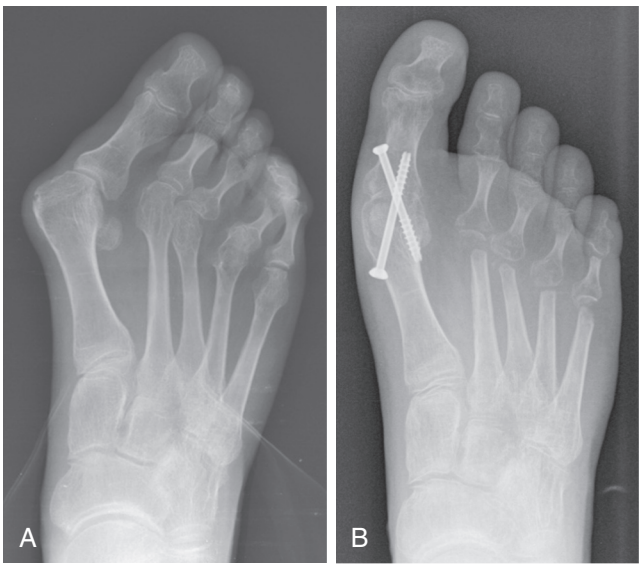



**Figure 49-3** Pre-operative **(A)** and post-operative **(B)** anteroposterior radiograph of hallux valgus deformity with lesser metatarsophalangeal joint erosions treated by fusion and lesser metatarsal head resections.

## NONOPERATIVE TREATMENT

Medical management remains the cornerstone of treatment for many forms of foot and ankle arthritis. In fact, many of the current recommendations for operative treatment

may soon be modified given the alteration of disease progression with current medical regimens for RA.[17] The most common medical management still consists of nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, and disease-modifying anti-rheumatic drugs (DMARDs) and, more recently, biologic therapies. Although each of these drug classes has done much to alleviate patient suffering, they are not without impact on the surgical management of rheumatic disease. There is concern about lower fusion rates in the setting of NSAIDs and increased infection rates in the setting of steroids or DMARDs.[18,19] Moreover, patients who have been on chronic steroids are at risk for post-operative adrenal insufficiency and may require perioperative corticosteroids.[20] Close communication and collaboration between the rheumatologist and surgeon are essential to good outcomes.

Footwear modification can often have profound benefits for patients. Shoes should be examined in the clinic to be sure that they can accommodate a patient's deformity. Patients often feel best in shoes with a deep, wide toe-box, a firm heel counter, and soft heel. Well-constructed walking or jogging shoes usually provide sufficient room for mild to moderate deformities. It is helpful to provide patients with a list of suitable manufacturers when making such recommendations.

Often it is necessary to prescribe a custom orthotic insert for those with more moderate deformities. Typically the insole of the shoe must be removed to make room for the orthotic. Again, most walking or jogging shoes will suffice. In general, custom orthotics can be divided into rigid, semi-rigid, and softer accommodative devices. Rigid and semi-rigid orthotics are usually used to correct supple deformities and should be used with caution in patients with RA.[21] More commonly, patients benefit from accommodative orthotics (i.e., orthotics made of softer material that can be molded to "accommodate" a deformity).[22] These can then be further modified by incorporating a "relief" under a deformity, thereby further unloading it. When referring patients to a specialist for orthotics, it is best to provide the orthotist with a prescription that includes the patient's precise diagnosis (e.g., RA or OA with metatarsalgia), as well as the type of orthotic and any modifications desired (e.g., a "custom accommodative orthotic with a relief under the lesser metatarsal heads").[23]

Finally, injections of a mixture of anesthetic and corticosteroid to areas of inflammation or bursitis are useful to treat both inflammatory and non-inflammatory conditions affecting the foot and ankle. In the foot and ankle, however, such injections must be judiciously used. Most important, injections into and around tendons should be avoided. Because of the forces associated with weight bearing and ambulation, these tendons are under substantial load. The injection of a corticosteroid directly into or even near a tendon can adversely affect the biomechanical properties of the tendon and can ultimately lead to rupture.[24] A further precaution is to avoid corticosteroid injections into the lesser MTP when there is evidence of joint instability (manifested by valgus or varus deviation on radiographs or sagittal plane instability on physical examination). Such injections can lead to further attenuation of the joint capsule and can result in frank joint dislocation.

## OPERATIVE TREATMENT

If symptoms persist despite nonoperative management, surgical intervention should be considered. Two important factors must be taken into account when deciding whether or not to proceed with surgery. First, the soft tissues and vascular status must be carefully assessed. Both may be compromised and could negatively affect outcome. Second, the ability of patients to comply with the post-operative regimen (e.g., the ability able to use crutches and not bear weight if necessary) must be considered. Even limited noncompliance can lead to a poor outcome, especially in fusion surgery.

As noted earlier, most surgical procedures fall into one of the following categories: arthrodesis (joint fusion), arthroplasty (joint replacement), corrective osteotomy, ostectomy, and synovectomy (joint or tendon).

### Arthrodesis

Arthrodesis remains a surgical cornerstone for the rheumatoid foot and ankle. With an arthrodesis procedure, the two sides of the joint are roughened with a burr or small chisel. Next, the two bones to be fused are compressed and fixed together, usually with one or more screws (see Figure 49-3B). In the weeks and months after surgery, the body is "tricked" into thinking that there is a fracture present at the fusion site and heals this with bone. As such, the two bones become one and are considered fused. Fusion surgery offers reliable pain relief in the majority of patients. One obvious concern with fusion surgery is the loss of motion. For the patient, however, this usually results in only mild functional compromise. Further, to the untrained eye, the patient has a remarkably minor change in gait.

Commonly performed fusions in patients with RA include ankle arthrodesis, isolated hindfoot fusions, triple arthrodesis, midfoot arthrodesis, and arthrodesis of the first MTP joint. A triple arthrodesis involves fusion of the talocalcaneal, talonavicular, and calcaneocuboid joints. Together, these joints allow coronal plane motion and thereby are most important when an individual is walking on uneven ground.

Arthrodesis is still commonly performed in patients with RA of the ankle. If there is minimal deformity and no loss of bone stock, ankle fusion surgery may be performed arthroscopically or through a "mini open" approach. These techniques involve less soft tissue dissection and stripping, thereby minimizing loss of bony perfusion. Nevertheless, the time period for which the patient must avoid bearing weight (from 6 to 12 weeks) remains the same. The success rate of ankle fusion surgery in patients with RA is generally 85% or greater. Although the osteopenia associated with the disease can compromise fixation, it can also theoretically enhance fusion because less sclerotic subchondral bone is present.

In the hindfoot, fusion surgery may be performed on one or more of the three joints of this part of the foot (i.e., the talocalcaneal, talonavicular, and calcaneocuboid joints). If only one of these joints is diseased, an isolated fusion of this joint is acceptable.[25] This reduces surgical morbidity and the extent of the procedure. Nevertheless, with fusion of just one of the joints of the hindfoot, motion in the other joints

is reduced.[26] If more than one joint is diseased, a "double" or "triple" arthrodesis is necessary.

In the midfoot, fusion surgery results in negligible loss of motion because the joints of the midfoot normally have less than 10 degrees of motion. In a patient with both OA and inflammatory arthritis, symptomatology is most often limited to the medial (first through third) TMT joints. The lateral (fourth and fifth) TMT joints are infrequently symptomatic, even in the setting of advanced radiographic changes.

Finally, in the forefoot, fusion surgery is indicated only for the first MTP joint. This procedure is used for both arthrosis and advanced hallux valgus (bunion) deformities. When the first MTP joint is fused, it is positioned in a slightly dorsiflexed position to assist ambulation. With MTP fusion in 47 patient feet, Coughlin[27] reported 96% good to excellent results and 100% fusion at an average 6.2-year follow-up.

In summary, fusion surgery generally provides reliable pain relief and a stable, plantigrade foot. Nevertheless, the loss of motion of the fused joint can lead to increased motion and altered biomechanics at adjacent joints. This result ultimately may lead to arthritic changes in these joints.[28] Further, fusion surgery may lead to subtle, albeit real, changes in gait.[29] Finally, the minimal ramifications of fusing just one joint may become much greater in the setting of a subsequent fusion in either the ipsilateral or contralateral limb.

## Arthroplasty

Concerns regarding fusion have driven many to work toward improving joint replacement surgery (arthroplasty) in the foot and ankle. Most notably, total ankle replacement surgery has evolved and is now a viable alternative to arthrodesis. Nevertheless, although there are many orthopedic surgeons who perform ankle replacement surgery, there are still some who do not or who do so on a limited basis. The U.S. Food and Drug Administration currently approves five ankle prostheses for implantation. Robust long-term survival data as published for hip and knee arthroplasty are not yet available.

The main advantage of ankle arthroplasty is preservation of motion. Its main two disadvantages are technical complexity and the difficulty with subsequent fusion if the procedure fails. In general, ankle replacement surgery is indicated for middle-aged and elderly individuals with low functional demands and minimal deformity. Two other indications especially pertinent in patients with ankle arthritis include (1) bilateral disease and (2) concomitant ipsilateral hindfoot disease or pre-existing arthrodesis. The paradox of ankle replacement surgery is that ankle replacement is contraindicated in young patients, for whom preservation of motion is most important. On the other hand, arthroplasty is more commonly performed in older patients, for whom preservation of motion is less important and who might do fine with a fusion. Nevertheless, total ankle replacement surgery continues to evolve, and reported success rates with modern designs continue to improve[30,31] (Figure 49-4).

In the foot, arthroplasty is performed by some surgeons for the first MTP joint. Results from the relevant literature are still somewhat conflicted, however. Although some encouraging early results with arthroplasty were achieved, other studies have shown high rates of implant failure and loosening secondary to synovitis from polymeric silicone (Silastic) particle wear.[32-34] Further, advanced deformity, often present in patients with RA, is considered to be a relative contraindication to first MTP joint arthroplasty. Nevertheless, new implant designs may hold increased promise. In general, these implants are lower profile and resect less bone, which also makes it easier to perform a subsequent fusion, if necessary.




**Figure 49-4** Anteroposterior radiograph of total ankle arthroplasty.

## Osteotomy

Corrective osteotomies are used for two primary reasons in the treatment of RA: to correct deformity, to redistribute forces on a joint or the terminal aspect of a bone, or both.

Examples of osteotomies to correct deformity include calcaneal osteotomies for pes planovalgus and metatarsal osteotomies for hallux valgus. Previously, patients with RA and concomitant pes planovalgus or hallux valgus underwent fusion surgery. However, with advances in medical management of the disease, it is not unreasonable to attempt joint preservation surgery in patients who have mild to moderate disease, healthy soft tissues, and flexible deformities.

Examples of osteotomies to redistribute forces include tibial osteotomies in the setting of eccentric ankle arthritis and metatarsal osteotomies in the setting of metatarsal overload and metatarsalgia. Patients requiring surgery for ankle RA previously underwent fusion surgery only, while patients requiring surgery for metatarsalgia underwent metatarsal head resection. Again, however, advances in medical management of the disease allow joint preservation osteotomies to be considered. This is especially the case for metatarsalgia, which is common in patients with RA, yet increasingly does not entail frank dislocation or articular erosion.

### Ostectomy

Although more commonly seen in patients with OA, some patients with RA may present with symptoms of mechanical ankle impingement arising from anterior bone spurs. In cases without global joint destruction, surgical resection of the spurs, or cheilectomy, is a reasonable treatment. Although no studies have examined cheilectomy in RA specifically, patients with less severe erosive changes tend to be more satisfied with the results of cheilectomy.[35]

### Synovectomy

For those patients with inflammatory arthritis resistant to medical management and nonoperative treatment, synovectomy can provide a period of pain relief for many patients.[36,37] It is thought that early synovectomy of either the affected joint or tendon may help halt the progress of joint destruction. Joint synovectomy is indicated in those who have failed medical management yet still have a relatively preserved articular surface. This may be performed arthroscopically or open through a traditional arthrotomy. Otherwise, synovectomy of the affected tendons allows some preservation of function.

## CONCLUSION

Foot and ankle pain is a prevalent and potentially debilitating problem. Unfortunately, many forms of arthritis, including RA, establish a vicious cycle of foot and ankle pain and altered biomechanics. Synovitis and articular erosions lead to both pain and deformity. A proper history and physical examination are essential for establishing an anatomic diagnosis. Although advanced imaging modalities such as MRI and CT can be useful as adjuncts, radiography remains the gold standard. Nonoperative modalities such as medications, bracing, physical therapy, orthotics, and footwear modification are able to relieve pain and maintain function for many. For recalcitrant symptoms, substantial relief may be afforded by surgical intervention in the form of arthrodesis, arthroplasty, osteotomy, ostectomy, and synovectomy.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 50



# Hand and Wrist Pain

Carrie R. Swigart • Felicity G. Fishman

**KEY POINTS**

- Patients with carpal tunnel syndrome typically present with nocturnal paresthesias associated with intermittent pain or paresthesia during the day.
- Ganglia are mucin-filled cysts arising from joint capsules or tendon sheaths. If they are particularly symptomatic, treatment with corticosteroid injections may be attempted, but surgical excision may be necessary.
- De Quervain's disease—that is, inflammation of the extensor pollicis brevis and abductor pollicis longus tendons in the first dorsal extensor compartment—is common in women and is associated with repetitive hand activities, such as caring for an infant.
- Painful osteoarthritis involving the carpometacarpal joint of the thumb can often be treated successfully with splinting.
- Trigger fingers, caused by thickening of the A1 retinacular pulley in the palm, usually can be treated with corticosteroid injections and splinting.

The multiple functions that the hand performs in daily life are usually taken for granted until the hand becomes affected by disease or injury. Depending on the nature of the disorder, patients have different adaptive capacities. Patients presenting with pain and dysfunction of the hand, wrist, or both represent a wide spectrum, diverse in age, occupations, and avocations. These patients have a broad range of medical conditions that may or may not be related to their current problem. Each patient has a different story to tell about his or her hand and wrist and why he or she is seeking treatment. It is the clinician's role to sort out these various factors, some of which may seem confounding, and determine the most appropriate diagnosis and course of treatment.

This chapter presents guidelines that are useful in the evaluation of patients presenting with hand and wrist pain. Complete coverage of all of the various conditions that can affect the hand and wrist is beyond the scope of this chapter. Instead, the conditions discussed include the most common disorders seen by general practitioners and hand surgeons. The conditions are grouped by their anatomic area to include pain localized to the volar, dorsal, radial, or ulnar wrist; the base of the thumb; and the palm and digits.

## PATIENT EVALUATION

### Anatomy

The complex anatomy of the hand and wrist involves many structures that interact in close proximity to one another. Several different disorders can manifest with similar symptom patterns despite varying diagnoses. A precise knowledge of the anatomy of the hand and wrist often eliminates several diagnostic considerations on the basis of the physical examination alone. The history of the illness and the examination also help narrow further investigation by enabling the physician to better choose appropriate additional diagnostic tests. Several common sites of pain in the hand and wrist and their corresponding leading diagnoses are illustrated in Figure 50-1. Pain in one location can have multiple causes depending on the patient profile and the history of the problem. A thorough review of the pertinent regional anatomy is important to help differentiate the many possible causes of hand and wrist pain.

### History

Important patient factors include age, sex, hand dominance, occupation, and hobbies or sports. When determining the history of the problem, a history of recent or distant trauma should be sought, and an estimation of the severity of the trauma should be noted. Next, questions about the duration and frequency of the pain and its intensity and quality should be addressed. The pain of degenerative arthritis is often described as a localized "toothache"-type pain that is always present at a low level and increases with activity, whereas the pain of tendinitis may be sharp, poorly localized, and present only with activity. Rheumatoid arthritis (RA) manifests initially with hand and wrist involvement in 25% of patients and is characterized by joint effusion, with bilateral hand and wrist involvement and morning stiffness. Nighttime symptoms of a burning-type pain in the hand and wrist that are exacerbated by arm position are often associated with nerve entrapment syndromes. Specific activities that either cause pain or alleviate it should also be noted. Arthritis at the base of the thumb, or first carpometacarpal joint, is often aggravated by opening jars, turning doorknobs, and doing needlework or other hobbies.

## PHYSICAL EXAMINATION

A thorough examination of the involved extremity and comparison with the uninvolved extremity are essential.

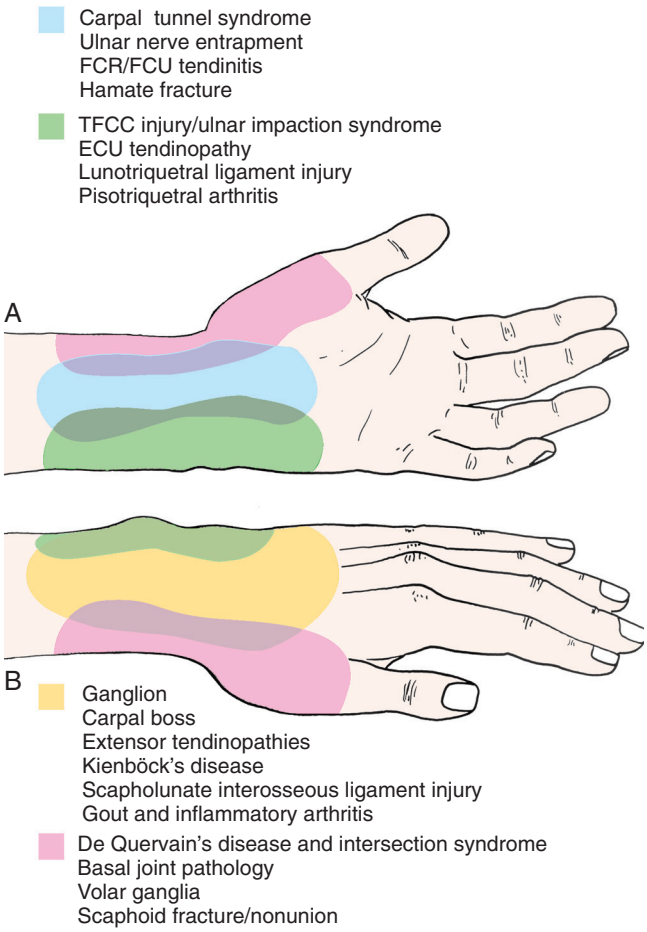



**Figure 50-1** **A,** Palmar and ulnar view of the hand and wrist with areas of pain and tenderness marked with their corresponding leading differential diagnoses. **B,** Dorsal and radial view of the hand and wrist with areas of pain and tenderness marked with their corresponding leading differential diagnoses. ECU, Extensor carpi ulnaris; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; TFCC, triangular fibrocartilage complex.

Attention should be directed to abnormalities of the more proximal joints of the elbow and shoulder and the cervical spine. As the differential diagnosis narrows, the examination should be tailored as needed to include or eliminate any possible systemic causes. As with other musculoskeletal examinations, the range of motion of the involved joints should be observed and compared with the opposite side. Any difference between active and passive motion should be noted. Careful palpation for the site of maximal tenderness is important in differentiating the source of the pain and is particularly important when trying to exclude possible factors of secondary gain. Measurements of grip and pinch strength are also helpful in many situations as a diagnostic aid and a baseline measurement to follow for improvement. Many provocative maneuvers are useful in differentiating causes of pain; these maneuvers are discussed with the specific disease with which they are associated.

## Imaging Studies

Technologic advances have increased the availability of imaging studies for the hand and wrist. Improvements in MRI resolution using small joint coils allow more precise imaging of small structures in the hand and wrist. Advancements in ultrasound technology have allowed this tool to be increasingly used in the diagnosis of musculoskeletal complaints. With the multitude of ancillary studies available, it is important to be selective in using these tools to establish or refute diagnostic possibilities. With cost containment in mind, imaging studies should be used most often to confirm a diagnosis rather than to find one. An understanding of the advantages and limitations of each tool is necessary to enable them to be used to their fullest potential.

Among the available tools, plain radiographs are the easiest to perform and most readily available. A routine hand or wrist series, including anteroposterior, lateral, and oblique views, is a useful screening tool but often lacks the required specificity. Depending on the suspected diagnosis, many special views are available. These views are discussed later in this chapter with the specific diagnoses to which they pertain.

When further detail of the bony anatomy is required, CT is currently the best available tool. The most common uses for CT in the hand and wrist include evaluation of intra-articular fractures of the distal radius and metacarpals, scaphoid fractures and nonunions, and intraosseous cysts or tumors.[1,2]

Advances in ultrasound and MRI technology have enhanced our ability to evaluate the soft tissue structures of the hand and wrist. Smaller ultrasound probes with higher resolution have made it possible to visualize and differentiate structures such as flexor tendons, ganglion cysts, and ligaments. MRI technology is constantly improving, providing new uses in the hand and wrist. By altering the parameters of this test, information about anatomy and physiology can be obtained.[3] Specific uses of these tests and others such as arthrography, ultrasound, and bone scans are addressed with the diagnoses for which they are most useful.

## Additional Diagnostic Tests

### Neurodiagnostic Tests

Neurodiagnostic tests, including nerve conduction studies and electromyography, are useful in the diagnosis of suspected neurologic disorders of the upper extremity. Specifying the type and nature of the examination required enhances the information gained by these studies. If a nerve compression syndrome such as carpal or cubital tunnel syndrome is suspected, nerve conduction studies may be sufficient without the added cost and patient discomfort of formal electromyographic testing. Nerve conduction studies evaluate the speed of conduction of motor and sensory nerves across a set distance at a specific location and compare this speed with established normal values. A decrease in the speed of nerve conduction, as evidenced by an increase in the latency, is seen with localized nerve compression and is shown in several different nerves concomitantly in demyelinating diseases such as multiple sclerosis. When more severe nerve injuries are suspected or if there is clinical evidence of muscle weakness or atrophy, an electromyogram can be useful to evaluate the extent of the process or rule out a myopathic process.[4]

### Injections and Aspirations

The use of injections and aspirations can be therapeutic and diagnostic. Corticosteroids can be administered selectively in conjunction with a local anesthetic for more lasting relief and in some cases can be curative.[5-11] Some of the most common sites for injection are the A1 pulley region of the finger for trigger finger, the carpal canal for carpal tunnel syndrome, and the first dorsal compartment of the wrist for de Quervain's disease.

Aspiration of joints or other fluid collections, such as ganglia, can yield vital diagnostic information and can be therapeutic. If infection is suspected, aspiration should be used to obtain a sample of joint fluid for gram stain, cell count, and culture. Diagnoses such as gout and pseudogout can be confirmed by crystal analysis under polarized light. Many ganglia and retinacular cysts can be treated temporarily or permanently with simple aspiration.[12,13]

The accuracy of injections and aspirations can be increased with the use of ultrasound. This modality can aid in the injection of symptomatic trigger fingers, de Quervain's tenosynovitis, and carpal tunnel syndrome. Ultrasound-guided injections were found to be in the tendon sheath 70% of the time versus 15% of the time when ultrasound was not used.[13a,13b] Joint injections or aspirations, as well as aspiration of ganglia, also can be greatly aided by ultrasound guidance.[13c]

### Arthroscopy

Direct visualization of a joint via arthroscopy can be an invaluable diagnostic tool. Despite the increasing sensitivity of imaging techniques such as MRI, arthroscopy provides a dynamic evaluation that static imaging cannot provide.[14] Since the first published report of a series of cases by Roth and colleagues in 1988,[15] it has become the "gold standard" for evaluation of chronic wrist pain.[16-18] With new surgical techniques being developed, surgeons often can proceed directly to the definitive treatment using arthroscopy entirely or in part.[19-24]

## COMMON CAUSES OF HAND AND WRIST PAIN

## Wrist Pain: Palmar

### Carpal Tunnel Syndrome

Carpal tunnel syndrome (CTS) is the most commonly diagnosed compression neuropathy in the upper extremity. It usually occurs as an isolated phenomenon, but symptoms of CTS can accompany many systemic diseases, such as congestive heart failure, multiple myeloma, and tuberculosis.[25-28] More commonly, CTS is associated with conditions such as pregnancy, diabetes, obesity, RA, and gout.[29-39]

The classic constellation of symptoms consists of nocturnal paresthesias in the affected digits; paresthesias or hypoesthesias in the thumb, index, and long fingers; and weakness or clumsiness of the hand. Patients often report forearm and elbow pain that is aggravated by activities but is poorly localized and aching in nature. Occasionally, more proximal symptoms such as shoulder pain are the main presenting complaint.[40] Past reports have indicated a 3:1 prevalence of CTS in women. Approximately half of patients are aged 40 to 60 years, although occasionally CTS has been diagnosed in children.[41,42]

The diagnosis of CTS is usually clinical. Tinel's sign, shown by radiating paresthesias in the median nerve distribution with gentle percussion over the volar wrist, indicates nerve irritation. Reproduction of symptoms with wrist flexion, as described by Phalen,[43] and with the carpal compression test, as described by Durkan,[44] has been shown to be more specific.[45] Decreased sensibility and thenar atrophy are late signs seen in persons with advanced median nerve entrapment. Bilateral electrodiagnostic tests, specifically nerve conduction velocity testing, should be used to confirm the diagnosis, particularly in patients claiming a compensable injury or in patients with atypical signs or symptoms. Prolonged motor and sensory latencies across the carpal canal confirm pathologic compression of the median nerve.[46-48] In patients with classic clinical findings, a study found that CTS could be diagnosed with a high degree of accuracy on clinical grounds alone and that the addition of electrodiagnostic tests did not increase the accuracy.[49] When attempting to differentiate CTS from more proximal nerve entrapments such as cervical root compression or thoracic outlet syndrome, the addition of electromyography of the cervical paraspinal muscles and proximal conduction tests (H reflex, f waves) can be useful.[50]

Conservative treatment for CTS consists of splinting of the wrist in a neutral position and consideration of oral nonsteroidal anti-inflammatory drugs (NSAIDs) for pain control. Splinting should be used sparingly during the workday to prevent secondary muscle weakness and fatigue and is best prescribed to prevent provocative wrist positioning at night. The splint should not hold the wrist in extension beyond 10 degrees (Figure 50-2). "Off the shelf" splints often need to be manipulated to neutral or 10 degrees of extension before being provided to the patient. Although splinting may be beneficial for relief of symptoms in cases of mild compression, its long-term effectiveness is limited.[51] The use of vitamin $B_6$ (100 to 200 mg/day) has been helpful in some cases, but its efficacy has not been confirmed in a randomized trial. The popularity of corticosteroid injections in the treatment of CTS has waxed and waned during the past half century. Although it has been shown to be quite



**Figure 50-2** Typical night splint used in the treatment of carpal tunnel syndrome.

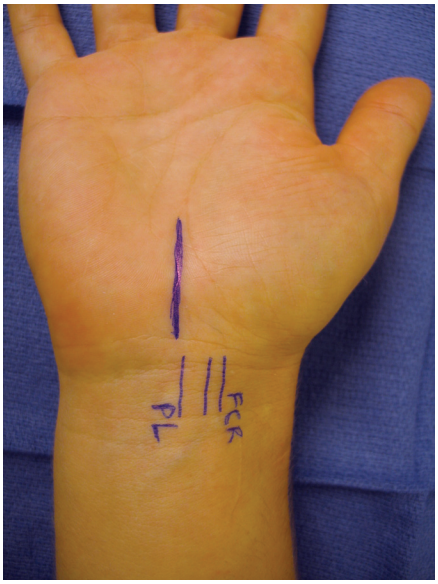



**Figure 50-3** Usual incision used for release of the carpal tunnel. Note its position just ulnar to the course of the palmaris longus tendon (PL) and the relative position of the flexor carpi radialis tendon (FCR).

effective in the short term, the long-term efficacy is mixed.[52-54] In addition, injections have been associated with exacerbation of the condition and permanent median nerve injury if performed incorrectly.[55,56] For these reasons, injections are most often indicated in cases when the condition is thought to be temporary, such as with pregnancy, or if surgery must be deferred because of a medical condition or major life event.

Surgical release is indicated for patients with confirmed CTS who have not responded to a course of conservative treatment. In patients who exhibit late findings of objective sensory loss or thenar atrophy, early surgery should be recommended. The incision for a modern carpal tunnel release is not more than 3 cm long and parallels the skin creases of the palm (Figure 50-3).

### Ulnar Nerve Entrapment: Cubital Tunnel Syndrome

Entrapment of the ulnar nerve as it passes through the cubital tunnel just posterior to the medial epicondyle of the elbow can manifest with symptoms localized to the ulnar border of the hand. Medial forearm pain and irritability of the ulnar nerve at the elbow may be present as well. Presenting symptoms usually consist of paresthesias, numbness, or both in the small and ring fingers. Percussion of the nerve in the cubital tunnel elicits Tinel's sign. Prolonged elbow flexion reproduces the symptoms. In contrast to CTS, it is not unusual for patients to present with early atrophy of the intrinsics, which is most easily appreciated in the first dorsal interosseous muscle.

Electrodiagnostic studies can help confirm the diagnosis and differentiate cubital tunnel syndrome from more distal compression of the ulnar nerve in Guyon's canal (discussed later). If malalignment of the elbow is present or the patient relates a history of childhood trauma, radiographs should be obtained to rule out a supracondylar or epicondylar malunion. So-called tardy ulnar nerve palsy can develop years after a supracondylar fracture of the elbow.[57]

Conservative treatment includes strategies to help the patient avoid having the elbow flexed for prolonged periods, particularly at night. Soft or semirigid elbow splints prevent elbow flexion beyond 50 to 70 degrees. NSAIDs can be beneficial in acute or traumatic cases. Surgical decompression of the nerve is indicated when a person does not obtain relief from splinting and activity modification or when clinical or electrodiagnostic evidence of muscle denervation is present.

### Ulnar Nerve Entrapment: Guyon's Canal

In 1861 Guyon[58] published a description of the contents of an anatomic canal at the wrist. The distal branches of the ulnar nerve and the ulnar artery pass through this space. As it exits the canal, the ulnar nerve divides into its sensory and motor branches. Compression of the nerve within or proximal to the canal usually manifests with a combination of sensory and motor symptoms in the ulnar nerve distribution. Patients report numbness and paresthesias of the palmar aspect of the ring and small fingers. Motor symptoms are usually described as a cramping weakness with grasping and pinching. As with median neuropathy, atrophy of the intrinsics and objective sensory loss are late findings.

In contrast to carpal tunnel syndrome, in which patients usually have an ill-defined onset of symptoms, ulnar nerve compression in the canal of Guyon is often of more acute onset. It can be associated with repeated blunt trauma,[59-61] a fracture of the hamate or the metacarpal bases, or occasionally a fracture of the distal radius.[62,63] Space-occupying lesions such as a ganglion, lipoma, or anomalous muscle also can cause compression.[64-68] Because of the difference in etiology, this nerve entrapment syndrome often is not amenable to conservative treatment. If an anatomic lesion such as a fracture or a mass is present, it must be addressed. If repetitive blunt trauma is the cause, without associated fracture or arterial thrombosis, splinting and activity modification can alleviate the symptoms.

### Flexor Carpi Radialis and Flexor Carpi Ulnaris Tendinitis

Similar to other tendinopathies around the wrist, irritation of the wrist flexors occurs with stress of the wrist in a particular position. Activities that require forced wrist flexion for prolonged periods or with repetition put patients at risk for inflammation around the flexor carpi radialis tendon,[69] the flexor carpi ulnaris tendon, or both. The condition manifests with tenderness along the course of the tendon, especially near its insertion. Wrist flexion against resistance with radial or ulnar deviation reproduces the symptoms. Treatment consists of splinting and rest, elimination of activities that cause pain, and use of oral NSAIDs. Injection of a corticosteroid into the flexor carpi radialis or flexor carpi ulnaris sheath may be curative. Sharp pain that is associated with an intense inflammatory localized reaction is suggestive of calcific tendinitis and is most commonly seen around the flexor carpi ulnaris tendon.[70,71] If calcific tendinitis is suspected, a plain radiograph can be useful in confirming the diagnosis, but the calcification may not

become apparent until 7 to 10 days after the onset of symptoms.

### Hamate Fracture

An uncommon and underdiagnosed cause of palmar pain in young, active persons is a fracture of the hook of the hamate. These fractures can occur from a fall on an extended wrist, from a "dubbed" golf shot, or from forcefully striking a ball with a club or bat. Plain radiographs of the wrist are usually read as normal. Pain in the base of the palm overlying the hamate is the most common presenting symptom. Often the pain is present only with the activity that caused the fracture. Because of the proximity of the ulnar nerve, patients also can have sensory and motor symptoms of distal ulnar neuropathy. Occasionally, in the acute setting, vascular complaints such as cold intolerance or frank ischemia from ulnar artery thrombosis can be the presenting condition. Fracture of the hamate hook has also been reported to cause rupture of flexor tendons if left untreated.[72]

A carpal tunnel radiographic view, obtained with the wrist in a hyperextended position, may show the fracture (Figure 50-4A). Alternatively, a selective CT scan through the hamate is a more accurate way to confirm the diagnosis (Figure 50-4B).[73] If diagnosed within 2 to 3 weeks of injury, casting should be attempted to allow the fracture to heal.[74] If casting fails to achieve healing or if the fracture is diagnosed late, surgical treatment is indicated; most authors favor excision of the hook, followed by a gradual return to activities.[75-78]

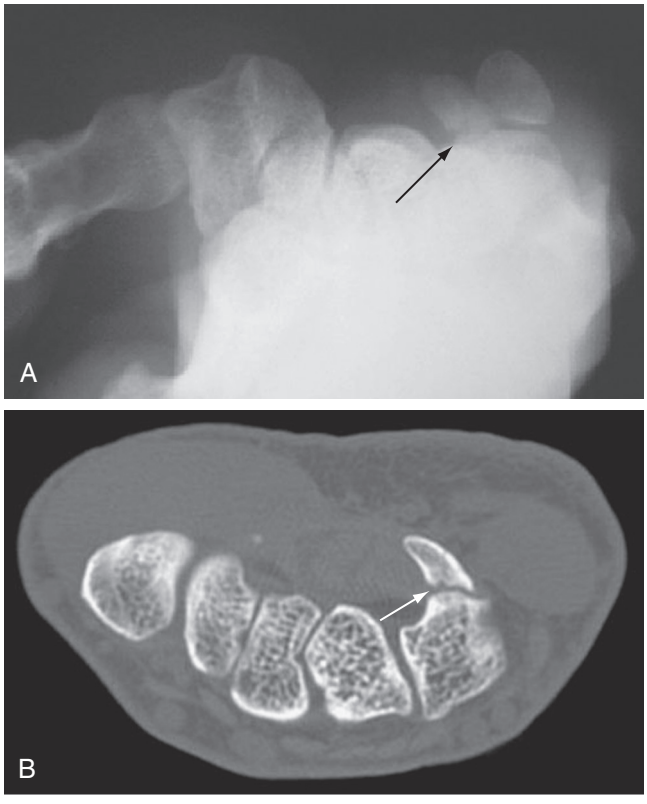



**Figure 50-4** **A,** Carpal tunnel view radiograph showing a hamate hook fracture *(arrow)*. **B,** Coronal computed tomography scan showing the same hamate hook fracture *(arrow)*.

## Wrist Pain: Dorsal

### Ganglion

Ganglia account for 50% to 70% of all soft tissue tumors of the hand and wrist. Of these, 60% to 70% occur around the dorsal wrist. These mucin-filled cysts usually arise from an adjacent joint capsule or tendon sheath. The most common site of origin in the wrist is the scapholunate ligament. Although most ganglia occur as a well-circumscribed and obvious soft mass, some are evident only with the wrist in marked volar flexion. As a result of their characteristic appearance, ganglia usually are not misdiagnosed but should be differentiated from the less well-demarcated swelling of extensor tenosynovitis, lipomas, and other hand tumors. Plain radiographs are typically normal but occasionally show an intraosseous cyst or an osteoarthritic joint. Some ganglia may not be clinically apparent and are known as "occult" ganglia. Ultrasound and MRI have been shown to be useful in the diagnosis of these ganglia.[79,80]

Not all ganglia are painful. Patients may present with reports of wrist weakness or simply because of the cosmetic appearance of the cyst. In approximately 10% of cases, evidence of associated trauma to the wrist is seen. The ganglia may appear suddenly or develop over many months. Intermittent complete resorption followed by reappearance months or years later is common.

Most conservative measures such as splinting and rest have only a temporary effect on ganglia. They tend to diminish in size with rest and enlarge with increased activity. Spontaneous rupture is common, and, at one time, attempting to rupture the cyst with a heavy object, such as a large book, was recommended as treatment. Aspiration can be performed but has mixed results because of the thick gelatinous nature of the fluid within the cyst. Even if adequate decompression of the cyst can be achieved, reaccumulation of the fluid usually occurs. Aspiration in conjunction with irrigation or injection of corticosteroids can be effective in alleviating the symptoms for varying periods.[12,13,81]

Occasionally a ganglion can interfere with the function of the wrist by limiting motion, especially in extension. Pressure of the mass on the terminal branches of the posterior interosseous nerve may be painful. Excision is generally curative but may result in short-term stiffness and some loss of terminal flexion as a result of surgical scarring. Occasionally a patient desires excision of the cyst for cosmetic reasons. With proper excision, recurrence is less than 10%,[82-84] but when the dissection is incomplete and fails to identify the origin of the cyst, recurrence rates can reach 50%. Arthroscopic resection has been shown to be a safe and effective method of treating dorsal wrist ganglia.[23,24]

### Carpal Boss

Often confused with a dorsal ganglion, the carpal boss is a bony, nonmobile prominence on the dorsum of the wrist. It is an osteoarthritic spur that forms at the second or third carpometacarpal joints.[85] The boss is most evident with the wrist in volar flexion. The vast majority are asymptomatic, but some persons may present with pain and localized tenderness over the prominence. The condition is twice as common in women as in men, and most patients are in their

20s to 30s. Radiographs are best taken with the hand and wrist in 30 to 40 degrees of supination and 20 to 30 degrees of ulnar deviation to put the bony prominence on profile (the "carpal boss view").[86] Conservative treatment consists of rest, immobilization, NSAIDs, and occasionally injection with corticosteroids. If persistently painful despite these measures, surgical excision of the boss may be necessary but is associated with a prolonged recovery and continued symptoms in a high percentage of patients.

### Extensor Tendinopathies

The extensor pollicis longus (EPL) tendon can be irritated as it passes around Lister's tubercle. In contrast to other tendinopathies around the wrist, this condition carries a significant risk of tendon rupture. Early diagnosis and sometimes urgent operative treatment are necessary to prevent this complication. Localized pain, swelling, and tenderness are the hallmarks of this condition, and similar to other tendinopathies, initial treatment consists of decreased activity and splinting. A short course of oral anti-inflammatory medication can be useful in decreasing symptoms. Diagnostic injections with lidocaine can help differentiate the condition from other causes of wrist pain, but corticosteroid injections are not routinely used in this condition because of a propensity for the EPL to rupture in chronic cases.

Patients may present with a rupture of the EPL without antecedent pain or swelling. It is well-known that EPL rupture is associated with fractures of the distal radius that likely occur as a result of a relative "watershed zone" of vascular supply within its tight retinacular sheath as it passes around Lister's tubercle of the distal radius. Tendon rupture most often occurs with minimally displaced or non-displaced fractures and can occur several weeks or months after the original injury.[87-90] Persons with RA and systemic lupus erythematosus are especially prone to rupture of the EPL and other tendons.

### Kienböck's Disease

Kienböck's disease is named for Dr. Robert Kienböck,[91] who in 1910 first described what he postulated were avascular changes in the lunate. Nearly a century later, the cause of this disease remains unclear; it is likely multifactorial. Kienböck's disease should be suspected when a young adult presents with pain and stiffness of the wrist and swelling and tenderness around the region of the dorsal lunate. An increased propensity of the disease occurs among patients with an ulna that is anatomically shorter than the radius (so-called ulnar negative variance). Radiographs are needed to confirm and stage the process. Kienböck's disease is staged by the degree of fragmentation and collapse of the lunate, associated osteoarthritis, and carpal collapse in a system originally proposed by Stahl.[92] In this system, the earliest sign of the disease is a linear or compression fracture in the lunate. Later stages show sclerosis of the lunate, followed by lunate collapse and a loss of carpal height. In the final stage, the carpus shows signs of diffuse osteoarthritis with complete collapse and fragmentation of the lunate (Figure 50-5). With the increased sensitivity of MRI, it is possible to identify avascular changes within the lunate before they become evident on plain radiographs. These avascular changes are referred to as "stage zero" Kienböck's disease.

The treatment for Kienböck's disease is largely surgical. Based on the stage of the disease and the postulated cause, several surgical procedures have been described. In early stages of the disease, when lunate collapse is minimal and no osteoarthritis is present, the goal of surgery is to "unload" the lunate by redistributing articular contact forces and allowing it to revascularize.[93-96] The most common procedure is a radial shortening osteotomy that is performed to neutralize the ulnar variance. In later stages, various intercarpal arthrodeses have been used to readjust and maintain carpal height and alignment.[97-99] More recently, microsurgical techniques have been used to revascularize the lunate with promising early results.[100]

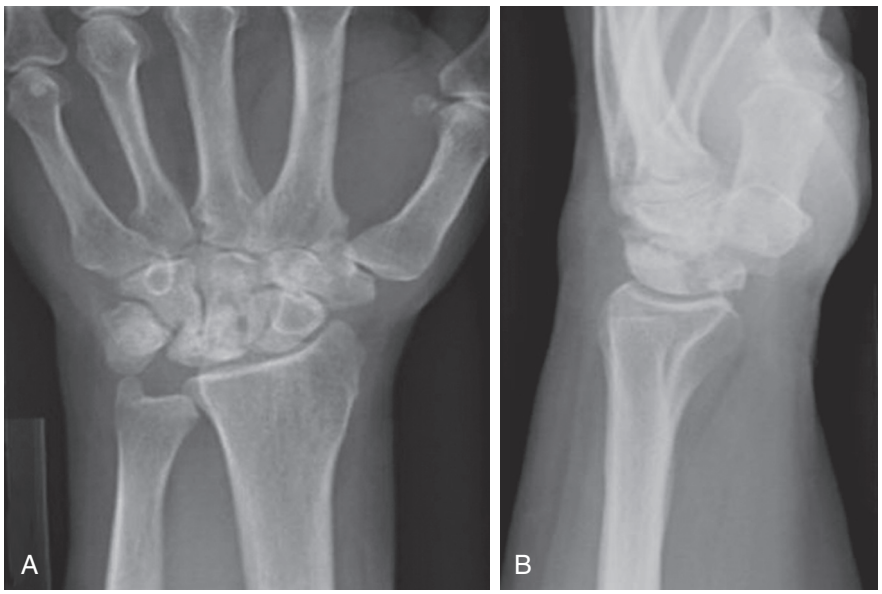



**Figure 50-5** Advanced Kienböck's disease, showing carpal collapse, intercarpal and radiocarpal arthrosis, and fragmentation of the lunate. **A,** Posteroanterior view. **B,** Lateral view.

### Scapholunate Interosseous Ligament Injury

The interosseous ligament between the scaphoid and the lunate is a stout structure, especially dorsally, and usually requires a significant force to cause disruption. The typical mechanism of injury is a fall onto the outstretched hand with the wrist extended. Early diagnosis is essential to prevent the late sequelae of carpal collapse. The key radiographic features of scapholunate dissociation (scapholunate interval widening) are shown in Figure 50-6. The anteroposterior view shows the scapholunate interval better than the posteroanterior view.[101] Early surgical intervention is recommended with the goals of maintaining carpal alignment and preventing an otherwise inevitable progression to carpal collapse and degenerative arthritis.

### Gout and Inflammatory Arthritis

All of the inflammatory arthritides, including the crystal arthropathies, can manifest as dorsal wrist pain. Approximately 25% of patients with a diagnosis of RA present initially with hand and wrist symptoms. The reader is referred to Chapters 94 to 96 for further details.

## Wrist Pain: Ulnar

### Triangular Fibrocartilage Complex Injury and Ulnocarpal Impaction Syndrome

One of the most complex and confusing areas of the wrist from a diagnostic standpoint is the articulation of the ulna with the carpus. The triangular fibrocartilage complex (TFCC), so named by Palmer and Werner,[102] comprises the articular disk itself and the immediately surrounding ulnocarpal ligaments. It can be injured by a variety of acute and chronic mechanisms. Hyperpronation and hypersupination of the carpus during forceful activities are the usual causes of acute injuries, whereas repetitive pronation and supination more often cause attritional changes in the TFCC.

The radius and ulna must remain congruent through a 190-degree arc.[103] Limitation of motion and pain with pronation and supination are consistent with a tear of the supporting ligaments and resultant distal radioulnar joint (DRUJ) instability. If a sufficient portion of the stability has been lost, the ulna appears clinically dislocated or subluxated, and forearm rotation is severely limited. Lateral radiographs of the wrist in neutral and full pronation and supination generally are not specific enough to confirm ulnar subluxation. To evaluate better the congruency of the DRUJ through its range of motion and to assess for subtle subluxations, CT can be performed on both wrists simultaneously in positions of neutral, full pronation, and full supination.[104-107]

Tears of the TFCC may manifest with painful clicking during wrist rotation. Patients generally have localized tenderness on the midaxial border of the wrist and directly beneath the extensor carpi ulnaris tendon. If forced ulnar deviation of the wrist or gripping or both reproduce the patient's symptoms, a degenerative tear of the central portion of the TFCC is more likely. The degenerative tear is frequently a component of the ulnocarpal impaction syndrome, a condition associated with higher than normal loads on the ulnar carpus secondary to a congenitally positive ulnar variance.

Plain radiographs are most useful in determining ulnar variance and for ruling out fractures or arthritis as a cause of ulnar wrist pain. Because of the variable relationship of the radius and ulna depending on forearm rotation, it is important to take standardized films when measuring ulnar variance.[108,109] A posteroanterior view of the wrist with the shoulder abducted to 90 degrees and the elbow flexed to 90 degrees shows the DRUJ in neutral forearm rotation and is easily reproducible (Figure 50-7). Because the ulna lengthens relative to the radius during power grip, a radiograph in



**Figure 50-6** Anteroposterior radiograph of the wrist showing scapholunate interosseous space widening *(arrow)* and scaphoid foreshortening associated with scapholunate interosseous ligament disruption.



**Figure 50-7** Posteroanterior radiograph of the wrist in neutral forearm rotation showing the method of measuring ulnar variance by drawing tangential lines to the distal ulna and distal radius. The space between these lines in millimeters is the ulnar variance. A positive value indicates that the ulnar length is greater than the radial length.

the same position during maximal grip best shows impaction of the ulna on the carpus.

Ancillary studies for TFCC tears include three-compartmental arthrography and MRI. In arthrography, sequential injections of radiopaque dye are performed into the carpal joint, midcarpal joint, and DRUJ. The test is considered positive when the dye is seen leaking from one compartment to another. The site of the leak determines the location of the torn structure.[110] However, several studies have shown that age-related attritional tears occur in the TFCC and other ligamentous structures of the wrist.[111-113] Technologic advancements in MRI have improved the ability to visualize and diagnose abnormalities in the TFCC. MRI can be combined with arthrography to better visualize the TFCC and the intrinsic wrist ligaments. Peripheral detachments and central degenerative tears of the TFCC can be visualized. MRI remains highly operator dependent and technique dependent, and the studies should be interpreted in the context of the findings on physical examination.[114]

Patients presenting with pain localized to the ulnar side of the wrist often respond to simple splinting and rest. This conservative treatment and NSAIDs can be used effectively while a workup is in progress. A course of rest and splinting, followed by a gradual return to activities, may completely alleviate ulnar-sided symptoms.

Despite the advancements in imaging techniques, there is often no substitute for direct visualization of the ulnocarpal joint, DRUJ, or both. Arthroscopy has become an invaluable diagnostic and surgical tool. Tears of the TFCC can be visualized and their clinical significance better determined. Arthroscopy, performed in conjunction with fluoroscopy, can assess for instability of the DRUJ, intercarpal joints, or both. Several surgical procedures can now be performed entirely or in part through the arthroscope.[115,116]

### Extensor Carpi Ulnaris Tendinitis and Subluxation

The extensor carpi ulnaris tendon can become irritated with forced pronation/supination activities, such as putting topspin on a tennis ball. In severe cases the tendon can become unstable around the ulnar head as its restraining dorsal retinaculum becomes increasingly lax. Patients report pain with forceful rotation of the forearm, and sometimes an associated snapping of the extensor carpi ulnaris (ECU) tendon is present. Early treatment consists of immobilization of the wrist and forearm to prevent rotation. Anti-inflammatory oral medication or a cortisone injection can help decrease the inflammation more quickly. After an adequate period of rest, if the acute inflammation resolves but the ECU tendon continues to be unstable, surgery may be indicated to reconstruct or release the sheath at the wrist.

### Pisotriquetral Arthritis

Degenerative changes in the pisotriquetral articulation are usually post-traumatic in nature. Patients may recall a fall onto the extended wrist with direct trauma to the ulnar side of the palm. Affected persons present with pain during passive wrist hyperextension and exacerbation with flexion against resistance. Tenderness and often crepitus occurs with palpation of the pisotriquetral joint. As with many joints, splinting, NSAIDs, and occasionally injection with corticosteroid and lidocaine are the mainstays of conservative treatment. If this approach is inadequate to control the symptoms, surgical resection of the pisiform is indicated.

## Wrist Pain: Radial and Thumb

### De Quervain's Disease and Intersection Syndrome

One of the most common sites of tendon irritation around the wrist is in the first dorsal extensor compartment, a phenomenon known as de Quervain's disease. The tendons involved are the extensor pollicis brevis (EPB) and the abductor pollicis longus (APL). At the level of the radial styloid, these two tendons pass through an osteoligamentous tunnel composed of a shallow groove in the radius and an overlying ligament. Anatomic studies have shown that a high percentage of patients have a divided first dorsal compartment, with separate compartments for the EPB and APL slips, and this feature can account for failure of conservative treatment and injections.[117-119]

Persons with de Quervain's disease are typically women in their 30s and 40s, although the condition can develop in men and women at any age. De Quervain's disease is the most common tendinopathy to develop in postpartum women because of the specific hand and wrist position requirements in the care of an infant. Any activity requiring repeated thumb abduction and extension in combination with wrist radial and ulnar deviation can aggravate this problem. Patients report pain along the course of these tendons with grasping activities. Clinically, tenderness is present along the affected compartment, and swelling may be present over the radial styloid. In severe cases, a creaking sound can be elicited with movement of the involved tendons. Finkelstein's test of forced ulnar deviation of the wrist with the thumb clasped in the fisted palm is pathognomonic of the condition.[120,121]

A less common condition that may occur in the same general location in the wrist is intersection syndrome. Although initially attributed to friction between the first and second dorsal compartment tendons, Grundberg and Reagan[122] subsequently showed that the condition represented a tendinopathy of the radial wrist extensors within the second dorsal compartment.

The primary treatment for de Quervain's disease and intersection syndrome is rest with splinting. For de Quervain's disease, the wrist should be held in slight extension and the thumb abducted in a thumb spica splint to the level of the interphalangeal joint. Immobilization of the wrist alone, in approximately 15 degrees of extension, is usually adequate for intersection syndrome. The addition of a 2- to 4-week course of anti-inflammatory medication also can be helpful. Phonophoresis with a cortisone cream and injection of the compartment with cortisone are second-line treatments if immobilization alone fails to provide adequate relief. Injection of corticosteroid into the affected first dorsal compartment is curative for de Quervain's disease in approximately 75% of patients.[123] Surgery may be indicated for patients who do not respond to a course of conservative treatment, including injection. For de Quervain's disease and intersection syndrome, surgery consists of releasing the stenotic retinacular sheath of the involved compartment.

### Basal Joint Arthropathy

Inflammation and pain related to the carpometacarpal joint of the thumb are common and can occur at any age. In younger patients, instability secondary to ligamentous laxity is associated with joint subluxation and abnormal cartilage wear and may lead to pain with mechanical activities. In women older than 45 years, studies show that 25% have radiographic evidence of degeneration of the basal joint.[124,125] Patients generally present with pain at the base of the thumb that is worsened by pinch activities. They often report difficulty with tasks such as opening jars and bottles or turning doorknobs and keys. The thumb carpometacarpal joint may be swollen and subluxed and is generally tender to palpation. The joint should be assessed for the presence of increased laxity by manual subluxation of the base of the metacarpal out of the trapezial "saddle" with radial and volar force. With advanced degenerative disease, crepitus is sometimes appreciated.

Radiographs should be obtained to determine the stage of the disease. The addition of a basal joint posteroanterior stress film, in which the patient presses the tips of the thumbs together firmly with the nail plates facing up, is helpful in assessing joint subluxation (Figure 50-8). The most commonly used staging system, which was developed by Eaton and Glickel,[126] is based on the degree of involvement of the trapeziometacarpal joint and whether the scaphotrapezial joint is involved.[126] Advancing stages show increased subluxation of the basal joint with development of joint space narrowing, osteophytes, and subchondral cysts.

Regardless of the stage of the disease, the first line of treatment is immobilization of the thumb carpometacarpal joint, leaving the interphalangeal joint free. Splinting has been shown to alleviate the symptoms of carpometacarpal joint inflammation in more than 50% of patients.[127] NSAIDs can be a useful adjunct. Injections of a corticosteroid are effective, usually for just a limited time. Although therapy for thenar muscle strengthening has been advocated, especially in early stages, its benefits are minimal, and occasionally it can aggravate the problem.

Many persons are able to manage their symptoms with a combination of splinting, medications, corticosteroid injections, and activity modification. The most effective splints are those that are custom made of a moldable plastic material. They may be hand based as shown in Figure 50-9 or forearm based to immobilize the wrist as well. If these various nonoperative treatments are insufficient, surgery may be indicated. In persons with advanced degenerative changes and those whose symptoms continue to interfere sufficiently with their daily activities, surgery to replace the joint with a prosthetic device or to excise the trapezium and reconstruct the soft tissue supports is indicated.

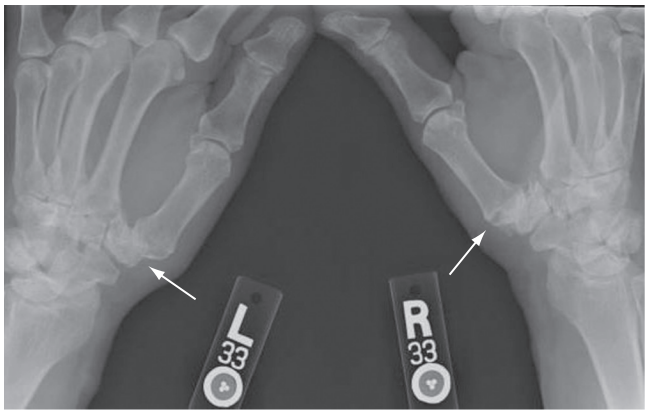



**Figure 50-8** "Basal joint stress" radiograph showing stage 3 degeneration of the left thumb and stage 4 degeneration of the right thumb.

### Thumb Metacarpophalangeal Joint Injuries and Instability

The metacarpophalangeal (MCP) joint of the thumb is primarily stabilized by the radial and ulnar collateral ligaments, which provide radioulnar and dorsal stability.[127a,127b] Ulnar collateral ligament (UCL) injuries result from a sharp radial deviation of the thumb MCP joint, whereas radial collateral ligament (RCL) injuries are generally the product of a sudden adduction of the MCP joint. A complete rupture of the UCL and retraction of the ligament proximal to the adductor aponeurosis is referred to as a "Stener lesion." In this situation, the UCL cannot reapproximate to the anatomic origin of the ligament. Acute UCL injuries are often termed "skier's thumb," whereas chronic attritional ruptures are described as "gamekeeper's thumb." RCL injuries occur less commonly than UCL injuries and are estimated to comprise only 10% to 42% of collateral injuries to the thumb.[127c]

Radiographic evaluation and physical examination remain the mainstays for diagnosis of an RCL or UCL injury. Stress views of the MCP joint can be performed and



**Figure 50-9** Typical hand-based custom-molded splint used in the treatment of symptomatic basal joint arthritis. The thumb interphalangeal joint and wrist are left free to improve the patient's function while wearing the splint.

may demonstrate asymmetry of the joint space. Physical examination, including stress testing of the MCP joint, can determine a partial versus complete tear. Physical examination in the acute setting may be aided by administration of plain lidocaine into the MCP joint to help decrease patient discomfort and anxiety. A 30-degree overall laxity of the affected thumb MCP joint or a 15-degree difference from the contralateral thumb is considered predictive of disruption of the collateral ligament.[127d,127e]

Nonoperative treatment is successful for the majority of acute UCL and RCL tears. Partial tears of the UCL, with a firm endpoint detected on physical examination, can be immobilized for 2 to 6 weeks in a thumb-spica cast or thermoplastic splint and results in a stable, minimally symptomatic joint. Partial or complete tears of the RCL can be similarly treated with immobilization. If gross instability of the MCP joint is present on stress testing with no firm endpoint or if a Stener lesion is suspected, these injuries are best treated with surgical intervention.[127b,127e]

### Volar Ganglion

Another common location for ganglia is the radial side of the volar wrist. Ganglia typically originate from the scaphotrapezial joint but become superficial and are clinically evident at or near the distal wrist crease over the flexor carpi radialis tendon. Volar ganglia can occur in close proximity to the radial artery and should be differentiated from a radial artery aneurysm. Aspiration, if attempted, should be performed carefully to avoid vascular injury, and surgery should be preceded by performance of an Allen test to document patent ulnar arterial flow. Volar ganglia are associated with a higher recurrence rate and a higher complication rate than their dorsal counterparts.[128]

### Scaphoid Fracture with Nonunion

Occasionally a young or middle-aged patient presents with a nonunited scaphoid fracture with no recollection of a traumatic incident. When evaluating a relatively young patient with pain at the base of the thumb, wrist swelling in the region of the anatomic snuffbox, and a decreased range of motion of the wrist, plain radiographs and a specialized ulnar-deviation "navicular" radiograph should be obtained to rule out scaphoid disease. In patients in whom a scaphoid nonunion has been present for a significant period, secondary changes in carpal alignment and joint degeneration have usually occurred. Although treatment with splint or cast immobilization can be attempted, surgical repair of the scaphoid or another wrist salvage procedure is usually required.

### Distal Radius Fractures

Distal radius fractures often occur as a result of a fall on an outstretched hand. Patients with a distal radius fracture generally present with a history of recent trauma with subsequent pain, swelling, ecchymosis, and often an obvious deformity of the affected wrist. The median nerve and carpal tunnel are in close anatomic proximity to the fracture site, and the patient may describe paresthesias in addition to pain. Radiographs of the wrist will demonstrate the degree of displacement and whether the fracture is extra-articular or intra-articular. Although many distal radius fractures can be treated successfully with closed reduction and immobilization (generally performed in the setting of the emergency department or urgent care facility), a fivefold increase in the operative treatment of distal radius fractures in elderly persons from 1996 to 2005 has recently been reported.[128a] The vast majority of distal radius fractures are still treated nonoperatively, but improvements in orthopedic implants have led to an increasing trend toward operative fixation of these injuries.

## Palm

### Trigger Finger

Painful clicking and locking of the digits in flexion is one of the most common causes of pain in the hand. This condition, caused by a thickening of the A1 retinacular pulley in the palm, is commonly known as *trigger finger*. The thumb is the most commonly affected digit, followed by the ring and long fingers.[129] Patients may present with isolated activity-related pain in the proximal interphalangeal joint without frank clicking or locking. Early clicking is felt as a snapping sensation during digital motion and is frequently worse upon awakening. As the condition progresses, the digital range of motion can be reduced and secondary proximal interphalangeal joint contractures develop. The final stage is a locked trigger finger that cannot be straightened actively.

Primary trigger finger is the most common type and is found most often in middle-aged persons. Triggering of the thumb is four times more frequent in women than in men.[5] Secondary triggering is seen in association with such diseases as RA, diabetes, and gout. In this type, trigger fingers are often multiple and can co-exist with other stenosing tendinopathies, such as de Quervain's disease or CTS. Congenital or developmental triggering can be identified in children and is much less common. Similar to its presentation in adults, the thumb is most commonly affected, but in contrast to adults, triggering often presents with the interphalangeal joint locked in flexion.

Nonoperative treatment of this condition consists primarily of splinting and local steroid injections. Splinting is most effective at night to prevent the digit from locking. In adults, injection of steroid into the tendon sheath has been shown to be quite effective (Figure 50-10).[5,6,130] Injection is used infrequently in infants or children. When nonoperative treatments fail to give lasting relief, surgical treatment consists of longitudinal division of the A1 pulley at the level of the metacarpal head, which is a simple procedure that yields reliable results with few complications.

### Retinacular Cysts

Retinacular ganglion cysts can occur in conjunction with a triggering digit or in isolation. These cysts are located at the base of the digit over the A1 pulley as a discrete, firm, pea-sized nodule. They originate from the flexor tendon sheath or annular pulleys and contain synovial fluid. Patients usually report pain when gripping objects or with direct pressure over the cyst. A retinacular cyst is most easily

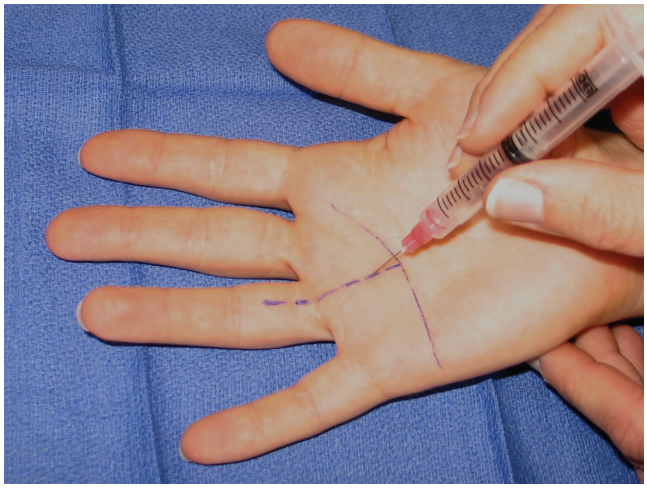


**Figure 50-10** Technique for injecting a trigger finger. The *solid line* denotes the distal palmar crease. The *dashed line* indicates the midline of the digit. The needle enters at an angle of between 45 and 60 degrees.

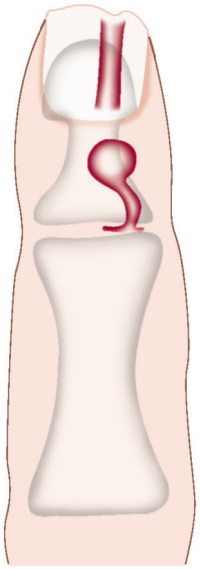


**Figure 50-11** Dorsal view of a digit with an as yet clinically inapparent mucous cyst and the corresponding groove deformity of the nail plate.

treated initially by needle decompression, with care to avoid injury to the sensory nerves that lie immediately adjacent to the flexor tendon and associated cyst. Approximately 50% recur after aspiration, and surgical resection may be required.

## Digits

### Mallet Finger

Mallet finger refers to a loss of terminal extension of the distal interphalangeal joint of the digit and can be classified as bony or soft tissue depending on where the disruption in the extensor mechanism occurred. Mallet fingers can occur with minimal trauma, such as tucking in bed sheets, and may not be recalled by the patient, which sometimes leads to a delay in diagnosis and treatment. When a person presents with a digit that droops at the distal interphalangeal joint and cannot be actively extended but has full passive motion, a radiograph should be obtained to determine if there is an associated fracture of the distal phalanx. An extension splint is the treatment of choice for bony and soft tissue mallet fingers. The distal interphalangeal (DIP) joint should be held in full extension, and care should be taken not to force the DIP joint into hyperextension to prevent dorsal skin ischemia and necrosis. Splinting is performed full time for 6 to 8 weeks. The patient should not remove the splint for showering or any other activity but may change the splint carefully for skin care, provided that the joint is maintained in extension. Proximal interphalangeal flexion exercises are initiated from the outset and are important to help reset the tension in the extensor mechanism. Gentle DIP flexion exercises are begun at 8 weeks, and splinting is decreased to nighttime between 8 and 10 weeks. Patients usually can expect a small extension lag, on the order of 5 degrees, and a return of most of their flexion.

### Osteoarthritis of the Digits

Osteoarthritis of the interphalangeal (IP) joints is extremely common in older persons and is most often manifested as Heberden's nodes of the DIP joint. Despite gross deformities, pain and dysfunction may be minimal. A mucous cyst may appear in association with degenerative arthritis. Mucous cysts appear on the dorsum of the joint and can cause nail growth deformities as a result of pressure on the germinal matrix (Figure 50-11). The changes in nail growth may precede clinical detection of the cyst. These cysts should not be aspirated with a needle because of the close proximity of the DIP joint and the risk of secondary joint infection. Treatment consists of DIP joint immobilization to control symptoms or surgical excision of the cyst and, in particular, the underlying osteophytic spurs.

### Tumors

Benign bone tumors such as simple bone cysts and enchondromas are common in the phalanges. These tumors usually cause no symptoms and frequently are diagnosed as incidental findings on routine hand radiographs. Enchondromas are most commonly located in the metaphysis of the proximal phalanx and may lead to fracture with minimal trauma as a result of weakening of the bone structure. If a pathologic fracture occurs, nonoperative treatment is indicated until the fracture heals. The bone tumor subsequently can be addressed with curettage and bone grafting. Occasionally, because of malalignment, earlier surgical intervention becomes necessary.

Many soft tissue tumors can occur in the hand and digits. Some common benign tumors are giant cell tumors of the tendon sheath, lipomas, and glomus tumors. Lipomas and giant cell tumors of the tendon sheath manifest clinically as painless, slow-growing masses in the palm and digits. Surgical excision is necessary for diagnosis. Glomus tumors arise from the pericytes in the fingertip or subungual area and typically present with intermittent sharp pain in the fingertip. These vascular tumors become intensely symptomatic when the hand is exposed to cold temperatures because of abnormal arteriovenous shunting through the

hypertrophic glomus system. Surgical excision is generally curative and should be preceded by MRI to rule out multifocal sites.

### Infection

The most common infection in the hand is the paronychia. It involves the fold of tissue surrounding the fingernail. *Staphylococcus aureus* is the usual pathogen, introduced by a hangnail, a manicure instrument, or nail biting. Patients present with an exquisitely painful and erythematous swelling involving a part of the nail fold. Occasionally, the infection can progress to surround the nail in a horseshoe fashion and undermine the nail plate. If seen early, within the first 24 to 48 hours, oral antibiotics and local treatment of the finger with warm soaks can be effective. Superficial abscesses can be drained with a sharp blade through the thin skin without requiring local anesthesia. Larger or more chronic infections require surgical drainage.

An infection of the distal pulp of the fingertip, known as a *felon*, is a particular problem in diabetic patients. This infection differs from other subcutaneous infections because of the vertical fibrous septa that divide and stabilize the pulp of the fingertip. Often patients have had some recent penetrating injury in the area. Because of the tightly constrained area of the infection, patients present with an intensely painful fingertip. There may be an area of "pointing" over the abscess. Surgical drainage is required, followed by soaks and oral antibiotics, and intravenous antibiotics are generally recommended in diabetic patients.

Although similar in appearance to a paronychia, herpetic whitlow is caused by herpes simplex virus and must be differentiated from other fingertip infections because of a radically different treatment protocol.[131,132] Whitlow was common among dental hygienists before the widespread use of gloves for all health care workers. It is now most commonly seen in children. As with bacterial infections, the area becomes painful and erythematous; local tenderness is much less severe, however. Diagnosis is ascertained by clinical presentation and history. Vesicles that are seen early can be ruptured for fluid analysis and viral culture. Nonoperative treatment with oral anti-viral agents is recommended.

Other hand and digit infections, such as suppurative flexor tenosynovitis, deep space infections of the palm, pyogenic arthritis, infections from bite wounds, and osteomyelitis, should be evaluated initially with radiographs of the hand and appropriate blood work. If possible, antibiotics should be withheld until definitive cultures are obtained from the affected area. Antibiotics should be administered intravenously, and the hand and wrist should be immobilized. Most infections of this nature require surgical drainage for definitive treatment.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 51



# Temporomandibular Joint Pain

Daniel M. Laskin

**KEY POINTS**

- Temporomandibular joint (TMJ) pain must be distinguished from pain that more commonly arises from the muscles of mastication (myofascial pain), which can produce similar signs and symptoms.
- TMJ pain also must be distinguished from pain that originates in the ear or parotid gland.
- TMJ pain and masticatory muscle pain usually are accompanied by limitation of mouth opening, but pain arising from the ear or parotid gland is not.
- Most major systemic arthropathies can also involve the TMJ and thereby give rise to pain and limited jaw movement.
- Displacement of the intra-articular disk in the TMJ produces pain that is accompanied by a clicking or popping sound or the sudden onset of jaw locking.

Pain in the temporomandibular joint (TMJ) region is a commonly encountered symptom that affects more than 10 million people in North America, and is universally recognized across populations worldwide as a significant health care burden. Because of its diverse causes, however, proper diagnosis and treatment may be difficult to achieve. Because of the proximity of the ear and parotid gland and the similar nature of pain arising from these areas, pathologic conditions involving these structures are often confused with conditions arising in the TMJ. Pain that occurs in the adjacent muscles of mastication, also a frequently encountered situation, not only is similar to TMJ pain in character and location, but also is associated with jaw dysfunction, a common finding with painful conditions directly involving the TMJ. For these reasons, knowledge of the various painful conditions occurring in the TMJ region is essential in establishing a correct diagnosis.

Because patients with primary TMJ disease often have secondary myofascial pain in the muscles of mastication, and because secondary TMJ disease can develop in patients with primary myofascial pain, the generally accepted term used to describe this overlapping group of conditions is *temporomandibular disorders*. These conditions are subdivided for purposes of diagnosis and treatment into conditions that primarily involve the TMJ (TMJ problems) and conditions that primarily involve the muscles of mastication (myofascial pain and dysfunction [MPD], masticatory myalgia). From a diagnostic standpoint, it is important to consider the numerous conditions that mimic the temporomandibular disorders or MPD by producing similar signs and symptoms (Tables 51-1 and 51-2).

Table 51-3 lists the various pathologic entities that commonly involve the TMJ. Although a variety of conditions are known, only three types are considered to generally produce pain: the various arthritides, derangements of the intra-articular disk, and certain neoplasms.

## ARTHRITIS OF THE TEMPOROMANDIBULAR JOINT

Arthritis is the most common painful condition affecting the TMJ. Although osteoarthritis and rheumatoid arthritis are encountered most frequently, cases of infectious arthritis, metabolic arthritis, and TMJ involvement as part of the spondyloarthropathies are also seen in practice. Traumatic arthritis is another relatively common occurrence.

### Osteoarthritis

Osteoarthritis is the most common type of arthritis involving the TMJ and the most frequent cause of pain in that region. Clinical symptoms of the disease have been reported in 16% of the general population,[1] but radiographic features have been found in 44% of asymptomatic individuals.[2] Although the TMJ is not a weight-bearing joint in the same sense as the joints of the long bones, the stresses associated with such parafunctional habits as clenching and grinding of the teeth are sufficient to contribute to similar degenerative changes in some patients.[3] Acute and chronic trauma and derangements of the intra-articular disk also are common causes of secondary degenerative arthritis.

#### Clinical Findings

Primary osteoarthritis, which usually is seen in older individuals, is insidious in its onset; it generally produces only mild discomfort, and individuals rarely complain about the condition. Secondary osteoarthritis usually occurs in younger patients (20 to 40 years old) and tends to be painful. In contrast to primary degenerative joint disease and rheumatoid arthritis, it often is limited to only one TMJ, although it may become bilateral in the late stages, and involvement of other joints is uncommon. The condition is characterized by TMJ pain that is increased by function, joint tenderness, limitation of mouth opening, and occasional clicking and popping sounds. In the late stages, crepitation may be noted in the joint.

**TABLE 51-1** Differential Diagnosis of Nonarticular Conditions Mimicking Temporomandibular Joint Pain or Myofascial Pain in the Masticatory Muscles

| Disorder | Jaw Limitation | Muscle Tenderness | Diagnostic Features |
|---|---|---|---|
| Pulpitis | No | No | Mild to severe ache or throbbing; intermittent or constant; aggravated by thermal change; eliminated by dental anesthesia; positive radiographic findings |
| Pericoronitis | Yes | Possible | Persistent mild to severe ache; difficulty swallowing; possible fever; local inflammation; relieved with dental anesthesia |
| Otitis media | No | No | Moderate to severe earache; constant pain; fever; usually history of upper respiratory infection; no temporomandibular joint tenderness |
| Parotitis | No | No | Constant aching pain, worse when eating; feeling of pressure in ears; absent salivary flow; ear lobe elevated; suppuration from duct |
| Sinusitis | No | No | Constant aching or throbbing; worse with change of head position; nasal discharge; often maxillary molar pain not relieved by dental anesthesia |
| Trigeminal neuralgia | No | No | Sharp stabbing pain of short duration; trigger zone; pain follows nerve pathway; older patient age group; often relieved by dental anesthesia |
| Atypical (vascular) neuralgia | No | No | Diffuse throbbing or burning pain of long duration; often associated autonomic symptoms; no relief with dental anesthesia |
| Temporal arteritis | No | No | Constant throbbing preauricular pain; artery prominent and tender; low-grade fever; may have visual problems; elevated erythrocyte sedimentation rate |
| Trotter's syndrome | Yes | No | Aching pain in ear, side of face, and lower jaw; deafness; nasal obstruction; cervical lymphadenopathy |
| Eagle's syndrome | No | No | Mild to sharp stabbing pain in ear, throat, and retromandible; provoked by swallowing, turning head, carotid compression; usually post tonsillectomy; styloid process >2.5 cm |

Modified from Laskin DM, Block S: Diagnosis and treatment of myofascial pain dysfunction (MPD) syndrome. *J Prosthet Dent* 56:75–84, 1986.

**TABLE 51-2** Differential Diagnosis of Nonarticular Conditions Producing Limitation of Mandibular Movement

| Disorder | Jaw Limitation | Muscle Tenderness | Diagnostic Features |
|---|---|---|---|
| Odontogenic infection | Yes | Yes | Fever; swelling; positive radiographic findings; tooth tender to percussion; pain relieved and movement improved with dental anesthesia |
| Nonodontogenic infection | Yes | Yes | Fever; swelling; negative dental findings on radiograph; dental anesthesia may not relieve pain or improve jaw movement |
| Myositis | Yes | Yes | Sudden onset; jaw movement associated with pain; areas of muscle tenderness; usually no fever |
| Myositis ossificans | No | No | Palpable nodules seen as radiopaque areas on radiograph; involvement of nonmasticatory muscles |
| Neoplasia | Possible | Possible | Palpable mass; regional nodes may be enlarged; may have paresthesia; radiograph may show bone involvement |
| Scleroderma | No | No | Skin hard and atrophic; mask-like facies; paresthesias; arthritic joint pain; widening of periodontal ligament |
| Hysteria | No | No | Sudden onset after psychological trauma; no physical findings; jaw opens easily under general anesthesia |
| Tetanus | Yes | No | Recent wound; stiffness of neck; difficulty swallowing; spasm of facial muscles; headache |
| Extrapyramidal reaction | No | No | Patient on antipsychotic drug or phenothiazine tranquilizer; hypertonic movement; lip smacking; spontaneous chewing motions |
| Depressed zygomatic arch | Possible | No | History of trauma; facial depression; positive radiographic findings |
| Osteochondroma coronoid | No | No | Gradual limitation; jaw may deviate to unaffected side; possible clicking sound on jaw movement; positive radiograph findings |

Modified from Laskin DM, Block S: Diagnosis and treatment of myofascial pain dysfunction (MPD) syndrome. *J Prosthet Dent* 56:75–84, 1986.

### Imaging Findings

The earliest radiologic feature of osteoarthritis of the TMJ, whether primary or secondary, is subchondral sclerosis in the mandibular condyle. If the condition progresses, condylar flattening and marginal lipping may be noted. In the later stages, erosion of the cortical plate, osteophyte formation, or both may occur. Breakdown of the subcortical bone occasionally may result in the formation of so-called *bone cysts*. Although changes in the articular fossa generally are not as severe as changes in the condyle, cortical erosion sometimes can be seen. Narrowing of the joint space also

**TABLE 51-3** Differential Diagnosis of Temporomandibular Joint (TMJ) Diseases

| Disorder | Jaw Limitation | Muscle Tenderness | Diagnostic Features |
|---|---|---|---|
| Agenesis | No | Yes | Congenital; usually unilateral; mandible deviates to affected side; unaffected side long and flat; severe malocclusion; often ear abnormalities; radiograph shows condylar deficiency |
| Condylar hypoplasia | No | No | Congenital or acquired; affected side has short mandibular body and ramus, fullness of face, deviation of chin; body of mandible elongated and face flat on unaffected side; malocclusion; radiograph shows condylar deformity, antegonial notching |
| Condylar hyperplasia | No | No | Facial asymmetry with deviation of chin to unaffected side; cross-bite malocclusion; prognathic appearance; lower border of mandible often convex on affected side; radiograph shows symmetric enlargement of condyle |
| Neoplasia | Possible | Yes | Mandible may deviate to affected side; radiographs show enlarged, irregularly shaped condyle or bone destruction, depending on type of tumor; unilateral condition |
| Infectious arthritis | Yes | No | Signs of infection; may be part of systemic disease; radiograph may be normal early, later can show bone destruction; fluctuance may be present; pus may be obtained on aspiration; usually unilateral |
| Rheumatoid arthritis | Yes | Yes | Signs of inflammation; findings in other joints (hands, wrists, feet, elbows, ankles); positive laboratory test results; retarded mandibular growth in children; anterior open bite; radiograph shows bone destruction; usually bilateral |
| Spondyloarthropathies | | | |
| Psoriatic arthritis | Yes | Yes | Presence of cutaneous psoriasis; nail dystrophy; involvement of distal interphalangeal joints; radiograph shows condylar erosion; negative for rheumatoid factor |
| Ankylosing spondylitis | Yes | Yes | Frequent involvement of the spine and sacroiliac joint; extra-articular manifestations of spondylitis include iritis, anterior uveitis, aortic insufficiency, and conduction defects; erosive condylar changes; TMJ ankylosis may occur |
| Metabolic arthritis | | | |
| Gout | Yes | Yes | Usually sudden onset; often monoarticular; commonly involves great toe, ankle, and wrist; joint swollen, red, and tender; increased serum uric acid; late radiographic changes |
| Pseudogout | Yes | Yes | Generally unilateral; TMJ may be only joint involved; joint frequently swollen; presence of intra-articular calcification; may be a history of trauma |
| Traumatic arthritis | Yes | Yes | History of trauma; radiograph normal except for possible widening of joint space; local tenderness; usually unilateral |
| Degenerative arthritis | Yes | Yes | Unilateral joint tenderness; often crepitus; TMJ may be only joint involved; radiograph may be normal or show condylar flattening, lipping, spurring, or erosion |
| Ankylosis | Yes | Yes | Usually unilateral, but can be bilateral; may be history of trauma; young patient may show retarded mandibular growth; radiographs show loss of normal joint architecture |
| Internal disk derangement | Yes | Yes | Pain exacerbated by function; clicking on opening or opening limited to <25 mm with no click; positive MRI findings; may be history of trauma; usually unilateral |

Modified from Laskin DM, Block S: Diagnosis and treatment of myofascial pain dysfunction (MPD) syndrome. *J Prosthet Dent* 56:75–84, 1986.

occurs in the late stages; this is indicative of concomitant degenerative changes and thinning of the intra-articular disk. Although changes in the TMJ usually can be seen on plain radiographs, sagittal and coronal CT scans are the preferred modality to image the bony structures.

## Diagnosis

The diagnosis of osteoarthritis is made on the basis of the patient's history and the clinical and radiographic findings. The patient often reports a history of trauma or parafunctional oral habits. Involvement is generally unilateral, and no significant changes are observed in any of the other joints. The pain tends to be well localized, and the TMJ is often tender to palpation.

## Treatment

Treatment of degenerative arthritis of the TMJ is usually medical, as in other joints of the body. Treatments involve patient use of nonsteroidal anti-inflammatory drugs, application of heat, eating a soft diet, limitation of jaw function, and use of a bite appliance to control parafunction if the patient has a chronic habit of clenching or grinding the teeth. Arthrocentesis has also been shown to be helpful.[4,5] Physical therapy with thermal agents, ultrasound, and iontophoresis also can be beneficial, and isotonic and isometric exercises are used to improve joint stability after the acute symptoms have subsided. The use of intra-articular steroid injections is controversial; they should be used only in patients with acute symptoms that do not respond to other

forms of medical management. Because of the potentially damaging effects of multiple steroid injections,[4,6] they should be limited to no more than three or four single injections given at 3-month intervals. Intra-articular injection of high-molecular-weight sodium hyaluronate given twice, 2 weeks apart, has been shown to have essentially the same therapeutic effect as a steroid injection, without the potential adverse effects.[5,7]

When the acute symptoms have been controlled, therapy is directed toward control of factors that might contribute to the degenerative process. Unfavorable loading of the joint is eliminated by replacement of missing teeth to establish a good, functional occlusion; correction of any severe dental malrelationships through orthodontics or orthognathic surgery; and continued use of a bite appliance at night to control teeth-clenching or teeth-grinding habits.[6,8]

For patients in whom medical management for 3 to 6 months fails to relieve the symptoms, surgical management may be indicated. Surgery involves removal of the minimal amount of bone necessary to produce a smooth articular surface. Unnecessary removal of the entire cortical plate, as occurs with the so-called *condylar shave* procedure or high condylotomy, can lead to continuation of the resorptive process in some instances, and should be avoided if possible.

## Rheumatoid Arthritis

More than 50% of patients with rheumatoid arthritis have involvement of the TMJ.[9] Although the TMJ may be affected early in the course of the disease, other joints in the body usually are involved first. The approximate female-to-male ratio is 3:1. TMJ involvement may also characterize juvenile inflammatory arthritis. In children, destruction of the mandibular condyle by the disease process results in growth retardation and facial deformity characterized by a severely retruded chin. Fibrous or bony ankylosis is a possible sequel at all ages.

### Clinical Findings

Patients with rheumatoid arthritis of the TMJ have bilateral pain, tenderness, swelling in the preauricular region, and limitation of mandibular movement. These symptoms are characterized by periods of exacerbation and remission. Joint stiffness and pain are usually worse in the morning and decrease during the day. The limitation in mandibular movement worsens as the disease progresses; an anterior open bite may also develop in the patient.

### Imaging Findings

Although radiographic changes may not be noted in the early stages of the disease, approximately 50% to 80% of patients show bilateral evidence of demineralization, condylar flattening, and bone erosion while the disease progresses, so the articular surface appears irregular and ragged. Erosion of the glenoid fossa also might also be seen. Narrowing of the joint space is caused by destruction of the intra-articular disk. With continued destruction of the condyle, loss of ramus height can lead to contact of only the posterior teeth and an anterior open bite. Increasingly, MRI is used as an additional modality for the detection of articular damage, synovitis, or both, or of involvement of adnexal structures.

### Diagnosis

Rheumatoid arthritis is diagnosed on the basis of the patient's history, clinical and radiographic findings, and confirmatory laboratory tests. Distinguishing features for rheumatoid arthritis and degenerative arthritis of the TMJ are shown in Table 51-3.

### Treatment

Treatment of rheumatoid arthritis of the TMJ is similar to that provided for other joints.[7,10] Anti-inflammatory drugs are used during the acute phases, and mild jaw exercises are used to prevent excessive loss of motion when acute symptoms subside. In severe cases, disease-modifying drugs, such as methotrexate, and biologic agents, including TNF inhibitors, abatacept, tocilizumab, and rituximab, may be used pending systemic presentation. Orthognathic surgery may be necessary in patients with an anterior open bite after the disease goes into remission, or in patients in whom ankylosis develops after that condition is corrected.

## Spondyloarthropathies

In addition to the adult and juvenile forms of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and reactive arthritis also can involve the TMJ.[8-13]

### Psoriatic Arthritis

Psoriatic arthritis occurs in approximately one-third of patients who have cutaneous psoriasis (see Chapter 77). This disorder has a sudden onset, can be episodic in nature, and may show spontaneous remission.[9,12] Often only one TMJ is involved. Symptoms include TMJ pain and tenderness, restricted jaw movement, and crepitation, mimicking the symptoms of rheumatoid arthritis.[9,12] Radiographic changes are nonspecific and cannot be distinguished easily from those of other types of arthritis, particularly rheumatoid arthritis and ankylosing spondylitis.[13,14] They usually involve erosive changes in the condyle and glenoid fossa associated with extreme narrowing of the joint space.[11,15,16] In severe cases, ankylosis may develop, reflected occasionally in new bone formation at earlier stages.[12,17]

The physician usually diagnoses psoriatic arthritis on the basis of the triad of psoriasis, radiographic evidence of erosive arthritis, and a negative serologic test for rheumatoid factor. Even in the presence of a rash, however, the diagnosis cannot be absolutely confirmed. The differential diagnosis always should include rheumatoid arthritis, reactive arthritis, ankylosing spondylitis, and gout.

Treatment of psoriatic arthritis of the TMJ is as described in Chapter 77 and is driven essentially by the imperative to treat the systemic inflammatory disease process.[13,18-21] Surgery is necessary if ankylosis occurs.

### Ankylosing Spondylitis

TMJ involvement develops in approximately one-third of patients with ankylosing spondylitis several years after onset of the disease. Pain and limitation of jaw movement are the most common symptoms, and ankylosis can develop in advanced cases.[8,11,14,22] On radiographic examination, approximately 30% of patients show erosive changes in the condyle and fossa and narrowing of the joint space.[15,23] In long-standing cases, a more florid osteophytic response is sometimes seen during quiescent periods. The severity of the changes seems to be related to the severity of the disease. Treatment of ankylosing spondylitis of the TMJ is generally medical and is part of the total management of the patient. Physical therapy is used to improve jaw mobility, and a bite appliance is used, when indicated, to reduce parafunctional stress on the joint. If ankylosis develops, surgery is the treatment of choice.[24]

### Reactive Arthritis

Reactive arthritis of the temporomandibular joint is more common in males than in females. It is characterized by recurrent pain, swelling, and limitation of mouth opening.[25] Radiographically, condylar erosion may be evident.[26] Treatment is similar to that of the other seronegative spondyloarthropathies, consisting of nonsteroidal anti-inflammatory drugs, intra-articular steroids, and disease-modifying drugs. If a specific triggering bacterial infection can be identified, an appropriate antibiotic should be prescribed.

## Traumatic Arthritis

Acute trauma to the mandible that does not result in a fracture can still produce injury to the TMJ. When this occurs in a child, it is essential to warn the parents about the possibility of future retardation of mandibular growth and associated facial deformity resulting from damage to the articular cartilage, which is an important growth site.[16,27]

Traumatic arthritis is characterized by TMJ pain and tenderness and limitation of jaw movement. The resultant inflammation and occasional hemarthrosis can lead to loss of tooth contact on the affected side. Frequently, bruises or lacerations are apparent at the site of the initial injury. No radiographic changes may be seen, or widening of the joint space may be produced by intra-articular edema or hemorrhage. In some instances, radiographs may show an intracapsular fracture that was not recognized on clinical examination.

Treatment of traumatic arthritis consists of the use of nonsteroidal anti-inflammatory drugs, application of heat, a soft diet, and initial restriction of jaw movement. When acute symptoms subside, range-of-motion exercises should be used to avoid fibrous ankylosis.

## Infectious Arthritis

Infectious arthritis rarely involves the TMJ. Although it can affect the joint as part of such systemic diseases as gonorrhea, syphilis, tuberculosis, and Lyme disease,[17,18,28,29] the most common way is by direct extension of an adjacent infection of dental, parotid gland, or otic origin.[19,30] Occasionally, it also may occur from localization of blood-borne organisms in the joint after a traumatic injury or by direct involvement through a penetrating wound.[20,30] The most common pathogens are *Staphylococcus aureus*, *Haemophilus influenzae*, and *Streptococcus* species.[31]

### Clinical Findings

Infectious arthritis generally results in unilateral pain, tenderness, swelling, and redness in the region of the TMJ. Chills, fever, sweating, and systemic findings characteristic of the specific type of infection also are present. Often the patient's teeth cannot be occluded because of swelling within the joint. In pyogenic forms of infectious arthritis, fluctuation may be noted in the joint region. Patients with Lyme disease show characteristic skin lesions and, often, positive serology.[18,29]

### Imaging Findings

Radiographic findings are usually normal in early stages of the disease because of lack of bony involvement, but the intra-articular accumulation of pus or inflammatory exudate may cause separation of articulating surfaces, which can be detected on MRI. Later, depending on the severity and chronicity of the infection, varying degrees of bony destruction, ranging from damage to the articular surface of the mandibular condyle to extensive osteomyelitis, may be seen. In the late stages, fibrous or bony ankylosis may occur. In children, infectious arthritis can affect growth of the condyle, resulting in facial asymmetry.

### Treatment

Treatment of infectious arthritis includes the use of appropriate antibiotics, proper hydration, control of pain, and limitation of jaw movement. Arthrocentesis with Ringer's solution one to three times weekly until acute symptoms subside has also been recommended.[32] Suppurative infections may require aspiration, incision and drainage, or sequestrectomy. When bone loss is extensive, reconstructive procedures may be necessary. In children in whom mandibular growth has been affected, a costochondral graft can be used to correct facial asymmetry and re-establish growth of the mandible.

## Metabolic Arthritis

Metabolic arthritis, which can accompany gout or pseudogout (calcium pyrophosphate dehydrate arthropathy), is rare in the TMJ.[21,33]

### Gout

Gouty arthritis of the TMJ occurs most frequently in men older than 40 years and usually is preceded by involvement of one or more joints of the feet or hands. The attack usually occurs suddenly, and the joint becomes swollen, painful, red, and tender. Recovery may occur in a few days, and remission can last for months to years.

When attacks are infrequent, radiographic changes may not be noted for a long time. Because so few cases have been

reported, the precise radiographic changes that occur have not been well documented. Calcified areas in the disk, destruction of the hard tissues of the joint, condylar exostoses and spurring, and the presence of tophi have been described.[21,33-35] The initial approach to treatment of gout involving the TMJ is medical. If symptoms are not controlled, however, surgical débridement of the joint and arthroplasty may be indicated.

### Pseudogout

Calcium pyrophosphate dehydrate arthropathy (pseudogout) in the TMJ clinically mimics gout, and the mandibular condyle may show degenerative and erosive changes radiographically. In the primary form, which usually is seen in older patients, intra-articular calcification is noted (chondrocalcinosis), and diffuse calcification occurs in the intra-articular disk.[21-25,36-39] Similar changes are seen in the secondary form, but it occurs in younger patients and frequently is preceded by a history of trauma. Fine-needle aspiration can help distinguish pseudogout from gout. In gout, under polarizing microscopy, the crystals are needle shaped and there is negative birefringence; in pseudogout the crystals are rhomboid/rod shaped and weakly birefringent.

Just as in gout of the TMJ, the initial treatment of pseudogout is medical, and surgery is reserved for patients in whom such treatment is ineffective.

## INTERNAL DERANGEMENTS

Internal derangements are a common cause of pain in the TMJ. They represent a disturbance in the normal anatomic relationship between the intra-articular disk and the condyle, resulting in interference with the smooth movement of the joint.

### Clinical Findings

Three stages of internal derangement have been identified: (1) a painless inco-ordination phase, in which a momentary catching sensation is felt during mouth opening; (2) anterior disk displacement with reduction into the normal position during mouth opening, which is characterized by a clicking or popping sound (Figure 51-1); and (3) anterior disk displacement without reduction on attempted mouth opening, which is characterized by restriction of jaw movement, or locking (Figure 51-2). Joint pain in patients with anterior disk displacement, with or without reduction, is caused by condylar compression of the highly innervated retrodiskal tissue that occupies the glenoid fossa while the intra-articular disk assumes a more forward position, and by the accompanying inflammation.

### Etiology

The three main causes of internal derangement of the intra-articular disk are trauma, abnormal functional loading of the joint, and degenerative joint disease.[26,40] Although some clinicians believe that occlusal factors also play a role in causing internal derangements, no conclusive studies have shown such a relationship.

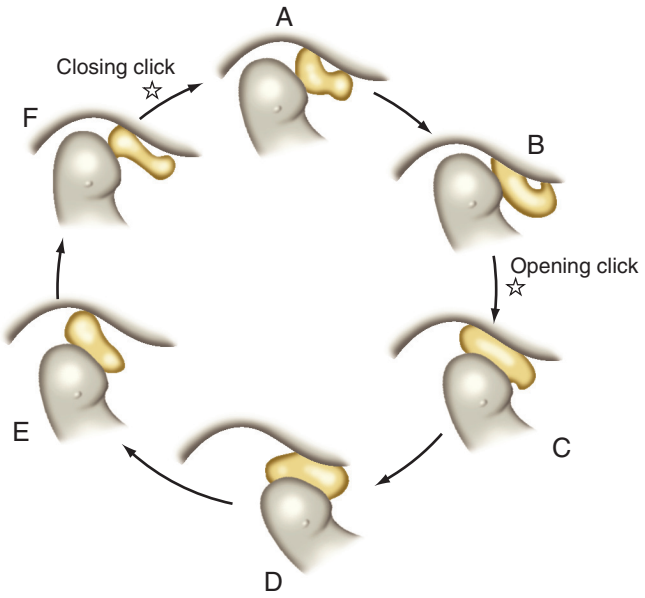



**Figure 51-1** Anterior displacement of the intra-articular disk with reduction on opening of the mouth. A clicking or popping sound occurs as the disk returns to its normal position in relation to the condyle. During closure, the disk again becomes anteriorly displaced, sometimes accompanied by a second sound (reciprocal click). *(Modified from McCarty W: Diagnosis and treatment of internal derangements of the articular disc and mandibular condyle. In Solberg WK, Clark GT, editors:* Temporomandibular joint problems: biologic diagnosis and treatment, *Chicago, 1980, Quintessence, p 155.)*

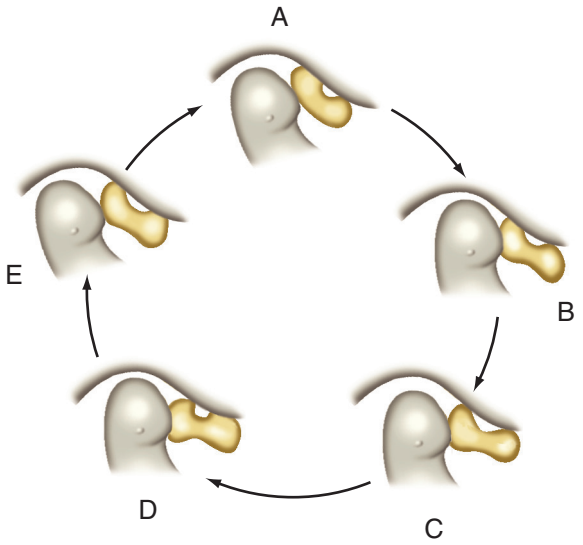



**Figure 51-2** **A-E,** Anterior displacement of the intra-articular disk without reduction on attempted mouth opening. The displaced disk acts as a barrier and prevents full translation of the condyle. *(Modified from McCarty W: Diagnosis and treatment of internal derangements of the articular disc and mandibular condyle. In Solberg WK, Clark GT, editors:* Temporomandibular joint problems: biologic diagnosis and treatment, *Chicago, 1980, Quintessence, p 151.)*

Acute macrotrauma is probably the most common cause of internal derangement. Among the events thought to cause macrotrauma are a blow to the jaw, endotracheal intubation, cervical traction, and iatrogenic stretching of the joint during dental or oral surgical procedures. Although whiplash injuries frequently have been implicated in the

development of internal derangement, a study of 155 patients with this type of injury showed that clicking in the TMJ immediately after the automobile accident developed in only one patient.[27,41] At 1 month of follow-up, two additional patients of the 129 contacted experienced clicking, but at 1 year, no additional patients of the 104 contacted had developed clicking. Although internal derangements of the TMJ can be caused by a whiplash injury, the incidence seems to be low.

Whether a patient manifests alterations in the articular surface leading to a catching or binding sensation, anterior disk displacement with reduction on mouth opening (clicking or popping), or anterior disk displacement without reduction during mouth opening (locking) after trauma to the TMJ depends on the severity of the injury. Although associated traumatic arthritis causes pain during function in each of these instances, the pain is more severe in the last two conditions because of compression of retrodiskal tissue, which is now located in the articular zone.

Functional overloading of the TMJ associated with the habit of chronic teeth clenching is another frequent cause of internal derangements. Although the TMJ is constructed for eccentric movements, it is not constructed for the constant isometric loading and unloading that occurs during this activity. Such parafunction affects the lubrication of the joint and alters the articular surfaces, introducing friction between the disk and the condyle that leads to degenerative changes in the articular surfaces and results in gradual anterior displacement of the disk.[26,28,40,42]

Degenerative joint disease may precede the development of an internal derangement, or it may occur after the development of an internal derangement. In the first instance, changes in the character of the articulating surfaces result in an inability of the parts to glide smoothly over each other, gradually leading to forward displacement of the disk, which normally rotates posteriorly during mouth opening. In the second instance, the displaced disk results in an altered relationship between articulating components of the joint, which leads to degenerative changes in these structures. In patients in whom the condition causing the degenerative joint disease is still active, whether primarily or secondarily, both the causative condition and the disk derangement must be treated for the problem to be resolved completely.

## Imaging Findings

Depending on the cause of the internal derangement and its duration, radiographs may or may not show any evidence of degenerative joint disease. MRI shows anterior disk displacement in the closed mouth position, as well as a return to a normal disk relationship during mouth opening, in patients with clicking and popping. However, in patients with locking, the disk remains in the anterior position on attempted mouth opening, and movement of the condyle is limited. In a small group of patients with locking, MRI showed the intra-articular disk in normal position when the teeth are in occlusion, rather than in anterior displacement, and no change in disk position occurs when the patient attempts to open the mouth.[29,43] In such cases, adhesion of the disk to the articular eminence prevents translation of the condyle. These patients differ from those with anteriorly displaced, non-reducing disks in that they do not have a history of TMJ clicking that precedes the sudden onset of locking.

## Treatment

Initial treatment of patients with painful clicking or popping in the TMJ consists of a nonsteroidal anti-inflammatory drug; a soft, nonchewy diet; and use of a bite-opening appliance to reduce compression of retrodiskal tissue (Figure 51-3). A muscle relaxant drug can be added to the regimen if the patient has associated myofascial pain. When the pain has stopped, no further treatment is generally necessary, although joint noise still may be present. However, in patients who have teeth-clenching and -grinding habits, use of a bite appliance is indicated at night to control these habits and prevent recurrence of the pain.

A long-term follow-up study (1 to 15 years) of 190 patients with a history of clicking treated by such conservative nonsurgical modalities, which are not directed specifically to the problems of joint noise or disk displacement, showed that the condition worsened in only 1% of patients, indicating that it is permissible to observe individuals with painless clicking as long as they remain otherwise asymptomatic.[30,44]

In patients with pain and clicking in the TMJ that is unresponsive to nonsurgical management, the disk should be repositioned arthroscopically or by open surgery (diskoplasty). Patients with parafunctional habits should continue the use of a bite appliance when sleeping. In patients with locking (anterior disk displacement without reduction), whether painful or not, treatment is urgent because if the condition is left untreated for a long time, subsequent management can be complicated by further degenerative changes in the disk and condyle that make disk salvage (diskoplasty) impossible. Initial treatment involves joint lavage and lysis of adhesions by arthrocentesis or arthroscopically. The former involves establishment of inlet and outlet portals in the upper joint space with hypodermic needles,

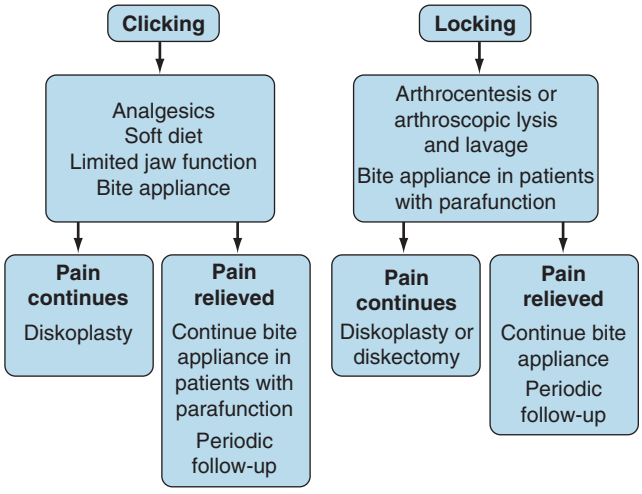



**Figure 51-3** Management of internal derangements of the temporomandibular joint. Patients with painful clicking or locking are treated medically initially, whereas patients with locking require surgical intervention.

irrigation with lactated Ringer's solution to remove inflammatory tissue breakdown products and cytokines, and lysis of adhesions by hydraulic distention and manual manipulation of the joint (Figure 51-4).[31,45]

The results of arthrocentesis parallel those achieved with arthroscopic lysis and lavage, and the procedure is less invasive. Although neither of these procedures restores the disk to its normal position, they do restore disk and joint mobility, and they reduce pain and improve function in most patients.[32,33,46,47] In these patients, the retrodiskal tissue within the joint undergoes fibrosis and acts as a pseudodisk. It is important that patients who have teeth-grinding or -clenching habits are prescribed a bite appliance postoperatively to wear while sleeping.

In patients who do not respond favorably to arthrocentesis or arthroscopy, the displaced disk should be repositioned by an open operation. If the disk is extremely deformed and cannot be repositioned, or if a large, nonreparable perforation in the disk or a tear in the retrodiskal tissue is present, the disk should be removed. Although autogenous auricular cartilage or dermal grafts, or temporalis muscle flaps, have been used as disk replacements, results have been unpredictable.[31,45,48] More recent long-term studies have shown that most patients can tolerate a diskless joint.[34,49] Currently, no acceptable alloplastic substitutes for the disk are available.

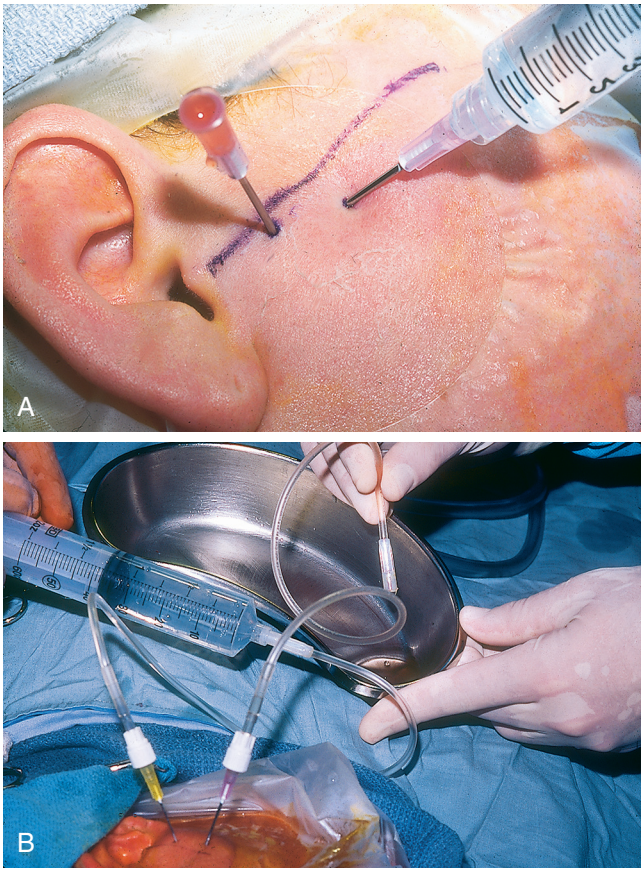



**Figure 51-4** Temporomandibular joint arthrocentesis. **A,** Hypodermic needles inserted into the upper joint space to allow lavage of the joint. **B,** Joint is being irrigated with lactated Ringer's solution.

## NEOPLASMS

Although primary neoplasms involving the TMJ are uncommon, they must be considered in the differential diagnosis of painful conditions affecting this region.[35,36,50,51] Chondroma, osteochondroma, and osteoma are the most frequently encountered benign tumors, but isolated cases of fibro-osteoma, myxoma, fibrous dysplasia, giant cell reparative granuloma, aneurysmal bone cyst, synovioma, synovial chondromatosis, chondroblastoma, osteoblastoma, glomus tumor, and synovial hemangioma have been reported. Malignant tumors of the TMJ are even rarer, with infrequent reports of fibrosarcoma, chondrosarcoma, synovial fibrosarcoma, osteosarcoma, malignant fibrous histiocytoma, malignant schwannoma, leiomyosarcoma, and multiple myeloma. The TMJ also can be invaded by neoplasms from the cheek, the parotid gland, the external auditory canal, and the adjacent ramus of the mandible. Metastasis to the condyle from distant neoplasms in the breast, lung, prostate, colon, thyroid gland, liver, stomach, and kidney has been described.

Tumors of the TMJ can cause pain, limitation of jaw movement, deviation of the mandible to the affected side on attempted mouth opening, and difficulty in occluding the teeth. Depending on the nature of the condition, radiographs may show bony deformation, apposition, or resorption. A biopsy is necessary to establish the definitive diagnosis.

## MYOFASCIAL PAIN AND DYSFUNCTION

MPD, or masticatory myalgia, is considered to be a psychophysiologic disease that primarily involves the muscles of mastication, and not the TMJ. Women are affected more frequently than men; the ratio in various reports ranges from 3:1 to 5:1. Although the condition can occur in children, the incidence seems to be greatest in adults 20 to 40 years old. MPD frequently is confused with painful conditions that affect the TMJ, such as degenerative arthritis or internal derangements; patients with primary MPD can develop these diseases secondarily, and patients with primary joint disease can develop secondary MPD. Enhanced understanding of the causes and pathogenesis of this condition makes its diagnosis easier and its treatment more effective.[37,38,52,53]

### Etiology

Psychological stress has been suggested as an important contributing factor in the development of MPD (psychophysiologic theory).[39,54,55] It is hypothesized that in most patients, stress-related, centrally induced increases in muscle activity, frequently combined with the presence of parafunctional habits such as clenching or grinding of the teeth, may result in associated muscle fatigue, pain, and limited mouth opening.[40,54] However, similar symptoms occasionally have been seen to result from muscle overextension, muscle overcontraction, or trauma (Figure 51-5). A counter-theory (the pain adaptation theory of Lund)[56] has been proposed to suggest that pain in the masticatory muscles leads to a reduction rather than an increase in muscle activity as a protective mechanism, and this reduction causes the

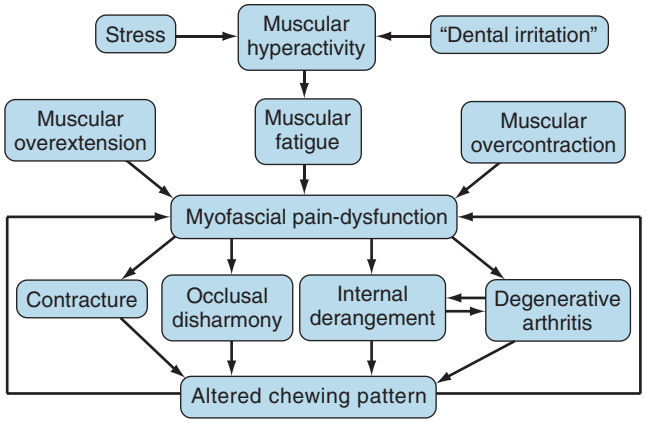



**Figure 51-5** Causes of myofascial pain and dysfunction. Although the diagram shows three pathways, the one involving psychological stress is most common. The mechanism by which stress leads to myofascial pain and dysfunction is termed the *psychophysiologic theory. (Modified from Laskin DM: Etiology of the pain-dysfunction syndrome.* J Am Dent Assoc *79:147–153, 1969. Copyright 1969 American Dental Association. Reprinted by permission of ADA Publishing Co.)*

limitation in mouth opening; however, this theory does not explain the origin of the pain. Despite extensive research, the cause of myofascial pain and dysfunction remains unknown.

## Clinical Findings

Pain of unilateral origin is the most common symptom of MPD. In contrast to the pain associated with joint disease, which is well localized, the pain of muscle origin is more diffuse. The patient generally is unable to identify accurately the specific site involved; this can serve as an important diagnostic criterion in distinguishing between muscle and joint disorders.

Depending on the muscle involved, the pain associated with MPD may be described by the patient in various ways. The masseter is the muscle most frequently involved, and the patient usually refers to the pain as a *jaw ache*. The temporalis is the next most commonly involved muscle; it produces pain on the side of the head, which is interpreted by the patient as a headache. Involvement of the lateral pterygoid muscle produces earache or a deep pain behind the eye, whereas medial pterygoid involvement causes discomfort on swallowing and the feeling of a painful, swollen gland beneath the angle of the mandible. Medial pterygoid involvement can cause stuffiness or a full feeling in the ear.

The pain associated with MPD is usually constant, but it is often more severe on arising in the morning or may worsen gradually as the day progresses. Pain generally is exacerbated by jaw function, especially during such activities as eating and excessive talking. Myofascial pain tends to be regional rather than local, and patients with a long-standing problem may complain that pain in the facial region has spread to the cervical area and later to the shoulders and back.

Tenderness in the muscles of mastication, another common finding, can be used to confirm the source of the pain in muscles that are accessible to palpation (masseter, temporalis, and medial pterygoid). Although muscle tenderness usually is not reported by the patient, this symptom can be elicited easily by the examiner. The most frequent sites of tenderness are near the angle of the mandible, in the belly and the posterosuperior aspect of the masseter, in the anterior temporal region, and over the temporal crest on the anterior aspect of the coronoid process. The location of some of the tender areas suggests that tendons also may be a source of pain and tenderness.

Limitation of mandibular movement is the third cardinal symptom of MPD. It manifests as an inability to open the mouth as wide as usual and as a deviation of the mandible to the affected side when mouth opening is attempted. Lateral excursion to the unaffected side is also reduced. The limitation of mandibular movement usually is correlated with the amount of pain present.

A clicking or popping sound in the TMJ is another finding in some patients with MPD. This is not a cardinal sign, however, because it occurs only in patients with a chronic teeth-clenching habit, which gradually produces frictional changes in the joint and subsequent disk displacement.[26,40] The presence of joint sounds alone is insufficient to allow a diagnosis of MPD. Joint sounds must be accompanied by myofascial pain and tenderness in the masticatory muscles that began before the onset of the joint noise. Such patients must be distinguished from patients with a primary internal derangement, in whom muscle splinting produces myofascial pain and tenderness after the onset of the joint noise. The history and differences in physical findings are helpful in making this distinction.

In addition to the three cardinal symptoms of pain, muscle tenderness, and limitation of mouth opening, patients with MPD usually have no clinical or radiographic evidence of pathologic changes in the TMJ. These negative characteristics are important for the physician in establishing the diagnosis because they confirm that the primary site of the problem is not the articular structures.

## Diagnosis

Because the cardinal signs and symptoms of MPD are similar to those produced by such organic problems involving the TMJ as degenerative joint disease and internal disk derangement and by a variety of nonarticular conditions (see Tables 51-1 and 51-2), diagnosis of this condition can be difficult. To make a diagnosis, the physician needs a careful patient history and a thorough clinical evaluation. Periapical radiographs of the teeth and a screening radiograph (panoramic) of the TMJs can be helpful to eliminate dental problems or gross joint disease. If screening views of the TMJs show some abnormality, CT scans are usually advisable for confirmation. MRI also can be useful in determining the position of the disk when an internal derangement of the TMJ is being considered. Depending on the suspected condition, other radiographic views of the head and neck and scintigraphy may be needed to establish a final diagnosis.

Certain laboratory tests may be helpful in some instances. These include a complete blood cell count if an infection is suspected; serum calcium, phosphorus, and alkaline phosphatase measurements for possible bone disease; serum uric acid determination for gout; serum creatinine and creatine kinase levels to detect muscle disease; and erythrocyte

**TABLE 51-4** Distinguishing Features of Myofascial Pain and Fibromyalgia

| | Myofascial Pain | Fibromyalgia |
|---|---|---|
| Patient age distribution | 20-40 years | 20-50 years |
| Gender distribution | Mainly women | Mainly women |
| Distribution of pain | Localized; usually unilateral | Generalized; bilaterally symmetric |
| Tender points | Few | Multiple |
| Trigger points | Uncommon | Common |
| Fatigue | Localized muscle fatigue | Generalized fatigue |
| Sleep disturbance | Common | Common |

sedimentation rate, rheumatoid factor, latex fixation, and anti-nuclear antibody tests for suspected rheumatoid arthritis. Electromyography can be used to evaluate muscle function. Psychological evaluation and psychometric testing are good research tools, but they have little diagnostic value other than to identify the presence of associated abnormal behavioral characteristics.

Fibromyalgia is a condition that sometimes is confused with myofascial pain, particularly when MPD involves several regions in addition to the face. Although fibromyalgia may eventually develop in a a small subset of patients with MPD, these are probably distinct conditions.[41,57] Table 51-4 lists the distinguishing characteristics of myofascial pain versus fibromyalgia.

## Treatment

Treatment of MPD is divided into four phases.[42,58] When a definitive diagnosis is made, phase I therapy should be started (Figure 51-6). Phase I therapy initially involves providing the patient with some understanding of the problem. Because patients often have difficulty accepting a psychophysiologic explanation for their condition, the physician's discussion with the patient should deal first with the issue of muscle fatigue as the cause of the pain and dysfunction, and delay consideration of the role of stress and psychological factors until the symptoms have improved and the patient's confidence has been gained. Associating the symptoms to the specific masticatory muscles from which they arise helps the patient understand the reason for the type and location of the pain—headache from the temporalis muscle, jaw ache from the masseter muscle, discomfort on swallowing and stuffiness in the ear from the medial pterygoid muscle, and earache and pain behind the eye from the lateral pterygoid muscle.

In addition to the initial explanation, the patient should be counseled regarding home therapy. This counseling includes recommendations to avoid clenching and grinding of the teeth, to eat a soft diet, to use moist heat and massage on the masticatory muscles, and to limit jaw movement. A nonsteroidal anti-inflammatory drug should be prescribed for the pain. In patients who have problems sleeping, a small dose of amitriptyline at bedtime is helpful to improve sleep and reducing parafunction.

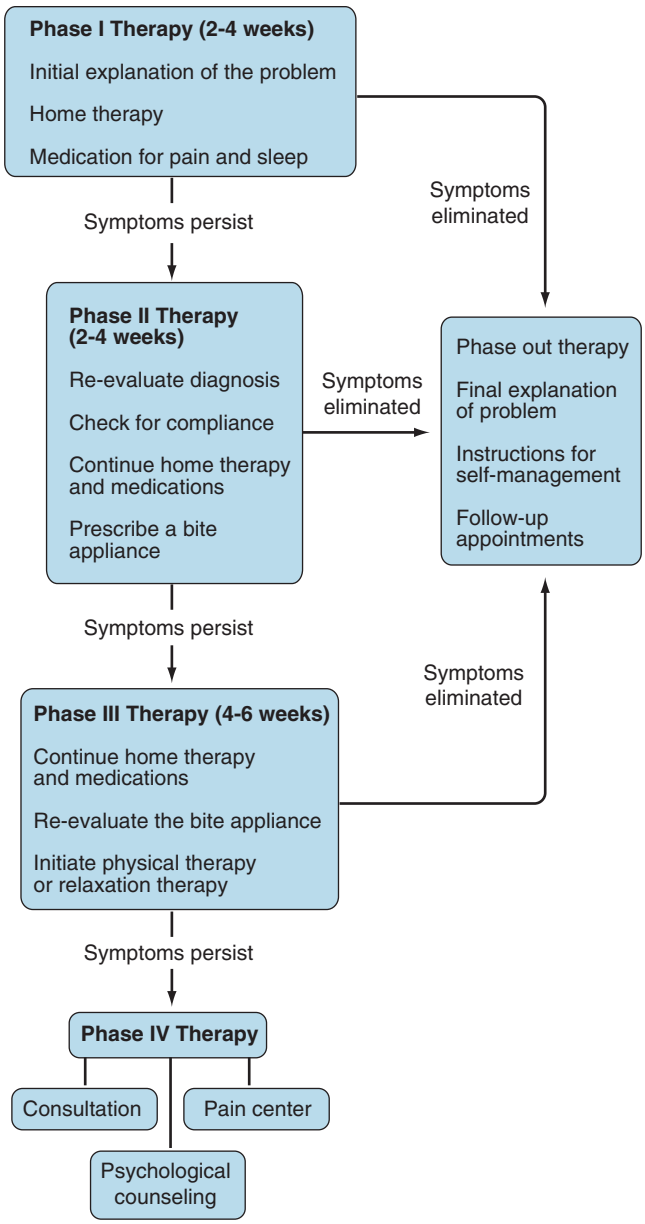



**Figure 51-6** Management of myofascial pain and dysfunction. The treatments are divided into four phases. If the symptoms are eliminated in any of the first three phases, the ongoing therapy is gradually phased out, and the patient is instructed in continued self-management of the condition. *(Modified from Laskin DM, Block S: Diagnosis and treatment of myofascial pain dysfunction [MPD] syndrome.* J Prosthet Dent *56:75–84, 1986.)*

Approximately 50% of these patients experience resolution of their symptoms within 2 to 4 weeks with phase I therapy. For patients whose symptoms persist, phase II therapy is initiated. Home therapy and medications are continued, and a bite appliance is made for the patient. Although numerous types have been used, the Hawley-type maxillary appliance is probably the most effective because it prevents contact of the posterior teeth and thus prevents most forms of parafunctional activity (Figure 51-7).[43,59] The appliance generally is worn at night, but it can be worn for 5 to 6 hours during the day, if necessary. The appliance

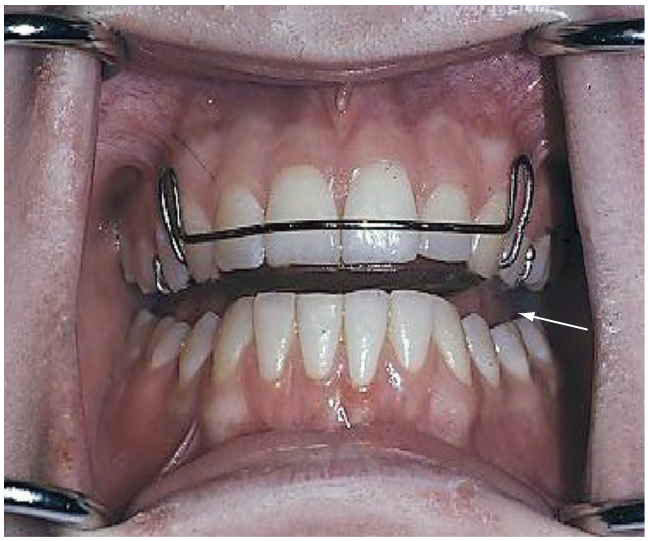


**Figure 51-7** Hawley-type maxillary bite appliance. Only the anterior teeth contact the appliance, and space between the posterior teeth is evident *(arrow)*.

should not be worn continuously, however, because the posterior teeth may supraerupt in some patients.

With phase II therapy, another 20% to 25% of patients become symptom free in 2 to 4 weeks. When the patient becomes symptom free, the medications are stopped first, and use of the bite appliance is discontinued next. If the patient has a return of symptoms, and the appliance is worn only at night, its use can be continued indefinitely.

Patients who do not respond to the use of a bite appliance are entered into phase III treatment for 4 to 6 weeks. In this phase, physical therapy (heat, massage, ultrasound, and electrogalvanic stimulation)[60] or relaxation therapy (electromyographic biofeedback and conditioned relaxation)[61] is added to the regimen. No evidence shows that one form of treatment is better than another, and either can be used first. If one is unsuccessful, the other can be tried. Phase III therapy usually helps another 10% to 15% of patients.

If all of these approaches fail, and no question arises about the correctness of the diagnosis, psychological counseling is recommended. This counseling involves helping patients to identify possible stresses in their lives and learning to cope with such situations. If the diagnosis is in doubt, the patient should be referred first for appropriate dental and neurologic consultation and re-evaluation. Another alternative is to refer patients with recalcitrant MPD to a TMJ center or pain clinic because such patients generally require a multidisciplinary approach for successful treatment.

## CONCLUSION

Successful management of patients with temporomandibular disorders depends on an accurate diagnosis of the disorder and use of the proper therapy based on the physician's understanding of the cause of the patient's condition. Of particular importance is distinguishing patients with MPD, who constitute the majority of patients encountered and who are not surgical candidates, from patients with TMJ disease, who frequently require surgical treatment. Even in the latter group, many commonly encountered conditions, such as arthritis and internal disk derangements, often respond to nonsurgical therapy, and this type of treatment should be given a fair trial before more aggressive management is considered.

The references for this chapter can also be found on Expertconsult.com.

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## WEBSITES

www.nicdr.nih.gov—General information, clinical trials, and sponsored research in TMJ and related areas.
www.aaoms.org—General information about TMJ surgery.
www.tmj.org—Advocate group that provides general information for patients.

CHAPTER 52



# Fibromyalgia

Leslie J. Crofford

**KEY POINTS**

- **Fibromyalgia (FM) is a disorder of centrally amplified and maintained musculoskeletal pain with evidence of altered pain processing in the spinal cord and brain.**
- **Although pain is the defining symptom of FM, fatigue, unrefreshing sleep, dyscognition, depression, and anxiety also have a significant impact on health-related quality of life.**
- **The diagnosis of FM relies on patient report using one of several valid criteria sets.**
- **Patients with other rheumatic diseases have a higher prevalence of FM than the general population, and the presence of comorbid FM affects assessment of disease activity.**
- **It is important to identify FM in order to develop an overall treatment plan that addresses the different mechanisms responsible for musculoskeletal pain.**

Fibromyalgia (FM) is a disorder characterized by chronic, widespread musculoskeletal pain often associated with debilitating fatigue, unrefreshing sleep, dyscognition, depression, and anxiety.[1] Patients with FM have evidence of disordered sensory processing as manifested by widespread allodynia (i.e., pain elicited by an innocuous stimulus that is not usually painful) and hyperalgesia (i.e., exaggerated pain elicited by a noxious stimulus). Patients often have a personal and family history of regional or visceral pain, such as migraine or tension headaches, temporomandibular disorder, irritable bowel syndrome, interstitial cystitis, pelvic pain syndromes, and depression or anxiety.[1-3] A careful history typically reveals a pain-prone phenotype with musculoskeletal pain as only one of the affected body regions.[1] The genetic vulnerability to FM and other disorders characterized by amplified pain is associated with polymorphisms in genes involving pain transmission, neurotransmitter, and stress-response pathways.[4-6] It is thought that in vulnerable persons, triggering events involving activation of sensory nociceptors lead to long-term changes in pain transmission and descending inhibitory pathways.[5] This concept of centrally maintained or amplified pain as the central physiologic alteration in FM has led to treatment strategies that differ from traditional anti-inflammatory or analgesic approaches to musculoskeletal pain.[1] Cognitive and emotional factors also play a significant role in the impact of FM on health, requiring the clinician to address the patient's beliefs and behaviors that augment pain symptoms. In some patients, a multidisciplinary approach to treatment will provide significant benefits.[7]

Because the diagnosis of FM relies solely on patient report and all of the symptoms of FM exist in a continuum in healthy persons, significant controversy has existed regarding the legitimacy of FM as a medical illness.[8] What is clear is that pain reports of persons with FM are associated with activation of areas in the brain associated with pain transmission, as determined by advanced neuroimaging studies.[9] Thus objective evidence of the veracity of the subjective report of pain exists. From a practical perspective, the treating clinician should be able to identify the symptoms of FM and sort through the differential diagnosis, understand its relationship to comorbid rheumatic diseases, assist the patient in distinguishing central pain from mechanical or inflammatory pain, and provide management approaches that address patient symptoms.

## HISTORICAL PERSPECTIVE

Descriptions of patients with symptoms of FM in the medical literature go back centuries. In early descriptions, the condition was often called "muscular rheumatism" to distinguish it from "articular rheumatism."[10] In 1815, Dr. William Balfour, a surgeon from Edinburgh, described nodules and suggested that inflammation in muscle connective tissue was the cause for nodules and pain. He also first reported focal tenderness, referred to as *tender points*, in 1824. Sir William Gowers coined the term *fibrositis* in 1904, sharing in the belief that patients experienced inflammation of fibrous connective tissue that led to tender points in patients with muscular rheumatism.[11]

An interesting historical treatise by Dr. Philip Hench and Edward Boland published in 1946 described the management of rheumatic diseases in U.S. Army soldiers and provides insights that remain relevant in more modern times.[12] They report that muscular rheumatism occurred in 13% of soldiers from the First World War (April 1, 1917 to December 31, 1919). Specialized rheumatism centers were established in the 1940s for soldiers of the Second World War. Hench and Boland published the incidence of rheumatic diseases in the first 1000 cases and differentiated "psychogenic rheumatism," occurring in 20%, from "fibrositis," which included regional syndromes such as bursitis and tendonitis, occurring in 13.4%. Hench and Boland noted that most of the patients sent to these specialized centers with muscular rheumatism did not have myositis or

fibrositis but psychogenic rheumatism, which they considered a psychoneurosis manifested by musculoskeletal complaints. They went on to state that primary fibrositis "puts its victims at the mercy of changes in external environment: thus weather, heat, cold, humidity, rest, exercise, etc. characteristically influences most of them for better or for worse." On the other hand, psychogenic rheumatism "puts its victims at the mercy of changes in the internal environment: thus their symptoms may vary with mood or psyche, pleasure, excitement, mental distraction, worry, or fatigue."

The description of psychogenic rheumatism included an attitude that was tense, anxious, defensive, and antagonistic. The chief symptoms were described as burning, tightness, weakness, numbness, tingling, or tired sensations that were often continuous day and night. They also describe severe fatigue causing disability, worsening of symptoms during and after exercise, and a "touch me not" reaction to examination. Psychotherapy was the preferred treatment approach for these patients. Patients with fibrositis, on the other hand, were treated with physical rehabilitation. In evaluating outcome, 82% of patients with primary fibrositis returned to duty, compared with only 64% of patients with psychogenic rheumatism.[12] In our current understanding, FM likely represents a combination of primary fibrositis and psychogenic rheumatism, with both conditions having a component of central pain amplification but diverging in the psychological and behavioral responses to pain.

Dr. Hugh Smythe provided the first modern description of widespread pain and tender points in the 1970s.[13] The term *fibromyalgia* was adopted soon thereafter because pathologic studies consistently fail to find evidence of inflammation in tender areas, calling into question the underlying hypothesis of the fibrositis construct. In 1981, Dr. Muhammad Yunus published a more comprehensive description of the symptoms and signs of FM,[14] which was recognized as a diagnosis by the American Medical Association in 1987. Yunus made the relevant comment that many patients with fibrositis syndrome, fibromyositis, fibromyalgia, myofibrositis, interstitial myofibrositis, myofascial pain syndrome, myofascitis, muscular rheumatism, nonarticular rheumatism, and tension rheumatism were also diagnosed with the separate entity of psychogenic rheumatism.

The American College of Rheumatology (ACR) published classification criteria in 1990 relying on the presence of widespread pain and tender points that allowed researchers to distinguish FM from other rheumatologic diseases and that had the effect of allowing researchers from around the world to identify a more homogenous group of patients for research.[15] A revision of the criteria published in 2010 eliminated tender points, placed less emphasis on widespread pain, and placed more emphasis on associated symptoms.[16] An additional modification to these criteria allow them to be used in epidemiologic studies.[17] Debate is ongoing regarding the most appropriate criteria for clinical and research uses, although in practice either these or other validated criteria sets will allow the clinician to make the diagnosis of FM.

Research intended to ascribe an etiology to FM began when Dr. Harvey Moldofsky reported abnormalities in polysomnographic studies of persons with FM in 1975, reporting intrusion of alpha or waking frequency waves in regions of delta or slow wave sleep now known to be nonspecific.[18] Since publication of the 1990 ACR classification criteria, investigators have identified a number of other potential mechanisms associated with FM that may be responsible for symptoms. In 2007, the U.S. Food and Drug Administration (FDA) approved pregabalin, an agent also approved for neuropathic pain, for treatment of FM. This step was followed by approval of the dual norepinephrine-serotonin reuptake inhibitors duloxetine and milnacipran for treatment of FM.[19]

Throughout the history of FM, controversy has existed regarding the veracity of patients' complaints and their reported impact on function.[8] The controversy has become somewhat contentious in the era of disability and the increased cost of FDA-approved medications. However, identifying FM and understanding the recent advances in mechanisms associated with development of central pain amplification has significant benefits that allow practitioners to educate patients about their symptoms and target pharmacologic and nonpharmacologic treatment to manage FM. With respect to the presence of psychogenic rheumatism in these patients, the most current approach to patients with FM who have excessive thoughts, feelings, and behaviors that are distressing and disruptive to daily life would be to consider an additional diagnosis of somatic symptom disorder, the new terminology in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) that replaces somatization disorder, hypochondriasis, pain disorder, and undifferentiated somatoform disorder. This way of approaching patients separates the neurobiologic changes resulting in widespread pain amplification from the emotional and behavioral response to these symptoms. Considering both these aspects of FM, which occur in differing proportions in individual patients, may assist the practitioner in developing a treatment plan.

## DIAGNOSTIC CRITERIA

All of the validated criteria sets for FM include a requirement for chronic musculoskeletal pain. The ACR 1990 criteria focus on the widespread nature of the musculoskeletal pain, requiring that pain be present on both sides of the body, above and below the waist, and including the neck, back, or chest. A demonstration of the presence of widespread allodynia also was required, which was accomplished by a physical examination of 18 defined areas that should be painful with mechanical pressure, at a minimum of 11 sites (Table 52-1).[15] The so-called tender point examination has been controversial for several reasons, including differences in the ability to detect tenderness in men compared with women when using this examination. Another criticism of the ACR 1990 criteria is the absence of other important symptoms, such as fatigue and unrefreshing sleep.

In 2010, a new preliminary criteria set was published that eliminates the requirement for a physical examination, relying on the patient's report of the number of painful areas to define a widespread pain index and incorporating a symptom severity score in recognition of the other symptoms of FM (Table 52-2).[16] A person can be diagnosed with FM with pain being the dominant symptom or with a predominance of other somatic symptoms. The criticism of the 2010 criteria, as well as a modification published in 2011 intended for use in epidemiologic studies,[17] is the loss of the

**TABLE 52-1** American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia*

**1. History of Widespread Pain**

*Definition:* Pain is considered widespread when all of the following are present: pain in the left side of the body, pain in the right side of the body, pain above the waist, and pain below the waist. In addition, axial skeletal pain (cervical spine or anterior chest or thoracic spine or low back) must be present. In this definition, shoulder and buttock pain is considered as pain for each involved side. "Low back" pain is considered lower segment pain.

**2. Pain in 11 of 18 Tender Point Sites upon Digital Palpation**

*Definition:* Pain, upon digital palpation, must be present in at least 11 of the following 18 sites:

- Occiput: bilateral, at the suboccipital muscle insertions
- Low cervical: bilateral, at the anterior aspects of the intertransverse spaces at C5-C7
- Trapezius: bilateral, at the midpoint of the upper border
- Supraspinatus: bilateral, at origins, above the scapula spine near the medial border
- Second rib: bilateral, at the second costochondral junctions, just lateral to the junctions on upper surfaces
- Lateral epicondyle: bilateral, 2 cm distal to the epicondyles
- Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle
- Greater trochanter: bilateral, posterior to the trochanteric prominence
- Knee: bilateral, at the medial fat pad proximal to the joint line

Digital palpation should be performed with an approximate force of 4 kg.

For a tender point to be considered "positive," the subject must state that the palpation was painful. "Tender" is not to be considered "painful."

*For classification purposes, patients will be said to have fibromyalgia if both criteria are satisfied. Widespread pain must have been present for at least 3 months. The presence of a second clinical disorder does not exclude the diagnosis of fibromyalgia.

From Wolfe F, Smythe HA, Yunus MB, et al: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 33:160-172, 1990.

**TABLE 52-2** American College of Rheumatology 2010 Preliminary Diagnostic Criteria for Fibromyalgia

**Criteria**

A patient satisfies diagnostic criteria for fibromyalgia if the following three conditions are met:

1. WPI ≥7 and SSS ≥5 or
   WPI between 3 and 6 and SSS ≥9.
2. Symptoms have been present at a similar level for at least 3 months.
3. The patient does not have a disorder that would otherwise explain the pain.

**Ascertainment**

1. WPI: Note the number areas in which the patient has had pain during the past week. In how many areas has the patient had pain? Score will be between 0 and 19.

| | | | |
|---|---|---|---|
| Shoulder girdle, L | Hip (buttock, trochanter), L | Jaw, L | Upper back |
| Shoulder girdle, R | Hip (buttock, trochanter), R | Jaw, R | Lower back |
| Upper arm, L | Upper leg, L | Chest | Neck |
| Upper arm, R | Upper leg, R | Abdomen | |
| Lower arm, L | Lower leg, L | | |

2. SSS:
   Fatigue
   Waking unrefreshed
   Cognitive symptoms

*For the each of the three aforementioned symptoms, indicate the level of severity during the past week using the following scale:*

0 = No problem
1 = Slight or mild problems; generally mild or intermittent
2 = Moderate; considerable problems; often present and/or at a moderate level
3 = Severe: pervasive, continuous, life-disturbing problems

*Considering somatic symptoms* in general, indicate whether the patient has:*

0 = No symptoms
1 = Few symptoms
2 = A moderate number of symptoms
3 = A great deal of symptoms

The SSS is the sum of the severity of the three symptoms (fatigue, waking unrefreshed, and cognitive symptoms) plus the extent (severity) of somatic symptoms in general. The final score is between 0 and 12.

*For reference purposes, here is a list of somatic symptoms that might be considered: muscle pain, irritable bowel syndrome, fatigue/tiredness, thinking or remembering problem, muscle weakness, headache, pain/cramps in abdomen, numbness/tingling, dizziness, insomnia, depression, constipation, pain in upper abdomen, nausea, nervousness, chest pain, blurred vision, fever, diarrhea, dry mouth, itching, wheezing, Raynaud's phenomenon, hives/welts, ringing in ears, vomiting, heartburn, oral ulcers, loss/change in taste, seizures, dry eyes, shortness of breath, loss of appetite, rash, sun sensitivity, hearing difficulties, easy bruising, hair loss, frequent urination, painful urination, and bladder spasms.

L, Left; R, right; SSS, Symptom Severity Score; WPI, Widespread Pain Index.

From Wolfe F, Clauw DJ, Fitzcharles MA, et al. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* 62:600-610, 2010.

requirement that the pain be widespread (as defined in the 1990 criteria) and the potential expansion of the prevalence of FM by including persons with a lower level of pain symptoms, with a possible bias toward persons with somatic symptom disorder. Other criteria sets have been published that include other configurations of symptoms and signs, but the basic clinical manifestations remain unchanged.[20,21] At the present time, it remains unclear if the presence of tenderness, identified by tender point examination or other methods that demonstrate allodynia or hyperalgesia, improves the specificity of the diagnosis. It also is not clear how to best define widespread pain; for example, is a high number of painful sites regardless of their distribution the same as having pain all over the body? Although it is clear that symptoms not related to pain occur frequently and are important to patients with FM, the inclusion of patients whose clinical picture is dominated by nonpain symptoms, such as fatigue, unrefreshing sleep, and dyscognition, remains controversial.

## EPIDEMIOLOGY

A number of epidemiologic studies have been performed using different ways to identify patients with FM. The prevalence depends on the method of ascertainment. Using the ACR 1990 criteria, the worldwide prevalence is estimated at approximately 2% of the population. The first major study was conducted in Wichita, Kansas, using a population-based mail screening followed by physician assessment.[22] In that study, the overall prevalence was 2%, with 3.4% of women and 0.5% of men diagnosed with FM. Subsequently, the prevalence in London, Ontario, Canada, was found to be 4.9% of women and 1.6% of men.[23] It is of interest that these same Canadian investigators evaluated an Amish community to determine if litigation or compensation availability had an influence on FM prevalence.[24] They found that the age-adjusted point prevalence of FM was 10.4% in women and 3.7% in men, which was greater than that found in rural or nonrural non-Amish adults and was interpreted as providing evidence that FM is present in communities that lack a financial incentive for having the disorder.

In France, also using a two-step method of ascertainment, the prevalence was 1.6%; gender was not specified.[25] In a Korean hospital study, the prevalence was 1.7%, with 3.2% of females and 0.6% of males being affected.[26] The prevalence of FM appears to increase with age to about age 70 years, after which it decreases slightly.[22,23] FM can be diagnosed in children—usually in adolescents, and more commonly in girls.[27] The prevalence in patients with rheumatic diseases is estimated at 11% to 30%, although a recent large study suggests that the range is between 6% and 13% for the most common rheumatologic conditions.[28]

When using a modified version of the ACR 2010 criteria developed for use in population studies and without physician examination, the prevalence in Olmstead County, Minnesota, was 6.4%: 7.71% in women and 4.48% in men.[29] A German study using these same criteria reported a prevalence of 2.1% overall, with 2.4% in women and 1.8% in men.[30] An internet survey in Japan also using the modified 2010 criteria reported an overall prevalence of 2.1%, with 2.3% in women and 0.85% in men.[31]

The prevalence of FM is generally greater in clinical settings than in epidemiologic studies. It was noted to be 5.7% in general medical clinics[32] and 2.1% in family practice settings.[33] In rheumatology clinics, FM prevalence was higher, with between 12% and 20% of new patients having this diagnosis.[14,34] The prevalence of FM in children is approximately 1.5% across studies, and it is usually diagnosed in adolescent girls.[27,35]

The incidence of FM is not easily determined; however, one study using an *International Classification of Diseases, Ninth Revision* (ICD-9) coding insurance claims database containing 62,000 nationwide enrollees per year between 1997 and 2002 found an age-adjusted incidence of 11.28 per 1000 person years for females and 6.88 cases per 1000 person-years in males.[36] In that study, patients with FM were between two and seven times more likely to have comorbid depression, anxiety, headache, irritable bowel syndrome, chronic fatigue syndrome, systemic lupus erythematosus, and rheumatoid arthritis. Contrast these data with the incidence of FM in patients with other rheumatic diseases: In a cohort of approximately 1000 patients in the early stage of arthritis who were followed up by rheumatologists, the incidence of FM was 6.77 per 100 person-years in the first year after the diagnosis. The incidence declined to 3.58 per 100 person-years in the second year.[37] This makes FM approximately 10-fold more common in patients with early arthritis than in a general claims dataset.

## CLINICAL FEATURES

In clinical settings, patients often report that they have pain "all over their body." They often have difficulty precisely localizing the pain and may describe it as moving from place to place. Patients often describe the pain as "deep," originating in muscles or bones. Patients may use many different pain descriptors, including "throbbing," "stabbing," and "burning." In a study comparing 100 patients who had FM and met 1990 ACR criteria with 76 control subjects who had widespread pain, four symptoms distinguished patients with FM from control subjects: pain severity, severe fatigue lasting 24 hours after minimal activity, weakness, and self-reported swelling of the neck glands.[38] Pain is typically present on most days almost all day, although the intensity may wax and wane. Patients often describe tenderness to light touch or pressure. Pain is typically exacerbated by physical activity, and some patients report that it is worsened with changes in the weather, although this phenomenon has not been confirmed in rigorous studies. In addition to pain, patients report muscular stiffness, tightness, and weakness.

The main purpose of the physical examination is to evaluate the patient for other conditions that cause musculoskeletal pain. Patients with FM exhibit tenderness that can be identified by tender point examination. Tender point sites represent specific areas of muscle, tendon, and fat pads that are more tender to palpation than surrounding sites. Sites selected as part of the ACR 1990 criteria represent tender point sites that best discriminated between patients with and without FM. To test for pain with digital palpation, the ACR 1990 criteria indicate that the examiner should press with an approximate force of 4 kg.[15] The patient should report pain in response to this level of pressure,

which in practice is approximately the force it takes for the thumbnail to blanche.

In addition to the usual musculoskeletal and connective tissue examination, it is useful to evaluate patients, particularly young patients, for joint hypermobility.[39] There are indications that FM may be more common among patients with these findings, and physical measures may be quite useful in management of patients with these associations. If regional pain disorders, such as bursitis, tendonitis, or arthritis, can be identified during the physical examination, treatment of these potentially exacerbating pain generators may help with the more widespread pain. Certainly, any mechanical or inflammatory conditions with the potential to cause musculoskeletal pain should be identified and treated.

The pain of FM is often accompanied by other pain amplification syndromes (Table 52-3). These syndromes often have been present for many years and, as with FM, they may wax and wane over time. It may be important for the patient to understand the genetic and physiologic relationships between these diagnoses. In addition, it is clinically important to determine which symptoms have the greatest impact on the patient. Symptom domains have been evaluated by patient and physician Delphi exercises and were generally concordant (Table 52-4).[40] Assessing these clinical concerns may help the clinician prioritize management strategies. For example, if sleep is a major contributor to symptoms, the patient may be counseled on sleep hygiene or, if appropriate, screened for restless leg syndrome or sleep apnea and then referred for evaluation and management of sleep disorder.[41] Similarly, if depression or anxiety is a major contributor to the overall symptom complex, then attention should be given to management of these concerns.[42] Dyscognition may be of major importance to patients, particularly in the workplace.[43,44] These complaints have validity when studied carefully, with abnormalities in executive function and divided attention. Fatigue may be the most difficult symptom to evaluate and treat because it may be due to deconditioning, depression, disrupted sleep, medication adverse effects, or comorbid conditions.

**TABLE 52-3** Fibromyalgia-Associated Pain Amplification Syndromes

| |
|---|
| Temporomandibular disorder |
| Tension and migraine headaches |
| Irritable bowel syndrome and other functional gastrointestinal disorders |
| Interstitial cystitis/irritable bladder |
| Dysmenorrhea and other pelvic pain syndromes |
| Vulvodynia |

**TABLE 52-4** Patient-Reported Outcomes (PRO) in Fibromyalgia

| PRO Concept | Patient Domains | Clinician Domains and Measures |
|---|---|---|
| Pain | Pain or physical discomfort<br>Joints aching or pain<br>Stiffness<br>Feeling tender where touched | Pain<br>Patient global status<br>Clinician global status<br>Tender point intensity |
| Treatment side effects | Problems with medication (e.g., medication adverse effects or reliance on medications)‡ | Adverse effects |
| Mobility | Difficulty moving, walking, or exercising | Physical function<br>HRQOL |
| Cognition | Problems with attention or concentration<br>Disorganized thinking<br>Memory problems | Dyscognition<br>HRQOL |
| Energy | Lack of energy or fatigue<br>Having to push yourself to do things | Fatigue<br>HRQOL |
| Impact on daily living | Limited in doing normal daily life and household activities<br>Ability to make plans, accomplish goals, or complete tasks<br>Being sensitive to outside factors<br>Unpredictability of symptoms | HRQOL |
| Emotional well-being | Depression<br>Having to push yourself to do things<br>Frustration‡<br>Irritability‡ | Depression<br>Anxiety<br>HRQOL |
| Sleep | Impact on sleep (e.g., difficulty falling asleep, saying asleep, or getting up in the morning) | Sleep quality<br>HRQOL |

*Most important fibromyalgia symptoms based on a Delphi exercise involving 100 patients with fibromyalgia and conducted at four different sites in the United States. The original 104 items were extracted from patient focus groups and then consolidated and reduced according to pretest rankings before being tested by Delphi.

†Clinician Delphi exercise involving 23 clinicians and outcome filtered through Outcome Measures in Rheumatology Clinical Trials VII (OMERACT VII) fibromyalgia workshop attendees.

‡Included based on pretest priority rankings.

HRQOL, Health-related quality of life; PRO, patient-reported outcomes.

From Mease PJ, Arnold LM, Crofford LJ, et al. Identifying the clinical domains of fibromyalgia: contributions from clinician and patient Delphi exercises. *Arthritis Rheum* 59:952-960, 2008.

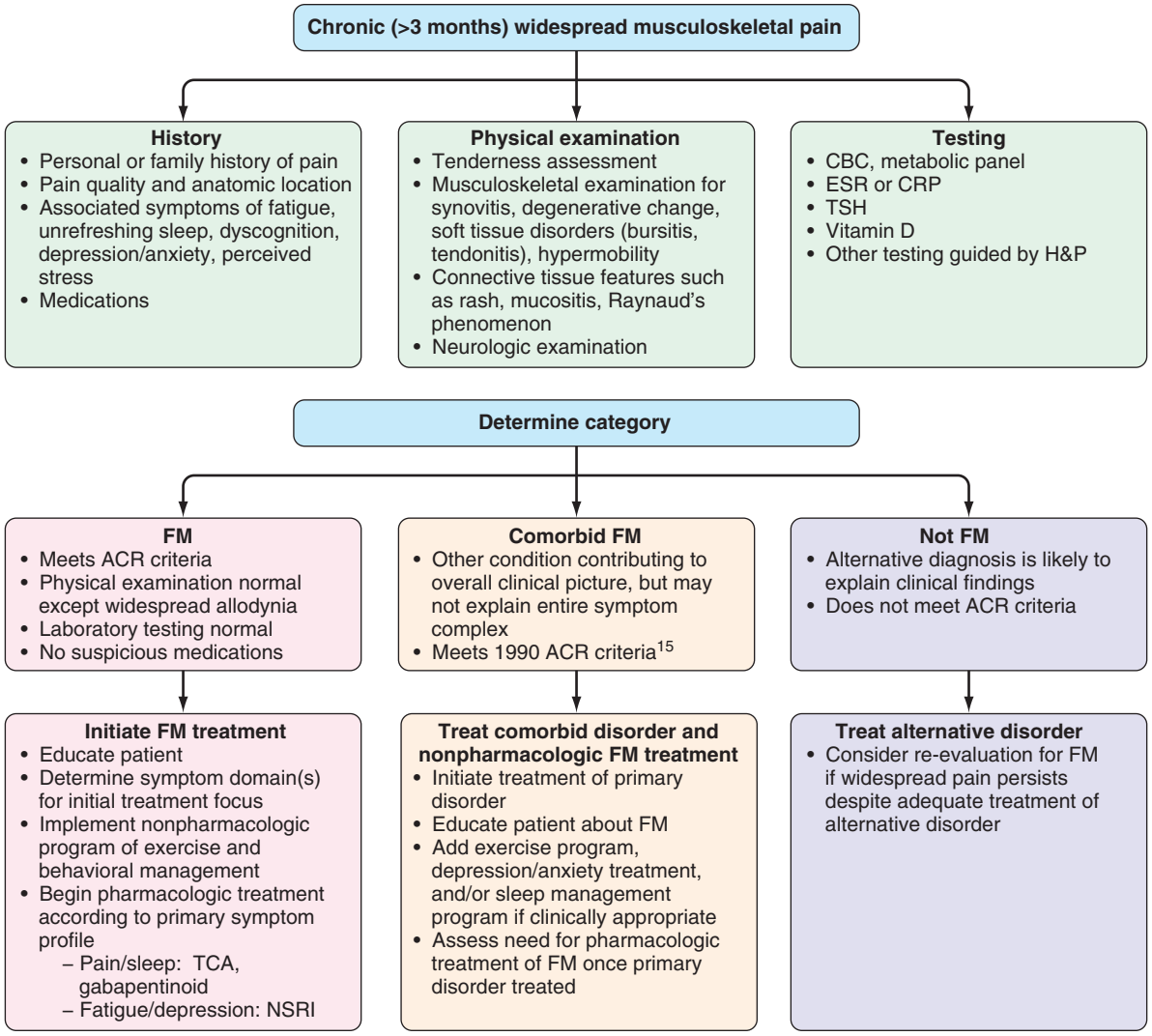



**Figure 52-1** Strategy for evaluation and initial management of patients with chronic widespread musculoskeletal pain. After initial evaluation, patients may be diagnosed with fibromyalgia (FM), FM comorbid with another diagnosis, or not FM. Strategies for initial management of each category are shown. ACR, American College of Rheumatology; CBC, complete blood cell count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; H&P, history and physical examination; NSRI, norepinephrine serotonin reuptake inhibitor; TCA, tricyclic anti-depressants; TSH, thyroid-stimulating hormone.

## DIFFERENTIAL DIAGNOSIS AND RHEUMATOLOGIC COMORBIDITIES

A strategy for diagnosing FM is shown in Figure 52-1.[45,46] Common conditions that should be considered in the differential diagnosis are shown in Table 52-5. Most of these conditions should be identified by careful history and physical examination with selected laboratory tests. Medications that may be associated with FM mimics, such as statins, should be noted. Physical findings that should prompt additional testing include prominent focal abnormalities on neurologic examination, such as weakness or numbness, joint inflammation, fever, rash, skin ulcers, or alopecia.[45] Hypothyroidism can be excluded using laboratory tests. Seronegative spondyloarthropathies may have elevated inflammatory markers and abnormal results of imaging studies. These patients also typically report that their pain improves rather than worsens with exercise. In older patients, polymyalgia rheumatic should be excluded using laboratory testing. Screening questions for major depressive disorder should be undertaken. If the history and physical examination suggest inflammatory arthritis or a systemic autoimmune disease, appropriate laboratory and serologic testing should be undertaken. It is not useful to perform repeat diagnostic testing, particularly if symptoms have been present for more than 1 to 2 years and if the symptoms do not change.

FM is frequently comorbid with other rheumatologic diseases. In a recent study of 835 patients, the prevalence of FM was 13.4% in 67 patients with systemic lupus erythematosus, 12.6% in 119 patients with ankylosing spondylitis, 12% in 25 patients with Sjögren's syndrome, 10.1% in 238 patients with osteoarthritis, 6.9% in 29 patients with polymyalgia rheumatica, and 6.6% in 197 patients with rheumatoid arthritis. Significant correlations were found between disease activity indexes and Fibromyalgia Impact

**TABLE 52-5** Differential Diagnosis of Diffuse Myalgias

| Diagnosis | Findings |
|---|---|
| **Inflammatory** | |
| Polymyalgia rheumatica | Elevated ESR and/or CRP |
| Seronegative spondyloarthropathies | Abnormal imaging |
| Connective tissue diseases | Positive serologies |
| Systemic vasculitis | Systemic inflammation, end-organ damage |
| **Infectious** | |
| Hepatitis C | Positive antibodies |
| Human immunodeficiency virus | Positive antibodies |
| Lyme disease | Positive antibodies |
| Parvovirus B19 | Positive antibodies |
| Epstein-Barr virus | Positive antibodies |
| **Non-inflammatory** | |
| Degenerative joint/spine disease | Abnormal imaging |
| Fibromyalgia | Widespread allodynia/ hyperalgesia |
| Myofascial pain | Localized allodynia/hyperalgesia |
| Joint hypermobility | Joint hypermobility |
| Metabolic myopathies | Abnormal muscle biopsy |
| **Endocrine** | |
| Hypo- or hyperthyroidism | Abnormal thyroid function tests |
| Hyperparathyroidism | Elevated serum calcium |
| Addison's disease | Abnormal serum cortisol |
| Vitamin D deficiency | Low serum vitamin D |
| **Neurologic Diseases** | |
| Multiple sclerosis | Abnormal neurologic examination and imaging |
| Neuropathic pain | Reasonable cause or abnormal imaging |
| **Psychiatric Diseases** | |
| Major depressive disorder | Positive depression screening |
| **Drugs** | |
| Statins | History of exposure |
| Aromatase inhibitors | History of exposure |

*Recommended routine testing includes ESR or CRP and thyroid-stimulating hormone. Other diagnostic testing should be guided by risk profile and history and physical examination. Repeated diagnostic testing is discouraged.

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Questionnaire (FIQ) scores for most rheumatologic patients.[28] Identifying central pain amplification has important implications for evaluation and treatment of comorbid conditions. For example, it is well known that the tender joint component and patient-reported symptoms of the Disease Activity Score (DAS)-28 are higher in patients with FM than in patients without FM. Using more objective criteria for synovitis in those patients, such as ultrasound, may be required to determine if treatment of rheumatoid arthritis should be accelerated.

Other comorbidities are frequent in patients with FM and do not vary a great deal with the clinical site in which the patient is seen. Patients with FM have a significantly higher prevalence of visceral pain syndromes, regional pain syndromes, and mood disorders. In one study using an ICD-9 coding insurance claims database containing 62,000 nationwide enrollees, patients with FM were between two and seven times more likely to have comorbid depression, anxiety, headache, irritable bowel syndrome, chronic fatigue syndrome, systemic lupus erythematosus, and rheumatoid arthritis.[36] In a study from the United Kingdom primary care dataset of persons eventually diagnosed with FM, visit rates were highest for depression, fatigue, chest pain, headache, and sleep disturbance.[47] A population-based study from the Swedish Twin Registry evaluating 44,897 persons found substantial co-occurrence for chronic widespread pain with chronic fatigue (odds ratio [OR], 23.53; 95% confidence interval [CI], 19.67 to 18.16), depressive symptoms (OR, 5.26; 95% CI, 4.75 to 5.82), and irritable bowel syndrome (OR, 5.17; 95% CI, 4.55 to 5.88).[48] Using a co-twin analysis, the associations remained for chronic fatigue and irritable syndrome but not for depressive symptoms. In a rheumatology practice, patients with FM were found to have high lifetime rates of migraine, irritable bowel syndrome, chronic fatigue syndrome, major depression, and panic disorder.[49]

FM is frequently identified in patients seen primarily for interstitial cystitis, irritable bowel syndrome, migraine and other forms of headache, temporomandibular disorder, multiple chemical sensitivities, and chronic fatigue syndrome. In a systematic review from 2001 of the aforementioned conditions, it was noted that there were many similarities in case definition and symptoms and that the proportion of patients with an unexplained clinical condition meeting criteria for a second unexplained condition was striking.[50] Psychiatric conditions associated with FM include major depressive disorder, bipolar disorder, anxiety disorders, including panic disorder, post-traumatic stress disorder, social phobia and obsessive compulsive disorder, and substance abuse disorder.[51,52] In a study of 108 persons with FM and 228 persons without FM, the odds ratio for persons with FM having bipolar disorder was 153 (95% CI, 26 to 902; $P$ <0.001); any anxiety disorder, 6.7 (95% CI, 2.3 to 20; $P$ <0.001); substance use disorder, 3.3 (95% CI, 1.1 to 10; $P$ = 0.040); and major depressive disorder, 2.7 (95% CI, 1.2 to 6.0; $P$ = 0.13).[51]

Should a patient with FM have multiple pain amplification disorders, the practitioner will need to determine if he or she should be treated as having one disorder of central etiology or whether there is a clinical reason for diagnosis of multiple different disorders. Many of the medications and nonpharmacologic treatments for these conditions overlap. To avoid use of multiple medications, if a single agent can be used—for example, an agent with anti-depressant activity—it may be possible to treat comorbidities and FM.

## ASSESSMENT OF SEVERITY

Symptom severity, physical function, and disability are key status and outcome variables in persons with FM. In the clinic, numeric ratings for pain and fatigue along with a measure of functional status, including those routinely used for rheumatic diseases, such as one of the Health Assessment Questionnaires (HAQ) instruments, work very well in patients with FM.[53,54] The revised FIQ (FIQ-R) addresses all of these core domains, as well as FM impact and several

**The revised Fibromyalgia Impact Questionnaire**

**Domain 1 directions:** For each of the following nine questions, check the one box that best indicates how much your fibromyalgia made it difficult to do each of the following activities over the past 7 days:

| | | | |
|---|---|---|---|
| Brush or comb your hair | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Walk continuously for 20 minutes | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Prepare a homemade meal | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Vacuum, scrub, or sweep floors | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Lift and carry a bag full of groceries | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Climb one flight of stairs | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Change bed sheets | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Sit in a chair for 45 minutes | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |
| Go shopping for groceries | No difficulty | □ □ □ □ □ □ □ □ □ □ □ | Very difficult |

**Domain 2 directions:** For each of the following two questions, check the one box that best describes the overall impact of your fibromyalgia over the past 7 days:

| | | | |
|---|---|---|---|
| Fibromyalgia prevented me from accomplishing goals for the week | Never | □ □ □ □ □ □ □ □ □ □ □ | Always |
| I was completely overwhelmed by my fibromyalgia symptoms | Never | □ □ □ □ □ □ □ □ □ □ □ | Always |

**Domain 3 directions:** For each of the following 10 questions, check the one box that best indicates the intensity of your fibromyalgia symptoms over the past 7 days:

| | | | |
|---|---|---|---|
| Please rate your level of pain | No pain | □ □ □ □ □ □ □ □ □ □ □ | Unbearable pain |
| Please rate your level of energy | Lots of energy | □ □ □ □ □ □ □ □ □ □ □ | No energy |
| Please rate your level of stiffness | No stiffness | □ □ □ □ □ □ □ □ □ □ □ | Severe stiffness |
| Please rate the quality of your sleep | Awoke rested | □ □ □ □ □ □ □ □ □ □ □ | Awoke very tired |
| Please rate your level of depression | No depression | □ □ □ □ □ □ □ □ □ □ □ | Very depressed |
| Please rate your level of memory problems | Good memory | □ □ □ □ □ □ □ □ □ □ □ | Very poor memory |
| Please rate your level of anxiety | Not anxious | □ □ □ □ □ □ □ □ □ □ □ | Very anxious |
| Please rate your level of tenderness to touch | No tenderness | □ □ □ □ □ □ □ □ □ □ □ | Very tender |
| Please rate your level of balance problems | No imbalance | □ □ □ □ □ □ □ □ □ □ □ | Severe imbalance |
| Please rate your level of sensitivity to loud noises, bright lights, odors, and cold | No sensitivity | □ □ □ □ □ □ □ □ □ □ □ | Extreme sensitivity |

**Scoring: Step 1.** Sum the scores for each of the three domains (function, overall, and symptoms).
**Step 2.** Divide domain 1 score by three, divide domain 2 score by one (that is, it is unchanged), and divide domain score 3 by two.
**Step 3.** Add the three resulting domain scores to obtain the total revised Fibromyalgia Impact Questionnaire score.

**Figure 52-2** The revised Fibromyalgia Impact Questionnaire (FIQR) assesses functional status as well as the overall impact and fibromyalgia symptoms.[55] The FIQR total score can be used as an outcome measure in clinical studies. The FIQR function score and the symptom scores can be used individually to determine severity. Paper and online versions perform similarly, and the FIQR performs similarly to the original version.

other symptoms (Figure 52-2).[55] The ACR 2010 criteria modified for epidemiologic studies provide another measure of FM severity; it is called the FM Symptom Scale and combines the Widespread Pain Index (WPI) and the modified Symptom Severity Score (SSS) into a continuous score.[16]

For research purposes, the Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) FM working group has recommended that the domains of pain, fatigue, sleep, depression, physical function, quality of life, multidimensional function, patient's global impression of change, tenderness, dyscognition, anxiety, and stiffness be assessed.[56] This assessment can be accomplished using the FIQ-R and the Medical Outcomes Scale SF-36.[57]

## MECHANISMS

The two intrinsic mechanisms associated with the risk of developing chronic painful musculoskeletal disorders, including FM, are pain amplification and psychological distress.[5] Pain amplification may be related to sensitization of afferent pathways in the peripheral or central nervous system that process coded pain information or impairment in the inhibitory systems of the central nervous system.[9] Psychological factors include enhanced somatic awareness or the perception and interpretation of sensory information, anxiety, depression, perceived stress, and catastrophizing.[58] It is likely that genetic vulnerability coupled with environmental triggers are required to produce the clinical phenotype. The specific environmental triggers may include nonspecific behavioral factors, such as smoking and obesity, stress exposures, and nociceptive musculoskeletal pain. The combination of risk factors is likely to vary among persons who meet the definition of FM.

### Genetic Risk

A number of studies have demonstrated the importance of genetics in the susceptibility of FM and other syndromes that are often comorbid with FM. A study of familial aggregation reported that first-degree relatives of FM probands are far more likely to have FM (OR, 8.5; 95% CI,

2.8 to 26; $P$ <0.001). Twin studies have estimated that the contribution of genetic factors to chronic widespread musculoskeletal pain load on two latent traits are best explained by both affective and sensory components.[59] These twin studies suggest a modest genetic influence with concordance for chronic widespread pain, with concordance for monozygotic female twins of 30%, and for dizygotic female twins of 16%.[60]

It is unlikely that a specific gene or set of genes is associated with FM. Rather, similar to autoimmune diseases, there are more general vulnerability genes that interact with environmental exposures to lead to clinical expression of pain conditions. Thus most of the genes associated with FM have also been reported in association with other clinically defined pain syndromes. For example, two major neurotransmitter pathways have been repeatedly associated with musculoskeletal pain.[5] The first is the adrenergic pathway, in which *COMT*, the gene encoding the enzyme catechol-*O*-methyltransferase that is responsible for the catabolism of catechol neurotransmitters, such as epinephrine, norepinephrine, and dopamine, is most frequently associated with chronic musculoskeletal pain conditions. Most studies of *COMT* report an increased risk of chronic pain associated with a Val159Met (rs4680) that encodes a protein with lower enzymatic activity.[5] More extensive studies have expanded the functional locus to three major haplotypes that modify expression and activity of the enzyme, thus conferring low-risk and high-risk phenotypes for acute pain sensitivity, as well as risk for developing chronic pain.[61] Additional genetic variation in the β2-adrenergic receptor gene (*ADRB2*; rs1042713 and rs1042714) has been associated with an increased risk of FM and chronic widespread pain. Haplotype variants that regulate β2-adrenergic receptor expression and internalization are associated with differences in susceptibility to chronic pain.[62]

The second pathway associated with chronic pain syndromes is the serotonin pathway. Specific genes include the 5-hydroxytryptamin receptor 2A (*HTR2A*) and 5HT transporter (*SLC6A4*).[63-65] A 44–base pair insertion/deletion polymorphism in the promoter region of *SLC6A4* is most frequently associated with risk of chronic pain conditions, including FM.[5]

Both of these genetic pathways are also associated with several "endophenotypes" or intermediate measurable phenotypes that are present in patients with FM. These endophenotypes include autonomic dysregulation, altered pain processing and modulation, sleep dysfunction, and anxiety, in the case of the adrenergic pathway.[5] Personality and affective traits, such as somatic awareness, depression, and anxiety, have been associated with genetic variation in the serotonin pathway and are associated with risk for chronic pain.[5]

Candidate gene analysis of patients carrying the diagnosis of FM identified pathways also including genes within the biogenic amine and adrenergic pathways though different from candidates more generally associated with chronic pain, as well as a cannabinoid receptor.[6] A genome-wide linkage scan study of families with FM identified chromosome 17p11.2-q11.2, a region that contains the serotonin transporter gene (*SLC6A4*) and the transient receptor potential vanilloid channel 2 gene (*TRPV2*).[66]

## Central Pain Amplification and Potential Peripheral Mechanisms

Several lines of evidence support the concept of central pain amplification (also called central sensitization), which is generally interpreted as a change in the relationship between stimulus intensity and the perception of the stimulus as noxious or painful.[67] In patients with FM, stimuli usually perceived as innocuous are perceived as painful (allodynia), and painful stimuli are given a higher pain rating (hyperalgesia).[68] During central sensitization, second-order and higher-order neurons exhibit transcriptional and translational events that lead to heightened sensitivity.

Most of the data supporting central sensitization are generated through psychophysical testing using different types of stimuli such as pressure or heat. The investigator may use either a subjective report of pain or an objective measure such as brain imaging. One experimental paradigm called wind-up or temporal summation of pain measures the change in pain intensity after repeated applications of a sensory stimulus.[68] Healthy persons will experience an increase in pain in response to identical stimulus intensity if the stimulus is presented with a short interstimulus interval. Patients with FM report the same wind-up phenomenon with longer interstimulus intervals, which is interpreted to mean that patients with FM have presensitized pain transmission neurons.[69,70] Patients with FM also have a delayed recovery to baseline, termed *aftersensations*, which correlates with clinical pain intensity.[70]

Studies have also implicated a deficiency in descending inhibitory control of noxious stimulation in persons with FM. Normally, endogenous inhibitory mechanisms are activated in response to nociceptive stimuli and involve serotonergic, noradrenergic, and opioidergic inhibitory pathways. Persons with FM do exhibit reduction in these pathways.[71] It is difficult to know whether activation of afferent pain pathways, deficiency of descending pathways, or both explain enhanced pain perception in a given person. Positron emission tomographic scanning has demonstrated alterations in mu-opioid receptor availability and increased dopamine activity in persons with FM, both of which could indicate alteration of normal pain inhibitory pathways.[72,73]

Disagreement exists regarding the role of peripheral nociceptive input in the cause and maintenance of FM, although the increased prevalence of FM in persons with other rheumatic diseases provides an argument that persistent peripheral pain generators may be an important risk factor contributing to the phenotypic changes in pain transmission neurons.[74] Other triggering events, such as certain types of infections, trauma, or psychological stress, have been proposed.[75,76] Pain is localized to muscle tissues in virtually all persons with FM. Abnormalities in muscle tissues have been reported, including altered metabolisms by phosphorus-31 magnetic resonance spectroscopy (MRS).[77] Some data implicate muscle microcirculation and muscle mitochondria in FM.[68,78,79] Reports have been made of small fiber neuropathies in persons with FM, which are thought potentially to be a source of persistent nociceptive input.[80-83] For example, in a recent study of persons with FM and control subjects, intraepidermal nerve fiber

density was reduced in 32.6% in the former.[80] Another study used microneurography to record C-fiber action potentials in persons with FM and control subjects.[83] Persons diagnosed with FM had abnormalities in the functioning of type 1B C nociceptors that are normally silent and mechanoinsensitive, including spontaneous activity, prolonged firing in response to mechanical stimulation, and/or a distinctive pattern of activity-dependent slowing of conduction velocity. Whether these data indicate that a subset of persons with FM may in fact have neuropathic pain associated with a non–length-dependent small fiber neuropathy or whether the key element that results in FM is that peripheral pain generators lead to central sensitization is not clear.

Altered peripheral and cerebrospinal fluid levels of neurotrophins, chemokines, and cytokines have been reported in persons with FM.[68] Mechanisms for the effects of these substances in explaining the pathophysiology of FM remain undetermined. However, the concept that activation of glial cells by cytokines and chemokines may accompany activation of pain transmission neurons can be demonstrated in animal models.[84]

Functional neuroimaging has contributed much to the understanding of FM and, because the outcome is objective, it has clearly confirmed the validity of patient reports. One of the first studies to address this issue demonstrated that greater regional cerebral blood flow receiving pain pathway input was related to the patient's report of pain rather than to the stimulus intensity.[85] Other neuroimaging techniques have emerged and have provided information in FM and other chronic pain states. Using proton MRS ($^{1}$H-MRS), persons with FM were found to have low levels of *N*-acetyl-aspartate, a metabolite believed to be a marker of neuronal density and viability, perhaps indicating loss of neural function and activity in the hippocampus.[86] Also using $^{1}$H-MRS, it was found that the main excitatory neurotransmitter, glutamate/glutamine, was elevated in the posterior insular cortex, and a decrease in the signal correlated with improvements in pain.[87,88] Other brain regions of persons with FM, including the amygdala, posterior cingulate, and ventral lateral prefrontal cortex, had elevated levels of glutamate/glutamine, suggesting a possible role for this neurotransmitter in pain and perhaps other symptoms of FM.[86]

### Stress Response Systems

A number of studies suggest abnormalities of stress-response systems—the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system—in persons with FM. It is unclear whether these abnormalities are a cause or a consequence of FM. Genetic studies implicating genes in these pathways suggest that lack of resiliency in these pathways may provide a measure of vulnerability, but other studies implicate exposure to acute or chronic stress as a triggering mechanism. One study of patients without chronic widespread pain but who were at risk based on high rates of psychological distress and somatization provided evidence that HPA axis dysfunction may precede the development of chronic pain[89] and that HPA axis dysfunction was associated with the subsequent development of chronic widespread pain.[90] There is a striking lack of consistency in the specific HPA axis measures associated with FM.[91]

Many studies, however, consistently demonstrate alterations of measures of autonomic function, especially heart rate variability. These alterations are most often said to demonstrate predominance of the sympathetic nervous system, a characteristic shared by other pain amplification syndromes.[92] Other studies are interpreted as demonstrating stronger parasympathetic decline, a finding associated with the tendency to exhibit defensive behaviors.[93]

## SOCIAL AND PSYCHOLOGICAL FACTORS

### Life Stress and Socioeconomic Factors

In many longitudinal epidemiologic studies, chronic pain and other somatic symptoms can be predicted by childhood abuse and traumas, low educational attainment, social isolation, depression, and anxiety.[94] In a population-based study to determine psychosocial factors that predicted new-onset chronic widespread pain, investigators identified a random sample of subjects from sociodemographically disparate backgrounds and then identified more than 3000 who did not have pain at baseline and more than 300 who had new widespread musculoskeletal pain at follow-up.[95] The strongest predictors were premorbid somatic symptoms, illness behaviors, and sleep problems. In another community-based study, perceived physical and emotional trauma as precipitating factors for FM were associated with health care seeking rather than pain severity.[96]

Lower socioeconomic status predicts greater symptom severity and functional impairment in persons with FM, even controlling for levels of pain, depression, and anxiety.[97] The biopsychosocial model of pain posits that pain experience and its impact on the individual is a function of interacting combinations of nociceptive input; psychological processes, including beliefs; coping repertoire; and mood; and environmental contingencies that would include family, community, and cultural rules or expectations.[98] All of these factors are likely to play a key role in the clinical expression and health impact of FM.

### Personality, Cognitive, and Psychological Factors

Persons with FM may have a specific personality profile characterized, for example, by high levels of neuroticism. However, many studies include state variables, such as catastrophizing, self-esteem, and motivation or coping strategies, with personality traits. It is sometimes unclear whether a personality feature is a state or a trait; for example, catastrophizing may be seen as a personality feature, a cognitive error, or a coping strategy.[99] A recent study using a standard five-factor personality scale (neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness) showed that patients with FM were not different from patients with other rheumatic pain conditions or other chronic illnesses.[100] Furthermore, the scores fell within the normal range for the general population. However, a cluster of patients with FM was identified with a personality profile (high neuroticism and lower extraversion) that reflects a proneness to experience emotional distress, a difficulty for

positive emotion, and a tendency to ineffective use of emotional regulation processes rather exhibiting rumination and maladaptive behaviors. The patients in this cluster also exhibited more psychosocial problems.[100]

Another way of stratifying patients is the psychological and behavioral response to chronic pain. Persons with FM have been characterized and divided into groups that predict outcome based on psychological characteristics.[101] Persons classified as "dysfunctional" exhibit the highest pain intensity, interference, and distress and the lowest control and activity levels. The "adaptive coper" groups report the lowest levels of pain and interference, as well as the highest activity levels. The "interpersonally distressed" patients report high levels of affective distress and more negative spousal responses to pain.

It is likely that important relationships exist between psychological and physiologic pathways in persons with FM. This likelihood is certainly not surprising because regulation of domains characterized as psychological and physiologic use common mediators. For example, in a recent study of persons with FM, investigators performed a cluster analysis based on pain characteristics and cognitive, affective, and behavioral responses to pain and stress.[102] The study demonstrated that psychophysiologic responses of blood pressure, heart rate, and skin conductance were associated with specific types of psychological coping and psychiatric diagnoses.[103]

## TREATMENT APPROACHES

### Education and Self-Management

Treatment guidelines for persons with FM typically emphasize the need to incorporate principles of self-management.[104,105] Making the diagnosis of FM provides an intellectual framework for educating patients about their symptoms and engaging them as an active participant in the treatment plan (Figure 52-3).[105] Providing the diagnosis can provide relief from health-related anxiety, and no studies have suggested that labeling with FM is harmful for health or health care expenditures.[106,107] Educating patients regarding the physiology of chronic pain in FM, as well as the importance of nonpharmacologic therapies for its management, is likely to be beneficial. One key goal is to assist the patient in differentiating peripheral pain generators from the centrally maintained diffuse pain. In addition, educating patients about which treatment is intended to target which pain symptom may help with adherence and satisfaction with treatment. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) or disease-modifying agents may be intended for peripheral mechanical or inflammatory pain but would not be expected to effectively treat FM pain.[104]

A comparison of network meta-analyses of different treatment strategies found that multicomponent therapies incorporating pharmacologic therapies with aerobic exercise

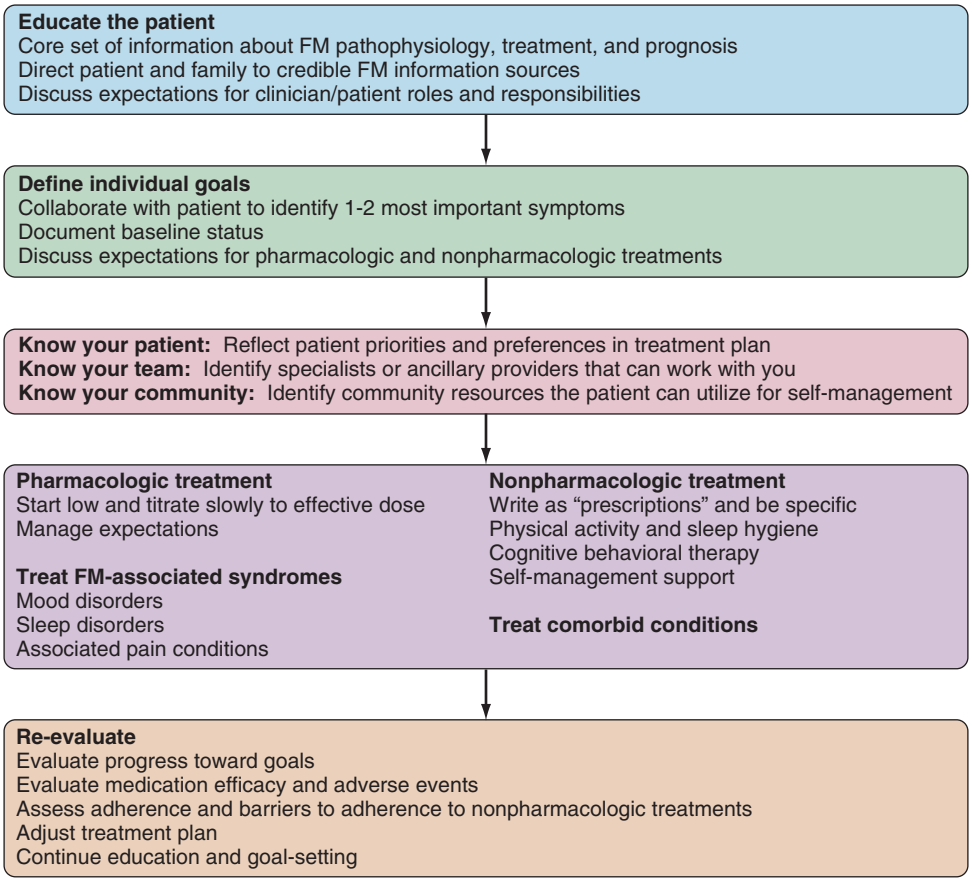



**Figure 52-3** Integrated approach to fibromyalgia (FM) management. *(Modified from Arnold LM, Clauw DJ, Dunegan LJ, et al: A framework for fibromyalgia management for primary care providers.* Mayo Clinic Proceedings. *87[5]:488–96, 2012.)*

and cognitive behavioral therapy (CBT) seems most promising.[108] Many of the nonpharmacologic strategies useful for FM may not be available locally but lend themselves to Internet-based programs or written materials.[105] The provider and patient should collaborate to prioritize individual treatment goals and develop a plan to achieve those goals. Focusing on longer term goals, while acknowledging that FM symptoms tend to wax and wane in response to both external and internal environmental stressors, is advised.[105]

## Exercise and Body-Based Therapies

A number of systematic reviews have been conducted to assess the benefits of exercise for persons with FM.[109-112] In evaluating exercise interventions, a recent umbrella systematic review synthesized physical activity interventions for adults with FM, focusing on four outcomes, including pain, multidimensional function, physical function, and adverse effects.[113] The researchers found positive results of diverse exercise interventions for all outcomes and no adverse effects. The variability of the interventions did not allow for recommendations regarding the mode of exercise or the frequency, intensity, and duration of treatments. It is not possible to rigorously determine whether land- or water-based exercise is superior. All major outcomes, including function, pain, stiffness, muscle strength, and fitness, improved after aquatic exercise training compared with control subjects, although the quality of the evidence was rated as low to moderate; however, land-based training was superior to aquatic training for improving muscle strength.[112] Tai chi, yoga, qigong, and a variety of other movement therapies have also been reported to be safe and effective for overall symptoms and physical functioning in persons with FM.[114] In general, active physical exercise strategies of any type will be helpful. Finding the exercise to which the patient is most likely to adhere will be the most important factor.

More passive strategies may be useful as an adjunct but should not replace active physical exercise. A meta-analysis of balneotherapy and hydrotherapy reported evidence for a small reduction in pain and a small improvement in health related to quality of life, although the investigators recommend that high-quality studies with larger sample sizes be performed.[115] A recent systematic review of massage suggested moderate evidence that myofascial release is beneficial for FM symptoms, but only limited evidence supports the application of connective tissue massage and Shiatsu. Swedish massage did not improve outcomes.[116] Acupuncture has been compared with sham acupuncture, and a meta-analysis including nine trials concluded that there was not enough evidence to prove the efficacy of acupuncture therapy compared with sham acupuncture.[117] Neurostimulatory therapies, including transcutaneous electrical nerve stimulation and central neurostimulatory therapies, may be helpful in persons with FM.[118,119]

## Cognitive Behavioral Therapy

CBT includes interventions that are based on the basic premise that chronic pain is maintained by cognitive and behavioral factors and that psychological treatment leads to changes in these factors through training in specific techniques.[120] These interventions would include cognitive restructuring and behavioral training, such as relaxation and social skills training. CBT provides benefit for reducing pain, negative mood, and disability in patients with FM.[121] CBT is typically delivered in group therapy over variable periods. Mindfulness-based stress reduction is a cognitive therapy that helps persons self-manage and reframe worrisome and intrusive thoughts through mindfulness meditation. This technique reduced perceived stress, sleep disturbance, and symptom severity, although there was no improvement in pain or physical functioning in a randomized, controlled clinical trial.[122] Operant behavioral treatment focuses on the modification of pain behavior by increasing activity levels, reducing health care–seeking behavior, and reducing pain-reinforcing behaviors in significant relationships.[120] Overall, the magnitude of benefit for CBT is small across the symptom domains of FM, with pain coping, depressed mood, and health care–seeking behavior having the most significant improvement.

## Pharmacologic Approaches

The major classes of drugs useful for patients with FM are anti-depressants, particularly those with mixed reuptake inhibition of serotonin and norepinephrine, such as duloxetine and milnacipran, and other agents useful for neuropathic pain, such as gabapentin and pregabalin (Table 52-6).[104] Evidence-based guidelines have been formulated by the American Pain Society (2005),[123] the European League Against Rheumatism (2007),[124] the Association of the Scientific Medical Societies in Germany (2008),[125] the Canadian National Fibromyalgia Guideline Advisory Panel (2012),[104] and the Israeli Rheumatology Association (2013).[126] These recent treatment guidelines and reviews emphasize the need for selecting a treatment approach based on predominant symptoms and initiating treatment in low doses with slow dose escalation.[1] Indirect comparison among the three agents approved by the FDA for FM with regard to efficacy and harms demonstrate that pregabalin, duloxetine, and milnacipran are superior to placebo for all outcomes of interest (pain, fatigue, sleep disturbance, depressed mood, and reduced health-related quality of life) except pregabalin for depressed mood, duloxetine for fatigue, and milnacipran for sleep disturbance. Adjusted indirect comparisons indicated no significant differences between these drugs for 30% pain relief and dropout rates due to adverse events.[127]

Drugs with anti-depressant activity have been a mainstay for FM treatment. Comparisons of older and newer agents have not found large differences in treatment effects, although adverse effect profiles differ.[128] Older agents, such as amitriptyline and cyclobenzaprine, may improve a wide range of symptoms, although adverse effects such as dry mouth, weight gain, constipation, and sedation may limit their tolerability.[128] Selective serotonin reuptake inhibitors typically have not been as useful as mixed reuptake inhibitors with respect to analgesic activity, although they are helpful for symptoms of depression and anxiety.[1] Older agents such as fluoxetine, paroxetine, sertraline, and venlafaxine that are not as serotonin selective thus are favored over newer, more serotonin-specific agents such as citalopram and escitalopram. These drugs are associated with nausea, sexual dysfunction, weight gain, and sleep

**TABLE 52-6** Evidence-Based Recommendations for Pharmacologic Treatments

| Class | Drug | Evidence Level | Adverse Effects |
|---|---|---|---|
| Tricyclic compounds | Amitriptyline, cyclobenzaprine | 1A | Dry mouth, weight gain, constipation, "groggy" feeling; avoid in older patients |
| Serotonin norepinephrine reuptake inhibitors | Duloxetine,† milnacipran† | 1A | Nausea, palpitations, headache, fatigue, tachycardia, hypertension |
| SSRI‡ | Fluoxetine, sertraline, paroxetine, venlafaxine | 1A | Nausea, sexual dysfunction, weight gain, sleep disturbance |
| Gabapentinoids | Gabapentin, pregabalin† | 1A | Sedation, dizziness, weight gain, peripheral edema |
| Cannabinoids | Nabilone, dronabinol | 3C | Sedation, dizziness, dry mouth |
| NSAIDs | | 5D | Gastrointestinal, renal, cardiovascular adverse effects |
| Opioids | Tramadol, other opioids | 2D, 5D | Sedation, constipation, addiction, opioid-induced hyperalgesia |

*As rated in the 2012 Canadian Fibromyalgia Guidelines.
†Approved by the U.S. Food and Drug Administration.
‡Highly selective SSRIs (e.g., citalopram and escitalopram) are not effective as analgesics.
NSAIDs, Nonsteroidal anti-inflammatory drugs; SSRI, selective serotonin reuptake inhibitor.

disturbance. The norepinephrine serotonin reuptake inhibitors duloxetine and milnacipran are approved by the FDA for treatment of FM. The addition of norepinephrine reuptake inhibition is important for achieving analgesic efficacy.[129] Time to effect for these drugs is generally 2 to 4 weeks, and thus dose adjustment and assessment of efficacy after institution of treatment should be performed within several months. Providers are cautioned to ensure that high doses or combinations of anti-depressants and concomitant medications or supplements do not result in the serotonin syndrome, which can cause agitation, tachycardia and hypertension, sweating/shivering, diarrhea, muscle rigidity, fever, seizures, and even death.

In general, agents tested and used for neuropathic pain have been more useful in FM than agents targeting peripheral mechanical and inflammatory sources of pain. Gabapentin and pregabalin are both approved by the FDA for use in postherpetic neuralgia and painful diabetic neuropathy.[130] Although only pregabalin has been approved by the FDA for use in FM, a study of gabapentin found that it also was effective.[131-133] Both gabapentin and pregabalin bind to a receptor subunit, the $\alpha 2\delta$ subunit of a calcium channel on the cell surface of neurons, to inhibit excitatory neurotransmitters.[129] There are pharmacokinetic and pharmacodynamic advantages for pregabalin that may make dosing somewhat simpler than for gabapentin, but the mechanism of actions for these drugs is the same. Both drugs may reduce pain, improve sleep, and improve health-related quality of life; however, they have substantial adverse effects, including dizziness, grogginess, weight gain, and edema of the extremities.[134] Other anticonvulsant agents have less evidence to support their use in persons with FM.

Cannabinoids are used by persons with many forms of chronic pain. A systematic review of smoked cannabis, cannabis extracts, nabilone, and dronabinol suggests modest efficacy in persons with FM and other chronic pain conditions.[135,136] NSAIDs are not effective treatments for central pain, although they may be helpful in treating inflammatory or mechanical peripheral pain generators that contribute to the overall burden of pain. Tramadol with or without acetaminophen has been studied in persons with FM, although there is increasing concern that opioids are less effective than previously thought, and their risk-benefit profile is worse than that of other classes of analgesics.[1,124] A Cochran review found no evidence to support the use of oxycodone for treatment of FM.[130] Treatment recommendations from professional societies uniformly advise against the use of strong opioids in persons with FM.[104] This recommendation is based, at least in part, on mechanistic studies demonstrating elevated cerebrospinal fluid enkephalins and evidence that mu-opioid receptors are either occupied or downregulated in the brain of persons with FM.[137] Low-dose naltrexone has been evaluated in very small trials and is thought to perhaps act on glial cells rather than on neurons.[138]

## Practical Advice

Persons with FM are likely to benefit from multimodal treatment that includes nonpharmacologic treatment, especially if one component includes exercise. In practice, it is crucial that patients be informed that there is no pharmacologic treatment that can alleviate the symptoms of FM. A general rule is that effective medications only improve pain by 30% to 50% in only 30% to 50% of patients, which includes the placebo response rate. Little information is available about combinations of approved medications, although patients with FM often use multiple medications. In general, efforts should be made to consolidate treatments and use the fewest number of medications possible. Patients should be involved in choosing nonpharmacologic strategies that will be most accepted and that are practical to employ. In addition to general nonpharmacologic approaches, it may be useful to target specific symptoms, such as sleep, with efforts to improve sleep hygiene and regulation of circadian rhythms.[105]

Many drugs and alternative therapies are used by persons who have FM and other forms of chronic musculoskeletal pain. Widespread use of nonapproved treatments reflects the generally poor effectiveness and tolerability of currently available drugs. For any treatment of FM, there is also likely to be a significant placebo effect in addition to the specific

efficacy of a therapeutic agent. It is certainly reasonable to work with patients on individual "n-of-1" trials, although it is important to assess both efficacy and harm and to discontinue use of ineffective medications or treatments with adverse effects.

## OUTCOME

The outcome of FM can be studied in terms of the change in the level of symptoms, use of services, and work disability. An early study of patients with FM who were followed up for 4.5 years found that an adequate physical activity level and increasing age predicted a positive outcome, while receiving a permanent disability pension or having experienced an excess of major negative life events predicted a negative outcome.[139] A six-center, 7-year study of 538 patients published in 1997 found that functional disability worsened slightly and health satisfaction improved slightly. Correlations between first and last assessment were often highly correlated with values for disease measures at the initial assessment predictive of final values. The authors concluded that little change in FM symptoms occurred over time.[140] A 2003 prospective study of patients with FM who were referred to a specialty clinic, 70 of 82 were reassessed after 3 years. Of these patients, 47% reported overall moderate to marked improvement, whereas the remaining 53% reported slight improvement, no change, or deterioration. The baseline predictors for a favorable outcome were younger age and less sleep disturbance.[141] Another study in the same year evaluated 27 of 48 patients who were reassessed over a 2-year time frame. In general, the patients showed no improvement in their symptoms over the observation period, regardless of the type of therapy they had received. No change occurred in either work capacity or disability-pension status. General satisfaction with quality of life improved, as did satisfaction regarding health status and the family situation.[142]

In a 2006 study intended to assess utilization of health services, 2260 patients who were seen in a primary care setting and newly diagnosed with FM were studied from 10 years before until 4 years after FM diagnosis.[47] Patients with FM had considerably higher rates of visits, prescriptions, and testing from at least 10 years prior to diagnosis compared with control subjects, and these rates accelerated to twice the numbers of visits and prescriptions at the time of diagnosis. Visit rates were highest for depression, fatigue, chest pain, headache, and sleep disturbance. After diagnosis, visits for most symptoms and health care use markers declined, but within 2 to 3 years, most visits rose to levels at or higher than those at diagnosis. The authors concluded that being diagnosed with FM may help patients cope with some symptoms but that the diagnosis has a limited impact on health care resource use in the longer term.

In a more recent longitudinal study of 1555 patients with FM during semiannual observations for up to 11 years, there was minimal improvement in symptoms overall. However, 25% reported at least moderate improvement in symptoms. These data suggested that the course of FM was one of continuous high levels of self-reported symptoms and distress.[143] Taken as a whole, although some patients improve, the data tend to suggest minimal improvement in most cases despite treatment.

A study of patients with juvenile FM (JFM) was recently reported. In this study, patients with JFM and healthy control subjects were assessed approximately 6 years after diagnosis and with an average age of 21 years. Patients with JFM had more pain, poorer physical function, and greater anxiety and depression than did healthy control subjects. They were more likely to be married and less likely to have a college education. More than 80% of patients with JFM continued to experience symptoms into adulthood, and more than half met the criteria for FM at follow-up. Those who met adult FM criteria exhibited the highest levels of physical and emotional impairment.[27]

Overall, patients with FM typically have a lifelong problem with chronic pain. Assisting patients in the self-management of their symptoms, reducing health care–seeking behaviors, and improving healthy behaviors are important goals of treatment. Perhaps the most important function of the rheumatologist is to confirm the diagnosis and determine if the patient has a comorbid rheumatic condition that should be treated. If FM is complicating another rheumatic condition, specific management of FM may improve overall health outcomes.


The references for this chapter can also be found on ExpertConsult.com.

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PART 7 DIAGNOSTIC TESTS AND PROCEDURES IN RHEUMATIC DISEASES

CHAPTER 53



# Synovial Fluid Analyses, Synovial Biopsy, and Synovial Pathology

Hani S. El-Gabalawy

**KEY POINTS**

- Analysis of synovial fluid samples by leukocyte count, cytology, polarized microscopy, Gram stain, and culture provides key diagnostic information, particularly in acute monoarthritis.
- Synovial biopsy performed by using closed needle techniques or arthroscopy may provide valuable diagnostic information, particularly in persistent monoarthritis.
- Although the histopathologic features of synovitis are generally nonspecific, some synovial diseases can be diagnosed with small synovial tissue biopsies.
- Analysis of synovial tissue by using immunohistology and other molecular techniques has been of great value in understanding the mechanisms of synovitis.
- Sequential analysis of synovial tissue samples in the context of therapeutic trials provides unique information regarding the effects of treatment on the target organ.

Analysis of synovial fluid and synovial tissue obtained from diseased joints provides important diagnostic information in specific clinical settings and is valuable in addressing a spectrum of research questions about of the pathogenesis and mechanisms of rheumatic diseases. Many peripheral joints are readily accessible to sampling of both synovial fluid effusions and synovial tissue, although the knee is the most frequently sampled joint. The techniques used to obtain and analyze synovial fluid and tissue samples are discussed in this chapter.

## SYNOVIAL FLUID ANALYSIS

### Synovial Fluid in Health

Under normal conditions, a small volume of synovial fluid is present in each joint, forming a thin interface between the surfaces of the articular cartilage, and providing for friction-free movement of these surfaces. In a large joint such as the knee, the volume of synovial fluid is estimated to be less than 5 mL. Moreover, intra-articular pressure is typically subatmospheric. Compositionally, normal synovial fluid is an ultrafiltrate of plasma to which proteins and proteoglycans are added by fibroblast-like synoviocytes in the lining layer. Most of the small-molecular-weight solutes such as oxygen, carbon dioxide, lactate, urea, creatinine, and glucose diffuse freely through the fenestrated endothelium of the synovium and are normally present at levels comparable with plasma levels. Evidence for active transport of glucose has been found. The total protein concentration of normal synovial fluid is 1.3 g/dL. The concentration of individual plasma proteins is inversely proportional to the molecular size, with small proteins such as albumin present at approximately 50% of plasma levels, and large proteins such as fibrinogen, macroglobulins, and immunoglobulins present at low levels. In contrast to this selective entry on the basis of size, clearance of synovial fluid proteins through the synovial lymphatics is unrestricted by size. Hyaluronan is the major proteoglycan synthesized by synovial cells and secreted into synovial fluid. Hyaluronan is highly polymerized and reaches molecular weights exceeding one million Daltons, which gives this fluid its characteristic viscosity. The hyaluronan also acts to retain small molecules in the synovial fluid. The lubricating capacity of the synovial fluid is attributed to a glycoprotein called *lubricin*.[1] This molecule has been fully characterized on the basis of the study of individuals with mutations of the *PRG4* gene, which encodes for its production.[2] These mutations result in an autosomal recessive loss-of-function disorder called the *camptodactyly–arthropathy–coxa vara–pericarditis syndrome*, which features a progressive, non-inflammatory arthropathy characterized by severe cartilage destruction associated with proliferation of synovial lining cells. The role of lubricin in maintaining the health of the cartilage has been further demonstrated in a murine knockout model.[3]

### Accumulation of Synovial Effusions

Synovial fluid and its contents are cleared through the synovial lymphatics by a process that is aided by joint motion. Excess fluid can accumulate in any diarthrodial joint as a result of many processes, including non-inflammatory, inflammatory, and septic disorders. In addition, overt hemarthroses can result from both traumatic and nontraumatic disorders. The most important mechanism contributing to the accumulation of joint effusions is an increase in synovial microvascular permeability. This permeability allows for an increase in the efflux of plasma proteins, particularly larger



Video available on the accompanying ExpertConsult website.

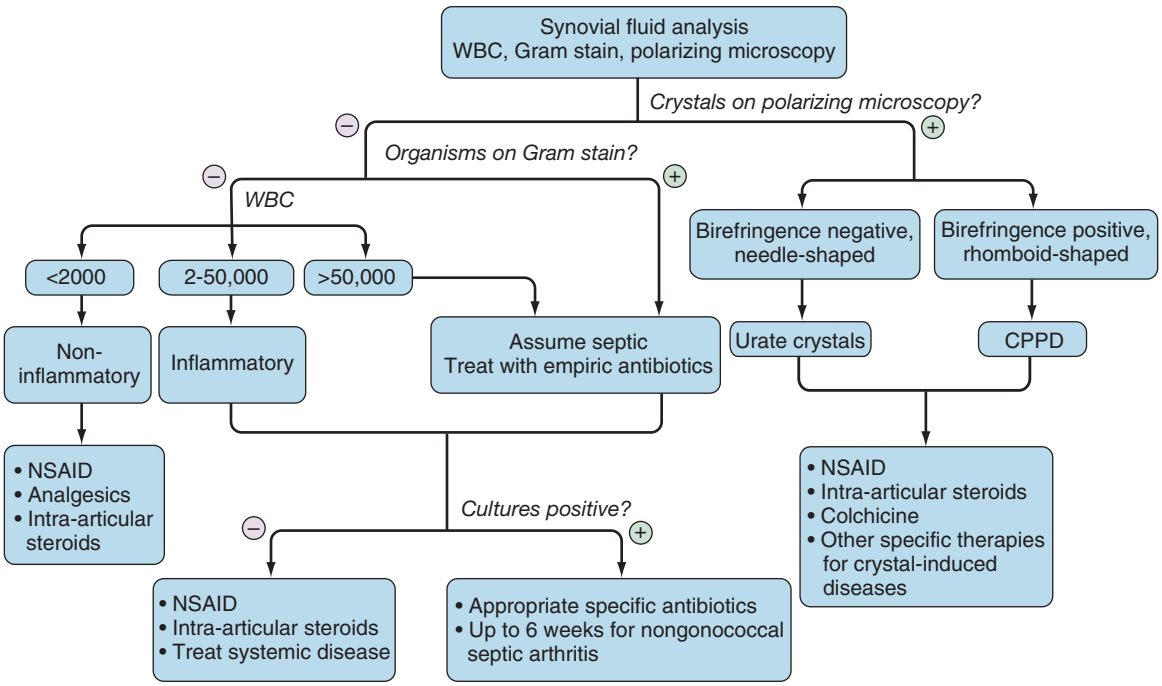



**Figure 53-1** A simplified algorithm for analyzing synovial fluid samples and initiating a plan of management. CPPD, Calcium pyrophosphate dihydrate; NSAID, nonsteroidal anti-inflammatory drug; WBC, white blood cell; –, negative test; +, positive test.

proteins, which in turn increases osmotic pressure and contributes to the effusion. Leukocytes accumulate in the fluid after transmigration through the endothelium, stimulated by chemokines produced in the synovium. The capacity of synovial lymphatics to clear proteins, cells, and debris is rapidly exceeded, which in turn contributes to their accumulation in the synovial compartment.

## Arthrocentesis

Most peripheral joints are readily accessible for diagnostic arthrocentesis, and the procedure can be performed in almost any ambulatory care setting equipped for sterile procedures. Joints that are less accessible because of their deeper location, such as the hip, may require an imaging technique that uses fluoroscopy or ultrasound to guide the needle and ensure accurate placement. Details of techniques used for arthrocentesis are described in Chapter 54. Because the ease with which joint fluid is aspirated depends on the gauge of the needle that is used, physicians should attempt arthrocentesis with a needle of adequate gauge, particularly in the larger joints. Moreover, high-suction gradients created by large syringes should be avoided, because they may actually reduce a physician's ability to successfully aspirate synovial fluid. Difficulty in aspiration of synovial fluid may stem from a number of intra-articular factors, including viscosity, the presence of debris such as rice bodies, and loculation of fluid into inaccessible areas. Instillation of a small amount of sterile saline may help to obtain enough fluid for culture in situations in which infection is highly suspected, yet direct aspiration is difficult.

Once obtained, it is important to analyze aspirated synovial fluid samples as quickly as possible to avoid spurious results. Ideally, leukocyte count and differential should be performed on fresh specimens. If the specimen cannot be analyzed quickly, and short-term storage is needed, the specimen should be kept at 4° C. An aliquot preferably should be placed in ethylenediaminetetraacetic acid (EDTA) to prevent clotting. Delays in analysis longer than 48 hours should be avoided. A simplified algorithm for analyzing synovial fluid samples is shown in Figure 53-1.

## Gross Examination

The physician can get a first impression of the nature of the synovial fluid while fluid enters the syringe during the arthrocentesis procedure itself. For example, the viscosity of the fluid is apparent during this step. As has been mentioned, normal synovial fluid is highly viscous because of its hyaluronan content and forms a long string when a drop is expressed from the end of the needle. With increasing levels of inflammation associated with recruitment and activation of leukocytes in the synovial cavity, the hyaluronan is digested, resulting in loss of viscosity that is appreciated as a reduction in the "stringiness" of the fluid. Large pieces of debris such as rice bodies, thought to arise from detached ischemic synovial villi, may be visible while they are aspirated. These can cause sudden arrests in the flow of fluid into the syringe, requiring manipulation and redirection of needle placement.

Inspection of the aspirated synovial fluid can yield other important diagnostic information. For example, floridly purulent fluid will be completely opaque because of the very high number of leukocytes present, but synovial fluid that is transparent to the point at which printed text can be read through it is seen in non-inflammatory settings. Inflammatory synovial fluid of the type that would be aspirated from an individual with active rheumatoid arthritis (RA), appears

cloudy and translucent. The degree of translucency depends on the intensity of the inflammatory response and the concentration of leukocytes in the sample. Synovial fluid from patients with ochronosis may have a speckled appearance, and particulate debris from joint prostheses may be visible on gross inspection.

During the arthrocentesis procedure, the physician may have an important challenge in determining whether the presence of blood in the aspirated synovial fluid indicates a hemarthrosis or, alternatively, is a result of trauma from the procedure itself. In the latter case, the blood may remain unmixed with the synovial fluid, and appear as red streaks in an otherwise yellow fluid In the case of hemarthroses, the synovial fluid is generally homogeneously bloody and does not form a clot. The causes of frank hemarthrosis are varied and include trauma, pigmented villonodular synovitis, tumors, hemophilia, and other bleeding disorders or anticoagulant therapy, Charcot joint, and, occasionally, intense inflammation from a chronic arthropathy such as RA or psoriatic arthritis.

## Leukocyte Count

Analysis of leukocyte counts and cytology provide important diagnostic information regarding the cause of a synovial effusion (Table 53-1). A fresh specimen should be placed in a heparinized tube for rapid analysis and, if the fluid is particularly viscous, it may need to be diluted in normal saline before counting. Normal synovial fluid contains fewer than 180 nucleated cells/mm$^3$, most of which originate as desquamated synovial lining cells. The leukocyte count broadly classifies synovial fluids as noninflammatory (<2000 cells/mm$^3$), inflammatory (2000 to 50,000 cells/mm$^3$), and septic (>50,000 cells/mm$^3$). These definitions provide broad guidelines to help narrow the differential diagnosis rather than representing inherent biologic properties of the fluid.

The most common causes of non-inflammatory synovial fluids are mechanical derangements of the joint and osteoarthritis. Other causes include endocrinopathies such as acromegaly and hyperparathyroidism; inherited disorders such as ochronosis, hemochromatosis (which can also present with hemarthrosis), Ehlers-Danlos syndrome, Wilson's disease, and Gaucher's disease; acquired disorders such as Paget's disease, avascular necrosis, and osteochondritis dissecans; and an uncommon condition called *intermittent hydrarthrosis*, in which joints become effused in a cyclic manner. At the other extreme, leukocyte counts of 50,000 to 300,000 cells/mm$^3$ are most commonly associated with septic arthritis and should prompt the clinician to empirically treat the individual as such until this diagnosis is excluded with a high degree of certainty, which typically requires definitive culture results and, possibly, repeat aspiration. Leukocyte counts exceeding 50,000 cells/mm$^3$ are often seen in acute crystal-induced arthritis, particularly gout. Inflammatory cell counts between 3000 and 50,000 cells/mm$^3$ are seen in a wide spectrum of articular disorders, including many cases of septic arthritis. Thus, most patients with acute attacks of gout and pseudogout, active RA, reactive arthritis, and psoriatic arthritis, as well as patients with gonococcal arthritis and other nonpyogenic forms of septic arthritis, will typically present with synovial fluid cell counts in this range (see Table 53-1).

## Synovial Fluid Cytology

Characterization of the cells present in synovial fluid is an important diagnostic step that the examiner can achieve initially by performing cytology on a wet mount of the synovial fluid. To perform the wet mount analysis, the examiner places a single drop of synovial fluid on a clean glass slide, which then is covered by a coverslip and examined under low- and high-power light microscopy. In addition to leukocytes, and in the case of traumatic taps or hemarthroses, or large numbers of erythrocytes, wet mount may reveal the presence of clumps of fibrin and crystals, cartilage and synovium fragments, and lipid droplets. These can all appear as amorphous material, and care should be

**TABLE 53-1** Characteristics of Synovial Fluid

| | Appearance | Viscosity | Cells per mm$^3$ | % PMNs | Crystals | Culture |
|---|---|---|---|---|---|---|
| Normal | Transparent | High | <200 | <10% | Negative | Negative |
| Osteoarthritis | Transparent | High | 200-2000 | <10% | Occasional calcium pyrophospate and hydroxyapatite crystals | Negative |
| Rheumatoid arthritis | Translucent | Low | 2000-50,000 | Variable | Negative | Negative |
| Psoriatic arthritis | Translucent | Low | 2000-50,000 | Variable | Negative | Negative |
| Reactive arthritis | Translucent | Low | 2000-50,000 | Variable | Negative | Negative |
| Gout | Translucent to cloudy | Low | 200->50,000 | >90% | Needle-shaped, negatively birefringent monosodium urate monohydrate crystals | Negative |
| Pseudogout | Translucent to cloudy | Low | 200-50,000 | >90% | Rhomboid, positively birefringent calcium pyrophosphate crystals | Negative |
| Bacterial arthritis | Cloudy | Variable | 2000->50,000 | >90% | Negative | Positive |
| PVNS | Hemorrhagic or brown | Low | — | — | Negative | Negative |
| Hemarthrosis | Hemorrhagic | Low | — | — | Negative | Negative |

PMNs, Polymorphonuclear neutrophils; PVNS, pigmented villonodular synovitis.

taken to avoid assuming their composition without further characterization.

Characterization of synovial fluid leukocytes is best achieved by staining a dried smear of the fluid. Wright stain is most commonly used for this purpose. The phenotype and morphology of the leukocytes can then be assessed under high power by using oil immersion. Septic-range synovial fluid containing more than 50,000 cells/mm$^3$ is almost always associated with a high preponderance of polymorphonuclear leukocytes, often greater than 90%. Monocytes and lymphocytes predominate in the synovial fluid of patients with viral arthritis, lupus, and other connective tissue diseases. Synovial fluid samples from patients with active RA, reactive arthritis, psoriatic arthritis, and acute attacks of crystal-induced arthritis typically demonstrate a preponderance of polymorphonuclear leukocytes, although fluids from patients with early-stage RA may have a low leukocyte count with primarily mononuclear cells. The presence of large numbers of "ragocytes," which are granulocytes that have engulfed immune complexes, is associated with active RA, and their presence may indicate an unfavorable prognosis in this disease.[4] Reiter's cells represent cytophagocytic mononuclear cells that have phagocytized apoptotic polymorphonuclear leukocytes. This may represent a pathway by which autolysis and release of damaging mediators from the latter cells are avoided.[5] The presence of Reiter's cells is not specific for reactive arthritis, nor indeed for spondyloarthropathies in general. Occasionally, eosinophils will predominate in the synovial fluid, which may be associated with parasitic infection, urticaria, or hypereosinophilic syndrome. It has been suggested that cytocentrifugation of synovial fluid is the optimum method for performing cytopathology, although the cost-effectiveness of this technique is questionable in most clinical settings.

## Wet Smear Analysis by Polarized Microscopy

A search for crystals by using polarized microscopy is particularly valuable in the diagnosis of acute monoarthritis or oligoarthritis, in which gout and pseudogout are often on the differential diagnosis. In such a clinical situation, if indeed the absence of pathogenic crystals in synovial fluid can be established, the likelihood of septic arthritis increases, prompting the initiation of intravenous antibiotics and potentially necessitating a hospital admission. Thus the rapid and accurate diagnosis of a crystal-induced process can prevent a costly and unnecessary sequence of events. It is helpful if the individual or the team that performs the arthrocentesis can also rapidly examine the specimen by polarized microscopy. For this procedure, the examiner requires a functional polarizing microscope, as well as adequate experience in the identification of crystals using this technique. This is particularly important in the case of calcium pyrophosphate crystals, which are notoriously difficult to detect.

The examiner should take care that the slide and the coverslip are free of dust, talc, and other particulate matter. Crystals present in the specimen rotate the light in such a way that they appear as bright objects in an otherwise dark field. Birefringent debris frequently are scattered throughout the slide and should not be mistaken for crystals.

The first-order red compensator is usually inserted immediately below the upper filter and serves to block out green light. Birefringent material in the specimen appears as a bright yellow or blue color in the red field generated by the first-order compensator. While birefringent crystals are rotated relative to the axis of the first-order compensator, the color changes from yellow to blue, or vice versa. Crystals that are yellow when oriented parallel to the axis of the compensator are negatively birefringent, and those that are blue are positively birefringent.

Identification of crystals in synovial fluid is greatly facilitated by detailed examination of the specimen, under both low and high power, by using the approach previously described. A combination of morphology and birefringence serves to identify the crystals. Monosodium urate (MSU) crystals, as shown in Figure 53-2, are the easiest to identify because the crystal load is typically high during an acute attack of gout. A good degree of concordance between laboratories in the identification of MSU crystals has been shown.[6-8] These crystals appear as strongly negatively birefringent needle-shaped objects, many of which are intracellular, having been phagocytized by synovial fluid leukocytes. In contrast, calcium pyrophosphate dihydrate (CPPD) crystals seen during attacks of pseudogout tend to be smaller, rhomboid-shaped objects that are weakly

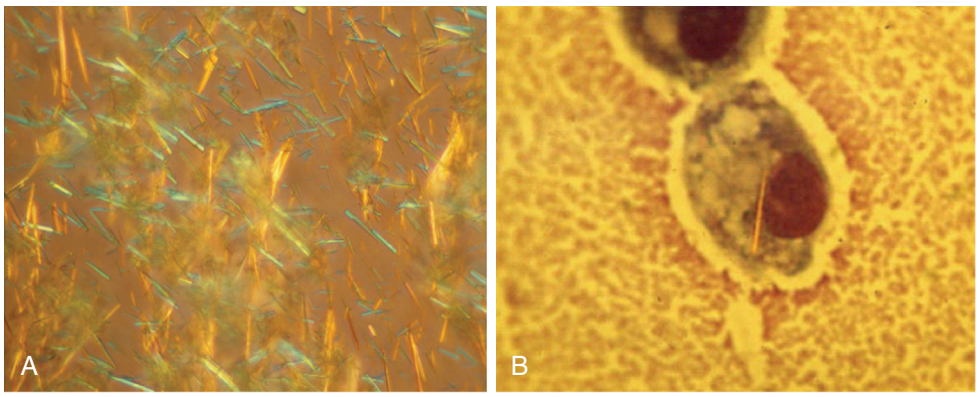



**Figure 53-2** **A,** Urate crystals in the tophus from a patient with gouty arthritis. Crystals are negatively birefringent and needle shaped. **B,** Intra-cellular urate crystal as seen on Wright stain. *(Courtesy H. Ralph Schumacher, Jr.)*

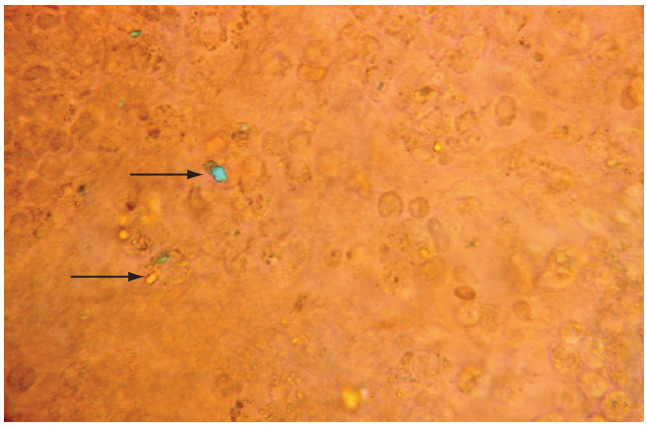


**Figure 53-3** Calcium pyrophosphate crystals in the synovial fluid from a patient with pseudogout. Crystals are positively birefringent and rhomboid shaped *(arrows). (Courtesy H. Ralph Schumacher, Jr.)*

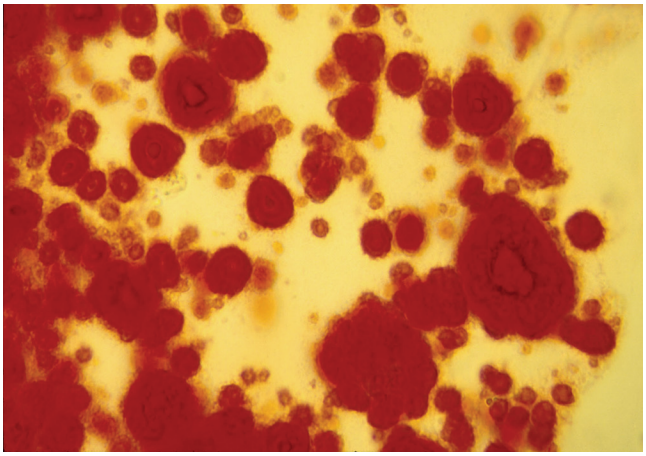


**Figure 53-4** Clumps of calcium hydroxyapatite crystals demonstrated by using alizarin red staining. Crystals are nonbirefringent. *(Courtesy H. Ralph Schumacher, Jr.)*

positively birefringent, as shown in Figure 53-3. Because the CPPD crystal load during an attack of pseudogout tends to be relatively low, and because CPPD crystals are only weakly birefringent, it is important to examine all areas of the specimen on the microscope slide, and possibly to prepare a second wet mount to exclude or confirm this diagnosis. Concordance between laboratories in the recognition of CPPD is substantially lower than in the case of MSU crystals.[6-8] A particularly challenging situation arises when intra-cellular crystals cannot be identified, yet birefringent extra-cellular objects resembling crystals are seen scattered throughout the slide. This may be caused by powder from gloves or dirt on the slides.

As with other analyses on the synovial fluid, the examiner should perform wet mount preparation and analysis as quickly as possible, although identification of crystals can still be successful after prolonged storage of specimens. The crystal load decreases substantially while the acute inflammatory attack subsides, thus making a specific diagnosis more difficult while the attack begins to subside. Urate crystals have been detected in synovial fluid between attacks of gout.

Deposits of hydroxyapatite or basic calcium phosphate are present within the joint and in periarticular locations such as around the shoulder area, and are associated with osteoarthritis. These crystals have been implicated in a particularly destructive syndrome that has been named *Milwaukee shoulder*.[9] Hydroxyapatite can be detected in synovial fluid, but because these crystals are generally nonbirefringent, it is not possible to detect them by polarized microscopy. A useful and rapid method with which to detect hydroxyapatite and other calcium-containing crystals such as octacalcium and tricalcium phosphate is to stain the fluid with alizarin red S stain and look for clumps of crystals under routine light microscopy (Figure 53-4). These crystals have also been identified by using electron microscopy, although this method is rarely available to the practicing clinician.

Synovial cholesterol crystals appear as flat, platelike structures with notched corners (Figure 53-5), and lipid crystals have the appearance of Maltese crosses. Both can be strongly birefringent, both negatively and positively.

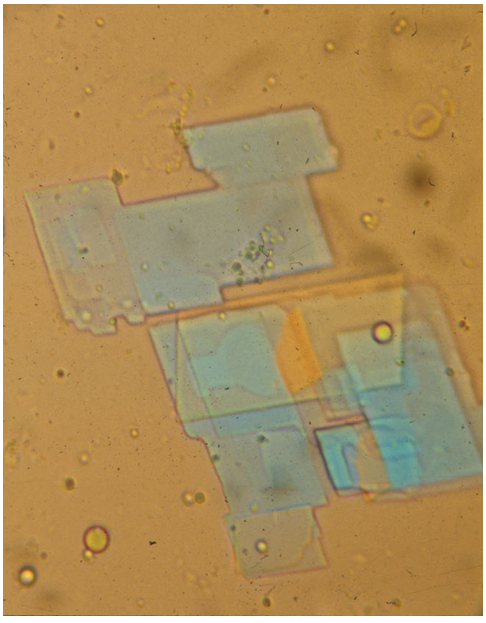


**Figure 53-5** Cholesterol crystals in a synovial fluid sample. *(Courtesy H. Ralph Schumacher, Jr.)*

Corticosteroid crystals can be highly birefringent and mimic urate or CPPD crystals. Large amounts of lipid in the synovial fluid can be visible on gross examination. The significance of these crystals in synovial fluid is unclear, but it is unlikely that they are pathogenic in most cases.

## Detection of Microorganisms by Gram Stain, Culture, and Polymerase Chain Reaction Analysis of Synovial Fluid

A wide spectrum of organisms can cause septic arthritis, although the most common pathogens are Gram-positive bacteria such as staphylococci and streptococci. Because septic arthritis causes rapid destruction of the joint, and because it can spread hematogenously to other areas and is

associated with significant mortality, it is imperative that a specific diagnosis be made as quickly as possible, and that empiric therapy with broad-spectrum antibiotics be started until this diagnosis can be confirmed or excluded.

A Gram stain performed on fresh synovial fluid will identify an organism in an estimated 50% of cases of septic arthritis,[10] with the highest sensitivity for Gram-positive organisms. Moreover, the specificity of a positive Gram stain approaches 100%. Clearly, this indicates that the positive predictive value for the Gram stain is very high, and that the negative predictive value is substantially lower. The gold standard for diagnosing septic arthritis is still bacteriologic culture, which has a sensitivity of 75% to 95% and a specificity of 90% in cases of nongonococcal septic arthritis.[11,12] The use of blood culture bottles further increases the yield of positive synovial cultures.[13] Bacteriologic cultures are the only studies that provide a guide for specific anti-microbial therapy. Because the sensitivity of bacteriologic cultures declines dramatically after antibiotic therapy is instituted, it is important that the clinician perform arthrocentesis before any antibiotics are administered. Cultures should be performed even when uric acid or other crystals are demonstrated in the synovial fluid because gout and septic arthritis can coexist.[14] In the case of gonococcal arthritis, the sensitivity of bacteriologic culture, even if performed on a sample collected by using appropriate media, is low, with an estimate of less than 10%.

Polymerase chain reaction (PCR) carries a high degree of sensitivity and specificity for the detection of microorganisms in synovial fluid and tissue, even in individuals who are culture negative.[15] Most bacteria can be detected on the basis of amplifying specific sequences in their ribosomal RNA (16S rRNA). PCR is now the procedure of choice for diagnosing gonococcal arthritis,[16,17] and is a highly sensitive and specific method of detecting tuberculous arthritis, although as discussed later, analysis of synovial tissue is better than analysis of synovial fluid for making this diagnosis.[18,19] PCR is also a method of verifying the successful elimination of the offending organism in cases of septic arthritis.[20,21]

The sensitivity and specificity of PCR in detecting synovial microorganisms should be balanced against the biologic significance of a positive test. Contaminants are easily detected by using this method, and highly stringent conditions for sample collection are required to prevent false-positive tests. Moreover, PCR studies of synovial fluid and tissue from a spectrum of chronic forms of arthritis, including RA, osteoarthritis, reactive arthritis, and undifferentiated arthritis, have indicated the presence of microorganisms in a significant number of specimens.[22,23] The biologic significance of these findings and the potential role of bacterial DNA or cell wall fragments in the pathogenesis of these arthropathies remain unclear.

## BIOCHEMICAL ANALYSIS OF SYNOVIAL FLUID

A number of widely available biochemical tests may add to the diagnostic impression of aspirated synovial fluid samples, although lack of specificity of these biochemical analyses tends to limit their value.[12,24] Testing for synovial fluid glucose, protein, and lactate dehydrogenase (LDH) has long been included in routine practice, and values obtained should be compared with serum values. Samples from septic arthritis typically exhibit very low glucose, low pH, and high lactate levels; these levels are indicative of a switch to anaerobic metabolism. Highly inflammatory synovial fluids from RA exhibit a similar profile, along with high protein and LDH levels. Levels of pressure of oxygen ($pO_2$) in the blood are often in the hypoxic range in RA synovial fluids and are correlated with increased lactate and levels of pressure of carbon dioxide ($pCO_2$) in the blood.[25,26] A prospective study conducted to evaluate these tests in a spectrum of inflammatory and non-inflammatory disorders demonstrated considerable variability in each diagnostic category, which limits their clinical utility.[24]

Serologic testing of synovial fluid to detect rheumatoid factor, anti-nuclear antibodies, and complement levels has been suggested as a method that can be used to confirm a diagnosis of RA or other connective tissue diseases. In particular, RA synovial fluids may be positive for rheumatoid factor even when serum is not,[27] and complement levels are typically low as a result of consumption by immune complexes. These findings are of insufficient sensitivity and specificity to be of value on a routine clinical basis.

### Synovial Fluid Analysis in Arthritis Research

The ease with which synovial fluid is aspirated from inflamed joints has allowed a wide spectrum of research studies to be conducted on this biologic material. In research settings, cells in synovial fluid samples are typically separated by centrifugation, and cellular and noncellular components of the fluid are analyzed separately. Detailed analysis of the phenotype and functional properties of synovial fluid leukocytes has been particularly informative in RA and reactive arthritis research, in which immunophenotyping of lymphocyte subpopulations has provided important clues to the pathogenesis of these diseases. In the case of reactive arthritis, in which triggering organisms are often identified, the proliferative and cytokine responses of synovial fluid lymphocytes to antigens derived from *Chlamydia*, *Yersinia*, and other pathogens have been elucidated.[28,29] Synovial fluid T cells from reactive arthritis patients are biased toward production of T helper (Th)2 cytokines such as interleukin (IL)-10 and IL-4, whereas synovial fluid T cells from patients with RA are Th1 biased and exhibit defects in Th2 differentiation.[30-32]

Analysis of the noncellular portion of synovial fluid has provided important information regarding a spectrum of soluble molecules, including cytokines and growth factors,[33] extra-cellular matrix proteins, autoantibodies, and therapeutic drug levels. Moreover, broad-based proteomic studies of synovial fluid with the use of fractionation techniques and mass spectrometry are beginning to provide novel approaches to understanding pathogenesis and prognosis in arthropathies such as RA.[34]

## SYNOVIAL BIOPSY

Sampling of synovial tissue is a direct approach to defining the pathologic processes that cause swollen, painful joints.. In clinical settings, it can be particularly valuable in evaluating an undiagnosed persistent monoarthritis when other

investigations, including synovial fluid analysis, have failed to provide a specific diagnosis. In research settings, analysis of synovial tissue samples has dramatically improved our understanding of the pathogenetic mechanisms underlying RA, spondyloarthropathies, and other chronic articular disorders. More recently, synovial biopsy has been explored as a method for defining the target tissue response to therapeutic agents, particularly targeted biologic therapies.

## Blind Percutaneous Synovial Biopsy

Percutaneous needle biopsy is most commonly performed according to the method originally described by Parker and Pearson,[35,36] utilizing a biopsy needle that now carries their name. Percutaneous synovial biopsy is most often performed on the knee joint, although the technique can readily be adapted for use in other joints such as the wrist, elbow, ankle, or shoulder. A modification of the original Parker-Pearson needle has facilitated synovial biopsy of small hand joints such as metacarpophalangeal and proximal interphalangeal joints.[37] The technique for Parker-Pearson synovial biopsy uses a 14 gauge needle with a lateral aperture just proximal to the inserted end of the needle. This lateral opening features a sharp cutting edge for severing trapped synovial tissue that is captured by applying suction with a 3 to 5 mL syringe. With this approach, multiple 1 to 3 mm samples are obtained by angling the trocar in several directions. This also serves to minimize the sampling error involved. Synovial samples are typically pink and are easily removed with a slight twisting motion. Because of the blind nature of the procedure, samples of fat, muscle, or fibrous tissue may be obtained and need to be separated from true synovial samples.

Percutaneous synovial biopsy is easily performed in most ambulatory care settings with the use of relatively inexpensive equipment, and recently ultrasound guidance has improved the yield. The overall morbidity of the procedure is low and is comparable with that of arthrocentesis, with perhaps a slightly higher rate of hemarthrosis. The risk of hemarthrosis can be minimized if the patient does not bear weight for a few hours after the procedure. The main disadvantage of the procedure is its blind nature. In comparison with visually guided arthroscopy, samples derived from the interface between synovium and adjacent cartilage are underrepresented when the blind procedure is used.[38,39] As discussed later, this drawback is particularly relevant to a number of research questions.

## Arthroscopically Guided Synovial Biopsy

Arthroscopy is widely used by orthopedic specialists for the diagnosis and treatment of a variety of articular disorders, particularly mechanical derangements of intra-articular structures such as cruciate ligaments and menisci. During the past two decades, the arthroscopic procedure has been adapted for diagnostic synovial biopsies in settings that do not require a fully equipped operating theater and general anesthetic. In most cases, intra-articular local anesthesia suffices for the procedure, although conscious sedation may be required in some individuals. The procedure is well tolerated and is associated with low morbidity, although the risks to the patient of hemarthrosis and infection after the procedure are slightly higher than that of percutaneous needle biopsy. The patient should be instructed to minimize weight bearing for 24 to 48 hours after the procedure.

The primary advantage of arthroscopy is its ability to visually guide the biopsy procedure. This permits macroscopic evaluation of the synovium and sampling of areas that appear to be severely affected by the pathologic process, and it allows for sampling of the interface between inflamed synovium and adjacent cartilage. This interface is an area of particular interest for understanding the pathogenesis of destructive arthropathies such as RA.[38] As with samples obtained by percutaneous synovial biopsy, individual samples are allocated for specific laboratory studies depending on the clinical or research question under investigation.

## Processing Synovial Tissue Samples

In all cases, an adequate number of individual synovial specimens need to be allocated for routine light microscopy with the use of formalin fixation and paraffin embedding. This provides the highest-quality sections for hematoxylin and eosin (H&E) histologic analysis, and it allows the most accurate delineation of pathologic processes within tissue. Although formalin-fixed sections can be used in some cases for immunohistology, formalin fixation alters the conformation of many protein antigens, making them inaccessible for specific identification by immunohistology. Many of the molecular markers used to analyze diseased synovium, including cell surface markers, cytokines, adhesion molecules, and proteases, require that tissue samples be snap frozen in a suitable mounting medium, such as optimal cutting temperature compound, and then sectioned with the use of a cryostat. The sections can be processed by using antigen-specific monoclonal or polyclonal antibodies, and color development achieved by one of several immunofluorescence or immunoperoxidase methods. Typically, a nuclear counterstain is also used to assist in orientation of the tissue—hematoxylin in the case of immunoperoxidase studies. If only formalin-fixed, paraffin-embedded tissue is available, an alternative method for detecting antigens that are sensitive to formalin fixation is antigen retrieval.

Several antigen retrieval methods are available, including enzymatic and thermal methods,[40] which have been used to successfully retrieve a spectrum of antigens from archival synovial tissue samples for immunohistologic studies, although the quality of the tissue sections often deteriorates after antigen retrieval. A number of double-staining immunohistology techniques have been developed for simultaneous evaluation of the expression of two markers in the same tissue section, although these techniques are labor intensive and often require considerable experimentation to generate good stains.[41] Formalin fixation dissolves crystals, and if this is a diagnostic consideration, the specimen should be fixed in ethanol.

The sensitivity and specificity of molecular DNA and RNA techniques provide unprecedented opportunities to explore the pathogenesis of synovial disorders. Although these studies can be carried out on very small quantities of tissue, examiners need to take great care in handling and processing tissue samples to prevent degradation of the nucleic acids, particularly with RNA when RNAase enzymes

are ubiquitous, and can rapidly degrade the small quantity of RNA present in a tissue sample. As discussed later, the search for microbial DNA and RNA has been of particular interest in attempts to understand the cause and pathogenesis of reactive arthritis, RA, and other forms of chronic synovitis of unknown cause. Techniques used to analyze human gene expression in small tissue samples have rapidly progressed. This has enabled the detection and quantitation of multiple mRNA transcripts in very small quantities of biopsy material, in many cases without the need for amplification.[42,43]

## SYNOVIAL PATHOLOGY

### Synovial Membrane in Healthy Individuals

A detailed description of the composition of normal synovium is provided in Chapter 2. Histologically, the normal synovial lining layer is one to three cells thick and is composed of closely associated macrophage-like (type A) and fibroblast-like synoviocytes (type B) that are not separated from the underlying stroma by a basement membrane, as is the case with a true epithelium. In many areas, visible gaps in this lining layer allow small molecules to easily diffuse through the extra-cellular matrix into the synovial fluid. The two types of lining layer synoviocytes are distinct and can be differentiated on the basis of ultrastructural and immunohistologic features. Macrophage-like synoviocytes are myeloid in origin, as they exhibit the morphologic characteristics of phagocytic cells and express macrophage markers such as CD68, CD14, and FcγRIIIa. Fibroblast-like synoviocytes are synthetic cells of mesenchymal origin that are the primary source of hyaluronan and other proteoglycans found in normal synovial fluid. They express CD55 (decay-accelerating factor [DAF]), high levels of vascular cell adhesion molecule (VCAM)-1, and the enzyme uridine diphosphoglucose dehydrogenase (UDPGD), which is involved in the synthesis of hyaluronan and has been detected by cytochemical methods (Figure 53-6). Fibroblast-like synoviocytes have also been shown to uniquely express cadherin-11, a specialized adhesion molecule that is involved in homotypic aggregation of these cells and that contributes to maintaining the integrity of the synovial lining layer.[44] Quantitatively, most of the cells in the normal synovial lining layer are type B cells. The underlying stroma features a rich network of capillaries with fenestrated endothelium in the immediate sublining area that serve to maintain the health and viability of adjacent cartilage. Larger arterioles and venules can be found deeper in the synovial stoma. The synovial microvasculature is surrounded by loose connective tissue, which also incorporates the synovial lymphatics that serve to drain this tissue. The synovium of completely asymptomatic individuals not uncommonly exhibits a modest infiltrate of T lymphocytes that are occasionally organized in perivascular aggregates, although B cells were not seen.[45]

### Synovial Histopathology in the Evaluation of Monoarthritis

Pathologic analysis of synovial tissue samples can be of considerable value in certain clinical settings. However, the histopathologic interpretation of synovial biopsy specimens is often nondiagnostic and lacks specificity.[46] Pathologic analysis of synovial samples from patients with undiagnosed monoarthritis may be of particular value. The presence of large numbers of neutrophils in the synovial tissue stroma is highly suggestive of septic arthritis, and in such cases Gram stain may reveal bacteria in the tissue. Because septic arthritis is usually acute in onset, synovial biopsy is rarely required, and the diagnosis can be made by analyzing synovial fluid as described previously. Gonococcal arthritis may require synovial biopsy for diagnosis (Figure 53-7). A mononuclear cell infiltrate, on the other hand, is more consistent with a chronic inflammatory process and has a wide differential diagnosis, as described previously. The presence of granulomas supports a diagnosis of tuberculous arthritis or sarcoidosis, both of which cause chronic monoarthritis. The synovial granulomas of tuberculosis (TB) may be caseating or noncaseating, and staining of the tissue for acid-fast bacilli, culture, and molecular probing can yield a definitive diagnosis in an estimated 50% of cases. Similarly, a spectrum of fungal infections can be diagnosed by using similar approaches, but special stains such as a Gomori stain may be required. The diagnosis of sarcoid arthropathy is suspected in synovial specimens with noncaseating granulomas in cases where mycobacterial or fungal infection has been excluded.

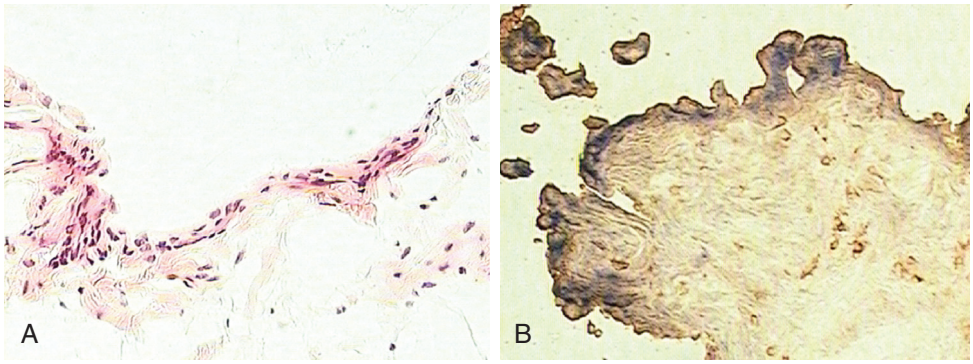



**Figure 53-6** Normal synovium. **A,** A lining layer one to two cells deep that is composed of macrophage-like synoviocytes (type A) and fibroblast-like synoviocytes (type B). **B,** Normal synovium stained for the enzyme uridine diphosphoglucose dehydrogenase, an indicator of hyaluronan synthesis by fibroblast-like synoviocytes.

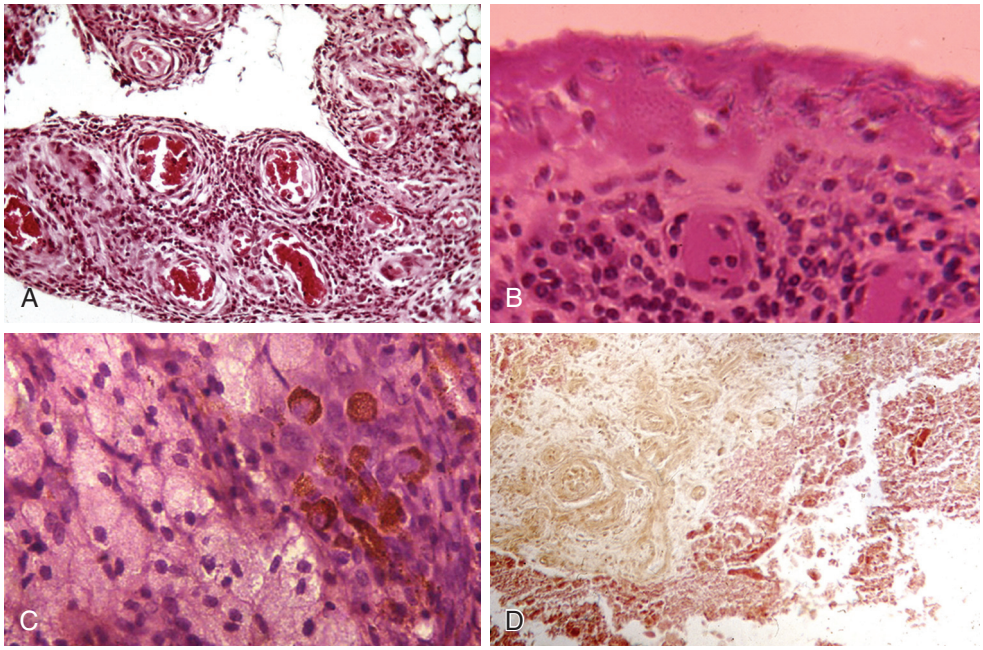



**Figure 53-7** **A,** Synovial pathology of gonococcal arthritis. A marked infiltrate with polymorphonuclear leukocytes and vascular congestion is present. **B,** Synovial pathology of scleroderma shows loss of the lining layer with surface fibrin deposition and mononuclear inflammation in sublining areas. **C,** Pigmented nodular synovitis with hemosiderin deposits and foamy cells. **D,** Amyloidosis with deposits on the synovial surface, Congo red stain. *(**A-D,** Courtesy H. Ralph Schumacher, Jr.)*

Pigmented villonodular synovitis is an important consideration in individuals with chronic monoarthritis of a large joint such as the knee or hip. This disorder has a characteristic MRI appearance caused by hemosiderin deposits in the synovium and large cystic lesions in adjacent bone. Histopathologic analysis of the synovium can confirm this diagnosis and demonstrates a diffusely hypervascular proliferative lesion with mononuclear cells of the monocyte/macrophage lineage, foamy multinucleated cells resembling osteoclasts, and hemosiderin deposits[47] (see Figure 53-7). Synovial sarcomas are rare tumors that must be diagnosed on the basis of synovial pathology.

## Synovial Histopathology in the Evaluation of Polyarthritis

In current clinical practice, the availability of well-validated diagnostic criteria and specific serologic tests, combined with a relative lack of specificity in synovial histopathologic features, limits the clinical utility of synovial pathology in the differential diagnosis of oligoarthritis and polyarthritis. On the other hand, analysis of synovial tissue samples obtained in the context of research studies from patients with RA and various spondyloarthropathies has dramatically enhanced our understanding of the cellular and molecular mechanisms of these disorders. This is reflected in a large body of literature published during the past three decades.[38,48]

RA synovium has been the most extensively studied histopathologically, and a detailed discussion of RA synovitis can be found in Chapter 69. The two characteristic features seen in RA synovitis are hyperplasia of the lining layer and infiltration of the sublining stroma with mononuclear cells (Figure 53-8). The surface of the lining layer is often covered with fibrin deposits generated from activation of the fibrinolytic system in inflammatory synovial fluid. Occasionally, the synovial lining layer is completely denuded and is replaced by a dense fibrin cap. In highly inflamed tissues, fibrin deposits extend deeply into the sublining stroma, which may be edematous because of the marked increase in vascular permeability. The earliest synovial changes in RA appear to feature microvascular abnormalities,[49] and mononuclear cell infiltrates have been detected in asymptomatic joints of patients with RA.[50,51] These features are nonspecific and are seen in the synovium of acutely inflamed joints from a spectrum of disorders, including reactive arthritis and psoriatic arthritis.

In RA, the mononuclear cell infiltrate in the sublining stroma can be diffuse but more commonly is arranged in perivascular aggregates that resemble lymphoid follicles (see Figure 53-8). Although the presence of lymphoid aggregates in the synovial membrane is typical of RA, this histopathologic feature is by no means unique to RA synovitis.[52-55] Lymphoid follicles are typically located near vessels with tall endothelium, which are termed *high endothelial venules;* these vessels specialize in the recruitment of lymphocytes (Figure 53-9). Multinucleated giant cells are occasionally seen in RA synovium (Figure 53-10), and some tissues demonstrate granuloma formation. Finally, synovial tissue obtained at the time of joint arthroplasty often exhibits extensive fibrosis and may be indistinguishable from arthroplasty samples obtained from patients with osteoarthritis.

The synovial histopathology of psoriatic arthritis, ankylosing spondylitis, and reactive arthritis has been compared with that of RA.[56,57] In all cases, a similar spectrum of inflammatory cell populations has been identified, but

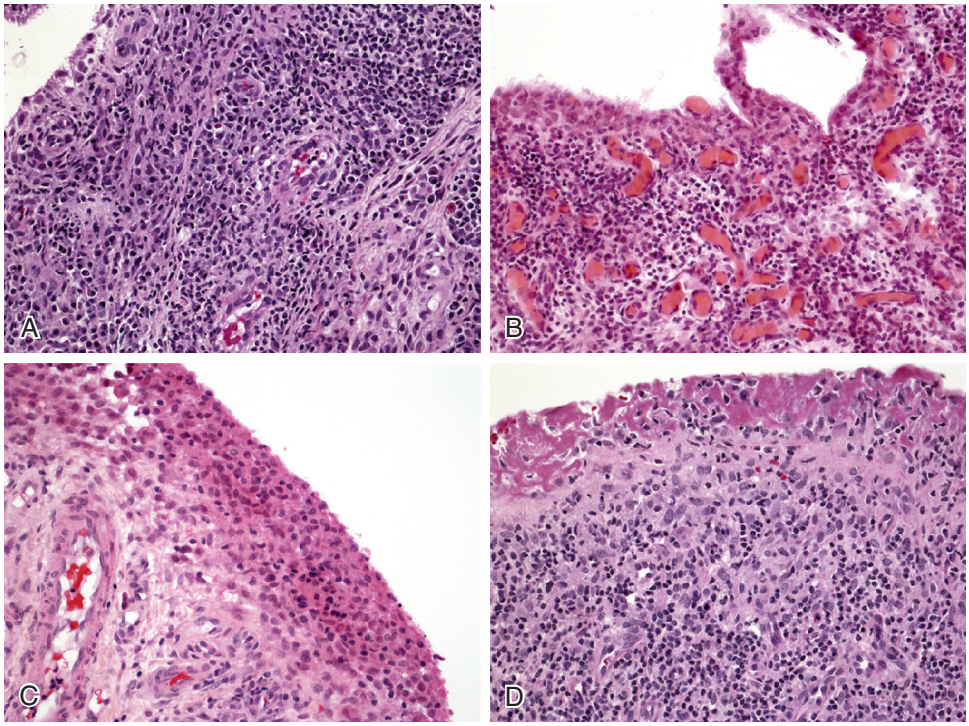



**Figure 53-8** Histopathology of rheumatoid arthritis synovitis. **A,** Lymphoid aggregate. **B,** Diffuse lymphocytic infiltrate. **C,** Hyperplasia of the lining layer. **D,** Fibrin cap replacing a denuded lining layer.

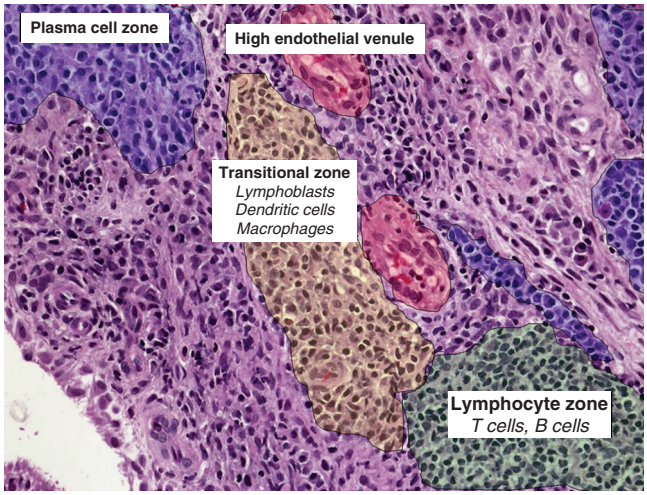



**Figure 53-9** Microarchitecture of rheumatoid arthritis synovial lymphoid aggregates.

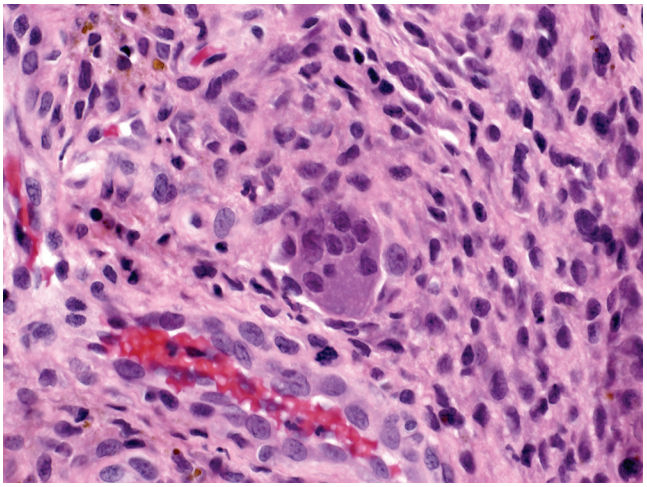


**Figure 53-10** Multinucleated giant cell in a patient with rheumatoid arthritis.

several subtle and potentially important differences have been observed. Overall, synovial histologic and immunohistologic features of psoriatic arthritis, both oligo- and polyarticular, resemble those of other spondyloarthropathies to a greater extent than RA (see Synovial Immunohistology section below).[57] Comparative studies have suggested that synovial lesions in psoriatic arthritis are more vascular than those of RA, with more tortuosity of the synovial microvasculature.[58,59] This is evident both macroscopically and microscopically. Moreover, lymphoid aggregates of various sizes were identified in 25 of 27 synovial tissue samples from patients with psoriatic arthritis, and 13 of 27 had large organized aggregates with all of the features of ectopic lymphoid neogenesis that have been associated with RA synovitis.[52] Studies of synovium from the peripheral joints of ankylosing spondylitis patients have revealed intense infiltrates of lymphocytes, plasma cells, and lymphocytic aggregates.[60,61] Comparisons made between the synovial lesions seen in patients with reactive arthritis and those seen in patients with early-stage RA of similar disease duration

suggest that reactive arthritis synovia are less infiltrated with B lymphocytes, plasma cells, and macrophages.[62,63] It should also be noted that synovium from patients with osteoarthritis often features the presence of lymphocyte aggregates, although these tend to be small and less well developed than those seen in RA.[54]

The synovium of lupus patients showed synovial hyperplasia, inflammatory infiltrates, vascular proliferation, edema and congestion, fibrinoid necrosis and intimal fibrous hyperplasia of blood vessels, and superficial fibrin deposits, although these changes were quantitatively modest compared with those of RA.[64] In early scleroderma, the lining layer was seen to be thin with deposits of fibrin and stromal lymphocytes and plasma cells,[65] and similar changes were seen in patients with dermatomyositis and polymyositis[66] (see Figure 53-7). A recent study comparing the immunopathologic features of early untreated Behçet's disease with those of psoriatic arthritis noted that although a similar degree of inflammation was seen in the two disorders, synovitis in Behçet's disease demonstrated higher numbers of neutrophils and T cells than were seen in psoriatic synovitis.[67]

In patients with chronic crystal arthropathies, large deposits of birefringent material can be detected in the synovium.[68] Amyloid arthropathy can be diagnosed by demonstrating amyloid deposits in the synovium with Congo red staining (see Figure 53-7). The synovium in ochronosis contains brownish shards of cartilage.[69] Multicentric reticulohistiocytosis can be diagnosed pathologically by the presence of large foamy cells and multinucleated cells in the synovium. In arthritis of hemochromatosis, the synovium exhibits brown hemosiderin deposits in the lining cells, and CPPD crystals can also be observed.[70]

## SYNOVIAL IMMUNOHISTOLOGY

### Sampling Error and Quantitative Analysis

Immunohistology utilizes specific monoclonal or polyclonal antibodies with well-defined molecular targets and is an effective tool for analyzing the cellular and molecular features of the synovium. While the field has progressed during the past two decades, it has become clear that algorithms for generating reproducible quantitative data from immunohistologically stained sections are required. Moreover, approaches are needed for minimizing the sampling bias that is inherent in biopsy-based studies.[71] Studies have suggested that if six or more individual specimens from different parts of the joint are examined, variance is reduced to less than 10% for T cell and activation markers.[72] Furthermore, synovial inflammatory features are similar in areas adjacent to and distant from the pannus cartilage junction, with the possible exception of macrophage numbers, which tend to be higher in adjacent areas.[73,74]

Various methods have been proposed by which quantitative data for immunohistologically stained synovial tissue sections can be generated.[75,76] The easiest and least costly method is to generate semiquantitative scores of staining intensity (e.g., on a 0 to 3 scale) from multiple areas of the tissue, and on the basis of these to obtain an average score for the entire tissue. The reliability and reproducibility of this method are increased if two observers score the tissue sections independently and a final average of the scores is generated. Computer-assisted image analysis involves capturing images from multiple areas of the tissue samples to which color-specific quantitative software algorithms are then applied. This method generates the greatest quantity of reproducible data but requires expensive equipment and certain levels of operator skill. Furthermore, differences in the background staining intensity of individual sections can make this type of analysis technically difficult.

### Synovial Lining Cell Layer

Compared with normal synovium, the lining layer in RA and other forms of chronic inflammatory arthritis is often hyperplastic, resulting from an increase in both type A and type B cells, as indicated by an increase in CD68 and CD55 staining, respectively (Figure 53-11). It is assumed that macrophage-like synoviocytes are recruited from the blood and then migrate through the synovial stroma and ultimately are retained in the lining layer in close association with fibroblast-like synoviocytes. It is currently proposed that the increase in fibroblast-like synoviocytes may be related more to defects in apoptosis than to recruitment or local proliferation. Expression of several families of adhesion molecules by both types of lining cells results in their close association and modulates their activation status. These include β1 and β2 integrins and their respective immunoglobulin supergene family ligands, particularly intercellular adhesion molecule (ICAM)-1 and VCAM-1.[77-79] Cadherin-11 expressed by fibroblast-like cells likely plays a key role in the adhesive interactions that sustain the lining layer hyperplasia.[44] This adhesion molecule is widely expressed in the lining layer of normal cells, as is shown in Figure 53-12. The relationship between fibroblast-like synoviocytes in the lining layer and other populations of mesenchymal cells in the sublining stroma remains uncertain. Immunohistology indicates that expression of CD55, VCAM-1, and cadherin-11 is primarily seen in the lining cell layer with minimal evidence of expression in sublining fibroblast populations. Similarly, our understanding of the relationship between lining layer macrophage-like cells and sublining macrophages is incomplete, and both express widely used macrophage markers such as CD68 and CD14. Seminal work from Edwards and associates has suggested that macrophage-like lining cells preferentially express FcγRIIIa receptors, which may serve to localize immune complexes to the synovium.[80]

Functionally, the lining cell layer in chronic inflammatory arthropathies such as RA and psoriatic arthritis typically has the appearance of being activated. Human leukocyte antigen (HLA)-DR is highly expressed, particularly by macrophage-like cells, which may suggest a role for these cells in antigen presentation.[81] Several studies have indicated that cells in the lining layer are the principal source of cartilage-degrading proteases, particularly matrix metalloproteinase (MMP)-1 and MMP-3[82,83] (Figure 53-13). The lining layer is generally less hyperplastic in spondyloarthropathies such as psoriatic arthritis and reactive arthritis compared with RA.[57,61,84] Less is known about the functional state of the lining cells in these disorders, although it is likely that differences compared with RA are quantitative rather than qualitative.

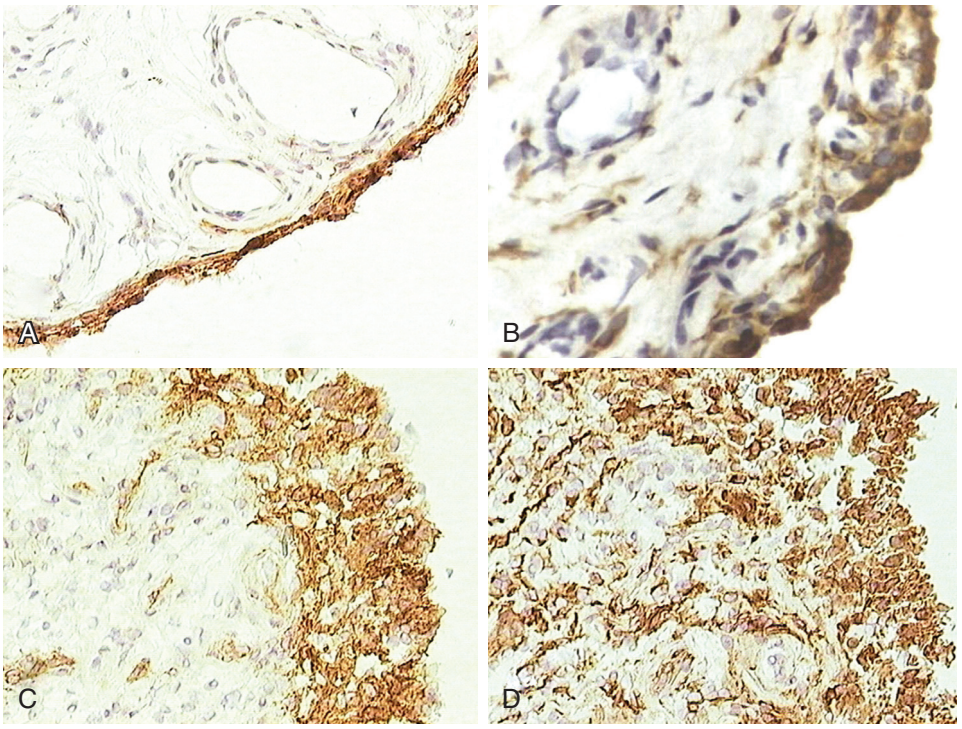



**Figure 53-11** **A-D,** Immunoperoxidase staining *(brown color)* of normal synovium and rheumatoid arthritis (RA) synovium for CD55 (fibroblast-like synoviocytes) and CD68 (macrophage-like synoviocytes). Both subsets of lining cells are increased in the hyperplastic lining layer of RA synovium.

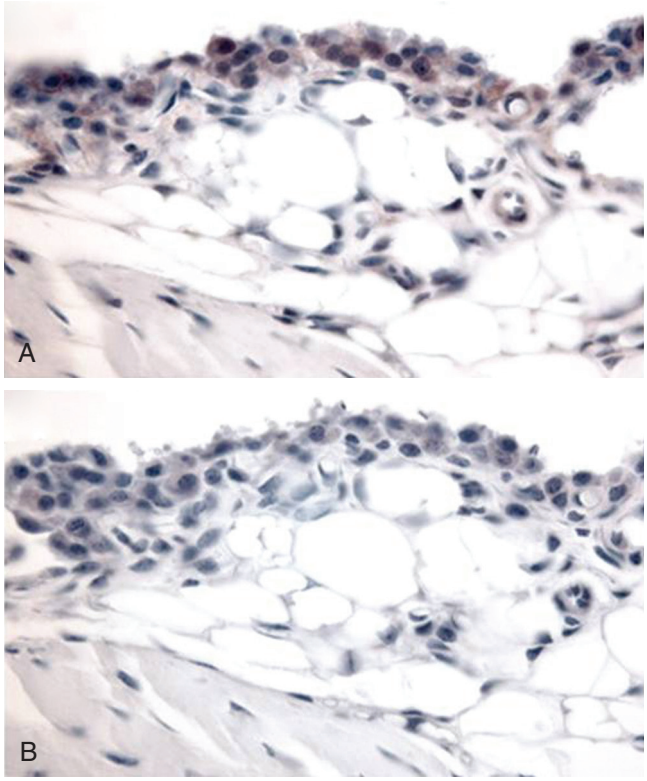



**Figure 53-12** Immunohistochemical staining for cadherin-11 in the normal synovial lining layer *(brown color)* **(A).** Control staining is shown in **B.** *(From Lee DM, Kiener HP, Agarwal SK, et al: Cadherin-11 in synovial lining formation and pathology in arthritis.* Science *315:1006–1010, 2007.)*

## Synovial Lymphocytes and Plasma Cells

A predominance of $CD3^+$ T cells, is found in the synovial tissues of patients with RA and spondyloarthropathies, and the CD4/CD8 ratio is 4:1 or greater in the lymphocytic aggregates but is lower in more diffuse infiltrates. Moreover, the CD4 cells in the aggregates also express CD27,[85] which facilitates B cell help. Considerable attention has been paid to whether the infiltrating T cells in RA and other arthropathies are primarily Th1 (interferon [IFN]-γ producing) or Th2 (IL-4 producing) biased, but the data in this area have been inconsistent. Until recently, it was suggested that T cells in synovium from patients with RA are more Th1 biased compared with those in synovium from patients with spondyloarthropathies, with a higher Th1/Th2 cytokine ratio.[86] Identification of a third subset of Th cells that express IL-17 and play a central role in chronic inflammatory disorders has necessitated a revision in the role that T cells play in synovitis.[87,88] The presence of IL-17, IL-1β, and tumor necrosis factor (TNF) in RA synovium was found to be predictive of progressive damage.[89] Furthermore, a subset of CD4 T cells expressing CD25 and the gene *FoxP3*, so-called *regulatory T cells* (Tregs), are now known to play a regulatory role in antigen-specific T cell expansion. Although Tregs are readily detected in the joints of patients with RA and other inflammatory arthropathies, their suppressor function appears to be defective in this microenvironment.[90-93] It has been suggested that CD8 T cells are needed to maintain the structure of ectopic lymphoid-like structures in RA synovium, even though the numbers of these T cells typically are substantially lower than the numbers of $CD4^+$ cells.[94]

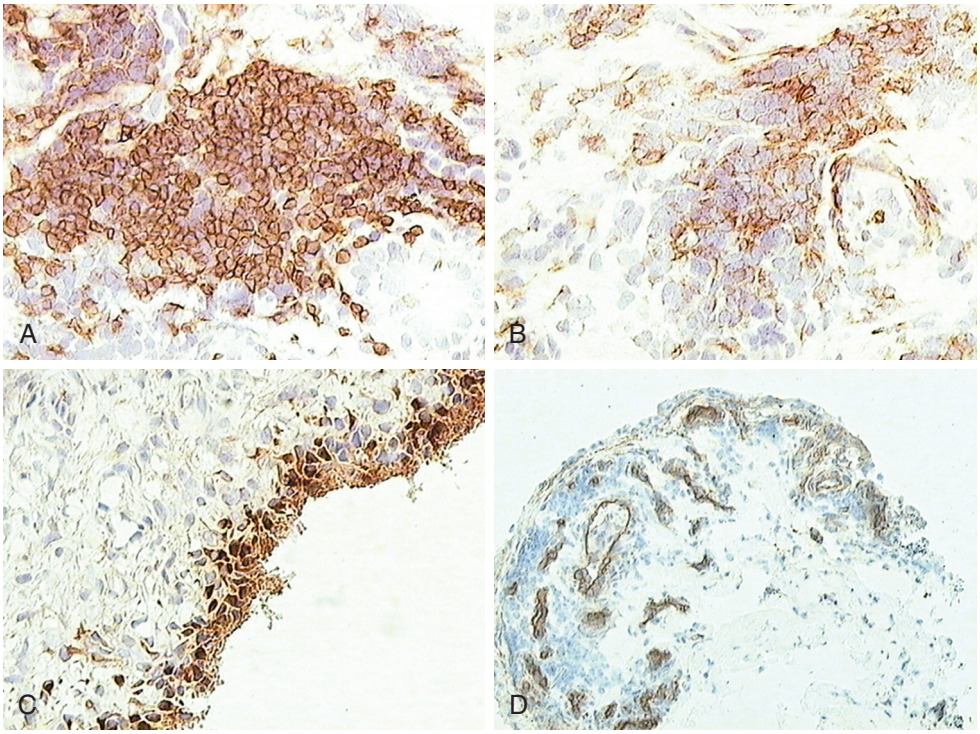



**Figure 53-13** Immunoperoxidase staining *(brown color)* of rheumatoid arthritis synovium. **A,** T lymphocytes. **B,** B lymphocytes. **C,** Matrix metalloproteinase-1. **D,** $\alpha_v\beta_3$ integrin (angiogenic vessels).

B cells are identified by expression of CD19 and CD20 and are particularly abundant in tissues that exhibit large lymphoid aggregates with germinal centers. B cells typically are found in close association with $CD4^+$ T cells in these aggregates (see Figure 53-9). Experiments in severe combined immunodeficiency (SCID) mice suggest that B cells may be critical for maintaining the microarchitecture of synovial lymphoid follicles and for T cell activation.[95] Memory B cells are efficient antigen-presenting cells, and rheumatoid factor–producing B cells are well suited for capturing a wide spectrum of antigens in immune complexes.

The areas surrounding the lymphoid aggregates are often densely infiltrated with sheets of $CD38^+$ plasma cells. Analysis of V gene variants and rearrangements in B cells and plasma cells in both RA and reactive arthritis synovium indicates that plasma cells from a particular aggregate are clonally related, suggesting that their terminal differentiation occurred in the synovial microenvironment.[96] Synovial plasma cells actively synthesize immunoglobulin, some of which result in the production of autoantibodies such as anticitrulline antibodies, which recognize local citrullinated antigens.[97-99] As stated previously, plasma cell infiltrates are also seen in psoriatic arthritis, ankylosing spondylitis, and reactive arthritis synovium, although a systematic analysis of synovial samples from patients with early arthritis has suggested that their presence is most suggestive of RA.[62] One study found that intra-cellular citrullinated proteins were detected in RA but not in spondylarthropathy synovium.[57] In contrast, another study found that the presence of citrullinated proteins was not specific for RA synovitis.[100]

The areas immediately adjacent to the dense lymphoid aggregates, which comprise primarily $CD4^+$ T cells and B cells, have been called *transitional zones*[101,102] (see Figure 53-9). These areas feature a lower CD4/CD8 ratio and appear to be particularly active immunologically. Transitional areas are rich in macrophages and interdigitating dendritic cells, both of which are highly efficient antigen-presenting cells. Lymphoblasts, in particular $CD8^+$ T cells, are seen to be present in close proximity to antigen-presenting cells.

Natural killer cells can be identified by cell surface markers, expression of granzymes, and functional assays. Several studies have suggested an expansion of subsets of natural killer cells in RA synovial tissue and synovial fluids.[103-105] Mast cells are abundant in RA synovium and co-localize with inflammatory mediators and proteases in the synovial microenvironment.[106,107]

## Synovial Sublining Macrophages and Dendritic Cells

Macrophages are present in the sublining areas of healthy and chronically inflamed synovium and are particularly abundant in the sublining stroma of RA synovium. Indeed, when markers such as CD68 and CD14 are used to study highly inflamed tissues, no clear distinction can be made between the sublining macrophage population and the macrophage-like synoviocytes present in the hyperplastic lining layer, although expression of complement receptor for C3b and iC3b was shown to be unique for lining macrophages.[108] Studies that used various macrophage markers suggest that recently migrated macrophages in perivascular areas express CD163 brightly, in addition to expressing CD68 and CD14, whereas macrophages in large lymphocytic aggregates and in the lining layer are less likely to

express CD163. $CD163^+$ macrophages, which have recently been called M2 macrophages, were found to be more abundant in spondylarthropathy than in RA synovium.[57] The functional correlates of these phenotypic differences remain unclear.[109-111] M1 macrophages, which produce TNF and IL-1β, are more abundant in RA and are under-represented in psoriatic arthritis and other spondyloarthropathies in which M2 macrophages are more abundant.[111] Furthermore, the number of macrophages in RA synovium, primarily of the M1 subset, correlates well with the destructive potential of the synovitis, as evidenced by erosive radiographic damage.[112-114] This may reflect the highly activated status of these cells, which serve as the principal source of synovial TNF and IL-1β. A body of evidence has suggested that populations of synovial macrophages serve as osteoclast precursors that mature in the synovial microenvironment and then directly mediate erosive damage to adjacent bone.[115,116]

Mature dendritic cells are the most efficient and potent of the antigen-presenting cells, and are found abundantly in inflamed synovium in close contact with T lymphocytes.[117,118] Two major subsets of dendritic cells have been described: myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). They can be identified by immunohistology as stellate cells with dendrites that express high levels of HLA-DR and co-stimulatory molecules such as CD80, CD83, and CD86. mDCs express CD11c and CD1c, and pDCs express CD304.[119] One study suggested that compared with psoriatic synovitis, RA synovium is particularly enriched in pDCs.[119] Detailed studies that have examined the expression of chemokines involved in dendritic cell migration and recruitment suggest that a substantial proportion of dendritic cells in the synovium arrive in an immature state and subsequently undergo maturation within the synovial microenvironment in a T cell–rich area.[117,118] Follicular dendritic cells in the germinal centers of large lymphocytic aggregates express the markers CD16, FDC, and VCAM-1.

## Synovial Microvasculature, Endothelium, and Stromal Mesenchymal Cells

The stromal elements in RA are often expanded in parallel with the inflammatory cell infiltration. The microvasculature appears to be markedly increased, particularly in the deep sublining areas, and this expansion is presumed to relate to local stimulation of angiogenesis (see Figure 53-13). Morphometric studies have suggested that the number of vessels immediately adjacent to the lining layer is actually reduced compared with normal tissue.[120] This situation, combined with the metabolic demands of this tissue, may actually produce a relatively ischemic and hypoxic environment, which is reflected in the biochemical properties of RA synovial fluid.[121] Immunohistologic studies have indicated that the molecular consequences of hypoxia, particularly expression of hypoxia-inducible factor-1α (HIF-1α), a key regulator of the cellular hypoxic response, are increased in RA synovitis.[122,123] Studies that have directly measured synovial tissue $pO_2$ by using arthroscopic probes have confirmed the hypoxic nature of RA synovitis.[124-126] The synovial endothelium in RA and other inflammatory arthropathies is activated by pro-inflammatory mediators in the microenvironment to express adhesion molecules such as E-selectin, ICAM-1, and VCAM-1, which are involved in the recruitment of inflammatory cells.[127]

## Synovium-Cartilage-Bone Interface

The interface between inflamed synovium and adjacent cartilage and bone in RA and other chronic arthropathies is a site of particular interest because much of the articular damage occurs in these areas. In RA, this destructive synovial tissue is called *pannus*, which may spread to cover most of the surface of the cartilage and invade the bone in bare areas at the joint margin (Figure 53-14). Pannus has been pathologically characterized primarily from samples obtained at the time of joint arthroplasty, although arthroscopic studies at earlier stages of disease have attempted to characterize synovial samples adjacent to this area. Immunohistology suggests that synovial macrophages and fibroblasts are abundant at the pannus-cartilage interface, and that high levels of proteases are expressed by these cells. At the interface between pannus and bone, substantial numbers of multinucleated osteoclasts can be identified morphologically and by specific markers such as calcitonin receptors, cathepsin K, and staining for tartrate-resistant acid phosphatase[128] (see Figure 53-14). Moreover, expression of receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL), a key cytokine in osteoclastogenesis, was prominent in these areas.[129]

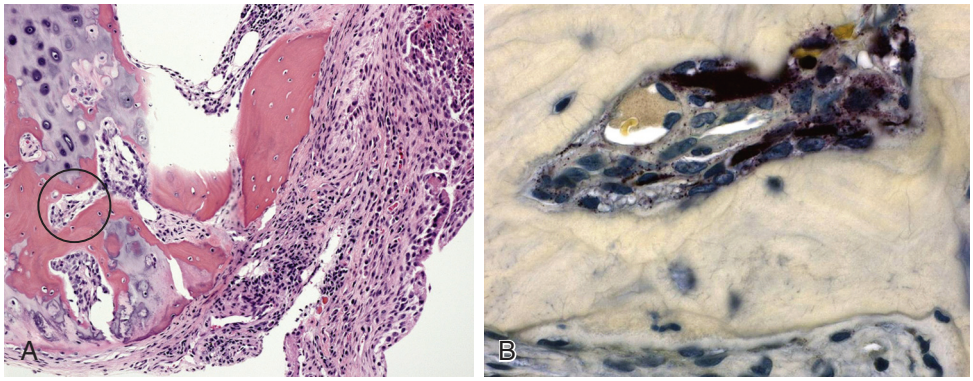



**Figure 53-14** Interface between pannus tissue and bone in a patient with rheumatoid arthritis. **A,** The synovial lesion is invading adjacent bone (see *circle*). **B,** Staining for tartrate-resistant acid phosphatase in the circled area demonstrates the presence of osteoclasts.

### Synovial Biopsy and Pathology as Research Tools for Clinical Biomarker Development

Multiple academic rheumatology centers have used arthroscopic or needle synovial biopsy and quantitative immunohistology as research tools with which to develop a better understanding of disease pathogenesis. In recent years, there has been a focus on the early clinical and preclinical stages of RA and other forms of chronic inflammatory arthritis, in the hope of devising interventions that could achieve long-term remissions and even disease prevention. In the case of RA, it is now well established that circulating autoantibodies such as RF and anti-CCP precede clinical disease onset, often by several years. [130] What is less clear is the stage where the synovium becomes engaged in the immune-inflammatory process. To address this question, synovial biopsy studies were undertaken in autoantibody-positive individuals at high risk for RA, but without clinical evidence of synovitis.[131,132] These studies failed to demonstrate significant inflammatory changes in these pre-clinical synovial samples, although there were subtle T cell infiltrates in the synovium of individuals who subsequently developed RA. These findings suggest that it is unlikely that a prolonged sub-clinical synovitis stage precedes clinical detectable joint inflammation in RA.

In individuals with established inflammatory arthritis, it has been hypothesized that analysis of the synovial lesions could provide informative biomarkers for predicting the impact of therapeutic interventions in RA and other forms of inflammatory arthritis. Such studies may be particularly valuable in predicting the effects of the expanding arsenal of targeted biologic therapies, in which the molecular target and the biologic basis of the mechanism of action are well defined.[133-146] To test this hypothesis, a recent study developed a classification paradigm for RA synovial phenotypes based on gene expression profiling and immunohistology, and found that these synovial phenotypes could be used as a source of biomarkers that correlated with response to biologic therapeutics.[142] Using a statistical classification algorithm derived from the synovial gene expression profile and concordant immunohistologic pattern of synovitis, the study identified four distinct phenotypes: lymphoid, myeloid, low inflammatory, and fibroid. These phenotypes, in particular the lymphoid versus myeloid patterns, served to predict response to infliximab treatment in a post-hoc analysis of a synovial biopsy-based clinical trial, with good responders significantly more likely to express the myeloid, M1 macrophage-dominated phenotype.[137,138] Representative soluble biomarkers were identified from the gene sets expressed by the myeloid versus lymphoid phenotype (the latter dominated by T and B lymphocyte-associated genes). These biomarkers were sICAM-1 for the myeloid phenotype and CXCL13 for the lymphoid phenotype. The serum levels of these biomarkers were found to be correlated with the clinical response to either adalimumab, a TNF inhibitor, or tocilizumab, an IL-6 receptor inhibitor, in a randomized clinical trial.[143] These results, derived from group level analyses, are encouraging, but it is too early to know how effective this approach will be in predicting response to therapeutic strategies on an individual level. They do demonstrate that synovial tissue analysis is a potentially important source of clinically relevant biomarkers.

This appealing proposition is currently hindered by several important considerations. First, the arthroscopic equipment, expertise, and infrastructure needed to undertake these studies remain limited to a small number of centers. Second, considerable concern has arisen regarding the issue of sampling bias in these studies, particularly because serial biopsies are compared in the same individual. As has been discussed, various approaches are used to minimize this bias, including systematic sampling of the same areas of the joint, computerized image analysis of multiple representative tissue samples, quantification of an adequate number of microscopic fields, and utilization of quantitative PCR and proteomic techniques to assess overall levels of specific molecules.

## CONCLUSION

Analysis of synovial fluid and tissue samples provides valuable diagnostic information in specific clinical settings. In cases in which septic or crystal-induced arthritis is suspected, as in acute monoarthritis, synovial fluid analysis is critical for the diagnosis. In cases of undiagnosed chronic monoarthritis, synovial biopsy may provide definitive evidence of conditions such as TB, sarcoidosis, and pigmented villonodular synovitis.

Systematic analysis of synovial tissue in RA and other forms of inflammatory arthritis, particularly with the use of immunohistology, has provided a wealth of information concerning the cellular and molecular mechanisms that sustain synovial lesions. Research protocols are currently exploring the utility of synovial biopsy in predicting response to anti-rheumatic therapies.


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CHAPTER 54



# Arthrocentesis and Injection of Joints and Soft Tissue

Ahmed S. Zayat • Maya Buch • Richard J. Wakefield

**KEY POINTS**

- Arthrocentesis (i.e., joint aspiration) is a key diagnostic procedure in rheumatology, especially when patients have an acutely hot and swollen joint on presentation.
- Injections of joint and soft tissues can be an effective primary or adjuvant therapeutic approach in some rheumatic and musculoskeletal disorders.
- Knowledge of joint anatomy and supervised training are essential for safe practice.
- The right indication, proper technique, and good postinjection care improve procedure outcome.
- Procedure technique can vary between clinicians. However, certain anatomic facts and best practice techniques should always be considered.
- Ultrasound guidance can be used if available and if a blinded procedure has failed to obtain a synovial fluid sample or improve symptoms. For some joints, however, an ultrasound-guided procedure may be the preferred first-line approach.

The ability to target a needle to the exact site of pathology is an essential aspect of patient management in musculoskeletal care. Needles do not only allow the accurate placement of local therapies (e.g., corticosteroids), but also allow fluid to be withdrawn for both diagnostic (e.g., excluding crystals and infection) and therapeutic reasons). Localized injections may be placed in joints or periarticular soft tissues. Different injectants have been used as therapies during the last century, but corticosteroid and local anesthetics are still the most common agents used by most clinicians around the world. Some clinicians continue to use hyaluronic acid and yttrium. To perform such interventional procedures, a good knowledge of anatomy and supervised experience is required to reduce potential complications and improve outcome. Although conventionally, needle interventions are done blinded (with the exception of some fluoroscopic-guided procedures), ultrasound-guided aspirations and injections are increasingly used by rheumatologists because this technique is increasingly available in clinics. Ultrasound guidance has the advantage of confirming the need for the procedure (by providing evidence of the presence of inflammation) and allowing real-time visualization of the needle site and therefore more accurate placement of drugs. However, this technique requires an appropriately skilled operator and more time than other procedures. It may therefore be reasonable to restrict the use of ultrasound for joints that do not respond to a blinded injection and those that are conventionally difficult to do with a blinded injection (e.g., the hip joint).

## INDICATIONS AND CONTRAINDICATION OF ARTHROCENTESIS AND SOFT TISSUE INJECTIONS

A wide range of indications exist for arthrocentesis and injections of joints and soft tissues. The first, most important, indication for arthrocentesis is diagnostic joint aspiration. This is a critical procedure to rule out septic arthritis in patients who are first seen with a hot swollen joint. Joint aspiration can provide fluid for both macroscopic inspection (e.g., pus or hemarthrosis) and laboratory analysis (Table 54-1). Synovial fluid laboratory analysis can confirm the presence of crystals (e.g., sodium monourate crystals in gout) or rule out infection. Joint aspiration and drainage have also a symptomatic relief effect by reducing pain from severe joint distension. Joints injections may be used to deliver pharmacologic agents locally to treat various musculoskeletal conditions. Joints injections can help to treat treat persistent synovitis/recurrent effusion in monoarthritis, oligoarthritis or polyarthritis when systematic treatments fail or are not indicated. In inflammatory conditions such as rheumatoid arthritis (RA) or crystal arthropathies, the effect of steroid joint injections tends to be better and longer lasting than in osteoarthritis (OA), in which effects may be short lived or nonexistent.[1] It helps reduce pain and swelling and improve range of movement. Steroid injections are also indicated for a range of soft tissue conditions, such as trigger fingers, tenosynovitis, enthesopathies (e.g., epicondylitis), bursitis, adhesive capsulitis, rheumatoid nodules, and entrapment neuritis (e.g., carpal tunnel syndrome). When performing procedures, it is essential to check for contraindications and warn about potential complications (Table 54-2).

## DRUGS AND PREPARATIONS USED FOR INJECTION

During the years, different preparations have been investigated for potential intra-articular use, ranging from the very early trials of Lipiodol (poppy seed oil) and Jodipin (product of sesame oil)[2] to the very recent intra-articular injections of cultured stem cells.[3] However, crystalline corticosteroid preparations appear to have the best evidence in improving joints inflammation and reducing pain.[4] Local anesthetics have been used in conjunction with corticosteroids to provide immediate relief of symptoms and to help identify

**TABLE 54-1** Macroscopic and Microscopic Appearances of Synovial Fluid in Different Conditions

| Diagnosis | Appearance | Viscosity | Special Findings | Cellularity |
|---|---|---|---|---|
| Normal | Clear-yellow | High | Nil | Few cells & little debris |
| Inflammatory arthritis | Cloudy | Low | | >90% polymorphs |
| Osteoarthritis | Clear-yellow | Very high | | < 50% polymorphs |
| Gout | Cloudy | Mild low | Monosodium urate crystals (needle-like) | >90% polymorphs |
| Pseudogout | Cloudy | Mild Low | Calcium pyrophosphate crystals (rhomboid) | >90% polymorphs |
| Septic arthritis | Turbid or purulent | Low | Culture positive | >90% polymorphs |
| Hemoarthrosis | red | High | Blood | Blood |

**TABLE 54-2** Important Considerations Prior to Joint Injection

| |
|---|
| Avoid performing joint injections in the following situations (if you have clinical suspicion, do not inject with corticosteroid): |
| • Prosthetic joint (needs to be done by orthopedic surgeon in a sterile environment) |
| • Cellulitis or leg ulcers on same limb, psoriasis, or eczema at injection site |
| • Systemic infection |
| • Raised internalized normalized ratio (INR) (e.g., because of warfarin [>2.5] or bleeding disorder) |
| • Drug allergy to corticosteroid or local anesthetics |
| Some orthopedics specialists prefer for patients not to have joint injections at least 3 months before joint replacement. |
| Always explain the following potential complications: |
| • Postinjection flare (uncommon and can occur after a few hours as long as 24 to 48 hours after injection) |
| • Septic arthritis (rare, incidence increases in the elderly or immunocompromised) |
| • Bleeding (review if the patient is taking warfarin; relatively safe if INR <2.5; avoid deep joints and use of large-gauge needles if patient is on warfarin) |
| • Tendon rupture (do not inject against resistance, avoid injecting Achilles tendon) |
| • Fat and skin atrophy and depigmentation of the skin around the injection site (avoid injecting superficial structures with potent corticosteroids preparations) |
| • Misplaced intravascular injection (always aspirate before injection) |
| • Neurovascular image (always know the anatomic landmarks of these structures before injecting) |
| • Cartilage damage (may occur with multiple frequent injections) |
| • Allergic reaction to local anesthesia (check allergy history) |
| • Flushing and/or palpitations (within 24 hours of injection from systematic absorption of steroid) |
| • Possible transient increase in blood glucose level if patient has diabetes mellitus |

the accurate placement of the injection, by observing whether the area to which the anesthetic is applied shows immediate relief of symptoms. Methylprednisolone and triamcinolone are the most commonly used crystalline corticosteroids for treating joints, with similar outcomes in some studies.[5] Hydrocortisone is a weak soluble preparation of corticosteroid that is often used, preferably for soft tissue or superficial injections, to reduce risk of skin depigmentation or fat atrophy; however, it tends to be less effective, and the effects are shorter lived than with methylprednisolone and triamcinolone. In this chapter, some suggestions for type of preparation and the dose are provided. However, the specific clinical circumstances and the relative risks and benefits of long acting and short acting agents should be considered for each patient.

Some clinicians prefer not to mix corticosteroid with local anesthetics because this may lead to precipitation of the corticosteroid or have a putative chondrotoxic effect in vitro.[6,7] However, anecdotal evidence suggests that mixing lidocaine with crystalline corticosteroid is a common practice, with no increased procedure risk. Premixed preparations are currently available from manufacturers. Lidocaine hydrochloride (1% and 2%) and bupivacaine HCl (0.25% and 5%) are the most commonly used local anesthetics. Lidocaine starts acting within 30 seconds, and the effects last as long as 1 hour. It is useful for painful procedures and for testing for accurate placement of the injection. However, if a longer local anesthetic effect is required (e.g., for a suprascapular nerve block), bupivacaine starts to act after 30 minutes, and the effect can last as long as 8 hours. This longer effect can also be useful if the patient has previously reported a postinjection pain flare up. Worldwide, many rheumatologists use hyaluronic acid derivatives to treat osteoarthritic joints. Hyaluronic acid derivatives are believed to act by replacing the synovial fluid in the joint to function as a lubricant, shock absorber, and an anti-inflammatory agent. They have been reported to be useful for relatively longer pain relief in OA.[8]

## PROCEDURES DESCRIPTION

Clinicians need to perform joint aspiration and injections using considered and specific approaches for good localization of the needle and reduced risk of complications (Table 54-3). Clinicians often use varied approaches and different preparations. These descriptions therefore reflect the authors' own practice, but the general principles described in the text should still apply for the procedure to be safe and effective. Ultrasound-guided arthrocentesis is not necessarily performed according to the traditional approach, but the approach is made upon the access window found in ultrasound, and the structures are visualized. The techniques described will be divided into procedures for

**TABLE 54-3** Elements Required for Successful Injection

| |
|---|
| Choose a quiet and uninterrupted environment. |
| Always use aseptic technique. Mark before cleaning. Once the injection area is cleaned, do not touch it unless complete asterile conditions apply. |
| Always aspirate before injecting to avoid intravascular placement of a large volume of lidocaine or crystalline steroid. |
| Never force an injection against resistance to avoid risk of tendon/ligament rupture. |
| If difficulty is encountered or there is resistance to injection, withdraw slightly and change the needle direction. |
| Always document procedure in patient notes. Documentation should include confirmation of verbal/written consent, patient understanding of potential complications, confirmation of aseptic approach, and drug used. |

intra-articular and for soft tissue injections. The technique for each procedure will be described for both upper and lower limbs, beginning with the distal and moving to the proximal joints. When ultrasound is commonly used in a technique, this approach will be described accordingly. The images shown in this chapter only show the hand that is used to hold the needle. In practice, the other hand is used to hold the position of the joint steady.

## UPPER LIMB INJECTIONS

### Proximal Interphalangeal Joint Injection

These joints are often injected for symptomatic OA or joints that do not respond to systemic treatment for RA. The aim of an intra-articular injection is to place the needle tip within the joint capsule, and not specifically within the joint space (i.e., directly between the bones).

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 5 to 10 mg (±0.2 mL 2% lidocaine).

*The procedure is as follows:*

1. Palpate and mark the proximal interphalangeal joint (PIP) joint space medial or lateral to the extensor tendon on the dorsal aspect of the finger.
2. Insert the needle obliquely to the skin from the dorsolateral or mediolateral position with the tip of the needle passing under the extensor tendon (Figure 54-1). Aspirate and then slowly inject the needle. The capsule of the joint should be felt to slowly distend. Follow the general postinjection care steps.

### Metacarpophalangeal Joint Injection

This injection can be indicated for solitary inflamed metacarpophalangeal (MCP) joints that are not responding to conventional or systemic therapy. Occasionally, they may also be used for symptomatic OA.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of methylprednisolone, 10 mg (±0.2 mL 2% lidocaine).

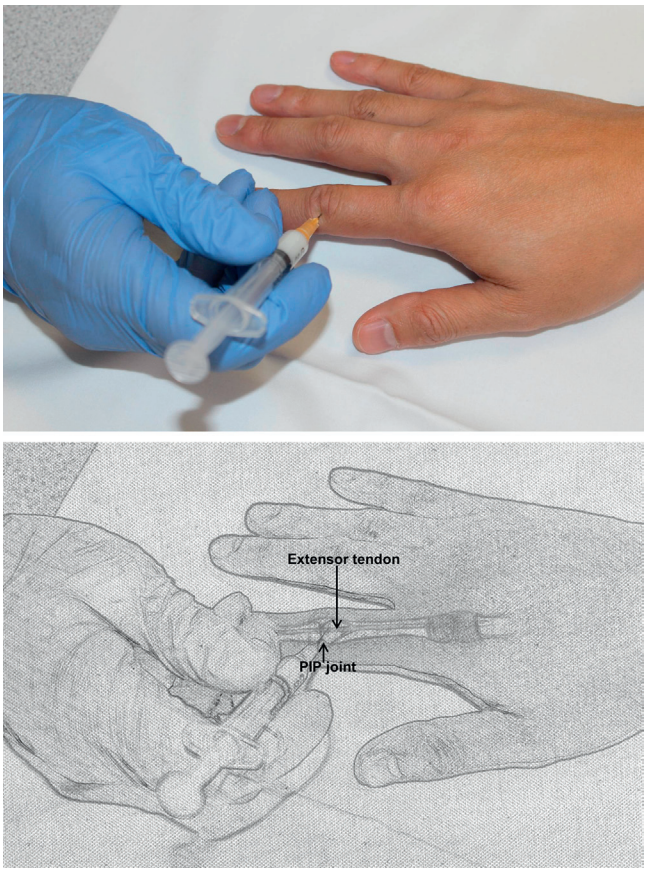



**Figure 54-1** Proximal interphalangeal joints (PIP) joint injection.

**TABLE 54-4** Considerations for Possible Crystal Arthropathies

| |
|---|
| If infection is considered, send fluid to laboratory in a plain universal container—mark as urgent, contact laboratory asking for urgent microscopy/culture and subsequently positively seek result. |
| Negative result for crystals from laboratory does not rule out crystal disease. |
| If you have access to a polarizing microscope, check for crystals yourself. Monosodium urate crystals are needle shaped, negatively birefringent while calcium pyrophosphate crystals are short, thick, and negatively birefringent. |

*The procedure is as follows:*

1. Palpate and mark the MCP joint space medial or lateral to the extensor tendon from the dorsal side. A radial approach is preferred for the second MCP joint.
2. With the joint slightly flexed, the needle is inserted below the extensor tendon inside the joint capsule, as with the PIP joint (Figure 54-2). Aspirate, and then slowly inject the syringe. Follow the general postinjection care steps.

### Wrist Joint Injection

Aspiration of the wrist can be useful in ruling out septic or crystal arthropathies. Table 54-4 has more information. In cases of symptomatic inflamed wrist joints, regardless of systematic therapy, steroid injection can be indicated. The

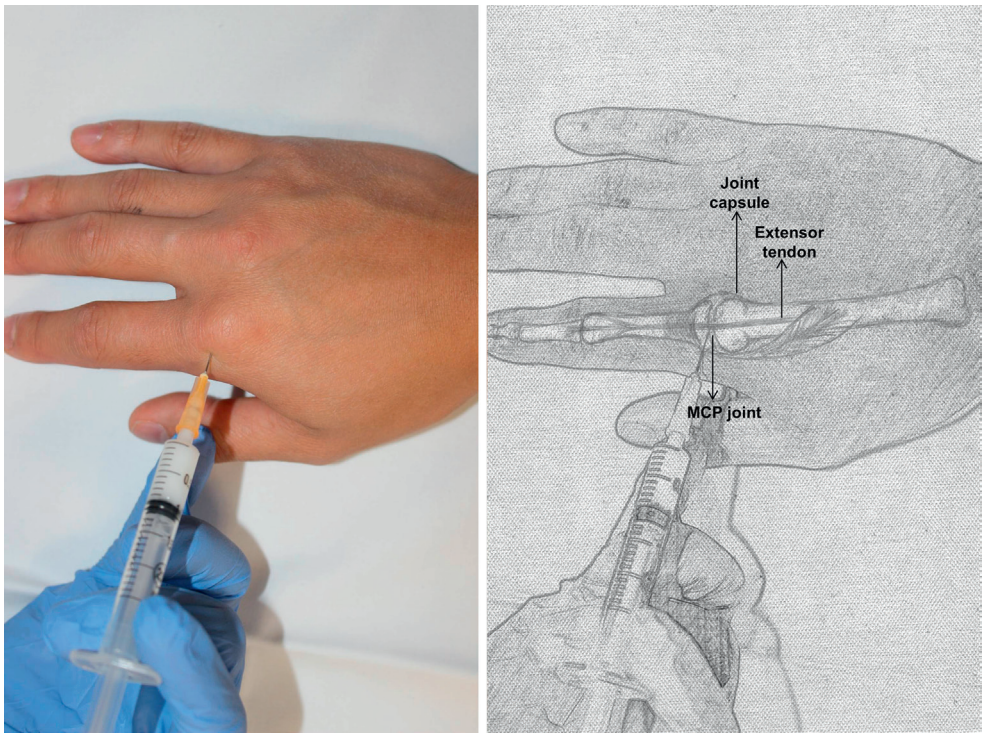



**Figure 54-2** Metacarpophalangeal (MCP) joint injection.

best entry for needle placement is the gap between the third (extensor pollicis longus) and fourth extensor tendon (extensor digitorum) compartments (Figures 54-3 and 54-4A).

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 10 mg (±0.2 mL 2% lidocaine).

*The procedure is as follows:*

1. Feel for the joint line by flexing and extending the wrist. The gap can be easily felt near midline just ulnar and slightly distal to the Lister's tubercle.
2. Insert the needle perpendicular to skin at the joint line. Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.

For ultrasound-guided injection, the gap between the fourth and fifth extensor tendon compartments can be used (Figure 54-4).

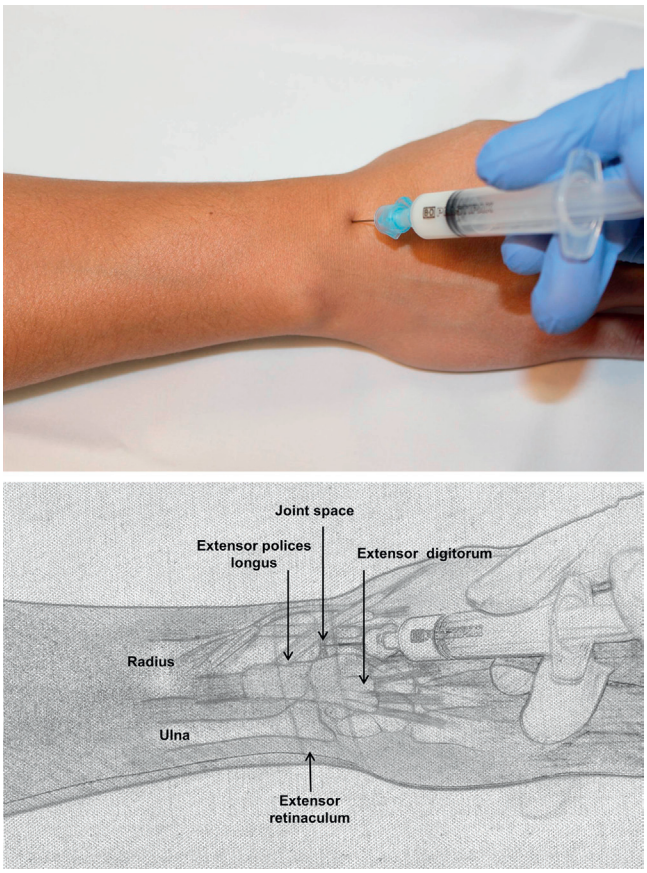



**Figure 54-3** Wrist joint injection.

## Elbow Joint Injection

This procedure is indicated for elbow monoarthritis or oligoarthritis. When you perform the procedure, you should be aware of the ulnar nerve, which lies medially (on the ulnar side) and passes in the ulnar groove between the medial epicondyle and olecranon process. Thus a radial approach is highly recommended.

Materials needed for the procedure are a 23-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 20 to 40 mg (±2 mL 1% lidocaine).

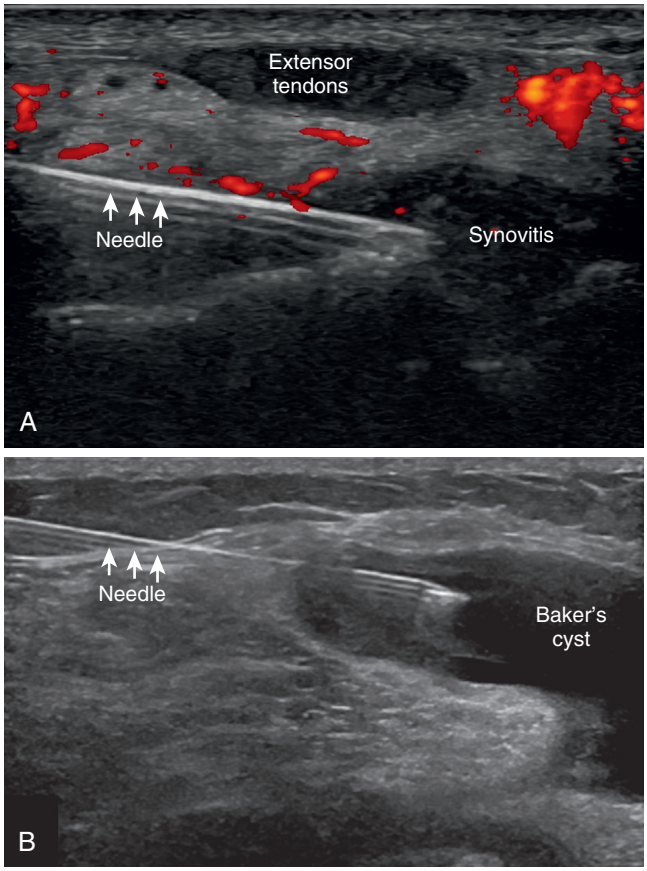



**Figure 54-4** Ultrasound image. **A,** Wrist joint. **B,** Baker's cyst injection.

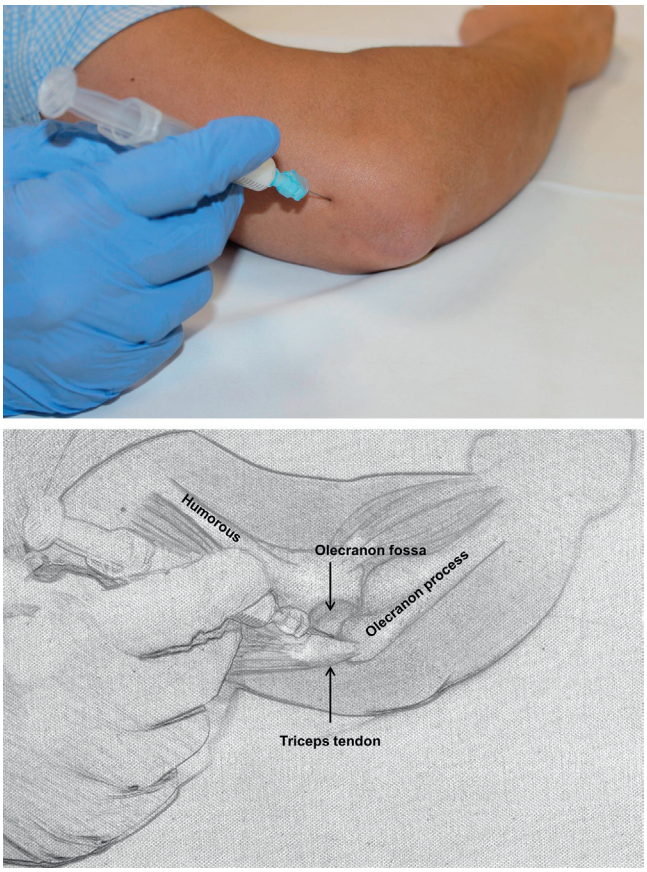



**Figure 54-5** Elbow joint injection.

*The procedure is as follows:*

1. Place the patient in the supine position with the elbow flexed 90 degrees and placed over the chest.
2. Palpate and mark the cleft between the lateral epicondyle and olecranon process. Insert the needle perpendicular to the skin radial to triceps tendon, aiming distally (Figure 54-5). Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.
3. Injection medial to the olecranon process should be avoided (caution: ulnar nerve).

## Shoulder Joint (Glenohumeral) Injection

Shoulder OA, RA, and frozen shoulder are the main indications that glenohumeral injections are needed. We recommend injecting from the posterior side because this approach has no overlying neurovascular structures (Figure 54-6).

Materials needed for the procedure are a 21-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 20 to 80 mg (±1 mL 1% lidocaine).

*The procedure is as follows:*

1. Place the patient in the sitting position with his/her back to you.
2. Feel the joint 2 to 3 cm inferior and medial to the acromial tip. Internally and externally rotating the joint may help confirm the joint line. Advance the needle in an anterior direction. Ensure the plunger has very little or no resistance when injecting. Try to aspirate before slowly injecting the syringe. Follow the general postinjection care steps.

## Acromioclavicular Joint Injection

The acromioclavicular joint (ACJ) is a synovial joint with a very small joint space; as a consequence, only a small amount of fluid can be injected. Direct or indirect ultrasound guidance can help to ensure the correct placement of the injection, especially if there is suspected osteophytosis and loss of joint space.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 10 mg (±0.2 mL 2% lidocaine).

*The procedure is as follows:*

1. Palpate and mark the ACJ.
2. Insert the needle directing inferiorly and slightly posteriorly, aiming toward the center of the joint space (Figure 54-7). The joint is surrounded by tough ligaments that may produce some initial resistance to the needle. Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.

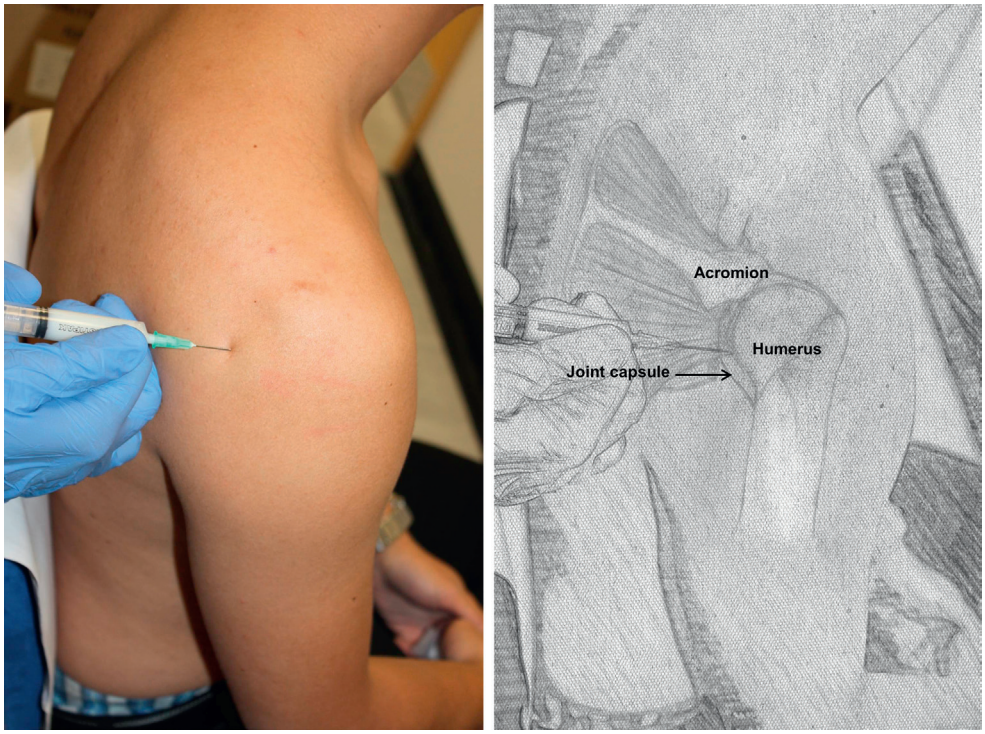



**Figure 54-6** Shoulder joint (glenohumeral) injection.

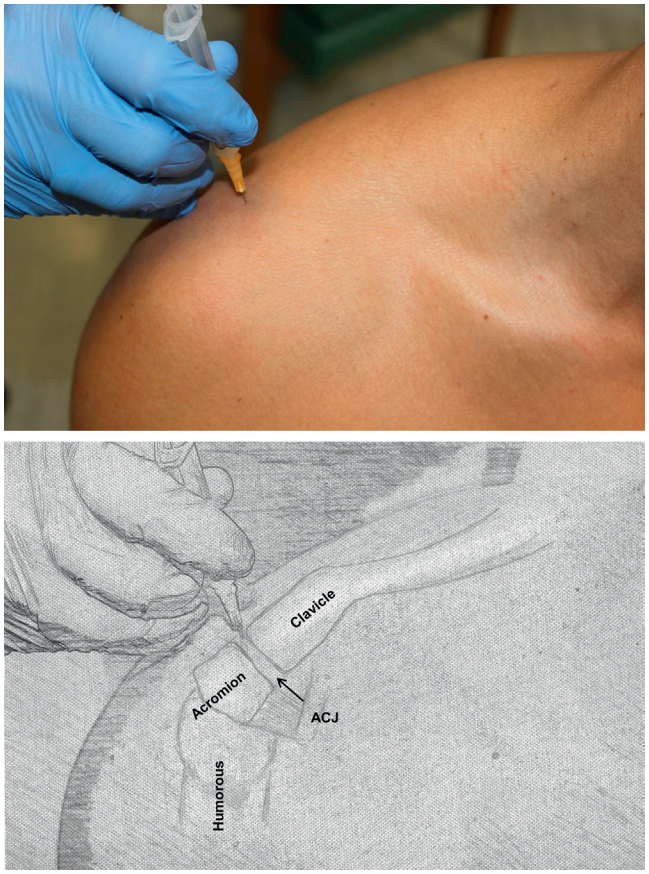



**Figure 54-7** Acromioclavicular joint injection.

# LOWER LIMBS

## Metatarsophalangeal Joint

Injection and aspiration of metatarsophalangeal (MTP) joints (including commonly the first MTP joint) can be used particularly for gout diagnosis and treatment and reduction of MTP joint OA symptoms.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 5 to 10 mg (±0.2 mL 2% lidocaine).

*The procedure is as follows:*

1. Palpate and mark the MTP joint space medial or lateral to the extensor tendon from the dorsal side. A medial approach is preferred for the first MTP procedure.
2. Insert the needle perpendicular to the skin, with some plantar flexion of the MTP joints. Aim to inject under the extensor tendon (Figure 54-8). Aspirate and then slowly inject the syringe. Follow the postinjection care steps.

## Midfoot Joints (Talonavicular and Navicular Cuniform)

Direct or indirect ultrasound guidance is preferred for these joints, because they are narrow and difficult to locate by palpation, especially if coexistent OA is present. In indirect placement, the place of entry can be identified and marked by using a surgical marker in both longitudinal and transverse ultrasound view. A cross-mark can be made where the two lines meet. After the probe is removed, the needle can be inserted into the center of the cross-mark.

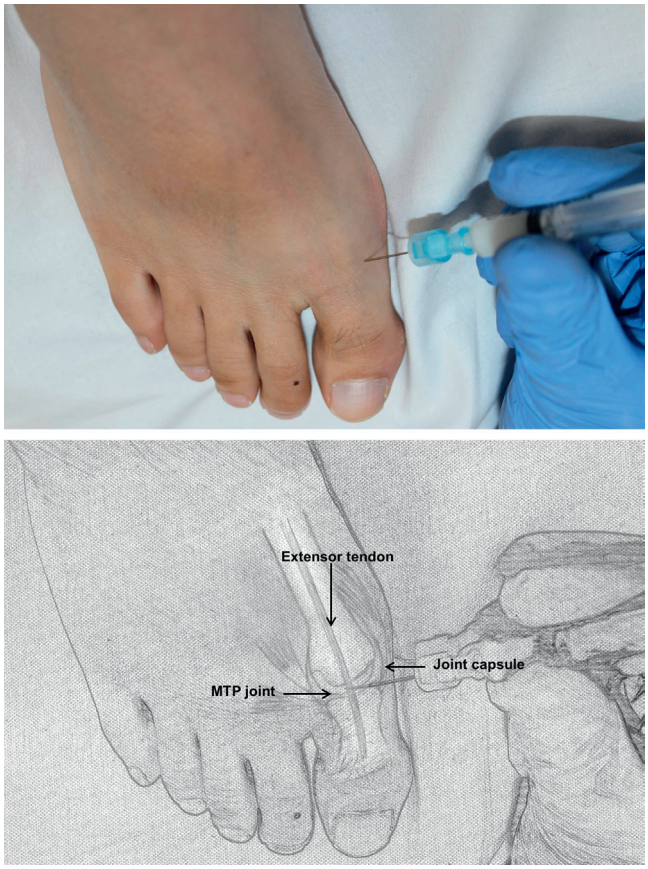



**Figure 54-8** Metatarsophalangeal (MTP) joint injection.

## Ankle (Tibiotalar) Joint

Both anterior and posterior blind approaches for ankle joint injection can be used. However, the anterior approach, which is easier and less painful, will be described in this chapter. The clinician performing the ankle injection needs to be aware of specific anatomic structures to help identify the correct location, reduce the risk of complications, and improve the outcome of the procedure. One important structure is the dorsalis pedis artery, which lies just lateral to the extensor hallucis tendon (EHL) at ankle level. The EHL can be identified when the big toe is extended. The clinician is advised to palpate the artery to avoid injecting it. The deep peroneal nerve, which innervates the leg muscles that raise the feet and toes during walking, runs medial to the EHL, crosses behind the tendon 1 to 1.5 cm proximal to the ankle, and is located lateral to EHL tendon at ankle joint space level. Because of these anatomic structures, we recommend making the injection medial to the EHL.

Materials needed for the procedure are a 23-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 20 to 40 mg (±2 mL 1% lidocaine).

*The procedure is as follows:*

1. Place the patient in the supine position with ankle in dorsal extension.
2. Identify the joint line by having the patient flex and extend the joint (the joint can be more proximal than expected by just looking at the surface anatomy; thus palpation is strongly recommended).
3. Insert the needle medial to the tibialis anterior tendon (the tendon just medial to the EHL). Direct the needle posteriolaterally tangent to the curve of the talus (Figure 54-9). Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.

## Subtalar Joint

The subtalar joint is the articulation between the talus and calcaneus tendon. This injection can be difficult if done blind, because the joint is very narrow and covered with thick capsule and cartilage. Thus ultrasound guidance is recommended. The lateral approach is preferred because of the lesser risk of damage to neurovascular structures and the ease of identifying the sinus tarsi. The talocalcaneal ligament is inside the joint; thus it can produce resistance to the needle during the procedure.

Materials needed for the procedure are a 23-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 20 to 40 mg (±2 mL 1% lidocaine).

*The procedure is as follows:*

1. Place the patient in the supine position and the ankle in the inversion position.
2. Identify the joint line by eversion and inversion of the joint. The joint line is anterior and inferior to lateral malleoli.
3. Insert the needle perpendicular to the direction toward the medial malleolus for approximately 1 inch (Figure 54-10). Insert the needle through the resistance of the ligament until you feel the tissue give; this indicates that you are in the joint space. Aspirate and then slowly inject the syringe. If you feel high resistance to the plunger, it means you are still in the ligament. Push the needle further until you can easily inject the plunger. Follow the postinjection care steps.

## Knee Joint Injection

This is one of the most common procedures performed. It is usually indicated for aspirations for diagnostic or therapeutic purposes to relieve the knee synovitis present with inflammatory arthritis and crystal arthropathy or secondary to OA. When performing the knee injection, the clinician should aim to inject the suprapatellar pouch (SPP) rather than the tibiofemoral joint. The SPP is a large bursa-like structure that extends behind the upper half of the patella and quadriceps tendon and is connected to the knee joint space.

Materials needed for the procedure are a 21-gauge needle, 5 mL syringe (a long needle is needed in cases of obesity), and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisolone, 20 to 80 mg (±3 mL 1% lidocaine).

*The procedure is as follows:*

1. Place patient in a supine position with the knee in a relaxed and slightly flexed position (the knee can be supported with a rolled towel or pillow to help with relaxing in the bent position).

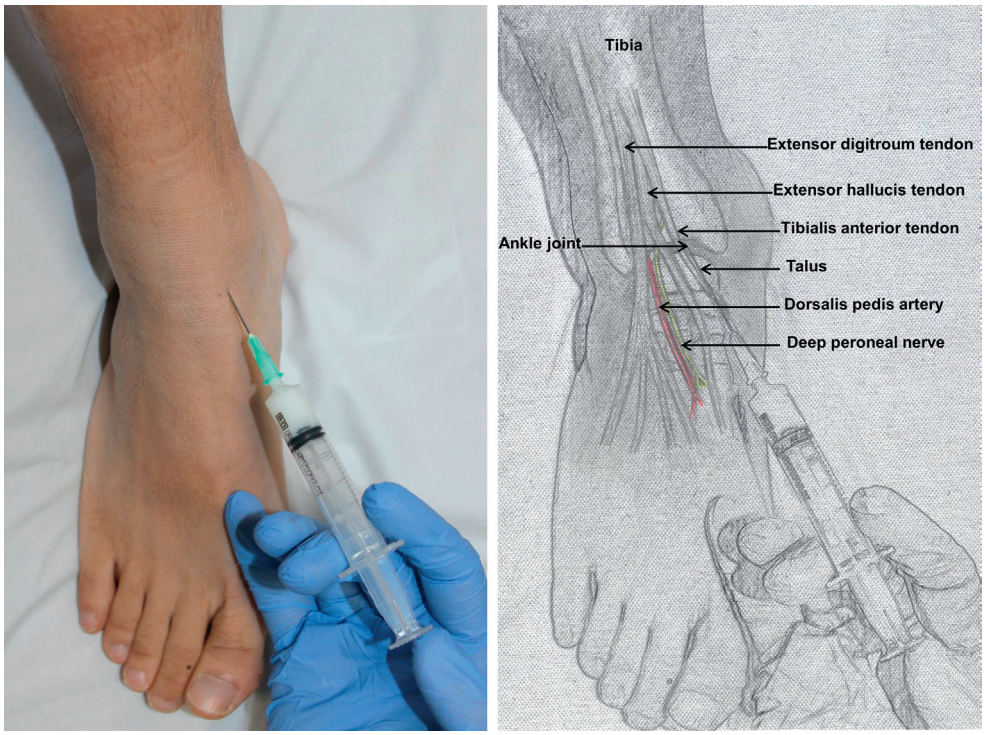



**Figure 54-9** Ankle (talotibial) joint injection.

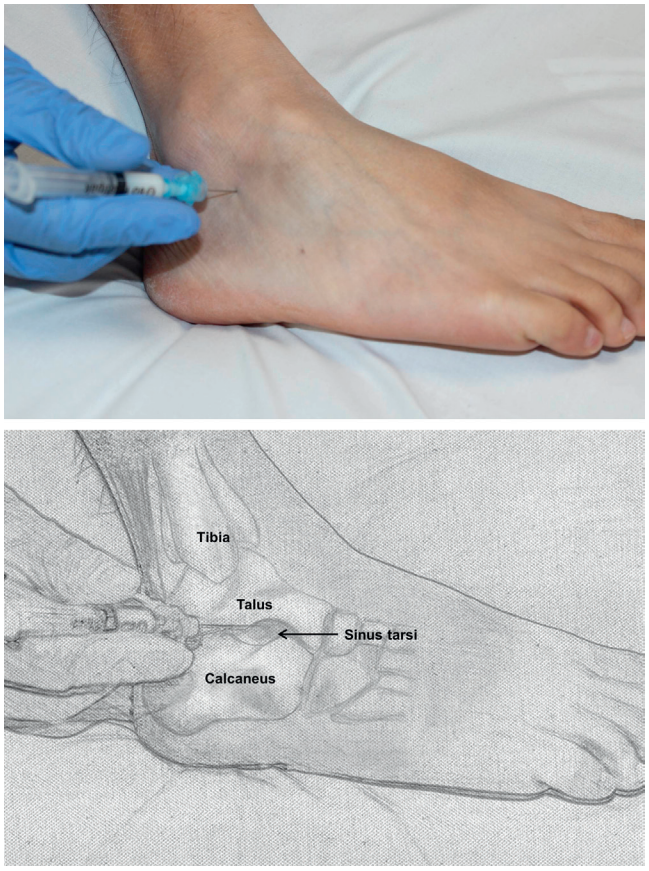



**Figure 54-10** Subtalar joint injection.

**TABLE 54-5** Considerations for Possible Septic Arthritis

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|---|
| Sometimes in septic arthritis, gout, or hemoarthrosis, the pus or synovial fluid can be very thick and therefore difficult to aspirate; in this case, use larger-bore needles and/or ultrasound guidance to allow the withdrawal of the knee content. |
| If no fluid is obtained and an infected joint is strongly suspected, consider injecting the joint with saline (lavage) and re-aspirating. |

2. Palpate either the lateral or medial border of the patella. Identify the point at which the proximal one-third meets the distal two-thirds. Insert the needle under the patella in a slightly cranial position toward the SPP, just proximal to the upper pole of the patella (Figure 54-11). Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.

For Baker's cysts, it is preferable to use ultrasound guidance so that the cyst can be easily visualized and the neurovascular structures can be avoided (see Figure 54-4B; Table 54-5).

## SOFT TISSUE INJECTIONS: UPPER LIMBS

### Trigger Finger and Tendon Sheath Injection

Digital flexor tenosynovitis is a common condition associated with inflammatory arthropathies and occasionally with OA. If the tendon sheath is thickened, it may lead to triggering. However, patients with tenosynovitis can be seen with swelling and pain of the finger that can be mistaken for joint synovitis or dactylitis. Use of ultrasound for diagnosis and procedure guidance is highly recommended.

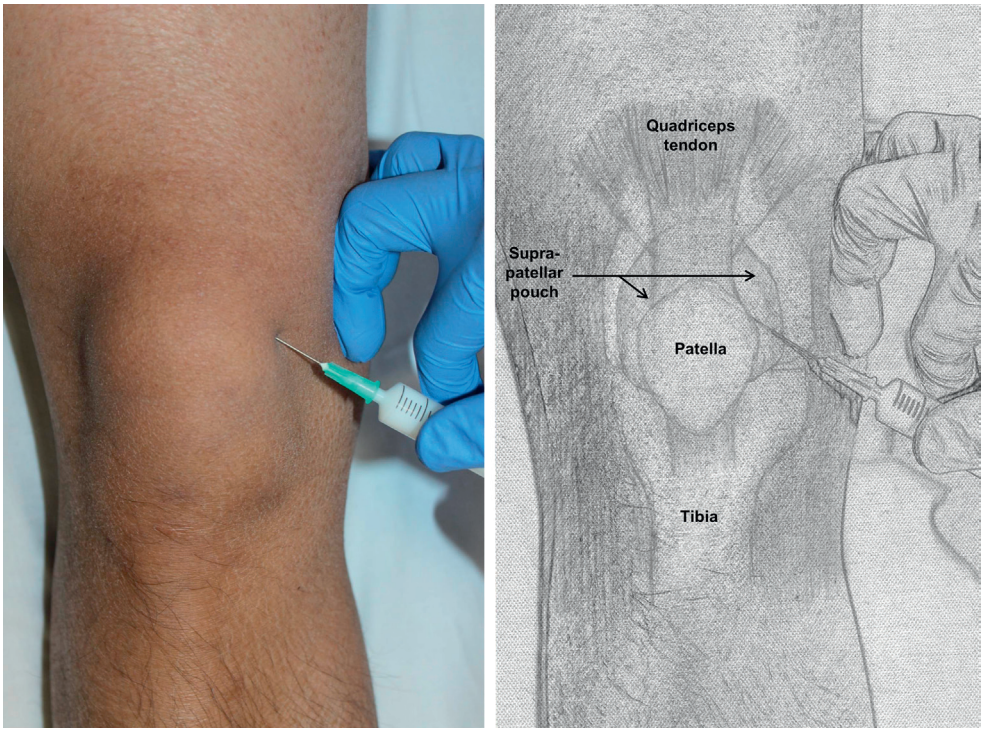



**Figure 54-11** Knee joint injection.

Materials needed are a 25-gauge needle, a 2 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide (5 mg) or hydrocortisone, 25 mg (±1 mL 1% lidocaine).

*The procedure is as follows:*

1. Position the patient's palm facing upward with the fingers extended.
2. Insert the needle with a 30 degree inclination distal to the crease over the MCP joint, and advance it proximally, aiming toward the tendon and reducing the angle between the needle and the skin, to as close to a parallel position as possible (without touching skin).
3. Try to inject generally while advancing. Once the needle is inside the tendon sheath, the resistance to the plunger will disappear.

For an ultrasound-guided procedure, visualize the tendon on longitudinal view (Figure 54-12). Insert the needle from a proximal (or distal) position to the probe, directing it to the region of interest where the whole length of needle can be visualized and the location of injection can be allocated in real time. Proceed as described above.

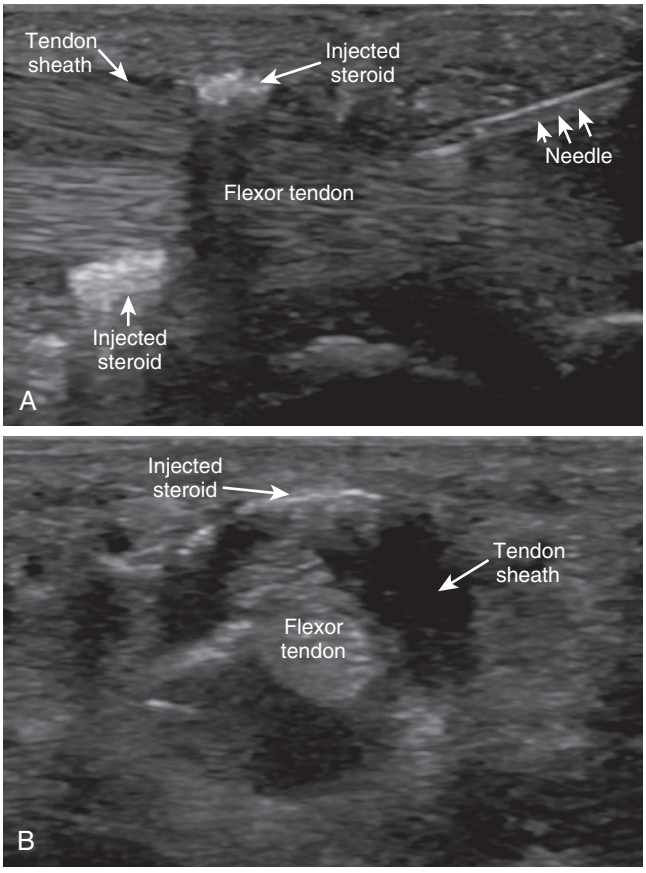



**Figure 54-12** Ultrasound-guided tendon sheath injection. **A,** Longitudinal view. **B,** Transverse view.

## Carpal Tunnel Injection

This procedure can be beneficial in cases of mild to moderate sensory carpal tunnel syndrome that do not respond to conservative therapies such as splints. It can also be indicated when surgical release cannot be done because of long wait times or patient preference. The median nerve lies below the palmaris longus tendon, which can be used as a landmark for this procedure. The clinician can visualize the palmaris longus tendon by asking the patient to oppose the thumb and little finger. It is anatomically absent in 14% of the population.[9]

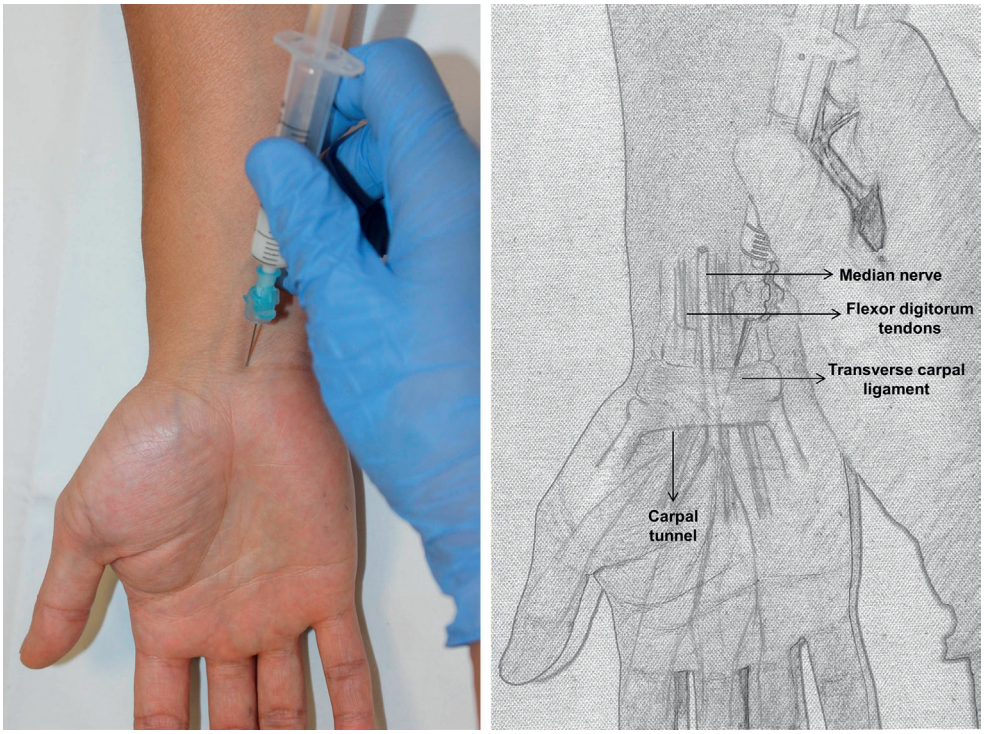



**Figure 54-13** Carpal tunnel injection.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide (5 to 10 mg) or hydrocortisone, 25 mg (±1 mL 1% lidocaine).

*The procedure is as follows*

1. Place the patient's palm facing upward.
2. Insert the needle at an angle of approximately 45 degrees at the distal palmar crease toward the index finger and below the palmaris longus tendon (from the ulnar side) (Figure 54-13). If the patient feels any paraesthesia, withdraw the needle slightly and reposition it as it can be an indication of penetrating the median nerve.

For ultrasound-guided procedures, put the median nerve on transverse view. Insert the needle from the ulnar side toward the median nerve. The clinician should be able to visualize the whole length of needle in real time. Be careful not to penetrate the nerve. Inject the corticosteroid close to the median nerve under flexor retinaculum.

## De Quervain Tendonitis Injection

This procedure is indicated for treatment of inflammation of the abductor polices longus and the extensor policis brevis common sheath. This procedure is best performed under direct ultrasound guidance.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide (5 to 10 mg) or hydrocortisone, 25 mg (±1 mL 1% lidocaine).

*The procedure is as follows:*

1. Locate and mark the tendons by asking the patient to extend and abduct the thumb. With de Quervain syndrome, the tendon sheath can be swollen, which directs the position of the needle.
2. Insert the needle just distal to radial styloid near the base of thumb and advance it proximally along the line of the tendon sheath, directing it toward the radial styloid.

For ultrasound guidance, the approach is similar to the tendon sheath injection described previously.

## Tennis Elbow Injection

This injection can be indicated for lateral epicondylitis (tendopathy of the forearm of common extensor origin) in combination with or after failure of conservative management treatment, such as physiotherapy.

Materials needed are a 23-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisone, 10 to 40 mg (±1 mL 1% lidocaine).

*The procedure is as follows:*

1. Place the patient in a supine position, with the elbow flexed to 90 degrees and placed over the chest.
2. Locate the most tender point in the common extensor tendon (Figure 54-14). Insert the needle until the bone surface is reached, withdraw slightly, and then inject.

## Subacromial Bursa

This injection can be indicated for treatment of subacromial bursitis, impingement syndrome, rotator cuff tendonitis, adhesive capsulitis, and calcific tendonitis. Both anti-inflammatory effects of steroid and hydrodistension of the capsule and bursa may have roles in relief of symptoms. Relief of symptoms after use of local anesthetics is helpful diagnostically as well.

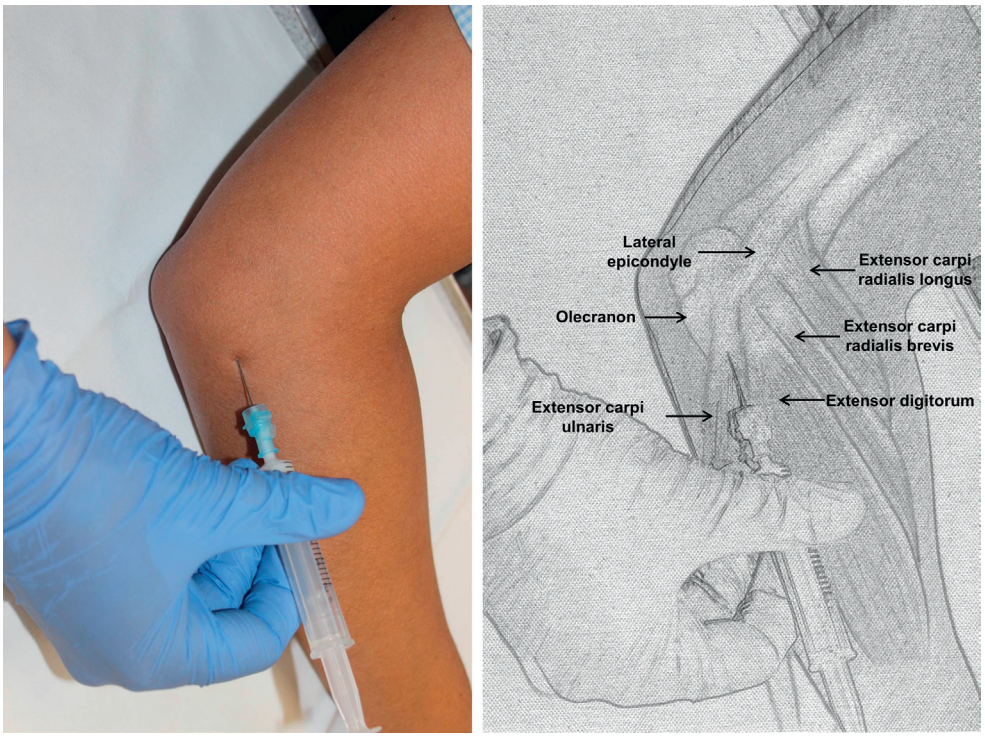



**Figure 54-14** Tennis elbow injection.

Materials needed for the procedure are a 21-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisone, 20 to 40 mg (±3 mL 1% lidocaine).

*The procedure is as follows:*

1. While the patient's arm is in internal rotation, feel the depression below the acromial process posteriolaterally with your thumb.
2. Insert the needle, aiming to position it slightly anterior and inferior to the acromial process, directing it toward the coracoid process (Figure 54-15). Little or no resistance should be encountered while injecting the plunger because the subacromial bursa has a potentially large space.

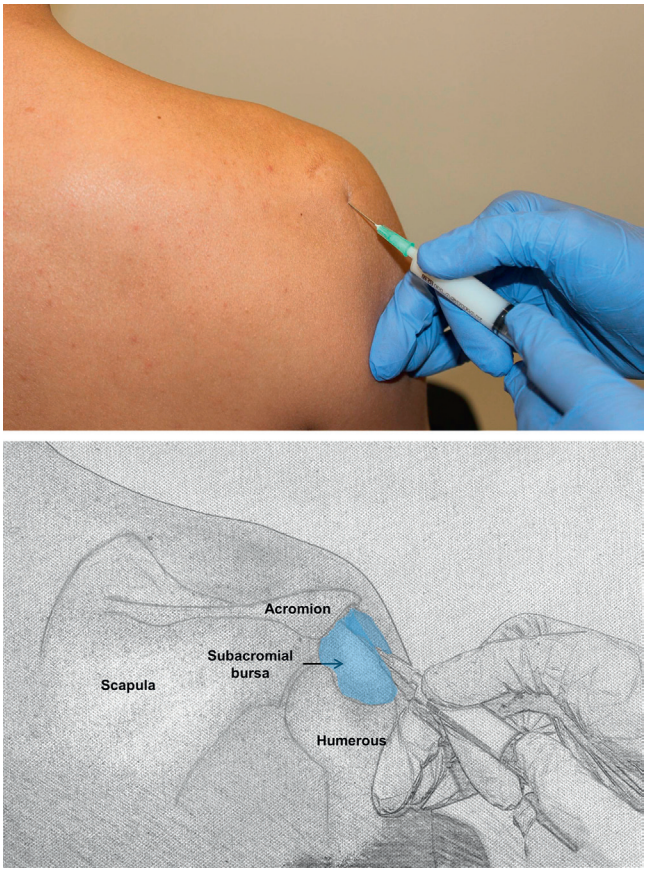



**Figure 54-15** Subacromial bursa.

## SOFT TISSUE INJECTIONS: LOWER LIMBS

### Morton's Neuroma Injection

This is a benign neuroma of an intermetatarsal plantar nerve. Patients are seen with a pain between the third and fourth toes (the most commonly affected area) and/or with a feeling similar to a pebble in the shoe. It can be confirmed with a click sound on MTP squeeze (Mulder's sign) or by ultrasound examination.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisone, 10 to 20 mg (±5 mL, 2% lidocaine).

*The procedure is as follows:*

1. Palpate and mark the place of entry, which should be halfway between the MTP heads and one-half

inch proximal from the web space from the dorsal side (Figure 54-16).
2. Insert the needle perpendicular to the skin, and advance it through the resistance of the transverse tarsal ligament. A give sensation is felt when the needle passes through the ligament.

## Plantar Fascia Injection

Plantar fasciitis injections are indicated if conservative measures, such as podiatrist interventions (e.g., stretching exercises and insoles), fail. The injection is usually very painful and may lead to fat pad atrophy, which reduces shock absorption. For this reason we do not recommend frequent injections or injection of the fat pad directly at the foot base. Rupture of plantar fascia is also a reported complication.

Materials needed for the procedure are a 23-gauge needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisone, 20 to 40 mg (±2 mL, 1% lidocaine).

*The procedure is as follows:*

1. Place the patient in the lateral decubitus position on the affected side, with the lower leg extended and the upper leg flexed at the hip and knee.
2. Palpate the medial calcaneal tuberosity, and mark the maximum tender point. Insert the needle medially perpendicular to the skin and slightly distal to the medial calcaneal tuberosity (Figure 54-17). Advance the needle until it touches the bony surface.
3. Aspirate and then slowly inject the syringe. Follow the postinjection care steps.

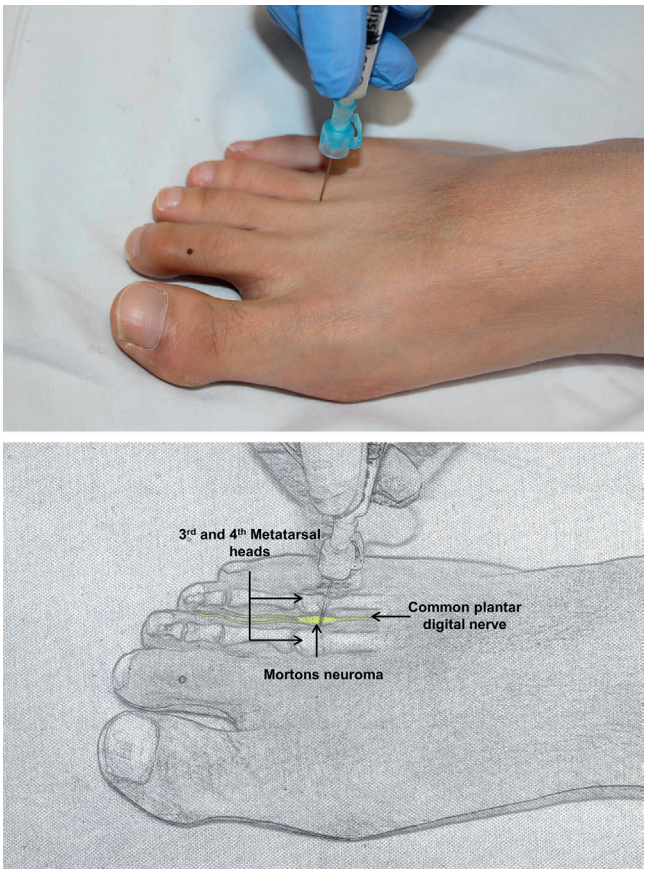



**Figure 54-16** Morton's neuroma injection.

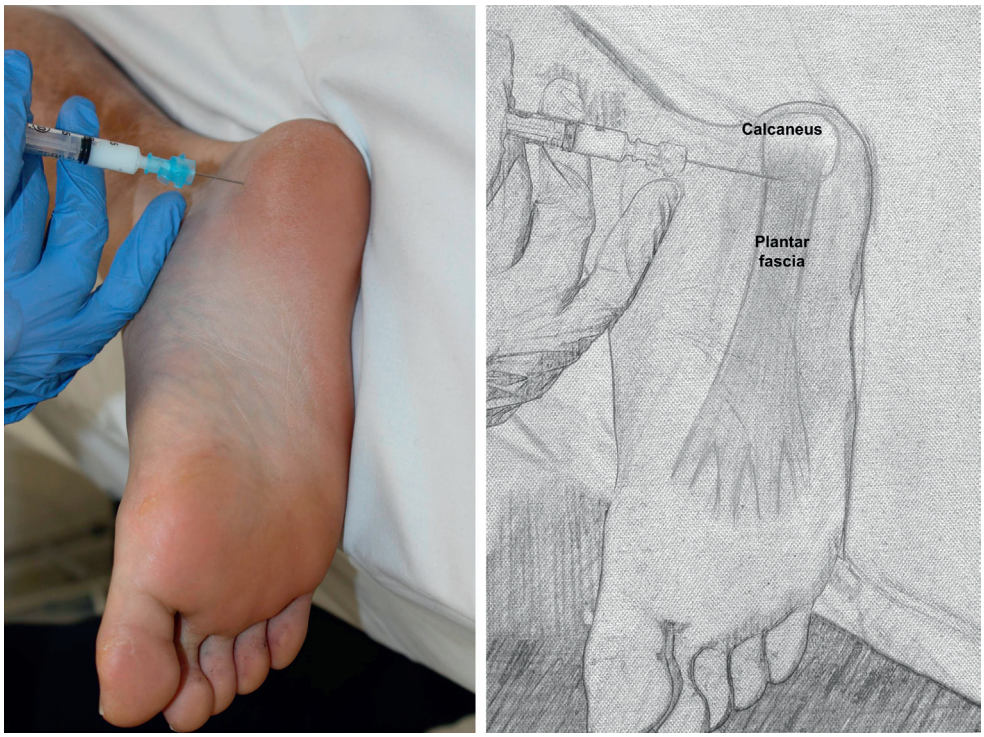



**Figure 54-17** Plantar fascia injection.

For an ultrasound-guided procedure, place the plantar fascia on transverse view at the area just distal to the calcaneal tuberosity. Insert the needle from the medial aspects where the whole length of needle can be visualized and the location of the injection can be visualized in real time. Proceed as described in the preceding text.

## Retrocalcaneal Bursa Injection

This procedure is better performed under ultrasound guidance. However, it still can be done without imaging assistance if ultrasound is not available.

Materials needed for the procedure are a 23-gauge needle, a 5 mL syringe, and a preparation of methylprednisone, 20 mg (±1 mL, 1% lidocaine).

*The procedure is as follows:*

1. Mark the spot in front of the Achilles tendon just proximal to the calcaneus.
2. Insert the needle from the medial or lateral side, perpendicular to the skin at the marked spot for approximately 1.5 cm (Figure 54-18). Never inject against resistance.

For ultrasound-guided procedures, place the Achilles tendon in a transverse view at the area where the retrocalcaneal bursa can be visualized. Insert the needle from the medial or lateral aspects where the whole length of needle can be visualized and the location of injection can be seen in real time. Proceed as described in the preceding text.

## Tibialis Posterior and Peroneal Tendon-Sheath Injection

This procedure is best done under ultrasound guidance. However, a blinded procedure can be performed if swelling of the tendon sheet is very visible and can be identified and injected.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of hydrocortisone, 25 mg/mL.

*The procedure is as follows:*

1. Palpate the medial malleolus and identify the pulsation of the posterior tibial artery. Mark the line of the posterior tibial artery to avoid it.
2. Insert the needle just posterior to the level of the distal end of the medial malleolus (Figure 54-19). The tibialis posterior tendon is the first structure posterior to the medial malleolus (lateral malleoli for the peroneal tendon sheath injection). The needle needs to be directed proximally in a 45 degree angle on an artificial line that extends from the big toe to the heel, following the path of the tendon. Aspirate and then slowly withdraw the syringe. The tendon sheath may form a sausage-like swelling depending on the injection volume. Follow the postinjection care steps.

For ultrasound-guided procedures, use direct visualization of the tibialis posterior (or peroneal) tendons and tendon sheath on longitudinal view and needle inside it before you aspirate and inject. The same anatomic description applies.

## Tarsal Tunnel Syndrome Injection

This injection is indicated for tarsal tunnel syndrome, which is an entrapment of the posterior tibial nerve in the tarsal tunnel. Patients with tarsal tunnel syndrome can be seen with numbness and tingling in the medial third of the plantar area. It can be confirmed with nerve conduction studies.

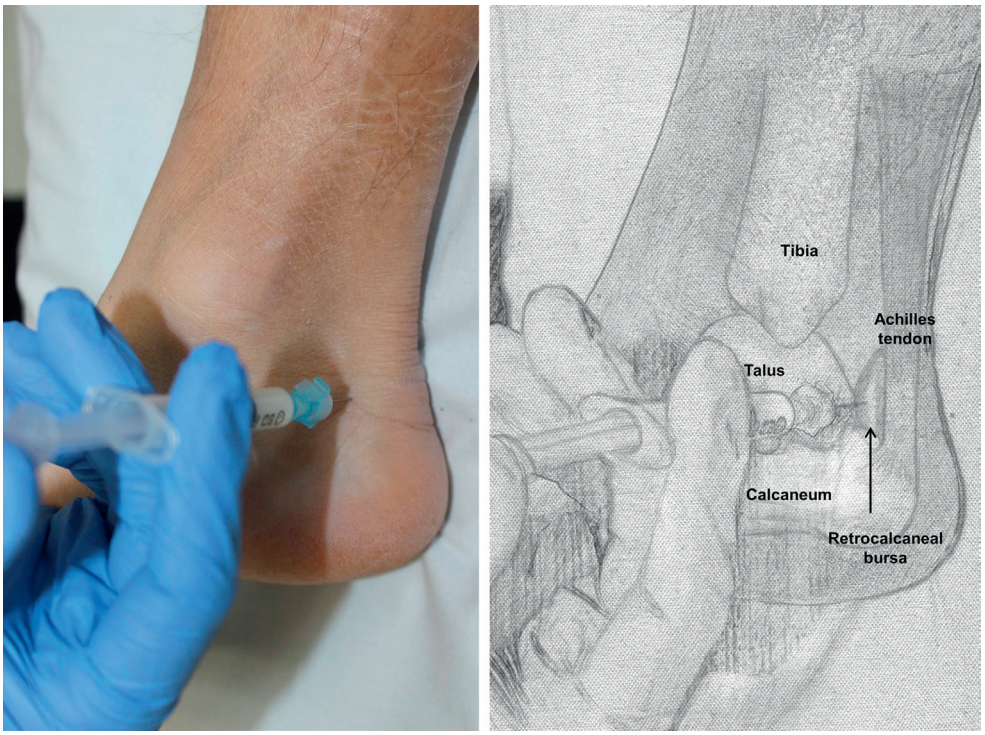



**Figure 54-18** Retrocalcaneal bursa injection.

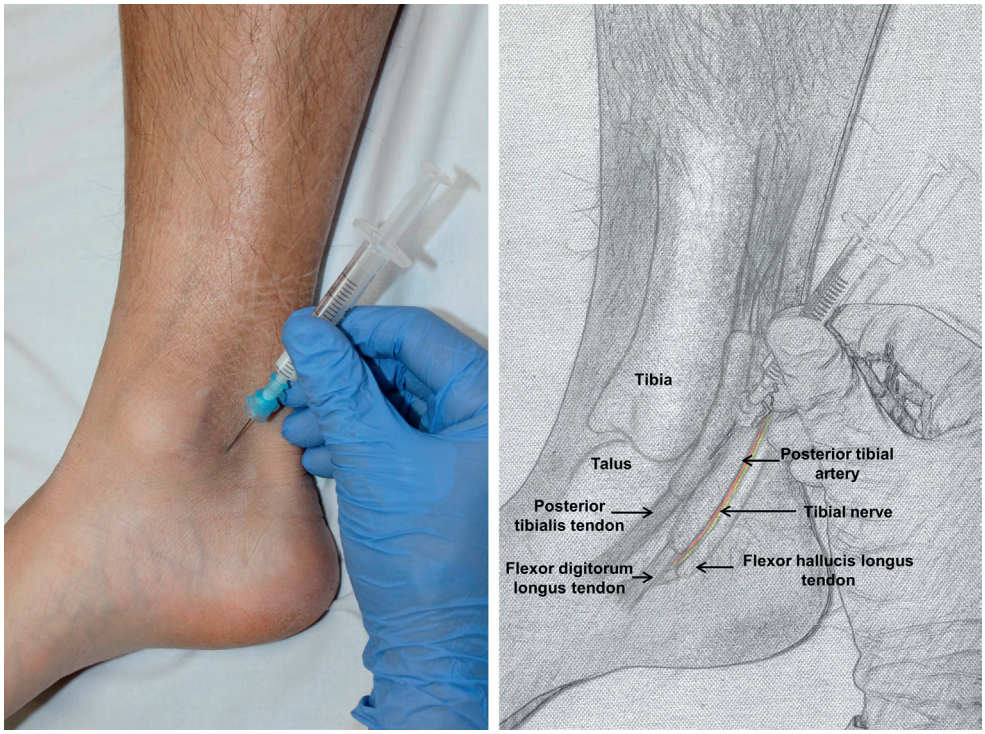



**Figure 54-19** Tibialis posterior and peroneal tendon-sheath injection.

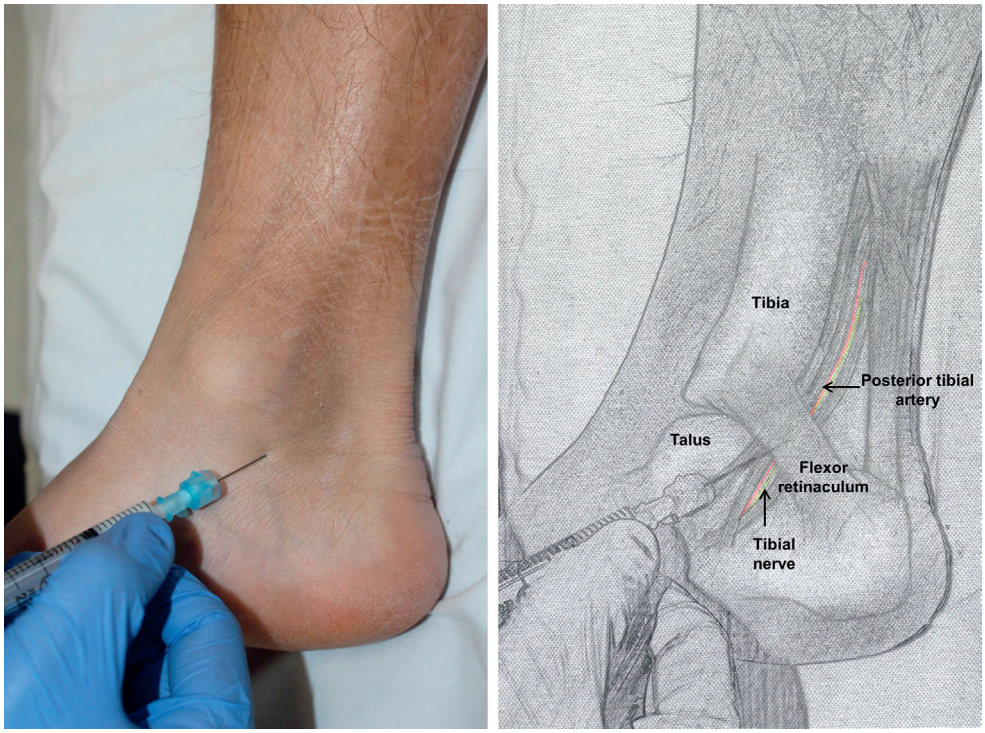



**Figure 54-20** Tarsal tunnel syndrome injection.

Materials needed for the procedure are a 25-gauge needle, a 2 mL syringe (an insulin syringe can be used as well), and a preparation of hydrocortisone, 25 mg/mL.

*The procedure is as follows:*

1. Palpate the medial malleoli and identify the pulsation of the posterior tibial artery. Mark the line of the posterior tibial artery to avoid it.
2. Insert the needle posterior to the medial malleoli and anterior to posterior tibial artery (Figure 54-20). The needle should be inserted parallel to the skin, directing distally in a 45 degree angle on an artificial line extending from the big toe to the heel. Aspirate and then slowly inject the syringe. Follow the general postinjection care steps.

### Trochanteric Bursa

Trochanteric bursitis or greater trochanteric pain syndrome is common, and patients can be seen with hip pain over the greater trochanter area associated with severe tenderness when they lie on the affected side. The greater trochanter can be identified as a bony prominence at the proximal lateral end of the femur.

Materials needed for the procedure are a 23-gauge (long 2-inch) needle, a 5 mL syringe, and a preparation of a longer-acting corticosteroid like triamcinolone acetonide or triamcinolone hexacetonide or methylprednisone, 40 mg (±2 mL, 1% lidocaine).

*The procedure is as follows:*

1. Ask the patient to lie on his or her side, with the affected side facing upward.
2. Palpate and mark the most tender point over the greater trochanter area.
3. Insert the needle perpendicular to the skin until it reaches the hard bony surface, withdraw the needle slightly, aspirate, and then inject. Follow the general postinjection care steps.

## CONCLUSION

Arthrocentesis and joint and soft tissue injections are a key part of rheumatology management with respect to diagnosis and treatment. It can be safe and very effective as long as certain key points are considered. The correct indication, knowledge of anatomy, right technique, and use of correct medications can lead to excellent outcomes. Meticulous documentation, patient education about potential complications, and postinjection care are very important to optimize the procedure outcome. Different techniques can be applied. However, with the introduction of musculoskeletal ultrasound, imaging-guided procedures have become more feasible.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 55

# Anti-nuclear Antibodies



Stanford L. Peng • Joseph E. Craft

## KEY POINTS

- Among the rheumatic diseases, the presence of anti-nuclear antibodies (ANAs) is characteristic of systemic lupus erythematosus (SLE), systemic sclerosis, inflammatory myositis, and Sjögren's syndrome. It is required for the diagnosis of some syndromes, such as mixed connective tissue disease or drug-induced lupus. Fluorescent ANA testing is appropriate as a first screening step when such diseases are clinically suspected.
- Testing of individual ANA specificities should be performed only in the context of clinical signs that correlate with antibody-disease associations (e.g., anti-DNA or anti-Smith [Sm] in the suspicion of SLE).
- Key ANA specificities in SLE include anti–double-stranded DNA, which corresponds to renal disease and overall disease activity; antiribosomal P, which corresponds to neuropsychiatric manifestations and renal disease; anti-Ro/SS-A and anti-La/SS-B, which associate with cutaneous and neonatal lupus; and anti-Sm, which is considered SLE-specific without clear clinical disease manifestation correlations.
- Key ANA specificities in systemic sclerosis include anti-kinetochore (anti-centromere), which corresponds to CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias) manifestations; anti–sclerosis (Scl)-70 (topoisomerase I) and anti–RNA polymerase III, which are associated with diffuse cutaneous disease and pulmonary fibrosis, and accelerated risk of cancer-associated systemic sclerosis; and anti–polymyositis (PM)-Scl (exosome), which is found in myositis–systemic sclerosis overlap.
- Key ANA specificities in inflammatory myositis include anti-synthetase, such as antihistidyl transfer RNA synthetase (e.g., Jo-1), which is associated with the poor-prognosis anti-synthetase syndrome, and anti–Mi-2 (nucleosome remodeling-deacetylase complex), which is associated with dermatologic manifestations.
- Key ANA specificities in Sjögren's syndrome (SS) include anti-Ro/SS-A and anti-La/SS-B, found in mothers of children with neonatal lupus, and antifodrin, which does not seem to have well-documented prognostic ramifications, but, similar to anti-Ro/SS-A and anti-La/SS-B, is observed at high frequency in the disease.
- Although many ANA specificities are generally considered disease or manifestation specific, exceptions are common, confounded by the observation that many autoantibodies are present at low frequencies in healthy people.
- In clinical use, ANA testing is insufficient to establish or refute diagnoses. Rather, it adds weight to diagnoses that rely heavily on other clinical information.

Anti-nuclear antibodies (ANAs) include a wide diversity of autoantibodies directed against multiple intra-cellular antigens, classically consisting of nuclear specificities such as DNA or small nuclear ribonucleoproteins (snRNPs).[1,2] The ANA diseases (Table 55-1) include syndromes characterized by an unusually high prevalence of ANAs, often screened for by the fluorescent anti-nuclear antibody (FANA) test: systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and mixed connective tissue disease (MCTD). The prevalence of ANAs in polymyositis (PM), dermatomyositis (DM), and Sjögren's syndrome (SS) have been reported to be somewhat lower than in the other ANA diseases, but they are often grouped together because they share similar target antigens and presumably similar etiologies. For decades, ANA testing has been an important diagnostic and prognostic tool for these connective tissue diseases (CTDs), and they have become routine assays in the evaluation of patients with suspected rheumatic disease. However, these autoantibodies also arise in a variety of infectious, inflammatory, and neoplastic diseases, as well as in healthy persons, and thus some knowledge regarding their intricacies and the limitations of the assays is required for appropriate clinical application.

## HISTORY

### KEY POINT

- ANAs have evolved from clinical pathologic observations to be tools in the study of cellular biochemical processes.

In the first formal description of an ANA-related phenomenon in 1948, bone marrow specimens from a patient with SLE were found to contain lupus erythematosus (LE) cells, which were subsequently found useful in the diagnosis of SLE and drug-induced lupus, as well as SS and rheumatoid arthritis (RA).[3] LE cells were soon discovered to be attributed to deoxyribonucleoprotein-specific autoantibody, which opsonized and/or induced apoptosis in free-cell nuclei, resulting in antibody sensitization and phagocytosis by polymorphonuclear neutrophils. In 1957, indirect immunofluorescence allowed the development of the FANA as a more sensitive assay for SLE and related diseases.[4] Finer distinction of autoantibody reactivities detected by FANA testing led to the description of Smith (Sm) antigen, nuclear ribonucleoprotein (nRNP), and Ro/Sjögren's syndrome (SS-A) and La/SS-B specificities, which gained further biologic prominence with the demonstration that

**TABLE 55-1** Diseases and Related Conditions Associated with Anti-nuclear Antibodies

| Condition | Patients with ANAs (%) |
|---|---|
| **Diseases for Which ANA Testing Is Helpful for Diagnosis** | |
| Systemic lupus erythematosus | 99-100 |
| Systemic sclerosis | 97 |
| Polymyositis/dermatomyositis | 40-80 |
| Sjögren's syndrome | 48-96 |
| **Diseases in Which ANA Is Required for the Diagnosis** | |
| Drug-induced lupus | 100 |
| Mixed connective tissue disease | 100 |
| Autoimmune hepatitis | 100 |
| **Diseases in Which ANA May Be Useful for Prognosis** | |
| Juvenile idiopathic arthritis | 20-50 |
| Anti-phospholipid antibody syndrome | 40-50 |
| Raynaud's phenomenon | 20-60 |
| **Some Diseases in Which ANA Is Typically Not Useful** | |
| Discoid lupus erythematosus | 5-25 |
| Fibromyalgia | 15-25 |
| Rheumatoid arthritis | 30-50 |
| Relatives of patients with autoimmune diseases | 5-25 |
| Multiple sclerosis | 25 |
| Idiopathic thrombocytopenic purpura | 10-30 |
| Thyroid disease | 30-50 |
| Patients with silicone breast implants | 15-25 |
| Infectious diseases | Varies widely |
| Malignancies | Varies widely |
| **Healthy ("Normal") Persons** | |
| ≥1:40 | 20-30 |
| ≥1:80 | 10-12 |
| ≥1:160 | 5 |
| ≥1:320 | 3 |

ANA, Anti-nuclear antibody.

Modified from Kavanaugh A, Tomar R, Reveille J, et al. Guidelines for clinical use of the anti-nuclear antibody test and tests for specific autoantibodies to nuclear antigens. American College of Pathologists. *Arch Pathol Lab Med* 124:71-81, 2000.

their autoantigens play prominent roles in cellular homeostasis (Table 55-2). Subsequent investigations have revealed many autoantigens, many of which are still largely uncharacterized. Thus ANAs have not only served as diagnostic markers but also have greatly aided studies on intra-cellular metabolism.

## RELEVANCE OF ANTI-NUCLEAR ANTIBODIES TO DISEASE PATHOGENESIS

**KEY POINT**

**ANAs and their respective autoantigens have been implicated in disease pathogenesis by producing directly toxic or other pro-inflammatory effects.**

Because of the characteristic presence of ANAs among the ANA diseases, ANAs have been speculated to play a role in the pathogenesis of rheumatic disease. For example, it is suspected that anti-DNA antibodies promote inflammation in SLE nephritis via immune complex deposition, direct binding to cross-reactive glomerular antigens, and/or intracellular penetration and induction of cellular toxicity.[5] Similarly, RNP antibodies such as anti-Ro/SS-A, anti-La/SS-B, and anti-Sm have been implicated in the pathogenesis of cutaneous or cardiac manifestations by penetrating live cells and/or binding to exposed antigens in the skin and/or heart.[6] Sera containing anti–sclerosis (Scl)-70 (topoisomerase I) activity can induce high levels of interferon (IFN)-α, correlating with diffuse cutaneous scleroderma and lung fibrosis,[7] and it has been demonstrated that anti–Jo-1$^+$ or anti-Ro/SS-A$^+$ sera from patients with myositis induce type I IFN and/or intercellular adhesion molecule-1 on endothelial cells.[8,9]

However, autoantibodies alone fail to account for disease pathogenesis. Induction of type I IFN activity by anti-Ro/SS-A–containing sera, for instance, appears restricted to patients with SLE or SS, not asymptomatic persons.[10] This phenomenon, interestingly, may reflect additional biologic issues among or effects of the autoantigens themselves, such as novel conformations or epitopes: for example, a proteolytically sensitive conformation of histidyl-transfer RNA (tRNA) synthetase, the target of pulmonary fibrosis–related Jo-1–specific antibodies, has been described in the lung,[11] and an apoptope (i.e., an epitope expressed on apoptotic cells) of Ro/SS-A may be specific to SLE, suggesting a unique role of apoptosis in disease pathogenesis.[12] The autoantigens themselves may have unique biologic functions: 60 kDa Ro/SS-A, for instance, may serve as a receptor for the anti-phospholipid-related β2-glycoprotein I, and this dynamic may account for differences in Ro antibody pathogenicity.[13] In addition, many autoantigens likely have intrinsic pro-inflammatory properties, such as the stimulation of innate inflammation by DNA and RNA via Toll-like receptors (TLRs) 3, 7, and 9 or other intra-cellular nucleic-acid binding receptors,[14] or the induction of smooth muscle responses by the centromere protein CENP-B via CCR3.[15] Interestingly, apparent remission of SLE in a patient has been correlated with loss of TLR responsiveness, antibody deficiency, and disappearance of anti-DNA, supporting such concepts.[16] Thus the pathogenesis of the connective tissue diseases appears to reflect a complex interplay between direct inflammatory or other biologic effects of the autoantigens, as well as the consequences of the autoantibody responses.

## METHODS OF DETECTION

**KEY POINTS**

**The gold standard screening test for ANAs is the fluorescent ANA test.**

**Many antibody tests, including ANA screening in some laboratories, are performed via enzyme-linked immunosorbent assay because it affords higher throughput testing; however, this technique may result in lower accuracy.**

**Optimal clinical interpretation of ANA tests requires knowledge of the technique(s) used in each specific case.**

### Immunofluorescence

The FANA provides a rapid yet highly sensitive screening method for ANA detection and remains the gold standard for initial clinical testing.[17] Here, test sera at varying

**TABLE 55-2** Diagnostic Characteristics of the Anti-nuclear Antibodies

| Specificity | Target Autoantigen(s) (Function) | ANA Pattern(s) | Other Tests | Primary Rheumatic Disease Associations |
|---|---|---|---|---|
| **Nuclear** | | | | |
| **Chromatin-Associated Antigens** | | | | |
| DNA | | | | |
| | dsDNA | Rim, homogeneous | RIA, ELISA, CIF, Farr | SLE |
| | ssDNA, dsDNA | Rim, homogeneous | RIA, ELISA, CIF | SLE |
| | ssDNA | Undetectable | ELISA | SLE, DIL, RA |
| Histone | (Nucleosome structure) | | IB, RIA, ELISA | |
| | H1, H2A/B, H3, H4 | Homogeneous, rim | SLE, DIL, RA, PBC, SSc | |
| | H3 | Large speckles | | SLE, UCTD |
| Kinetochore (centromere) | CENP-A, -B, -C, and/or –D (mitotic spindle apparatus) | Speckled* | IF, ELISA | SSc, SLE, SS |
| Ku | Regulatory subunit (Ku 70/80) of DNA-dependent protein kinase (DNA break repair) | Diffuse-speckled nuclear or nucleolar* | ID, IPP, IB | SLE, PM/SSc overlap |
| PCNA/Ga/LE-4 | PCNA (DNA scaffold) | Nuclear/nucleolar speckles* | ELISA, ID, IB, IPP | SLE |
| **Spliceosome Components** | (Splicing of pre-mRNA) | Speckled | ID, ELISA, IB, IPP | |
| Sm | Sm core B′/B, D, E, F, and G | | | SLE |
| RNP, nRNP | U1 snRNP 70K, A, and C | | | SLE, MCTD |
| | U2 snRNP | | | SLE, MCTD, overlap |
| | U4/U6 snRNP | | | SS, SSc |
| | U5 snRNP | | | SLE, MCTD |
| | U7 snRNP | | | SLE |
| | U11 snRNP | | | SSc |
| | SR | | ELISA, IB, IPP | SLE |
| **Other RNPs** | | | | |
| Ro/SS-A | Ro (ribosomal RNA processing) | Speckled or negative | ID, ELISA, IB, IPP | SS, SCLE, NLE, SLE, PBC, SSc |
| La/SS-B/Ha | La (ribosomal RNA processing) | Speckled | ID, ELISA, IB, IPP | SS, SCLE, NLE, SLE |
| RNA helicase A | RNA helicase A | ? | IP | SLE |
| TIA-1, TIAR | TIA-1, TIAR | ? | IB, IPP | SLE, SSc |
| Mi-2 | NuRD complex (transcription regulation) | Homogeneous | ID, IPP | DM |
| p80-coilin | Coiled bodies | Speckled | | SS |
| MA-I | Mitotic apparatus | Speckled* | | SS, SSc |
| **Nucleolar** | | | | |
| RNAP | (RNA transcription) | Punctate | IPP, IB | |
| | RNAP I | Nucleolar | | SSc |
| | RNAP II | Nuclear/nucleolar† | | SSc, SLE, overlap |
| | RNAP III | Nuclear/nucleolar† | | SSc |
| Ribosomal RNP | Ribosomal RNPs (protein translation) | Nucleolar, cytoplasmic | ID, IB, IPP, ELISA | SLE |
| Topoisomerase I (Scl-70) | Topoisomerase I (DNA gyrase) | Diffuse, grainy nuclear or nucleolar | ID, IB, ELISA | SSc |
| Topoisomerase II | Topoisomerase II (DNA gyrase) | ? | ELISA | SSc |
| U3 snoRNP (fibrillarin) | U3 snoRNP (ribosomal RNA processing) | Clumpy | IB, IPP | SSc |
| Th snoRNP (RNase MRP) | RNase MRP (mitochondrial RNA processing) | Diffuse with sparse nuclear | IPP | SSc |
| NOR 90 (hUBF) | hUBF (ribosomal RNA transcription) | 10-20 discrete spots or nuclear* | IB, IPP | SSc |
| PM-Scl (PM-1) | Exosome (RNA processing/ degradation) | Homogeneous nuclear or nucleolar | ID, IPP, IB | PM, DM, SSc, overlap |
| Nucleobindin-2 (Wa) | Nucleobindin-2 | ? | ELISA | SSc, SLE, PM/DM |

*Continued*

**TABLE 55-2** Diagnostic Characteristics of the Anti-nuclear Antibodies—cont'd

| Specificity | Target Autoantigen(s) (Function) | ANA Pattern(s) | Other Tests | Primary Rheumatic Disease Associations |
|---|---|---|---|---|
| **Cytoplasmic** tRNA Synthetases | (Translational machinery) | | | |
| Jo-1 | $tRNA^{His}$ | Diffuse | ID, IPP, IB, ELISA, AAI | PM, DM |
| PL-7 | $tRNA^{Thr}$ | Diffuse | ID, IPP, IB, ELISA, AAI | PM, DM |
| PL-12 | $tRNA^{Ala}$ | Diffuse | ID, IPP, IB, ELISA, AAI | PM, DM |
| EJ | $tRNA^{Gly}$ | Diffuse | ID, IPP, IB, ELISA, AAI | PM, DM |
| OJ | $tRNA^{Ile}$ | Diffuse | ID, IPP, IB, ELISA, AAI | PM, DM |
| KS | $tRNA^{Asn}$ | Diffuse | ID, IPP, IB, ELISA, AAI | UCTD, ? |
| Mas | $tRNA^{[Ser]Sec}$ | ? | IPP | Myositis |
| Fodrin | α- and/or β-Fodrin (cytoskeletal component) | Diffuse subplasmalemmal | ELISA | SS |
| Signal recognition particle | Signal recognition particle (transmembrane protein handling) | ? | IPP, IB | PM |
| KJ | Translational apparatus | ? | ID, IB | Myositis |
| Elongation factor 1α (Fer) | Elongation factor 1α (protein translation) | ? | IPP | Myositis |

*Cell cycle–dependent.

†May also stain nucleoli because of an association with antibodies to RNA polymerase I.

AAI, Aminoacylation inhibition; ANA, anti-nuclear antibody; CENP, centromere protein; CIF, *Crithidia luciliae* immunofluorescence; DIL, drug-induced lupus erythematosus; DM, dermatomyositis; ds, double stranded; ELISA, enzyme-linked immunosorbent assay; Farr, Farr radioimmunoassay; hUBF, human upstream binding factor; IB, immunoblot; ID, immunodiffusion; IF, immunofluorescence; IPP, immunoprecipitation; MCTD, mixed connective tissue disease; mRNA, messenger RNA; NLE, neonatal lupus erythematosus; NOR, nuclear organizer region; nRNP, nuclear ribonucleoprotein; NuRD, nucleosome remodeling-deacetylase; overlap, overlap syndromes; PBC, primary biliary cirrhosis; PCNA, proliferating cell nuclear antigen; PM, polymyositis; PM-Scl, polymyositis scleroderma; RA, rheumatoid arthritis; RIA, radioimmunoassay; RNAP, RNA polymerase; RNase, ribonuclease; RNP, ribonucleoprotein; SSc, systemic sclerosis; SCLE, subacute cutaneous lupus erythematosus; SLE, systemic lupus erythematosus; Sm, Smith; SnRNP, small nuclear ribonucleoprotein; SR, serine/arginine splicing factors; ss, single stranded; SS, Sjögren's syndrome; tRNA, transfer RNA; TIA-1, T cell intracytoplasmic antigen 1; TIAR, TIA-1–related protein; UCTD, undifferentiated connective tissue disease.

Modified from Fritzler MJ: Immunofluorescent antinuclear antibody test. In Rose NR, De Macario EC, Fahey JL, et al, editors: *Manual of clinical laboratory immunology,* Washington, DC, 1992, American Society for Microbiology, p 724.

dilutions (typically serially increasing by twofold) are incubated with substrate cells, and bound antibodies are detected by fluorescein-conjugated anti-human IgG, followed by visualization via a fluorescence microscope. Results typically are reported by two parameters: pattern and titer, with any pattern of reactivity at a titer of 1:40 or greater considered positive. The former parameter includes one or more morphologic descriptors that typically reflect the localization of the respective autoantigen(s) (see Table 55-2; Figures 55-1 and 55-2). Titer is generally reported as the last dilution at which an ANA pattern is detectable, but such an assessment has been considered somewhat imprecise and subjective, and interlaboratory standardization has not been widely instituted; attempts to standardize the protocol have included computer-based fluorescent image quantification, subjective optical scales, and the use of standardized sera to define international units (IU/mL), which vary by laboratory.

As such, FANA results must always be interpreted in light of the techniques used by the individual laboratory. Cultured cell lines such as HEp-2 cells have remained a gold standard substrate because of their higher concentration of nuclear and cytoplasmic antigens and standardization of use, but some laboratories continue to use heterogeneous substrates, such as rodent liver or kidney tissues, that possess the advantage of eliminating interference from blood-group antibodies, heterophile antibodies, or passenger viruses but may exhibit lower sensitivity of some cell cycle–dependent antigens, such as Ro/SS-A. Additional issues that may contribute to variability in FANA results include differences in reagents and instruments, such as the quality of the fluorescein-conjugated anti-human IgG, specific reference sera, and the microscope used.[18,19]

## Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assays (ELISAs) provide highly sensitive and rapid techniques for the detection of autoantibodies. They are commonly used for the detection of specific ANAs, such as anti-DNA and extractable nuclear antigen (ENA) autoantibodies (anti-Sm, anti-Ro/SS-A, anti-La/SS-B, and anti-RNP), often in a "reflex" manner upon detection of a positive screening FANA test. With this technique, test sera are incubated in wells precoated with purified target antigen, and bound antibodies are detected via an enzyme-conjugated anti-human immunoglobulin antibody, followed by color visualization with the appropriate enzyme substrate. The popularity of this technique has further resulted from the commercial availability of ELISA kits and the ability to perform these

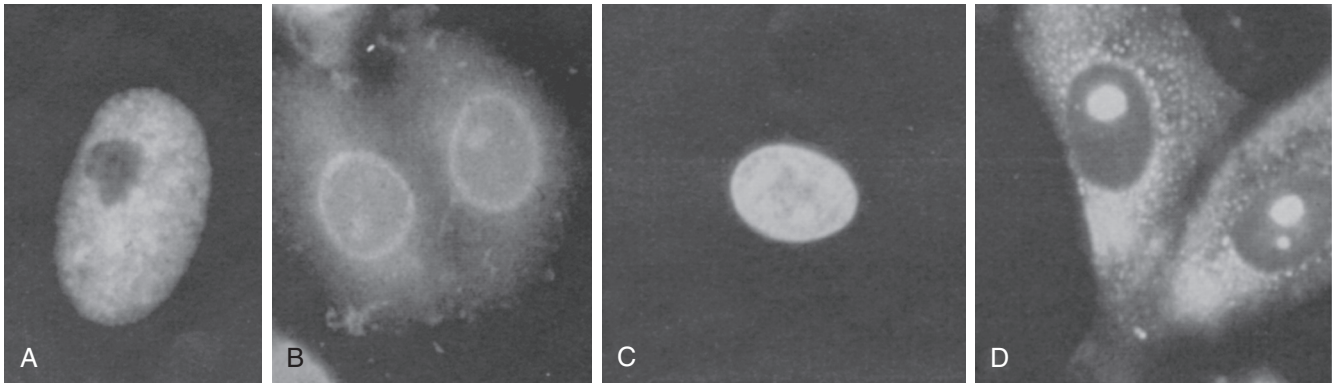



**Figure 55-1** The fluorescent anti-nuclear antibody test: specificities of systemic lupus erythematosus. **A,** Speckled nuclear pattern of anti-Sm antibodies. **B,** Nuclear rim pattern of anti–DNA antibodies. **C,** Homogeneous nuclear pattern of anti-DNA antibodies. **D,** Discrete cytoplasmic and nucleolar pattern of antiribosome antibodies.

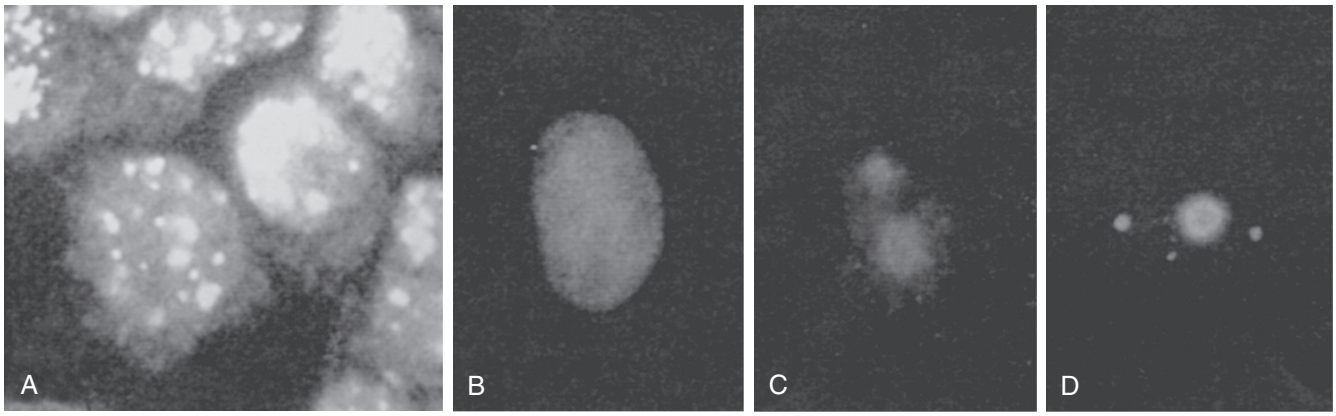



**Figure 55-2** The fluorescent anti-nuclear antibody test: specificities of systemic sclerosis. **A,** Discrete speckled nuclear pattern of anti-kinetochore (centromere) antibodies. **B,** Grainy nuclear and nucleolar pattern of anti-topoisomerase I (sclerosis [Scl]-70) antibodies. **C,** Diffuse nucleolar and sparse nucleoplasmic pattern of anti-Th (ribonuclease mitochondrial RNA processing complex, 7-2) antibodies. **D,** Punctate nucleolar staining of anti–RNA polymerase antibodies. (***A,*** *From the Clinical Slide Collection on the Rheumatic Diseases, copyright 1991; used by permission of the American College of Rheumatology.)*

assays on a multiplex platform, enabling large numbers of clinical specimens to be processed quickly at reasonably low cost. As a result, many laboratories also use such solid-phase immunoassays instead of FANA for the screening ANA test; however, this practice is limited by the number of displayed autoantigens (typically 8 to 10), resulting in reduced sensitivity compared with FANA.[20] Conversely, because the ELISA technique can denature autoantigens, ELISAs may produce false-positive results, and confirmation may warrant further testing, which is not always clinically available. Recognition of the local technique used for the detection of ANAs is therefore critically important for their clinical application in diagnosis and/or prognosis.

## Anti-DNA Antibody Tests

Anti-DNA antibodies warrant special consideration because of their wide range of autoantigenic epitopes and their assay difficulties.[5] Antibodies that recognize denatured single-stranded (ss)DNA, which are less specific for rheumatic disease, bind the free purine and pyrimidine base sequences; SLE-specific antibodies that recognize native, double-stranded (ds)DNA bind the deoxyribose phosphate backbone or the rarer, conformation-dependent left-handed helical Z-form. Two methods to ensure the use of native dsDNA in anti-DNA tests include digestion with S1 nuclease, which removes overhanging ssDNA ends, or chromatography on a hydroxyapatite column, which separates ss segments from dsDNA. Unfortunately, despite such efforts, native DNA may spontaneously denature, especially when bound to plastic ELISA plates; this effect may account for some reports of a relative lack of specificity of anti-dsDNA antibodies for SLE.

The Farr radioimmunoassay, which remains the gold standard for DNA antibody testing; involves the binding of autoantibodies to radiolabeled dsDNA in solution. Precipitation of the antibody-DNA complexes by ammonium sulfate allows a quantification of the percentage of incorporated (antibody-bound) radioactive dsDNA. Normal sera typically bind a small fraction of added DNA (usually less than 20%), whereas SLE sera often bind nearly 100% of added DNA. However the specificity of this assay still depends on the quality of dsDNA and the removal of contaminating ssDNA. Also, because of the involvement of radioactivity, the Farr assay is not routinely used in clinical laboratories.

The *Crithidia* test provides an inherently reliable dsDNA substrate that is more often clinically available. Here, the hemoflagellate *C. luciliae* serves as a substrate for indirect immunofluorescence. Its kinetoplast, a modified giant mitochondrion, contains a concentrated focus of stable, circularized dsDNA, without contaminating RNA or nuclear proteins, providing a sensitive and specific immunofluorescence substrate by which to establish anti-dsDNA activity. Thus, together, ELISAs, *C. luciliae* immunofluorescence, and possibly Farr radioimmunoassay tests provide effective, complementary mechanisms to distinguish anti-ssDNA and anti-dsDNA.

### Other Assays

Several additional assays for the determination of ANA specificity include the immunodiffusion and counterimmunoelectrophoresis techniques, two relatively insensitive assays used in many clinical studies associating ANA specificities (especially ENAs) with disease manifestations and outcome; immunoprecipitation and immunoblot, two sensitive and specific assays predominantly confined to research settings; and enzyme inhibition assays (e.g., inhibition of topoisomerase I by anti–Scl-70 and inhibition of RNA splicing by anti-snRNP), which include highly specialized techniques to characterize ANAs functionally.[21] Such assays have not achieved widespread use in clinical diagnostic laboratories because of their cumbersome and/or highly specialized natures.

## INTERPRETATION OF THE FANA

**KEY POINT**

**Although the FANA pattern and titer may provide some insight into the specific autoantigen(s) targeted, as well as the potential likelihood of connective tissue disease, in a given patient, such correlations should only guide, not absolutely determine, clinical decisions.**

### Pattern

Patterns of staining by FANA are often reported as homogeneous, speckled, or rim/peripheral when nuclear staining is present but may also be reported as cytoplasmic, centromere, or nucleolar, reflecting the intra-cellular localization of the target antigen(s) (see Table 52-2; see Figures 55-1 and 55-2). The presence of unusual patterns may be particularly helpful in appropriate clinical settings, such as the presence of a centromere pattern in a patient with features of SSc, suggesting anti-kinetochore antibodies, or a cytoplasmic pattern in a patient with features of myositis, suggesting anti-tRNA synthetase antibodies.[22] However, the presence or absence of patterns is not always highly accurate in predicting specificity, and non-nuclear patterns may not be reported at all by some laboratories, including rare patterns such as nuclear dot, Golgi, or antimitochondrial antibodies, whose clinical significance is uncertain.[23] Furthermore, the role of the FANA pattern in predicting target autoantigen specificities has been largely supplanted in some clinical laboratories by widely available autoantigen-specific ELISAs. As a result, the presence of any such pattern is evidence in an appropriate clinical setting of non–organ-specific autoimmunity, which may warrant further evaluation; however, a specific pattern may only be available and useful in certain cases.

### Titer

Although the widely accepted cutoff for FANA positivity has remained 1:40, greater clinical significance has generally been thought to correlate with higher titers.[18] Healthy persons, usually older and female persons, or relatives of persons with connective-tissue diseases may produce positive FANAs at a frequency sometimes exceeding 30% (see Table 55-1).[24,25] Although these persons often possess titers of less than 1:320 with homogeneous staining patterns, many subjects possess higher titers yet remain clinically asymptomatic for years, and occasional patients with SLE may demonstrate negative FANAs, which perhaps is a more frequent observation if they possess isolated anti-Ro or anti-ssDNA antibodies and/or if the laboratory uses rat or mouse tissues.[26] As a result, the presence of high- versus low-titer FANA results may not be of sufficient clinical significance to warrant differential subsequent evaluation. Rather, a positive screening FANA of any titer requires clinical correlation.

## DISEASES ASSOCIATED WITH ANTI-NUCLEAR ANTIBODIES

**KEY POINTS**

**Key ANA specificities in SLE include anti-dsDNA, which corresponds to renal disease and overall disease activity; antiribosomal P, which corresponds to neuropsychiatric manifestations and renal disease; anti-Ro/SS-A and anti-La/SS-B, which are associated with cutaneous and neonatal lupus; and anti-Sm, which is considered SLE specific without clear clinical disease manifestation correlation.**

**Key ANA specificities in systemic sclerosis include anti-kinetochore (anti-centromere), which corresponds to CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias) manifestations; anti–Scl-70 (topoisomerase I) and anti-RNA polymerase III, which are associated with diffuse cutaneous disease and pulmonary fibrosis; and anti–PM-Scl (exosome), which is found in myositis–systemic sclerosis overlap.**

**Key ANA specificities in inflammatory myositis include antihistidyl-tRNA synthetase (e.g., Jo-1), which is associated with the poor prognosis anti-synthetase syndrome, and anti–Mi-2 (nucleosome remodeling-deacetylase complex), which is associated with dermatologic manifestations.**

**Key ANA specificities in Sjögren's syndrome include anti-Ro/SS-A and anti-La/SS-B, found in mothers of children with neonatal lupus, and antifodrin, which does not seem to have well-documented prognostic ramifications, but, similar to anti-Ro/SS-A and anti-La/SS-B, is observed at high frequency in the disease.**

### Systemic Lupus Erythematosus

ANAs remain a hallmark of SLE; although past studies have reported FANA frequencies as low as 90%, the test is

positive in more than 99% of patients with use of current methods.[27] SLE often evokes autoantibodies against a seemingly endless range of antigens in many cellular locations, but the majority of SLE autoantigens reside in the nucleus and may be broadly categorized into chromatin-associated versus RNP antigens (Tables 55-2 and 55-3), which, interestingly, may facilitate disease subclassification and prognosis.[28]

## Chromatin-Associated Antigens

**Anti-DNA.** Although antibodies against DNA remain one of the most widely recognized specificities in SLE, antibodies against its more physiologic forms—such as nucleosomes or chromatin—are more prevalent in and probably relevant to pathogenesis.[29,30] Nonetheless, most clinical literature remains linked to classic anti-dsDNA antibodies (see anti-DNA antibody tests discussed earlier); many diseases exhibit anti-ssDNA activity, but only SLE sera characteristically possess high-titer anti-dsDNA and/or anti–Z-DNA activity, as characterized by positive Farr or *Crithidia* assays, seen in approximately 73% of patients, in contrast to low titers seen often in SS, RA, other disorders, and healthy persons.[31] In SLE, anti-DNA antibodies strongly correlate with nephritis and disease activity, in contrast to other ANA specificities.[5,31] In some settings, drug-induced anti-DNA antibodies are observed, such as during therapy with some tumor necrosis factor (TNF) inhibitors, although they do not necessarily correlate with clinical manifestations of CTD or response to TNF inhibitor therapy.[32]

Some anti-DNA antibodies may cross-react with other autoantigens, explaining correlation with other end-organ manifestations, such as the neuronal *N*-methyl-D-aspartate (NMDA) receptor or ribosomal P antigens for central nervous system disease.[33,34] Such findings suggest that the immunologically relevant antigen for anti-DNA antibodies may not in fact be DNA. As a result, the presence of anti-DNA activity should always prompt consideration of renal disease, but the presence of anti-DNA activities does not always indicate lupus nephritis, and vice versa. Indeed, in inconsistent clinical settings, anti-dsDNA antibodies likely have low prognostic value.[35]

**TABLE 55-3** Anti-nuclear Antibodies in Systemic Lupus Erythematosus*

| Antibody Specificity | Prevalence (%) | SLE-Specific? | Major Disease Associations |
|---|---|---|---|
| **Chromatin-Associated Antigens** | | | |
| Chromatin | **80-90** | In high titer | |
| dsDNA | **70-80** | In high titer | **Renal LE, overall disease activity** |
| Histone | **50-70**<br>H1, H2B > H2A > H3 > H4 | No | **Drug-induced lupus, anti-DNA** |
| Ku | **20-40** | No | Overlap |
| RNA polymerase II | 9-14 | Relatively (SLE and overlap) | |
| Kinetochore | 6 | No | |
| PCNA | 3-6 | No | |
| **RNP Components** | | | |
| snRNPs | | | |
| Sm core | **20-30** | Yes | |
| U1 snRNP | **30-40** | No | |
| U2 snRNP | 15 | | |
| U5 snRNP | ? | | |
| U7 snRNP | ? | | |
| Ro/SS-A | **40** | No | **Cutaneous LE**<br>**Neonatal LE and CHB** |
| La/SS-B | **10-15** | No | **Neonatal LE** |
| Ribosomes | | | |
| P0, P1, P2 protein | **10-20** | Yes | **Neuropsychiatric LE** |
| 28S rRNA | ? | | |
| S10 protein | ? | | |
| L5 protein | ? | | |
| L12 protein | ? | | |
| SR proteins | 50-52 | | |
| Proteasome | 58 | | |
| TNF TRs | 61 | | Nephritis |
| RNA helicase A | 6 | | |
| RNA | ? | | |
| Ki-67 | ? | | |

*Shown are the major anti-nuclear antibody specificities described in systemic lupus erythematosus, along with estimated prevalences and disease associations (***bold*** indicates data supported by multiple studies). See text for details.

CHB, Congenital heart block; dsDNA, double-stranded DNA; LE, lupus erythematosus; PCNA, proliferating cell nuclear antigen; rRNA, ribosomal RNA; SLE, systemic lupus erythematosus; Sm, Smith; snRNP, small nuclear ribonucleoprotein; SR, serine/arginine splicing factors; SS, Sjögren's syndrome; TNF TRs, tumor necrosis factor translational regulators, including T cell intracytoplasmic antigen-1 and TIA-1–related protein.

**Anti-histone (Nucleosome).** Anti-histone antibodies target the protein components of nucleosomes, the DNA-protein complexes that form the substructure of transcriptionally inactive chromatin. They are common in SLE, associate with anti-dsDNA, and are particularly characteristic of and sensitive for drug-induced lupus, where they associate with anti-ssDNA.[30] However, they are commonly seen in other rheumatic diseases, including myositis and SSc, as well as chronic infections, such as Epstein-Barr virus,[21] and as a result, clinical correlations for anti-histone antibodies have not been consistent.

**Other Chromatin-Associated Autoantigens.** Other chromatin-associated autoantigens in SLE include several specificities also observed in other rheumatic and nonrheumatic diseases with still somewhat undefined clinical significance. For instance, autoantibodies against Ku, the catalytic subunit of the DNA-dependent protein kinase implicated in DNA repair and V(D)J recombination, have been associated with a number of clinical manifestations, but only inconsistently.[36] Other chromatin-associated autoantigens include proliferating cell nuclear antigen (PCNA), which participates in a scaffold to facilitate DNA replication, recombination, and repair, and RNA polymerase II, which transcribes some snRNA genes, as well as all protein-encoding genes—both of which remain of uncertain clinical correlation.[21]

### Ribonucleoproteins

**Anti–Small Nuclear Ribonucleoproteins.** In SLE, the most well-described snRNP autoantibodies include the Sm and U1 RNP specificities, which target the RNAs or proteins of the spliceosome, a complex of RNP particles involved in the pre–messenger (m)RNA splicing.[37] These particles include the U1, U2, U4/U6, U5, U7, U11, and U12 snRNPs, each of which consists of its respective uridine-rich ("U") snRNA and a set of polypeptides, including a common core of "Sm" polypeptides (B/B′, D1, D2, D3, E, F, and G), as well as particle-specific polypeptides.[38] Anti-Sm antibodies, which target proteins of the Sm core, the B/B′, and one of the D polypeptides, as well as the Sm-like LSm4, appear in only 20% to 30% of patients with SLE but are considered specific for the diagnosis[39]; however, their presence has only inconsistently been associated with specific disease activity and/or prognosis. In contrast, anti–U1 snRNP (nuclear RNP or U1 RNP) autoantibodies, which target the 70K, A, or C polypeptides specific to the U1 snRNP, occur in 30% to 40% of patients with SLE but are not specific for SLE and likewise have been only variably associated with several disease manifestations.[21,40] Several other snRNP antibodies have been described in SLE, often in overlap syndromes—for example, U2, U5, or U7 snRNP–specific—although their clinical correlations also remain inconsistent.[38]

**Anti-Ro/SS-A and La/SS-B.** The RNP particles anti-Ro/SS-A and La/SS-B, which are part of a macromolecular complex that predominantly processes RNA polymerase III transcripts, often have been associated with SS and the neonatal lupus syndrome, as well as ANA$^-$ SLE (especially anti-Ro: See the previous discussion of FANA). Some, but not all, studies have indicated that anti-Ro may segregate among rheumatic diseases based on subunit specificities; for example, Ro52 without Ro60 specificities correlates with SS, whereas Ro60, perhaps specifically a Ro60 apoptope, with or without Ro52 specificities, correlates with other CTDs, including SLE.[41] In SLE, anti-Ro associates with several manifestations, especially skin disease (cutaneous lupus, chilblains, and photosensitivity) and sicca symptoms, but also the neonatal lupus syndrome, including congenital heart block, anti-La, rheumatoid factor, pulmonary disease, complement (especially C4) deficiencies, thrombocytopenia, lymphopenia, and cardiac fibroelastosis.[21] In comparison, anti-La correlates with late-onset SLE, secondary SS, the neonatal lupus syndrome, and protection from anti-Ro–associated nephritis.[42]

**Antiribosome.** The most well-studied antiribosome antibodies in SLE, antiribosomal P protein (anti-P), target the P0, P1, and P2 proteins of the large 60S ribosome subunit. Although they occur in only a minority of patients, they are considered highly specific for SLE and are particularly specific for neuropsychiatric lupus, classically psychosis.[43] Correlations with active disease, renal disease, liver and hematologic disease, alopecia, anti-Sm, anti-DNA, and anti-cardiolipin antibodies have also been reported.[44] Other less prevalent antiribosomal antibodies target ribosomal (r)RNA, such as the 28S rRNA, or other ribosomal proteins, such as the S10, L5, and L12 subunit proteins, although their clinical significance remains unclear.[45]

**Other ANAs in SLE.** Many other SLE ANA specificities have been described, some of which apparently are quite prevalent, such as the SR splicing factors, proteosome, and TNF translational regulator or RNA helicase A specificities. Many such specificities continue to lack clear clinical context, although preliminary analyses indicate some correlation, such as Ki-67 with sicca or RNA with overlap syndromes. Others remain of interest because of their connection with other diseases, such as perinuclear anti-neutrophil cytoplasmic antigens, topoisomerase I, or kinetochore specificities.[21]

## Systemic Sclerosis (Scleroderma)

ANAs against nucleolar antigens characterize the autoantibody response in SSc. Positive FANAs, sometimes speckled in appearance, appear in up to 97% of sera, although percentages vary depending on the substrate used for detection. Unlike SLE sera, however, SSc sera typically, but not always, contain monospecific autoantibody specificities, targeting such structures as the kinetochore, topoisomerase I, or RNA polymerases (Tables 55-2 and 55-4).[46,47]

### Anti-Kinetochore (Centromere) and Anti-Topoisomerase I

Anti-kinetochore (centromere) and anti-topoisomerase I specificities constitute major diagnostic tools in the subclassification of SSc. Originally named anti-centromere, anti-kinetochore targets at least four centromere (kinetochore) antigens (CENPs) of the mitotic spindle apparatus that promotes chromosome separation during mitosis: CENP-B (the predominant kinetochore autoantigen), CENP-A, CENP-C, and CENP-D. As such, these specificities require mitotically active cells for robust detection, accounting for some ANA$^-$ SSc findings (see the previous discussion

**TABLE 55-4** Anti-nuclear Antibodies in Systemic Sclerosis*

| Antibody Specificity | Prevalence (%) | SSc-Specific? | Mutually Exclusive? | Major Disease Associations |
|---|---|---|---|---|
| Kinetochore (centromere) | **22-36** | Relatively | Yes | **CREST** |
| Topoisomerase I | **22-40** | Relatively | Yes | **Diffuse cutaneous disease**<br>**Pulmonary fibrosis** |
| Topoisomerase II | 22 | | | |
| RNA polymerases | **4-23** | | | |
| RNA polymerases I | | Relatively | Yes | Renal crisis |
| RNA polymerase II | | No | | Overlap |
| RNA polymerases III | | Relatively | Yes | Renal crisis, diffuse disease |
| B23 nuclear phosphoprotein | 11 | | | |
| U3 snoRNP (fibrillarin) | 6-8 | | | |
| Th snoRNP (RNase MRP, 7-2 RNA) | **4-16** | | | Limited cutaneous disease |
| U11/U12 RNP | 3 | | | Pulmonary fibrosis |
| PM-Scl | **2-5** | No | | **Myositis-SSc overlap** |
| Sp1 | ? | No | | |
| NOR 90 (hUBF) | ? | No | | |

*Shown are the major anti-nuclear antibody specificities described in systemic sclerosis, along with estimated prevalences and disease associations (***bold*** indicates data supported by multiple studies). Anti-nuclear antibody specificities whose incidences are thought to be "mutually exclusive" of each other in systemic sclerosis are indicated. See text for details.

CREST, Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias; hUBF, human upstream binding factor; MRP, mitochondrial RNA processing complex; NOR, nucleolar organizer region; PM-Scl, polymyositis scleroderma; RNase, ribonuclease; snoRNP, small nucleolar ribonucleoprotein; SSc, systemic sclerosis.

regarding FANA). Their clinical significance has been extensively studied and is heavily associated with Raynaud's phenomenon (RP) and CREST (calcinosis, RP, esophageal dysmotility, sclerodactyly, and telangiectasias), in which up to 98% of patients have anti-kinetochore antibodies. In contrast, anti-topoisomerase I (Scl-70) autoantibodies, which predominantly target the catalytic region of DNA helicase topoisomerase I, generally predict diffuse cutaneous disease with proximal skin involvement—pulmonary fibrosis.[48] However, approximately 40% of all patients with SSc lack either antibody,[49] and a minority (<1%) possess both antibodies,[47] such that, although clinically useful for disease classification and prognosis, they may not be used for definitive diagnoses.

### Anti-RNA Polymerases

Anti–RNA polymerase (RNAP) antibodies, which target the eukaryotic RNA polymerases (see the section on SLE: anti–RNAP), are associated with diffuse cutaneous involvement. Although anti–RNAP II antibodies appear in other diseases, such as SLE or overlap syndrome, and may be associated with other autoantibody specificities against Ku or RNPs, anti–RNAP I and III antibodies appear to be specific for SSc, and they may be useful for the prediction of renal crisis. RNAP III antibodies in particular may predict diffuse cutaneous SSc, including higher skin score, tendon friction rubs, and renal crisis, and are associated with an increased risk of cancer, particularly of the lung.[50,51]

### Anti–Polymyositis Scleroderma

Anti–polymyositis scleroderma (anti–PM-Scl) antibodies target the PM-Scl-75 and PM-Scl-100 components of the exosome, an exoribonuclease complex that regulates rRNA.[52] Responses against PM-Scl-75 alone appear more common among patients with diffuse SSc, whereas overlap syndromes are typically associated with responses against both components.[53] Its presence associates with myositis-SSc overlap without SLE features: 50% of anti–PM-Scl antibody–positive patients have the overlap, whereas 25% of the patients with the overlap have the antibody.[54,55]

### Other SSc-Related ANAs

Several other specificities have been described in SSc with possible prognostic implications, including (1) anti-fibrillarin, which targets a component of the U3 small nucleolar (sno) RNP and may associate with diffuse disease, including internal organ or skeletal muscle involvement or pulmonary hypertension; (2) anti-topoisomerase II, which appears to associate with pulmonary hypertension, as well as localized scleroderma; (3) anti-Th (Th snoRNP, mitochondrial RNA processing ribonuclease [RNase]), which may predict pulmonary hypertension, limited cutaneous disease, puffy fingers, small-bowel involvement, hypothyroidism, and reduced arthritis or arthralgias; (4) antibodies against the nuclear phosphoprotein B23, which appear to associate with pulmonary hypertension and anti-fibrillarin antibodies; (5) antibodies against the RNAP II transcription activator Sp1, which seem to correlate with RP and other signs of undifferentiated CTDs; (6) TNF translational regulator specificities, which may associate with lung involvement; (7) anti-U11/U12 RNP antibodies, which appear to correlate with pulmonary fibrosis; and (8) anti–nucleolar organizer region (NOR) 90 (human upstream binding factor).[56] Other ANAs described in SSc include several specificities characteristically observed in other CTDs, such as histone, Ku, Ro, tRNA, snRNP, and anti-neutrophil cytoplasmic antibody (ANCA), although their clinical relevance in SSc remains unclear.

## Inflammatory Muscle Diseases

Inflammatory muscle diseases comprise a diverse group of illnesses often characterized by autoantibody responses against cytoplasmic antigens. Although between 40% and 80% of patients with polymyositis/dermatomyositis (PM/DM) have positive ANA, as many as 90% of patients with all types of inflammatory muscle diseases have autoantibodies to cellular antigens.[57] Myositis autoantibodies are generally categorized into myositis-specific autoantibodies (MSAs), which are considered exclusive to inflammatory myositis, and those associated with overlap syndromes that include myositis (Tables 55-2 and 55-5).

### Myositis-Specific Autoantibodies

The most well-characterized MSAs include the anti-synthetases, which target different aminoacyl-tRNA synthetases.[58] Some of these antibodies target the tRNA anticodon loop, enabling them to inhibit enzymatic activity. The individual prevalences of these antibodies vary, but their clinical associations remain similar: Although PM appears more commonly with anti–Jo-1, and DM is more common with the other synthetases, these specificities together correlate with the "anti-synthetase syndrome," which includes interstitial lung disease, arthritis, RP, "mechanic's hands," hyperkeratotic lines, sclerodactyly, facial telangiectasia, calcinosis, and sicca, generally with a relatively poor prognosis.[59] Recent studies have suggested that anti–Jo-1 antibody levels may correlate with disease activity, as well as the IFN-γ–inducible chemokines CXCL9 and CXCL10.[60] Nonetheless, other disease associations have been reported that are distinct from the anti-synthetase syndrome; for example, one study has associated anti–threonyl-tRNA synthetase antibodies with fetal loss and severe relapsing myositis.[61] A relatively rare activity, anti-KS, has been described against the asparaginyl-tRNA synthetase in a few patients with interstitial lung disease and inflammatory arthritis or undifferentiated CTD,[62] and PL-7 antibodies may correlate with milder muscle disease.[63]

Other MSAs include specificities against Mi-2, a component of the nucleosome remodeling–deacetylase (NuRD) complex involved in chromatin remodeling and transcription regulation, which associates with DM and dermatologic manifestations such as the "shawl" and "V" signs and ultraviolet light exposure[64]; and Mas, a UGA suppressor serine tRNA that carries selenocysteine ($tRNA^{[Ser]Sec}$). Antibodies against the signal recognition particle, the cytoplasmic RNP that translocates nascent proteins across the endoplasmic reticulum, have been reported to be MSAs that associate with acute, severe, treatment-resistant disease; however, findings of recent studies are conflicting.[65] A few recently described specificities, anti-p155 and anti-p140 (MJ, nuclear matrix protein NXP-2) and anti–CADM-140, may represent novel MSAs and may be useful in the distinction of cancer-associated myositis.[56]

### Myositis Overlap Autoantibodies

The most well-described myositis overlap–associated ANAs include snRNP and PM-Scl specificities. In myositis, anti-snRNP antibodies typically target U1 snRNP, although a few anti-Sm and anti–U2 snRNP specificities have been described (see SLE: anti-snRNP). The former tend to associate with features of MCTD, including SLE-myositis overlap, myositis-SSc overlap, and undifferentiated features (Raynaud's phenomenon, puffy fingers, and arthritis) later progressing to myositis, possibly responding to corticosteroids,[66] whereas anti–U2 snRNP associates with myositis and sclerodactyly, sometimes with SLE, and usually without interstitial lung disease.[38] Anti–PM-Scl antibodies associate with myositis-SSc overlap without SLE features (50% of anti–PM-Scl antibody–positive patients have overlap, whereas 25% of patients with overlap have the antibody), and they also associate with arthritis, DM skin lesions, calcinosis, mechanic's hands, and eczema.[54] Other antibodies associated with myositis in overlap syndromes include several specificities found in other diseases, such as Ku, found more commonly in SLE and SSc, and U3 snoRNP (fibrillarin), which is associated with myositis in SSc, especially of the diffuse type. Several other specificities have also been described in inflammatory myositis, although their clinical significance remains largely undefined (see Table 55-5).[21]

## Sjögren's Syndrome

In persons with SS, reported incidences of positive FANAs range widely, reflecting differences in study populations and disease criteria, and they depend heavily on the inclusion or exclusion of secondary, CTD-related disease, which increases the likelihood and amplitude of positive tests.[67] Thus, although ANA positivity as low as 40% has been reported, many studies report frequencies of 90% to 96%,[68] with a diverse range of autoantibodies, including both ubiquitous (Tables 55-2 and 55-6) and tissue-specific reactivities, such as anti-thyroid, gastric parietal cell, and muscarinic receptor. Such issues hinder interpretation of disease associations and disease specificities of ANAs in SS.

Of the SS ANA specificities, the most well-characterized remain Ro (SS-A) and La (SS-B), two nuclear RNPs involved in RNA metabolism (see SLE: anti-Ro/SS-A and anti-La/SS-B),[69] as well fodrin (nonerythroid spectrin), a cytoskeletal heterodimer composed of α and β subunits that are structurally and functionally similar to erythroid spectrin. Anti-Ro antibodies appear in approximately 40% to 95% of patients with SS, are associated with a number of extraglandular manifestations, including serologic association with anti-La and rheumatoid factor, and, interestingly, may result from genetically linked alternative mRNA processing of Ro.[70] Similarly, anti-La antibodies appear in as many as 87% of patients with SS and are also associated with extraglandular manifestations and serologic association with anti-Ro and rheumatoid factor.[21] Antibodies against α-fodrin have been detected in 64% to 67% of patients,[71] but they are relatively uncommon in other CTDs such as SLE. Preliminary analyses suggest some extraglandular and serologic associations. In contrast, antibodies against β-fodrin have been described in as many as 70% of patients, but clinical associations have not been reported.[72] Several other specificities have been reported in SS in a significant proportion (>3%) of patients, including antibodies against MA-I, a 200 kDa protein localized to the mitotic apparatus in dividing cells (which may be identical to

**TABLE 55-5** Anti-nuclear Antibodies in Inflammatory Muscle Diseases

| Antibody Specificity | Prevalence (%) | Disease Specificity | Major Disease Associations |
|---|---|---|---|
| Anti-tRNA synthetases | | | **Anti-synthetase syndrome** |
| Histidyl (Jo-1) | **20-30** | Myositis | |
| Threonyl (PL-7) | 1-5 | Myositis | |
| Alanyl (PL-12) | 1-5 | Myositis | |
| Glycyl (EJ) | 1-5 | Myositis | |
| Isoleucyl (OJ) | 1-5 | Myositis | |
| Asparaginyl (KS) | ? | Overlap | |
| Selenocysteinyl (Mas) | 1-2 | Myositis | ? |
| Mi-2 | **8 (15-20% of DM)** | Myositis* | **Dermatologic involvement** |
| Signal recognition particle | 4 | No | |
| KJ | <1 | Myositis* | |
| Proteasome | 62 | No | |
| Histone | 17 | No | |
| RNPs | | No | |
| U1 snRNP | 12 | | MCTD features |
| U2 snRNP | 3 | | |
| Ro | 10 | | |
| La | ? | | |
| PM-Scl | 8 | Overlap | Overlap |
| Elongation factor 1α (Fer) | 1 | No | |
| Histone | ? | No | |
| Ku | ? | Overlap | |
| U3 snoRNP | ? | Overlap | |

Shown are the major anti-nuclear antibody specificities described in inflammatory myositis, along with estimated prevalences and disease associations (***bold*** indicates data supported by multiple studies). See text for details.

*Often referred to as myositis-specific autoantibodies (MSAs).

DM, Dermatomyositis; MCTD, mixed connective tissue disease; RNP, ribonucleoprotein; snRNP, small nuclear ribonucleoprotein; snoRNP, small nucleolar ribonucleoprotein; tRNA, transfer RNA.

**TABLE 55-6** Anti-nuclear Antibodies in Sjögren's Syndrome*

| Antibody Specificity | Prevalence (%) | SS-Specific? | Major Disease Associations |
|---|---|---|---|
| Ro/SS-A | 40-95 | No | Neonatal LE and CHB |
| La/SS-B | 80-90 | No | Neonatal LE |
| Fodrin | 64-100 | Possibly | |
| α-Fodrin | 64-67 (100 in pediatric?) | | |
| β-Fodrin | 70 | | |
| Proteasome | 39 | No | |
| Pyruvate dehydrogenase | 27 | No | |
| p-ANCA | 11-40 | No | |
| MA-I | 8 | Possibly | |
| Mitochondrial | 6.6 | No | |
| pp75 (Ro-associated protein) | 6 | No | |
| Kinetochore | 4 | No | |
| p80-coilin | 4 | Possibly | |

*Shown are the major anti-nuclear antibody specificities described in Sjögren's syndrome, along with estimated prevalences and disease associations (***bold*** indicates data supported by multiple studies). See text for details.

CHB, Congenital heart block; LE, lupus erythematosus; p-ANCA, peripheral anti-neutrophil cytoplasmic antibody; SS, Sjögren's syndrome.

NuMA); p80-Coilin, an 80 kDa protein associated with nuclear coiled bodies (although this antibody might not be CTD specific); as well as specificities characteristically found in other rheumatic diseases, such as kinetochore and perinuclear ANCAs, although their clinical importance remains uncertain (see Table 55-6).[56]

## Mixed Connective Tissue Disease and Overlap Syndromes

Although overlap syndromes of CTD remain matters of nosological debate, virtually all investigators concur that ANAs are universal in these conditions.[73] Indeed, since the initial formal description of MCTD in 1972, the presence of anti–U1 snRNP antibodies has been consistently required for classification and/or diagnosis, with associated ANA titers typically exceeding 1:1,000 and often 1:10,000.[74] However, several investigations have noted that many such patients experience manifestations, both clinical and serologic, that allow the diagnosis of a defined CTD, such as SLE, RA, SSc, or PM/DM,[75] and therefore the accuracy of specific clinical associations of specific ANAs in "overlap" settings is hindered by issues of disease classification. Therefore, in such instances, it seems most reasonable to base the importance of individual autoantibody specificities upon their primary disease association, despite the lack of definitive, well-codified evidence (e.g., anti-topoisomerase I as predictive of eventual diffuse SSc-like skin disease or pulmonary fibrosis, or anti-dsDNA for lupus-like glomerulonephritis).

## Other Conditions

In contrast to the traditional ANA diseases, the presence of a positive ANA in other diseases remains largely unhelpful for diagnosis, although in RP, juvenile rheumatoid arthritis (JRA), and anti-phospholipid antibody syndrome (APS), it can aid prognosis (see Table 55-1). In RP, a positive result increases the likelihood, from 19% to 30%, of the development of a systemic rheumatic disease, including SLE, RA, and SSc, whereas a negative result decreases the likelihood to approximately 7%, which is often helpful for patient reassurance.[76] In JRA, ANA positivity may predict the development of uveitis,[77] and in APS, it may predict the development or presence of underlying SLE.[78] Other conditions in which ANAs have been found include other rheumatic diseases such as the vasculitides or sarcoidosis; autoimmune diseases such as multiple sclerosis or inflammatory bowel disease; and an ever-growing list of additional conditions, including dermatologic, infectious, psychiatric, neurologic, and cardiovascular diseases.[21]

## CLINICAL UTILITY OF ANTI-NUCLEAR ANTIBODY TESTING

The ANAs encompass a wide range of nuclear, nucleolar, and cytoplasmic autoantigen specificities. Within the ANA diseases, including SLE, SSc, PM/DM, SS, and MCTD, many autoantibodies possess unique rheumatologic associations, but the specificity of these associations has diminished as the sensitivity of clinical studies has increased the detection of these specificities in both rheumatic and nonrheumatic diseases. Consequently, tests for ANAs can greatly aid in the clinical evaluation of patients in the context of their particular disease associations, but these studies retain only an adjunct role in rheumatologic diagnosis.

Figure 55-3 describes an algorithm for the rheumatologic evaluation of a patient for ANAs. In most clinical laboratories, the FANA serves as a screening test, with a positive result often prompting "cascade" testing by the laboratory, ordering physician, or both of ELISAs or other assays for specific autoantibody specificities, such as anti-dsDNA, anti-Ro/SS-A, anti-La/SS-B, anti-RNP, and anti-Sm.[17] A negative or low-titer FANA in the setting of a low clinical suspicion of rheumatic disease usually indicates the absence of significant ANAs and argues against the diagnosis of one of the ANA diseases (see Table 55-1)[79]; however, if the clinical picture strongly suggests CTD, further investigation may involve specific assays for antigens that are often FANA negative, such as Ro, Jo-1, or phospholipids. On the other hand, because some specific ANAs possess diagnostic significance, positive FANA results usually warrant follow-up with specialized assays, but only in the setting of strong clinical suspicion, because the positive predictive value of an ANA in the absence of other clinical signs of CTD is low, in part because ANAs may precede clinical disease by many years,[80] and in part because of the relatively high incidence of ANA in healthy persons.[24] Thus, if SLE features are present, further work may focus on anti-DNA, anti-Sm, anti–U1 snRNP, and anti-Ro antibodies. Similarly, if MCTD, SS, SSc, or polymyositis are suspected, the serum may be tested, respectively, for anti–U1 snRNP; anti-Ro or anti-La; anti-topoisomerase I, anti-centromere, or antinucleoli; or anti–tRNA synthetases. If these tests are negative in the setting of high clinical suspicion, repeat testing at a later date may be warranted because titers of such autoantibodies can fluctuate over time, irrespective of disease course.[81] Positive results in these more specialized assays do not alone signify specific diseases, but rather add weight to diagnoses that should throughout the evaluation rely heavily

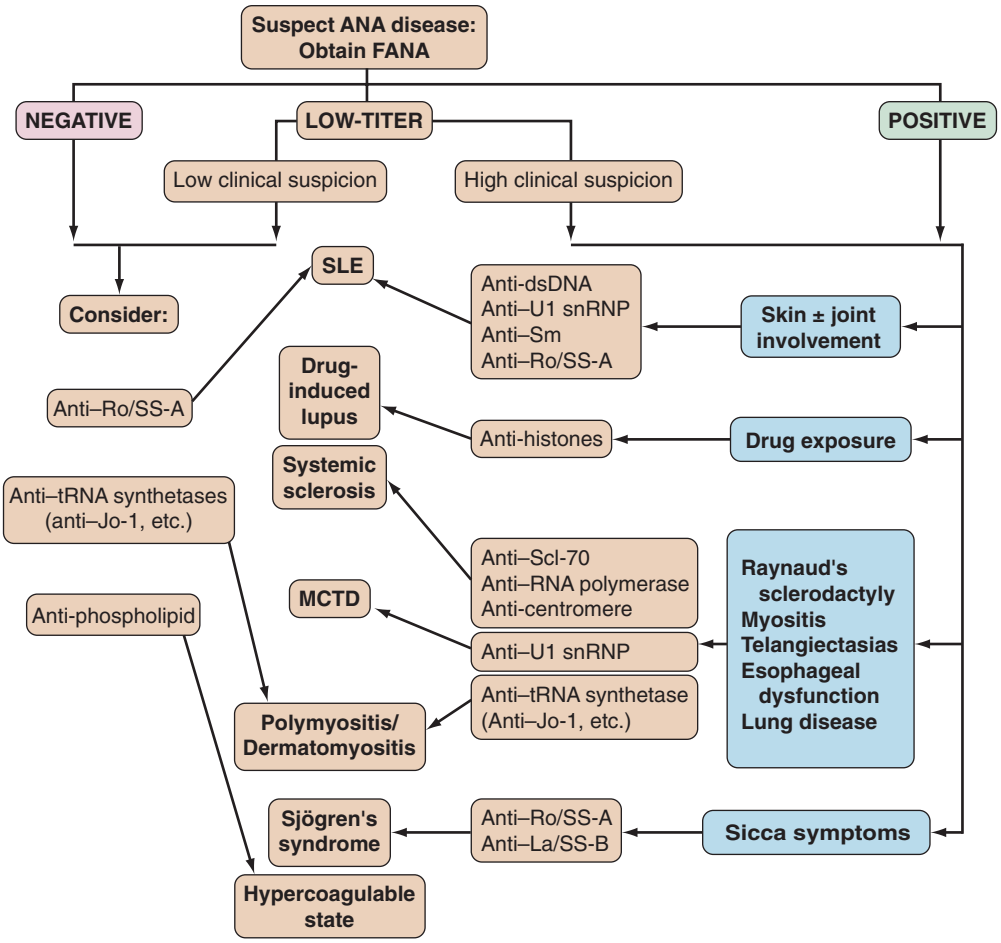



**Figure 55-3** Algorithm for the use of anti-nuclear antibodies (ANAs) in the diagnosis of connective tissue disorders. See text for details. Anti-dsDNA, Anti–double-stranded DNA; anti-tRNA, anti–transfer RNA; FANA, fluorescent anti-nuclear antibody; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; snRNP, small nuclear ribonucleoprotein; SS, Sjögren's syndrome.

on other clinical information. Indeed, many clinical and research studies upon which autoantibody-disease associations have been developed often utilized highly refined detection methods, such as immunoprecipitation or immunoblot, which are often unavailable in routine clinical laboratory testing. Thus the relevance of many antibody-test results in specific clinical settings continues to require careful, individualized interpretation by the referring physician.[82]



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 56

# Autoantibodies in Rheumatoid Arthritis



Felipe Andrade • Erika Darrah • Antony Rosen

**KEY POINTS**

- Autoantibodies in rheumatoid arthritis (RA) are important tools for diagnosis and for defining pathogenic pathways in this disease.
- Rheumatoid factors (RFs) are autoantibodies that recognize the Fc portion of immunoglobulin G molecules. They were the first autoantibodies described in RA.
- RFs have numerous causes in addition to RA and therefore have limited specificity for RA.
- Autoantibodies targeting citrullinated proteins are hallmarks of the immune response in RA.
- The anti–cyclic citrullinated peptide (anti-CCP) assay broadly detects antibodies against anti-citrullinated protein antibodies (ACPAs) and has high specificity and excellent sensitivity in RA.
- ACPAs precede the onset of clinical RA and are associated with more severe disease, making them useful as diagnostic and prognostic tools.
- Peptidylarginine deiminase enzymes, which catalyze the conversion of peptidyl-arginine to peptidyl-citrulline, play important roles in generating citrullinated autoantigens in RA.
- Genetic factors such as "shared epitope" human leukocyte antigen–DR subregion alleles appear to interact with environmental factors, such as smoking and infection, to initiate and drive autoimmunity in RA.

Autoantibodies have proven to be very useful tools for diagnosis and prediction in autoimmune rheumatic diseases. Emerging data pertaining to several autoimmune rheumatic diseases have demonstrated that the clinical evolution of disease from the pre-clinical phase to overt clinical disease is marked by a change in the specificity of the immune response, with autoantibodies directed against distinct antigenic targets at different disease phases. Whereas numerous autoimmune rheumatic diseases traditionally have been marked by highly phenotype-specific autoantibody responses (e.g., anti–double-stranded DNA antibodies in systemic lupus erythematosus [SLE] and high-titer anti–topoisomerase-1 antibodies in diffuse scleroderma), the discovery of highly specific autoantibody markers of rheumatoid arthritis (RA) lagged significantly behind these other diseases. During the past decade, enormous progress has occurred in this area, largely fueled by the discovery that citrullinated proteins are specific targets of autoantibodies in RA. This finding has highlighted post-translational modifications (PTMs) as critical determinants targeted by autoantibodies in RA, which has led to the identification of novel autoantibody systems and pathogenic mechanisms in this disease (Table 56-1). In this chapter we will review the autoantibodies in RA, with emphasis on rheumatoid factors (RFs), anti-citrullinated protein autoantibodies (ACPAs), and the anti–peptidylarginine deaminase (PAD) autoantibodies. The implications of these specificities for diagnosis and prediction of outcome, as well as for understanding pathogenic events in the disease, will be highlighted.

## RHEUMATOID FACTOR

The earliest assays (later called the Rose-Waaler agglutination test) that suggested the existence of autoantibodies in RA were developed in the early 1940s, when sera from patients with RA were shown to cause agglutination of sheep blood cells that had been sensitized with subagglutinating doses of rabbit anti–sheep erythrocyte antibodies.[1,2] These assays were later shown to be detecting immunoglobulin (Ig)M antibodies from patients with RA that recognized the Fc portion of IgG.[3] Numerous modifications of the agglutination assay evolved, particularly the use of IgG-coated latex beads instead of sheep erythrocytes.[4,5] Subsequent developments established RF assays in radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and nephelometry formats, with increased convenience but similar performance in terms of sensitivity and specificity. RFs may be positive in healthy control subjects (1% in younger persons and up to 5% in persons older than 70 years) and in patients with numerous other non-RA diseases (including other rheumatic diseases such as Sjögren's syndrome and cryoglobulinemia), as well as chronic infections.[6-8] The pretest probability of diagnosing RA therefore greatly influences the performance of the RF test, with sensitivities and specificities both in the 50% to 90% range, depending on the patient groups studied.[9,10] The existence of IgG and IgA RFs and evidence of somatic hypermutation have suggested that some RFs in RA are T cell dependent.[11,12] Although some studies have suggested that IgG and IgA RFs increase the specificity of RFs for RA,[13] a recent meta-analysis showed that assays of the different RF isotypes performed very similarly in terms of sensitivity and specificity and therefore do not add much compared with standard RF assays.[14]

Importantly, patients with RA also vary in terms of timing of the appearance of RF, with RF preceding symptomatic disease in a significant subpopulation[15-18] and following disease onset with variable kinetics in other patients.

**TABLE 56-1** Native and Modified Targets of Autoantibodies in Rheumatoid Arthritis

| Type of Antigen | Type of PTM | Antigen Generation | Protein | Prevalence of Autoantibodies |
|---|---|---|---|---|
| Native | None | Unknown | IgG (Fc)<br>hnRNP-A2 (RA33)<br>G6PI<br>PAD4 | 50%-90%<br>35%<br>12%-29%<br>30%-40% |
| Post-translationally modified | Citrullination | Enzymatic reaction mediated by PADs; arginine residues are changed to citrulline | Vimentin, fibronectin, actin, HSP90, histones, α-enolase, eEF1a, CAP-1, CapZalpha-1, asporin, cathepsin D, histamine receptor, PDI, ER60 precursor, ALDH2, collagen type I and II, eIF4GI, aldolase, PGK1, calreticulin, HSP60, FUSE-BP1 and 2, ApoE, MNDA | Up to 80% |
| | Carbamylation | Nonenzymatic reaction of cyanate with lysine residues; lysine is changed to homocitrulline | Unknown | 45% |
| | MAA adducts | Nonenzymatic reaction; peroxidation of membrane lipids generate reactive aldehydes that modify lysine residues, generating MAA adducts | Unknown | 29%-93% |

ALDH2, Mitochondrial aldehyde dehydrogenase; ApoE, apolipoprotein E; CAP1, adenylyl cyclase–associated protein-1; CapZalpha-1, F-actin capping protein alpha-1 subunit; eEF1a, elongation factor 1-alpha; eIF4GI, eukaryotic translation initiation factor-4G1; FUSE-BP, far upstream element binding protein; G6PI, glucose-6-phosphate isomerase; IgG, immunoglobulin G; hnRNP, heterogeneous nuclear ribonucleoprotein; HSP, heat shock protein; MAA, malondialdehyde-acetaldehyde; MNDA, myeloid nuclear differentiation antigen; PAD, peptidylarginine deaminase; PDI, protein disulfide-isomerase; PGK1, phosphoglycerate kinase-1; PTM, post-translational modification.

Indeed, the earlier onset of RF in patients with RA has been associated with more severe disease, highlighting a possible contribution of these antibodies to amplification.[19] The observation that RF only appears after disease onset in some patients suggests that it may mark distinct, sequential events in pathogenesis, which are very close together in the subgroup in whom RF occurs early, or are separated in time in patients in whom RF follows symptoms. The mechanisms that drive the production of RF in persons with RA are not fully understood. The presence of somatic hypermutation in RFs and the evidence that these antibodies are produced by plasma cells in the rheumatoid synovium have suggested that the RF response is driven locally by antigen.[11,12] However, it is still unclear how the IgG becomes self-immunogenic in the rheumatoid joint. In contrast to other autoantigens in RA, there is no evidence that the IgG targeted by RFs requires modification (e.g., PTM) for antibody recognition. Nevertheless, it is still possible that the initial event that breaks tolerance to IgG may be initiated through an abnormally modified molecule, as occurs for other autoantigens in RA. The possible mechanisms whereby RF may play an amplifying role in RA are numerous and include amplification of antigen capture, signaling, and effector functions, among others.[20]

## ANTIBODIES AGAINST CITRULLINATED ANTIGENS

### Antikeratin Antibodies and Antiperinuclear Factor: Initial Insights into a Novel Group of Autoantibodies in Rheumatoid Arthritis

Although RFs have been clinically useful in diagnosis of RA—particularly in cases in which the index of clinical suspicion is high—these autoantibodies have two shortcomings: (1) their lack of specificity for RA and (2) an unclear kinetic and mechanistic connection to pathogenesis. Thus defining additional autoantibodies specific for RA has been a major priority. A significant discovery in this regard was made initially by Nienhuis and Mandema in 1964[21] (Figure 56-1). These investigators recognized an autoantibody that stained keratohyalin granules surrounding the nucleus in cells of human buccal mucosa, which they called antiperinuclear factor (APF). These APF antibodies were found in 49% to 91% of patients with RA,[21-25] and they have reported specificities of 73% to 99%.[21,24-27] The assay was difficult to standardize because not every donor of buccal mucosal cells demonstrated the staining pattern,[28-31] and thus the assay did not find its way into routine clinical practice, but the specificity for RA suggested that the assay might be reporting on a potentially important pathogenic pathway. A second research group subsequently demonstrated that RA sera recognized antigens in the stratified squamous layer of esophageal epithelium—a staining pattern they termed antikeratin antibodies (AKAs).[32] Subsequent iterations of this assay utilized other forms of stratified squamous epithelium, including human skin.[33] Although this AKA assay also had very good specificity properties, it was of limited sensitivity[34-37] and was not particularly convenient or easy to standardize. Thus neither assay was broadly used in the clinical diagnostic arena. Like the LE cell in SLE, however, the assays provided a framework for antigen discovery that would later lead to highly specific and convenient tools for RA diagnosis and classification.

### Discovery of Autoantibodies That Recognize Peptidylcitrulline

The initial discovery of antibodies that recognized keratinized squamous epithelium in patients with RA prompted

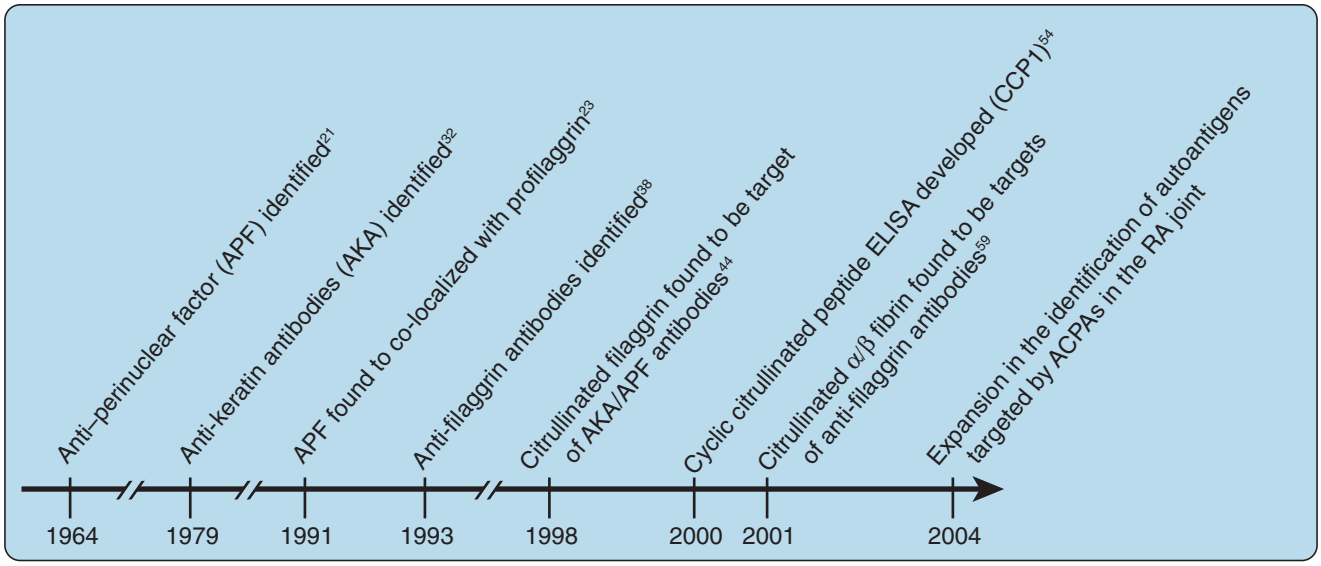



**Figure 56-1** A timeline of the discovery of anti-citrullinated protein antibodies (ACPAs) in rheumatoid arthritis (RA). ELISA, Enzyme-linked immunosorbent assay.

studies to define the target of these antibodies. It was initially shown that APF only stained a fully differentiated squamous epithelial layer and that APF staining was identical to the pattern produced by a monoclonal antibody specific for human (pro)filaggrin in indirect immunofluorescence of permeabilized squamous epithelial cells.[23] Although the antigen could not be definitively identified using this co-localization alone, it suggested that the differentiation state might affect antigen recognition. Subsequently, RA sera were shown to recognize a 40 kDa protein extracted from human epidermis, which was identified as a neutral/acidic isoform of filaggrin.[38] IgG from RA sera affinity-purified against the 40 kDa filaggrin protein was reactive in both the APF and AKA tests, demonstrating that these autoantibodies were very similar or identical.[39]

Filaggrin (filament-aggregating protein) is produced during the late stages of terminal differentiation of epithelial cells. It is synthesized as a heavily phosphorylated precursor protein (profilaggrin) that consists of 10 to 12 filaggrin repeats.[40] Profilaggrin is deposited in granules, and filaggrin is released by proteolytic cleavage during differentiation of the cells by proteases that remain to be fully defined. Coincident with cleavage, the protein is dephosphorylated, and a significant proportion (~20%) of the arginine residues are deaminated (i.e., converted to citrulline),[41,42] a PTM mediated by the PAD enzymes (see later section Citrullinated Antigen Generation in Rheumatoid Arthritis).[43] Because RA sera appeared to specifically target the neutral isoform of filaggrin (which is present and citrullinated in fully differentiated squamous epithelia), van Venrooij and colleagues addressed whether RA sera that were positive in the AKA/APF assays specifically recognized citrullinated peptides.[44] Thus regions within the deduced amino acid sequence of human profilaggrin with a high antigenicity index and the largest number of arginine residues were selected to generate synthetic peptides where arginine residues were substituted with citrulline. Using these citrullinated peptides, van Venrooij and colleagues demonstrated that AKA/APF antibodies are directed against citrullinated filaggrin. It is important to highlight that antibodies against citrullinated proteins in RA do not recognize free citrulline but only citrulline residues (i.e., peptidylcitrullines) within the context of peptides or protein sequences.

These initial studies became the basis for the major discovery that protein sequences containing citrulline residues are one of the most prominent targets for autoantibodies in RA. However, because the epidermis is not a target of rheumatoid inflammation and there is no evidence that (pro)filaggrin is expressed in articular tissues, this molecule cannot be the autoantigen that drives the AKA/APF response in the joint. The fact that anti-citrullinated filaggrin antibodies were enriched in synovial tissue compared with the serum or synovial fluid[45] and that these antibodies are synthesized locally by plasmocytes within the rheumatoid pannus[46] strongly suggests that anti-citrullinated filaggrin cross-reacts with an antigen enriched in the RA joint/synovial tissue. Because citrullination is a very frequent event in different tissues,[47-51] great caution must be exercised when attempting to ascribe a primary role for a specific antigen in driving the antipeptidylcitrulline antibody response. The relevance of filaggrin in RA is therefore largely historic and unlikely to be of pathogenic relevance. Instead, its conformation and high content of citrulline residues make it an excellent surrogate with which to detect antibodies against citrullinated molecules. Many other citrullinated autoantigens found in the rheumatoid joint that are currently being characterized are much more likely to be of pathogenic relevance (see later section Anti-citrullinated Protein Antibodies).

## Anti–Cyclic Citrullinated Peptide Antibodies

In the initial characterization of AKA/APF antibodies, van Venrooij and colleagues used a single C-terminal peptide derived from filaggrin (amino acids 306 to 324) to generate nine variants in which five arginine residues were changed to citrullines, either individually or in pairs, and these peptides were all assayed by ELISA.[44] Interestingly, although

the peptides were almost identical (the only difference being that the citrulline residues had different positions within the peptide), remarkable differences were found in the serum reactivity patterns toward each peptide (from 20% to 48% positivity), suggesting that although citrullination plays a critical role in antigen recognition by AKA/APF antibodies, the modification per se is not the only determinant that confers antibody binding. Instead, the data suggested that AKA/APF represent a pool of antibodies that recognize citrulline residues depending on the context of their surrounding amino acids. Indeed, when data from all peptides were pooled, the sensitivity increased to as much as 76%.[44]

Because peptides often adopt a β-turn conformation within the antibody–peptide complex[52] and cysteine-bridged cyclic peptides have been shown to mimic the β-turn structure of the original antigenic determinant and can bind with enhanced affinity to antibodies,[53] van Venrooij and colleagues engineered a cyclic peptide (substituting the terminal serine residues with cysteines and cyclizing the peptide through the formation of a disulfide bond) to which anti–citrullinated peptide antibodies reacted with higher affinity.[54] Using this cyclic citrullinated peptide (later named CCP1) and its linear counterpart, it was demonstrated that the cyclic structure increased the sensitivity of the assay (68% vs. 49%, without affecting specificity),[44,54] although it was still less sensitive than the assays using the combination of linear peptides (i.e., 76% vs. 68%).[44] Nevertheless, CCP1 became the antigen for the first generation of ELISAs designed to detect antibodies against citrullinated autoantigens.

To improve the CCP1 test, libraries of citrullinated peptides were used to construct the second-generation anti-CCP assay (CCP2), which was broadly adopted for clinical use.[55,56] In 2005, a third generation of anti–cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrullinated epitopes that are not identifiable with the second-generation CCP assays. In studies directly comparing second- and third-generation anti-CCP assays, the assays performed very similarly,[57,58] with a slightly increased sensitivity of CCP3 in some studies (e.g., sensitivities of 82.9% vs. 78.6% for CCP2, with specificities in the 93% to 94% range).[58]

## ANTI-CITRULLINATED PROTEIN ANTIBODIES

The finding that autoantibodies in RA recognize peptide sequences containing citrulline residues and that filaggrin was unlikely to be the physiologic target of these antibodies prompted the search for the primary protein targets of the antibodies detected by the CCP assay. It was believed that the identification of these antigens may provide additional information with regard to patient subtyping (compared with the general anti-CCP assay), as well as novel insights into disease etiology and pathogenesis. With the initial discovery of some of these citrullinated antigens, the name *anti-citrullinated protein antibodies* (ACPAs) was introduced to refer to RA autoantibodies for which detection was performed using citrullinated proteins/peptides from putative RA-associated autoantigens instead of filaggrin-derived or commercial CCPs.

Using protein extracts from rheumatoid synovial membranes and affinity purified anti-citrullinated filaggrin antibodies, Masson-Bessière and colleagues showed that RA synovial tissue contains several citrullinated proteins and defined the α and β chains of fibrin(ogen) as the first local targets for ACPAs in RA synovial tissue.[59] Because circulating fibrinogen is not citrullinated, the presence of citrullinated fibrin in the rheumatoid synovium strongly suggested that after fibrin deposition, citrullination occurs in situ through the activity of locally expressed PAD. This proposal (i.e., that the joint is the site for autoantigen citrullination in RA) has been further supported and extended to many other citrullinated autoantigens in RA.[60]

To date, several different approaches have been used to identify potential ACPA targets in RA. Although these approaches all utilize protein sequencing to identify autoantigens detected by ACPAs using immunoblotting assays, they vary in terms of the source of the antigens. Thus, in addition to RA pannus, identification of citrullinated autoantigens have been extended to rheumatoid synovial fluid, as well as to cell lysates in which purified PADs have been used to generate in vitro citrullinated proteins.

Using these approaches, several citrullinated autoantigen candidates have also been identified. These candidates include vimentin[61,62]; fibronectin[63]; actin[64]; heat shock protein (HSP)90[65]; histones[66]; α-enolase, elongation factor-1α, and adenylyl cyclase–associated protein-1[67]; F-actin capping protein α-1 subunit, asporin, cathepsin D, histamine receptor, protein disulfide-isomerase, ER60 precursor, and mitochondrial aldehyde dehydrogenase[68]; collagen type I[69] and II[70]; eukaryotic translation initiation factor 4G1[71]; aldolase, phosphoglycerate kinase-1 (PGK1), calreticulin, HSP60, and the far upstream element binding proteins (FUSE-BP) 1 and 2[72]; and apolipoprotein E and myeloid nuclear differentiation antigen.[73] Interestingly, some of these molecules (e.g., vimentin, α-enolase, collagen type II, HSP60, aldolase, and calreticulin) had been previously identified as autoantigens in RA, before it was recognized that protein citrullination played a role in their recognition by autoantibodies.[67,74] Although RA sera containing ACPAs may recognize both the native and citrullinated forms of the antigen, the modified form is usually preferentially recognized. Although this group of citrullinated antigens represents proteins that are recognized by RA antibodies, the number of citrullinated proteins found in the rheumatoid joint is more extensive and includes several dozen molecules that have not yet been characterized.[73,75,76] Whether unique ACPAs exist for each one of these molecules or whether only a few citrullinated autoantigens are responsible for driving the complete ACPA response in RA is still unknown.

Among the citrullinated candidate antigens present in the joint, the best characterized clinically and pathogenically are fibrin(ogen), vimentin, collagen type II, and α-enolase. ELISA-based assays have been developed using citrullinated proteins (e.g., fibrinogen) or citrullinated peptides (e.g., vimentin, collagen type II, and α-enolase) derived from these putative RA autoantigens to detect ACPAs. In the case of vimentin, the citrullinated sequence from a mutated isoform isolated from RA synovial fluid (named mutated citrullinated vimentin [MCV]) has been more commonly used instead of the native sequence.[77] Although it was greatly anticipated that the detection of

ACPAs targeting single antigens would provide additional diagnostic information compared with the anti-CCP ELISA, studies in this regard are inconclusive. Thus, in the absence of convincing evidence that measuring individual ACPAs provides any additional information compared with the anti-CCP assay, it appears that the use of CCPs is still a more reliable test to confirm the presence of antibodies against citrullinated proteins. In the case of patients who are anti-CCP negative, the use of antigen-specific assays may provide an additional tool to detect ACPAs.

Because single ACPAs appear to have limited utility in RA, further assays have been introduced to measure more than one ACPA simultaneously. For example, autoantibodies targeting putative RA-associated citrullinated autoantigens are measured using custom Bio-Plex bead-based autoantibody assays (Bio-Rad Laboratories, Hercules, Calif.) in which antigens (usually peptide sequences) are conjugated to spectrally distinct beads.[78] Autoantibody binding to antigen-coated beads is detected with anti–human phycoerythrin–labeled antibody and analysis with a Luminex 200 instrument (Luminex, Austin, Tex.). This assay is slightly more sensitive than the CCP ELISA, detecting ACPAs in approximately 10% of patients negative for anti-CCP antibodies (representing 3.7% of the total patient cohort),[79] a difference that might be explained by the array of peptides used on the assay. In addition, the bead-based multiplex assay has been useful to define the epitope spreading of ACPAs in pre-clinical RA.[78] However, although the multiplex assay provides additional information with regard to unique ACPA specificities present in the patient, knowing these specificities has not yet demonstrated any advantages for diagnosis or prognosis in RA.

## Anti–Cyclic Citrullinated Peptide Antibodies/ Anti-citrullinated Protein Antibodies: Clinical Relevance

The clinical importance of autoantibodies against citrullinated antigens in RA stems from the following favorable features (Figure 56-2):

1. *Anti-CCP antibodies have high specificity for the diagnosis of RA.* In systematic reviews and meta-analyses, anti-CCP antibody positivity has been shown to be as sensitive as but more specific than RF in distinguishing RA from other forms of inflammatory arthritis.[14]
2. *The presence of anti-CCP autoantibodies is an important predictor of development of RA.* RA developed within 3 years in more than 90% of the patients with undifferentiated arthritis who tested positive for anti-CCP, in contrast to only 25% of the patients who tested negative for anti-CCP.[80]
3. *Anti-CCP positivity has been associated with a more severe and destructive disease course, although the independent role of the antibody in comparison with RF has not yet been confirmed.* Some investigators have shown that both RF and anti-CCP antibodies are independent predictors of severity, whereas others have shown that anti-CCP antibodies rather than RF are the better predictor of radiographic progression,[81-88] particularly in patients who were seronegative for RF.[88]
4. *Anti-CCP/ACPAs are associated with RA-related interstitial lung disease (ILD) and cardiovascular disease (CVD).*[89-95] ILD and CVD are extra-articular manifestations with high mortality in persons with RA. Although the mechanistic significance of these associations is still unclear, one possibility is that these antibodies are markers of systemic inflammation that contributes to lung and cardiovascular damage. Alternatively, it is noteworthy that citrullination has been found in lung, myocardium, and atherosclerotic plaque,[51,96-98] suggesting the possibility that these antibodies are directly pathogenic by targeting extra-articular tissues containing citrullinated antigens. The presence of anti-CCP antibodies in patients who have ILD but do not have RA is intriguing and has focused attention on the lung as a possible extra-articular site where RA may be initiated[99,100] (see section Potential Environmental Factors in Rheumatoid Arthritis Pathogenesis).

The early and accurate diagnosis of RA, together with effective treatment with disease-modifying anti-rheumatic

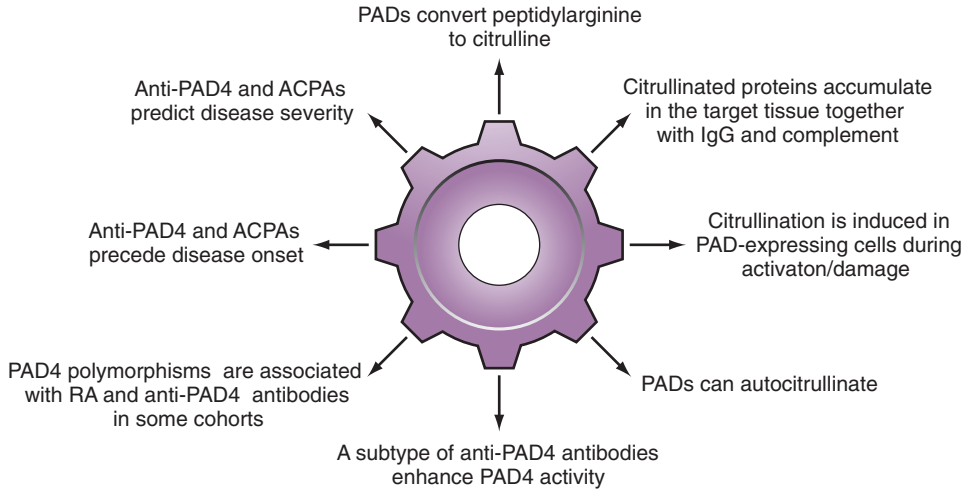



**Figure 56-2** Protein citrullination as a hub in rheumatoid arthritis (RA) pathogenesis. ACPAs, Anti-citrullinated protein antibodies; PAD, peptidylarginine deiminase.

drugs, decreases accrual of joint damage in RA. Because anti-CCP/ACPAs are markers of disease severity and are detectable early in the disease course, they are powerful tools to classify patients with early stage inflammatory arthritis who will benefit from treatment.[101-103]

## Kinetics of Appearance of Anti–Cyclic Citrullinated Peptide Antibodies/ Anti-citrullinated Protein Antibodies in Rheumatoid Arthritis

Recent studies have demonstrated that autoantibodies may precede the onset of clinical disease in both tissue-specific and systemic autoimmune diseases.[17,104-110] Defining events prior to clinical onset is challenging, and several different study designs have been used to define the appearance of autoantibodies and their relationship to disease. The two general categories include (1) stored blood samples collected prior to disease onset (blood banks or military cohorts) and (2) prospective studies examining emergence of autoantibodies and disease in high-risk persons (often relatives of affected individuals).[111] In RA, the former approach has clearly shown that autoantibodies precede RA diagnosis in many persons in whom seropositive RA subsequently develops, often by 2 to 6 years.[109,110,112,113] In several studies, 20% to 60% of patients with RA were RF positive prior to diagnosis, and 30% to 60% of patients were positive for anti-CCP.[109,110,112,113] With one exception,[113] the prevalence of anti-CCP prior to diagnosis was almost twice as high as RF. Interestingly, whereas RF remained present consistently, anti-CCP could vary over time, even disappearing in some persons. In one study, pre-clinical anti-CCP positivity was strongly associated with the development of erosive RA (odds ratio [OR], 4.64; 95% confidence interval, 1.71 to 12.63; $P < 0.01$), whereas RF was not ($P = 0.60$).[113] The evolution of autoantibody epitope spreading in pre-clinical RA has been addressed using a multiplex assay. The study confirmed that ACPA positivity predicts progression to RA in asymptomatic persons and showed that the number of ACPA specificities accumulate over time during the pre-clinical phase of the disease.[78] Moreover, the expansion of the ACPA response precedes the elevation of many inflammatory cytokines, including tumor necrosis factor (TNF), interleukin (IL)-6, IL-12p70, and interferon (IFN)-γ, suggesting that ACPAs may be part of the driving inflammatory process in RA.[78]

Together, these data highlight that the development of autoimmunity against citrullinated antigens is often asymptomatic and generally precedes the onset of clinical disease. This presymptomatic phase is potentially of great importance, because it may identify persons in whom important precursor conditions for the subsequent development of RA have been satisfied. The subsequent events that convert this RA precursor into a chronic, self-sustaining process are not yet known, but defining them is of great importance.

## Genetic Associations with Anti–Cyclic Citrullinated Peptide Antibodies/ Anti-citrullinated Protein Antibodies

Although the association of specific human leukocyte antigen–DR region (HLA-DR) alleles and RA has been known for decades, the relationship between genetics and the development of RA autoantibodies is just beginning to be elucidated.[114] A subset of HLA-DR alleles termed the "shared epitope" (SE) alleles includes HLA-DR*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, and *1402 and is united by a conserved sequence of amino acids (QRRAA, QKRAA, or RRRAA) on the alpha helix of the DR-β chain peptide-binding groove.[115] SE alleles are strongly associated with the development of anti-CCP/ACPA but are not independently associated with RF.[80] Furthermore, there appears to be a gene dosage effect on the relative risk of anti-CCP development with an OR of 3.3 to 4.7 for patients with one SE allele and an OR of 11.8 to 13.3 for patients with two SE alleles.[80,116,117] Analysis of individual SE alleles has revealed that anti-CCP/ACPAs are predominantly associated with HLA-DR4 rather than HLA-DR1 SE alleles.[118] Interestingly, smoking has been shown to skew the genetic association of SE alleles with anti-CCP development, with HLA-DR1 and HLA-DR10 SE alleles being more important in this patient group (see Anti-citrullinated Protein Antibodies in Rheumatoid Arthritis: Insights into Disease Mechanism section).[119]

In addition to SE alleles, a single nucleotide polymorphism in the *PTPN22* gene (1858C/T) is also associated with anti-CCP antibodies with an OR of 3.80.[120] *PTPN22* encodes for lymphoid protein tyrosine phosphatase and has been shown to be associated with several autoimmune disorders, including RA.[121] The combination of *PTPN22* 1858C/T genotype and anti-CCP antibodies was 100% specific for RA and confers a relative risk of 130.03. The 1858C/T polymorphism was not associated with RF isotypes and appeared to act independently of SE alleles.[120] Genetic factors such as the SE and *PTPN22* may therefore play a role in the pre-clinical phase of RA, predisposing individuals to the generation of anti-CCP antibodies and susceptibility to the overt disease phenotype.

## Citrullinated Antigen Generation in Rheumatoid Arthritis

### The Peptidylarginine Deiminase Enzymes

Citrulline is not part of the natural pool of amino acids used for protein synthesis, and therefore citrulline residues need to be generated post-translationally once the protein has been synthesized. This PTM, termed *arginine deamination* or *citrullination*, is mediated by the calcium-dependent PAD enzymes. PADs target arginine residues in proteins and mediate hydrolysis of the arginine guanidinium side chain, resulting in the generation of citrulline residues and ammonia (Figure 56-3A). The PADs belong to a larger group of guanidino-modifying enzymes called the amidinotransferase (AT) superfamily.[122,123] This superfamily of enzymes is expressed both in prokaryotes and eukaryotes and includes the arginine deaminases, the dimethylarginine dimethylaminohydrolases, and the dihydrolases.[123] PADs are found in numerous species from bacteria to humans. Interestingly, the bacterial PADs are not dependent on calcium for activity and only contain an approximately 40 kDa catalytic domain, whereas vertebrate mammals have larger multidomain enzymes (~75 kDa) whose activity is regulated by calcium. Five PAD enzymes have been

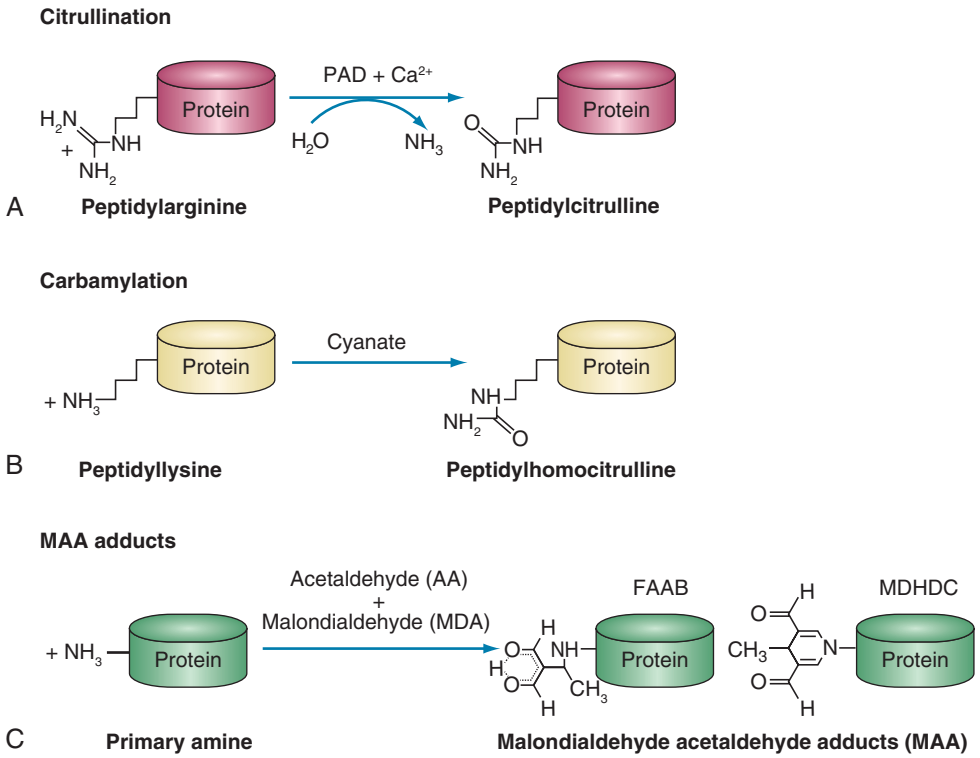



**Figure 56-3** Post-translational modifications targeted by antibodies in rheumatoid arthritis (RA). **A,** During citrullination, the calcium-dependent peptidylarginine deiminase (PAD) enzymes target arginine residues in proteins, resulting in the generation of citrulline residues and ammonia. **B,** Carbamylation results from the reaction of cyanate with the primary amine group of peptidyllysine that is converted to peptidylhomocitrulline. **C,** Malondialdehyde-acetaldehyde (MAA) adducts (FAAB and MDHDC) are generated from the reaction of malondialdehyde (MDA) and acetaldehyde (AA) with primary amine groups of amino acid residues, preferentially peptidyllysine. FAAB, 2-formyl-3-(alkylamino)butanal; MDHDC, 4-methyl-1, 4-dihydropyridine-3,5-dicarbaldehyde.

identified in mammals, and the *PADI* genes are located at a single cluster on chromosome 1p36.1.[43,124] For historical reasons, these isozymes have been designated PAD1 to PAD4 and PAD6. Human PAD4 was initially named PAD5 but was later renamed PAD4 to reflect the fact that it is a true ortholog of this isoform.[125] Although initial evidence suggested that PAD4 was the only nuclear PAD,[126] recent evidence suggests that some PAD2 may also reside in the nucleus.[127]

The PADs are highly conserved and share 50% to 55% sequence identity with each other,[128] with different PADs having distinct substrate preferences and expression in specific tissues. PAD1 is primarily expressed in uterus and skin. PAD2 is more widely expressed in muscle, skin, brain, spleen, secretory glands, monocytes, and neutrophils. PAD3 is expressed in skin and neutrophils, PAD4 is expressed in hematopoietic cells (e.g., neutrophils and monocytes), and PAD6 is broadly expressed in germ cells, peripheral blood leukocytes, lung, small intestine, liver, spleen, and skeletal muscle.[43,61,124,125,129-131] Because of their prominent expression in rheumatoid synovial tissue and fluid,[60,132,133] PAD2 and PAD4 have gained prominence as potential candidates that drive citrullination of self-antigens in RA.

## Peptidylarginine Deiminase Structure, Activity, and Regulation

The three-dimensional structure of PAD4[128,134] and PAD2[135] has been solved and is likely representative of the broader family. PAD2 and PAD4 are dimers formed by head-to-tail contact between the N-terminal domain of one molecule and the C-terminal domain of the second. The N-terminal domain includes two immunoglobulin-like subdomains (named subdomain 1 and 2) that are proposed to mediate protein-protein interactions and/or substrate targeting.[128] Five and six $Ca^{2+}$-binding sites were identified in the structure of PAD4 and PAD2, respectively, with $Ca^{2+}$ binding inducing conformational changes required to generate the active site cleft. Interestingly, the calcium binding residues are highly conserved among PADs, except for PAD6, which lacks some of these sites; the catalytic cysteine is also different in PAD6, and together these findings have suggested that PAD6 is not a deaminating enzyme.

PAD1 to PAD4 rely strongly on the presence of $Ca^{2+}$ for activity and require millimolar amounts of calcium (~2 to 5 millimolar) to be activated in vitro.[136] The resting concentration of calcium in the cytoplasm is normally maintained in the range of 50 to 100 nM and only rises to 200 to 800 nM upon cell activation with physiologic stimuli.[137-140] The large discrepancy between the in vitro and cellular calcium requirements for PAD activation suggests that additional mechanisms, such as allosteric effects of PTMs or partner binding, may regulate PAD activity in vivo by modifying the calcium requirement of the enzyme.[136] It is also important to note that whereas PAD activation provides one level of regulation, the activity of the PADs may also be negatively regulated through autocitrullination. This is not dissimilar to other enzymes, where automodification (e.g., autophosphorylation) modifies enzymatic function. Autocitrullination has been described for PADs 1, 2,

3, and 4.[141,142] In the case of PAD4, direct citrullination of arginines surrounding the active site cleft appears to have major impact on activity and substrate binding.[141] Besides cell activation, it has been proposed that PADs may also be activated under conditions where intra-cellular concentrations of calcium approach extra-cellular concentrations (~1 to 1.4 millimolar), which may occur in dying cells experiencing secondary necrosis or as result of membranolytic damage.[75]

### Structural and Functional Implications of Protein Citrullination

Arginine residues play a central role in the maintenance of secondary and tertiary protein structure. The positively charged guanidino group allows the formation of intramolecular hydrogen bonds to backbone carbonyl oxygens and also intermolecularly between different proteins.[143] During the process of citrullination, the net charge of the protein is reduced by the loss of a positive charge per citrulline residue, producing changes in protein structure and affecting intramolecular and intermolecular interactions.[144]

It is likely that these structural changes also have important functional implications. Indeed, deamination has been implicated in several physiologic processes. In the skin (which expresses PADs 1, 2, and 3[48,130,145]), deamination of filaggrin (a component of the cornified cell envelope) is critical for its degradation into free amino acids, which act as a natural moisturizing factor in the stratum corneum.[48,145] Interestingly, PAD2-deficient mice have normal development and show no abnormalities in the skin,[146] suggesting functional redundancy in mouse development. Whether PAD2 functions in the skin during injury or environmental perturbations is currently unknown. In the immune system, PAD2 (and/or potentially other PADs) citrullinates chemokines, a process that modulates chemokine activity.[147-150] Thus PAD2 may play important roles in controlling effector functions related to environmental triggers.

Human PAD4 (first called PAD5) was first found in human myeloid leukemia HL-60 cells induced to differentiate into granulocytes by retinoic acid[125] and later described in peripheral blood granulocytes.[129] Histones H2A, H3, and H4 and nucleophosmin/B23 were the first PAD4 substrates to be identified.[151] PAD4 appears to function as a transcriptional co-regulator, mediated by its ability to catalyze the deamination of specific residues present in the N-terminal tails of histones.[152-154] This function, together with the finding that PAD4 is expressed during granulocyte differentiation, initially suggested that this enzyme may have a role during granulopoiesis. However, mice deficient in PAD4 have normal development,[155] suggesting that this enzyme has no essential roles in steady-state cellular functions, granulopoiesis, or other developmental processes. Recent studies have demonstrated that PAD4-mediated citrullination of histones is required for neutrophil extra-cellular trap (NET) formation and bacterial clearance,[154,155] suggesting a role for this enzyme in immune and inflammatory effector functions.

PAD6, the most recently defined member of the PAD family, was initially cloned from mouse oocytes and named egg PAD (ePAD),[156] although the enzyme is widely expressed in different tissues. PAD6 is essential for the formation of cytoplasmic lattices, and female mice deficient in PAD6 are infertile but otherwise normal.[131] The function of PAD6 in other organs/tissues besides ovaries is unknown.

### Peptidylarginine Deaminases in Rheumatoid Arthritis

Multiple PADs are likely responsible for autoantigen citrullination in RA, particularly PADs 2 and 4.[60,132,133] Although PAD4 has some unique characteristics that have focused significant attention on this protein, it is important to be aware that no evidence exists to suggest that pathologic citrullination in RA is exclusively or preferentially mediated by this isoform.

It is of interest that several polymorphisms in PAD4 have been genetically associated with RA development, particularly in Asian populations.[157-160] In this regard, two common haplotypes of the *PADI4* gene were initially identified[157] and designated "susceptible" or "nonsusceptible" based on their relative frequency in patients with RA versus control subjects. In the initial population, the OR for the susceptible haplotype was almost 2; this effect was not observed in most white populations. Changes in messenger RNA stability of the susceptible *PADI4* reported in the initial paper were not associated with changes in enzyme level. Furthermore, although the susceptible haplotype generates a PAD4 molecule containing three amino acid substitutions in the N-terminal region (i.e., Gly55-Ser, Val82-Ala, and Gly112-Ala), current evidence suggests that these changes have no effect on the function of the protein.[141,161] Indeed, they appear to affect conformation only within the N-terminal domain but not the active site located in the C-terminal domain.[161] The fact that PAD4 acts as a head-to-tail dimer may nevertheless allow conformational differences in the N-terminus of one molecule to influence the C-terminal domain of another and influence catalysis or even inhibition by autocitrullination. It has been proposed that the PAD4 genotype may exert its effects on RA disease susceptibility as a consequence of its immunogenicity, rather than its enzyme activity.[141] Consistent with this hypothesis is the observation that, in addition to its ability to citrullinate RA autoantigens, PAD4 itself is an RA autoantigen (see next section Anti–peptidylarginine deiminase autoantibodies).[162,163] Moreover, it is noteworthy that the *PADI4* susceptible gene appears to contribute to the development and progression of RA regardless of ACPA status,[164,165] supporting the notion that the snp variant may drive susceptibility through other mechanisms besides the production of citrullinated autoantigens.

## ANTI–PEPTIDYLARGININE DEIMINASE AUTOANTIBODIES

In addition to its prominent enzymatic role in the generation of citrullinated autoantigens, PAD4 itself is targeted by autoantibodies in a subset of patients with RA.[162,163] These antibodies recognize the unmodified form of PAD4, but it has been suggested that autocitrullinated PAD4 may be more immunogenic in some persons.[141] As has been observed for anti-CCP and RF, anti-PAD4 antibodies are frequently present prior to the onset of any disease symptoms.[107] Interestingly, in a cohort of white patients with RA who had established disease, the susceptible PAD4 haplotype was

strikingly associated with PAD4 autoantibodies, even though the association of the susceptible haplotype with RA development has not been observed in white populations.[163] The sensitivity of PAD4 antibodies for RA is in the 30% to 40% range, with a specificity of greater than 95%. PAD4 autoantibodies are associated with anti-CCP antibodies and are independently associated with more severe, erosive RA that persists despite treatment with TNF inhibitors.[166,167] Recently, a subset of anti-PAD4 antibodies identified by their cross-reactivity to the related protein PAD3 has been identified and appears to be the major driver of the previously seen associations with erosive disease severity and progression despite treatment.[136] These antibodies were also associated with anti-CCP antibodies, as well as the SE alleles. Furthermore, patients with PAD3/PAD4 cross-reactive antibodies are at the highest risk for the development of RA-associated ILD, an effect significantly augmented in patients who were past or present cigarette smokers (OR, 61.4).[168] The causal relationship between PAD3/PAD4 antibody development and cigarette smoking is unknown but suggests that environmental factors such as cigarette smoke may trigger the development of RA-associated autoantibodies in susceptible persons (see later section Potential Environmental Factors in Rheumatoid Arthritis Pathogenesis).

## MECHANISMS OF CITRULLINATED AUTOANTIGEN PRODUCTION IN RHEUMATOID ARTHRITIS

Two potential mechanisms have been implicated in aberrant PAD activation and the production of citrullinated autoantigens in the rheumatoid joint. One mechanism involves the hyperactivation of PADs intra-cellularly, and the second relies on extra-cellular autoantigen citrullination. Importantly, these mechanisms are not mutually exclusive but are complementary. Initial immunohistochemical studies of synovial tissue focused attention on synoviocytes and inflammatory cells as potential sources of PADs and citrullinated antigens in the RA joint.[132,133] However, whereas functional studies to address PAD expression, activity, and production of citrullinated autoantigens in isolated synoviocytes are limited and inconclusive,[169] the study of neutrophils has provided important insights into mechanisms of autoantigen citrullination in RA.[64,75,170] These cells represent one of the most abundant cell types in RA synovial fluid and constitutively express several PADs.[64] Furthermore, they can hypercitrullinate and generate citrullinated autoantigens when activated with calcium ionophores,[64] and they are highly enriched in citrullinated autoantigens when freshly isolated from the RA joint.[75] Two mechanisms have been proposed to trigger PAD activation and citrullinated autoantigen production in neutrophils in RA: (1) formation of neutrophil extra-cellular traps (NETs), an antimicrobial system in which granule proteins and chromatin are extruded from the cell and form extra-cellular fibers that bind pathogens,[170] and (2) membranolytic damage mediated by complement or perforin.[75]

During NET formation, PAD4-mediated histone citrullination is important for chromatin unfolding.[171] Activation of PAD4 during this process is believed to result in citrullination of other molecules, resulting in autoantigen generation. Alternatively, it has been proposed that PADs may be released during NET formation, contributing to extracellular citrullination. Although NETs may be an important source of inflammatory signals and some citrullinated autoantigens, the contribution of this process to generating the whole set of citrullinated molecules found in the rheumatoid joint (i.e., the RA citrullinome) is unclear.[170] Instead, recent studies have found that immune pathways that form pores in cell membranes and that are active in the RA joint (e.g., the complement system's membrane attack complex [MAC] and perforin released from cytotoxic cells) are potent activators of PADs and efficient inducers of the RA joint citrullinome.[75] The membranolytic damage induced by these pathways is associated with high intra-cellular calcium flux,[172] which likely explains the hyperactivation of PADs in the damaged cell. It is also possible that other forms of cell death may play a role in mediating enhanced citrullination in the RA joint, particularly if these death pathways (e.g., pyroptosis or necroptosis) result in extra-cellular leakage of PADs. The discovery that fibrinogen is heavily citrullinated in the RA joint provided initial evidence that citrullination can indeed occur extra-cellularly.[59] Furthermore, soluble PAD2 and PAD4 have been detected in the synovial fluid from patients with RA,[60,173] suggesting that these enzymes can gain access to the extra-cellular space during inflammation.

Although the extra-cellular calcium concentration in synovial fluid (0.5 to 1 mM) is more than 1000-fold higher than intra-cellular levels, it is still not optimal for maximal PAD activity in vitro. Recent studies have demonstrated that anti-PAD3/PAD4 cross-reactive autoantibodies are able to enhance PAD4 activity at calcium concentrations found extra-cellularly.[136] These anti-PAD3/PAD4 autoantibodies decrease the enzymatic requirement for calcium into the physiologic range and greatly augment PAD4 activity. Thus PAD4 released by neutrophils during NET formation or other forms of cell deaths may encounter anti-PAD4 antibodies in the synovial fluid with the capacity to activate enzymatic function, contributing to extra-cellular citrullinated autoantigen generation in RA.

## NOVEL POST-TRANSLATIONAL MODIFICATIONS TARGETED BY ANTIBODIES IN RHEUMATOID ARTHRITIS

### Anti–Carbamylated Protein Antibodies

Proteins that contain homocitrulline (i.e., carbamylated proteins) recently have been recognized as an important target of autoantibodies in RA, with IgG and IgA antibodies recognizing carbamylated antigens identified in more than 40% of patients with RA.[174] In contrast to citrullination, carbamylation is a nonenzymatic PTM in which homocitrulline is generated by the reaction of cyanate with the primary amine of lysine residues (Figure 56-3B). Carbamylation therefore accumulates in conditions that enhance cyanate levels, such as uremia, inflammation, and cigarette smoking.[175] In RA, inflammation is likely the most important source of carbamylated proteins. Here, myeloperoxidase (MPO) released from neutrophils promotes the conversion of thiocyanate to cyanate, further enhancing carbamylation.[175,176] Although homocitrulline structurally

resembles citrulline (homocitrulline is one methylene group longer), the finding that anti–carbamylated protein (anti-CarP) antibodies are present in approximately 35% of ACPA⁻ patients and that these antibodies are predictive of a more severe disease course independent of ACPA status has suggested that these antibodies are not cross-reactive between the two PTMs.[174] Moreover, anti-CarP antibodies appear many years before the diagnosis of RA and predict the development of RA independent of anti-CCP antibodies in patients with arthralgia.[177-179] Despite the growing interest in anti-CarP antibodies, a major caveat is that their primary protein targets in RA are still unknown. Indeed, carbamylated–fetal calf serum and carbamylated-fibrinogen are used as surrogate antigens for anti-CarP detection. Furthermore, carbamylated proteins have not yet been described in the RA joint.

### Anti–Malondialdehyde-Acetaldehyde Adducts Antibodies

Malondialdehyde-acetaldehyde (MAA) adducts are the most recently described target of anti-PTM antibodies in RA.[180] Similar to carbamylation, the generation of MAA protein adducts is nonenzymatic and involves the preferential modification of the primary amine of lysine residues. During oxidative stress caused by a variety of stimuli, including inflammation and exposure to alcohol, reactive oxygen species (ROS) trigger peroxidation of membrane lipids, resulting in the formation of malondialdehyde (MDA) and acetaldehyde (AA),[181-184] which react with proteins, resulting in the formation of stable MDA-AA protein adducts, referred to as MAA (Figure 56-3C).[185,186] These adducts have been implicated in contributing to inflammation and cellular injury associated with cardiovascular and alcoholic liver disease.[181-183] Interestingly, RA synovial tissue, but not tissue from patients with osteoarthritis, is enriched in MAA-modified proteins, and patients with RA have antibodies targeting this modification when they are detected using MAA-modified albumin as a surrogate antigen.[180] Whereas anti-MAA antibodies are strongly associated with anti-CCP and RF, it is interesting that anti-MAA antibodies of the IgG isotype are found in 88% of anti-CCP⁻ patients, suggesting that these may be useful biomarkers, particularly in persons with ACPA⁻ RA. However, whether this antibody system is clinically relevant in RA remains to be defined. In this regard, it is important to highlight that anti-MAA adduct antibodies are not specific for RA but have also been found in patients with alcohol-induced liver disease, diabetes, and CVD.[187-189] Therefore these antibodies may not exclusively be markers of joint damage but may be generated as a result of comorbidities associated with RA. Similar to carbamylation, the primary targets of anti-MAA adduct antibodies in RA are not yet known.

### Anti-citrullinated Protein Antibodies, Anti–Carbamylated Protein, and Anti–Malondialdehyde-Acetaldehyde Antibodies in Rheumatoid Arthritis: Insights into Pathogenesis

The finding that persons with RA have antibodies targeting different PTMs is intriguing and unlikely to be accidental. Because neutrophils produce all components (i.e., PADs, MPO, and ROS) required to generate the current set of modified autoantigens in RA (i.e., ACPAs, anti-CarP, and anti-MAA), it has been suggested recently that these distinct antibody systems may indeed represent markers of a common pathogenic event driven by neutrophils, potentially placing these cells at the center of autoantigen production in RA pathogenesis.[190] Confirming this hypothesis would have important implications for future anti-neutrophil therapies in this disease.

## OTHER AUTOANTIBODY SPECIFICITIES IN RHEUMATOID ARTHRITIS RECOGNIZING NONMODIFIED AUTOANTIGENS

Anti-RA33 antibodies were discovered in 1989 as a novel autoantibody specificity recognizing a nuclear protein with an apparent molecular mass of 33 kDa.[191] These autoantibodies initially were detected in 35% of patients with RA but also were detected in a small number of patients who had other autoimmune or degenerative rheumatic disorders. The antigen was termed RA33, and it was the first description of a nuclear antigen apparently specific for RA. Later, the antigen was identified as the A2 protein of the heterogeneous nuclear ribonucleoprotein complex (hnRNP-A2).[192] Further characterization of this antibody showed that it was not strictly specific for RA but was also found in approximately 20% of patients with SLE and in 40% to 60% of patients with mixed connective tissue disease (MCTD).[193,194] However, in SLE and MCTD, they usually occur together with antibodies to U1–small nuclear ribonucleoproteins (snRNPs) or Smith (Sm) antigen. Therefore anti-RA33 without concomitant anti–U1 snRNP autoantibodies were found to have specificity of 96% for RA.[193] Additionally, anti-RA33 antibodies in RA, SLE, and MCTD are distinguished by their recognition of different conformation-dependent epitopes in hnRNP-A2.[195] Interestingly, despite their limited specificity, in the absence of RF and ACPAs, anti-RA33 antibodies in patients with very early arthritis (<3 months' duration) are associated with a relatively mild nonerosive disease course, potentially identifying patients with a good prognosis who will respond well to treatment with disease-modifying anti-rheumatic drugs (DMARDs).[196] In studies in human samples, stimulation assays using hnRNP-A2–induced T cell proliferation in almost 60% of the patients with RA, but only 20% of the control subjects, with substantially stronger responses observed in patients with RA.[197] Interestingly, immunohistochemical analyses revealed pronounced overexpression of hnRNP-A2 in synovial tissue of patients with RA, placing the antigen at sites of disease in RA.

Another autoantigen of potential interest in RA is glucose-6-phosphate isomerase (G6PI). Arthritis in the K/BxN mouse model results from pathogenic immunoglobulins that recognize G6PI,[198-200] a glycolytic enzyme residing in the cytoplasm of all cells. Moreover, antibodies directed against G6PI can, alone, transfer arthritis to healthy recipients.[201] The pathogenic potential of this antibody prompted its study in human RA. However, although initial studies reported a high frequency of such antibodies in sera of patients with RA,[202] these results have been the subject of debate.[203-205] Overall, anti-G6PI antibodies in patients with

RA range between 12% to 29% in different cohorts, with a higher prevalence in patients with active disease. Patients with psoriatic arthritis, undifferentiated arthritis, and spondylarthropathy also displayed anti-G6PI antibodies at similar frequencies (12% to 25%), and similar titers are detected in a proportion (5% to 10%) of control subjects or patients with Crohn's disease or sarcoidosis.[205] The potential relevance of these antibodies in RA is still uncertain.

## ANTI-CITRULLINATED PROTEIN ANTIBODIES IN RHEUMATOID ARTHRITIS: INSIGHTS INTO DISEASE MECHANISM

Although our understanding of the pathogenesis of RA remains incomplete, current models employ a similar construct to other autoimmune rheumatic diseases—that is, in the setting of a complex genetic predisposition, environmental and stochastic events function to initiate an autoimmune process recognizing specific self-antigens, where the immune response itself participates in immune amplification and tissue injury (Figure 56-4). The striking association of RA (and particularly ACPAs) with particular class II major histocompatibility complex (MHC) alleles underscores at least one component of this framework. Whether RA autoantibodies are directly pathogenic or whether they are markers of inflammation and/or damage remains uncertain, particularly for RFs. However, ACPAs have several features that suggest direct involvement in RA pathogenesis (Figure 56-3):

1. The targets of these antibodies (i.e., citrullinated proteins) are abnormally expressed and highly enriched in synovial tissue and fluid of patients with RA.[60,68,133]
2. The antibody response directed against these citrullinated antigens precedes RA onset[17,104,110,206] and is highly specific for RA and predictive of disease progression.[83-87,207]
3. ACPA positivity is closely linked with the best-known predisposing genetic risk factors for the development of RA: the HLA-DR SE alleles,[116,120,208,209] in particular, HLA-DRB1*04,[118,210,211] linking genetic predisposition and autoantibody production.
4. Peptidylcitrulline contained in peptides derived from aggrecan and vimentin is preferred to arginine in the electropositive P4 pocket of the RA-susceptible HLA-DRB1*04:01/04, and HLA-II tetramers containing these peptides identified circulating citrullinated epitope–specific $CD4^+$ T cells.[212]
5. Plasma cells producing antibodies against citrullinated proteins are present in RA synovial tissue.[46]
6. About one-half of the anti-$CCP^+$ RA patients have circulating immune complexes containing citrullinated fibrinogen, and immunohistochemical staining has shown co-localization of the fibrinogen, immunoglobulin, and complement component C3 in RA pannus tissue.[213]
7. Immune complexes containing citrullinated fibrinogen can also activate monocyte-derived macrophages to produced TNF via engagement of Fcγ receptors and Toll-like receptor 4.[214,215] Similar observations implying overlapping pathogenic properties are likely also operative in the case of RFs in RA. Recent evidence also indicates the that distinct glycosylation patterns in ACPAs may influence pathogenicity of these antibodies.[216,217]

## Potential Environmental Factors in Rheumatoid Arthritis Pathogenesis

Although abundant citrullination is found in RA synovial tissue once disease is established, no studies have addressed whether abnormal synovial citrullination precedes the onset of clinical RA (a requisite to explain the presence of antibodies against citrullinated proteins long before clinical disease). Such evidence will be very challenging to obtain. At this stage, it is therefore important to be aware that the joint is not the only possible site of abnormal citrullination that might initiate the RA-specific immune responses to citrullinated autoantigens. It is possible that the joint becomes targeted secondarily after the ACPA immune response has been initiated at another site, as a consequence of an inflammatory event triggered by a common environmental exposure, such as periodontal infection/inflammation and/or smoking (Figure 56-4). Although no evidence exists that viral proteins are citrullinated in vivo, a subset of $ACPA^+$ RA sera has been demonstrated to cross-react with in vitro citrullinated peptides from Epstein-Barr virus EBNA-1 and human papilloma virus (HPV)-47 $E2_{345–362}$ proteins.[218,219] Defining the kinetics of appearance of these antibodies during disease development, and demonstrating directly that these modified proteins are generated during natural infection, will be important to determine if this cross-reactivity is relevant to RA pathogenesis.

Tantalizing similarities and epidemiologic associations exist between periodontitis and RA, including the presence of bone erosions, association with similar class II MHC alleles and smoking, and enrichment of severe periodontal disease in patients with RA.[220-224] These connections have focused attention on the study of oral pathogens that express enzymes with PAD activity, specifically *Porphyromonas gingivalis*,[225] a Gram-negative bacterium commonly present as a biofilm in the gingival crevice and intra-cellularly in oral epithelial cells. *P. gingivalis* expresses its own citrullinating enzyme named PPAD, which produces ammonia that likely has negative effects on neutrophil function.[226] PPAD is not an ortholog of mammalian PADs, having no sequence homology with mammalian PADs, and showing significant differences in citrullination activity: (1) PPAD can citrullinate free L-arginine in addition to protein-bound arginine, (2) the enzyme does not require calcium or any other metal ion for activity, and (3) it has preference for citrullination of carboxy-terminal arginines. *P. gingivalis* also produces arginine gingipains, endopeptidases that cleave substrates after arginine residues, a process that generates C-terminal arginine residues in substrates, which are then citrullinated by PPAD.[226] Two mechanisms have been proposed by which PPAD may be relevant to RA pathogenesis: (1) by generating citrullinated bacterial and host proteins and (2) through autocitrullination. The initial finding that antibodies to CEP-1 (the immunodominant peptide from citrullinated human α-enolase) cross-react with *P. gingivalis* enolase after citrullination with rabbit PAD opened the possibility that ACPA development might be initially driven by citrullinated bacterial products, which subsequently cross-react with citrullinated endogenous proteins

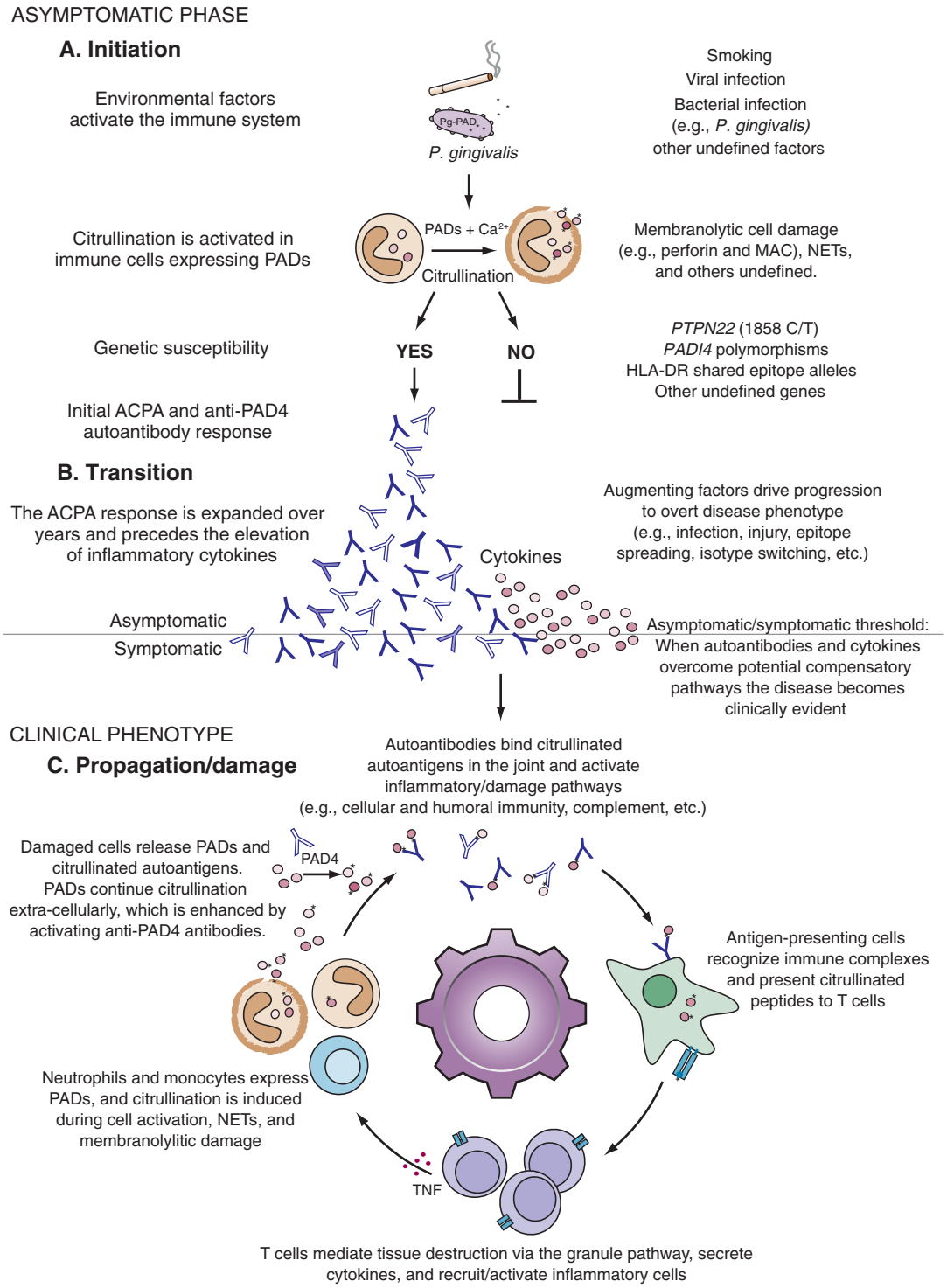



**Figure 56-4** A feed-forward model of rheumatoid arthritis (RA) pathogenesis: a pivotal role for protein citrullination. **A,** During disease initiation, immune pathways activated against environmental factors (e.g., perforin-induced cytotoxicity, neutrophil extra-cellular traps (NETs), and complement activation) generate the production of citrullinated proteins in peptidylarginine deiminase (PAD)-expressing cells. Although this process is likely normal and self-limited in the majority of the cases, some genetically susceptible persons may have the risk of developing autoantibodies against components involved in the process of citrullination (e.g., PAD4 and citrullinated proteins). This phase is likely asymptomatic because the autoantibodies are in low titer, they may not be pathogenic, and they target a limited number of citrullinated antigens.[78,217] The presence of autoantibodies may persist for several months to decades, during which a transition phase may occur. In some cases, autoantibody positivity may never progress to RA (i.e., anti-citrullinated protein antibody [ACPA]$^{+}$, RA$^{-}$ persons).[99,100] **B,** Depending on the exposure to augmenting factors that promote citrullination (e.g., infections, smoking, and injury), a transition phase occurs in which the ACPA response is amplified. This phase is characterized by an accumulation of multiple ACPA specificities, reflecting the process of epitope spreading.[78] The expansion of the autoantibody response closely correlates with the appearance of pre-clinical inflammation that includes the elevation of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-6, IL-12p70, and interferon γ.[78] Moreover, ACPAs acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of RA.[217] Together, the changes in the autoantibody response and the elevation in cytokines predict the imminent onset of clinical RA.[78,217] It is likely that compensatory pathways might be able to sustain a "healthy state" during the process of transition. However, when autoantibodies and cytokines overcome the threshold of compensation, the clinical phase is likely initiated. **C,** Once initiated, the immune response drives a feed-forward loop of target tissue destruction and disease propagation, resulting in the clinical phenotype. Although the citrullinating events that initiate the ACPA response may occur outside the joint, during disease propagation, citrullinated autoantigens are generated in the synovium by inflammatory cell PADs, leading to amplification of the immune response within the target tissue. Citrullinated antigens are indicated (*). HLA-DR, Human leukocyte antigen–DR region; MAC, membrane attack complex.

in the microenvironment of the inflamed joint.[227] Subsequently, it was found that arginine gingipain and PPAD, respectively, cleave and citrullinate human fibrinogen and α-enolase in vitro, extending this hypothesis that host proteins citrullinated by the bacterial enzyme may also play a role in initiating the ACPA response in RA.[228] Although this model is intriguing, the citrullination of bacterial enolase by PPAD or by a human host PAD during bacterial lysis of phagocytic cells has not been demonstrated. In addition, it is unclear whether the C-terminal citrullinated human fibrinogen and α-enolase fragments generated by gingipains and PPAD are indeed recognized by ACPAs in RA. An alternative mechanism that has been proposed for the involvement of PPAD in RA pathogenesis is that autocitrullinated PPAD may act as the inciting citrullinated antigen that may trigger the ACPA response in RA.[229] Although this theory is provocative, it remains controversial because PPAD autocitrullination appears to be an artifact generated when the protein is artificially expressed in *Escherichia coli*.[230,231]

Another environmental factor with potential relevance in initiating production of ACPAs is cigarette smoking. Interestingly, recent evidence has demonstrated (at least in some populations) a strong interaction between smoking and SE alleles in the development of ACPA$^+$ RA.[119,232-234] However, whereas primarily HLA-DRB1*04 alleles have been associated with ACPA production,[118,210,211] in smokers, HLA-DRB1*0101, *0102, and *1001 alleles showed the closest association with positive anti-CCP.[119] The observation that cigarette smoking induces increased expression of PAD2 and protein citrullination in lungs,[51] that anti-CCP antibodies of the IgA class are found in about one-third of patients with recent-onset RA,[235] and that anti-CCP antibodies can be found in patients with ILD who do not have RA[99,100] has suggested that the lung or other mucosal surfaces may play a role in generating or sustaining autoantibodies to citrullinated proteins. These gene-environment interactions, playing out in microenvironments outside the joint, add important dimensions to the framework within which RA pathogenesis must be investigated and understood. Although it is possible that none of the detailed mechanisms currently defined represent the unifying mechanism of pathogenesis in RA, the broader view of potential immunizing environments and the search for a frequent worldwide environmental exposure is of great importance.


The full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 57



# Acute Phase Reactants and the Concept of Inflammation

César E. Fors Nieves • Bruce N. Cronstein • Amit Saxena

**KEY POINTS**

- Inflammation comprises a complex and highly variable set of processes that represent a response to tissue damage from infection or injury.
- The acute phase response, a major accompaniment of inflammation, is induced by inflammation-associated cytokines and includes a reorchestration of acute phase protein synthesis by the liver.
- The erythrocyte sedimentation rate (ESR), the most commonly used clinical measure of inflammation, depends on numerous physical and chemical characteristics of blood, many of which are not related to inflammation.
- The quintessential acute phase protein, C-reactive protein (CRP), not only offers biomarker utility in the clinic, but also plays a role in host defense by recognition of biologic substrates, activation of the complement pathway, and by binding to leukocytes.
- Cytokines, chemokines, adhesion molecules, and other products of activated inflammatory cells are secreted during inflammation and play roles in the inflammatory response, but several problems limit the clinical usefulness of their quantitation for routine clinical purposes.
- CRP and ESR may reflect disease activity and correlate with disease prognosis in rheumatoid arthritis but generally are not helpful for differential diagnosis.
- Although CRP and ESR correlate with clinical activity in many inflammatory rheumatic diseases, the absent or modest CRP response seen in some patients with active systemic lupus erythematosus remains unexplained.
- CRP or ESR is elevated in more than 80% of patients with polymyalgia rheumatica and in approximately 95% of patients with giant cell arteritis. Both are useful for disease follow-up, but their imperfect correlation with disease activity indicates that these measures cannot supplant sound clinical judgment.
- Minor elevations of CRP, within the normal population reference range, are associated with increased risk of myocardial infarction but are nonspecific (especially in patients with rheumatic disease). Minor CRP elevation is associated with well-recognized risk factors for cardiovascular disease, which may explain its predictive value.

The inflammatory response is the body's natural defense against unchecked tissue damage from infection or injury. It occurs during the acute phase of an inciting event and, if the stimulus is not eliminated, in a chronic, putatively healing stage. When excessive or uncontrolled, these responses have the potential to cause significant harm to the host through processes such as autoimmune diseases, allergic reactions, and septic shock.

The concept of inflammation itself has evolved over thousands of years. Celsus first described the cardinal signs of redness, swelling, heat, and pain in the first century AD. The fifth sign, loss of function, is often attributed to Galen's work in second century.[1] Since that time, countless advances have been made in the understanding of inflammation, from histologic findings of inflammatory cells within tissues, to the discovery of hematologic and soluble mediators such as cytokines and complement. Also, the molecular signaling pathways that drive both its protective effects and the inappropriate injurious responses have been recently elucidated.

With increasing insight into mechanisms of the inflammatory response has come an appreciation of its significant complexity. Molecular and microscopic processes are different during acute and chronic stages, and diverse responses are induced by various types of exogenous and endogenous stimuli (e.g., bacteria, viruses, parasites, crystals, allergens, and ischemia). More recently, the role of the milieu of the inflammatory response, especially at the level of the vascular endothelium, has taken on considerable importance.[2] Furthermore, inherent redundancies of functions have been noted, as have interactions between the mediators of inflammation that allow for a broad, effective response, but these redundancies make it difficult to understand the pathways of inflammation in a linear fashion. These intricacies have resulted in a vague definition and on continued reliance on the final downstream macroscopic cardinal signs to tie together all of the ongoing processes. It is these same intricacies that have made evaluation of inflammation through laboratory tests imprecise.

Basic hematologic abnormalities may give clues to the presence of inflammation, but different patterns are often associated with underlying causes and are not universally found. Leukocytosis can be seen in infections, acute crystal diseases, and some autoimmune disorders such as adult Still's disease. Anemia is often associated with certain diseases that cause chronic inflammation, such as rheumatoid arthritis (RA). Reactive thrombocytosis occurs secondary to the release of cytokines after an inciting infectious or inflammatory event, and the role of platelets and platelet-derived mediators in stimulating inflammation has been described at the molecular level.[3]

Laboratory tests most commonly used by physicians to obtain information about the extent of inflammation are those that measure the acute phase reaction. In response to injury, local inflammatory cells secrete cytokines that

influence the liver to increase or decrease production of various proteins. The erythrocyte sedimentation rate (ESR) has been the classic marker of inflammation, with serum C-reactive protein (CRP) taking on an increasingly prominent role. Other novel inflammatory markers such as procalcitonin have been recognized, but their clinical utility has yet to be fully determined.

CRP elevations, especially minor ones, have been noted in numerous conditions that traditionally have not been considered inflammatory, most significantly involving the cardiovascular system. This has shed light on subclinical inflammation as a possible factor in the pathogenesis of a great number of diseases.

## ACUTE PHASE RESPONSE

Within minutes of tissue injury, activation of the innate immune system induces cytokine production that results in a multisystem acute phase response involving the liver, vascular system, bone marrow, and central nervous system.[4,5] Many elements of the reaction can be regarded as part of the innate response and are defensive or adaptive in nature.[6] Mouse studies have shown that as much as 7% of the regulatory gene pool undergoes significant changes in expression during inflammation, and that induction of liver acute phase genes is mediated by the transcription factor signal transducer and activator of transcription 3 (STAT3).[7-9]

Although the acute phase response can trigger numerous neuroendocrine, hematopoietic, and metabolic effects, the changes in plasma proteins synthesized by hepatocytes are monitored as signs of underlying inflammation (Tables 57-1 and 57-2). An acute phase protein is one in which the plasma concentration changes from baseline by at least 25% during inflammation; responses vary in terms of concentration and kinetics (Figure 57-1).[10] CRP and serum amyloid A (SAA) levels increase more than 1000-fold during acute infection and peak at 2 to 3 days. Concentrations of other proteins peak at longer periods and can range from a 50% increase in complement and ceruloplasmin, to a several-fold amplification in haptoglobin, fibrinogen, α1-proteinase inhibitor, and α1-acid glycoprotein. Other proteins are negative acute phase proteins, the concentrations of which fall during the inflammatory response. These include antithrombin III, protein S, prealbumin, albumin, transferrin, and apolipoprotein A-I.[4,5]

Hepatic stimulation of acute phase proteins is induced by cytokines released by activated monocytes, macrophages, neutrophils, natural killer (NK) cells, and endothelial cells acting at the front lines of the inflammatory response. The main cytokine influencing the liver is IL-6, once called the "hepatocyte-stimulating factor." It likely mediates protein expression via the Janus kinase (JAK) and STAT3 pathways, as well as C/EBP family members and Rel proteins (nuclear factor-κB [NF-κB]).[9,11] During initial stages, IL-1 and TNF synergize with IL-6 and trigger further IL-6 production, but their roles are limited.[12] The soluble IL-6 receptor amplifies IL-6 effects both locally and systemically. IL-6 also performs a protective role during disease, inducing the expression of an IL-1 receptor antagonist.[13]

Acute phase protein levels are not uniform in their expression; this is likely related to the underlying pathophysiologic state and is regulated by different combinations and interactions of cytokines.[5] The roles of the acute phase proteins themselves will be discussed throughout the chapter but have been found to include direct involvement in host defense by activation of the complement, proteinase inhibition, and antioxidant activity.[14] However, some of the described in vitro effects of proteins may not be relevant in vivo.

**TABLE 57-1** Human Acute Phase Proteins

| |
|---|
| **Plasma Proteins that Increase in Inflammation** |
| Complement System |
| C3 |
| C4 |
| C9 |
| Factor B |
| C1 inhibitor |
| C4b-binding protein |
| Mannose-binding lectin |
| Coagulation and fibrinolytic system |
| Fibrinogen |
| Plasminogen |
| Tissue plasminogen activator |
| Urokinase |
| Protein S |
| Vitronectin |
| Plasminogen-activator inhibitor 1 |
| Anti-proteases |
| α-Protease inhibitor |
| α-Anti-chymotrypsin |
| Pancreatic secretory trypsin inhibitor |
| Inter-α-trypsin inhibitors |
| Transport proteins |
| Ceruloplasmin |
| Haptoglobin |
| Hemopexin |
| Participants in inflammatory responses |
| Secreted phospholipase A2 |
| Lipopolysaccharide-binding protein |
| Interleukin-1–receptor antagonist |
| Granulocyte colony-stimulating factor |
| Others |
| C-reactive protein |
| Serum amyloid A |
| α-Acid glycoprotein |
| Fibronectin |
| Ferritin |
| Angiotensinogen |
| **Plasma Proteins that Decrease in Inflammation** |
| Albumin |
| Transferrin |
| Transthyretin |
| α-HS glycoprotein |
| Alpha-fetoprotein |
| Thyroxine-binding globulin |
| Insulin-like growth factor I |
| Factor XII |

HS, Heremans-Schmid.

From Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999.

### Erythrocyte Sedimentation Rate

Although ESR is an indirect screen for elevated concentrations of acute phase proteins, it has been the most widely used marker of inflammation for almost a century. Measurement of ESR is performed when blood is placed in a vertical

**TABLE 57-2** Other Acute Phase Phenomena

| |
|---|
| **Neuroendocrine Changes** |
| Fever, somnolence, and anorexia |
| Increased secretion of corticotropin-releasing hormone, corticotropin, and cortisol |
| Increased secretion of arginine vasopressin |
| Decreased production of insulin-like growth factor I |
| Increased adrenal secretion of catecholamines |
| **Hematopoeitic Changes** |
| Anemia of chronic disease |
| Leukocytosis |
| Thrombocytosis |
| **Metabolic Changes** |
| Loss of muscle and negative nitrogen balance |
| Decreased gluconeogenesis |
| Osteoporosis |
| Increased hepatic lipogenesis |
| Increased lipolysis in adipose tissue |
| Decreased lipoprotein lipase activity in muscle and adipose tissue |
| Cachexia |
| **Hepatic Changes** |
| Increased metallothionein, inducible nitric oxide synthase, heme oxygenase, manganese superoxide dismutase, and tissue inhibitor of metalloproteinase-1 |
| Decreased phosphoenolpyruvate carboxykinase activity |
| **Changes in Nonprotein Plasma Constituents** |
| Hypozincemia, hypoferremia, and hypercupremia |
| Increased plasma retinol and glutathione concentrations |

From Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999.

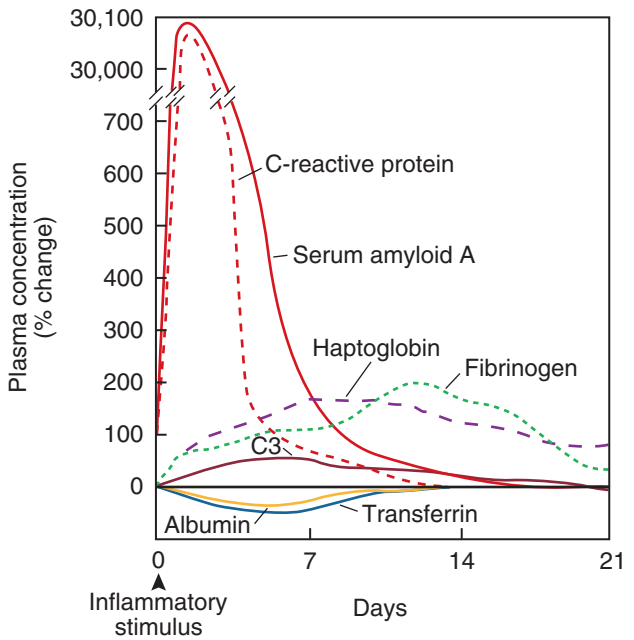



**Figure 57-1** Typical plasma acute phase protein changes after a moderate inflammatory stimulus. Several patterns of response are seen: major acute phase protein, increase 100-fold (e.g., C-reactive protein, serum amyloid A); moderate acute phase protein, increase twofold to fourfold (e.g., fibrinogen and haptoglobin); minor acute phase protein, increase 50% to 100% (e.g., complement C3); and negative acute phase protein, decrease (e.g., albumin, transferrin). *(Modified from Gitlin JD, Colten HR: Molecular biology of the acute-phase plasma proteins. In Pick E, Landy M, editors:* Lymphokines*, vol 14, San Diego, 1987, Academic Press, pp 123–153.)*

tube and the rate of fall of erythrocytes is measured. The ancient Greeks recognized increased red blood cell (RBC) sedimentation as a way to detect "bad bodily humors," but our modern understanding and use of RBC sedimentation as a test date back to the German scholar Fahraeus in 1918.[15] He determined that certain plasma proteins, especially fibrinogen, are able to lower the electrostatic charge on RBC surfaces so they can aggregate, form rouleaux, and fall faster.

Several factors are involved in acceleration of ESR. Asymmetric plasma proteins, such as fibrinogen and, to a lesser extent, alpha$_2$, beta, and gamma globulins decrease the negative charge of erythrocytes (zeta potential) that prevents rouleaux formation. Red cell factors also play a role in that changes in plasma ratios in anemic states also favor rouleaux. However, microcytosis, polycythemia, and abnormally shaped RBCs (e.g., sickle cells and spherocytes) hinder aggregation and lower the ESR.[16] Conditions that elevate fibrinogen, even if they are not necessarily considered inflammatory, can raise ESR. These include pregnancy, diabetes, end-stage renal disease, and heart disease. Major increases in the concentration of a single molecular species, such as a monoclonal immunoglobulin in multiple myeloma, also cause increased sedimentation.[17] The ESR is elevated in obesity, as is CRP, presumably as a result of IL-6 secretion by adipocytes.[18] Factors such as glucocorticoids, cryoglobulins, hypofibrinogenemia, and hyperviscosity lower the value.[4] The physiochemical dynamics that allow for sedimentation has been a continued source of debate, with disparate models presented to explain how proteins on cell surfaces interact to cause RBC aggregation.[19]

Although novel and rapid tests for ESR have proven promising, the International Committee for Standardization in Hematology continues to recommend the Westergren technique of testing anti-coagulated blood.[20,21] The usual accepted upper limits of normal are 15 mm/hr for males and 20 mm/hr for females; however, the ESR increases with age and varies by race, which calls the reliability of the test into question. A simple formula for calculating the upper limit of normal ESR at any age has been used regularly. In men, age in years divided by 2; in women, 10 plus age in years divided by 2. Despite the ability to control for age, other limitations of the test have been noted and are listed in Table 57-3. The relative virtues of CRP determination have diminished some of the importance of ESR, but it remains an easy, inexpensive test with a wealth of background literature. Therefore the sedimentation rate continues to play a prominent role in clinical practice.

## C-Reactive Protein

C-reactive protein is an acute phase protein, the serum concentration of which reflects ongoing inflammation better than other tests in most, but not all, diseases.[22] CRP was identified in 1930, when sera obtained from patients with *Streptococcus* pneumonia infection were found to contain a protein that could bind to the "C" polysaccharide of the bacterial cell wall. This protein circulates as a 115 kDa pentamer of noncovalently linked 23 kDa subunits, which has been highly conserved during hundreds of millions of years of evolution. In contrast to immunoglobulins and complement components, CRP deficiency in humans has

**TABLE 57-3** Comparison of Erythrocyte Sedimentation Rate and C-Reactive Protein

| | Erythrocyte Sedimentation Rate | C-Reactive Protein |
|---|---|---|
| Advantages | Much clinical information in the literature<br>May reflect overall health status | Rapid response to inflammatory stimuli<br>Wide range of clinically relevant values are detectable<br>Unaffected by age and gender<br>Reflects value of a single acute phase protein<br>Can be measured on stored sera<br>Quantitation is precise and reproducible |
| Disadvantages | Affected by red blood cell morphology<br>Affected by anemia and polycythemia<br>Reflects levels of many plasma proteins, not all of which are acute phase proteins<br>Responds slowly to inflammatory stimuli<br>Requires fresh sample<br>May be affected by drugs | Not sensitive to changes in SLE disease activity |

not been described. Genome-wide associated studies performed recently have shown that at least seven distinct loci are involved in the basal expression of CRP,[23-25] which is upregulated upon stimulation by the transcription factors C/EBP and Rel.[26] It is present in trace concentrations in the plasma of all humans (roughly 1 mg/L, with higher concentrations in women and the elderly). Plasma C-reactive protein is synthesized by hepatocytes, although other sites of local production and possibly minimal secretion have been suggested.

The precise function of CRP is unknown and may be varied, but it exhibits important recognition and activation capabilities, and it binds to numerous ligands.[27] CRP recognizes phosphocholine, phospholipids, fibronectin, chromatin, and histones, all of which are exposed at sites of tissue damage and by apoptotic cells; CRP may target them for clearance.[28] C-reactive protein bridges the gap between innate and adaptive immunity by activating the classic complement pathway and interacting with cells of the immune system through binding of Fcγ receptors.[29,30] CRP induces inflammatory cytokines, tissue factors, and shedding of the IL-6 receptor, all of which result in a complement-dependent increase in tissue damage.[28] Other CRP functions are anti-inflammatory, including promotion of the non-inflammatory clearance of apoptotic cells and prevention of neutrophil adhesion to the endothelium.[31,32] Thus CRP may play many pathophysiologic roles during the course of the inflammatory process.[14,33]

After an acute inflammatory stimulus, CRP concentration increases rapidly and peaks at 2 to 3 days at levels that reflect the extent of tissue injury. If the stimulus has been removed, serum CRP levels drop rapidly, with a half-life of roughly 19 hours.[34] Persistent elevations in CRP are seen in chronic inflammatory states such as active RA, pulmonary tuberculosis, or extensive malignant disease.

Immunoassays and laser nephelometry are used at modest cost to quantify serum CRP levels. Most healthy adults have levels less than 0.3 mg/dL. The significance of minor elevations in CRP is a subject of debate and will be discussed subsequently. However, usual methods of CRP determination are less precise at concentrations in the range of 0.3 to 1 mg/dL, so high-sensitivity (hs) CRP methods are used to accurately measure these levels. Generally, concentrations greater than 1 mg/dL reflect clinically significant inflammatory disease.[35,36] Concentrations of 1 to 10 mg/dL can be considered to represent moderate increases, and concentrations greater than 10 mg/dL show marked increases. Most patients with extremely high levels (e.g., >15 mg/dL) have bacterial infection. One study found that in patients with CRP concentrations greater than 50 mg/dL, infection was present in 88% of participants.[37] Clinical conditions associated with varying degrees of elevation of CRP are listed in Table 57-4, and the range of CRP concentrations in many rheumatologic diseases is shown in Figure 57-2.

**TABLE 57-4** Conditions Associated with Elevated C-Reactive Protein Levels

| |
|---|
| **Normal or Minor Elevation (<1 mg/dL)** |
| Vigorous exercise |
| Common cold |
| Pregnancy |
| Gingivitis |
| Seizures |
| Depression |
| Insulin resistance and diabetes |
| Several genetic polymorphisms |
| Obesity |
| **Moderate Elevation (1 to 10 mg/dL)** |
| Myocardial infarction |
| Malignancies |
| Pancreatitis |
| Mucosal infection (bronchitis, cystitis) |
| Most systemic autoimmune diseases |
| Rheumatoid arthritis |
| **Marked Elevation (>10 mg/dL)** |
| Acute bacterial infection (80% to 85%) |
| Major trauma |
| Systemic vasculitis |

Several limitations associated with the use of C-reactive protein measurement must be acknowledged. No uniformity in reporting concentrations has been noted between laboratories, and values can be conveyed in mg/L, μg/mL, or mg/dL. Similar to ESR, population studies show a skewed, rather than gaussian, distribution, which renders parametric statistical tests inappropriate for interpretation of CRP data. Population differences in CRP levels in the United States have been reported between sexes and among racial groups. This is presumably also prevalent as an issue in practice in other populations globally. Elevation of C-reactive protein in the elderly may represent age-related disorders, the pathogenesis of which may involve low-grade inflammation, which complicates the issue of what levels are considered normal.[38]

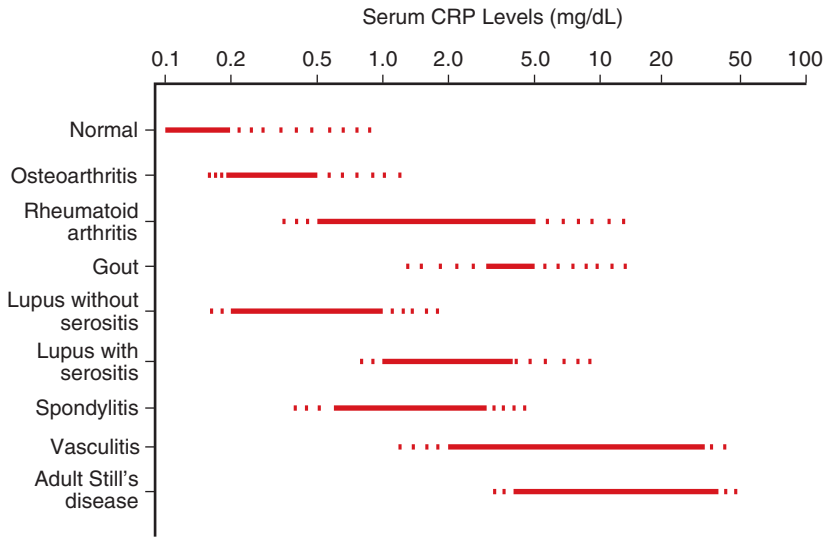



**Figure 57-2** Range of C-reactive protein (CRP) levels in rheumatic disease. Authors' estimates of expected levels of CRP (mg/dL) in certain rheumatic diseases.

## Procalcitonin

Procalcitonin (PCT), a propeptide of calcitonin, has recently received increased attention as a useful measure to differentiate between acute bacterial infection and other inflammatory and febrile syndromes. Studies have shown that elevated serum levels of PCT have a significantly higher specificity for infection than ESR and CRP.[39-42] PCT has been studied extensively as a clinical tool to facilitate the decision as to when to start and stop anti-bacterial agents in patients with pneumonia,[43,44] and it has also shown promise in differentiating between infectious and noninfectious causes of fever after orthopedic surgery, in which ESR and CRP levels can be difficult to interpret.[45,46] For all of these reasons, it remains an intriguing clinical tool for use in patients with fevers in the setting of autoimmune disease who are commonly on immunosuppressive medications and at higher risk of infection.

Normally produced in the C-cells of the thyroid gland, PCT is usually cleaved into calcitonin, katacalcin, and an N-terminal residue. Under normal circumstances, PCT is not released into the bloodstream; however, during severe infection plasma, levels can increase dramatically.[47,48] Interestingly, this does not lead to an increase in plasma calcitonin levels or activity. At this time, the exact site of PCT production, as well as its pathophysiologic role during sepsis, remains uncertain.[49]

In healthy humans, PCT levels are undetectable or very low (<0.1 ng/mL). During systemic bacterial infection, PCT levels can increase to higher than 100 ng/mL. Currently available diagnostic tests measure the calcitonin/N-ProCT part of the protein and therefore only a fragment of the 114 to 116 amino-acid chain of the prohormone. A useful reference range to exclude sepsis and systemic inflammation is less than or equal to 0.2 ng/mL. Plasma levels of greater than or equal to 0.5 ng/mL should be interpreted as abnormal and suggest sepsis. After reaching peak levels, circulating PCT has a half-life of approximately 22 to 35 hours in serum, and its concentration declines with a 50% plasma-disappearance rate of roughly 1 to 1½ days. It is worth noting that in patients with severe renal dysfunction these rates may be prolonged, but accumulation does not occur.[50]

As a biomarker for infection in autoimmune diseases, the role of PCT has not yet been fully established. In contrast to CRP, PCT levels are not elevated in in the majority of cases of noninfectious inflammation or nonbacterial infection. However, exceptions to this rule include some vasculitis syndromes such as Kawasaki's disease, Goodpasture's syndrome, adult-onset Still's disease[51,52] and granulomatosis with polyangiitis,[53] for which observational studies have demonstrated elevated levels of PCT in patients without evidence of bacterial infection. In systemic lupus erythematosus, data are mixed, and no definitive statement regarding the usefulness of PCT can be made at this time.[54-56]

## Other Acute Phase Proteins

Measurement of other acute phase proteins has been of limited value clinically because their responses to tissue injury are often slower, and the magnitude of concentration change is smaller than with CRP. Serum amyloid A (SAA), a circulating family of proteins produced by hepatocytes, adipocytes, macrophages, and fibroblast-like synoviocytes has been correlated with disease activity in a number of inflammatory disorders.[57] However, reliable testing for acute phase SAA is not widely available, and data about levels expected in disease are limited. Serum ferritin is moderately increased, triggered by cytokines such as IL-1, IL-6, IL-18, and TNF. Levels are frequently high in adult-onset Still's disease and systemic lupus erythematosus (SLE), and they correlate with disease activity.[58,59] Hepcidin, a liver-derived anti-microbial peptide and regulator of iron homeostasis, is induced by inflammation, and by IL-6 in particular.[60] Its levels rise in parallel with ferritin, and it is important in the development of anemia of chronic disease, acting as a negative regulator of iron absorption and macrophage iron release teleologically, to deprive microbes of iron.[61] Transferrin, which binds and transports iron, is a negative acute phase protein.

Apolipoprotein A-I, the principal protein constituent of high-density lipoprotein (HDL), is another negative acute phase protein. In chronic inflammatory diseases such as RA and SLE, decreased levels may contribute to increased risk of thrombotic events.[62,63] Serum albumin and prealbumin (transthyretin) are also negative acute phase proteins, although their measurement is not more helpful in diagnosis or prognosis than standard tests.[64] Serum complement fractions become depressed when the system is activated in certain autoimmune disorders but otherwise rise during the acute phase response.

## Cytokines

Although not acute phase proteins in the classic sense, cytokines display the most striking acute phase behavior of any circulating proteins. IL-6 responds dramatically to tissue injury, with concentration changes that are faster and greater than those of CRP or SAA. Acute inflammation and chronic inflammation have been associated with increases in IL-6, and serum levels of this cytokine have been correlated with the severity and course of disease in RA, juvenile arthritis, ankylosing spondylitis, and polymyalgia rheumatica (PMR).[65,66] It is also more sensitive than ESR for detecting disease activity in giant cell arteritis (GCA).[67] IL-6 levels may be useful in monitoring inflammation if hepatocytes are damaged to the point that they are not able to synthesize acute phase proteins.[4] Knowledge of soluble IL-6 receptor levels may also be helpful in this regard. The importance of cytokines such as TNF and IL-1 has been inferred by the successful reduction of inflammation by their therapeutic inhibitors. Certain diseases, such as the TNF receptor–associated periodic syndrome (TRAPS) and the autoinflammatory syndromes that involve mutations of the inflammasome controlling IL-1, point to the importance of these cytokines.

Increased levels of several other cytokines and circulating cytokine receptors have been associated with inflammation or disease activity as well (Table 57-5).[67,68] Different patterns of cytokine responses have been reported in different diseases, suggesting that cytokine determinations are potentially useful clinically.[69,70] However, their quantitation presents several problems related to their short plasma half-lives, the presence of blocking factors and natural inhibitors, and other technical considerations.[71] At present, high costs, limited availability, and the absence of standardization discourage the measurement of plasma cytokines and their receptors in clinical practice.

**TABLE 57-5** Products of Inflammatory, Endothelial, and Resident Target Tissue Cells/Matrix

| |
|---|
| **Cytokines and Related Molecules** |
| Cytokines: |
| IL-1 |
| IL-6 |
| IL-12 |
| IFN-α |
| TNF |
| Granulocyte-macrophage colony-stimulating factor |
| IL-1 receptor antagonist |
| **Products of Inflammatory and Endothelial Cells** |
| Calprotectin |
| von Willebrand factor |
| Soluble adhesion molecules (e.g., sVCAM and sE-selectin) |
| Hyaluronic acid |
| Collagen and aggrecan degradation products |
| Osteocalcin |

sVCAM, Soluble vascular cell adhesion molecule.

# ACUTE PHASE REACTANTS IN THE MANAGEMENT OF RHEUMATIC DISEASES

Measurement of ESR and CRP has no role in the diagnosis of any particular disease, including RA, osteoarthritis, SLE, PMR, GCA, or other inflammatory arthropathies. However, these measurements can be clinically helpful in three ways: (1) in evaluating the extent or severity of inflammation, (2) in monitoring changes in disease activity over time, and (3) in assessing prognosis.

## Rheumatoid Arthritis

ESR and CRP cannot be used in the diagnosis of RA, because 45% of patients may have normal serum levels at presentation,[72] although these values represent part of the diagnostic syndrome or classification criteria sets. These tests are more appropriately applied in RA for monitoring disease activity and response to therapy. Although ESR traditionally has been more widely used for these purposes, many studies have suggested that CRP levels correlate better with disease activity.[73] Some recent reports state that CRP levels may overestimate disease response compared with ESR; others claim that differences between the two are minimal.[73-75] The existence of patients with depressed CRP concentrations caused by carrying low-CRP–associated genetic variants must be taken into account when this test is used universally.[76] Matrix metalloproteinase (MMP)-3, pro-MMP-3, and soluble E-selectin have also been proposed as markers for RA disease activity. Their measurements correlate with CRP levels but do not provide more information than is provided by standard tests.[77-79]

CRP levels average 2 to 3 mg/dL in adult patients with RA who have moderate disease activity.[80] However, variation is considerable. At least 5% to 10% of patients have values in the normal range, whereas a few patients with severe disease activity have levels greater than 10 mg/dL. ESR values have been found to remain stable over the years.[81] ESR and CRP have long been used to follow the response to therapy; in general, effective disease-modifying anti-rheumatic drug therapy decreases CRP levels by approximately 40%. Inhibition of joint damage by these agents usually is accompanied by marked improvement in acute phase reactants. Progression of joint damage can occur while the patient is on therapy, however, despite decreases in ESR and CRP.[82] Even more striking improvement has been seen with biologic agents introduced since the 1990s, providing objective laboratory support for the encouraging clinical responses observed. In early reports of anti-TNF therapy, CRP and SAA levels declined by 75% and 85%, respectively, in approximately 1 week.[83] Treatment with abatacept, the T cell CD80/CD86:CD28 co-stimulation modulator, resulted in significant decreases in CRP at both 90 and 360 days of therapy.[84] Tofacitinib, a JAK inhibitor, also appeared to effectively decrease DAS28-ESR scores in RA patients in clinical trials; therefore it may

be a useful tool to monitor disease activity.[85] In one study, failure to suppress CRP levels 2 weeks after initiation of infliximab therapy identified most patients who would prove to be clinical nonresponders after 12 weeks.[86] In contrast to traditional disease-modifying anti-rheumatic drugs, TNF inhibitors have been found to inhibit joint damage even while clinical activity, reflected by CRP levels, remains high.[87] Tocilizumab, a human IL-6 receptor antibody, improves RA by inhibiting effects of the cytokine. However, because of its mechanism of action, inflammatory markers such as ESR and CRP drop to negative values, so that tracking them may not reflect the actual effect of the drug; care must be taken when monitoring disease activity during tocilizumab therapy.[88,89]

ESR and CRP also have value as prognostic indicators in RA. Elevated acute phase reactant levels are associated with early synovitis and erosions as detected by magnetic resonance imaging, with inflammatory cellular infiltrates in synovium, and with osteoclastic activation and reduced bone mineral density.[90-92] CRP predicts radiographic progression, as do ESR and the matrix metalloproteinases MMP-3 and MMP-1.[77,78,93-95] Finally, and perhaps most important, acute phase reactants correlate with work disability on long-term follow-up and predict progression to major joint replacement.[96,97] As in the normal population, CRP levels are associated with death from cardiovascular disease.[98] In patients with RA in whom heart failure developed, ESR was higher during the 6-month period immediately preceding the onset of heart failure than earlier in tits course.[99]

Serum or synovial fluid levels of many other tissue products (see Table 57-5) have been correlated with clinical measures of disease activity, severity, and radiographic damage.

Newer quantitative and objective assays that incorporate some of the measures previously mentioned as well as other novel ones, have recently come into the market to aid in the management and earlier diagnosis of patients with RA. Multibiomarker disease activity (MBDA) tests are increasingly popular and could play a significant role the future management and study of RA; however, more studies are required to determine their clinical usefulness across different mechanistic interventions.

## Systemic Lupus Erythematosus

Although serum levels of CRP often parallel disease activity in autoimmune disorders, it has been recognized that SLE is an exception.[100] Although marked CRP responses are seen in subsets of patients, such as those with serositis or chronic synovitis, many (e.g., patients with nephritis) show mild or no elevation during periods of activity.[101-103] Serum levels of SAA are also relatively low in comparison with those of patients with RA, which may explain why rates of secondary amyloidosis are decreased in these patients[104] In contrast, ESR correlates with disease activity and accrued tissue damage in SLE.[105] Fibrinogen levels increased over time in patients, regardless of disease activity.[106] Data are insufficient to evaluate the potential use of some of the other newer markers described previously, including PCT, but many SLE patients with normal CRP levels show elevated IL-6 concentrations.[107] Therefore a deficiency in IL-6 does not explain the muted CRP response in SLE.

Although the concomitant decrease in SAA may point against it, several investigations have raised the possibility that low CRP levels may be related to the pathogenesis of SLE: (1) An association has been noted between SLE and a genetic polymorphism associated with low CRP levels; (2) it has been observed that low CRP levels may contribute to defective clearance of autoantigens during apoptosis; and (3) the therapeutic efficacy of CRP has been reported in mouse models of SLE.[101,108-111] Recent studies have also raised the possibility that type I interferon (IFN), which is expressed significantly in SLE, may inhibit CRP expression.[112,113]

Substantial CRP elevation in SLE patients is more likely to result from superimposed infection than from activation of lupus. CRP levels greater than 6 mg/dL in these patients should serve as an impetus to exclude the possibility of infection, just as they should in other diseases.[10] Such levels should not be regarded as proof of infection, however; as indicated earlier, marked CRP elevation related to active SLE can be seen in the absence of infection.

Carotid plaque and intima-media wall thickness, correlates of atherosclerotic vascular disease, have been found in association with minor CRP elevation in women with SLE, as they have in patients with RA.[114,115]

## Polymyalgia Rheumatica and Giant Cell Arteritis

The diagnosis of PMR or GCA is supported by an elevated ESR, often greater than 100 mm/hr. However, such elevation is no longer regarded as a sine qua non of these disorders; continuing reports suggest that 10% to 20% of patients with PMR can have "normal" ESRs, depending on which value is taken as the limit of normal. Such patients tend to have fewer systemic symptoms and less severe, less frequent anemia.[116] They have the same frequency of positive temporal artery biopsy results, however, as patients with elevated ESR.[117,118]

Only approximately 5% of patients with GCA had ESR values less than 40 mm/hr; these patients had fewer visual and systemic symptoms than patients with high ESR values.[119] In contrast to these findings, ESR and CRP were found to be significantly lower in patients with ocular involvement, most commonly in the range of 70 mm/hr to 100 mm/hr. Patients with ESR greater than 100 mm/hr had decreased incidence of visual ischemic events.[120-122]

In PMR and GCA, CRP and ESR have been regarded in the past as equally valuable in assessing disease activity. However, recent reports suggest that CRP is more sensitive for both conditions and should be included routinely in the diagnostic workup.[118,123,124] The report that IL-6 is more sensitive than ESR for indicating disease activity in GCA is of particular interest.[125] Subsets of patients with PMR who have persistently elevated levels of CRP and IL-6 despite corticosteroid treatment have a higher risk of relapse.[126] A polymorphism at the IL-6 gene promoter has characterized these PMR patients with persistently elevated levels of the cytokine.[127] Clinical manifestations of disease, even in the presence of a normal ESR or CRP level, should not be ignored. Extreme elevation of the ESR in the absence of symptoms of PMR or GCA should raise suspicion of other disorders, such as infection, malignancy, or renal

disease. PCT levels in patients with PMR or GCA are usually normal according to a French prospective study. Elevated PCT levels in these patients with a normal temporal artery biopsy would be suggestive of an alternate diagnosis.[128]

Numerous markers of endothelial perturbation, although not acute phase reactants in the strict sense, are elevated in plasma in various inflammatory disorders of vessels, particularly PMR, GCA, and other vasculitides.[129] These molecules include von Willebrand factor, thrombomodulin, some vasoactive prostanoids, and a variety of adhesion molecules, such as vascular cell adhesion molecule-1.

### Adult-Onset Still's Disease

Markedly elevated concentrations of ferritin, disproportionately high compared with those of other acute phase reactants, have long been noted in adult-onset Still's disease but are not specific.[130] Only a small percentage, commonly less than 20%, of ferritin is glycosylated in adult-onset Still's disease, a criterion included in recently proposed classification criteria for this condition.[131,132] Extremely elevated ferritin levels have been found in macrophage activation syndrome, and 40% of individuals with this condition meet the criteria for adult-onset Still's disease, suggesting to some that the two disorders are not distinct entities.[133-135] Concentrations of serum IL-18 were extremely elevated in patients with active adult-onset Still's disease compared with those of patients with other connective tissue diseases or of healthy individuals and were correlated with serum ferritin values and disease severity.[136] The cytokine profile in the sera of patients suggests a type 1 T helper (Th) cell response, with significantly higher levels of TNF, IL-6, and IL-8, in addition to IL-18.[137] The role of IL-1 has been inferred from significant improvement in disease activity by the IL-1 receptor antagonist, anakinra.[138,139] It has been suggested that IFN-α may be responsible for the hyperferritinemia of adult-onset Still's disease.[130] CRP levels are usually markedly elevated in this disease, and PCT levels have also been found to be disproportionately elevated without evidence of acute bacterial infection in these patients.[51,52]

### Ankylosing Spondylitis

Ankylosing spondylitis ordinarily does not lead to a substantial increase in ESR or CRP. Median ESR and CRP levels are 13 mm/hr and 1.6 mg/dL, respectively, in patients with only spinal involvement, and 21 mm/hr and 2.5 mg/dL in patients with peripheral involvement or associated inflammatory bowel disease.[140] Treatment with infliximab led to an average decrease of 75% in CRP concentration after 12 weeks. Patients with lower CRP levels showed little improvement, however, raising the possibility that patients with higher CRP values show better responses to anti-TNF treatment than patients with lower CRP levels.[141-143] High-sensitivity CRP may better correlate with clinical disease activity compared with standard CRP testing.[144] It has been reported that levels of IL-8, IL-17, and IL-23 are elevated in the serum of patients with active ankylosing spondylitis, and polymorphisms in the IL-23 receptor gene are associated with the disease.[145-147]

### Osteoarthritis

Minor CRP elevations of 0.3 to 1.0 mg/dL have been reported in patients with osteoarthritis, particularly those with progressive joint damage.[148] However, no evidence supports this association independent of body mass index (BMI), because obesity is a common accompaniment of osteoarthritis.[149] Although local inflammation likely plays a role in the pathogenesis of osteoarthritis, systemic inflammation likely does not. However, CRP levels are higher in patients with erosive osteoarthritis of the hand than in those with nonerosive osteoarthritis.[150]

### Other Rheumatic Diseases

Acute phase markers are elevated in numerous rheumatic diseases while the inflammatory cascade commences. Elevated ESR and CRP can be found in systemic vasculitides, crystal arthropathies, psoriatic and reactive arthritides, and infectious joint diseases.[151-154] Monitoring for elevated SAA levels has been proposed as a tool for diagnosis and medication adjustment in familial Mediterranean fever,[155] although this test is not widely available, limiting its utility. CRP is often normal in patients with primary Sjögren's syndrome, and those with elevated responses do not differ clinically from patients with normal levels.[156] Oligoarticular-onset juvenile idiopathic arthritis is classically considered as not associated with elevated inflammatory markers, although they are present in patients most at risk for systemic disease.[157]

## PRACTICAL USE OF ACUTE PHASE REACTANTS

In the past, rheumatologists were found to use the ESR more than twice as frequently as they did CRP levels,[158] although ESR reflects many complex, poorly understood changes in the physical and chemical characteristics of blood not associated with inflammation. As indicated earlier, the reference normal values for ESR are unclear. It is well established that mean ESR values increase substantially with age and differ between men and women. Difficulties associated with interpretation of the ESR and an increasingly positive clinical experience with CRP suggest that rheumatologists may benefit from relying more on CRP than ESR testing.[159] No single ideal test can be used to evaluate the acute phase response, however. The relative virtues of these tests in following patients with RA have been discussed.[74,160]

Discrepancies between ESR and CRP may result from effects of blood constituents that are not related to inflammation, but that can influence the ESR. In addition, patterns of acute phase protein changes differ in different conditions.[5] ESR may be markedly elevated in many patients with active SLE, whereas the CRP is normal. Undoubtedly, numerous other clinical situations exist in which similar discrepancies occur. Although a falsely high ESR has many non-inflammatory physicochemical causes (some known, and many unknown), CRP values greater than 1 mg/dL almost invariably reflect a clinically significant inflammatory process. In light of these considerations, many authors believe that several tests, rather than a single test, should be performed and interpreted in their clinical context. It

has been suggested that ESR, which is associated with anemia and immunoglobulin levels, may reflect general severity in RA, whereas CRP is a better test of active inflammation per se.[161]

## C-REACTIVE PROTEIN AND HEALTH: ASSOCIATIONS WITH NONRHEUMATOLOGIC CONDITIONS

Although most ostensibly healthy individuals have CRP concentrations of 0.3 mg/dL or less, some have concentrations greater than 1 mg/dL. Such minor CRP elevation has long been attributed to trivial tissue injury or to minimal inflammatory processes, such as gingivitis. However, recent data indicate that CRP concentrations between 0.3 and 1 mg/dL have clinical relevance. This finding has led to an explosion of published literature measuring CRP levels in cardiac, neurologic, neoplastic, pulmonary, and even psychiatric disease.[162-166] The foundation of these investigations is the well-established notion that when a high-sensitivity CRP assay is used, serum CRP levels greater than 0.3 mg/dL indicate increased the relative risk of atherogenesis and future myocardial infarction.[167] The statistical strength of this is as robust as, but not more robust than, established risk factors such as hypertension, diabetes, and hypercholesterolemia.[168-170] The primary unanswered questions remain: Why does CRP predict myocardial infarction? Is it a pathogenic mediator itself?

The observation that CRP is associated with many noninflammatory conditions (e.g., low levels of physical activity, low intake of fruits and vegetables, a variety of other "unhealthy" diets, smoking, hypertension, obesity, sleep deprivation, and low alcohol intake) indicates that classic inflammation does not invariably underlie a CRP response.[171] Many of these CRP-associated conditions are known to be risk factors for cardiovascular disease, confounding the situation and suggesting that CRP predicts myocardial infarction because of its association with these risk factors. In addition, elevated CRP may not reflect inflammation, but rather a response to the presence of distressed, metabolically disturbed cells.[171] This reverse causation offers a potential explanation of the laboratory result, because atherosclerosis might trigger an elevation in CRP.

Nevertheless, CRP has been known for many years to bind to low-density lipoprotein (LDL) and to activate complement—a potentially pro-inflammatory response; it has also been detected in atherosclerotic plaques.[29,172-174] These observations have raised the possibility that CRP may play a direct causal role in coronary heart disease. Proatherogenic effects secondary to CRP injections given to mice, however, may have been caused by contaminants in commercial CRP preparations, not by CRP itself.[175] Because statin drugs lower CRP and LDL, some consider their effects to provide indirect evidence of a causal role of CRP. In the justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) trial, patients with normal LDL and elevated CRP were prescribed rosuvastatin, 20 mg daily, or placebo, and were followed prospectively. Those in the treatment arm had significant reductions in major cardiovascular events.[176] However, whether the effects of the study can be solely dependent on CRP reduction remains unclear; alternative explanations have been suggested, including the question of the importance of lowering LDL even among patients with healthy levels.[177] Whether to target CRP levels in cardiovascular disease is an ongoing topic of intense debate.

Because no CRP inhibitor drugs are available to directly measure the effect of lowering the protein, recent studies have centered on observing patients with genetic variations that result in different baseline levels of CRP. To date, results have been conflicting with regard to the role of genetically determined CRP levels in coronary heart disease.[178-182]

Epidemiologic studies that describe an association of CRP levels with morbidity and mortality in many chronic diseases, and even in normal aging, have become a cottage industry. It is important to remember that these are observational population studies. Although such associations may have broad and intriguing implications, particularly at a societal level, they reflect probabilities. This limits their clinical value when they are applied to individual patients.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 58



# Imaging in Rheumatic Diseases

Mikkel Østergaard • Robert G.W. Lambert • Ho Jen • Walter Grassi

## KEY POINTS

**Practical Use of Imaging in Inflammatory Joint Diseases**

**A. Peripheral joints**

**Use in clinical practice:**

- **To establish a diagnosis of rheumatoid arthritis (RA): radiography, magnetic resonance imaging (MRI), ultrasonography**
- **To assist with the diagnostic workup in suspected, but not definite, inflammatory joint disease and early, unclassified inflammatory joint disease (by detection of the presence or absence of synovitis, enthesitis, or bone erosions, among other conditions): radiography, MRI, ultrasonography**
- **To monitor disease activity: MRI, ultrasonography**
- **To monitor structural joint damage: radiography, MRI**
- **To assist with the prognostic stratification of patients with early RA: radiography, MRI, (ultrasonography*), (DXR*)**
- **To help define the presence or absence of true remission: MRI, ultrasonography**
- **To guide aspirations and injections in joints, bursae, and tendon sheaths: ultrasonography**

**Use in clinical research:**

- **To assess structural joint damage in RA trials: radiography, MRI**
- **To assess the anti-inflammatory effectiveness of a new compound: MRI, ultrasonography**
- **For pretrial selection of the patients most likely to progress: radiography, MRI**

**B. Axial joints**

**Use in clinical practice:**

- **To establish a diagnosis of ankylosing spondylitis (AS)/spondyloarthropathies (SpA): radiography/radiography and MRI**
- **To monitor disease activity: MRI**
- **To monitor structural joint damage: radiography, MRI, (CT†)**

**Use in clinical research:**

- **To assess structural progression in AS/SpA trials: radiography, MRI**
- **To assess the anti-inflammatory effectiveness of a new compound: MRI**
- **For pretrial selection of the patients most likely to progress: radiography, MRI**

*Promising, but more data needed.
†CT allows this but cannot be used because of radiation exposure.

For decades, imaging in rheumatology has been synonymous with conventional radiography (CR). However, new imaging modalities, including magnetic resonance imaging (MRI) and ultrasonography, have dramatically increased the amount and scope of information obtainable by imaging. In rheumatology, imaging may be used for multiple reasons that include establishing or confirming the diagnosis, determining the extent of disease, monitoring change in disease (e.g., activity and structural damage), selecting patients for specific therapies (e.g., surgery or injections), identifying complications of disease or treatment, and assessing therapeutic efficacy in trials. These different contexts may require different imaging approaches.

This chapter focuses on the inflammatory joint diseases, such as rheumatoid arthritis (RA), psoriatic arthritis (PsA), ankylosing spondylitis (AS), other types of axial spondyloarthritis (SpA), gout, and osteoarthritis (OA). Other aspects, including nuclear medicine and capillaroscopy in connective tissue diseases and vasculitides are also briefly discussed. The reader is referred to the chapters on the individual diseases for imaging aspects of other rheumatologic diseases, and to textbooks of musculoskeletal radiology[1] for more detailed descriptions of the different imaging modalities, including the technical aspects. This chapter outlines the virtues of CR and describes its current major importance in diagnosis and follow-up of rheumatologic diseases, but also, by putting emphasis on newer imaging modalities, particularly MRI and ultrasonography, stresses that rheumatology has entered a time of exciting and expanding therapeutic as well as imaging possibilities.

## CONVENTIONAL RADIOGRAPHY

### KEY POINTS

**Radiography is relatively inexpensive, easily available, and reliable.**

**Radiography provides information on bone damage and, indirectly through joint space narrowing, on cartilage damage, although radiography is neither sensitive nor specific for soft-tissue change.**

**Radiography findings are important parts of the classification criteria for many rheumatic disease, including RA, AS, SpA, PsA, and OA.**



Videos available on the accompanying ExpertConsult website.

**Radiography should most often be the first imaging investigation in arthritis because the significance of positive and negative findings is well known.**

**Radiography can be used to follow structural damage progression in inflammatory and degenerative joint diseases but is less sensitive to change than MRI.**

**The main disadvantage of radiography is its low sensitivity, particularly for soft tissue changes.**

The first roentgen ray image was an "x-ray" of the hand. Since then, imaging of musculoskeletal structures has been an important part of conventional radiography (x-ray).[1] The simple radiography is relatively cheap, available worldwide, and produces an image that is almost identical, regardless of technical parameters or whether the image is analogue or digital. The reliability of the image means that despite the limitations of radiography, advances in clinical practice can rely on the good old x-ray, knowing that it is largely independent of technologic advances. For many years to come, radiographs will appear much the same.

## Technical Aspects

The conventional radiograph is a two-dimensional summation image that is dependent on variable absorption of x-rays by different tissues for its inherent contrast. It has a very high spatial resolution that is rarely surpassed by other modalities, but radiography only offers high-contrast between a limited number of structures: bone (calcium), soft tissue, and air. Fat is visible as a separate density, but the distinction between soft tissue and fat is often subtle, and radiography cannot distinguish among the other soft tissues because cartilage, muscle, tendon, ligament, synovium, and fluid all appear to have the same density. These characteristics point to the radiograph's inherent advantages and disadvantages.[1,2]

In its favor, radiographs show skeletal structure very well, and because they are summation images, they allow excellent overall assessment of skeletal trauma and alignment. The limited number of images produced facilitates rapid review, and the high bone-soft tissue contrast often produces radiographic manifestations of disease in specific patterns that make the test particularly useful in daily clinical practice. The greatest disadvantage of radiography is its inherent lack of soft tissue contrast, which makes it insensitive for the detection of soft tissue abnormalities. The first radiographic sign of inflammatory arthritis may be permanent structural damage (bony erosion); in degenerating joints, cartilage damage is not usually visualized until sufficient overall cartilage loss allows approximation of the bone ends, resulting in joint space narrowing.

Although radiographs use ionizing radiation, they should be regarded as relatively safe, especially in older patients. An exception may be spine radiography, in which higher doses are used to penetrate the trunk, and in a younger population, MRI offers a safer and more informative alternative. In most examinations, two or more projections are required to adequately visualize the joint in question, and radiographic quality is improved by strict adherence to standard imaging protocols.

## Rheumatoid Arthritis

RA is the archetypal inflammatory joint disease, and it primarily targets synovium and peripheral joints. It is a systemic inflammatory disorder in which the typical clinical manifestations are usually symmetric, and thus the radiographic signs usually follow this pattern. As in all of radiology, the observed distribution of disease is often characteristic of the underlying cause. In RA, the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints of the hands and the wrists, and the metatarsophalangeal (MTP) joints of the feet are most often involved.[1,2]

Juxta-articular osteoporosis is a characteristic feature of RA, best seen in early disease in the small peripheral joints. In later stages of disease, generalized osteoporosis is usually present and is exacerbated by joint disuse. Erosion of bone (Figure 58-1) is a characteristic feature of RA that usually appears first at the margins of the joint, where the proliferating synovium lies directly on the surface of the bone between the edge of the cartilage-covered articular surface and the capsular attachment—the "bare areas." These marginal erosions may be subtle and first appear as disruptions of the thin white cortical line, especially at the radial aspect of the metacarpal heads, where they may be best seen on dedicated radiographic projections, such as the ball-catcher's view of the hands. These erosive changes are a good indication of the aggressiveness of the arthritis. In large joints, synovial proliferation may be very severe before bony erosion is detectable on radiography. This is especially notable in the knee, where pain and swelling caused by arthritis and bursitis occur long before erosion occurs. In addition to bare-area erosions, two other types of erosions have been described in RA. *Compressive erosion* refers to remodeling of inflamed and osteoporotic bone, with gradual invagination of one bone into another typified by protrusio acetabuli of the hip (see Figure 58-1E). *Surface erosion* of bone may also be seen, usually as a result of inflammation of an adjacent tendon sheath. A typical location for this is at the outer margin of the ulnar styloid process, secondary to extensor carpi ulnaris tenosynovitis.

Joint space involvement is characteristic in RA.[1,2] In many cases, the inflammatory processes result in progressive destruction of articular cartilage, which in turn causes the radiographic finding of concentric joint space narrowing. This condition is usually diffuse in RA as all of the cartilage is involved at the same time, and this feature may allow for differentiation from the focal or asymmetric type of joint space loss that occurs with degenerative disorders. Occasionally, cartilage destruction precedes synovial erosion of bone. Continued cartilage damage may result in partial or complete fibrous ankylosis, but progression to bony ankylosis is uncommon, although it may occur in end-stage disease in the wrist or midfoot. Subchondral radiolucent areas are common in RA and are often referred to as *cysts*, *geodes*, or *pseudocysts*. They may develop as a result of intraosseous extension of pannus, injury to the bone of any kind, or true intraosseous rheumatoid nodules. Mechanical factors accentuate their development, and very large cystic lesions may be seen in the elbow, femoral neck, or knee, and occasionally will precipitate pathologic fractures.

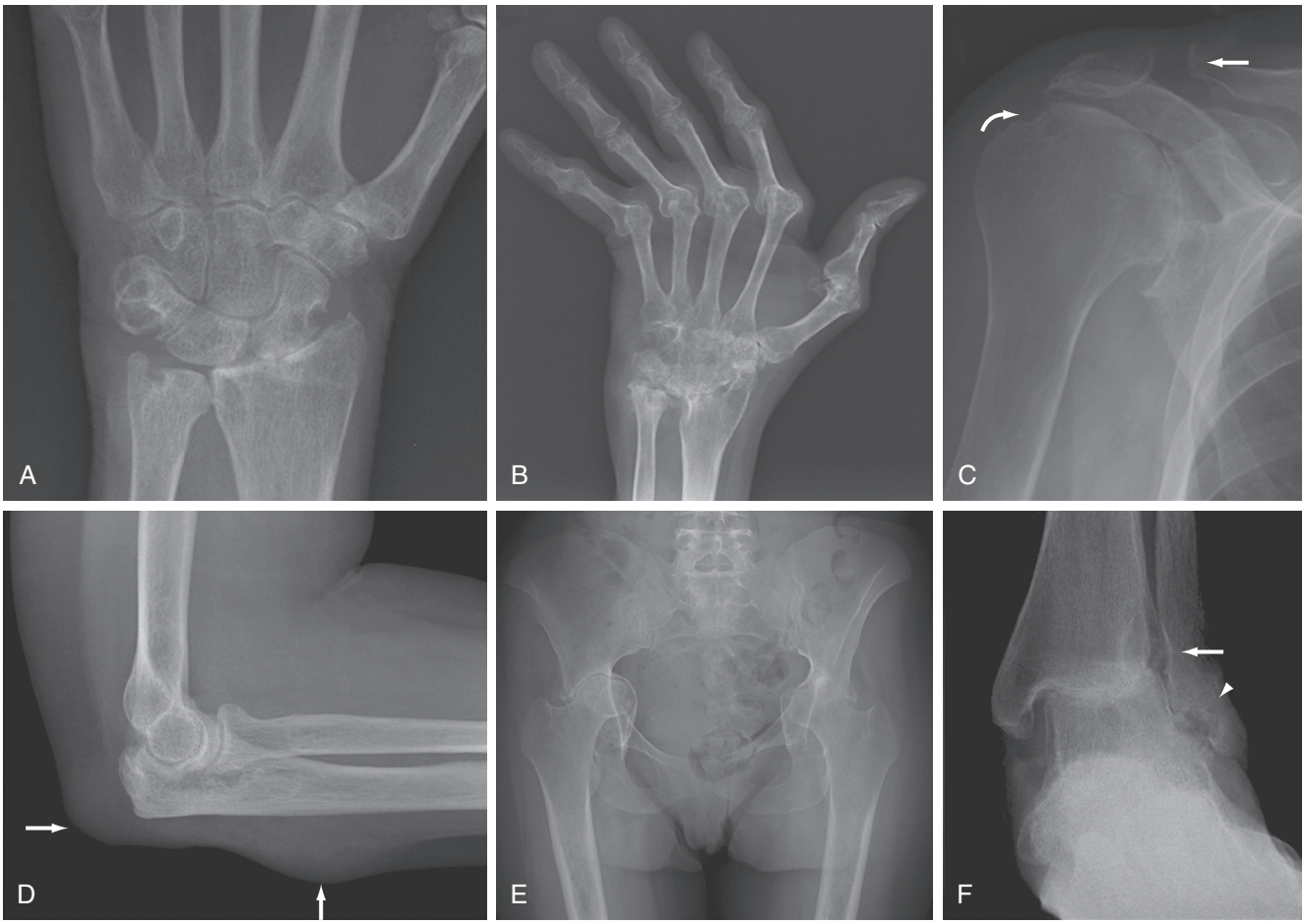



**Figure 58-1 Rheumatoid arthritis (radiographs). A,** Multiple typical bone erosions are seen (e.g., in triquetrum, pisiform, scaphoid, radius, and ulnar styloid process). Diffuse cartilage loss also is evident in the radiocarpal compartment. **B,** Severe ulnar deviation is present at the metacarpophalangeal joints with extensive erosions. Severe erosion and bony ankylosis are seen in the wrist. **C,** Posterior oblique view of a shoulder shows severe glenohumeral joint space narrowing with marginal erosion and cystic change of the humeral head adjacent to the greater tuberosity *(curved arrow)*. Elevation of the humeral head with respect to the glenoid indicates chronic rotator cuff tear. Tapering of the distal end of the clavicle and widening of the acromioclavicular joint *(straight arrow)* are also evident. **D,** Rheumatoid nodules appearing as lobulated subcutaneous soft tissue swellings *(arrows)* at the extensor surface of the elbow and forearm. **E,** Bilateral protrusio acetabuli. The medial acetabular margins protrude into the pelvis. Severe accompanying cartilage loss has occurred. **F,** Ankle with diffuse loss of cartilage space with erosions of the fibula (*arrow* and *arrowhead*). The hindfoot is in valgus alignment.

Symmetric soft tissue swelling around the small joints of the hands or feet is often the first clinical and radiographic sign of RA. The soft tissue swelling is caused by joint effusion, synovial proliferation, and periarticular inflammation, but the radiographic findings are nonspecific. Radiographs are much less sensitive for such changes than MRI and ultrasonography. Eccentric soft tissue swelling may be the result of adjacent bursitis, tenosynovitis, or rheumatoid nodules. Involvement of tendons and tendon sheaths is common; however, the soft tissue changes are usually poorly visualized and thus are of lesser diagnostic importance.

Joint malalignment and deformity (see Figure 58-1) are very common in RA and occur because of laxity and disruption of capsule, ligaments, and tendons. The deformities are most prevalent in the hand, wrist, foot, and neck. Note that these malalignments may be transient and can be reduced depending on the positioning for the radiographs. Late-stage disease is associated with severe deformity, as seen in arthritis mutilans.[1,2]

## Spine

Cervical spine involvement is common in RA and merits specific attention. It occurs mainly after several years of disease in rheumatoid factor $(RF)^+$ patients with severe peripheral RA, and may cause severe pain, instability and, ultimately, spinal cord compression.[3] Prevalences of radiologic cervical involvement as high as 70% have been reported, but are much lower in recent studies in accordance with the fact that intensive disease-modifying antirheumatic drug (DMARD) therapy has been shown to reduce cervical radiological progression.[4]

The upper cervical region is most affected, although abnormalities throughout the cervical spine are frequent (Figure 58-2). Upper cervical changes include odontoid process (dens) erosion and atlantoaxial subluxations (anterior, vertical [known as cranial settling], lateral, and posterior), whereas subaxial manifestations include abnormalities of apophyseal and discovertebral joints, subluxation, and dislocation.

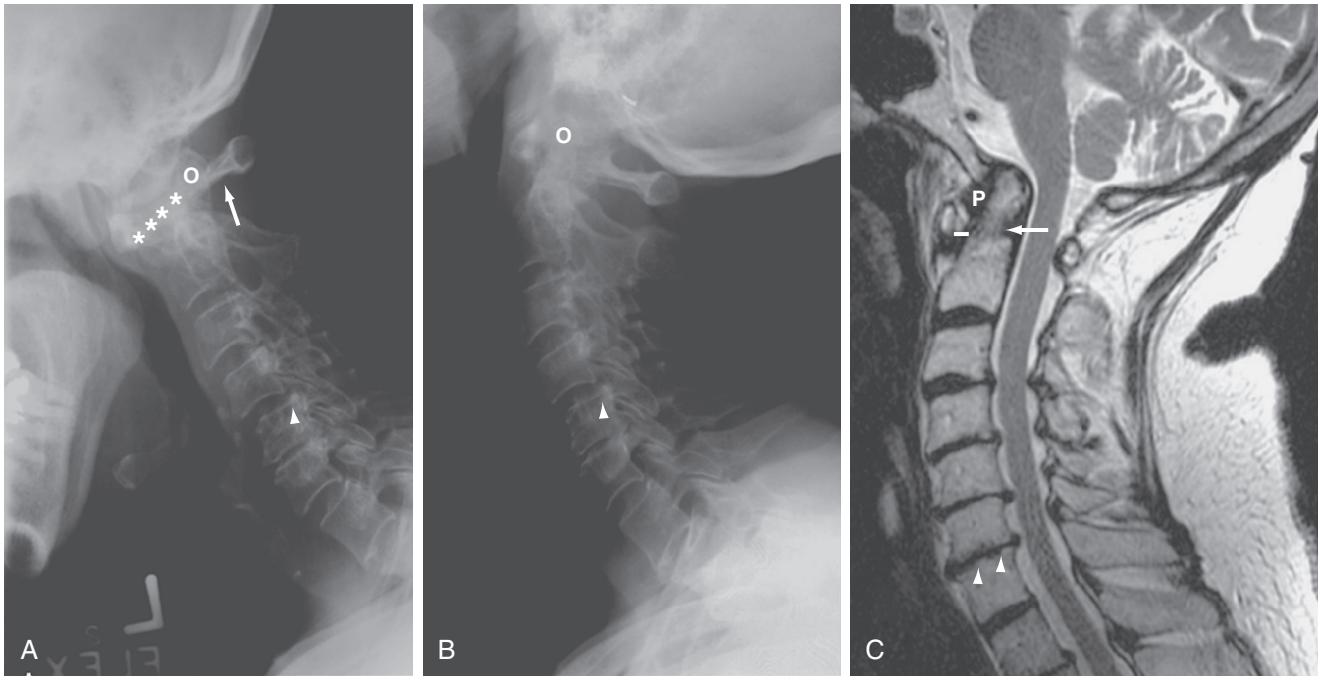



**Figure 58-2 Cervical spine in rheumatoid arthritis (RA) (radiography and MRI). A** and **B,** Radiography shows anterior atlantoaxial subluxation in RA. **A,** Lateral radiograph in flexion shows severe anterior atlantoaxial subluxation with a wide anterior atlantodental interval *(asterisks)* and a decreased posterior atlantodental interval *(arrow)*. **B,** Almost complete reduction of subluxation is noted on the lateral view in extension. There also is subaxial subluxation at the level of C4-C5 *(arrowhead)* with erosive changes in various facet joints. **C,** T2-weighted sagittal magnetic resonance (MR) image in RA shows low signal periodontoid pannus *(P)*. The odontoid *(O)* process appears irregular secondary to erosions *(arrow)*. The atlantodental distance shows mild widening *(solid line)*. There also is vertical subluxation without signs of cord compression. The anterior subarachnoid space is compromised by disk protrusions at multiple levels. Small erosions *(arrowheads)* are seen at the vertebral end plates at the C6-C7 level.

Adequate radiographic evaluation of the cervical spine requires lateral views in flexion and extension and should be performed in all patients with RA with neck pain. The flexion-extension views are especially important for demonstration of the degree of atlantoaxial instability.

Atlantoaxial subluxation is caused by laxity or rupture of the transverse ligament. The characteristic finding is anterior subluxation (i.e., abnormal separation between the anterior arch of the atlas and the odontoid process of the axis). Reports of the exact lower limit of abnormal distance vary, but a distance of greater than 2.5 mm is considered abnormal, or if the distance changes significantly between flexion and extension.[1] Cranial settling is diagnosed by radiographic measurement of the relationship between the tip of the odontoid process and landmarks at the base of the skull.[1] It may lead to protrusion of the dens through the foramen magnum and compression of neurologic structures. Significant atlantoaxial subluxations, particularly when accompanied by clinical findings, may require surgery.

Subaxial involvement is also frequent, and involves varying degrees of bone erosion, joint space narrowing and subluxation of facet joints, as well as disk space narrowing and spinous process erosions. Subaxial subluxations may also lead to neurologic deficits.[1]

MRI is useful for supplementary information on cervical spine involvement (see Figure 58-2C), including spinal cord compression (see MRI section).

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** Characteristic radiographic findings are part of the American College of Rheumatology (ACR) 1987 classification criteria for RA.[5] Radiography also has a role in the recent ACR/European League against Rheumatism (EULAR) 2010 classification criteria because patients who display bone erosions typical for RA plus at least one clinically swollen joint fulfill the criteria and are classified as having RA.[6,7] Radiography can help to differentiate RA from other joint conditions, including OA, PsA, and neoplasm.[2] EULAR recommendations for the use of imaging, including radiography, in the clinical management of RA have recently been published (Table 58-1).[8]

**Monitoring.** In routine clinical management and in clinical trials of RA, radiographic evaluation focuses on joint space narrowing and bone erosions in hands, wrists, and forefeet as measures of structural joint damage.[9-11] Validated scoring methods of radiologic damage (the Larsen method and the Sharp method and their modifications) are available and extensively used in clinical trial.[10-12] The van der Heijde and Genant modifications of the Sharp score are generally considered the methods most sensitive to change, but are also the most time consuming.[13] For clinical practice, the less time-consuming Simple Erosion Narrowing Score (SENS), based on counting joints with bone erosion and joints with joint space narrowing, is available,[14,15] but it is seldom used.

**TABLE 58-1** EULAR Recommendations for the Use of Imaging in the Clinical Management of Rheumatoid Arthritis*

| |
|---|
| When there is diagnostic doubt, conventional radiography, ultrasonography, or MRI can be used to improve the certainty of a diagnosis of RA in addition to clinical criteria alone.† |
| The presence of inflammation seen with ultrasonography or MRI can be used to predict the progression to clinical RA from undifferentiated inflammatory arthritis. |
| Ultrasonography and MRI are superior to clinical examination in the detection of joint inflammation; these techniques should be considered for more accurate assessment of inflammation. Conventional radiography of the hands and feet should be used as the initial imaging technique to detect damage. However, ultrasonography and/or MRI should be considered if conventional radiographs do not show damage and may be used to detect damage at an earlier time point (especially in early-stage RA). |
| MRI bone edema is a strong independent predictor of subsequent radiographic progression in early RA, and should be considered as a prognostic indicator. Joint inflammation (synovitis) detected by MRI or ultrasonography as well as joint damage detected by conventional radiographs, MRI, or ultrasonography can also be considered for the prediction of further joint damage. |
| Inflammation seen on imaging may be more predictive of a therapeutic response than clinical features of disease activity; imaging may be used to predict response to treatment. |
| Given the superior detection of inflammation by MRI and ultrasonography versus clinical examination, they may be useful in monitoring disease activity. |
| The periodic evaluation of joint damage, usually by radiographs of the hands and feet, should be considered. MRI (and possibly ultrasonography) is more responsive to change in joint damage and can be used to monitor disease progression. |
| Monitoring of functional instability of the cervical spine by lateral radiograph obtained in flexion and neutral positions should be performed in patients with clinical suspicion of cervical involvement. When the radiograph is positive or specific neurologic symptoms and signs are present, MRI should be performed. |
| MRI and ultrasonography can detect inflammation that predicts subsequent joint damage, even when clinical remission is present and can be used to assess persistent inflammation. |

*Recommendations are based on data from imaging studies that have mainly focused on the hands (particularly wrists, metacarpophalangeal and proximal interphalangeal joints). There are few data with specific guidance on which joints to image.[8]

†In patients with at least one joint with definite clinical synovitis, which is not better explained by another disease.

EULAR, European League against Rheumatism; MRI, magnetic resonance imaging; RA, rheumatoid arthritis.

**Predicting Outcome.** Early bone erosion correlates with poor long-term radiographic and functional outcome,[16] and early progression in radiographic erosion is related to future impairment in physical function.[17] In early undifferentiated arthritis, the presence of radiographic erosion increases the risk for persistent arthritis.[18] However, radiographic erosions are only present in a minority of patients with early-stage RA, with reported prevalences of 8% to 40% at 6 months,[19-23] and the absence of erosion in early disease is not necessarily associated with good outcomes. Thus, at this stage radiography is not effective for identifying future "non-progressors," (i.e., patients who will not show increasing structural joint damage).[24]

## Ankylosing Spondylitis

AS is the archetypal inflammatory joint disease that primarily targets fibrocartilage and the axial skeleton. Cartilaginous joints and sites of enthesis (tendon and ligament insertion) are involved early, and the characteristic involvement of the axial skeletal includes a predilection for the sacroiliac joints and all the articulations and entheses of the spine.

### Sacroiliac Joints

Erosion and ankylosis of the sacroiliac joints are the hallmarks of SpA.[1,25] Sacroiliitis is usually the first manifestation and is characteristically bilateral and symmetric in AS.[26,27] Early radiographic findings predominate on the iliac side of the cartilage compartment, with erosion of subchondral bone causing loss of definition of the articular surfaces, usually accompanied by varying degrees of adjacent osteoporosis and surrounding reactive sclerosis. Bone erosion may result in the radiographic observation of focal joint space widening (Figure 58-3A) and, as the disease progresses, definition of the joint is completely lost, with radiographic superimposition of erosion, sclerosis, and new bone formation which fills in the erosions and the original cartilaginous joint space. The joint may disappear completely in late-stage disease, with ankylosis and remodeling of the bone (see Figure 58-3B). The ligamentous compartment of the SI joint is frequently affected by bony erosion and entheseal proliferation, although these may be hard to see radiographically.[26,27]

### Spine

Traditional descriptions of the initial sites of spinal involvement are enormously influenced by the inability of radiography to adequately visualize many parts of the spine. Radiographic reports indicate that the lumbosacral and thoracolumbar junctions are first affected, whereas the MRI literature clearly indicates a predilection for early involvement of the midthoracic spine—an area that is extremely difficult to evaluate radiographically. The cervical spine is rarely affected first, but this can occur occasionally in women. Spinal disease rarely occurs in the absence of significant SI joint involvement.

The early radiographic manifestations of AS in the spine are most often caused by enthesitis at the edges of the discovertebral joints.[26] Focal sclerosis (the "shiny corner") and erosion (the "Romanus lesion") develop at the attachment of the annulus fibrosis to the anterior corner of the vertebral end plate and are characteristic features of early-stage AS (Figure 58-4A).[28] The anterior borders of vertebrae may appear straight or "squared" because of periosteal proliferation of new bone filling in the normal concavity or erosion at the anterosuperior and anteroinferior vertebral margins. This observation is much easier to make in the lumbar spine, where normal vertebrae are always concave anteriorly in comparison with the thoracic and cervical spine, where the normal contour is much more variable and may be square or occasionally convex.

The hallmark of spinal disease in AS is the development of characteristic bony spurs: syndesmophytes (see Figure

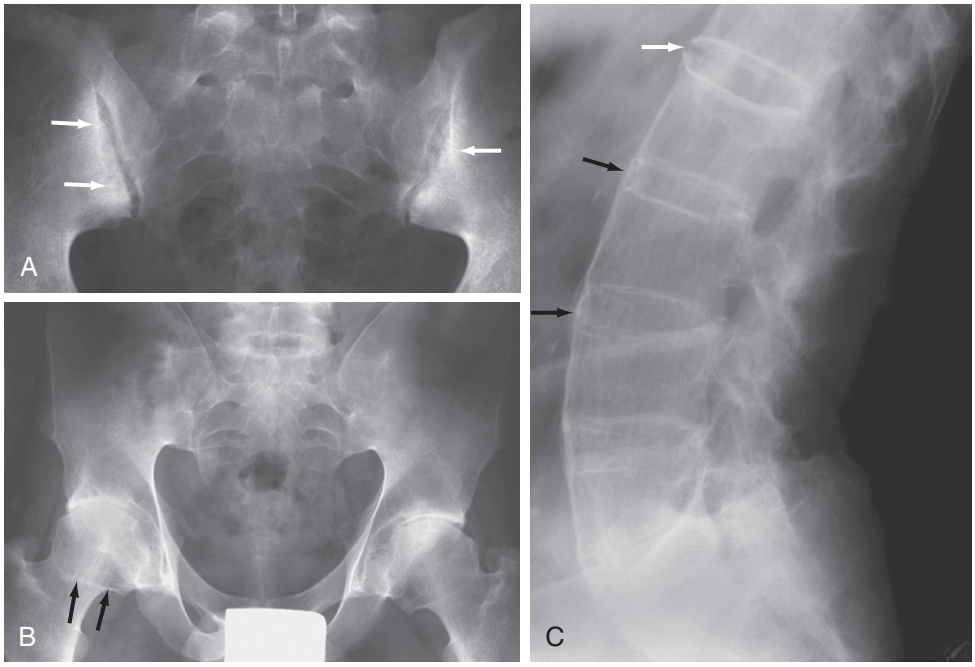



**Figure 58-3 Ankylosing spondylitis (radiograph). A,** Sclerosis *(arrows)* is seen along the iliac sides of the sacroiliac joints along with loss of portions of the iliac subchondral bone, indicating erosion. **B,** Bilateral hip joint space narrowing is present. A ring of osteophytes is noted at the synovial insertion *(arrows)* on each femoral head. The sacroiliac joints are fused (ankylosis). **C,** Syndesmophytes (*black* and *white arrows*), some of which extend from the edge of one vertebral body to the next (bridging syndesmophytes, ankylosis; *black arrows*), are seen is this lateral view of the spine.

58-3C). These spurs start as thin, vertically oriented projections of bone that develop from ossification within the outer fibers of the annulus fibrosus of the intervertebral disk. Syndesmophytes are radiographically visible on the anterior and lateral aspects of the spine starting from the corner of the vertebra. Progressive growth will bridge the intervertebral disk, causing ankylosis. Extensive syndesmophyte formation produces a smooth, undulating spinal contour—the "bamboo spine." The syndesmophytes that characterize AS must be differentiated from other spinal and paraspinal bone formations. Degenerative bony spurring in spondylosis deformans arises several millimeters from the discovertebral junction, is typically triangular in shape, and has a horizontally oriented segment of variable length at the point of origin. In diffuse idiopathic skeletal hyperostosis (DISH), bone formation in the anterior longitudinal ligament results in a flowing pattern of ossification that is usually thick, and the sacroiliac joints are not involved.

Erosion of the vertebral end plate is common in later stages of AS and may be focal or diffuse. It is also seen when pseudoarthrosis develops after a fracture in a previously ankylosed spine. Changes in the apophyseal joints are common and start with ill-defined erosion and sclerosis but may be hard to see. Capsular ossification or intra-articular bony ankylosis frequently occurs in late-stage disease (Figure 58-4). The ankylosed spine is very susceptible to fractures (see Figure 58-4C and D), which should always be suspected in case of unexplained pain exacerbations. Enthesitis at the interspinous and supraspinous ligamentous attachments is very common with bone formation causing whiskering and interspinous ankylosis.

### Use in Diagnosis, Monitoring, and Predicting Outcome

Although the modified New York Criteria are actually classification criteria, these criteria are the most commonly used to diagnose AS and are based on clinical features and radiographic sacroiliitis.[25] According to these criteria, AS may be diagnosed if, in addition to one clinical criterion, grade 2 sacroiliitis (minimal sacroiliitis: loss of definition of the joint margins, minimal sclerosis, joint space narrowing, and erosions) or higher occurs bilaterally, grade 3 (moderate sacroiliitis: definite sclerosis on both sides of the joint, erosions, and loss of joint space), or grade 4 (complete bony ankylosis) occurs unilaterally.[25] Because of the requirement for these radiographic structural changes, the duration of disease before diagnosis has been a median of 7 to 10 years.[29] The definitions of radiographic changes according to the New York Criteria are included in the Assessment of Spondyloarthritis International Society (ASAS) classification criteria for axial SpA,[30] the European Spondyloarthritis Study Group (ESSG) criteria for SpA,[31] and in the modified New York Criteria.[25] EULAR recommendations for the use of imaging, including radiographs, in the diagnosis and clinical management of SpA have recently become available (Table 58-2).[32]

Radiography of the spine is not included in the classification criteria but may be useful to follow structural disease progression in patients with spinal involvement. The bone changes seen in patients with axial SpA develop slowly and are often not present in patients with early-stage disease. In general, only minor changes can be observed in the first several years. Different scoring methods, all based on

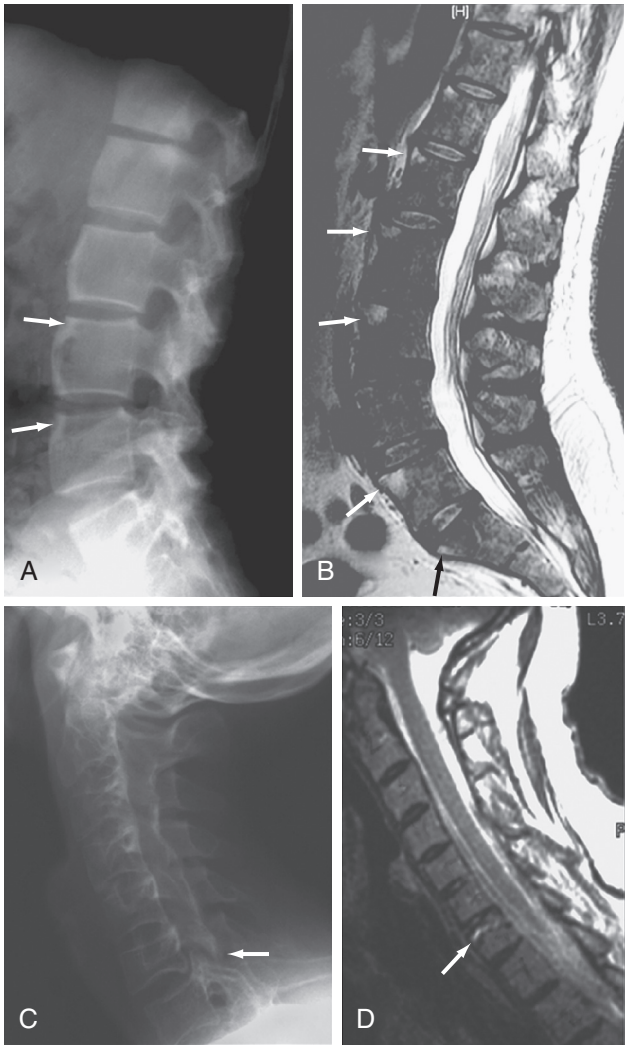



**Figure 58-4 Ankylosing spondylitis (radiography and MRI). A,** Lateral radiograph of the spine shows marked erosion at the vertebral margins has produced straight or slightly convex anterior vertebral surfaces ("squaring"). New bone formation has resulted in "shiny corners" *(arrows)*. The facet joints are fused. **B,** Sagittal fast spin echo T2-weighted MR image in another patient shows bone marrow edema at the anterosuperior corners of the vertebral bodies *(arrows)*, corresponding to early osteitis. **C** and **D,** Fracture in ankylosing spondylitis. **C,** Radiography shows disruption of the previously fused C6-C7 facet joints *(arrow)* and slight anterior subluxation. **D,** Sagittal T2-weighted MR image confirms a high signal fracture line *(arrow)* through the superior aspect of C7.

assessment of lateral views, have been developed to quantify changes in the spine of patients with AS: the Stoke AS Spine Score (SASSS), Bath AS Radiology Index (BASRI), and the modified Stoke AS Spine Score (mSASSS). A comparative study of the three methods concluded that all measures were reliable, but mSASSS was more sensitive to change.[33] These spine scores are primarily used in clinical research.

## Psoriatic Arthritis

The presentation of PsA is quite variable but is often distinctive. Peripheral joint involvement is common, and as many as half of patients with PsA have evidence of joint damage on radiographs within 2 years of presentation.[34] The hand and wrist are most often involved and are affected in as many as three quarters of patients with PsA. However, the pattern of joint involvement varies from patient to patient and also with time in individual patients. Radiographic appearances are usually asymmetric and may have a ray distribution, with the affected joints involving a single digit, including the DIP joints (Figure 58-5), which are seldom involved in RA. Clinically, this manifests as dactylitis, and is a common early feature of PsA. Osteoporosis is frequently absent, with the propensity for bone proliferation, which is a distinguishing feature of PsA. This may be in the form of periostitis of the shafts of the phalanges, which is sometimes the first radiographic manifestation, or irregular bony spurring at joints or entheses. Fluffy new bone formation adjacent to marginal joint erosions may result in a particularly characteristic "whiskering" appearance. Severe marginal erosion of the heads of metacarpals or phalanges may produce the appearance of a whittled pencil and, if combined with deep central erosion of phalangeal bases, is referred to as the "pencil-in-cup" appearance. Ankylosis of joints occurs frequently in advanced disease. Large joints are less often affected when the findings are usually similar to those of RA. Spinal involvement is frequent in patients with PsA. It may be seen in early-stage disease, and sacroiliitis may be demonstrable in as many as 75% of patients.[35] The changes may be extensive, and are more likely to be asymmetric than in AS.

### Use in Diagnosis, Monitoring, and Predicting Outcome

Although RA is characterized by mainly osteo-destructive lesions, in PsA both osteo-destructive and osteo-proliferative manifestations occur, which may even co-exist, not only in the same patient, but even in the same joint.[36] In particular, the osteo-proliferative lesions on radiography are characteristic, and are included in the new classification criteria (CASPAR) for PsA.[37] The presence of juxtaarticular new bone formation appearing as ill-defined ossification near joint margins (but excluding osteophytes) on radiographs of the hand or foot is one of five criteria.[37]

Structural joint damage on conventional radiography is an important outcome measure in PsA. Different radiographic scoring methods of peripheral joints have been developed (e.g., the Sharp–van der Heijde modified scoring method for PsA, which is a detailed scoring system for evaluating erosions and joint space narrowing) whereas osteolysis and pencil-in-cup phenomena are assessed separately.[13] Scoring systems are primarily used in clinical trials. No specific scoring systems for spine and sacroiliac joints in PsA exist, and can be monitored as in AS (see the subsequent text).

## Gout

A variety of microcrystals can deposit in and around the joints and induce an inflammatory response. In this chapter, the characteristics of gout (i.e., arthritis related to monosodium urate deposition) and of calcium pyrophosphate dihydrate (CPPD) crystal deposition disease will be discussed. The reader is referred to chapters on the individual diseases for description of the remaining conditions, such as calcium

**TABLE 58-2** EULAR Recommendations for the Use of Imaging in the Diagnosis and Management of Spondylarthritis in Clinical Practice

| |
|---|
| **Axial SpA: Diagnosis**<br>A. In general, conventional radiography of the SI joints is recommended as the first imaging method to diagnose sacroiliitis as part of axial SpA. In certain cases, such as young patients and those with short symptom duration, MRI of the SI joints is an alternative first imaging method.<br>B. If the diagnosis of axial SpA cannot be established based on clinical features and conventional radiography, and axial SpA is still suspected, MRI of the SI joints is recommended. On MRI, both active inflammatory lesions (primarily bone marrow edema) and structural lesions (such as bone erosion, new bone formation, sclerosis, and fat infiltration) should be considered. MRI of the spine is not generally recommended to diagnose axial SpA.<br>C. Imaging modalities other than conventional radiography and MRI are generally not recommended in the diagnosis of axial SpA.* |
| **Peripheral SpA: Diagnosis**<br>When peripheral SpA is suspected, ultrasonography or MRI may be used to detect peripheral enthesitis, which may support the diagnosis of SpA. Furthermore, ultrasonography or MRI might be used to detect peripheral arthritis, tenosynovitis, and bursitis. |
| **Axial SpA: Monitoring Activity**<br>MRI of the SI joints and/or the spine may be used to assess and monitor disease activity in axial SpA, providing additional information on top of clinical and biochemical assessments. The decision on when to repeat MRI depends on the clinical circumstances. In general, STIR sequences are sufficient to detect inflammation and the use of contrast medium is not needed. |
| **Axial SpA: Monitoring Structural Changes**<br>Conventional radiography of the SI joints and/or spine may be used for long-term monitoring of structural damage, particularly new bone formation, in axial SpA. If performed, it should not be repeated more frequently than every second year. MRI may provide additional information. |
| **Peripheral SpA: Monitoring Activity**<br>Ultrasonography and MRI may be used to monitor disease activity (particularly synovitis and enthesitis) in peripheral SpA, providing additional information on top of clinical and biochemical assessments. The decision on when to repeat ultrasonography/MRI depends on the clinical circumstances. Ultrasonography with high-frequency color or power Doppler is sufficient to detect inflammation, and the use of ultrasonography contrast medium is not needed. |
| **Peripheral SpA: Monitoring Structural Changes**<br>In peripheral SpA, if the clinical scenario requires monitoring of structural damage, then conventional radiography is recommended. MRI and/or ultrasonography might provide additional information. |
| **Axial SpA: Predicting Outcome/Severity**<br>In patients with AS (not nr-axSpA), initial conventional radiography of the lumbar and cervical spine are recommended to detect syndesmophytes, which predict development of new syndesmophytes. MRI (vertebral corner inflammatory or fatty lesions) may also be used to predict development of new radiographic syndesmophytes. |
| **Axial SpA: Predicting Treatment Effect**<br>Extensive MRI inflammatory activity (bone marrow edema), particularly in the spine in AS patients, might be used as a predictor of good clinical response to anti-TNF treatment in axial SpA. Thus, MRI might aid in the decision of whether to initiate anti-TNF therapy, in addition to clinical examination and CRP. |
| **Spinal Fracture**<br>When spinal fracture in axial SpA is suspected, conventional radiography is the recommended initial imaging method. If conventional radiography is negative, CT should be performed. MRI is an additional imaging method to CT, which can also provide information on soft tissue lesions. |
| **Osteoporosis**<br>In axial SpA patients without syndesmophytes in the lumbar spine on conventional radiography, osteoporosis should be assessed by hip DXA and AP-spine DXA. In patients with syndesmophytes in the lumbar spine on conventional radiography, osteoporosis should be assessed by hip DXA, supplemented by either spine DXA in lateral projection or possibly QCT of the spine. |

*CT may provide additional information on structural damage if conventional radiography is negative and MRI cannot be performed. Scintigraphy and ultrasonography are not recommended for diagnosis of sacroiliitis as part of axial SpA.

AP-spine, Anterior-posterior spine; AS, ankylosing spondylitis; CRP, C-reactive protein; CT, computed tomography; DXA, dual-energy x-ray absorptiometry; EULAR, European League against Rheumatism; MRI, magnetic resonance imaging; nr-axSpA, nonradiographic axial spondyloarthritis; SIJ, sacroiliac joints; SpA, spondyloarthritis; SOR, strength of recommendation; STIR, short tau inversion recovery; TNF, tumor necrosis factor.

From Mandl P, Navarro-Compan V, Terslev L, et al: EULAR recommendations for the use of imaging in the diagnosis and management of spondyloarthritis in clinical practice. *Ann Rheum Dis* 74:1327–1339, 2015.

hydroxyapatite crystal deposition disease, hemochromatosis, ochronosis, and Wilson's disease.

Radiography is not helpful to diagnose acute gouty arthritis because the findings are limited to the soft tissues and are nonspecific. Chronic tophaceous gout is an asymmetrical arthritis that frequently affects the feet, hands, wrists, elbows, and knees. The most common site of involvement is the first metatarsophalangeal (MTP) joint (Figure 58-6). The radiologic changes in long-standing gout are related to tophi and their effect on soft tissue and bone.[38]

Tophi appear as eccentric, nodular soft tissue masses around the joints with an amorphous increased density or patchy calcification.[38] Intraosseous tophi cause well-marginated subchondral cystic lucencies or intraosseous calcifications. Erosion of the adjacent bone is common at either the periosteal or endosteal surface. The erosions may be intra-articular, para-articular, or remote from the joint and are typically well defined with smooth sclerotic borders. An overhanging margin of bone at the edge of the erosion is a characteristic feature of gout. The joint space and bone density are usually preserved until late in the disease. Bursal inflammation commonly produces soft tissue swelling around the olecranon and in a prepatellar location.

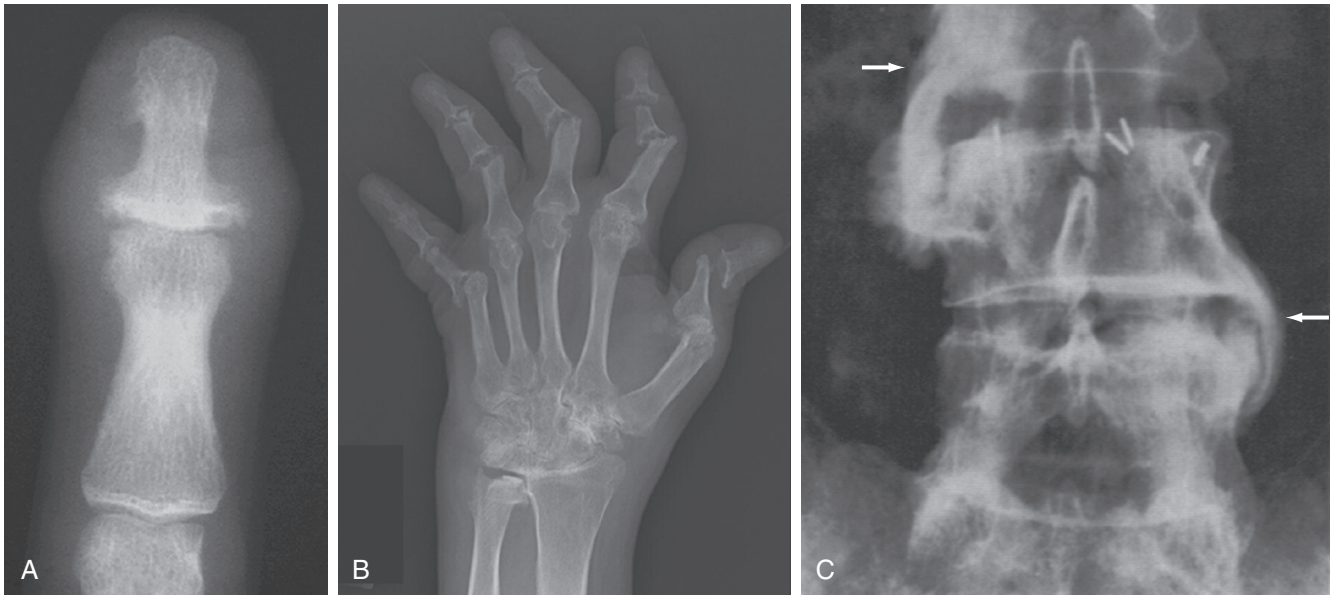



**Figure 58-5 Psoriatic arthritis (radiography). A,** Distal interphalangeal joint with classic radiographic findings, including soft tissue swelling, bone erosions with accompanying bone proliferation, and lack of osteoporosis. **B,** Arthritis mutilans resulting from psoriatic arthritis with destructive changes and joint deformity of the hand and pancompartmental ankylosis of the wrist. **C,** Psoriatic spondylitis. Thick asymmetric paravertebral ossifications *(arrows)*, which are characteristic of psoriatic spondylitis and reactive arthritis. *(From Resnick D, Niwayama G:* Diagnosis of bone and joint disorders, *Philadelphia, 1988, WB Saunders.)*

### Use in Diagnosis, Monitoring, and Predicting Outcome

The 1977 Criteria for the Classification of Acute Arthritis of Primary Gout[39] include the conventional radiographic features of asymmetric swelling and subcortical cysts without erosion. However, conventional radiographic features of acute gout have low clinical utility because of lack of sensitivity and specificity. In the chronic phase the characteristic pattern with asymmetrical, erosive polyarticular disease and tophi appearing as nodular soft tissue masses with amorphous increased density or patchy calcification may be diagnostically useful.

Recently a specific gout radiographic scoring method has been developed and validated and may improve sensitivity to change in longitudinal studies.[40] However, radiography is not a sensitive method for monitoring gout manifestations, compared with ultrasound and MRI, because inflammatory changes and tophi volumes cannot be assessed.[41]

## Calcium Pyrophosphate Dihydrate Crystal Deposition Disease

The term *CPPD crystal deposition disease* refers to a variety of clinical situations, including asymptomatic crystal deposition, pseudogout, pyrophosphate arthropathy, and other, less frequent presentations, such as pseudo-RA. Chondrocalcinosis is a general term that refers to calcification of hyaline or fibrocartilage regardless of its etiology (Figure 58-7).

The acute attack of pseudogout is often a monoarthropathy, and typical radiographic findings of soft tissue swelling and joint effusion are non-specific, mimicking gout and infection. Often chondrocalcinosis is not radiographically visible. The radiographic changes in more chronic pyrophosphate arthropathy are very similar to OA with joint space narrowing, sclerosis, and subchondral cysts, with or without intra-articular or periarticular calcinosis.[42]

The patellofemoral joint and, in contrast to OA, the radiocarpal and the second and third MCP joints are typical sites of involvement in pyrophosphate arthropathy. The distribution is usually bilateral and may be symmetrical. In addition to the usual target sites, arthritic changes may be observed in non-weight-bearing joints and the sacroiliac joints.[43]

Pyrophosphate arthropathy may be associated with extensive and rapid subchondral bone collapse and fragmentation with intra-articular loose bodies, resembling neuropathic osteoarthropathy (pseudo-Charcot). Tumorous CPPD crystal deposits that resemble gouty tophi are observed occasionally, most frequently in digits, and are referred to as *tophaceous pseudogout*.[44]

## Septic Arthritis

Septic arthritis is usually monoarticular and typically associated with rapid onset of symptoms, systemic illness, obvious local clinical signs, and laboratory evidence of acute inflammation. The earliest clinical sign is symmetrical soft tissue swelling around a joint because of soft tissue edema, hypertrophied synovium, and joint effusion. However, this may be hard to discern on radiography, and periarticular osteopenia may be the first radiographic feature. Initial widening of the joint space may be seen, such as in septic arthritis of the pediatric hip. Marginal erosions develop quickly and have a similar appearance to other forms of erosive inflammatory arthritis. While the infection progresses, hyaline cartilage and the subchondral cortex are rapidly destroyed with progressive narrowing of the joint space (Figure 58-8). In late stages, ankylosis of the joint may be seen. Adjacent




**Figure 58-6** **Tophaceous gout (radiography, computed tomography [CT], and MRI). A,** Radiography of forefoot in tophaceous gout. Extensive bone destruction is seen at the first metatarsophalangeal (MTP) joint with overhanging edges *(arrowhead)* and soft tissue swelling. Smaller erosions are present involving the first tarsometatarsal and second MTP joints *(arrows)*. **B,** CT scan shows hyperdense tophus around the extensor digitorum muscle *(arrow)*, with smaller deposits around the other extensor tendons. Axial precontrast **(C)** and coronal short tau inversion recovery **(D)** MR images show a large tophus *(arrow)* medial to the first metatarsal bone. Ca, Calcaneus; Na, navicular. *(**C** and **D**, Courtesy Professor Fiona McQueen, Auckland, New Zealand).*

osteomyelitis occurs with increasing frequency while the infection becomes chronic. With granulomatous infections, the characteristic triad (Phemister's triad) of radiographic findings are marked osteoporosis, marginal erosions, and absent or mild joint space narrowing. Periostitis and bone production are less common.

### Use in Diagnosis, Monitoring, and Predicting Outcome

Radiography should be routinely performed but is insensitive for diagnosis. When there is clinical suspicion of septic arthritis, joint aspiration should be performed without delay.

The utility of radiography is limited in septic arthritis with immediate joint aspiration (directly or by image guided techniques) required for early diagnosis and more advanced imaging techniques required for assessment of associated osteomyelitis or other complications.[45] However, radiography should still be routinely performed because it may assist in interpretation of other studies and still offers a satisfactory overall assessment of bone morphology and background disease.

**Osteoarthritis.** OA primarily targets hyaline cartilage, and its distribution is profoundly influenced by mechanical factors. OA is typically asymmetrical in distribution both within and between joints. The most characteristic sites of osteoarthritis include the hips, knees (Figure 58-9), proximal and distal interphalangeal joints of the hand, first carpometacarpal and trapezioscaphoid joints of the wrist (Figure 58-10), and first metatarsophalangeal joints.[1]

Joint space narrowing caused by thinning of hyaline cartilage is a key feature of OA. Unlike the inflammatory

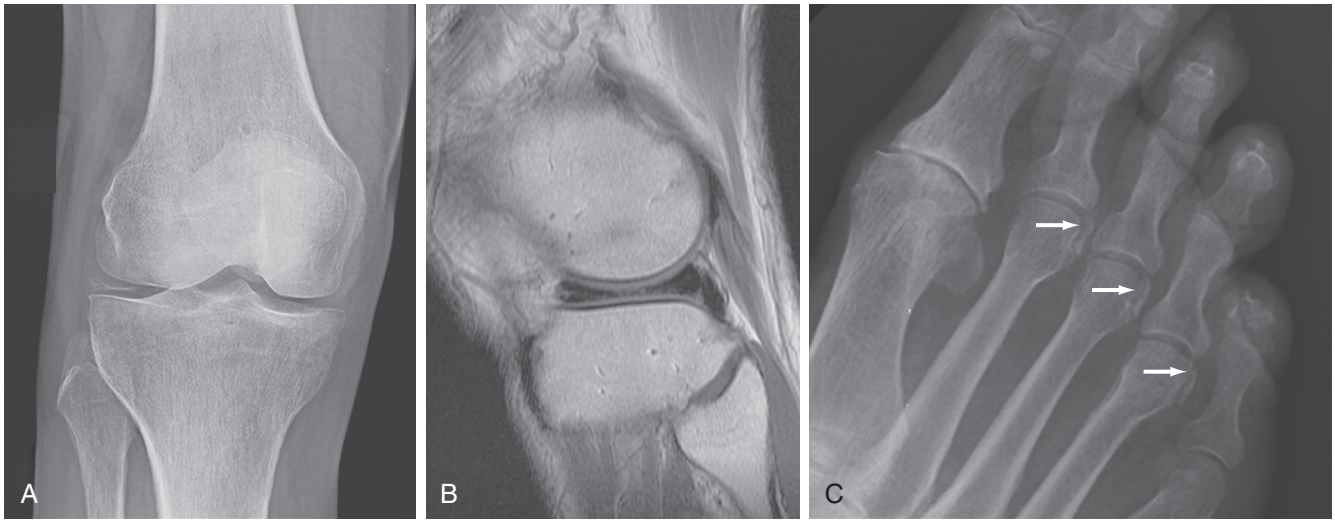



**Figure 58-7 Calcium pyrophosphate dihydrate crystal deposition disease (radiography and MRI).** Radiography of the knee **(A)** shows linear calcification within the lateral meniscus, whereas sagittal proton density MRI **(B)** of the same patient shows increased signal within the lateral meniscus, corresponding to chondrocalcinosis. **C,** Radiography of forefoot shows cartilaginous and capsular calcifications *(arrows)* within second through fourth metatarsophalangeal joints. A nondisplaced fracture of the fourth metatarsal neck is noted.

arthropathies, the resultant narrowing of the radiographic joint space is asymmetric, with cartilage thinning more pronounced in areas subject to greater mechanical stress, such as in one compartment of the knee. Accurate assessment of cartilage loss is hugely influenced by radiographic projection, particularly in the lower limbs. Sensitive detection of cartilage thinning requires apposition of the most affected surfaces, which requires the joint to be optimally positioned with sufficient mechanical stress to bring the surfaces into contact. In the knee, for example, angular deformity tends to reduce on supine radiography and severe cartilage loss may not be detected at all (see Figure 58-9). Bilateral weight-bearing projections are more consistent for the detection and measurement of cartilage loss and the erect extended anterior-posterior (AP) view is has been established as the preferred projection for diagnostic utility. However, no view is completely reliable until there is global loss of cartilage in late-stage disease. Weight-bearing in flexion is the most sensitive projection for cartilage thinning in the knee and the most reliable for detection of change, although the ideal degree of flexion for a specific individual varies depending on the distribution of cartilage loss (see Figure 58-9).[46,47]

Osteophytes are the most characteristic abnormality of OA, being more specific than joint space narrowing, and are often the first radiographic sign of the disease. They start as osseous metaplasia of the hyaline cartilage best seen at the articular margins but also occurring centrally on the articular surface. They can become large and often dominate the radiographic appearance. Thus, osteophytes are the key criterion to define presence of OA.[48]

Bone density is usually normal or sclerotic. Bone erosion does not occur except at the articular surface in late disease or occasionally earlier in the interphalangeal (IP) joints in inflammatory OA.

The radiographic appearance of periarticular soft tissues is relatively normal in OA. Synovitis and effusion do occur but only rarely dominate the radiographic presentation.

Some deformity is common, but tends to occur in particular joints such as the thumb and knee. The deformity is primarily caused by the asymmetric loss of cartilage and bone, resulting in malalignment or subluxation.

### Use in Diagnosis, Monitoring, and Predicting Outcome

Conventional radiography is the standard method for diagnosis and follow-up of OA. Radiographic changes are key in the ACR criteria for classification of OA. A patient with radiographic osteophytes and knee pain, and who is older than 50 years, has had stiffness for less than 30 minutes, or has crepitus should be classified with OA.[48] However, newer imaging modalities, in particular MRI, now provide additional information which is used in clinical trials and to understand the disease.

Various scoring systems have been developed to grade the severity of OA. Among the most well-known is the Kellgren and Lawrence five-point scale, which has been widely used in OA research studies.[49] Further atlas-based scoring systems have been developed, which provide more accurate discrimination among individual radiographic features of OA.[49,50] Whichever system is used, the reliability is variable. In addition, quantitative measurement of joint space width as a surrogate for cartilage thickness has been used in research studies, particularly in cases of knee OA but also in the hip and hand, and is subject to the limitations discussed previously.

## COMPUTED TOMOGRAPHY

**KEY POINTS**

**Computed tomography (CT) is a tomographic radiographic imaging technique that visualizes calcified tissue with high resolution. CT is a standard reference to detect bone destruction.**

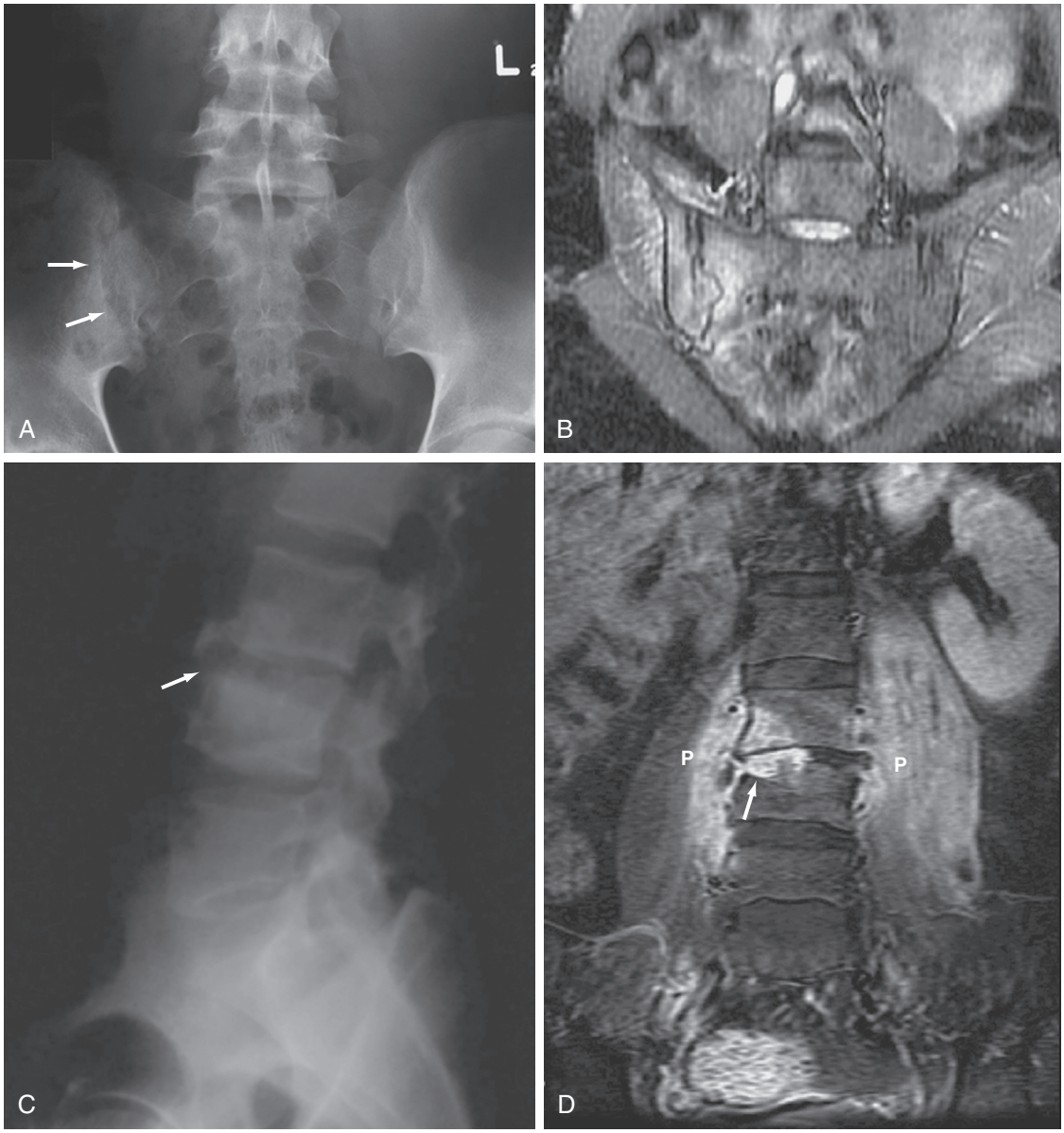



**Figure 58-8** **Septic arthritis (radiography and MRI). A,** Radiography in anteroposterior view of sacroiliac joints shows destruction of the right sacroiliac joint as part of septic arthritis *(arrows)*. **B,** In another patient, short tau inversion recovery (STIR) coronal MRI through sacroiliac joints shows erosive changes in the right sacroiliac joint with extensive bone marrow edema. **C** and **D,** Tuberculous spondylitis. **C,** Radiography of the lumbar spine in lateral view shows disk space narrowing at the L3-L4 level with destructive changes involving the superior end plate of the L4 vertebral body *(arrow)*. **D,** Coronal STIR MRI of the lumbar spine of the same patient confirms focal destruction of the L4 vertebral body *(arrow)*. Enlargement of both psoas muscles *(P)* with increased signal is noted, resulting from paraspinal extension of the infection.

**In rheumatology, CT is mainly useful to detect bone abnormalities in the axial skeleton (e.g., erosions, sclerosis, new bone formation, and fractures). CT is more sensitive for these purposes than radiography, and in most occasions MRI.**

**The role of CT of the axial skeleton is limited by the use of a relative high amount of ionizing radiation.**

**In practice, CT is rarely used unless radiography is unclear and MRI unavailable.**

**The main disadvantages of CT are the low sensitivity for soft tissue changes and the high patient exposure to ionizing radiation**

CT now has been available for 40 years, and each decade has brought further development in computing and engineering that has resulted in remarkable changes in the technology. Although still limited in soft-tissue contrast, CT offers fast and reliable acquisition, high resolution, and multiplanar capabilities that have enhanced its use in recent years. The modern CT scanner is a remarkable tool that will likely have an increasingly important role in arthritis imaging in the future.

## Technical Aspects

CT image acquisition is no longer restricted to the axial plane of imaging, and its multiplanar capability is so

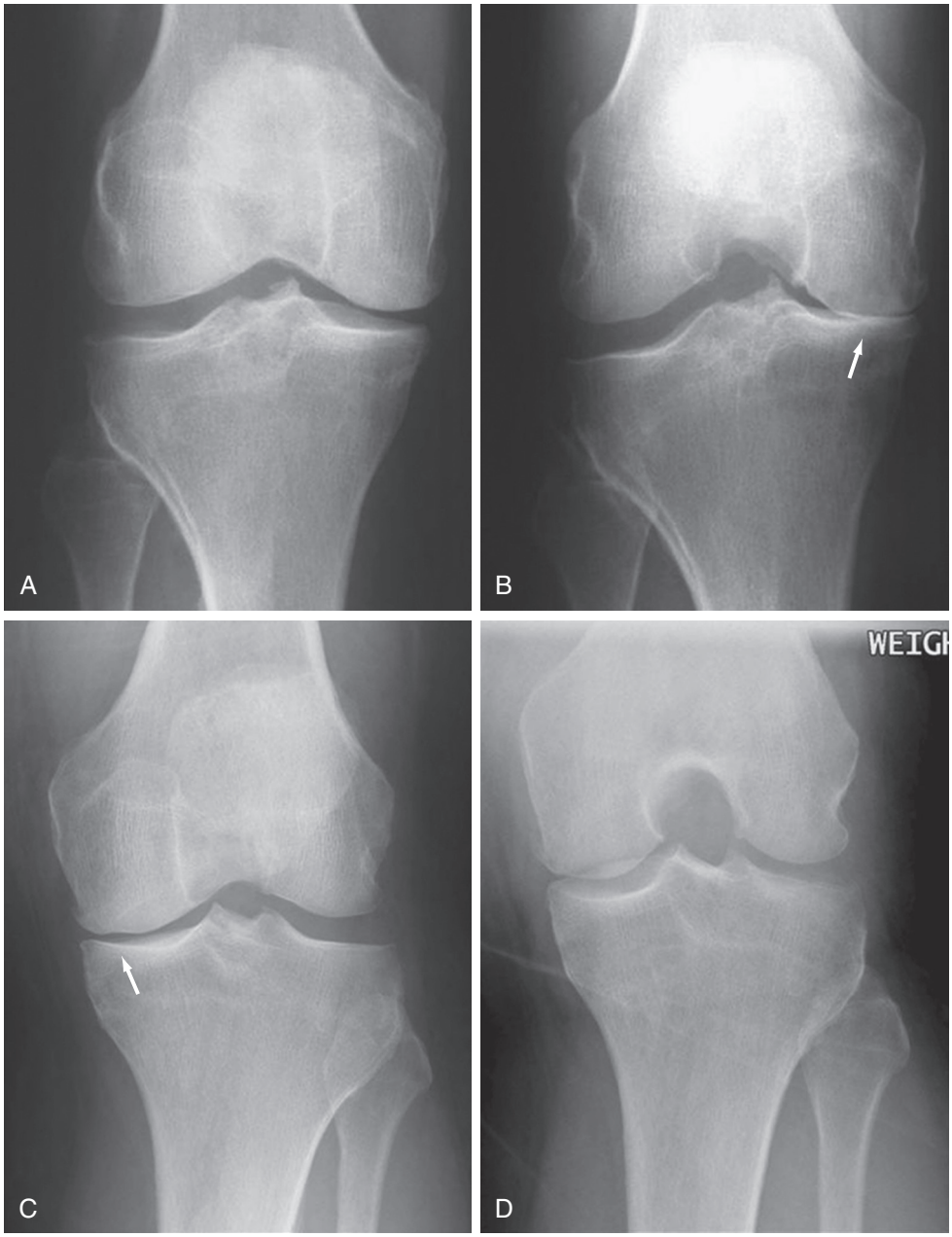



**Figure 58-9 Osteoarthritis of the knee (radiographs).** Non-weight-bearing radiographs of the knee frequently underestimate the severity of cartilage loss. A 59-year-old man with pain and suspected osteoarthritis had supine anteroposterior (AP) **(A)** and erect weight-bearing AP **(B)** radiographs of the knee. The supine view demonstrates mild osteophytosis and possible subtle joint space narrowing in the medial compartment, whereas on the weight-bearing view, severe medial joint space narrowing is present, indicating severe cartilage damage *(arrow)*. Usually the weight-bearing semi-flexed view is most sensitive to joint space narrowing (cartilage loss) but this is unpredictable: In a 53-year-old man, the erect AP view **(C)** shows more severe joint space narrowing *(arrow)* than is seen on the weight-bearing semi-flexed view **(D)**.

versatile that many CT scans of the body are now interpreted primarily from coronal or sagittal images in the same way as MRI. The data acquisition is so fast that patient motion is rarely a problem because CT scans are now routinely acquired in a few seconds. In fact, CT is often faster than multiple radiographs. Complex requirements for patient positioning in musculoskeletal CT are now largely irrelevant because almost all studies can be reconstructed to eliminate positioning problems. Patient tolerance is excellent and, unlike MRI, there are no absolute contraindications to CT. Spatial resolution is high, usually higher than MRI, and contrast resolution between soft tissue and bone is unsurpassed by any other modality. However, despite all the advantages listed above, the application of CT in arthritis imaging still has two fundamental flaws that prevent its universal application. First, CT is constrained in the same way as radiography by its limited soft tissue contrast capability. Second, ionizing radiation is used with increasing dose proportional to size of the body part and requirements for spatial detail. Although this is not a problem with the more distal extremities because the exposure doses are smaller and the tissue more radioresistant, it remains an issue for spine,

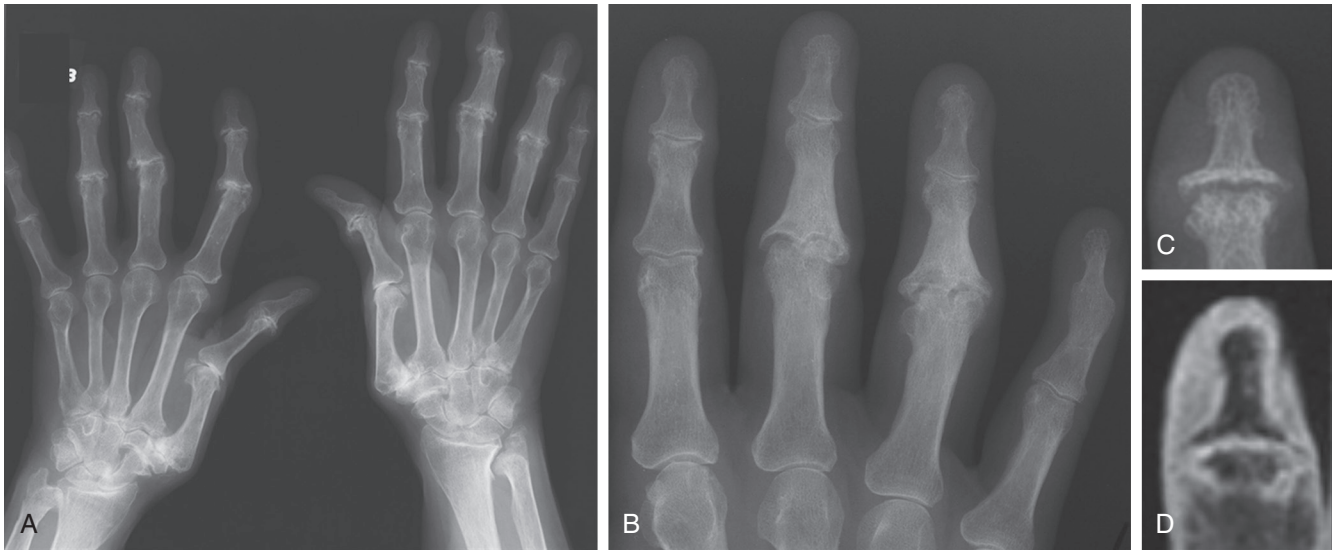



**Figure 58-10 Osteoarthritis of the hand (radiography and MRI). A,** Radiography shows asymmetric cartilage space narrowing at the proximal and distal interphalangeal joints, the first carpometacarpal joints, and the scaphoid-trapezium-trapezoid articulations. Subluxation at the first carpometacarpal joint and secondary hyperextension at the metacarpophalangeal joints are characteristic deformities of osteoarthritis. **B,** "Gull wing" deformities in erosive osteoarthritis: Cartilage loss and bone remodeling at the third and fourth proximal interphalangeal joints produce a gull wing appearance. Radiography **(C)** and coronal MRI **(D)** of distal interphalangeal joint show osteophyte and central erosions/collapse of the bone plate, representing severe osteoarthritis. *(**C** and **D,** Courtesy Dr. Ida Haugen, Oslo, Norway.)*

hip, and shoulder CT. Consequently, in most routine clinical situations, radiography provides sufficient information of a similar nature to CT for clinical decision making and is cheaper than CT and readily available. Ultrasonography is a better and cheaper way to visualize and quantify superficial soft tissue pathology; and MRI offers superior soft tissue contrast and bone marrow imaging. However, the technology continues to evolve, and low-dose CT is becoming more common, with iterative reconstruction techniques which reduce the radiation exposure by approximately 80% and bring the CT dose much closer to conventional radiographic exposure.[51]

## Rheumatoid Arthritis

The exquisite inherent contrast between bone and soft tissue that is afforded by CT makes it a gold standard reference for the detection of bone erosion and, as such, CT is ideally suited to the investigation of erosion in inflammatory arthritides. Modern CT with isotropic voxel acquisition and three-dimensional visualization allows accurate detection and quantification of bone erosion with good intra-observer agreement, whereas radiography is limited by its two-dimensional projection and superimposition of structures. However, CT is very limited in its ability to visualize soft tissue changes and, even with contrast enhancement and complex subtraction techniques, CT is still inferior to MRI and ultrasound for assessment of synovial changes, such as thickening and hyperemia. Furthermore, detection of erosion on CT and MRI shows very good agreement, although CT is slightly more sensitive.[52,53]

### Use in Diagnosis, Monitoring, and Predicting Outcome

CT is not currently used in routine clinical practice for the diagnosis of RA. However, it is potentially useful for diagnosis because CT appears to be the most sensitive technique for detection of erosion,[52,53] and CT of the feet is very simple with a very low radiation dose. CT is used for problem solving in specific cases, such as examination of the cervical spine if MRI is unavailable or contraindicated.

The use of CT for longitudinal assessment of damage progression has potential merit.[53,54] A problem with CT in comparison with MRI or ultrasonography is its inability to demonstrate improvement in soft tissue pathology. No CT data are available for prognostication in RA. The current use of CT in RA is very limited, although its ability to define erosion so clearly offers opportunities for future developments in early diagnosis and clinical trials research.

## Ankylosing Spondylitis/Axial Spondyloarthritis

CT allows for visualization of the same pathologic processes as conventional radiography—erosion, osteoporosis/sclerosis, and new bone formation/ankylosis, with the added benefit of multiplanar imaging free from superimposition of overlying structures (Figure 58-11).

In AS, the pathologic processes start in bone marrow and at sites of enthesis. However, CT has poor ability to detect soft tissue change and the imaging results are usually normal until structural damage is present. CT can detect osteoporosis or osteosclerosis quite well, but these changes are nonspecific. The primary value of CT in AS is its ability to detect and clearly define erosion of bone at any joint or enthesis. New bone formation is also well visualized in the form of syndesmophytes, ligamentous ossification, and periarticular and intra-articular ankylosis, but the use for CT in this regard is limited. CT can show these findings equally well in the axial and peripheral skeleton, but is used primarily in areas where radiographic visualization or interpretation is problematic.

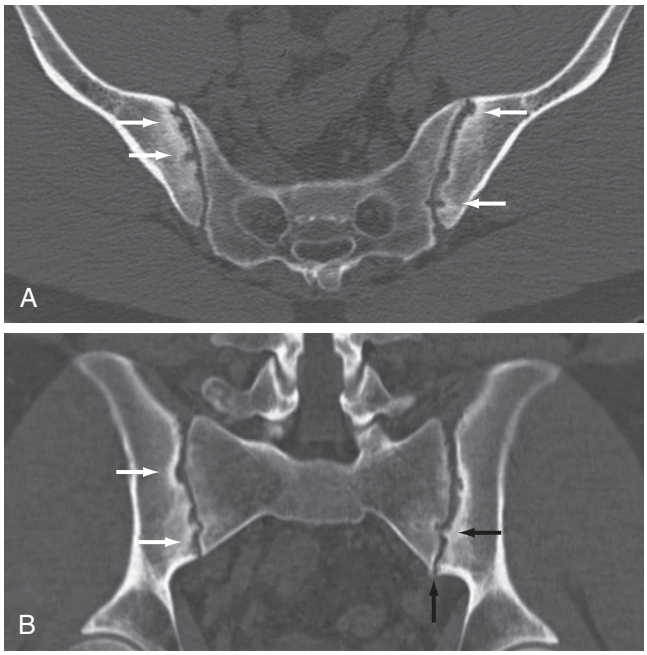



**Figure 58-11 Bone erosion in sacroiliac joints in spondyloarthritis (computed tomography [CT]).** Coronal **(A)** and axial **(B)** CT images of the sacroiliac joints in a patient with spondyloarthritis. Bone erosions *(white arrows)* and mild sclerosis and new bone formation *(black arrows)* are seen.

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** The diagnosis of AS is primarily based on the radiographic observation of bilateral moderate or unilateral severe sacroiliitis. When good quality radiographs of the sacroiliac joints are normal, or radiographic changes meet diagnostic criteria, there is no role for CT. Early detection of AS is better investigated by MRI and, if clearly defined structural damage is present on radiography, then there is no additional diagnostic utility for CT. However, CT of the sacroiliac joints is much easier to interpret than radiography, which is notoriously subject to poor observer reliability. When the radiographic findings are unclear, CT will usually resolve this uncertainty. Since CT shows bone erosion in exquisite detail, CT may also have a role to play in the further investigation of MRI equivocal findings. It should be noted that classification criteria for AS depend on radiographic findings and more recently MRI but not CT specifically. In the spine, CT is useful in the diagnosis of complications of late disease such as spondylodiskitis or spinal fracture, when patients may be unable to tolerate MRI because of pain or spinal deformity.

**Monitoring Disease Activity and Damage.** CT has no role in monitoring disease activity or damage at this time, because CT cannot show active inflammation and has a relatively high radiation dose. However, recent studies have shown that quantitative measurement of syndesmophyte formation in the spine is reliable and significantly more sensitive to change than assessment by radiography.[55] If these results can be reproduced at lower radiation exposure levels, low-dose CT could have an important future in the monitoring of structural damage progression in the spine.

**Predicting Outcome.** The prognostic value of CT findings of sacroiliitis require further investigation.

## Psoriatic Arthritis

CT is rarely used for the investigation of PsA, and few data are published on this topic. It has been confirmed that, in comparison with radiography, CT is much more sensitive for the detection of small foci of erosion or proliferation of the small bones of the hand.[56,57] CT was highly reliable for the detection of erosion but less so for proliferation, and neither radiography nor CT detected significant change in the bone findings in this short trial. In the peripheral skeleton, it would be expected that the application of CT for PsA would be similar to RA and in the axial skeleton, use of CT would be similar to AS. In both cases, use of CT is limited by the inability to directly visualize inflammation in either bone or soft tissue and, although it has superior ability versus radiography to detect bone erosion and proliferation, in most circumstances the extra radiation dose is not warranted.

### Use in Diagnosis, Monitoring, and Predicting Outcome

CT visualizes bone erosion and bone proliferation, but no systematic studies of specific diagnostic utility or prognostic value have been performed. Because of radiation concerns, CT is not used for disease monitoring in PsA.

## Gout

CT allows for clear visualization of bone erosion, and recent developments in technology offer exciting prospects for crystal imaging. Acute and chronic tophaceous gout is associated with deposition of urate crystals in soft tissues and the development of tophi. Chronic tophi are often partially calcified and all CT scanners are sensitive for the deposition of calcium in soft tissues (see Figure 58-6). The condition frequently involves intraosseous deposition of crystals, and this is much better visualized on CT than radiography.[58] CT shows soft tissue swelling caused by tophi quite well, and because anatomic bone detail is very good on CT, erosions are clearly delineated. Dual-energy CT (DECT) is a new technological development whereby images can be reconstructed based on a two-material decomposition algorithm that can separate calcium from monosodium urate (Figure 58-12).[59] DECT has been used to show that monosodium urate crystals are frequently present in joints affected by radiographic damage in gout, which supports the concept that MSU crystals interact with articular tissues, contributing to the development of structural joint damage.[60]

### Use in Diagnosis, Monitoring, and Predicting Outcome

CT visualizes bone erosions and tophi, but few systematic studies of specific diagnostic utility have been performed. Until recently, CT had no role in the diagnosis of acute gout before the development of bone erosions or tophi because it does not provide imaging of synovitis, tenosynovitis, or osteitis. However, CT scanning with dual-energy acquisition (DECT) is a recent technical development which can accurately detect the presence of monosodium urate with approximately 90% sensitivity and 80% specificity and with excellent reader reliability.[61,62] The sensitivity is less in patients with acute gout, but DECT is quite specific in this

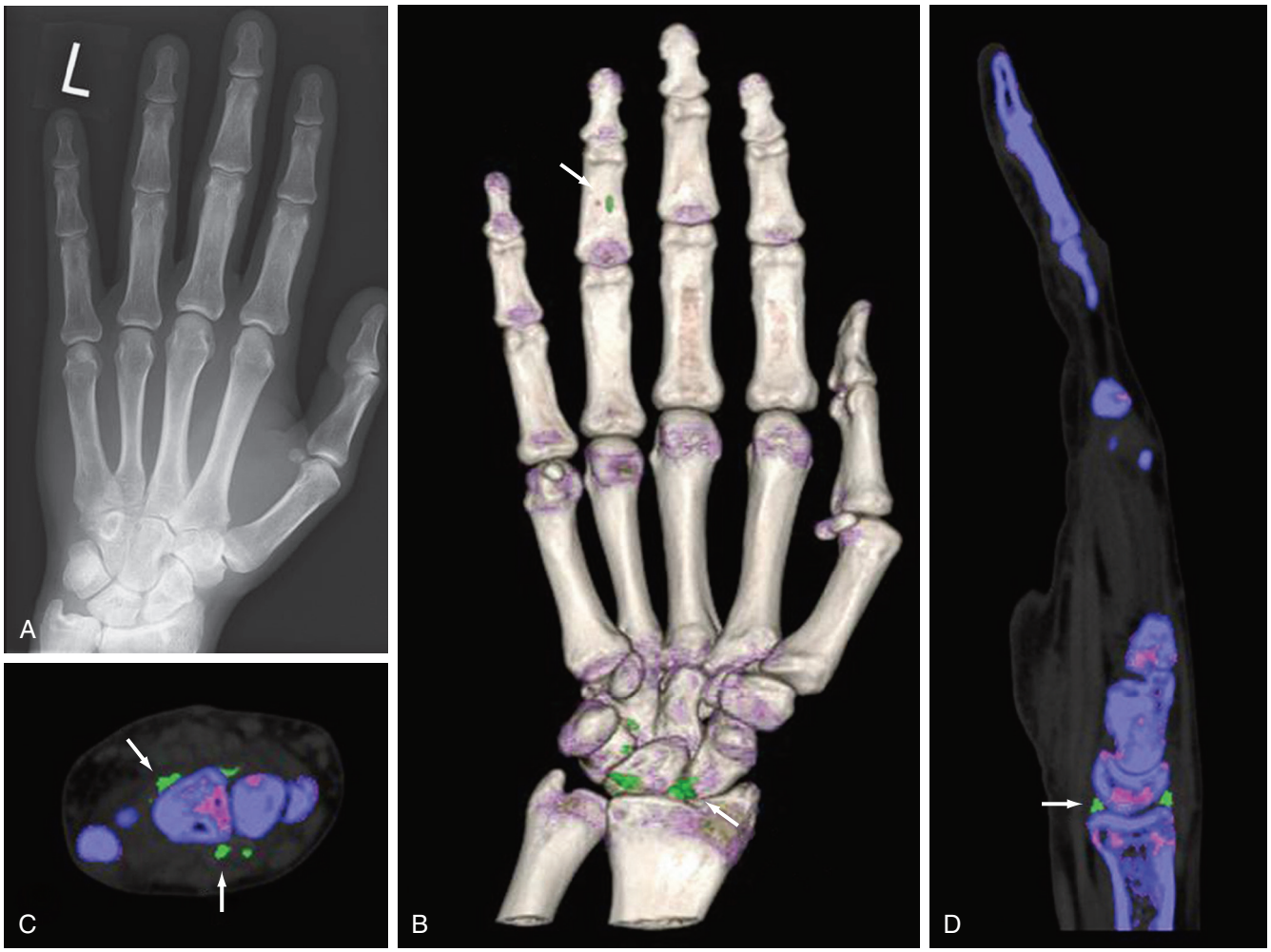



**Figure 58-12 Gout (radiography and dual-energy computed tomography [CT] scan).** Radiography **(A)** and dual-energy CT scans (**B** through **D**) of the left hand and wrist of a 39-year-old male patient with recent onset bilateral pain in hands and wrists. Radiography **(A)** and laboratory investigations were all normal. Dual-energy CT was performed and confirmed the presence of sodium urate crystals in the left wrist and one finger. Left wrist aspiration was attempted but was unsuccessful. The patient responded well to treatment for gout. Imaging findings: After processing of CT data with an algorithm specific for detection of urate crystals (Siemens, Erlangen), the urate crystals are displayed green *(arrows)* on three-dimensional anterior **(B)**, two dimensional transverse **(C)**, and two-dimensional sagittal **(D)** reconstructions.

group, whereas the specificity is less when OA is present. The greatest diagnostic utility of DECT would appear to be in cases of acute gout in small joints, in which the differential diagnosis is septic arthritis and joint fluid is unobtainable, or fluid microscopy fails to demonstrate monosodium urate crystals. Unfortunately, the technology is limited to CT scanners that have dual-source technology, and these scanners are very expensive and not currently widely available.

CT, including DECT, is reliable for monitoring tophus size.[41,63] A bone erosion scoring method has recently been developed.[64] These methods may be useful in trials.

## Calcium Pyrophosphate Dihydrate Crystal Deposition Disease

CT is not used in the management of uncomplicated peripheral CPPD, although calcifications can be visualized. CPPD can involve the cervical spine and cause neck pain. In such patients, CT may visualize CPPD deposition at the craniovertebral junction, often in the transverse ligament of atlas (crowned dens syndrome).[65,66] Calcific deposits may also be found in other periodontoid structures and the ligamenta flava in some patients, in addition to osseous abnormalities of the odontoid process, such as subchondral cysts or erosions. CT may also be used to verify the presence of calcifications at other rare locations (e.g., the temporomandibular joints).[67] In peripheral joints, CT can accurately depict the distribution of CPPD crystals, and has confirmed that, in fact, soft tissue deposition outside cartilaginous structures is common, especially in ligaments and joint capsules.[68]

## Septic Arthritis

CT is rarely used for the investigation of septic arthritis. Acute septic arthritis of large joints is a surgical emergency and although radiography, fluoroscopy, and ultrasound may be useful for either diagnosis or image-guided aspiration, CT is not useful in the peripheral skeleton. In chronic septic arthritis and infectious discitis, CT may be used to investigate complications, particularly osteomyelitis. However,

MRI or a variety of scintigraphic studies are generally preferred.[45] In complex cases in which MRI scanning is problematic, contrast-enhanced CT can be very useful to detect abscess formation in deep soft tissues that are not easily assessed by ultrasound, and CT guidance may be preferred for spine biopsy.

## Osteoarthritis

Even though CT can detect bone changes such as osteophytes more accurately than radiography,[69] overall it offers little additional diagnostic utility in peripheral joints. It generally has poor soft tissue contrast capability, hyaline cartilage cannot be directly visualized, and spatial resolution of bone detail is inferior to radiography. It may be used in specific problem-solving situations in which anatomy is complex and/or poorly visualized on radiography, such as in the lumbar spine facet joints, but this is not usually the case in the peripheral joints. CT is occasionally used to assess the extent of disease, such as in the orthopedic assessment of large joints, where detailed evaluation of bone morphology may be required pre-operatively. CT scanning with intra-articular contrast material (CT arthrography) may be the single most accurate modality for detection of cartilage thinning, fissuring, flaps, or volume[70] (Figure 58-13) and loose intra-articular bodies. This requires both a minimally invasive procedure and radiation exposure and is not widely used. Experimentally it has been shown that CT arthrography can detect changes related to alteration of the glycosaminoglycan (GAG) content of hyaline cartilage[71]) and that it correlates well with gadolinium-enhanced MRI estimates of cartilage damage, but neither modality correlates with arthroscopic findings in early disease.[72] CT arthrography may be used as a problem-solving tool in patients who are not able to undergo MRI.

### Use in Diagnosis, Monitoring, and Predicting Outcome

CT is not currently used in routine clinical practice for the diagnosis of OA, has no role for monitoring disease because it cannot quantify change in the target tissue (cartilage) without an invasive procedure, and no published data are available on the independent prognostic use of CT because good-quality radiography usually provides the same or more information.

# MAGNETIC RESONANCE IMAGING

**KEY POINTS**

**MRI allows sensitive visualization and assessment of peripheral inflammatory and destructive joint and soft tissue involvement in a variety of degenerative and inflammatory rheumatic diseases.**

**MRI is by far the best available method for detecting and monitoring inflammation in the spine and sacroiliac joints in AS and other spondyloarthritides.**

**MRI allows monitoring of inflammatory soft tissue changes (e.g., synovitis, tenosynovitis, and enthesitis) during treatment of patients with peripheral inflammatory joint diseases.**

**Through detection of early inflammatory changes, MRI can contribute to earlier diagnosis of RA and SpA.**

**MRI bone edema is uniquely visualized by MRI, and provides prognostic information in RA, SpA, OA, undifferentiated inflammatory arthritis and, possibly, other rheumatic arthritides**

**The main disadvantages of MRI are costs and lack of availability.**

MRI provides multiplanar tomographic imaging with unprecedented soft tissue contrast, without the use of ionizing radiation, and allows assessment of all the structures involved in musculoskeletal diseases. MRI is more sensitive than clinical examination and radiography for detection of inflammation and damage in inflammatory and degenerative rheumatologic disorders. Disadvantages of MRI include higher costs and lower availability than radiography, longer examination times and restriction to a limited anatomic area per session. It should be remembered, however, that the costs of MRI are only a small fraction of the cost of biologic treatment or of the indirect costs of sick leaves and early retirements.

Preceded by a section on key technical aspects, the characteristics of MRI (with respect to diagnosis, monitoring, and prognostication) and its clinical utility in RA, SpA, PsA, gout, and OA will be described in subsequent text.

## Technical Aspects

T1-weighted (T1w) imaging sequences are characterized by relatively short imaging times, good anatomic detail and the ability to visualize tissues with high perfusion and permeability, including the inflamed synovium, after intravenous contrast (paramagnetic gadolinium [Gd] compounds) injection. Fat and Gd-enhanced tissues have a high signal intensity on T1w images (Figure 58-14). T2-weighted (T2w) images depict both fat and fluid/edematous tissues with a high signal intensity. These are used together with T1w images in the diagnosis of degenerative spine disease. In inflammatory conditions T2w images with fat-saturation (FS) (Figure 58-15) are particularly useful because the signal from fat is suppressed in such sequences. This allows the detection of edematous tissue/fluid located in areas with fatty tissue (e.g., bone marrow edema). FS requires a homogenous field and a high magnetic field strength, not available on low field (<1.0 T) MRI units. The only fat-suppressed sequence possible on low-field MRI, the STIR (short tau inversion recovery) technique, based on relaxation time differences, can provide information on bone marrow edema,[73] even though with less detail.

Whereas MRI examination of axial joints require whole-body MRI units, MRI of the peripheral joints can be performed with whole-body MRI-units or dedicated extremity MRI-units (E-MRI). E-MRI increases the potential for widespread rheumatologic use, through markedly lower costs, more comfortable patient positioning, and elimination of claustrophobia.

Some E-MRI units may provide information on synovitis and bone destructions not markedly inferior to what is obtained by standard sequences on high-field units,[73,74] but the performance of different machines varies. For some

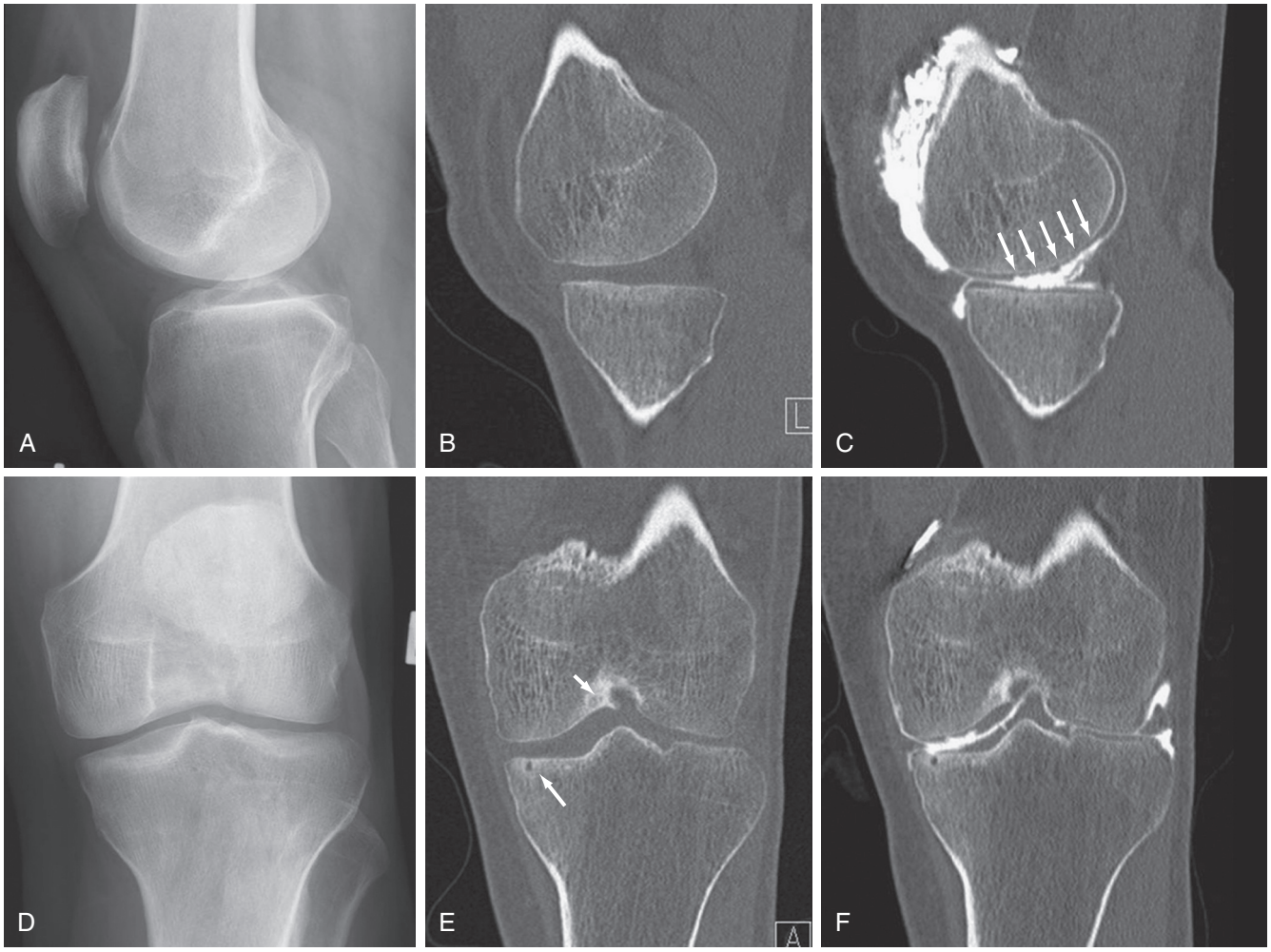



**Figure 58-13** Radiography, computed tomography (CT), and CT arthrogram of the knee in a 46-year-old male patient with unexplained pain and locking. Initial radiographs (**A** and **D**) appear near normal with preserved joint space, a tiny osteophyte arising from the medial tibial spine, and no other definite abnormality. CT scan with sagittal **(B)** and coronal **(E)** reconstructions confirms the presence of osteophytosis *(short arrow)* and a small subchondral cyst *(long arrow)* but show no definite chondral changes. CT arthrogram with sagittal reconstruction **(C)** demonstrates several focal hyaline cartilage thinning *(arrows)* and a meniscal tear. On the coronal reconstruction **(F)**, the tibial subchondral cyst is associated with complete loss of overlying hyaline cartilage.

units smaller field of view, longer imaging times, and lack of certain imaging techniques (particularly FS at low field) should be considered.[75]

The majority of MRI studies of the sacroiliac joint have used only one imaging plane (semi-coronal, i.e., parallel with the axis of the sacral bone). A supplementary T1w FS sequence may improve the evaluation of erosions,[76] and sequences designed for cartilage evaluation (e.g., 3D gradient echo sequences), may also be added.[77] To be maximally sensitive for changes in the ligamentous portion of the sacroiliac joints, imaging in the semiaxial plane is required.[76] This sequence may therefore be recommended when MRI is used for diagnostic purposes, although it is probably not essential when used as an outcome measure in trials. Although MRI on some indications (e.g., suspected disk herniation) should include axial images, MRI of the spine in SpA generally only involves sagittal images, but these should extend sufficiently lateral to include the frequently involved facet, costovertebral, and costotransversal joints.[78] Bone marrow abnormalities in both the sacroiliac joints and spine are detected almost equally well with the STIR and contrast-enhanced T1w FS sequences in patients with SpA, thus contrast injection is generally not needed.[79,80] In contrast, contrast injection increases the sensitivity for synovitis in peripheral joints.[81] To evaluate structural (sometimes referred to as *chronic*) changes, such as bone erosion, new bone formation, and fat infiltrations, T1-weighted images are mandatory. A supplementary T1w FS sequence may improve the evaluation of erosions.[76] In peripheral as well as axial joints, specific sequences designed for optimal cartilage evaluation can be applied (e.g., 3D gradient echo sequences).[77]

New MRI techniques are being developed all the time, and a variety of sequences are now available for the detection of increased water content in bone marrow such as occurs with inflammation—so-called *bone marrow edema* (BME). These include T2 with fat-saturation (T2 FS), T2 with water-excitation (WE), STIR, hybrid sequences, and chemical shift–based fat-water separation with the Dixon technique.[82] All of these sequences have advantages and

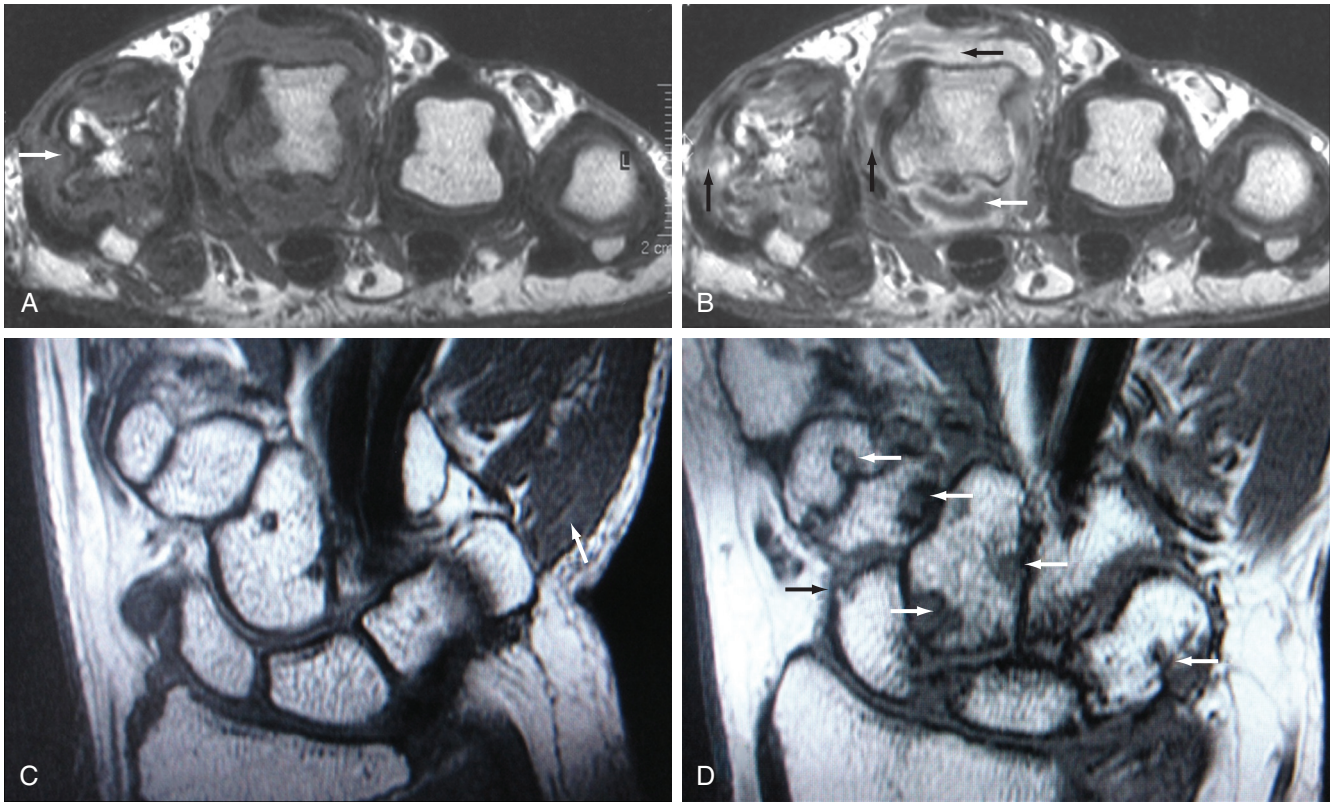



**Figure 58-14 Rheumatoid arthritis (MRI).** Axial T1-weighted precontrast **(A)** and postcontrast **(B)** MRI of the second *(left)* through fifth *(right)* fingers at the level of the metacarpophalangeal joints in a patient with rheumatoid arthritis (RA). Synovitis (*black arrows* in **B**), joint effusion (*white arrow* in **B**), and severe bone erosion (*arrow* in **A**) are seen. Coronal T1-weighted precontrast MRI of the wrist **(C)** in another patient with RA shows no bone erosions. Similar images 1 year later **(D)** show severe erosive progression (bone erosions marked by *arrows*).

disadvantages, and at this time it would appear that none of them consistently alters the diagnostic properties of MRI for the detection of BME. Most centers continue to use the more traditional T2 FS or STIR sequences because almost all MRI scanners can perform these two sequences, they are generally reliable, and fairly consistent across different manufacturers and magnetic field strengths.

The potential for molecular imaging with MRI is an exhilarating prospect for arthritis research and the future of clinical practice. Most of the work in this field is still at an experimental stage, but numerous techniques are under development for evaluation of the chemical and biophysical structure of musculoskeletal tissues, and articular cartilage in particular. Many publications now attest to the capabilities of T2 or T2* relaxation time mapping,[83,84] delayed gadolinium–enhanced MRI of cartilage (dGEMRIC).[85,86] T1ρ imaging (T1rho–T1 properties in the rotating frame of reference),[87,88] glycosaminoglycan chemical exchange saturation transfer (gagCEST),[89,90] diffusion tensor imaging (DTI),[91,92] and sodium MRI.[93,94] All of these MRI developments are highly technical, require dedicated software and/or hardware, and are very challenging to perform consistently. Several have been proposed for use in clinical practice, but none have established unequivocal utility beyond a standard set of high-quality sequences. Of all these new developments, DTI may have the most promise (Figure 58-16). It is a noninvasive technique in which the signal produced is dependent on water movement. One component of the DTI produces a result that is primarily dependent on the speed of water movement independent of direction—apparent diffusion coefficient (ADC)—creating an ADC map, whereas another component of the DTI, fractional anisotropy (FA), produces a result that is primarily dependent on the direction of water movement and is much less dependent on speed, creating an FA map. This technique is interesting because in cartilage the ADC is affected mostly by glycosaminoglycan concentration and distribution, whereas the FA is heavily dependent on the orientation and degree of organization of the collagen fibers. The DTI sequence would therefore seem to be capable of supplying all, or most, of the information available from all the other technologies combined.

Whole-body MRI is another technique in development. It allows imaging of the entire body in one examination, and has been introduced as a potential method for simultaneous assessment of peripheral and axial joints and entheses in both RA (Figure 58-17) and SpA/PsA (Figure 58-18).[95,96] The method still needs improved image quality and more validation, and it is not yet ready for clinical use. However, the method seems extremely promising, especially in PsA, because of the diverse manifestations of the disease.[96-98]

MRI is very safe. It involves no ionizing radiation and thereby no associated increased risk of malignancies. Adverse reactions to gadolinium-based contrast media (GBCM) are infrequent. In 2006 it became clear that that there is a link between exposure to some less stable GBCM and the development of nephrogenic systemic fibrosis (NSF) in patients with reduced renal function or on

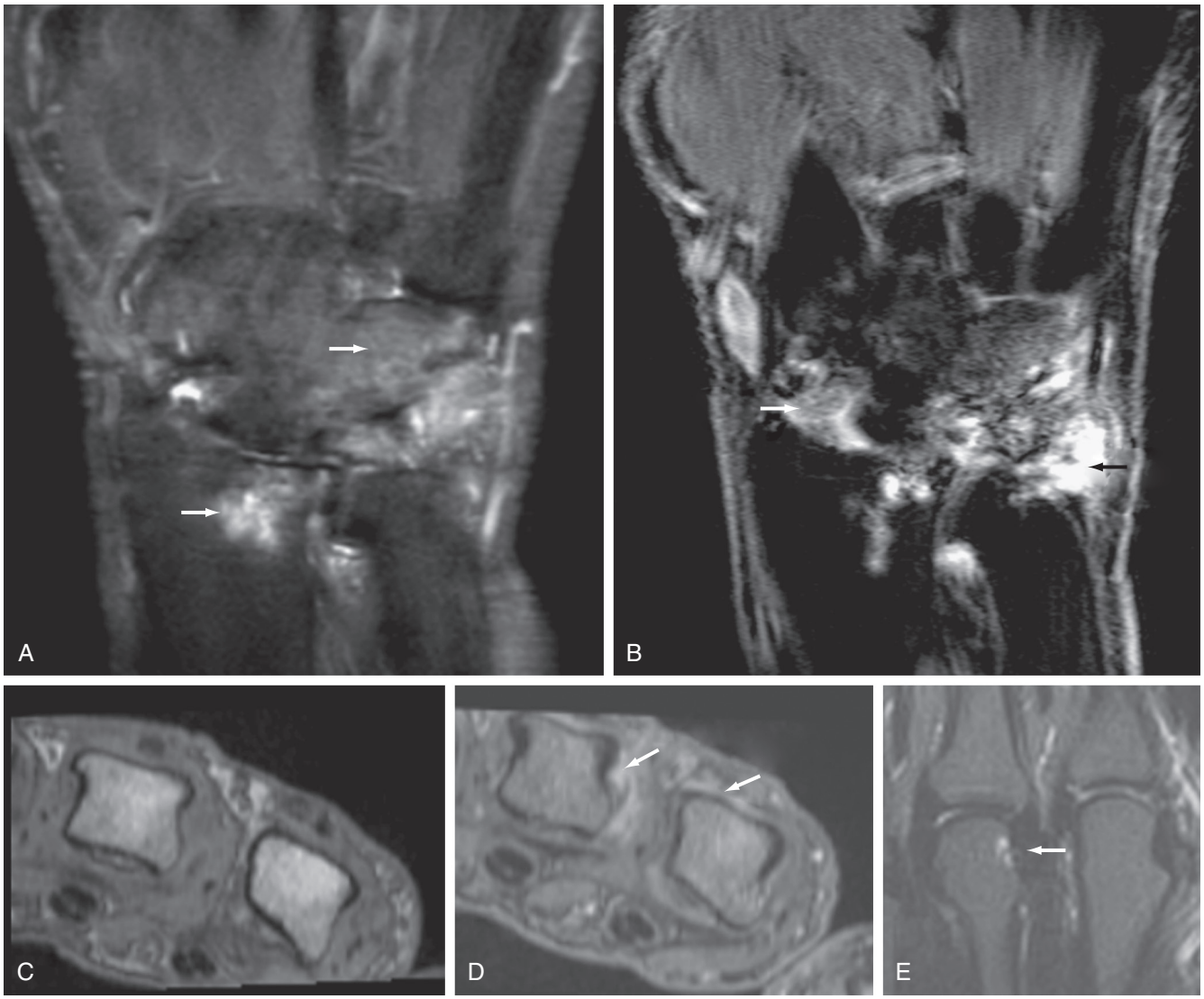



**Figure 58-15 Rheumatoid arthritis (RA) in clinical remission (MRI) and undifferentiated arthritis (MRI). A** and **B**, Patient with RA in clinical remission. Despite clinical remission, coronal short tau inversion recovery (STIR) **(A)** and postcontrast T1-weighted fat-suppressed **(B)** MRI show marked bone marrow edema (osteitis, *arrows* in **A**) and synovitis (*arrows* in **B**). **C** through **E,** Patient with undifferentiated arthritis. Axial T1-weighted precontrast **(C)** and postcontrast **(D)** T1-weighted MRI views show synovitis in second and third metacarpophalangeal joints *(arrows)*, whereas the STIR sequence **(E)** shows bone edema *(arrow)*. Radiography was normal. One year later the patient had developed RA, according to the American College of Rheumatology 1987 criteria. *(Courtesy Dr. Anne Duer-Jensen, Glostrup, Denmark.)*

dialysis.[99,100] NSF is a rare fibrosing condition which causes thickening and hardening of the skin, and sometimes involves organs such as liver, lungs, and heart.[101,102] In 2007, the European Medicines Agency (EMA) contraindicated the use of the least stable GBCM (gadoversetamide, gadodiamide, and gadopentetate) in patients with severely reduced renal function (glomerular filtration rate [GFR] <30 mL/min/1.73m$^2$) or on dialysis and required that they be used with caution in patients with moderately reduced renal function (GFR, 30 to 60 mL/min/1.73m$^2$). In 2010 the U.S. Food and Drug Administration (FDA) also contraindicated the three agents.[103] Since 2009 no new cases have been published, and NSF may have been eliminated by no longer using the less stable gadolinium-based contrast agents in patients with reduced renal function, or, in some countries, by not using the less stable agents at all.[104]

The European Society of Urogenital Radiology (ESUR) guidelines recommend that one should never deny any patient a well-indicated, enhanced MRI. Furthermore, one should use the lowest diagnostic dose of GBCM and agents with the highest stability (lowest risk of NSF) (e.g., gadoterate meglumine, gadoteridol, and gadobutrol).[105] The pharmacokinetics and diagnostic performance of these agents are similar to those contraindicated in patients with poor renal function. Therefore, it seems logical to use the low-risk agents in every patient, independent of renal function.

## Rheumatoid Arthritis

The majority of MRI studies in patients with RA have investigated knee, wrist, and finger joints. Although the

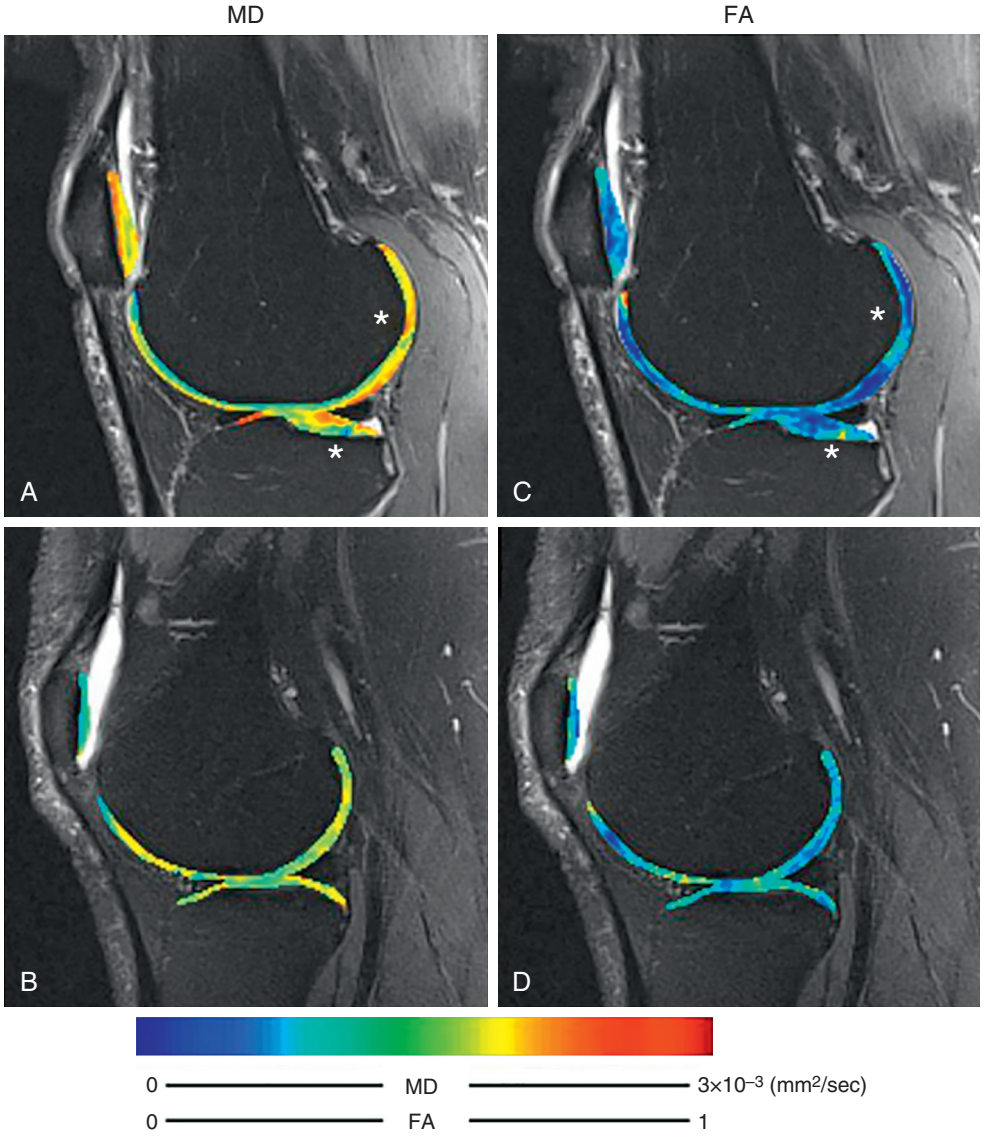



**Figure 58-16 Diffusion tensor imaging (DTI) of hyaline cartilage: sagittal MRI of the knee performed at 3 tesla by using a RAISED (radial spin echo diffusion tensor imaging) sequence.** Diffusion tensor imaging is an MRI technique that displays intensity of signal, often by using color scales, to represent specific properties of water within a tissue. It has two primary components that relate to water movement at the microscopic level. Mean diffusivity (MD) (**A** and **C**) is the mean of the apparent diffusion coefficients (ADC) and is representative of the degree (speed) of water movement (brighter = faster). Anisotropy is the property of being directionally dependent and fractional anisotropy (FA) (**B** and **D**) records the degree to which the direction of water movement in a tissue is constrained by the microscopic architecture of the tissue (brighter = more directionally dependent and less random). Subject 1 (**A** and **B**) is a patient with Kellgren Lawrence grade 2 osteoarthritis (OA). Some of the water in the hyaline cartilage (adjacent to *asterisks*) is less tightly bound, and thus the MD of the cartilage appears more in the red end of the scale in comparison with subject B, who is a healthy volunteer. Damage to the cartilage in early OA results in proteoglycan loss, and the water in the cartilage is less tightly bound and thus it can move faster. The structure of the collagen network may also be damaged in early OA, and the FA map in subject 1 shows that some of the cartilage has less fractional anisotropy and appears as a darker blue (adjacent to *asterisks*) compared with the healthy volunteer (subject 2) (**C** and **D**). While the highly organized collagen network starts to break down, the motion of the water becomes more random and less directionally dependent. By using these measurements, DTI has the unique capacity to separately detect damage in hyaline cartilage to both collagen and proteoglycan. *(Courtesy Dr. José Raya, New York, New York.)*

knee is an excellent model for methodological studies, the clinical value of MRI is mainly dependent on its power to evaluate wrists, hands and feet, which is also the primary focus of this section. Reports on other peripheral joints are few and not essentially different. Definitions of key pathologies in RA and PsA are provided in Table 58-3.

MRI allows for assessment of all the structures involved in RA ( i.e., synovial membrane, intra- and extra-articular fluid collections, cartilage, bone, ligaments, tendons, and tendon sheaths; see Figure 58-14). MRI, histopathologic, and mini-arthroscopic signs of synovial inflammation are closely correlated.[106,107] MRI bone marrow edema (see Figure 58-14) represents inflammatory infiltrates in the bone marrow (i.e., osteitis, as demonstrated by comparison with histologic samples obtained at surgery in patients with RA).[108,109] Whereas erosions reflect bone damage that has already occurred, bone marrow edema appears to represent the link between joint inflammation and bone destruction.




**Figure 58-17 Whole-body MRI of patient with rheumatoid arthritis (RA) and osteoarthritis and degenerative disk disease.** Short tau inversion recovery MRI of the shoulder girdle **(A)**, the right hand **(B)**, the hips **(C)**, the left hand **(D)**, the right **(E)** and left **(F)** knee, the right **(G)** and left **(H)** ankle and midfoot, the right **(I)** and left **(J)** foot, and the upper **(K)** and lower **(L)** spine. MR images show mild inflammation of the right acromioclavicular joint (*arrow* in **A**); inflammation of the wrist, first carpometacarpal joints, and several metacarpophalangeal and interphalangeal joints (*arrows* in **B** and **D**); a Baker's cyst in the right knee (*arrow* in **E**); inflammation in the right medial tarsometatarsal joint (*arrow* in **G**); tenosynovitis of the left flexor hallucis longus tendon (*arrows* in **H**); inflammation in the right first metatarsophalangeal joint (*arrow* in **I**); and several disk protrusions (*arrows* in **K** and **L**), but no spinal inflammation. The images were obtained in less than 45 minutes on a 3 Tesla MRI unit.

A high level of agreement for detection of bone erosions in wrists of patients with RA and MCP joints (concordance at 77% to 90% of sites) between MRI and CT, which is the gold standard reference for detection of bony destruction, documents that MRI erosions represent true bone damage.[52,110]

In the cervical spine, the primary imaging modality is CR, but MRI can provide detailed information on bone and soft tissue abnormalities, which can be a valuable supplement to radiographic evaluation[111-117] (see Figure 58-2C). MRI is able to directly visualize the pannus tissue (e.g., around the odontoid process). Cord compression on MRI seems a better predictor for deterioration than initial clinical and plain radiographic features, evidence that MRI is the imaging method of choice for evaluation of spinal cord affection in RA.[118]

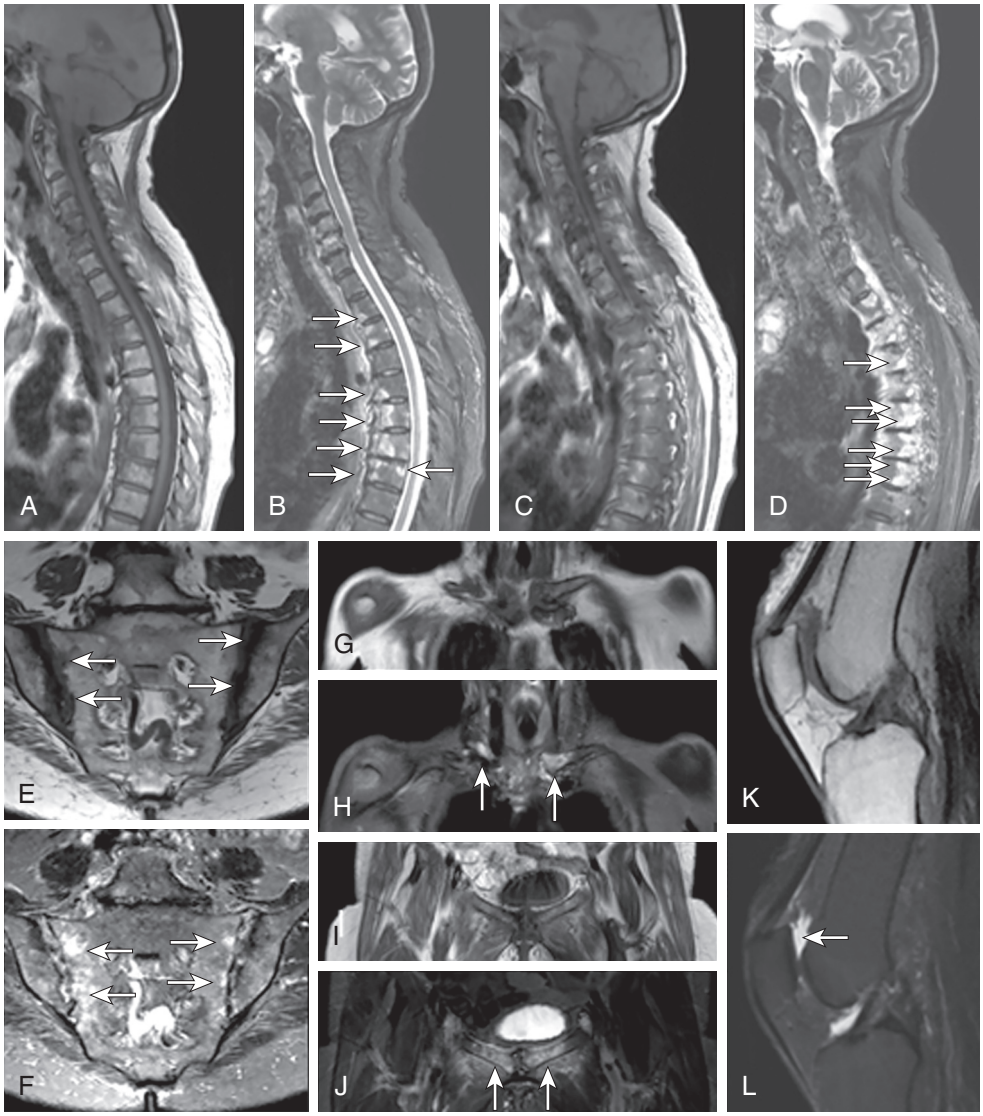



**Figure 58-18 Whole-body MRI of patient with spondyloarthritis (SpA).** T1-weighted (**A**, **C**, and every second image thereafter) and short tau inversion recovery (**B**, **D**, and every second image thereafter) at before (**A** through **L**) and 4 months after (**M** through **Y**) initiation of anti-TNF therapy. Before anti-TNF initiation, MR images of the central (**A** and **B**) and lateral (**C** and **D**) cervical and thoracic spine shows anterior corner inflammatory lesions (*arrows* in **B**) and inflammation in the pedicles and costovertebral joints (*arrows* in **D**). MRI of the sacroiliac joints (**E** and **F**), shoulder girdle (**G** and **H**), anterior pelvic girdle (**I** and **J**) and right knee (**K** and **L**), shows severe sacroiliac bone erosion (*arrows* in **E**), active sacroiliitis (*arrows* in **F**), sternoclavicular joint inflammation (*arrows* in **H**) and inflammation of the pubic symphysis (*arrows* in **J**), and mild knee joint effusion/synovitis (*arrow* in **L**).

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** Two large follow-up studies of undifferentiated arthritis have documented an independent predictive value of MRI in the diagnosis of RA.[119,120] Presence of bone edema (see Figure 58-15) had a positive predictive value of 86.1% for subsequent development of RA,[119] and a prediction model, including clinical hand arthritis, morning stiffness, positive RF, and MRI bone edema score in MTP and wrist joints correctly identified the development of RA or non-RA in 82% of patients.[120]

In the recent ACR/EULAR 2010 criteria for RA,[6] classification as definite RA is based on the presence of definite clinical synovitis (swelling at clinical examination in one or more joints, absence of an alternative diagnosis that better explains the synovitis, and achievement of a total score of 6 or more (of a possible 10) from the individual scores in four domains. In the joint involvement domain, which can provide as many as 5 of the 6 points needed for an RA diagnosis, MRI and ultrasound synovitis count. In other words, MRI and ultrasonography can be used to determine the joint involvement.[6,121,122]

**Monitoring Disease Activity and Structural Damage.** To be valuable for monitoring joint inflammation and destruction, a method must be reproducible and sensitive to change. MRI allows quantitative (volume or, for synovitis, early contrast enhancement after intravenous contrast injection) as well as less detailed (qualitative: presence/absence; semiquantitative: scoring) evaluation of synovitis, bone edema, and bone erosions. In observational and randomized clinical trials, semiquantitative scoring by the Outcome Measures in Rheumatology (OMERACT) RA




**Figure 58-18, cont'd** After 4 months of anti-TNF therapy, the corresponding images show almost inflammatory activity in the spine (almost absent corner inflammatory lesions in **N**; less but still present costovertebral inflammation in **P**); and the sacroiliac joint (only minimal bone marrow edema in **R**). The inflammation in the sternoclavicular joints (*arrows* in **T**) and pubic symphysis (*arrows* in **V**) is by and large unchanged and the amount of knee joint effusion/synovitis is somewhat increase (*arrows* in **Y**). The images were obtained in less than 45 minutes on a 3 Tesla MRI unit.

MRI (RAMRIS) scoring system has been the most frequently used system. It involves semiquantitative assessment of synovitis, bone erosions, and bone edema in the hands and wrists of patients with RA, based on consensus MRI definitions of important joint pathologies and a core set of basic MRI sequences.[123] The OMERACT erosion scores are closely correlated with erosion volumes estimated by MRI and CT. Good intra- and inter-reader reliability and a high sensitivity to change has been reported, demonstrating that the OMERACT RAMRIS system, after proper training and calibration of readers, is suitable for monitoring joint inflammation and destruction in patients with RA.[124] A EULAR-OMERACT RA MRI reference image atlas has been developed, providing an easy-to-use tool for standardized RAMRIS scoring of MR-images for RA activity and damage by comparison with standard reference images.[125] An MRI joint space narrowing scoring system to be used to assess cartilage damage as an adjunct to the RAMRIS system, has subsequently been developed and validated.[126,127]

MRI allows more sensitive monitoring of inflammation[128] and bone erosion (see Figure 58-14) [129-131] than clinical and radiographic assessments. A large study of 318 methotrexate-naïve patients demonstrated that inhibition of erosive progression by biologic therapy compared with placebo can be demonstrated by MRI by using half of the patients and half of the follow-up time as by radiography.[31] Several randomized controlled trials have documented the superior ability of MRI to discriminate the ability of different therapies to inhibit progressive structural bone and cartilage damage.[132-134]

**Predicting Outcome.** Several studies have demonstrated a predictive value of MRI pathology in wrist and/or MCP joints to radiographic progression. In particular, bone

**TABLE 58-3** MRI Definitions of Inflammatory and Structural Lesions in Rheumatoid Arthritis, Psoriatic Arthritis, and Axial Spondyloarthritis

| |
|---|
| **A. Peripheral Joints in Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA)[1]** |
| **Inflammatory Lesions** |
| **Synovitis:** An area in the synovial compartment that shows increased postgadolinium (post-gadolinium [Gd]) enhancement* of a thickness greater than the width of the normal synovium.<br>**Enhancement (signal intensity increase) is judged by comparison between T1-weighted (T1w) images obtained before and after intravenous (IV) Gd contrast.* |
| **Tenosynovitis**: Signal characteristics consistent with increased water content* or abnormal post-Gd enhancement† adjacent to a tendon, in an area with a tendon sheath.<br>**High signal intensity on T2-weighted (T2w) fat-saturated (FS) and short tau inversion recovery (STIR) images, and low signal intensity on T1w images.*<br>*†Enhancement is judged by comparison between T1w images obtained before and after IV Gd contrast.* |
| **Periarticular inflammation:** Signal characteristics consistent with increased water content* or abnormal post-Gd enhancement† at extra-articular sites. including the periosteum ("periostitis") and the entheses ("enthesitis"), but not the tendon sheaths.‡<br>**High signal intensity on T2w FS and STIR images.*<br>*†Enhancement is judged by comparison between T1w images, obtained before and after IV Gd-contrast.*<br>*‡Defined as tenosynovitis.* |
| **Bone marrow edema:** A lesion* within trabecular bone, with signal characteristics consistent with increased water content† and often with ill-defined margins.<br>**May occur alone or surrounding an erosion or other bone abnormalities.*<br>*†High signal intensity on T2wF.S and STIR images, and low signal intensity on T1w images.* |
| **Structural Lesions** |
| **Bone erosion:** A sharply marginated bone lesion, with typical signal characteristics,* which is visible in two planes with a cortical break seen in at least one plane.†<br>**On T1w images: loss of normal low signal intensity of cortical bone and loss of normal high signal intensity of marrow fat.*<br>*†This appearance is nonspecific for focal bone loss. Other lesions such as bone cysts may mimic erosions.* |
| **Joint space narrowing:** Reduced joint space width compared with normal, as assessed in a slice perpendicular to the joint surface. |
| **Bone proliferation:** Abnormal bone formation in the periarticular region, such as at the entheses (enthesophytes) and across the joint (ankylosis). |
| **B. Axial Disease: Ankylosing Spondylitis and Other Spondyloarthritides[2]** |
| **Inflammatory Lesions**<br>**Bone marrow edema:** Increase in bone marrow signal* on STIR images. |
| **Structural Lesions** |
| **Bone erosion:** Full-thickness loss of dark appearance of the cortical bone and change in normal bright appearance of adjacent bone marrow on T1-weighted images† |
| **Fat infiltration:** Focal increased signal in bone marrow on T1-weighted images.† |
| **Bone spur:** Bright signal on T1w images extending from the vertebral endplate towards the adjacent vertebra (spine) |
| **Ankylosis:** Bright signal on T1-weighted images extending across the SI joints (sacroiliac joints) or extending from one vertebra being continuous with the adjacent vertebra (spine)<br>**Reference point for bone marrow signal on STIR images: Sacroiliac joints: the center of the sacrum at the same craniocaudal level; spine: the center of the vertebra, if normal. If not normal, the signal in the center of the closest available normal vertebra.*<br>*†Reference point for bone marrow signal on T1-weighted images: Sacral bone: the center of the sacrum at the same craniocaudal level; Iliac bone: normal iliac marrow at the same craniocaudal level; spine: the center of the vertebra, if normal. If not normal, the signal in the center of the closest available normal vertebra.* |

[1]OMERACT (Outcome Measures in Rheumatology) MRI in inflammatory arthritis task force recommendations for MRI definitions of important pathologies in rheumatoid arthritis and peripheral psoriatic arthritis.[123,172]

[2]Canada-Denmark MRI Working Group and MORPHO group recommendations for MRI definitions of important pathologies in spine and sacroiliac joints in axial spondyloarthritis.[148,149,151]

marrow edema (see Figure 58-14) has been established as a strong independent predictor of subsequent radiographic progression in early-stage RA.[135-136] Regression analyses in 3-year and 5-year follow-up in the two respective cohorts have documented that bone edema found by MRI is a strong predictor of long-term radiographic progression.[137-138] Recent clinical trial data have confirmed this predictive value, and have also confirmed that early treatment-induced changes in bone marrow edema and synovitis predict the rate of future radiographic progression.[139] Small studies have indicated a relationship of baseline MRI findings with long-term functional disability[140] and tendon rupture at 6 years.[141]

Another issue of high clinical importance is whether MRI is useful to predict the disease course in patients in clinical remission (see Figure 58-15). MRI and ultrasound findings of synovitis are found frequently in patients in clinical remission,[142,143] and several studies have reported that baseline MRI and/or ultrasound findings of inflammation are significantly related to subsequent progressive structural damage.[144-146] These studies encourage further exploration of MRI and ultrasound for predicting the disease course and for evaluating disease status, including defining remission.

## Ankylosing Spondylitis/Axial Spondyloarthritis

MRI allows direct visualization of the abnormalities in peripheral and axial joints and entheses that occur in AS,

PsA, and other forms of SpA. AS, which is thought to be the most common and most typical form of SpA, is dominated by axial disease manifestations in the spine and sacroiliac joints. In accordance with this, this section will mainly focus on the axial manifestations, whereas the PsA section below will deal with peripheral manifestations.

Through its ability to detect inflammatory changes in bone and soft tissues, MRI is the most sensitive imaging modality for recognizing early spine and sacroiliac joint changes in AS.[28,32,147] MRI findings indicating active disease in the sacroiliac joints (sacroiliitis) include juxta-articular bone marrow edema and enhancement of the bone marrow and the joint space after contrast medium administration, while visible chronic changes include bone erosions, sclerosis, periarticular fatty tissue accumulation, bone spurs and ankylosis (Figure 58-19). Typical lesions of the spine, which indicate active disease, are spondylitis, spondylodiskitis (Figure 58-20), and arthritis of the facet, costovertebral, and costotransverse joints (Figure 58-21).[28,147,148] Chronic changes, such as bone erosions, focal fat infiltration, bone spurs and/or ankylosis (see Figure 58-21),[149] frequently occur. Enthesitis is also common, and may affect the interspinal and supraspinal ligaments and the interosseous ligaments in the retroarticular space of the sacroiliac joints. Some patients also have disease manifestations in peripheral joints and entheses, and these can be visualized by MRI.[28,147] Definitions of key pathologies in SpA are provided in Table 58-3. Table 58-2 lists the recent EULAR recommendations for use of imaging in the diagnosis and management of SpA.[32]

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** The introduction of MRI has resulted in a major improvement in the evaluation and management of patients with SpA. Diagnosis was previously dependent on the presence of bilateral moderate or unilateral severe radiographic sacroiliitis, as part of the modified New York criteria for AS.[25] This frequently delayed the diagnosis by 7 to 10 years.[29] Now, through the recent ASAS (Assessment of Spondyloarthritis) classification criteria for axial SpA, MRI forms an integral part because patients with active sacroiliitis on MRI (Figure 58-22) plus one clinical feature (e.g., psoriasis, enthesitis, or uveitis [see Reference 30 for complete list]), should be classified with axial SpA.[30] A consensus-based definition of the requirements to constitute active sacroiliitis (i.e., fulfill the MRI criterion of the ASAS criteria: a positive MRI) has been formulated: bone marrow

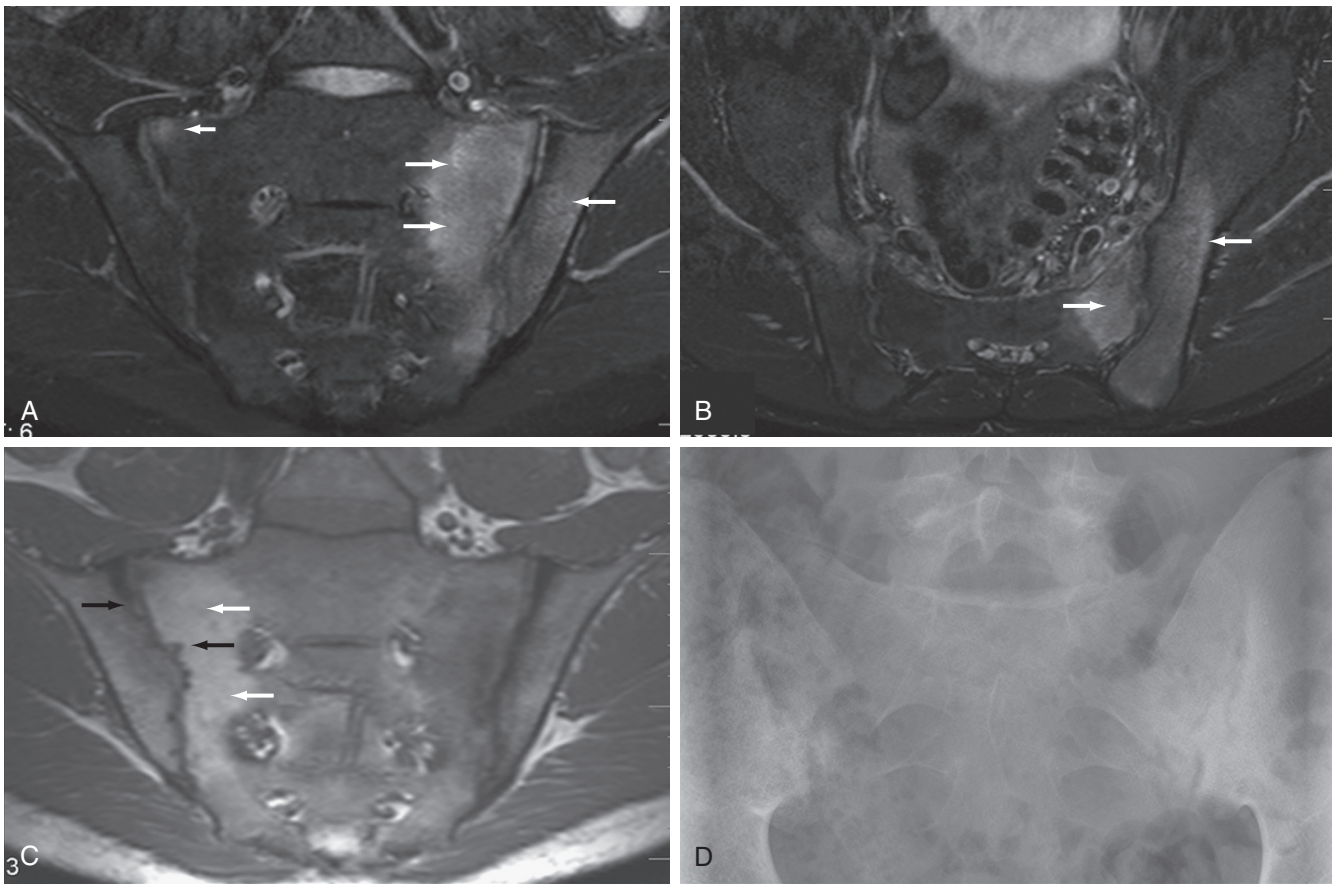



**Figure 58-19 Sacroiliitis in spondyloarthritis (MRI and radiography).** MRI (**A** through **C**) and radiography (**D**) of the sacroiliac joints of 26-year-old male with inflammatory back pain for 4 years. Semicoronal **(A)** and semiaxial **(B)** short tau inversion recovery images show massive iliac and sacral bone edema around the left sacroiliac joint (i.e., demonstrate severe active sacroiliitis) (*long arrows* in **A** and **B**). Inflammation (sacroiliitis) is also present in the right sacroiliac joint (*short arrow* in **A**). T1-weighted semicoronal image shows fat infiltration *(white arrows* in **C**) and bone erosion (*black arrows* in **C**). Anteroposterior radiograph **(D)** shows bilateral mild sclerosis on the iliac side, and bilaterally the articular surface is less well defined than normal, consistent with erosion. However, this feature is exaggerated by overlying bowel gas. *(Courtesy Dr. Susanne Juhl Pedersen, Glostrup, Denmark.)*

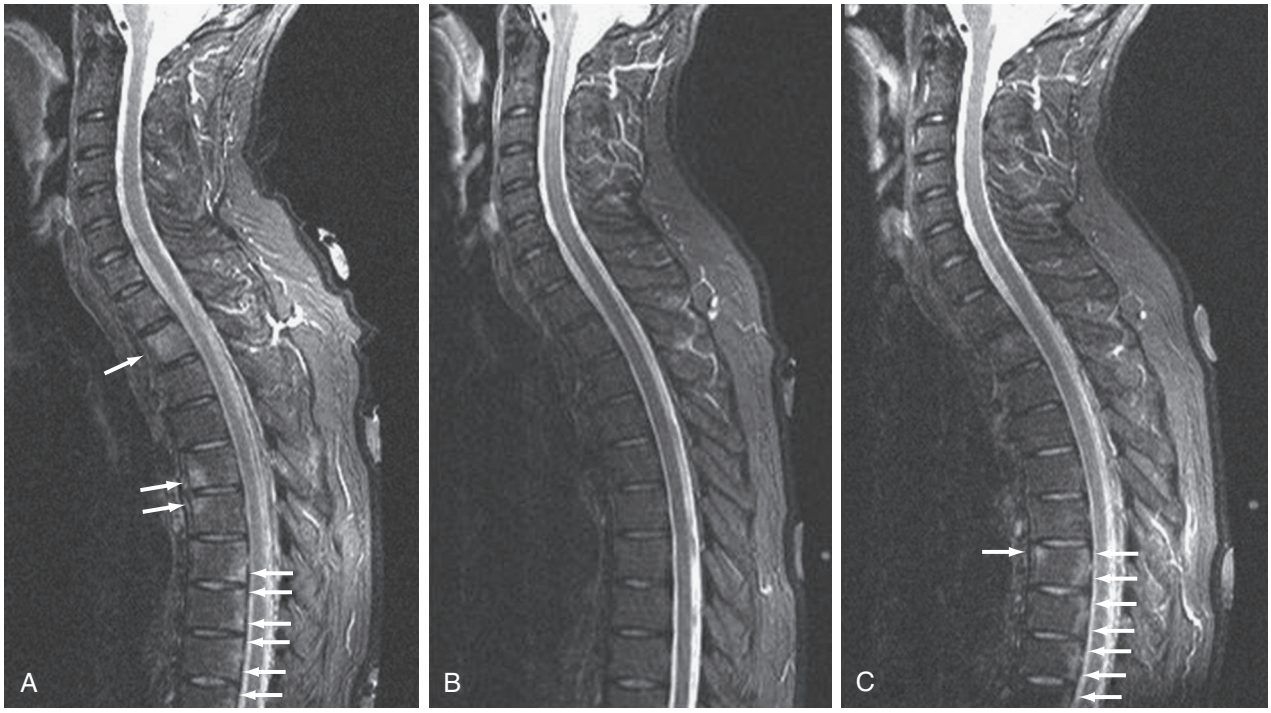



**Figure 58-20 Spine inflammation in ankylosing spondylitis (MRI).** A 43-year-old man with HLA-B27 positive ankylosing spondylitis with deteriorating symptoms, including inflammatory back pain, had an MRI scan before starting biologic therapy. Baseline sagittal short tau inversion recovery (STIR) MRI **(A)** shows diffuse increased signal (edema) in the T2 vertebral body and multiple foci of corner inflammation anteriorly at T5 and 6, and posteriorly at T7, T8, T9 and T10 *(arrows)*. Other images confirmed extensive active inflammation in the spine. The patient responded very well, and after 6 months of therapy, a repeat STIR MRI **(B)** showed complete resolution of bone marrow inflammation. Subsequently, the patient experienced recurrence of symptoms, and a third MRI **(C)** was performed (2 months after anti-TNF therapy was stopped). This MRI shows no edema at T5-T6, a conspicuous new lesion anteriorly at T7, and recurrent inflammation posteriorly in the lower thoracic spine *(arrows)*.

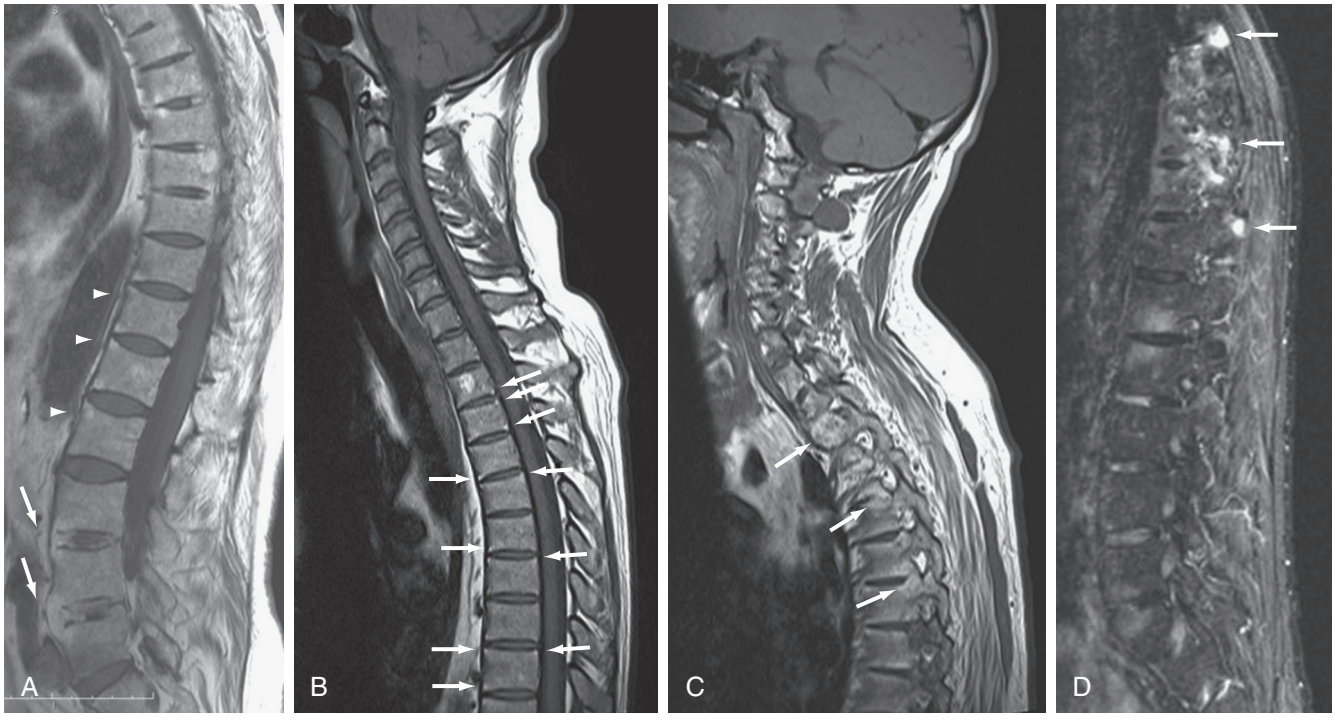



**Figure 58-21 The spine in ankylosing spondylitis (MRI). A** through **C,** T1-weighted sagittal MRI of three different patients with AS shows different structural lesions. **A,** Fat infiltration *(arrowheads)* in the bone marrow of several lumbar vertebral corners and anterior fusion *(arrows)* at L3-L4 and L4-L5. **B**, Fatty infiltration *(arrows)* in the bone marrow of multiple vertebral corners in the cervical spine, indicative of the diagnosis of spondyloarthritis. **C,** Extensive increased marrow fat signal *(arrows)* crossing the costovertebral joints (thoracic ankylosis) is seen, as are changes in several facet joints and other posterior elements. **D,** Sagittal short tau inversion recovery MRI shows intense inflammation in the bone marrow of the transverse processes and facet joints of the lower thoracic spine *(arrows)*, as well as less intense inflammation in several discovertebral joints.

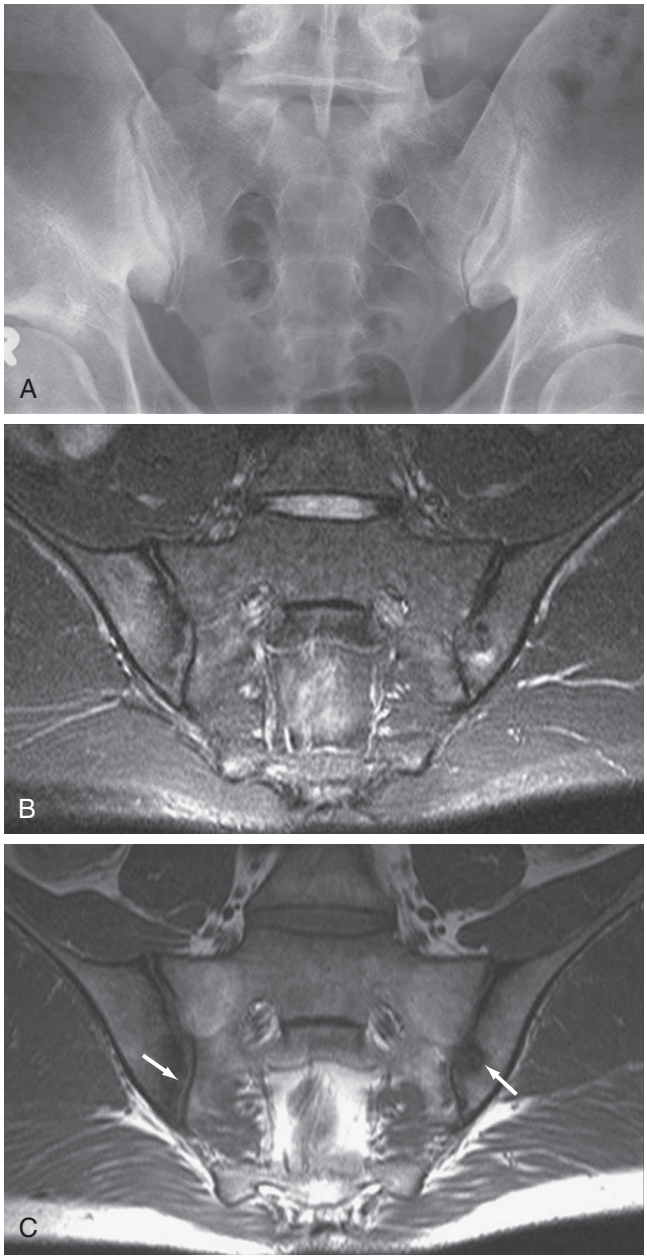



**Figure 58-22 Inflammatory back pain (radiography and MRI).** A 31-year-old man with inflammatory back pain. **A,** Radiography of the sacroiliac joints demonstrates only very subtle findings with possible sclerosis on the iliac side of the right sacroiliac joint and subtle spur formation at the inferior margin. **B** and **C,** MRI at the same time shows obvious findings of spondyloarthritis with mild bilateral iliac inflammation on short tau inversion recovery **(B)** and multiple findings on T1-weighted images **(C)**, including sclerosis most prominent on the right side and subchondral erosion *(arrows)*, which are very small on the right side and obvious on the left side.

edema located in two or more sites and/or in two or more slices.[150]

Subsequent data demonstrate that incorporating structural damage lesions (erosions) into the criteria would improve the diagnostic utility of MRI.[151-154] In contrast, sacroiliac joint fat lesions and/or spine lesions have not been found to improve the diagnostic utility of MRI.[155,156] An update of the ASAS MRI criterion has not yet been published.

**Monitoring Disease Activity and Damage.** MRI can provide objective evidence of currently active inflammation in patients with SpA (see Figure 58-20).[28,147] Until the introduction of MRI, disease activity assessment was restricted to patient-reported outcomes, such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Functional Index (BASFI), because disease activity could not be assessed in a sensitive manner by biochemical (mainly C-reactive protein [CRP]) or physical evaluation.

Several systems for assessment of disease activity in the sacroiliac joints and in the spine have been proposed (see Reference 157 for details). Reproducible and responsive methods are available.[158] The sensitivity to change and discriminatory ability of the three most used spine scoring systems (The Ankylosing Spondylitis spine Magnetic Resonance Imaging-activity [ASspiMRI-a] score, the Berlin modification of the ASspiMRI-a score, and the Spondyloarthritis Research Consortium of Canada (SPARCC) scoring system[159-161] have been demonstrated in clinical trials, and they have been tested against each other by the ASAS/OMERACT MRI in AS group.[158] All methods were feasible, reliable, sensitive to change, and discriminating. The SPARCC method had the highest sensitivity to change as judged by Guyatt's effect size, and the highest reliability as judged by the inter-reader intra-class correlation coefficient (ICC).[158] Separate MRI assessment of different inflammatory and structural findings at distinct anatomic locations in each discovertebral unit will allow for study of the temporal and spatial course of the disease development.[148,149] MRI is much less established for the assessment of structural changes (often referred to as *chronic changes*) than inflammatory changes. Because MRI provides otherwise inaccessible information on inflammatory activity, an equal effectiveness of MRI and radiography in assessing structural damage is a step forward because radiography and the ensuing need for two examinations and exposure to ionizing radiation could then be avoided. Scoring methods assess erosions, sclerosis, fat deposition, and/or bone bridges separately or as a global score.[149,159,162] The validation of methods for damage assessment is limited and their value is not yet clarified.

**Predicting Outcome.** Three spine studies have documented an association between the presence of bone marrow edema at the anterior corners of the vertebrae on MRI and subsequent development of syndesmophytes on radiography after 2 years of follow-up. Presence as opposed to absence of MRI anterior corner inflammation suggested a relative risk of 3 to 5 for a new anterior radiographic syndesmophyte at that level.[163-165] The association was even more pronounced in vertebral corners in which the inflammation had resolved following institution of anti-TNF therapy. This might be explained by TNF in an active inflammatory lesion restricting new bone formation, whereas reduction of TNF by application of a TNF-antagonist allows tissue repair to manifest as new bone formation.[164,165] Fat infiltration in the vertebral corners seems to be a key intermediary in the process because its development is linked to resolution of previous inflammation and to future development of syndesmophytes.[166,167]

One study suggests that in early inflammatory back pain, MRI images of severe sacroiliac bone marrow edema, with human leukocyte antigen (HLA)-B27 positivity is a strong

predictor of future AS, whereas mild or no sacroiliitis, irrespective of HLA-B27 status, was a predictor of that AS would not develop in the future.[168] Data on the value of MRI for predicting therapeutic response in SpA are very limited. A high spine MRI inflammation score and short disease duration have been reported as statistically significant predictors of clinical response (BASDAI improvement >50%) to anti-TNF therapy.[169,170] Further and larger studies are needed to clarify the role of MRI in the prediction of disease course and therapeutic response in clinical practice.

## Psoriatic Arthritis

The clinical appearance of PsA is very diverse, involving the spine, SI joints, peripheral joints and/or entheses. Thus MRI findings are variable. PsA shares clinical manifestations with RA and SpA, and this also applies to its features on MRI.[171] Peripheral PsA synovitis and erosions do not have disease-specific features on MRI, and MRI bone edema can involve any bone. A general agreement on which joints to image to assess PsA activity and damage is not established, and possibly needs to be individualized, based on the disease pattern. Whole-body MRI, allowing imaging of both peripheral and axial joints and entheses of the entire body in one examination, may be a future solution to this, but still requires more validation.[96-98]

MRI can visualize both peripheral and axial musculoskeletal anatomy and PsA disease manifestations. Findings include synovitis, tenosynovitis, periarticular inflammation, enthesitis, bone edema, bone erosion, and bone proliferation (Figure 58-23).[172-175] As in other types of SpA, enthesitis, dactylitis and spondylitis can be seen. Dactylitis has been shown on MRI to be caused by tenosynovitis with effusion, sometimes associated with diffuse soft tissue edema and/or synovitis in nearby finger or toe joints (see Figure 58-23).[176,177] There are few MRI studies in axial PsA, but findings are similar to AS findings, although more frequently asymmetric.[178,179]

The entheses has attracted attention as a possible primary location of disease.[180] Nail disease is common in PsA, and DIP joint inflammation seen on MRI has been described to extend to the nail bed.[181]

PsA can be clinically silent. In patients with psoriasis without arthritic signs or symptoms, pathologic findings on MRI (including periarticular edema, tendon sheath effusion, intra-articular effusion, synovial pannus, bone erosion, bone cysts, subchondral changes, and joint subluxation) have been reported in more than two thirds (68% to 92%) versus none to 1/12 or less of healthy controls.[182-184] The clinical importance of these findings is not yet clarified.

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** As described previously, MRI can detect the different pathologies involved in PsA. However, no studies have documented that MRI in an early undifferentiated arthritis cohort can be used to differentiate PsA from other arthritides.

**Monitoring.** Data on monitoring activity and damage are limited. Most studies only report qualitative MRI assessments of the different pathologies of PsA.[171] Quantitative assessment of contrast enhancement has been reported[185-186] but is insufficiently validated for clinical use. Scoring systems of inflammation and damage have been developed.[172,187,188] The OMERACT Psoriatic Arthritis Magnetic Resonance Image Scoring System (PsAMRIS) is the best

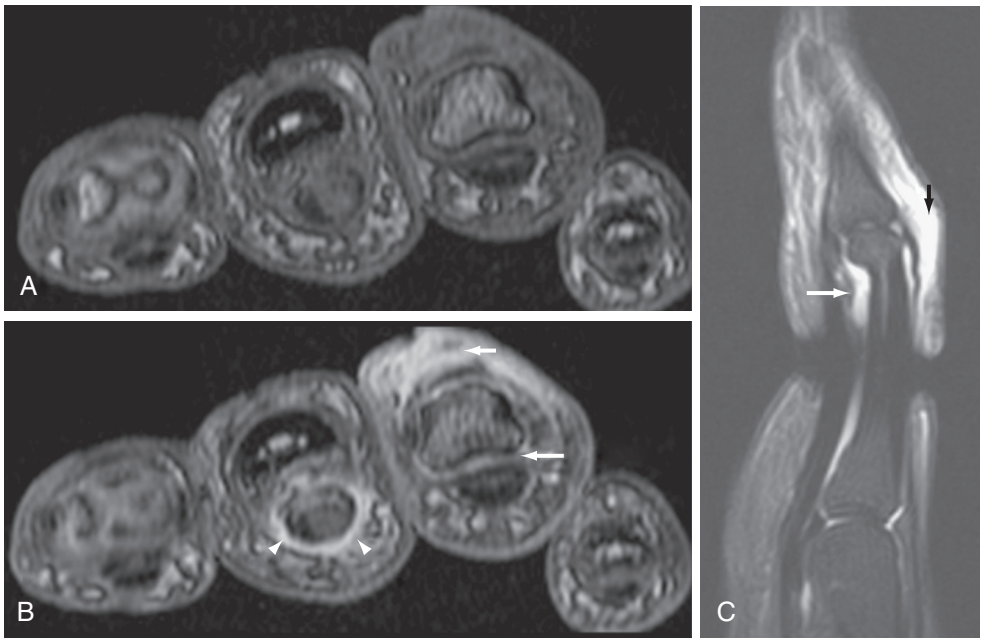



**Figure 58-23 Psoriatic arthritis (MRI).** Axial T1-weighted precontrast **(A)** and postcontrast **(B)** MRI of the second *(left)* through fifth *(right)* fingers at the level of the fourth proximal interphalangeal joint and sagittal short tau inversion recovery MRI **(C)** of the fourth finger show mild synovitis (*long arrows* in **B** and **C**) and considerable periarticular inflammation (*short arrows* in **B** and **C**) around the fourth distal interphalangeal joint and tenosynovitis (*arrowheads* in **B**). *(Courtesy Rene Poggenborg, Glostrup, Denmark.)*

validated and has a documented good intra- and inter-reader reliability and, for inflammatory parameters (synovitis, tenosynovitis, periarticular inflammation), sensitivity to change.[172,189,190] Its usefulness in clinical trials and practice needs further testing.

**Predicting Outcome.** No longitudinal studies of the prognostic value of MRI findings in PsA are available.

## Gout

MRI can directly visualize the inflammatory (synovitis, tenosynovitis, bone edema, and soft tissue inflammation) and destructive (bone erosion) aspects of gout arthropathy.[41,191-193] MRI can also visualize tophi and provide information on the inflammatory nature of these lesions which cannot be appreciated from radiography or CT. On MRI, tophi typically exhibit low signal on T1w images, with varying postcontrast enhancement, and medium-high signal on T2w images, indicating the presence of cellular tissue surrounding or infiltrating the crystalline mass (see Figure 58-6).[191,193] Calcification within the tophus can lead to regions of low signal on T2w images. Tophi are not always clinically detectable if the location is deep to the skin surface.

### Use in Diagnosis, Monitoring, and Predicting Outcome

The diagnostic accuracy of MRI-documented synovitis, bone erosion, and/or tophi against the gout classification criteria[39] has not yet been determined. However, MRI can detect tophi, and the presence of these strongly suggests a diagnosis of gout, but joint aspirate confirmation of monosodium urate crystals is usually required because the differential diagnosis includes infection or other space-occupying lesions.

Monitoring gout by MRI could include assessment of joint inflammation, erosion progression, and extent/size of tophi (see Figure 58-6). Tophus volume assessment by MRI has been reported to have a good intra- and inter-reader reproducibility and may be useful for this purpose in trials.[194] In contrast to RA, AS, and PsA, no MRI scoring system for overall assessment of gouty arthritis has yet been developed.

## Calcium Pyrophosphate Dihydrate Crystal Deposition Disease

MRI detects calcification of hyaline cartilage as an area of low signal intensity, especially on gradient echo images; meniscal chondrocalcinosis exhibits increased signal on T1-weighted and proton density images, and may simulate meniscal degeneration or tear (see Figure 58-7).[195] As in other diseases, MRI is a sensitive method for imaging subchondral cysts and inflammatory changes such as synovitis and effusion.

## Septic Arthritis

The MRI appearance of septic arthritis is nonspecific, and similar findings can be observed in other inflammatory arthritides. Thus, in case of clinical suspicion of septic arthritis, a joint aspiration must be performed without delay to avoid irreversible articular damage. Characteristic findings are synovitis, joint effusion, and soft tissue edema (see Figure 58-8) followed by bone erosion and cartilage destruction. MRI may be helpful in diagnosing complications of septic arthritis, such as abscesses and osteomyelitis. It should be remembered that most of the individual findings on MRI are not specific, and bone marrow edema alone does not necessarily denote osteomyelitis.[196] Contrast-enhanced MRI is particularly useful to identify abscess/necrosis in either soft tissue or bone that may confirm the diagnosis and/or requirement for surgical débridement. Investigation of the painful diabetic foot is a specific clinical presentation for which MRI may now be routinely performed to help distinguish between infection and trauma, and to identify nonviable tissues, although scintigraphic studies also play an important role in these complex patients.[45]

## Osteoarthritis

Based on the tomographic nature and ability to visualize cartilage, bone, and various soft tissues, MRI is very well-suited for assessment of inflammatory changes, structural and compositional changes in the cartilage, and other structural lesions in OA. The majority of studies have been undertaken in knee joints (Figure 58-24) or, less frequently, hip joints, but recently also in small joints in the hand (see Figure 58-10) in generalized OA.

MRI allows direct assessment of the thickness, surface contour, and internal architecture of articular cartilage in OA[197-199] (see Figure 58-24), making the staging and monitoring of OA development possible. Osteophytes at the joint margins or beneath the articular cartilage may be seen. Subchondral changes include bone edema, sclerosis, and bone cysts. MRI bone marrow lesions (sometimes referred to as *bone edema lesions*), which are areas with inhomogeneous, intermediate-low signal on T1w and high signal on water-sensitive techniques (STIR/T2 FS) (Figure 58-25), have, by comparison with histologic samples obtained by surgery in advanced OA, shown trabecular microfracture and bone marrow fibrosis and/or necrosis, but limited interstitial edema.[200-203] Synovitis is seen frequently by MRI in OA, albeit to a lesser degree than in RA.[204] Synovitis scores obtained by contrast-enhanced MRI have demonstrated a good correlation with arthroscopic and microscopic synovitis scores.[205]

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** The classification criteria for OA are based on clinical and conventional radiographic findings.[48] However, the use of MRI in the diagnosis of OA offers the advantages described in preceding text (i.e., that MRI can sensitively depict all the involved pathologic changes).

**Monitoring.** Various quantitative and semiquantitative techniques have been used to measure structural abnormalities and changes on MRI in OA.[197] Quantitative measurements apply computer-aided image processing to quantitate various aspects (e.g., volumes of cartilage, bone, bone marrow lesions, menisci, or synovium. These have been reported to have excellent reproducibility).[206] Measures of cartilage composition are also available (e.g., quantification of glycosaminoglycan content).[198,199] See the previous paragraph on the technical aspects of MRI, for other potential

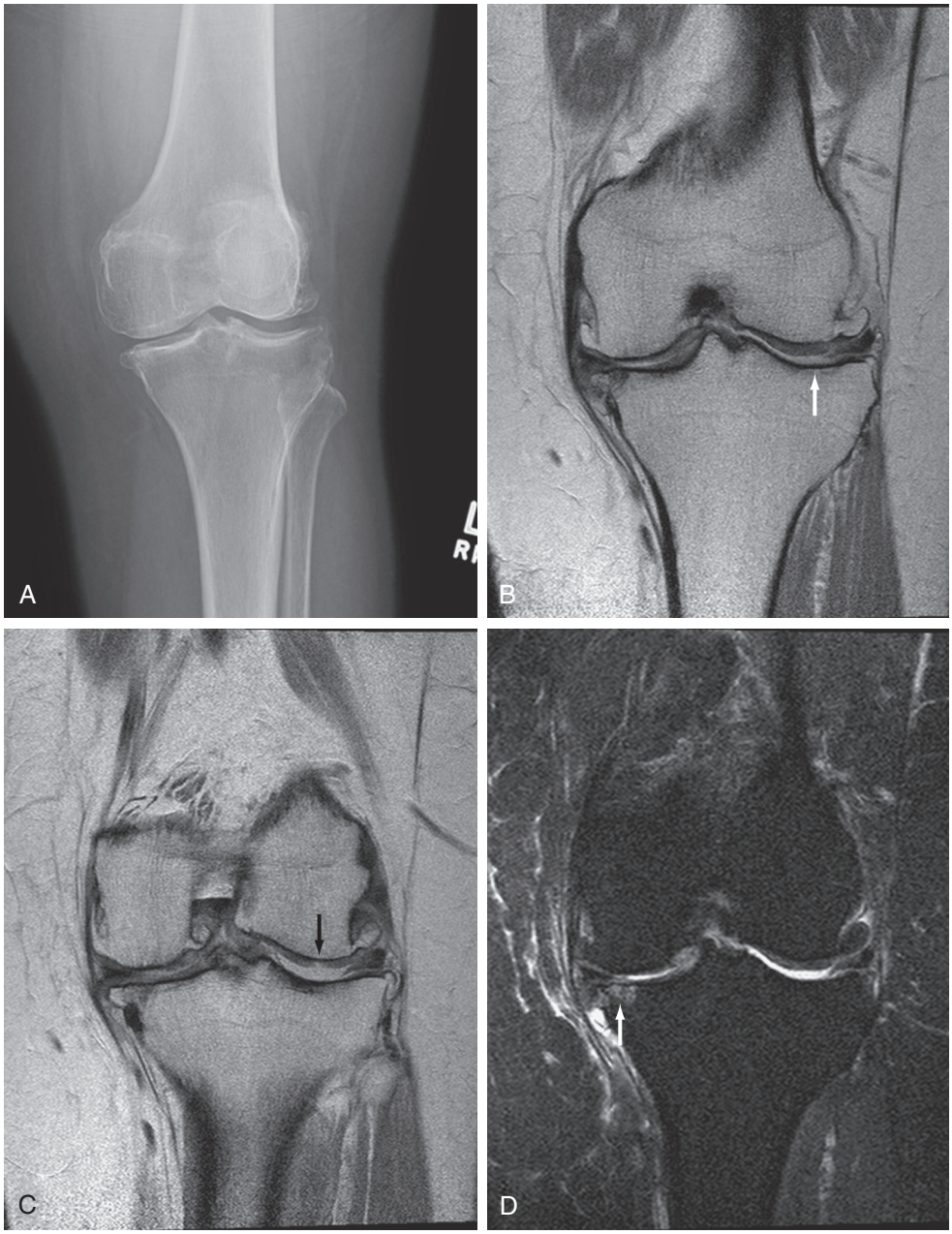



**Figure 58-24 Osteoarthritis of the knee (radiography and MRI). A**, Anteroposterior radiographic view of the left knee shows large osteophyte of the medial and lateral tibiofemoral joint and mild medial joint space narrowing. **B** and **C,** Coronal proton density-weighted MRI confirms the presence of large osteophytes in the tibiofemoral joint, shows diffuse cartilage loss of the medial tibia and femur, and discloses a denuded bone with complete loss of cartilage in the lateral tibial plateau (*arrow* in **B**). A small focal cartilage defect of the lateral femoral condyle (*arrow* in **C**) is also evident. Both lateral and medial menisci are partially macerated, and a subluxation of the medial meniscus is seen, along with severe cartilage loss on the medial and lateral tibial plateau and the medial femoral condyle. **D**, Coronal T2-weighted fat-suppressed MRI shows medial tibial plateau subchondral bone marrow edema (*arrow* in **D**). *(Courtesy Professor Ali Guermazi, Boston, Mass.)*

methods. Semiquantitative methods have been used to provide semiquantitative "multiple feature" ("whole-organ") assessments in the knee, based on conventional MRI acquisitions.[207-210] These measures have high reliability and better sensitivity to change than radiographic methods.[206] A system for systematic assessment of hand OA has also been developed and validated.[211]

**Predicting Outcome.** In a systematic literature review[212] quantitative cartilage volume change and presence of cartilage defects or bone marrow lesions (bone edema) in three of three studies was significantly related to subsequent total knee replacement (i.e., a predictive value was demonstrated).[213-215]

Furthermore, enlargements in bone marrow lesions have been demonstrated to be related to increased pain (and improvements in bone marrow lesions to decreased pain) in follow-up studies.[216,217] In contrast, an inconsistent and generally weak association between cartilage loss and symptom change, and a weak association between change in synovitis and change in pain have been reported. Finally, the presence of meniscal damage, cartilage defects, and/or BMLs predicts subsequent MRI-visible progression of disease.[212]

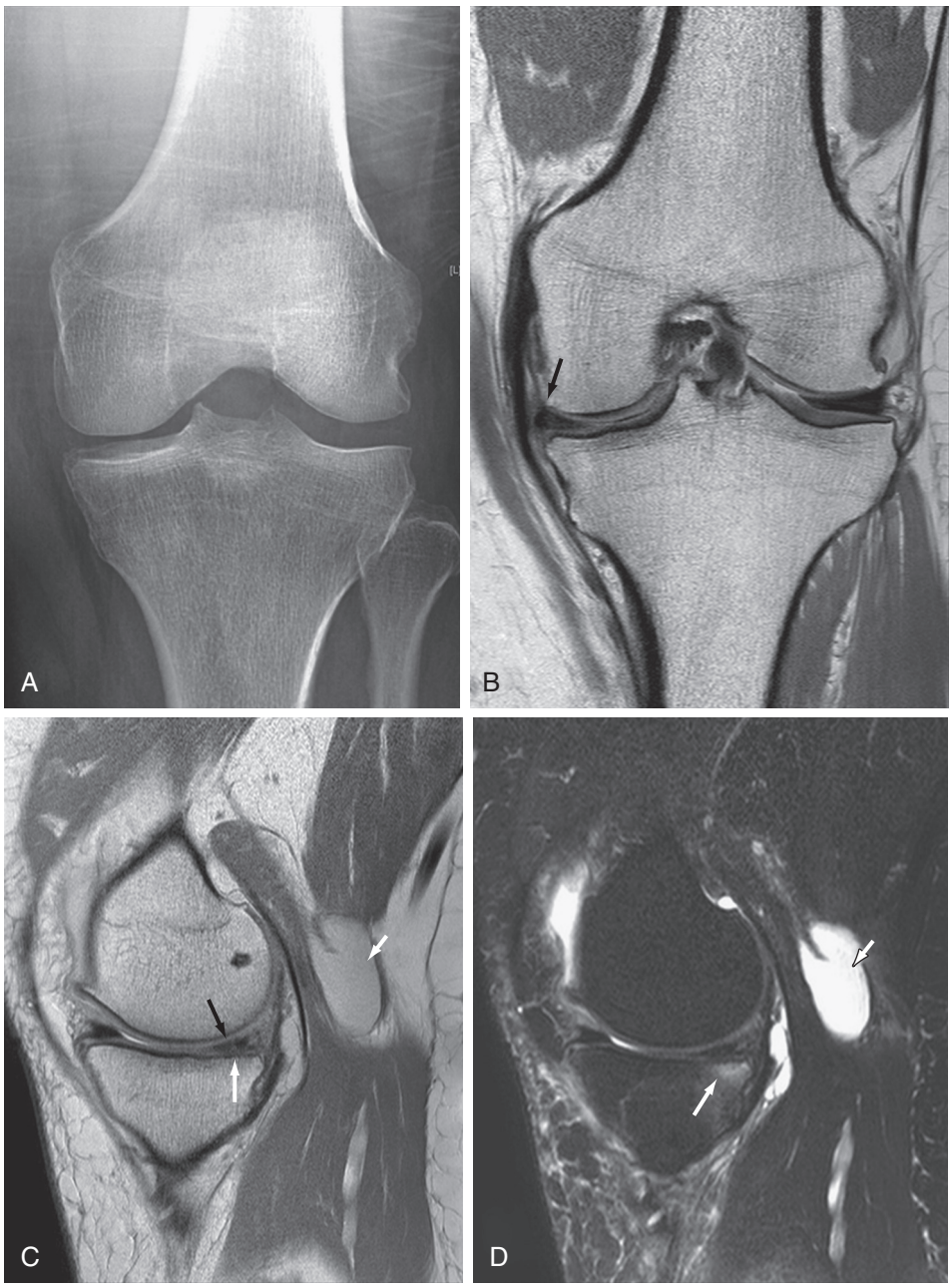



**Figure 58-25 Early osteoarthritis (OA) of the knee (radiography and MRI).** Radiography **(A)** and MRI (**B** through **D**) of the knee with early OA. **A,** Anteroposterior radiographic view shows an almost normal knee. The only possible abnormality is a tiny osteophyte at the medial tibial plateau. **B,** Coronal proton density-weighted MRI shows a minimal medial meniscus subluxation *(arrow)*. **C**, Sagittal proton density-weighted MRI shows partial maceration of the posterior horn of the medial meniscus *(black arrow)*, thinning of the cartilage at the posterior medial tibial plateau *(long white arrow)*, and a moderate-sized Baker's cyst *(short white arrow)*. **D,** Sagittal fat-suppressed T2-weighted MRI confirms the Baker's cyst *(short arrow)* and also shows subchondral bone marrow edema *(long arrow)* subjacent to the loss of cartilage at the posterior subregion of the medial tibial plateau. *(Courtesy Professor Ali Guermazi, Boston, Mass.)*

## ULTRASONOGRAPHY

### KEY POINTS

- Ultrasonography allows sensitive visualization and assessment of peripheral inflammatory and destructive joint and soft tissue involvement in a variety of degenerative and inflammatory rheumatic diseases.
- Ultrasonography can readily be performed by trained rheumatologists in relation to the clinical examination.
- Ultrasonography can, through detection of early inflammatory changes, contribute to earlier diagnosis of RA.
- Grey scale and power Doppler ultrasonography allow physicians to monitor inflammatory soft tissue changes (e.g., synovitis, tenosynovitis, and enthesitis) during treatment of patients with peripheral inflammatory joint diseases.
- Ultrasonography allows guidance of invasive procedures, making possible precise needle positioning for aspirations and injections (e.g., in joints, bursae, or tendon sheaths, or at entheses).
- The main disadvantages of ultrasonography are the need for a skilled operator interreader and interscanner variability, and the need for an "acoustic window."

Ultrasonography is an evolving imaging technique increasingly used by rheumatologists in daily clinical practice. This fact is confirmed by extensive publications and a wide body of literature during the last few years.[218,219] In some countries, including Belgium, Italy, Germany, Spain, and the United States, ultrasonography has become an integral part of rheumatology training. Absence of radiation, good visualization of the joint cavity, low running costs, multiplanar imaging capability, quantification of soft tissue abnormalities and real-time assessment are the main advantages of ultrasonography, compared with other imaging techniques. Moreover, ultrasonography is rapidly performed, readily accepted by patients, and increasingly used for guidance of invasive procedures (biopsy, joint aspiration, and injection).[220] It has been reported that ultrasonography increased the confidence in physicians' recommendations in 88.4% of RA patients.[221]

Ultrasonography provides a clear view of the anatomic damage induced by inflammatory or degenerative conditions and allows an accurate assessment of joint and soft tissue involvement in a variety of rheumatic diseases including RA, juvenile idiopathic arthritis, AS, and other spondyloarthritides, crystal deposition diseases, septic arthritis, SLE, entrapment neuropathies, systemic sclerosis, polymyositis, Sjögren's syndrome, vasculitis, OA, and regional pain syndromes (Figure 58-26).

Ultrasonography is rapid, safe, and very useful as guidance for invasive procedures.[220,222,223] Ultrasound-guided needle placement within the selected target area is an accurate and safe approach for patients requiring aspiration of synovial fluid, injection therapy, or biopsies. Under ultrasound guidance, the progression of the needle in the soft tissues toward the selected target area can be carefully controlled, therefore avoiding injury induced by the tip of the needle and/or complications such as subcutaneous or intratendinous injection of corticosteroid which has a high risk of tissue damage. Moreover, ultrasound-guided injections

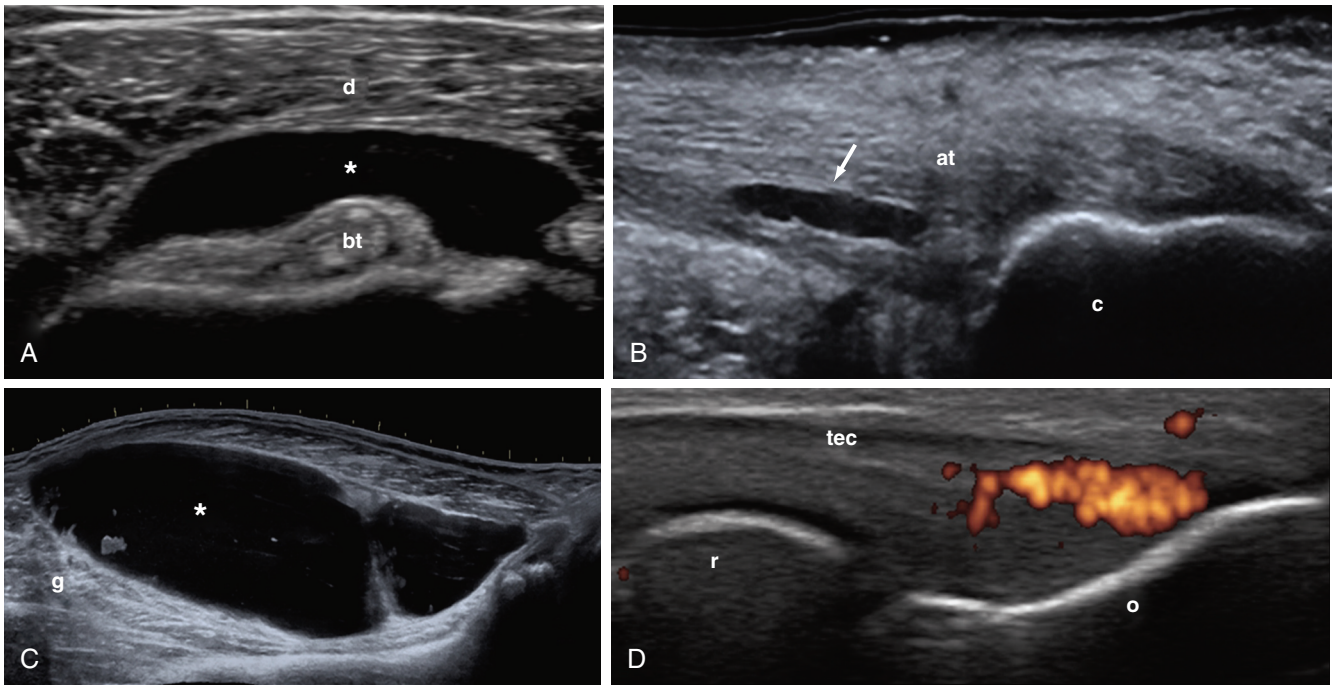



**Figure 58-26 Various regional pain syndromes (ultrasonography). A,** Shoulder (anterior transverse scan): fluid collection in the subdeltoid bursa *(asterisk)*. **B,** Achilles tendon *(at)* (longitudinal scan): large tendon tear *(arrow)*. **C,** Knee (posterior longitudinal scan): massive fluid collection inside the semimembranosus gastrocnemius bursa (popliteal cyst) *(asterisk)*. **D,** Elbow: epicondylitis characterized by intense power Doppler signal within the common extensor tendon of the fingers (tec). *bt*, Long head of the biceps tendon; *c*, calcaneous; *d*, deltoid muscle; *g*, gastrocnemius muscle; *o*, lateral epicondyle; *r*, radial capitellum.

for inflammatory arthritis are significantly less painful than palpation-guided models, and even though recent studies have not been able to demonstrate significantly improved clinical outcomes,[224,225] it is likely that ultrasound guidance will ultimately be shown to improve clinical outcomes and enhance cost effectiveness.

Ultrasonography can be used in daily rheumatology practice for a wide spectrum of indications (see Key Points for ultrasonography). In a recent study, the additional information obtained by ultrasonography led to changes in diagnosis in 60% of areas examined (compared with diagnoses made without ultrasonography, based solely on clinical examination) and changes in treatment in 28% of patients.[226]

## Technical Aspects

Musculoskeletal ultrasonography requires high-quality equipment. Low-frequency transducers (3.5 to 5 MHz) are useful to explore deep targets (e.g., the hip and sacroiliac joints). Medium-frequency transducers (6 to 13 MHz) are the best choice to explore large joints (the shoulder, knee, and elbow). High-frequency transducers (14 to 20 MHz) have a low penetration power of the ultrasonic beam but allow for an accurate assessment of superficial structures (tendons and finger joints), reaching a resolution power of less than 0.1 mm.

Ultrasonography is an operator-dependent technique. Thus, the quality of the pictures and the interpretation of sonographic images depend on the quality of the ultrasonography equipment, the technical conditions of the examination, and the skills and experience of the examiner.

Color Doppler and power Doppler ultrasonography explore blood perfusion and may play a key role in the monitoring of inflammatory disease activity and the assessment of response to therapy.[227] Color Doppler provides information on the direction of blood flow. Power Doppler has greater sensitivity in detecting blood flow, but is not able to determine either flow direction or velocity. The use of ultrasonography contrast agent enhances the detection of tissue vascularity with Doppler ultrasonography, which may have an effect on the detection of subclinical synovitis.[228] However, the use of ultrasonography contrast agents for the assessment of rheumatic diseases remains restricted to the research field, both for ethical and cost/efficacy reasons.

Ultrasonography is a continuously evolving technique, and recent advances in technology promise further improvements, especially in the field of 3D, four-dimensional (4D) ultrasonography, elastosonography, and fusion imaging.[229] 3D ultrasonography has the potential to dramatically reduce the duration of ultrasonography examination and to improve the standardization of the technique. Longitudinal, transverse, coronal planes, and 3D reconstruction of the target area can be acquired in a few seconds.[229] Elastosonography is a new technique to assess tissue elasticity. Its main potential applications in rheumatology include skin assessment in systemic sclerosis, evaluation of tendon stiffness, and differential diagnosis of subcutaneous nodules. Fusion imaging allows simultaneous comparison and mapping of ultrasound images onto another preacquired imaging modality, such as CT or MRI.

## Examination Technique

Ultrasonography examination of the musculoskeletal system should be performed with multiplanar scans. The EULAR guidelines were designed to standardize the approach to musculoskeletal ultrasonography in rheumatology.[230]

The patient position is critical for a correct ultrasound examination. For example, examinations to detect synovial fluid in a patient's knee joint should be performed with a suprapatellar scan of the knee in 30 degree flexion.[231] Particular attention must be paid to the angle of the ultrasound beam, which must be perpendicular to the examined structure to avoid anisotropy-dependent artifacts (spatial asymmetry). These artifacts may generate diagnostic errors, especially when the physician is studying fibrillar or fascicular structures such as tendons and nerves. Both longitudinal and transverse scans should be performed by slightly moving the transducer from the radial to ulnar and from the proximal to distal sides to enable maximum coverage of the anatomic surface area.

Operator dependency is the main shortcoming of ultrasonography. Adequate supervised training is essential to ensure the correct use of ultrasonography and appropriate interpretation of results. Another limitation of ultrasonography is in the comprehensive assessment of some anatomic areas (i.e., the spine, sacroiliac joint, and hip) resulting from the lack of adequate "acoustic windows." Pitfalls and errors are mostly related to misinterpretation of normal anatomy, inadequate regulation of the ultrasonography beam, and inappropriate examination techniques, mainly depending on pressure, position, and inclination of the probe. Grey scale and Doppler findings require a careful analysis that should also take account of the pharmacologic treatment. Glucocorticosteroids and biologic agents have the strongest potential to modify ultrasonography findings, but even nonsteroidal anti-inflammatory drugs may decrease grey scale and power Doppler synovitis scores.[232] Ultrasonography findings provide a wide range of useful information concerning the main tissue changes occurring in patients with rheumatic diseases. However, limited standardization still exists about scanning methodology and definitions of basic sonographic abnormalities. Questions such as which joints should be scanned or which scoring system should be used are still a matter of discussion.[233] The most widely accepted definitions for sonographic pathology in patients with RA, were published in 2005 by the OMERACT special interest group (Table 58-4).[234] An expert consensus paper on ultrasonography definition and scoring of tenosynovitis, that used a four-grade semiquantitative scoring system, was recently published, describing a four-grade semiquantitative scoring system which has shown high interobserver agreement (grey scale agreement, 87.5%; Doppler agreement, 96.3%).[235] Criteria of ultrasound remission are still not well defined. Power Doppler seems to be the most promising candidate method, even though the minimal acceptable level of power Doppler activity is unknown.[233]

## Rheumatoid Arthritis

RA is the inflammatory condition most frequently studied by ultrasonography. Ultrasound abnormalities detected in patients with RA include joint space widening, fluid

collection, synovial hypertrophy, cartilage defects, bone erosions, tendon sheath widening, and tendon tears.[236] Joint cavity widening is the earliest characteristic ultrasound finding of synovitis. Even minimal intra-articular fluid collection and synovial proliferation may be easily revealed by ultrasonography. Fluid collection shows a typical anechoic pattern that can easily be distinguished from the soft echogenicity of synovial hypertrophy. This appears as a homogeneous thickening of the synovial layer or as irregularly shaped clusters of echoes (bushy and villous appearance). Doppler signal may be particularly intense inside the areas of synovial hypertrophy in patients with active synovitis. Doppler signal is associated with the extent of inflammation detectable in the synovial biopsies in patients with RA.[237]

**TABLE 58-4** Definitions of Ultrasonographic Pathology in Inflammatory Joint Disease*

| |
|---|
| **Bone erosion.** An intra-articular discontinuity of the bone surface that is visible in two perpendicular planes. |
| **Synovial fluid.** Abnormal hypoechoic or anechoic (relative to subdermal fat, but sometimes isoechoic or hyperechoic) intra-articular material that is displaceable and compressible, but does not exhibit Doppler signal. |
| **Synovial hypertrophy.** Abnormal hypoechoic (relative to subdermal fat, but sometimes isoechoic or hyperechoic) intra-articular tissue that is nondisplaceable and poorly compressible and which may exhibit Doppler signal. |
| **Tenosynovitis.** Hypoechoic or anechoic thickened tissue with or without fluid within the tendon sheath, which is seen in two perpendicular planes and which may exhibit Doppler signal. |
| **Enthesopathy.** Abnormally hypoechoic (loss of normal fibrillar architecture) and/or thickened tendon or ligament at its bony attachment (may occasionally contain hyperechoic foci consistent with calcification), seen in two perpendicular planes that may exhibit Doppler signal and/or bony changes, including enthesophytes, erosions, or irregularity. |

*From OMERACT (Outcome Measures in Rheumatology) ultrasonography task force.[234]

The assessment of perfusion of synovial tissue may prove to be the most important part of evaluation of joints in patients with RA. Differentiation between inactive and persistent inflammation in the rheumatoid joint is one of the most important problems for the clinician because it may have a great impact on clinical decision making.

The detection of a highly perfused synovial pannus in a patient with early-stage arthritis should be regarded as a potential predictor of future anatomic damage (Figure 58-27). Ultrasonography can also be used to assess cartilage damage at the metacarpophalangeal joint level in patients with RA (Figure 58-28). The level of grey scale synovitis correlates with disease duration, whereas the presence of power Doppler is independent of disease duration, and thus may be a better marker of inflammation at any given time.[233]

The high spatial resolution of ultrasonography allows for the recognition of even minimal bone erosions. The sensitivity is highest in easily ultrasound-accessible areas, including the frequently involved second metacarpophalangeal

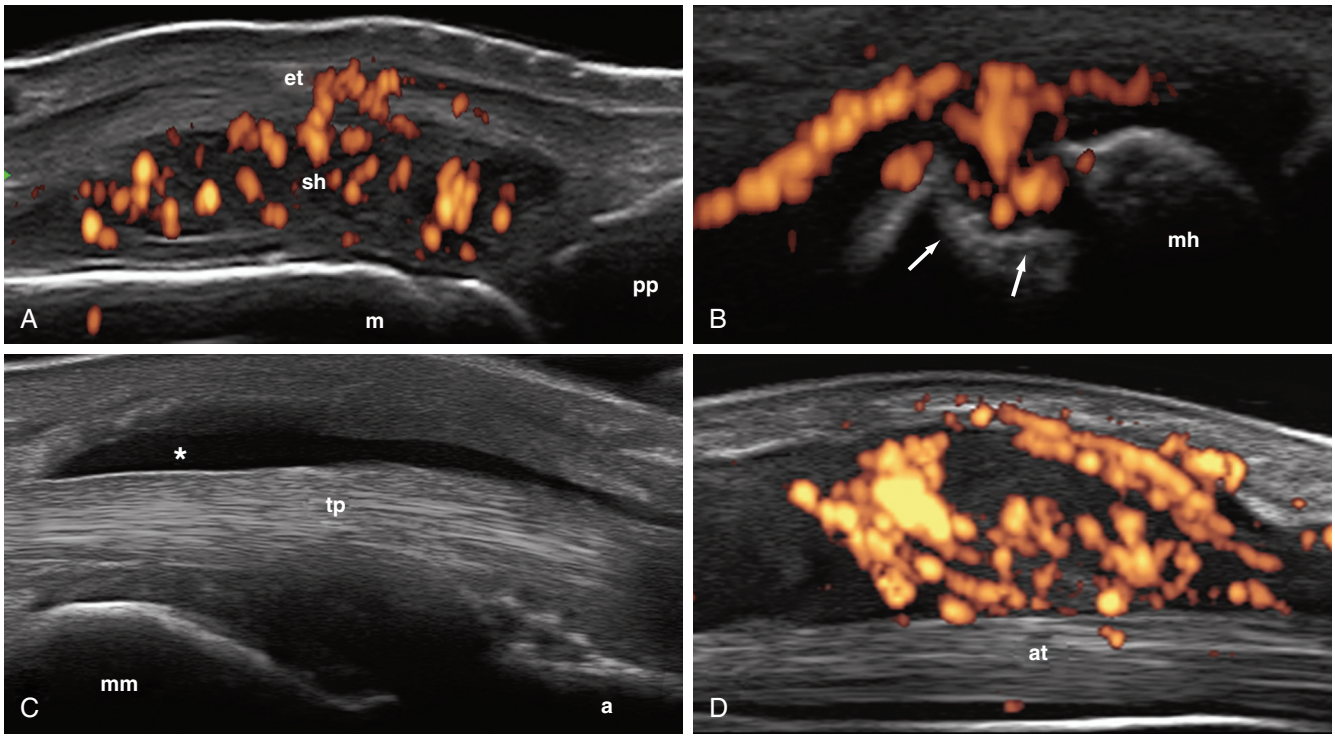



**Figure 58-27 Rheumatoid arthritis (ultrasonography). A,** Metacarpophalangeal joint (dorsal longitudinal scan): joint cavity widening, synovial hypertrophy *(sh)*, and intense power Doppler signal. **B,** Fifth metatarsophalangeal joint (lateral longitudinal scan): large bone erosion *(arrows)* associated with power Doppler signal inside the erosion. **C,** Tibialis posterior tendon *(tp)*: tendon sheath widening with homogeneous anechoic aspect of the content (fluid collection) *(asterisk)*. **D,** First compartment of the finger extensor tendons (longitudinal scan): chronic tenosynovitis characterized by synovial hypertrophy and intense power Doppler signal. *a*, Astragalus; *at*, abductor pollicis longus tendon; *et*, common extensor tendon of the finger; *m*, metacarpal bone; *mh*, metatarsal head; *mm*, medial malleolus; *pp*, proximal phalanx.

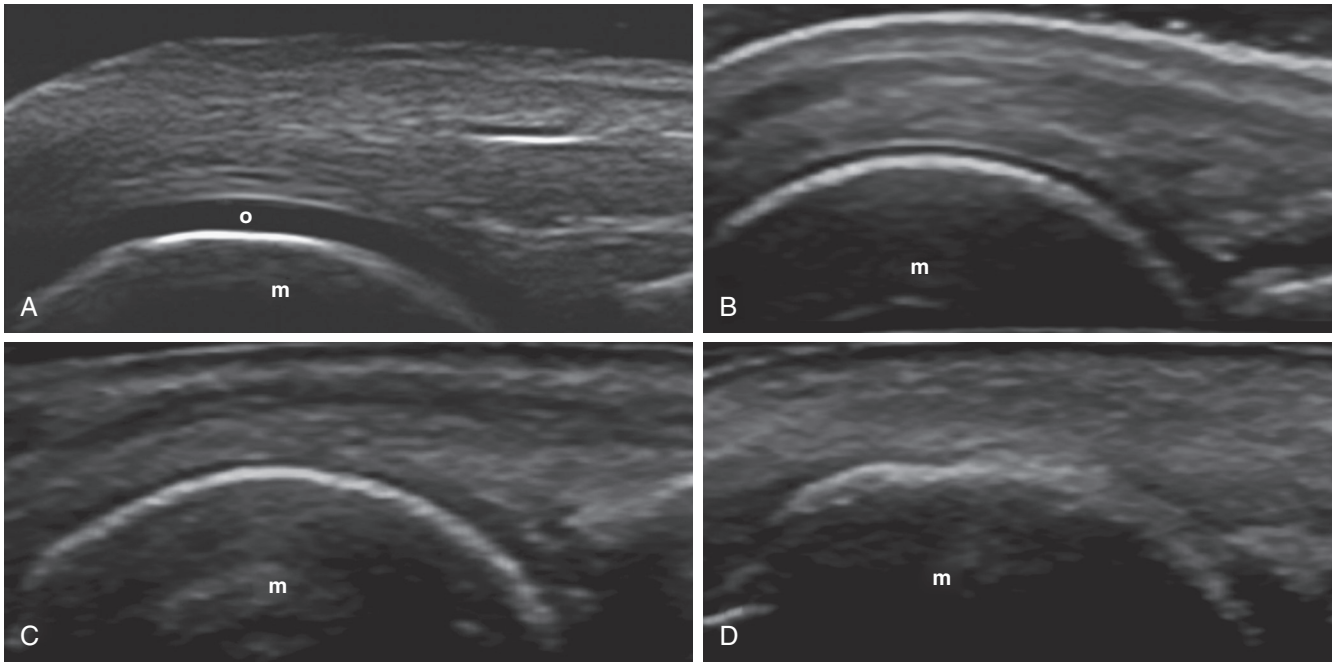



**Figure 58-28 Cartilage in rheumatoid arthritis (ultrasonography).** Hyaline cartilage *(circle)* of the metacarpal head *(m)* in healthy participant **(A)** and in patients with early **(B)** and advanced (**C** and **D**) rheumatoid arthritis. Loss of cartilage is homogeneous in different stages of the disease. Early **(B)**, late **(C)**, very late **(D)**.

and fifth metatarsophalangeal joint.[52,238,239] Ultrasound bone erosions are most frequently found at the fifth metatarsal head in patients with early-stage RA.[240] Bone erosions are viewed on ultrasound as an interruption of the sharp hyperechoic bone profile. Ultrasonography depicts the wall and the floor of the erosions, which in most cases are filled by a hyperperfused synovial pannus. It has been demonstrated that ultrasonography is able to detect more erosions than radiography, especially in early-stage RA, and that erosions detected with ultrasonography are true erosions compared with those detected with CT as the gold standard.[52,53,241] However, some of the smallest bone erosions detected by ultrasonography do not correspond to cortical bone breaks seen on micro-CT. These false-positive findings correspond to bone channels and forceps-like osteophytes.[242]

Tendon involvement is frequent and characteristic in patients with RA. The spectrum of pathologic changes detectable in tendons by ultrasonography is wide and heterogeneous. Tendon sheath widening, loss of the normal fibrillar echotexture and loss of definition of tendon margins, and partial or total tears are the abnormalities that characterize tenosynovitis.[236] Circumscribed or extensive collections of anechoic fluid within the synovial sheath is the most characteristic feature of exudative tenosynovitis. The presence of echoes within the tendon sheath can be associated with synovial proliferation and/or aggregates of cells and proteins.

Very subtle changes in tendon structure can be detected in patients with early-stage RA. The predictive value of these changes in detecting the more aggressive or rapidly progressive forms of disease has still not been clearly defined, and follow-up investigations are needed. Tenosynovitis at the extensor carpi ulnaris tendon seems to predict the development of erosions.[243] Early circumscribed tendon tears appear as minimal fragmentation of small groups of contiguous fibrils, which determines a characteristic loss of the normal fibrillar echotexture of the tendon. In the more advanced stages of structural damage, tendons can exhibit large partial tears or complete rupture. A three-grade semiquantitative score (normal, partial, and complete rupture) seems to be the best approach to the ultrasound assessment of tendon damage in patients with RA.[244] If the ultrasound beam and the major axis of the tendon are not perfectly perpendicular, certain areas of the tendon appear anechoic (anisotropy artifacts) and thus may be mistaken for possible tendon ruptures.

Ultrasonography is more sensitive than clinical examination for the detection of arthritis. A significantly higher number of inflamed joints are found by ultrasonography than by clinical assessment. However, ultrasound examination of 78 joints requires 70 minutes, and the time needed for even a seven-joint set can be challenging in daily clinical practice.[245,246]

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** The ability of ultrasonography to visualize intra- as well extra-articular changes suggests that it could be used to assist clinicians in reaching a specific diagnosis (e.g., in patients with early undifferentiated arthritis). The data describing the differential diagnostic value in patients with undifferentiated arthritis are, however, very limited. In the seronegative (RF and anti-citrullinated protein antibody [ACPA] negative) subgroup of 50 early undifferentiated inflammatory arthritis patients, certain clinical and radiographic features (elevated CRP, swollen joints, and radiographic erosion) raised the probability of persistent

inflammatory arthritis from 6% to 30%, whereas further addition of 1 to 3 specific ultrasound features (grade 3 grey-scale synovial hypertrophy, at least grade 2 synovial power Doppler signal, and at least one erosion) increased the probability to 50% to 94%, suggesting that combining ultrasonographic evaluation with routine assessment in seronegative early arthritis may markedly increase the certainty of diagnosis.[247] In seropositive disease (RF and/or ACPA positive), ultrasonography had no predictive value. In another study, ultrasonography was recently demonstrated to improve, compared with clinical joint examination, the accuracy of the 2010 ACR/EULAR criteria for identifying patients with a disease that requires methotrexate treatment.[248] The exact position of ultrasonography in the diagnosis of RA needs further clarification.

**Monitoring Disease Activity and Damage.** Although no international consensus yet exists on the best possible scoring system and which joints to score in the assessment of synovitis and other joint pathologies in RA,[246,249] the OMERACT ultrasonography task force has developed definitions for various joint pathologies, which can be used for further development and validation. Ultrasound measures of synovitis (Doppler signal and B-mode synovial membrane thickness), decrease in parallel with other markers of disease activity when glucocorticoids[227,228,250-252] or TNF-antagonists[253-258] are administered, indicating their potential for monitoring joint inflammation in RA.[259] Ultrasonography visualizes cartilage changes and bone erosion in accessible areas,[260-263] but data are still lacking on reproducibility and sensitivity to change.

Thus, ultrasonography is probably a valid method for monitoring synovitis and, in accessible areas, damage progression. However, the relative advantage of ultrasonography versus radiography for showing damage progression remains unclear, and more data on reproducibility (e.g., probe positioning, selection of site for the picture, machine selection, machine/pressure adjustments) and sensitivity to change when the entire ultrasound examination is repeated, are needed.

**Predicting Outcome.** Data on the predictive value of ultrasound findings are ambiguous. One study has found ultrasound synovitis to be predictive of future radiographic progression in patients treated with conventional DMARDs but not with anti-TNFs,[256] whereas another found no significant correlation between the baseline clinical, laboratory, functional, and ultrasound parameters, and the 1-year follow-up disease activity score (DAS)28, Health Assessment Questionnaire (HAQ) score, and radiographic scores in DMARD treated patients.[264]

The Leeds group has reported that ultrasound and MRI signs of joint inflammation are frequent in patients in clinical remission.[144,265-268] Baseline ultrasound synovial hypertrophy, ultrasound power Doppler, and MRI synovitis scores in individual joints were significantly associated with progressive radiographic damage. Furthermore, there was a significant association between the power Doppler score at baseline and structural progression during 12 months in asymptomatic MCP joints and 12 times higher odds of structural progression in joints with increased power Doppler signal.[142,268] Subsequent studies have supported the value of ultrasonography as an independent predictor of radiographic progression in patients with RA in remission.[269,270] Regarding whether ultrasonography findings in RA patients in clinical remission can predict clinical outcome, one study reported that ultrasonography power Doppler signal was a predictor of flare during continued DMARD therapy,[271] whereas two studies of RA patients in clinical remission on biologic therapy presented conflicting reports on whether ultrasonography could predict who could stop biologic therapy without clinical flare.[272,267] It remains to be determined whether ultrasonography can predict long-term clinically significant disease progression and preservation of clinically important function better than traditional clinical or serological scores, and whether ultrasonography and/or MRI should be incorporated in future treat-to-target strategies.[273]

## Ankylosing Spondylitis/Axial Spondyloarthritis

Despite the fact that contrast-enhanced Doppler ultrasonography has been reported to have a high negative predictive value for the detection of sacroiliitis,[274] the role of ultrasonography in assessment of sacroiliac and spine involvement in AS and other types of axial SpA is minimal.

AS and other types of axial SpA frequently involve peripheral joints and entheses. Ultrasonography of peripheral involvement in SpA is described in the section on PsA that follows.

## Psoriatic Arthritis

Ultrasonography assessment of patients with PsA should be focused on joints, tendons with and without a synovial sheath, entheses, skin, and nails (Figure 58-29).[275] Basic sonographic changes of joint and synovial tendons can be similar to those of RA. However, a highly hypervascularized pannus seems to be more prominent in PsA patients, both in small and large joints. This feature appears to be related to histopathologic findings.[276] Tendons without synovial sheaths are characteristically involved in PsA. Inflammatory changes detectable by ultrasonography include loss of fibrillar echotexture, focal, or diffuse hypoechoic tendon thickening caused by intratendinous edema, with or without power Doppler signal. Moreover, a peritendinous inflammation may appear as a hypoechoic swelling of the soft tissue surrounding the tendon which may show power Doppler signal.[277]

Ultrasonography can detect a wide range of abnormalities of the enthesis. These consist of entheseal thickening, focal hypoechogenicity, loss of the homogeneous fibrillar echotexture, irregularity of the bone profile (enthesophytes and/or bone erosions), and power Doppler signal. There is evidence to support the use of ultrasonography as a very sensitive tool to detect subclinical enthesopathy in patients with psoriasis and no clinical signs of arthritis and/or enthesitis. An international consensus-based ultrasonography definition of enthesitis has recently been published.[278] The hyperechoic thickening of the epidermis with or without acoustic shadowing and the hypoechoic swelling of the dermis with evident power Doppler signal are the main ultrasound features of psoriatic plaque. Both the nail plate and bed can be accurately depicted by ultrasonography, and a wide range of abnormalities can be detectable, including thickening and loss of the typical trilaminar aspect of the

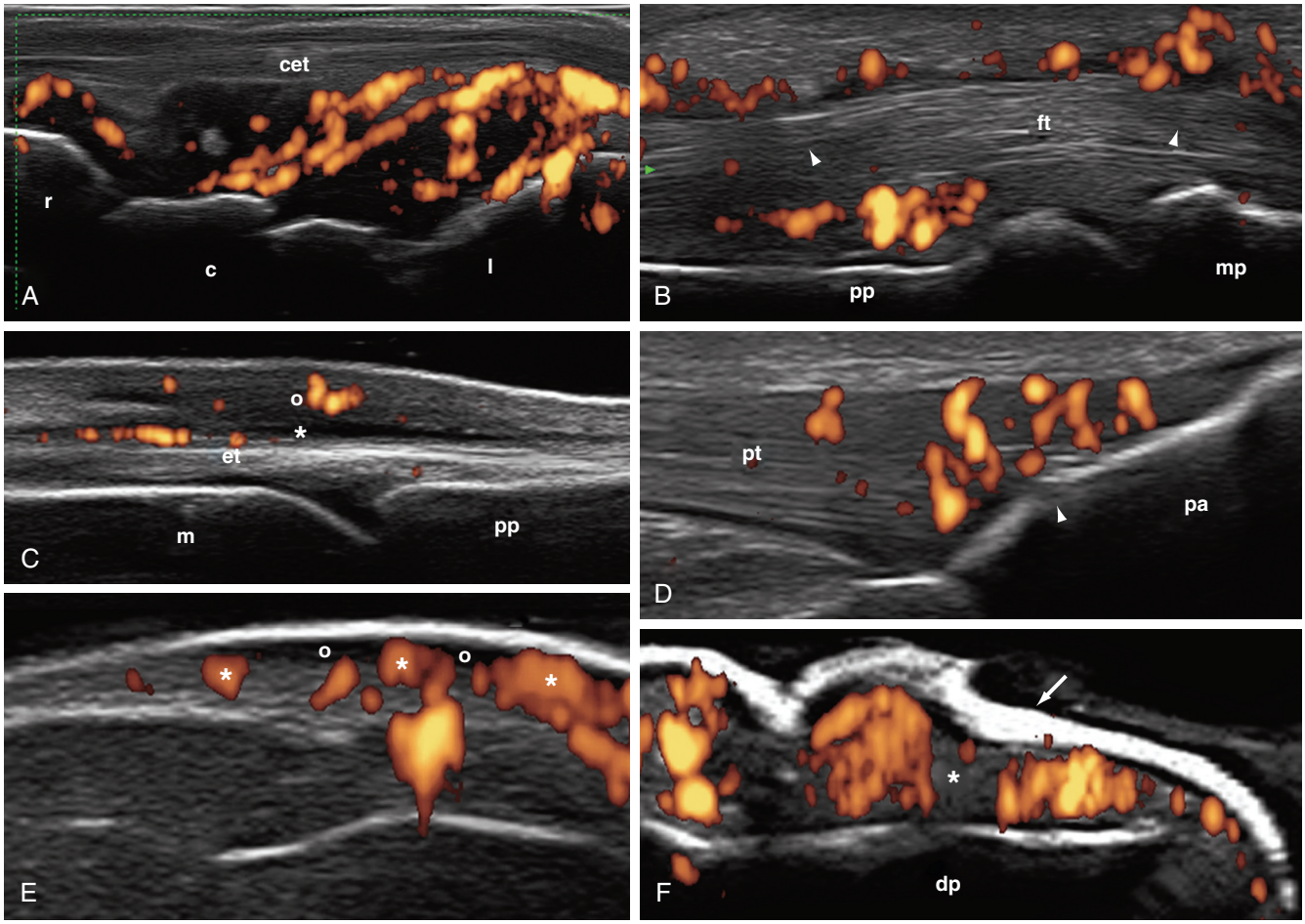



**Figure 58-29** **Psoriatic arthritis (ultrasonography). A**, Wrist (dorsal longitudinal scan): marked joint cavity widening of both radiocarpal and intercarpal joints with intense power Doppler signal. **B**, Proximal interphalangeal joint (longitudinal volar scan): chronic tenosynovitis with areas of low tendon echogenicity *(arrowheads)* and power Doppler signal. **C,** Metacarpophalangeal joint (dorsal longitudinal scan): peritendinous inflammation characterized by hypoechoic swelling surrounding the extensor digitorum tendon *(asterisk)* associated with power Doppler signal and subcutaneous edema *(circle)*. **D**, Distal patellar enthesis (longitudinal anterior scan): thickening of the enthesis associated with intense power Doppler signal and bone erosion *(arrowhead)*. **E**, Psoriatic plaque: hypoechoic subepidermal thickening *(circle)* with intense power Doppler signal *(asterisk)* indicating vasodilatation and angiogenesis. **F**, Psoriatic onychopathy: loss of the normal trilaminar aspect of the nail plate *(arrow)*, thickening of the nail bed *(asterisk)*, and intense power Doppler signal. *c*, Capitate; cet, common extensor digitorum tendons; *dp*, distal phalanx; *et*, common extensor tendon of the finger; *ft*, flexor finger tendons; *l*, lunate; *m*, metacarpal bone; *mp*, middle phalanx; *pa*, patella; *pp*, proximal phalanx; *pt*, patellar tendon; *r*, radius.

nail plate with or without increased Doppler signal at the nail bed.[275]

Power Doppler synovitis is correlated with PsA-specific composite scores such as the Disease Activity Index for Psoriatic Arthritis (DAPSA) and the Composite Psoriatic Disease Activity Index (CPDAI). Conversely, ultrasound signs of enthesitis, dactylitis, tenosynovitis, and perisynovitis are not associated with clinical composite scores.[279]

### Use in Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** Ultrasonography is more sensitive than clinical examination for detection of synovitis, tenosynovitis, and enthesitis in patients with PsA[280-282] but no studies have yet documented that ultrasonography in an early undifferentiated arthritis cohort can be used to differentiate PsA from other arthritides. Because enthesitis is prominent in SpA patients and may precede joint symptoms, it has been of interest to evaluate the entheses by use of ultrasonography as a means of diagnosing SpA and therefore also PsA.[283]

A recent study indicates that early ultrasonography enthesitis will predict development of SpA.[284] Further work is needed to clarify the role of ultrasonography in the diagnosis of PsA.

**Monitoring.** Most studies which aim to monitor treatment response have applied semiquantitative scoring systems for grey-scale and/or Doppler changes.[285,286] TNF blocker treatments have provided significant reduction in both grey-scale and Doppler semi-quantitative synovitis scores,[285] in entheseal morphologic abnormalities, power Doppler signal, and in bursitis.[287] Several systems to assess enthesitis have been proposed.[288-290] No system has been sufficiently validated to be used for monitoring PsA or other spondyloarthritides. More research is needed before standardized, reliable, and responsive ultrasonography outcome measures in PsA are available.

**Predicting Outcome.** No studies currently exist that evaluate the role of ultrasonography for prognosticating PsA. Entheseal involvement in patients with psoriasis, but without clinical PsA, indicate that enthesitis may be a

predictor of development of PsA.[291] However, longitudinal studies are required to clarify this.

## Gout

Ultrasonography can detect monosodium urate crystal deposition on the cartilage surface, inside the joint cavity, and around and inside tendons. The spectrum of ultrasound appearance of urate crystal aggregates can vary from homogeneously punctate to sharply defined hyperechoic densities of variable size and, eventually, to dense tophaceous material with posterior acoustic shadows.[292] Joint cavity widening is the most frequent ultrasound finding in patients with acute gout. The sensitivity of ultrasonography is comparable to that of DECT for the diagnosis of gouty arthritis, but ultrasonography is more cost effective and does not expose the patient to radiation.[293]

Monosodium urate crystal deposition on the surface of the articular cartilage results in hyperechoic enhancement of the chondrosynovial margin, which can range from homogeneous thickening of the chondrosynovial interface to areas of focal deposition.[294] Bone erosions are frequently detectable in patients with chronic gout.

Asymptomatic and symptomatic involvement of tendons is common in patients with gout. Urate crystals aggregates of variable size and shape are frequently detectable, especially at the patellar and the Achilles tendons. The normal fibrillar echotexture of tendons can be completely altered by the presence of intratendinous monosodium urate deposits, which appear as areas of inhomogeneous echogenicity sometimes generating an acoustic shadow (Figure 58-30).[294]

### Use in Diagnosis, Monitoring, and Prognostication

The detection of tophi could be helpful in diagnosing gout, especially when these lesions are not detectable clinically. Detection of a nodule with characteristic appearance on

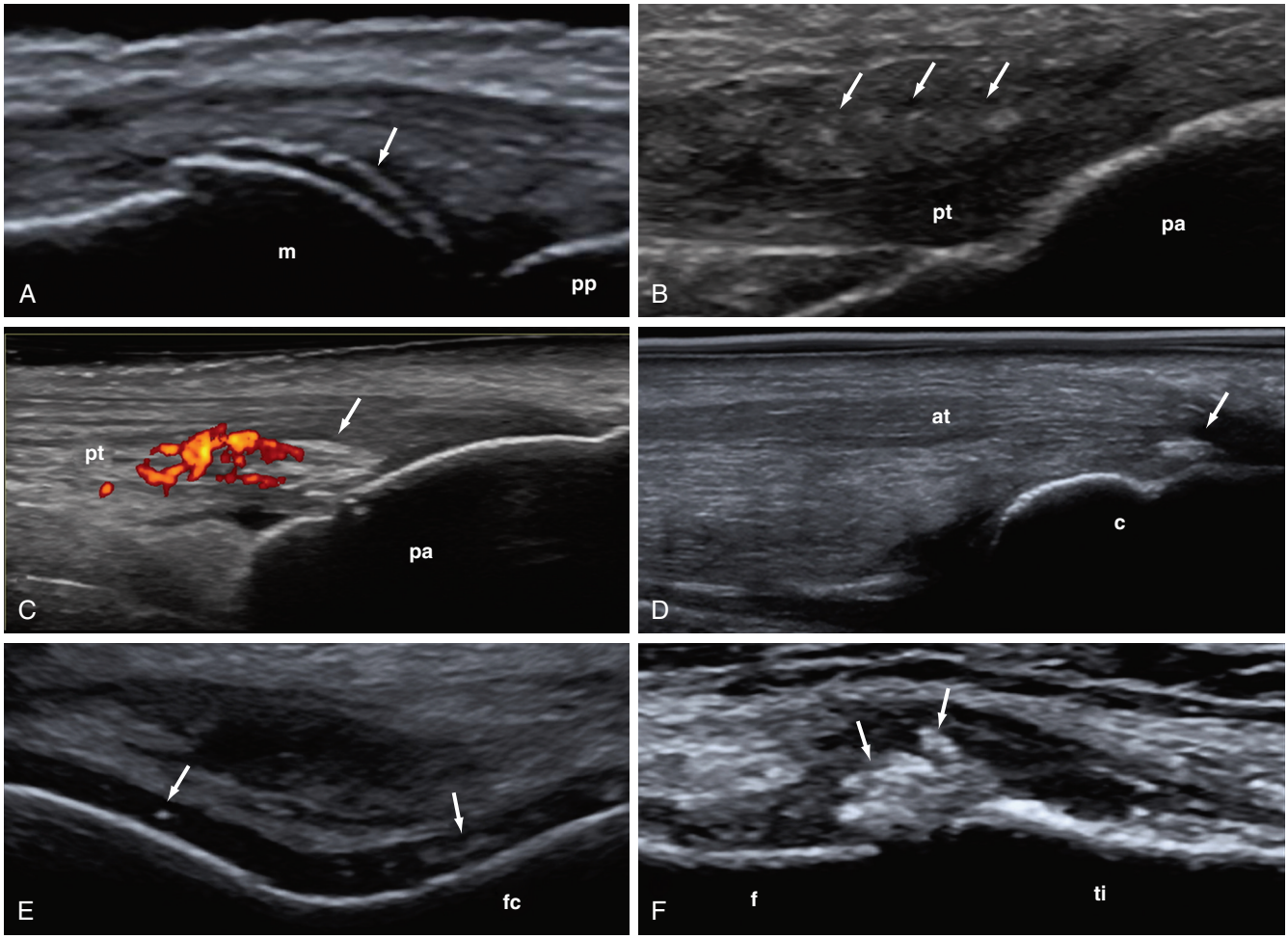



**Figure 58-30 Gout and calcium pyrophosphate dihydrate (CPPD) crystal deposition disease (ultrasonography). A** through **C,** Gout. **A,** Chronic gout. Metacarpophalangeal joint (longitudinal scan with fingers in full flexion): hyperechoic enhancement of the chondrosynovial interface *(arrow)* caused by monosodium urate crystal deposition. **B,** Distal patellar enthesis (longitudinal anterior scan): intratendinous monosodium urate deposits *(arrows)*. **C,** Distal patellar enthesis (longitudinal anterior scan): tophaceous deposits *(arrow)* surrounded by power Doppler signal. **D** through **F**, CPPD crystal deposition disease. **D,** Achilles tendon *(at)* (longitudinal scan): intratendinous linear hyperechoic deposits without acoustic shadow *(arrow)*. **E**, Femoral hyaline cartilage (transverse scan): calcium pyrophosphate deposits within the hyaline cartilage *(arrows)*. **F,** Knee (longitudinal lateral scan): diffuse meniscal calcification *(arrows)*. *c*, Calcaneus; *f*, femur; *fc*, femoral condyle; *m*, metacarpal head; *pa*, patella; *pp*, proximal phalanx; *pt*, patellar tendon; *ti*, tibia.

ultrasound, MRI, or CT is highly suggestive of gout, and the double contour sign, expected to represent urate crystals on the cartilage surface, has been shown to have diagnostic value.[295,296] Nevertheless, joint aspirate confirmation of monosodium urate crystals is usually required to rule out infection or other space-occupying lesions. Tophus size can be reliably assessed by ultrasonography and may be useful for monitoring treatment efficacy in clinical trials.[194,292] An ultrasonography scoring system for overall assessment of gout has not yet been formalized but is under development.[296] The prognostic value of ultrasonography findings is not known.

## Calcium Pyrophosphate Dihydrate Crystal Deposition Disease

Ultrasonography has been found to be highly sensitive in detecting aggregates of CPPD crystals.[294,297] CPPD aggregates range from tiny circumscribed hyperechoic spots to extended deposits with or without acoustic shadow. Based on case control studies,[297,298] a EULAR task force for the development of recommendations for calcium pyrophosphate deposition disease has concluded that "US [ultrasonography] can demonstrate CPPD in peripheral joints, appearing typically as thin hyperechoic bands within hyaline cartilage and hyperechoic sparkling spots in fibrocartilage. Sensitivity and specificity appear excellent and possibly better than those of conventional x-rays."[299] Moreover, floating aggregates of crystals can be observed inside fluid collections (see Figure 58-30).

## Septic Arthritis

Septic arthritis is a major emergency in rheumatology. The role of ultrasonography is to provide early findings which can raise the suspicion of joint infection and, potentially, aid in the collection of fluid. Synovial fluid in septic arthritis may show a variable degree of reflectivity. The effusion can range from hyperechoic to hypoechoic, with a diffuse pattern of low-level echoes. Power Doppler signal is generally intense, mostly in periarticular soft tissues. Sonographic-guided aspiration of even minimal collections of synovial fluid may play a key role in early differential diagnosis of acute monoarthritis. Bone and cartilage damage may be detectable in a few days if an aggressive treatment is delayed.

## Osteoarthritis

Ultrasonography is exquisitely sensitive in detecting structural changes of the articular cartilage.[300] Irregularity and/or loss of the thin and sharp contour of the outer border of the hyaline cartilage and/or increased cartilage echogenicity with patchy or diffuse loss of clarity are the earliest ultrasound features of OA. These changes seem to reflect structural alterations, such as fibrillation of cartilage and cleft formation. Variable loss of cartilage is detectable in patients with more advanced disease. Alteration of the subchondral bone, ranging from a tiny irregularity of the bone profile to multiple erosions, can be detected in areas that appear normal on conventional radiography in both erosive and non-erosive OA. A slight increase of cartilage thickness caused by inflammatory edema in the early phases of OA has been described.

Osteophytes are the most frequent and characteristic abnormalities of the bone profile in patients with OA, appearing as irregularities of the bone contour with posterior acoustic shadow. Joint effusions are commonly found in patients with OA. Minimal fluid collections that are not detectable on clinical examination are easily demonstrated by ultrasonography. The inhomogeneous echogenicity of synovial fluid can be related to proteinaceous material, cartilage fragments, aggregates of crystals, and calcified loose bodies. Popliteal cysts are frequent in patients with knee OA. Ultrasonography provides structural details about the content of the cyst, its communication with the joint space, and the possible compression of adjacent vascular structures. Cyst sizes and shapes vary widely, ranging from small (<1 cm) to giant multiloculated formations. Synovial hypertrophy in OA may show sonographic features similar to those observed in patients with RA but without the invasive properties of the rheumatoid pannus. Color/power Doppler ultrasonography is a useful tool to distinguish inflammatory from non-inflammatory knee swelling.[301] It has recently been demonstrated that inflammatory signs are more frequent in erosive osteoarthritis than in nonerosive osteoarthritis, not only in erosive joints but also in nonerosive joints. This finding suggests an underlying systemic cause for erosive evolution.[302]

Ultrasonography-detected capsule distension, synovial inflammation, and effusion are common in painful knee OA and correlate significantly with knee synovitis, effusion, and clinical parameters suggestive of an inflammatory flare. As in RA, ultrasonography detects more joints with inflammation than does clinical assessment. Color/power Doppler ultrasonography is a useful tool to distinguish inflammatory from non-inflammatory knee swelling.[301]

### Diagnosis, Monitoring, and Predicting Outcome

**Diagnosis.** The classification criteria for OA are based on clinical and conventional radiographic findings, not ultrasonography.[48] However, the use of ultrasonography in the diagnosis of OA is favored by the fact that ultrasonography can visualize the pathologic changes described in the preceding text.

**Monitoring.** Ultrasonography can demonstrate changes in synovitis thickness, effusion size, and popliteal cyst size.[303] With respect to structural change, including cartilage damage, the sensitivity to change remains to be determined. Reproducibility data are scarce and no consensus on scoring systems exists. Quantitative assessment of cartilage is restricted to thickness, as total volumes cannot be measured. More work is required to develop standardized definitions of pathology and to demonstrate the validity of ultrasonography in OA.[304]

**Predicting Outcome.** In patients with OA of the hand the presence of ultrasound inflammatory features such as power Doppler signal, synovial thickening and effusions are strongly associated with radiological progression in the subsequent 2 years.[302] Overall, the significance of ultrasonographically detected pathologic conditions in OA with regards to symptoms, prognosis, outcome, and response to therapy remains to be determined.[305]

# OTHER IMAGING MODALITIES

## Digital X-ray Radiogrammetry

**KEY POINTS**

**Digital x-ray radiogrammetry (DXR) performs automated radiogrammetry from conventional hand x-rays and provides an approximated bone mineral density (BMD).**

**Bone loss as measured by DXR-BMD may be useful for monitoring and predicting bone damage in RA.**

Three types of bone loss are early features of RA, focal articular bone erosion, periarticular osteopenia, and systemic osteoporosis. Periarticular bone loss is frequently the earliest radiographic feature of RA and is associated with disease activity and is known to precede bone erosions.[306] Radiographic osteopenia is, however, only detected if there is bone loss greater than 30%.[307]

Digital x-ray radiogrammetry (DXR) is a fully automated technique for performing radiogrammetry from standard hand radiographs to bridge the gap between radiogrammetry and bone densitometry. The DXR technology is based on a combined computerized radiogrammetric and textural analysis of the narrowest parts of the second, third, and fourth metacarpal bones. Based on cortical thickness (cm), porosity index, and an assumption of a constant bone density and elliptical bone, DXR calculates an approximated bone mineral density (BMD; g/cm$^2$).[308] Short-term precision of DXR-BMD (coefficient of variation [CV] and long-term precision have been reported as 0.28% and 0.25%, respectively, and the reproducibility as 0.05% to 0.27%.[309,310]

DXR is highly correlated with periarticular BMD measurements, and can therefore reflect periarticular bone loss even though it measures midshaft cortical bone.[311] DXR-BMD shows greater loss in patients with RA with high levels of inflammation, positive RF, or positive ACPA. Further, measurements of DXR-BMD changes are significantly associated with progression of structural damage in patients with early-stage RA.[312] Losses seen during 1year with DXR-BMD are predictive of subsequent erosive development.[309] However, so far only changes in DXR-BMD have been shown to be of predictive value, limiting its clinical applicability. DXR-BMD changes have also been shown to be responsive to treatment interventions. During disease modifying treatment, DXR-BMD losses are less severe in patients responding to treatment than those who do not.[309,311,312] This suggests that DXR-BMD may become a useful outcome measure and predictor of joint damage in RA clinical trials. However, its clinical usefulness and applicability has yet to be established.

## Nuclear Medicine

**KEY POINTS**

**Nuclear imaging provides not only morphologic but also physiologic information regarding the metabolic state of tissues.**

**Nuclear medicine techniques can be fused with other imaging modalities (e.g., SPECT/CT) providing combined functional and anatomic information.**

**PET can be useful for diagnosis and management of vasculitides, fever of unknown origin, and malignancies.**

**Other imaging modalities are generally better suited than nuclear medicine to investigate inflammatory and degenerative rheumatic diseases in clinical practice**

### Bone Scintigraphy (Planar)

Bone scintigraphy imaging can be planar (i.e., whole body and spot views) or tomographic (i.e., single photon emission tomography [SPECT]). This is analogous to the relationship between planar radiography (conventional x-rays) and CT.

As with all other modalities in diagnostic nuclear medicine, classical bone scintigraphy uses a physiologic/functional approach to imaging bone or joint pathology. By this method, an organic analogue of phosphate (methylene diphosphonate [MDP]) is tagged with a standard radionuclide ($^{99m}$Tc). The combined tracer, $^{99m}$Tc-MDP, is then administered intravenously. This organic phosphate analogue is then distributed with a whole-body distribution proportional to regional perfusion. It is adsorbed onto the surface of osteoid, at the sites of active mineralization (calcium phosphate formation). Throughout the entire diagnostic imaging test, from the onset of tracer administration to the final delayed images (from 3 to 24 hours), nuclear medicine gamma cameras detect photons emitted by the decaying radionuclide, forming diagnostic whole-body or spot images which demonstrate the concentration of the administered tracer. In the first few minutes after administration, the tracer distribution is a reflection of regional perfusion and relative blood pool distribution. By 3 hours, tracer distribution represents true incorporation into osteoid, reflecting local osteoblastic bone metabolism. Tracer which is not incorporated into bone is eliminated by renal excretion. Residual free tracer within plasma and extra-cellular space is proportional to blood pool and soft tissue distribution. At 24 hours, further clearance of physiologic tracer distribution (both bone and soft tissue) amplifies retention of tracer from pathologic accumulation. Radionuclide decay of $^{99m}$Tc (6-hour half-life) precludes any further imaging beyond 24 hours. Effectively, the delayed bone images (3 to 24 hours) provide an indirect measure of osteoblastic activity. Bone scintigraphy provides an imaging map of active bone metabolism.[313,314] Diagnostically, bone scintigraphy allows an opportunity to detect a pathologic condition that is associated with abnormal bone metabolism: specifically, where there is increased (or possibly decreased) osteoblastic activity. In theory, most bone and joint pathologies are associated with some degree of abnormal osteoblastic activity, even those which are primarily destructive or lytic. The sensitivity is limited by the volume of pathologic tissue, the relative intensity of the bone reaction, and physical constraints of photon detection which limit the spatial resolution.

Traditionally, bone scintigraphy has been used to diagnose and characterize a variety of bone and joint pathologies (traumatic, inflammatory, neoplastic, metabolic) (Figure 58-31), particularly in the setting of negative plain radiographs. It has relatively high sensitivity in this regard.[315] Although many traditional uses for bone scintigraphy have been supplanted by anatomic modalities with greater spatial

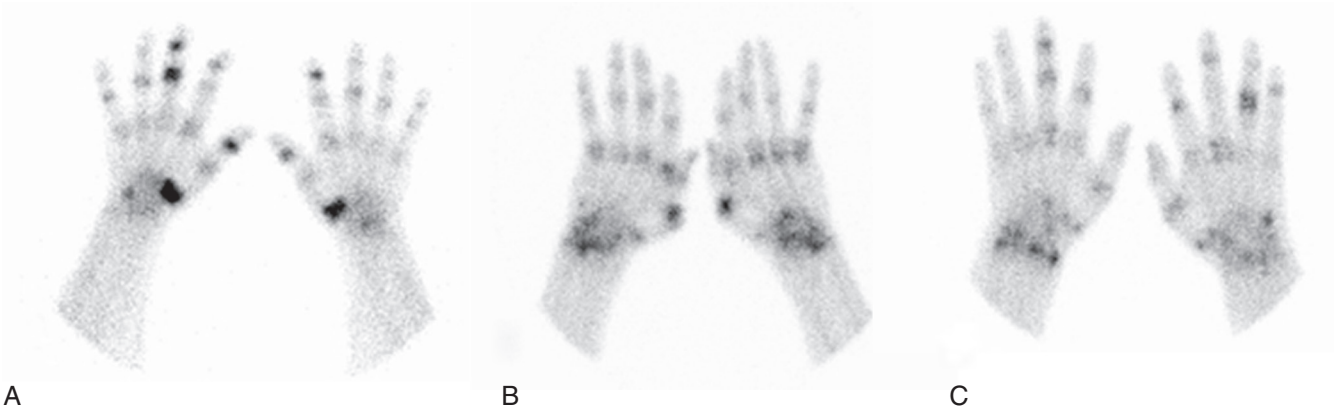



**Figure 58-31 Distributions of activity in osteoarthritis (OA), rheumatoid arthritis (RA) and psoriatic arthritis (PsA), on planar bone scintigraphy.** **A,** *Primary OA.* Severe activity is seen in the first carpometacarpal joint bilaterally and in several proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints. Mild/moderate activity is seen in the index DIP and thumb IP joints bilaterally. **B,** *RA.* Symmetric activity is seen in the radiocarpal joints, and all MCP and PIP joints. **C,** *PsA.* Bilateral asymmetric distribution is seen, in the right radiocarpal and in bilateral PIP and DIP in different fingers. Note the ray distribution in the right third finger.

resolution such as MRI and CT, scintigraphy is still the imaging modality of choice for certain specific clinical problems (e.g., follow-up of osteoblastic bone metastases, diagnosis of shin splints, demonstration of reflex sympathetic dystrophy). Bone scintigraphy is complementary to anatomic modalities such as CT and MRI because it provides an alternate, functional view of bone pathology that may not be anatomically apparent.

Whole body bone scintigraphic images are still the most efficient means of observing systemic bone pathology such as widespread bony metastatic disease, metabolic bone disease, or bone marrow expansion. A traditional three-phase planar bone scan is also efficient at demonstrating certain regional pathologies.[316]

From a rheumatology perspective, bone scintigraphy can demonstrate evidence of active arthritis (see Figure 58-31).[317] The whole-body surveillance capability easily documents the specific distribution of an active systemic arthritis or other concurrent systemic pathology. This information can augment or complement a clinical rheumatologic impression. In the presence of anatomically established arthropathy, bone scintigraphy can distinguish a metabolically active from an inactive joint.[318] From an orthopedic standpoint, bone scintigraphy has the ability to demonstrate bone stress reactions, periosteal reactions, or fractures. This is sometimes valuable for orthopedic surgical management.[319] In the evaluation of arthritides, scintigraphy can be a useful adjunct to clinical assessment, but should not be used routinely. Bone scans are more sensitive than conventional radiographs for detecting active bone pathology, but MRI is usually more sensitive and specific than bone scintigraphy, with the added advantage of not using ionizing radiation.[260,320,321] However, bone scintigraphy can provide a useful complementary role to MRI and other modalities for several clinical situations.

In addition to the use of ionizing radiation, costs, and limited spatial resolution, traditional planar bone scans have limited anatomic resolution, depending on the body part. Because the location of active pathology is often as important as its presence, this limitation has often reduced the specificity and overall accuracy of traditional planar bone scans.

### Single-Photon Emission Computer Tomography with and without Computed Tomography

SPECT (single-photon emission tomography) is the generation of cross-sectional scintigraphic images by processing multiple planar images obtained over a large range of camera positioning relative to the patient. The relationship of SPECT to planar scintigraphic imaging is analogous to the relationship between CT and plain radiography. Tomographic bone scintigraphy imaging provides improved sensitivity for the detection of pathologies such as active facet arthritis or spondylolysis.[322] However, precise anatomic location of the active pathology seen on conventional SPECT images (i.e., without concurrent CT), can be very difficult. This reduces its specificity. For example, facet arthritis versus active spondylolysis or other pathology in the posterior elements of a vertebral segment may be difficult to distinguish on SPECT imaging alone.

SPECT/CT is an example of modern fusion imaging. Other examples include positron emission tomography (PET)/CT and PET/MRI (see subsequent text). SPECT/CT is the acquisition of a CT study concurrent with the acquisition of a nuclear medicine SPECT study, while the patient remains in the same body position. This is performed by a single machine: a SPECT/CT capable gamma camera. The high resolution, anatomically specific CT images are then fused with the functional images from SPECT. The combined, fused images appear to provide far greater diagnostic accuracy than either alone. It is an effective marriage of tomographic imaging between functional (SPECT) and anatomic (CT) images.

SPECT/CT has, as PET/CT, revolutionized nuclear medicine imaging. The traditional weakness of nuclear medicine imaging, lack of anatomic resolution, has been largely corrected by its fusion with CT. In addition to identifying a functionally active abnormality, SPECT/CT now allows its precise anatomic localization to the extent allowed by

modern CT. The effect of this anatomic precision is a dramatic increase in the anatomic and overall diagnostic specificity of bone scintigraphy (and all other nuclear medicine modalities) when augmented with SPECT/CT. This improved specificity has been demonstrated for multiple medical problems such as infection, trauma, and tumor imaging.[323,324]

There is an approximate congruence between bone marrow edema seen on MRI and low to medium-grade patches of bone reaction.[325,326] As a result, fused bone SPECT/CT images approach the diagnostic capabilities of MRI in certain situations. Specific soft tissue pathologies, which are not traditionally diagnosed with bone scintigraphy (e.g., severe tendinopathy [Figure 58-32], subtle enthesitis, ligamentous injuries) can now be suggested by adjacent bone reaction combined with soft tissue findings on CT.

To assess arthropathy, SPECT/CT can occasionally diagnose a specific arthritis by the combination of an appropriate distribution of metabolically active arthropathy and specific anatomic findings on CT (Figures 58-33 and 58-34). When SPECT/CT, as CT, is used in clinical practice, the radiation dose should be considered.

The current literature for SPECT/CT imaging is deficient, with a significant lag behind technological advancement. Multiple musculoskeletal pathologies now visible on bone SPECT/CT which were previously visible only with conventional anatomic imaging (CT and MRI) have not yet been adequately documented in the nuclear medicine imaging literature. Multiple new signs and patterns of bone reaction which can offer indirect diagnoses of soft tissue pathologies have yet to be documented and fully assessed with respect to the sensitivity and specificity of their detection by bone SPECT/CT imaging. Furthermore, many current practicing interpreters of bone scintigraphy may need further education and experience with diagnostic CT for musculoskeletal pathology. Therefore, the full diagnostic capability of bone scintigraphy with SPECT/CT is not optimally used in current practice.

SPECT/MRI, another fusion imaging modality, is a theoretical possibility, but the potential utility of such an imaging modality has yet to be defined.

Various other tracers besides $^{99m}$Tc-MDP are available to detect inflammatory pathologies. Options include $^{67}$Ga citrate scan, $^{111}$In white blood cell (WBC) scan, $^{99m}$Tc WBC scan with or without concurrent bone marrow imaging (Figure 58-35), and $^{18}$F-fluorodeoxyglucose ($^{18}$FDG)-PET.[315,327] These may assist with imaging workup of osteomyelitis or soft tissue infection.

Similar to planar bone scintigraphy, SPECT/CT technology can be used for each nuclear medicine tracer,

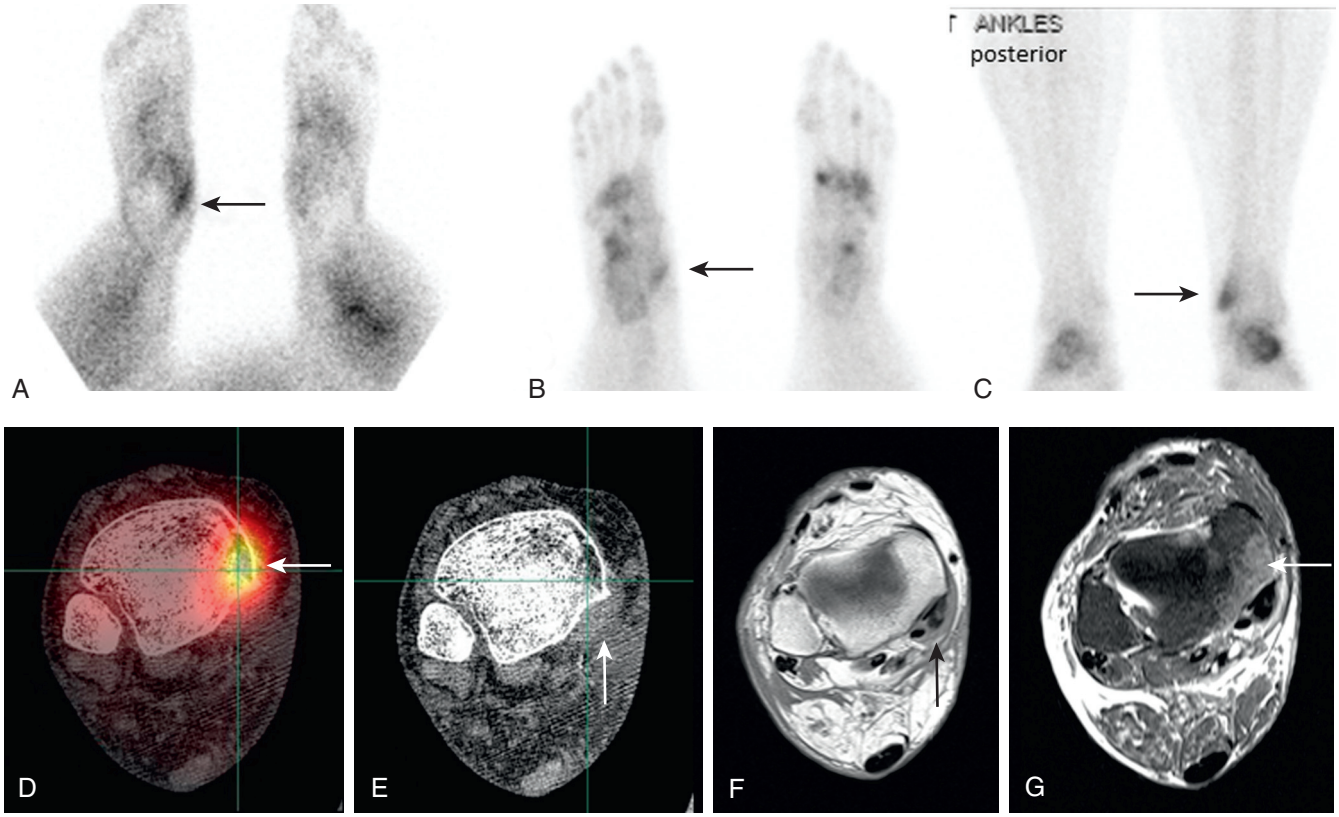



**Figure 58-32 Tibialis posterior tendinopathy demonstrated by bone single photon emission tomography/computed tomography (SPECT/CT).** A 74-year-old female with chronic pain in the medial malleolus. **A** and **C,** Planar bone scintigraphy. Initial planar blood pool images **(A)** show hyperemia in the vicinity of the right medial malleolus. Delayed bone images (**B** and **C**) show low grade bone reaction within the medial malleolus. **D,** SPECT/CT confirms the presence of a bone reaction in the medial malleolus *(arrow)*. **E,** The CT component of SPECT/CT demonstrates a thickened tibialis posterior tendon with abnormal contour *(arrow),* suggesting severe tendinopathy with an adjacent bone reaction. **F** and **G,** MRI confirms these findings because a proton density-weighted axial MR image **(F)** shows a longitudinal split tear and tenosynovitis of the tibialis posterior tendon (*arrow* in **F**), and the corresponding short tau inversion recovery MR image **(G)** with reactive bone marrow edema in the adjacent medial malleolus (*arrow* in **G**).

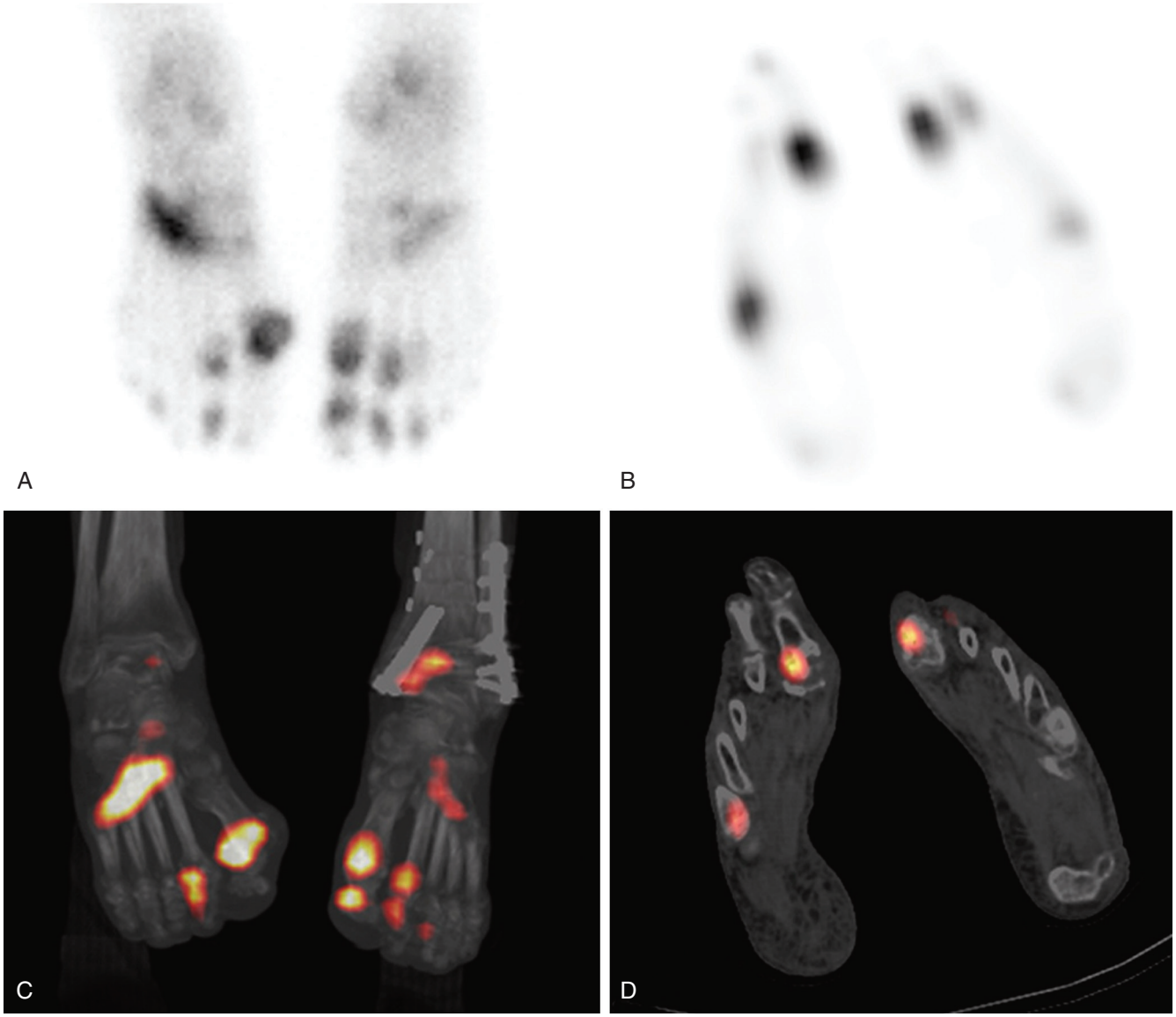



**Figure 58-33 Gout (single-photon emission computed tomography [SPECT]).** A 72-year-old female patient with known gout and prior trauma was investigated for possible complications. Planar **(A)** and transverse SPECT **(B)** images show multifocal increased uptake. Three-dimensional fused SPECT-CT **(C)** and transverse two-dimensional SPECT-CT **(D)** images provide superior localization of scintigraphic activity corresponding to the joints most affected by gout.

providing the same degree of improved anatomic and diagnostic specificity.

The choice of imaging agent depends on the specific clinical scenario: native versus violated bone, axial versus peripheral skeleton, prosthetic infection, acute versus chronic infection, fever of unknown origin. A discussion of optimal inflammatory imaging for each clinical condition is beyond the scope of this discussion.

## Positron Emission Tomography

PET is a functional imaging technique that enables metabolic mapping of tissues in vivo with positron-emitting radionuclides. $^{18}$FDG is the most commonly used radiopharmaceutical in PET because of its availability, favorable 110-minute half-life, and high uptake in most metabolically active tissues. When injected into the body, $^{18}$FDG demonstrates sites of increased glucose metabolism, thus identifying sites of metabolically active pathology in both bone and soft tissue. The metabolically active pathology can be malignant, inflammatory, or infectious. A PET scan is inherently tomographic and has a slightly higher spatial resolution than a SPECT scan. A PET/CT scan acquires both a PET and CT scan concurrently on the same machine, allowing for fusion of the two sets of images. Similar to SPECT/CT, the CT component of PET/CT provides a precise anatomic map which is fused with the functional images of glucose metabolism provided by PET.

$^{18}$FDG PET/CT is most often used in oncology, in which it has a well-established role for the diagnosis and clinical management of several malignancies. But $^{18}$FDG PET/CT imaging is also useful for a variety of clinical problems in cardiology, neurology, and infectious diseases. For example,

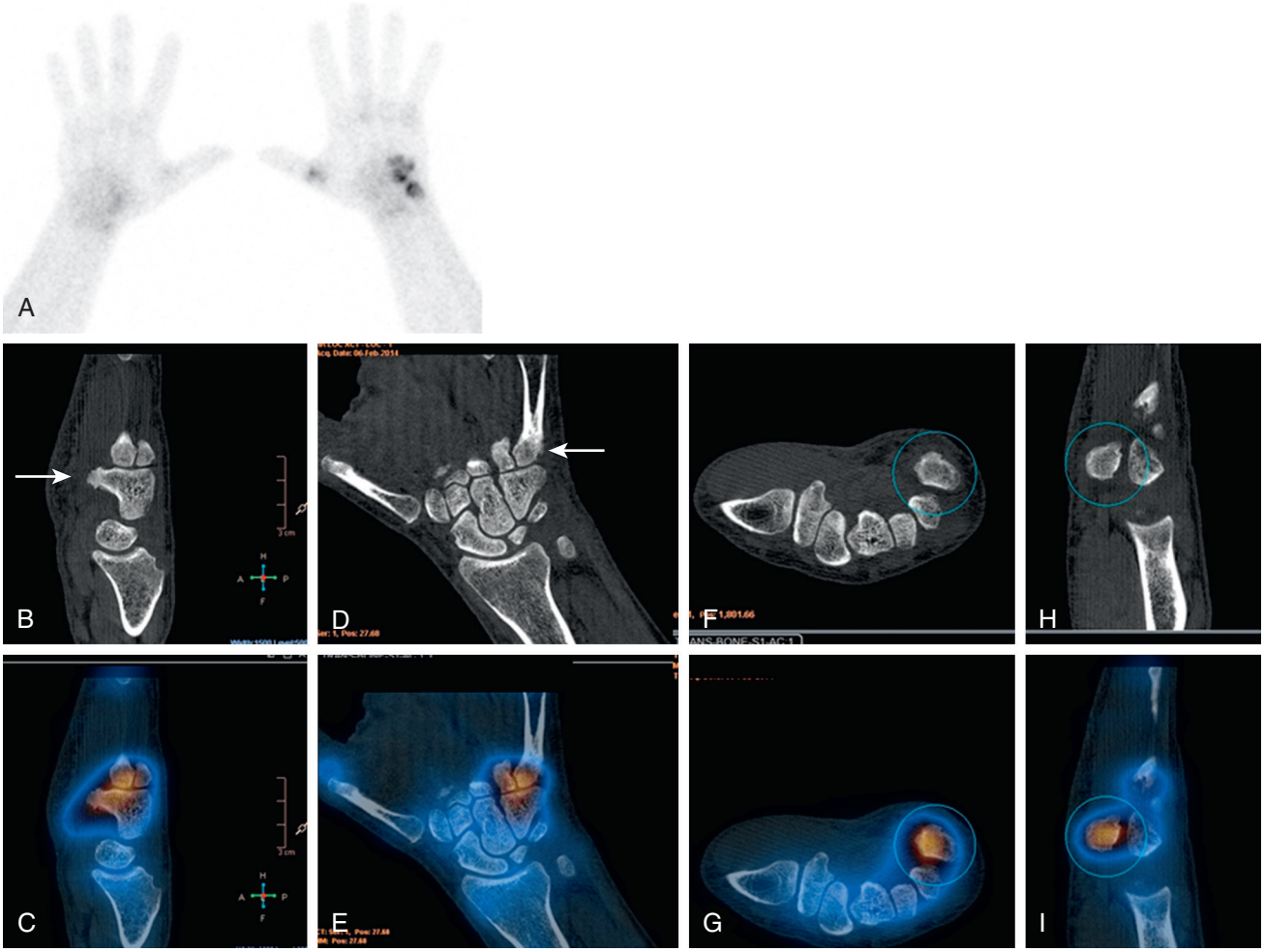



**Figure 58-34 Radiographically occult seronegative arthritis on single-photon emission tomography/computed tomography (SPECT/CT).** A 43-year-old man with pain in hands and feet for years, and normal radiographs. **A,** Planar bone scintigraphy of the hands show a nonspecific pattern of bone reaction within the pisiform and several ulnar-sided carpal bones including the fourth and fifth carpometacarpal (CMC) joints. Planar bone scintigraphy images of the feet (not shown) suggested enthesopathy in the base of the left fifth metatarsal bone and synovitis in the right ankle. **B** through **I,** SPECT/CT demonstrates active arthritis in the fourth and fifth CMC joints **(C, E)** and the pisiform **(G, I)**, with glowing orange signal. Images **B, D, F,** and **H** are CT images in the sagittal **(B, H)**, coronal **(D)** and axial **(F)** planes, and **C, E, G,** and **I** are the corresponding fused SPECT/CT images. Bone reactions on SPECT/CT include the hook of the hamate (*arrow* in **B**), the base of the fifth metacarpal (*arrow* in **D**), and the pisiform at the attachment of flexor carpi ulnaris tendon (*arrow* in **F**), where the CT shows fine, fluffy, whisker-like enthesopathy. The distribution of findings on planar images and on SPECT/CT suggest the presence of a seronegative arthritis, which was subsequently serologically confirmed.

$^{18}$FDG PET is now considered by many to be the diagnostic imaging test of choice to assess fever of unknown origin.[328] $^{18}$FDG PET has the highest overall sensitivity and specificity for the diagnosis of chronic osteomyelitis compared with bone scintigraphy, CT, and MRI.[329]

The uptake of $^{18}$FDG, similar to many other nonspecific inflammatory tracers, can conveniently demonstrate the presence and distribution of systemic disease.[330] In rheumatology, $^{18}$FDG-PET can be useful for the diagnosis and management of large-vessel vasculitis such as Takayasu's arteritis and that associated with granulomatosis with polyangiitis, polyarteritis nodosa, giant cell arteritis, and polymyalgia rheumatic (Figure 58-36).[327,331,332] $^{18}$FDG uptake is strongly correlated with MRI synovitis in RA,[333] but the role, if any, in routine clinical management of inflammatory arthritides remains to be determined.

## CONCLUSION

Imaging is an integral part of management of patients with rheumatic diseases. This chapter has outlined the status of imaging in rheumatic diseases, with a particular focus on inflammatory joint diseases. Please see the key point boxes for main messages. The chapter has explained the important role of conventional radiography, but also the exciting new opportunities to be gained with newer imaging techniques. The last decade has brought a vast amount of new knowledge that has dramatically changed the way we manage our patients with rheumatic diseases. It is exciting that with continued dedicated research and the rapid technical development it is likely that even larger improvements may occur in the decade to come, for the benefit of our patients.

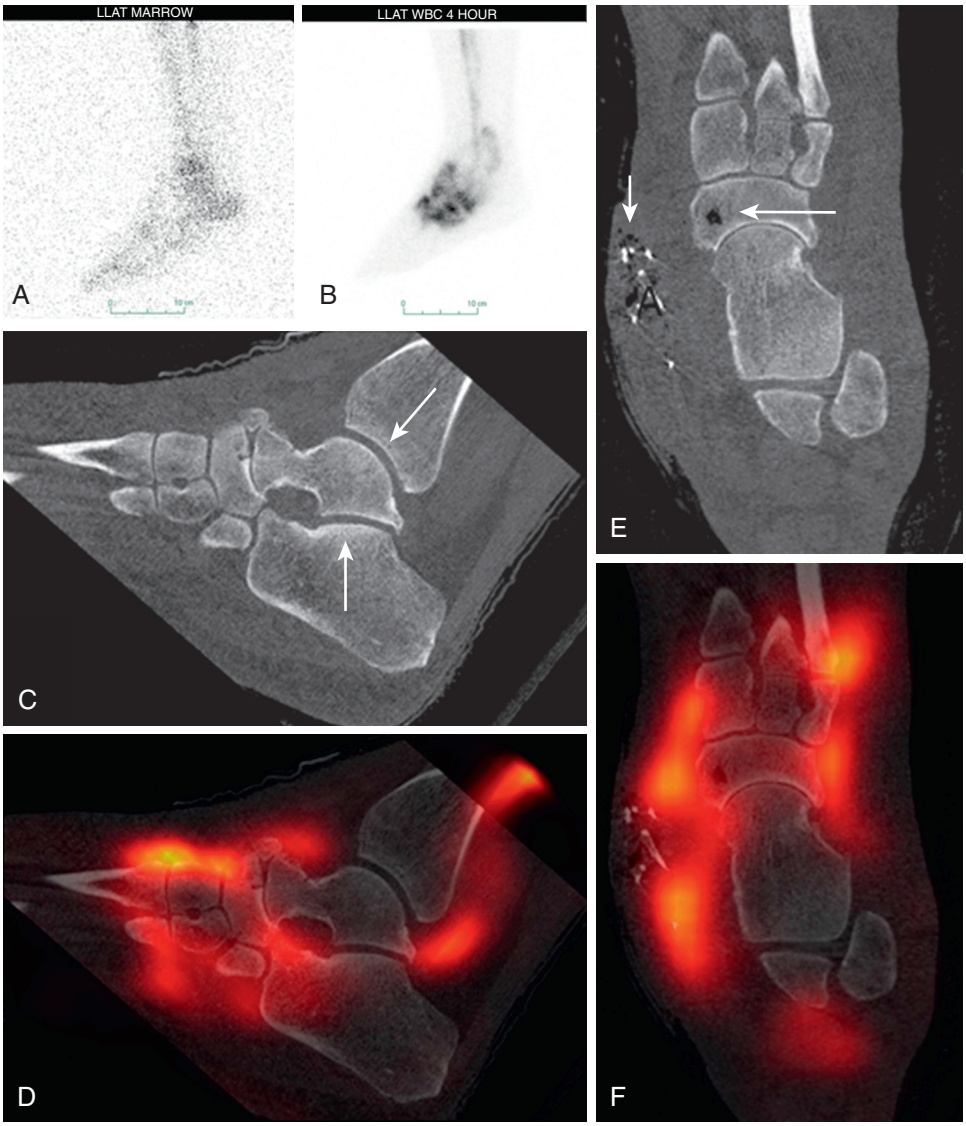



**Figure 58-35 Extensive septic arthritis of the midfoot demonstrated by planar white blood cell (WBC) bone scintigraphy and with single photon emission tomography/computed tomography (SPECT/CT).** A 41-year old male with soft tissue infection after minor surgery for removal of a small skin lesion in the dorsal/medial aspect of the midfoot. Extensive cellulitis was apparent clinically, along with a soft tissue abscess which was surgically drained. Standard planar bone marrow image, by using 99m technetium-sulfur colloid tracer **(A),** and WBC **(B)** image show intense, extensive WBC activity throughout the entire midfoot, with a bone and joint (rather than superficial soft tissue) distribution **(B)**. This is grossly incongruous with the distribution of bone marrow **(A),** suggesting that the WBC distribution represents extensive septic arthritis. **C** through **F,** Sagittal **(C)** and axial **(E)** CT and the corresponding SPECT/CT images **(D, F)** (i.e., CT fused with WBC SPECT). SPECT/CT confirms deep midfoot infection, with septic arthritis and osteomyelitis. WBC activity (**D** and **F**) has an articular (faint), periarticular and deep soft tissue distribution. The CT **(E)** shows medial soft tissue gas and speckled high density *(short arrow)* in the site of surgical débridement. Intraosseous gas in the navicular (*long arrow* in **E**) demonstrates at least one site of contiguous osteomyelitis. WBC uptake is seen within most joints of the midfoot (**D** and **F**). Note the sparing of the ankle and posterior subtalar joints (*thin arrows* in **C**), because they do not anatomically communicate with the midfoot joints.

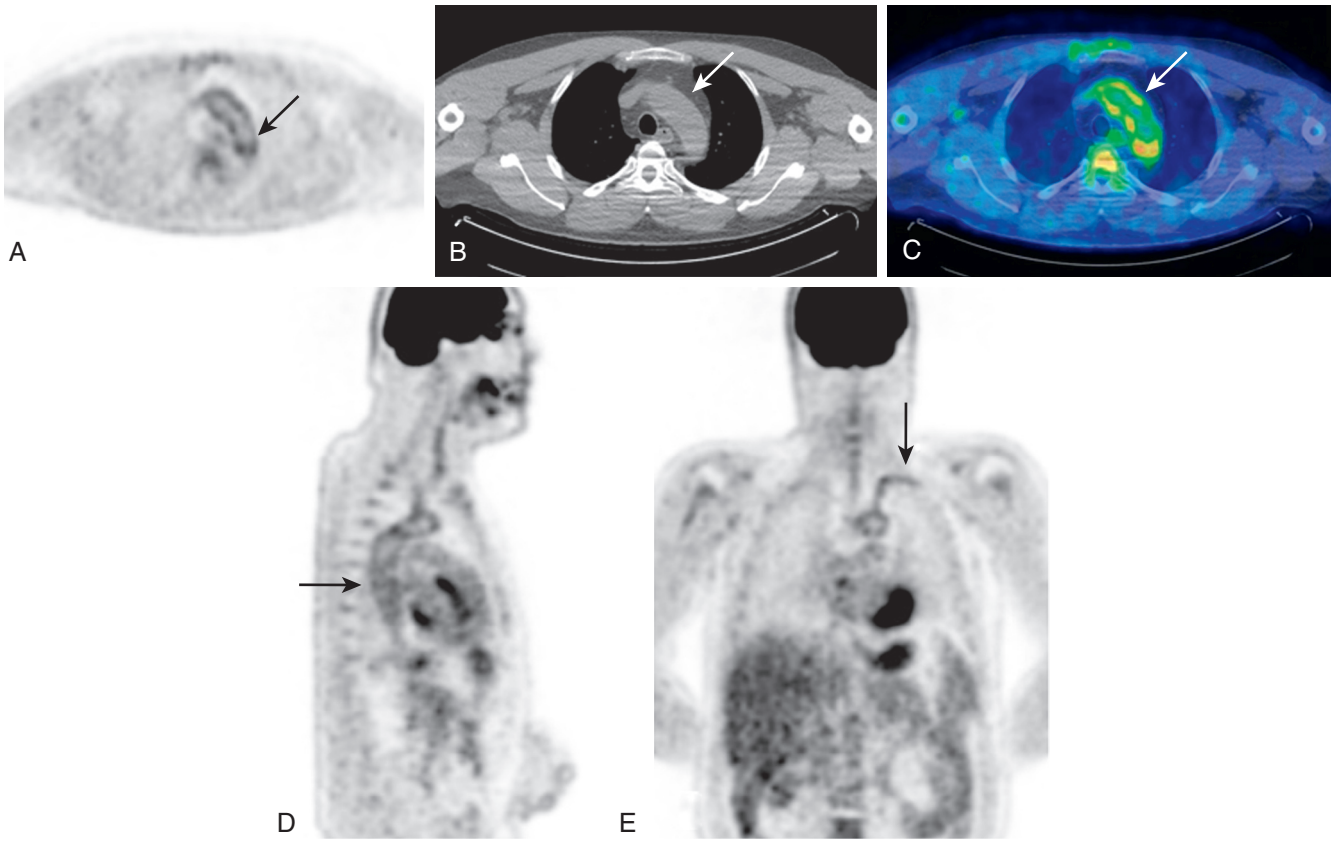



**Figure 58-36 Takayasu's arteritis, visualized by positron emission tomography/computed tomography (PET/CT).** A 46-year-old male with Takayasu arteritis receiving glucocorticoid therapy. An $^{18}$F-fluorodeoxyglucose PET/CT scan was requested to document active vasculitis because suboptimal therapy was suspected. **A** through **E,** Axial PET **(A),** CT **(B),** PET/CT **(C),** and sagittal **(D)** and coronal **(E)** PET images. Moderately intense inflammation is present in the aortic arch (**A, B,** and **C**) and descending aorta (*arrow* in **D**). Inflammation extends to the great vessels, including the left subclavian artery (*arrow* in **F**). These suggest persistent, active vasculitis.

Full references for this chapter can be found on ExpertConsult.com.

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PART 8 PHARMACOLOGY OF ANTI-RHEUMATIC DRUGS

CHAPTER 59



# Biology and Therapeutic Targeting of Prostanoids

Leslie J. Crofford

**KEY POINTS**

- NSAIDs are effective anti-inflammatory, antipyretic, and analgesic compounds.
- There is little difference in the efficacy of the various NSAIDs, but the pharmacologic characteristics of individual drugs, including potency, half-life, and relative inhibition of cyclooxygenase (COX)-1 and COX-2, play important roles in toxicity.
- Aspirin is an NSAID used in low doses to prevent cardiovascular disease. Taking aspirin and NSAIDs together is associated with increased toxicity in the gastrointestinal (GI) tract, and concomitant use of some NSAIDs with aspirin may be associated with aspirin resistance.
- NSAIDs are associated with risk for GI ulceration and bleeding. Patient-specific risk factors for toxicity should be recognized so risk-reduction strategies can be implemented.
- NSAIDs are associated with an elevated risk for cardiovascular disease. Clinicians should be aware of cardiovascular risk factors and either avoid prescribing NSAIDs or use intermittent dosing, low doses, or drugs with a short half-life.
- Periodic assessment of blood pressure, hemoglobin, electrolytes, renal function, and liver function is advisable, particularly in elderly patients.

The use of NSAIDs is ubiquitous in the practice of medicine because of their effectiveness as anti-inflammatory, analgesic, and antipyretic agents. NSAIDs differ widely in their chemical class but share the property of blocking production of prostaglandins (PGs). This effect is accomplished by inhibiting the activity of the enzyme PG G/H synthase (PGHS), also called *cyclooxygenase* (COX).

The clinical effects of NSAIDs are evaluated not only by their specific pharmacologic properties but also in terms of their effects on the different COX isoforms, COX-1 and COX-2. These isoforms serve different biologic functions in that COX-1 is expressed under basal conditions and is involved in the biosynthesis of PGs that serve homeostatic functions, whereas COX-2 expression is increased during inflammation and other pathologic situations. Inhibition of COX-2 by NSAIDs blocks PG production at sites of inflammation, whereas inhibition of COX-1 in certain other tissues, most importantly platelets and the gastroduodenal mucosa, can lead to common adverse effects of NSAIDs such as bleeding, bruising, and gastrointestinal (GI) ulceration.

In addition to their use in people with rheumatoid arthritis (RA) and osteoarthritis, NSAIDs are widely used in the symptomatic management of other rheumatic diseases characterized by chronic musculoskeletal pain and diverse forms of acute pain. Aspirin, which has unique properties among NSAIDs, is used by millions of people for primary and secondary prevention of cardiovascular thrombosis. In light of the widespread use of these drugs for common diseases, which are likely to increase in prevalence with the aging of the population, it is critically important to appreciate the potential adverse events and drug interactions associated with NSAIDs.

This chapter analyzes aspirin and other NSAIDs on the basis of their chemical structure, pharmacologic properties, and relative inhibition of COX-1 and COX-2. Particular attention to potential adverse events of specific NSAIDs in individual patients will facilitate use of these drugs in the safest possible manner. Acetaminophen (known as *paracetamol* outside the United States), an antipyretic and analgesic drug without anti-inflammatory activity, inhibits COX enzymes via a different mechanism than NSAIDs and is also discussed. Colchicine possesses anti-inflammatory characteristics similar to NSAIDs in some situations and is discussed in this chapter, although this drug differs in its mechanism of action and profile of adverse effects.

## HISTORY

Botanicals containing salicylates have been used since antiquity to treat pain, inflammation, and fever. About 3500 years ago, the Egyptian Ebers Papyrus recommended use of a decoction of dried myrtle leaves to be applied to the abdomen and back for relief of rheumatic pains. A thousand years later, Hippocrates recommended poplar tree juices for treatment of eye disease and willow bark to alleviate fever and the pain of childbirth. Throughout Roman times, the use of botanical treatments, including willow bark for pain and inflammation, was widespread. Plants containing salicylate were used medicinally in China and other parts of Asia. In addition, the curative effects of other botanicals were known to the indigenous populations of North America. Extracts of the autumn crocus plant

containing colchicine were used for treatment of acute gout as early as the sixth century AD.[1]

The first modern description of the therapeutic application of plants containing salicylates was reported to the Royal Society of London by the Reverend Edward Stone, who provided an account of the successful use of dried willow bark for fever.[1] In this first "clinical trial," a pound of bark was dried, pulverized, and put into the tea, beer, or water of 50 people with fever. Stone found that one dose (1 dram = 1.8 g) cured their fever. In 1763, Stone wrote, "I have no other motives for publishing this valuable specific, than that it may have a fair and full trial in all its variety of circumstances and situations, and that the world may reap the benefits accruing from it."

In 1860 salicylic acid was chemically synthesized, which led to its widespread use as an external antiseptic, antipyretic, and analgesic.[1] The bitter taste of salicylic acid prompted the chemist Felix Hoffman to synthesize the more palatable acetylsalicylic acid (ASA). After a demonstration of its anti-inflammatory effects, Dr. Heinrich Dreser of Bayer introduced this compound into medicine in 1899 as aspirin, and it remains the most widely used drug in the world.[1] Salicylate was identified as the active ingredient of willow bark in 1929.

Phenylbutazone came into clinical practice in 1949 and was followed by indomethacin, fenamates, naproxen, and others. Despite the diversity of their chemical structures, these drugs shared therapeutic properties with aspirin. Furthermore, adverse events including gastric upset, GI ulceration and bleeding, hypertension, edema, and renal damage were shared by all these drugs. In 1971 it was discovered that these drugs all acted by inhibiting PG biosynthesis, thereby providing a unifying explanation of their therapeutic actions and a rationale for grouping them together as NSAIDs.[1]

COX was isolated in 1976 from the endoplasmic reticulum of PG-forming cells.[2,3] However, several groups of investigators speculated that there must be a second COX enzyme on the basis of observed biology. In 1990, investigators demonstrated that bacterial lipopolysaccharide (LPS) increased PG synthesis in human monocytes in vitro and in mouse peritoneal macrophages in vivo, but only the LPS-induced increase was inhibited by dexamethasone and required the de novo synthesis of "new" COX protein.[4] This observation was the foundation of the concept for "constitutive" and "inducible" forms of COX. Soon thereafter, a number of investigators working in different systems reported the discovery of an inducible second form of COX.[3] Investigators who proceeded to clone the gene and deduced its structure found that the gene product was homologous to COX but to no other known protein. The observation that glucocorticoids inhibited the expression of COX-2 after a pro-inflammatory stimulus represented a link between the anti-inflammatory actions of NSAIDs and corticosteroids.

Because of the prediction that inhibition of COX-2 would block PG biosynthesis participating in the inflammatory response but was not required for homeostasis, there was a tremendous effort to develop drugs that selectively inhibit COX-2 without effect on COX-1 in the belief that these medications would provide clinical efficacy without adverse effects.[2,5] Identification of new drugs that differentially inhibited COX-2 over COX-1 was accomplished quickly as existing NSAIDs were tested on the two COX isoforms and crystal structures revealed differences in the protein structures on which new drug development could be based.[5,6]

One hundred years after aspirin was introduced and 10 years after the discovery of COX-2, the selective COX-2 inhibitors, celecoxib and rofecoxib, were developed. In clinical trials, the safety and efficacy profiles of these and related drugs showed promise, and the U.S. Food and Drug Administration (FDA) subsequently approved these COX-2–selective NSAIDs for treatment of arthritis and pain. After their introduction into clinical practice, however, it became clear that the most highly COX-2–selective NSAIDs, particularly rofecoxib, were more likely than traditional NSAIDs to be associated with adverse cardiovascular events.[7] This finding led to the voluntary withdrawal of rofecoxib and several other COX-2–selective NSAIDs from the market. Debate surrounding the relative risks of different NSAIDs to specific organ systems continues to the present day.

## CYCLOOXYGENASE BIOLOGY AND BIOACTIVE LIPIDS

Therapeutic and adverse effects of NSAIDs are best understood in the context of COX biology. The COX enzymes are the first committed step in the synthesis of PG from arachidonic acid (AA; Figure 59-1). AA is an omega-6 polyunsaturated fatty acid (PUFA) commonly found at the *sn*-2 position of cell membrane glycerophospholipids and cleaved from cell membranes by one of several different phospholipase $A_2$ enzymes.[8] Once generated, AA may enter several different pathways to generate bioactive lipids. It can be metabolized by COX to $PGG_2$ and then $PGH_2$ via its COX and peroxidase enzyme activities; by lipoxygenases (LOX) to hydroxyeicosatetraenoic acids (HETEs), hydroperoxyeicosatetraenoic acids (HPETEs), or leukotrienes; or by the cytochrome P450 family of enzymes to HETEs, HPETEs, or epoxyeicosatrienoic acids (EETs).[9,10] These bioactive lipids have diverse biologic activities in normal physiology and in pathologic conditions, including inflammation, pain, cardiovascular disease, and cancer.

In the COX pathway, the unstable intermediate $PGH_2$ spontaneously rearranges or is enzymatically converted by specific synthases to biologically active PG, of which there are many isoforms.[3] Although phospholipase $A_2$ activity is required to initiate PG synthesis, the overall regulation of the type and amount of PG produced in a given cell or tissue is determined by the expression levels of COX-1, COX-2, and terminal synthase enzymes.

Bioactive lipids synthesized via the COX and LOX pathways are important mediators of inflammation, but alternative substrates and pathways can generate anti-inflammatory lipids and lipids important in the resolution of inflammation (Figure 59-2).[11,12] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are omega-3 PUFAs, can also serve as substrates for COX and LOX, and their metabolic pathways lead to generation of resolvins, protectins, and maresins, which are bioactive lipids associated with resolution of inflammation.[12,13] It is hypothesized that generation of anti-inflammatory lipids and lipids that accelerate

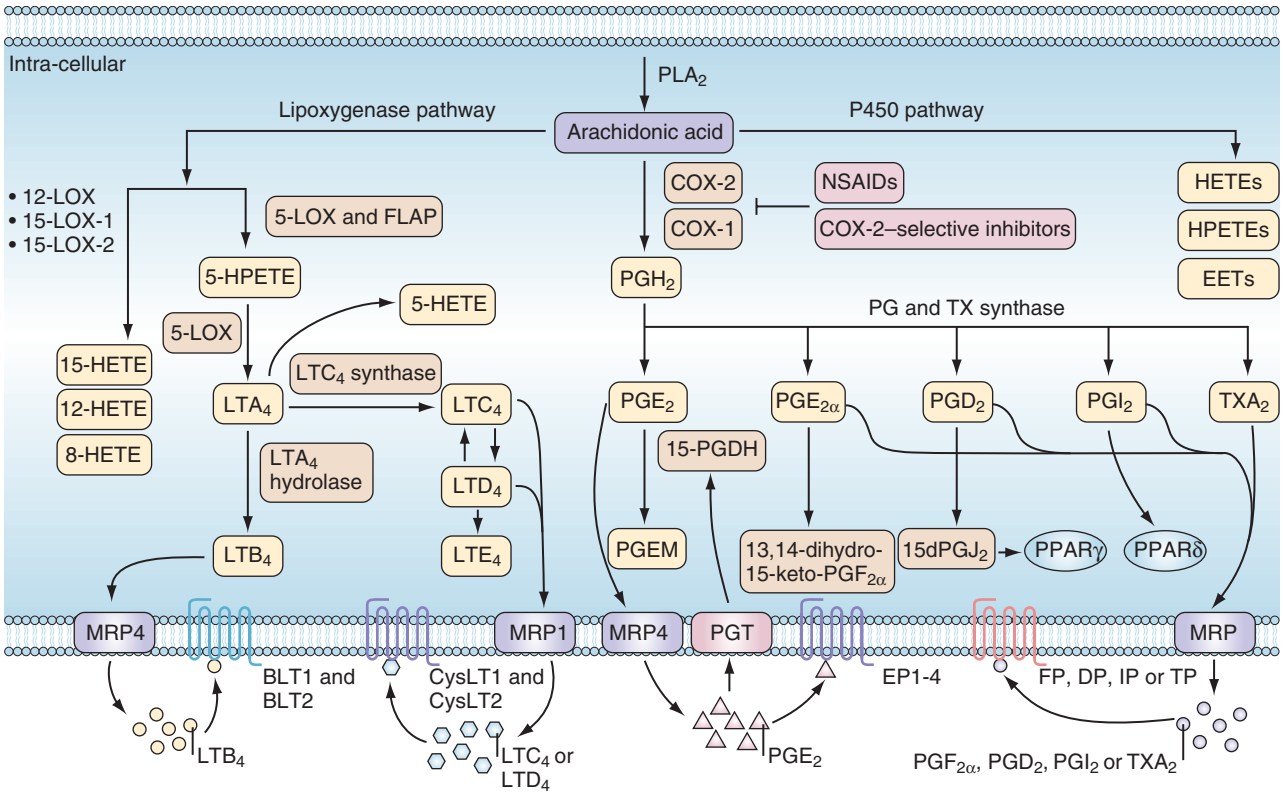



**Figure 59-1** An overview of human eicosanoid synthesis pathways. Arachidonic acid (AA) is a polyunsaturated fatty acid that constitutes the phospholipid domain of most cell membranes and is liberated from the cellular membranes by cytoplasmic phospholipase $A_2$ ($PLA_2$). Free AA can be metabolized to eicosanoids through three major pathways: the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 monooxygenase pathways. In the COX pathway, the key step is the enzymatic conversion of AA to the intermediate prostaglandin $G_2$ ($PGG_2$), which is then reduced to an intermediate $PGH_2$ by the peroxidase activity of COX. $PGH_2$ is subsequently metabolized to prostanoids, including PGs and thromboxanes (TX), by specific synthases. LOX converts AA to biologically active metabolites such as leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs). P450 metabolizes AA to epoxyeicosatrienoic acids (EETs). In the 5-LOX pathway, AA is converted to an intermediary 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which is converted to $LTA_4$. $LTA_4$ is subsequently converted to 5-HETE, $LTB_4$, $LTC_4$, $LTD_4$, and $LTE_4$. Each of the PGs and LTs exerts its biologic effects by binding to its cognate G protein–coupled receptor. $PGI_2$ can transactivate the nuclear peroxisome proliferator-activated receptor δ (PPARδ), and a $PGD_2$ dehydration product, $15dPGJ_2$, is a natural ligand for PPARδ. The multidrug resistance–associated protein (MRP) gene family is composed of efflux transporters for both PG and LT. PG transporter (PGT) is an influx transporter for PG. Hydroxyprostaglandin dehydrogenase 15-(NAD) (15-PGDH) mainly metabolizes intra-cellular $PGE_2$ and $PGF_{2\alpha}$ to stable 13,14-dihydro-15-keto-PGs. BLT, Leukotriene B(4) receptor; CysLT, cysteinyl leukotriene; DP, prostaglandin $D_2$ receptor; FLAP, 5-lipoxygenase-activating protein; FP, prostaglandin F receptor; IP, prostacyclin receptor; PGEM, prostaglandin $E_2$ metabolite; PGT, prostaglandin transporter; TP, thromboxane $A_2$ receptor. *(From Wang D, Dubois RN: Eicosanoids and cancer.* Nat Rev Cancer *10:181–193, 2010.)*

resolution of inflammation may explain why diets high in omega-3 fatty acids are associated with reduced inflammatory and cardiovascular diseases.[11] In addition, ASA possesses the capacity to alter COX-2 activity such that an "ASA-triggered" alternative catalytic cascade also results in synthesis of anti-inflammatory products, including lipoxins, protectins, and resolvins (see Figure 59-2).[11]

## Prostaglandin Production and Action

PGs act as autocrine and paracrine mediators with effects limited to the immediate vicinity of their synthesis. That PG actions are limited to their site of synthesis reflects their ephemeral nature and predisposition to metabolic inactivation to regulate promptly their potent biologic activities. Thus the enzymes required for production of a specific prostanoid must be co-localized and adjacent to the receptor mediating the action of prostanoids. Most cell types produce only one or a few prostanoid species; however, transcellular metabolism of unstable intermediates of COX may mediate qualitative and quantitative changes in the profile of eicosanoids produced in a given tissue.[14] PGs are exported from cells via the multidrug resistance–associated protein family of efflux transporters, and the PG transporter mediates influx of PG and metabolism to stable products via hydroxyprostaglandin dehydrogenase (see Figure 59-1).[10]

$PGE_2$, the most abundant PG at sites of inflammation, can be produced by many different cell types via at least three different PGE synthases.[15] Expression of the inducible form of PGE synthase, microsomal PGE synthase 1 (mPGES-1), is similar to expression of COX-2. mPGES-1 acts in concert with COX-2 to produce high levels of $PGE_2$ during inflammation. mPGES-1 expression is blocked by NSAIDs, and experiments demonstrate that $PGE_2$ itself, along with pro-inflammatory stimuli, is required for mPGES-1 upregulation.[16] This positive regulatory loop contributes to the high levels of $PGE_2$ at sites of inflammation and likely to the efficacy of NSAIDs. Other terminal PG synthases are not known to be highly regulated, and levels of other important bioactive PGs are stably produced or their levels are increased when phospholipase activity and COX-2 levels are increased.

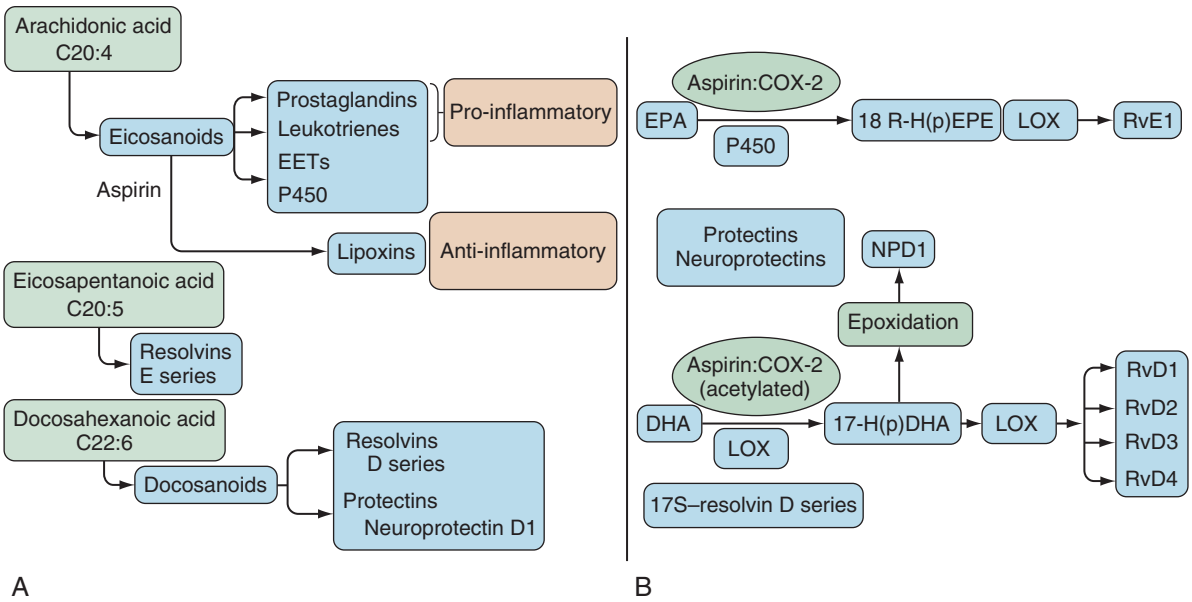



**Figure 59-2** Biosynthesis of anti-inflammatory lipids. Function of essential polyunsaturated fatty acids in the production of families of bioactive lipid mediators. **A,** Arachidonic acid is the precursor of metabolites that function as pro-inflammatory mediators. Prostaglandins and leukotrienes play pivotal roles in the progression of inflammation. Through cell-cell interactions, exemplified by platelet leukocytes in the vasculature or polymorphonuclear cell–mucosa interactions, or both, lipoxins are generated. They serve as "stop signals" and promote resolution. They also serve as endogenous anti-inflammatory mediators self-limiting the course of inflammation. The essential omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid (C20:5 and C22:6) are converted to new families of lipid mediators that are pivotal in promoting resolution (as in **B**). Resolvins of the E series such as RvE1 are generated from eicosapentaenoic acid, and resolvins of the D series such as RvD1 and the protectins such as neuroprotectin D1 (NPD1) are generated from docosahexaenoic acid, for which neural systems are enriched. **B,** Aspirin affects the formation of resolvin E1 by acetylating cyclooxygenase (COX)-2 in vascular endothelial cells, which, in a "stereoselective" way, can generate 18 R-H(p)EPE (hydroperoxyeicosapentaenoic acid), which is picked up through transcellular metabolism by leukocytes and converted by a lipoxygenase (LOX)-like mechanism to resolvin E1. Aspirin also affects the formation of D-series resolvins and catalytically switches COX-2 to a 17 R-LOX–like mechanism that serves to generate 17 R-series resolvin D. Aspirin also affects the formation of protectins and neuroprotectins by a similar mechanism and generates compounds carrying the 17 R epimer at the alcohol at carbon-17 in neuroprotectin D1 and other protectins. DHA, Docosahexaenoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; H(p)DHA, hydroperoxydocosahexaenoic acid. *(Modified from Serhan CN, Savill J: Resolution of inflammation: the beginning programs the end.* Nat Immunol *6:1191–1197, 2005.)*

The actions of PG are mediated by cell surface G protein–coupled receptors (GPCRs). At least nine PG receptors are known to exist, with additional splice variants (see Figure 59-1).[17] The PG receptors belong to three clusters within a distinct subfamily of the GPCR superfamily, with the lone exception of one of the $PGD_2$ receptors ($DP_2$), which belongs to the chemokine receptor subfamily. The *relaxant receptors* for prostacyclin (IP), $PGD_2$ ($DP_1$), and $PGE_2$ ($EP_2$ and $EP_4$) signal through $G_s$-mediated increases in intracellular cyclic adenosine monophosphate (cAMP). The *contractile receptors* for thromboxane $A_2$ (TP), $PGF_{2\alpha}$ (FP), and $PGE_2$ ($EP_1$) signal through a $G_q$-mediated increase in intra-cellular calcium. An *inhibitory receptor* for $PGE_2$ ($EP_3$) couples to $G_i$ and decreases cAMP formation. Note that $PGE_2$ has at least four different receptors with a broad range of potential actions. $EP_4$ in particular appears to mediate many of the pro-inflammatory activities of $PGE_2$.[18] Given the great diversity of PG receptors expressed by different cell types, PG signaling pathways constitute an enormously complex network that controls many biologic actions. Much work must be completed for us to understand fully all of the cellular signaling mechanisms by which PGs and their receptors elicit their respective biologic actions. This is particularly true because antagonists for many of these receptors show promise as novel targets for drug development.[18]

## Biochemistry and Structural Biology

COX-1 and COX-2 are bifunctional enzymes that mediate a COX reaction whereby arachidonate plus two molecules of $O_2$ are converted to the cyclic endoperoxide $PGG_2$, followed by a hydroperoxidase reaction in which $PGG_2$ undergoes a two-electron reduction to $PGH_2$.[8] COX enzymes are integral membrane proteins that sit within the inner leaflet of the lipid bilayer of intra-cellular phospholipid membranes of the nuclear envelope and the endoplasmic reticulum. The crystal structures of COX-1 and COX-2 have been identified, and they have essentially identical domain structures.[6] The COX enzymes are homodimers, with each monomer consisting of three structural domains. The N-terminal, epidermal growth factor–like domain is involved in dimerization via hydrophobic interactions. The membrane-binding domain is composed of four amphipathic α helices lodged into half of the lipid bilayer to form a hydrophobic channel in the center of the large catalytic domain that contains the COX and peroxidase active sites and that constitutes about 80% of the protein. The catalytic domain is globular with two distinct intertwining lobes. The interface of these lobes creates a shallow cleft on the upper surface of the enzyme where the peroxidase active site is located and where heme is bound.

COX and hydroperoxidase reactions occur at distinct but structurally and functionally interconnected sites. The COX reaction is peroxide dependent and requires that the heme group at the peroxidase site undergo a two-electron oxidation. A tyrosine residue (tyrosine 385) located at the COX active site is involved as a reaction intermediate. The physiologic heme oxidant in vivo is not known, but it has been shown that the COX activity of COX-2 can be activated at 10-fold lower concentrations of hydroperoxide than that of COX-1.[8]

NSAIDs function by blocking access of AA to the COX active site within a long, narrow, dead-end, hydrophobic channel whose entrance is framed by the four amphipathic helices of the membrane-binding domain. The channel extends into the globular catalytic domain and is about 8 Å wide. Significant narrowing of the channel occurs where arginine 120 protrudes into the channel. Arginine 120 is essential for binding both AA substrate and most carboxylate-containing NSAIDs in COX-1. By virtue of other differences in the hydrophobic channel, this residue is not essential for binding AA in COX-2, but it remains critical for binding of most carboxylate-containing NSAIDs.[19,20] Serine 530 is required for inhibition of COX by the phenylacetic acid NSAID diclofenac. Serine 530 is also the residue transacetylated by ASA and, along with valine 349, seems to govern the stereochemistry of the pocket such that after exposure to aspirin, AA is sterically blocked from a functional interaction with the catalytic domain in COX-1 and catalytic activity is completely inhibited.

A crucial structural difference between COX-1 and COX-2 is the substitution of the small amino acid valine in COX-2 for the isoleucine with a bulky side chain in COX-1 at position 523 that opens a side pocket in the hydrophobic channel in COX-2.[6] Overall, COX-2 has a wider and somewhat more flexible interior channel, a structural feature that has been exploited with respect to the development of COX-2–selective NSAIDs, as shown in Figure 59-3.[21]

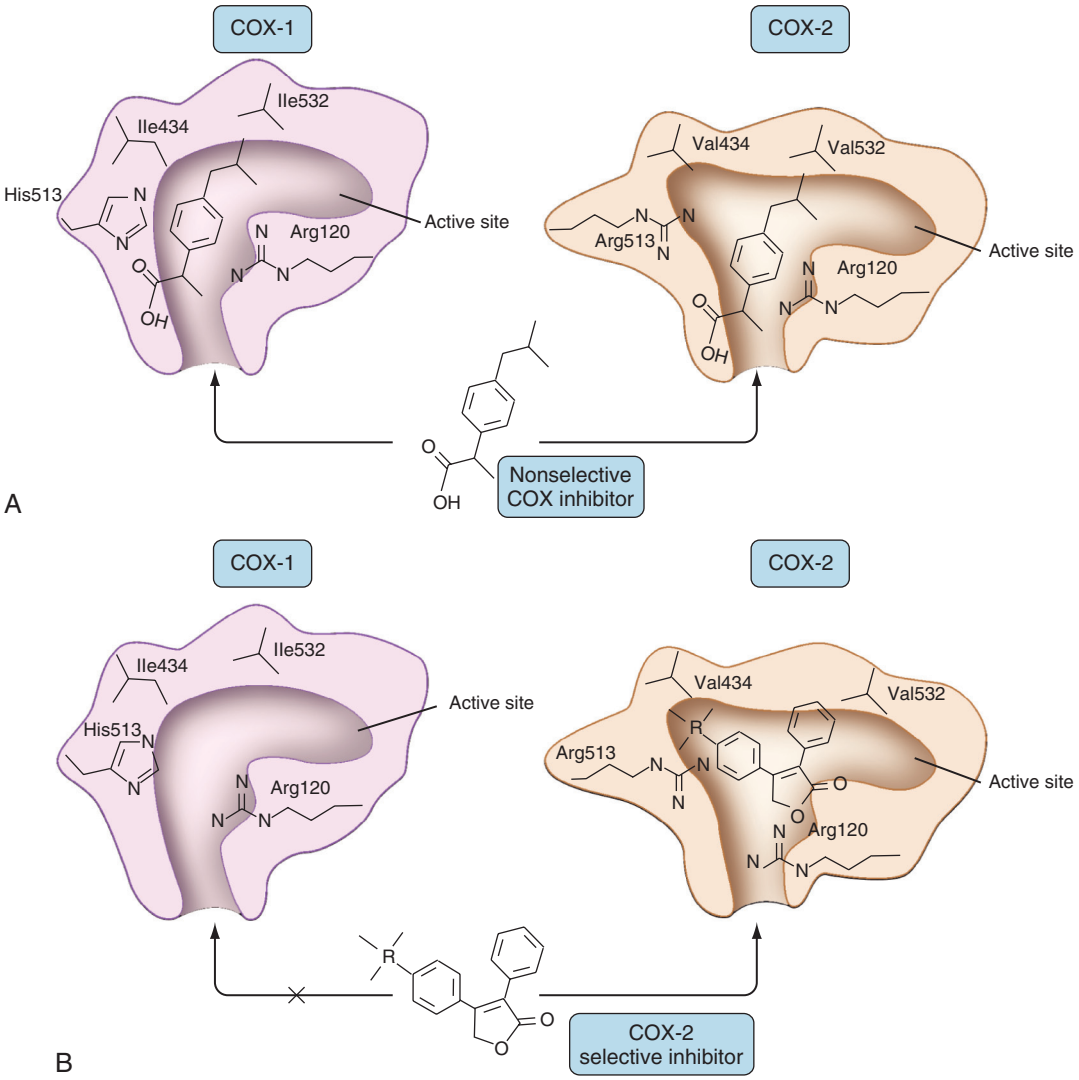



**Figure 59-3** Cyclooxygenase (COX)-1 and COX-2 substrate-binding channels. A schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues, Val434, Arg513, and Val523, form a side pocket in COX-2 that is absent in COX-1. **A,** Nonselective inhibitors have access to the binding channels of both isoforms. **B,** The more voluminous residues in COX-1, Ile434, His513, and Ile532, obstruct access of the bulky side chains of the coxibs. *(From Grosser T, Fries S, FitzGerald GA: Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities.* J Clin Invest *116:4–15, 2006.)*

Both enzymes form homodimers, but the monomers often behave asymmetrically as conformational heterodimers during catalysis and inhibition.[22] That is, when a fatty acid binds to one monomer, the other monomer becomes catalytically active and only one monomer is catalytically active at any one time. The specific fatty acid bound to the noncatalytic monomer can regulate catalytic activity.[23] Different NSAIDs interact differently with respect to allosteric inhibition of COX enzymes as one facet of their pharmacology.[22] ASA acetylation occurs in only one of the two COX monomers, which completely inhibits the activity of COX-1. However, COX-2 retains the ability to form a reduced amount of $PGH_2$ and alternative aspirin-triggered lipoxins from AA. The anti-inflammatory resolvins may be synthesized by ASA-acetylated COX-2 from omega-3 PUFA.[22]

### Molecular Biology

In addition to the differences in their structures relevant for the pharmacology of COX-1 and COX-2 inhibition, physiologically relevant differences exist with respect to expression and regulation.[2,3] Generally, COX-1 is constitutively expressed in most cells, and its expression is minimally altered by inflammatory stimuli. The promoter region of COX-1 has the characteristics of a gene that is continuously transcribed and stably expressed. COX-1 activity is regulated by substrate (AA) availability. When an increase in substrate mobilization occurs via phospholipase $A_2$ activation, a concordant increase occurs in PG synthesis mediated by COX-1. COX-1 is the only isoform expressed in mature platelets and is the most highly expressed COX isoform in normal gastroduodenal mucosa.[24] Because COX-1 is inhibited by nonselective NSAIDs, these physiologic properties may explain some of the common adverse effects of these drugs such as bleeding and GI ulceration.

In contrast, the COX-2 gene has the structure of a highly regulated product with binding sites for transcription factors such as nuclear factor-κB (NF-κB), cAMP-responsive element, and activator protein (AP)-1 which rapidly increase transcription in response to inflammatory signals.[2] COX-2 expression is highly induced by pro-inflammatory cytokines such as TNF and IL-1β, microbial products, and mitogens[2,8] and is inhibited by glucocorticoids.[2] COX-2 messenger RNA (mRNA) stability is a key regulator of COX-2 levels. The potential for instability of the COX-2 message is due to the presence of multiple adenine-uracil$_3$-adenine (AUUUA) instability sequences in the 3′ region that mediate a rapid degradation of mRNA, which ultimately suppresses COX-2 protein synthesis and PG production. Conversely, some stimuli, including IL-1β, may interfere with mRNA degradation and increase COX-2 levels and PG production.[25] COX-1 and COX-2 can be post-translationally modified. COX-1 is glycosylated at three asparagines involved in proper protein folding of the enzyme, whereas COX-2 can be glycosylated at four asparagines.[25,26]

The generalization that COX-1 expression is constitutive and COX-2 expression is inducible has its limitations, given that COX-2 is expressed constitutively in several organ systems and regulated by physiologic, as well as pathologic, stimuli. COX-2 is basally expressed in the brain, kidney, pancreas, and blood vessels and therefore plays an important role in normal reproductive, renal, cardiovascular, and skeletal physiology.[2,21,27]

## MECHANISM OF ACTION

### Cyclooxygenase Inhibition

All of the NSAIDs are synthetic inhibitors of the COX active site, but subtle mechanistic differences in the manner in which individual NSAIDs interact and bind with the active site are responsible for some of the differences in their pharmacologic characteristics.[28] ASA is the only covalent, irreversible modifier of COX-1 and COX-2, whereas all of the other NSAIDs are competitive inhibitors, competing with AA for binding in the active site. The competitive inhibitors are subdivided further on the basis of whether they bind to the COX active site in a time-dependent or time-independent manner.

Crystallographic studies have shown how ASA acetylates serine 530 of COX-1. Similar to other NSAIDs, ASA diffuses into the COX-1 active site at the mouth of the channel and travels to the constriction created by arginine 120, where it is in the best orientation to transacetylate serine 530, leading to the complete and irreversible inhibition of COX-1.[29] In COX-2, the channel of the active site is larger than COX-1, the orientation of ASA for serine 530 attack is not as good, and transacetylation efficiency for COX-2 is 10-fold to 100-fold less than for COX-1. ASA can also "trigger" COX-2 to alter its catalytic activity to produce 15 R-HETE and lipoxins from AA and to generate anti-inflammatory lipids from omega-3–PUFA.[11]

The time it takes for an NSAID to inhibit the COX active site relative to how long it takes for it to leave the COX channel is a crucial factor in the inhibition of COX.[30] Drugs such as ibuprofen exhibit such rapid rates that they essentially inhibit COX instantly but can be removed from the COX active site just as quickly when drug levels decrease. Both COX monomers must be inhibited by ibuprofen to block catalytic activity.[22] Conversely, indomethacin and diclofenac are time-dependent allosteric inhibitors that require seconds to minutes to bind to the COX active site and need only block one of the COX monomers to completely inhibit catalytic activity.[22] These NSAIDs also need hours to exit the COX active site. Initially, most traditional time-dependent NSAIDs form a loose complex with the COX active site before a stronger interaction is established. This complex is limited by the time it takes the drug to become properly oriented within the COX channel at arginine 120, the constriction site in the COX channel. This orientation may involve a change in conformation to the "open state" to allow the drug to access the upper part of the COX catalytic site.

Drugs such as flurbiprofen and indomethacin form a salt bridge between the carboxylate moiety of the NSAID and the guanidinium moiety of arginine 120. Hydrophobic interactions between the aromatic rings and the hydrophobic amino acids in the channel aid binding. Such interactions at the constriction point of the channel completely block the entry of substrate to the active site.[31] Diclofenac interacts with serine 530, not arginine 120, but also blocks entry of substrate.[32]

## COX-2 Selectivity

NSAIDs such as meloxicam, nimesulide, and etodolac show some selectivity for inhibiting COX-2 compared with COX-1. After the discovery of COX-2, efforts to further enhance COX-2 selectivity led to the development of celecoxib, rofecoxib, valdecoxib, etoricoxib, and lumiracoxib. The prototypical COX-2-selective NSAIDs, celecoxib and rofecoxib, are diaryl compounds containing a sulfonamide (celecoxib) and methylsulfone (rofecoxib) rather than a carboxyl group. Both drugs are weak time-independent inhibitors of COX-1 but strong time-dependent inhibitors of COX-2 that require their entry into and stabilized binding in the catalytic pocket. Because these drugs lack a carboxyl group, arginine 120 is not involved, but multiple sites of hydrogen and hydrophobic binding stabilize drugs at the catalytic site. The sulfur-containing phenyl ring of COX-2–selective NSAIDs plays a pivotal role in binding stability by occupying the hydrophobic side pocket characteristic of the COX-2 catalytic site. If this side pocket is removed by mutagenesis, all isozyme selectivity is lost.[6]

Most commonly, COX isozyme selectivity is defined by using the concentration of drug required to inhibit PG production by 50% in a particular assay system (inhibitory concentration [IC]50). Ratios using values obtained for COX-1 IC50s compared with COX-2 IC50s can be calculated and used as a standard measure for comparing the degrees of selectivity of a particular NSAID for one or the other COX isoform.[33] PG assay systems can vary widely, however, making it difficult to directly compare results from studies using different assay systems. To circumvent such problems, most clinicians have accepted the use of the in vitro whole-blood assay to compare NSAID selectivities. In this system, COX-1 inhibition is assessed as a function of the reduction of thromboxane made by platelets after clot formation. Inhibition of COX-2 is based on the inhibition of $PGE_2$ production in a heparinized blood sample after LPS stimulation. A COX-2–selective NSAID lacks inhibitory effect on platelet COX-1 at concentrations at or above those that maximally inhibit COX-2.[5,34]

## Cyclooxygenase-Independent Mechanisms of Action

At high, nonphysiologic concentrations, some NSAIDs seem to elicit effects on cellular pathways in vitro that do not involve the inhibition of COX. Because of the high doses of drug required and the use of in vitro systems, the relevance of these effects to in vivo activity is uncertain. Some NSAIDs inhibit phosphodiesterases associated with the metabolism of cAMP, leading to increased intra-cellular cAMP levels and the subsequent general inhibition of peripheral blood lymphocyte responses to mitogen stimulation, monocyte and neutrophil migration, and neutrophil aggregation.[35] NSAIDs scavenge free radicals, inhibit superoxide production by polymorphonuclear neutrophils, reduce mononuclear cell phospholipase C activity, and inhibit inducible nitric oxide (NO) synthase activity. Sodium salicylate and ASA inhibit NF-κB activation, as do certain inactive enantiomers of flurbiprofen. Some reports indicate that other cell signaling molecules such as mitogen-activated protein kinases and the transcription factor AP-1 also may be modulated by NSAIDs. Some NSAIDs bind to and activate members of the peroxisome proliferator-activated receptor (PPAR) family and other intra-cellular receptors. PPAR activation is thought to mediate anti-inflammatory activities. Nonselective acidic NSAIDs, including salicylate, ibuprofen, and diclofenac, but not nonacidic drugs, activate AMP-activated kinase.[36] Selective COX-2 inhibitors may have unique structural features that promote COX-independent activities such as cell-cycle regulation, apoptosis, and antiangiogenesis.[37]

## Mechanism of Acetaminophen and Other Analgesic Antipyretic Drugs

Acetaminophen (paracetamol) and dipyrone relieve pain and fever, but they are not anti-inflammatory. The precise mechanisms by which these drugs elicit their effects remain unclear. In the 1970s, it was proposed that acetaminophen worked by means of a "central" action by inhibiting COX activity primarily in the brain and not in peripheral tissues because they were not acidic and could cross the blood-brain barrier.[38] Acetaminophen does inhibit COX-1 and COX-2, but variably, in a manner dependent on cell and tissue type. Acetaminophen does not appear to inhibit by interacting with the COX active site; rather, it serves as a reducing co-substrate for the peroxidase site. The peroxide tone of cells and tissues in vivo may be responsible for inhibitor specificity, with platelets and activated macrophages being resistant to the action of acetaminophen and vascular endothelial cells being sensitive to its inhibitory effects on COX. Additionally, the inhibitory potency of acetaminophen is determined by the concentration of the COX enzyme.[38] This phenomenon may be an additional factor for the lack of clinical anti-inflammatory effects, because inflammation is associated with a markedly increased expression of the COX-2 enzyme. With the discovery of a COX-1 splice variant and studies showing that it is both highly expressed in brain and more sensitive to inhibition by acetaminophen, some investigators proposed that the analgesic and antipyretic actions of acetaminophen could be explained by its ability to inhibit the COX-1 splice variants (called COX-3 by some investigators, despite the fact that this variant does not arise from a unique gene).[39] However, more recent studies have rejected this mechanism as explanatory for the effects of acetaminophen.[38,40]

Salicylate has analgesic, antipyretic, and anti-inflammatory activity, but similar to acetaminophen and in contrast to ASA, it is a poor COX inhibitor. Salicylate also inhibits COX activity if substrate levels are low, and it is also dependent on the oxidative state of the enzyme, suggesting that this drug may inhibit COX by redox-related mechanisms.[41]

# PHARMACOLOGY AND DOSING

## Classification

NSAIDs are generally grouped according to their chemical structures, plasma half-life, and COX-1 versus COX-2 selectivity (Table 59-1 and Figure 59-4). Table 59-1 presents a representative compilation of common NSAIDs, formulations, dosages, half-lives, and precautions. Structurally, most

**TABLE 59-1** Common NSAIDs

| Drug | Brand Names | Available Formulations (mg) | Maximal Daily Dose (mg) | Tmax (hr) | Half-life (hr) | Dose Adjustment or Special Precautions |
|---|---|---|---|---|---|---|
| **Salicylic Acids** | | | | | | |
| Acetylsalicylic acid | Aspirin | Tablets: 81, 165, 325, 500, 650<br>Children's: 81<br>Suppository: 120, 200, 300, 600 | 3000 | 0.5 | 4-6 | Decrease dose by 50% in patients with renal failure and hepatic insufficiency |
| Salsalate | Disalcid<br>Amigesic<br>Salflex | Capsule: 500<br>Tablet: 500, 750 | 3000 | 1.4 | 1 | |
| Diflunisal | Dolobid | Tablets: 250, 500 | 1500 | 2-3 | 7-15 | Decrease dose by 50% in patients with renal failure |
| **Acetic Acids** | | | | | | |
| Diclofenac | Voltaren<br>Voltaren XR<br>Cataflam | Tablets: 25, 50, 75<br>Extended release: 100 | 225 | 1-2 | 2 | Incidence of increased transaminase levels higher than with other NSAIDs |
| Diclofenac + misoprostol | Arthrotec | Tablets: 50 or 75 plus misoprostol 200 µg | 200 | 1-2 | 2 | Incidence of increased transaminase levels higher than with other NSAIDs |
| Indomethacin | Indocin<br><br>Indocin SR | Caps: 25, 50<br><br>Sustained release: 75<br>Oral suspension: 25 mg/5 mL<br>Suppositories: 50 | 200 | 1-4 | 2-13 | Approved for treatment of patent ductus arteriosus |
| Sulindac | Clinoril | Tablets: 150, 200 | 400 | 2-4 | 16 | Prodrug metabolized to active compound<br>Decrease dose in patients with renal and liver disease and in elderly patients |
| Ketorolac | Toradol | IM/IV: 15 or 30 mg/mL<br><br>Tablets: 10 | 120 IV/IM<br><br>40 mg PO | 0.3-1 | 4-6 | Decrease dose by 50% in patients with renal failure and in elderly patients<br>Do not use >5 days |
| Tolmetin | Tolectin | Tablets: 200, 600<br>Caps: 400 | 1800 | 0.5-1 | 1-1.5 | |
| Etodolac | Lodine<br>Lodine XL | Caps: 200, 300<br>Tablets: 400<br>Extended release: 400, 500, 600 | 1200 | 1-2 | 6-7 | |
| **Propionic Acids** | | | | | | |
| Ibuprofen | Motrin<br>Advil<br>Nuprin<br>Rufen | Tablets: 200 (OTC), 300, 400, 600, 800 | 3200 | 1-2 | 2 | Avoid use in patients with severe hepatic disease |
| Naproxen | Naprosyn<br><br>Aleve<br>Anaprox<br>EC-Naprosyn<br>Naprelan | Tablets: 125 (OTC), 250, 375, 500<br><br>Sustained release: 375, 500<br>Suspension: 125 mg/5 mL | 1375 | 2-4 | 12-15 | Decrease dose in patients with renal and liver disease and in elderly patients |
| Fenoprofen | Nalfon | Caps: 200, 300, 600 | 3200 | 1-2 | 2-3 | Idiosyncratic nephropathy more frequent than with other NSAIDs |
| Ketoprofen | Orudis<br>Oruvail | Tablets: 12.5 (OTC)<br>Caps: 25, 50, 75<br>Sustained release: 100, 150, 200 | 300 | 0.5-2 | 2-4 | Decrease dose in patients with severe renal disease and hepatic disease and in elderly patients |
| Flurbiprofen | Ansaid | Tablets: 50, 100 | 300 | 1.5-2 | 3-4 | |
| Oxaprozin | Daypro | Tablets: 600 | 1800 or 26 mg/kg/day | 3-6 | 49-60 | Decrease dose in patients with renal failure and patients who weigh <50 kg |

**TABLE 59-1** Common NSAIDs—cont'd

| Drug | Brand Names | Available Formulations (mg) | Maximal Daily Dose (mg) | Tmax (hr) | Half-life (hr) | Dose Adjustment or Special Precautions |
|---|---|---|---|---|---|---|
| **Fenamic Acids** | | | | | | |
| Meclofenamate | Meclomen | Caps: 50, 100 | 400 | 0.5 | 2-3 | |
| **Oxicams** | | | | | | |
| Piroxicam | Feldene | Caps: 10, 20 | 20 | 2-5 | 3-86 | Decrease dose in patients with hepatic disease and in elderly patients |
| Meloxicam | Mobic | Tab: 7.5, 15 | 15 | 5-6 | 20 | |
| **Nonacidic Compounds** | | | | | | |
| Nabumetone | Relafen | Tablets: 500, 750 | 2000 | 3-6 | 24 | Food increases peak concentration<br>Reduce dose in patients with renal disease<br>Avoid in patients with severe liver disease<br>Limit dose to 1 g/day in elderly patients |
| **COX-2 Selective Inhibitors** | | | | | | |
| Celecoxib | Celebrex | Caps: 100, 200, 400 | 400 (800 mg in FAP) | 3 | 11 | Contraindicated in people with a sulfonamide allergy |
| Etoricoxib* | Arcoxia | Tablets: 60, 90, 120 | 120 | 1-1.5 | 22 | Contraindicated in patients with severe renal or liver disease<br>Caution in patients with mild to moderate disease |

*Not approved by the U.S. Food and Drug Administration.
FAP, Familial adenomatous polyposis; IM/IV, intramuscular/intravenous; OTC, over the counter; PO, oral.

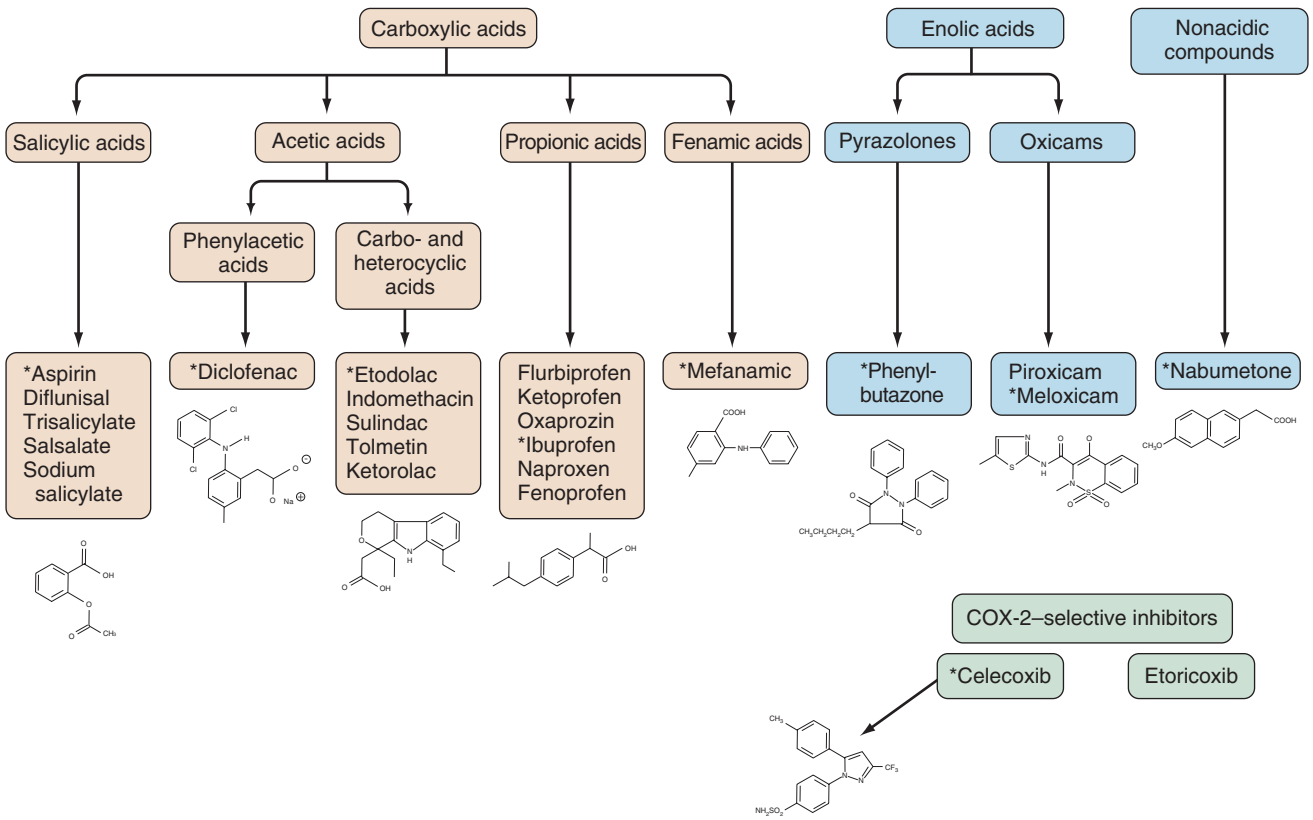



**Figure 59-4** Classification and representative structures of the traditional NSAIDs and cyclooxygenase-2 (COX-2)–selective NSAIDs. *Selected NSAID structure from each subclass.

NSAIDs are organic acids with low pK values that lend themselves to their accumulation at sites of inflammation—areas that often exhibit lower pH values than do uninvolved sites. The persistence of NSAIDs in the synovial fluid is associated with a sustained therapeutic effect despite relatively rapid clearance from the plasma, vascular wall, and kidneys.[42] Usually a direct relationship exists between low pK and a short half-life, but there are exceptions, such as nabumetone, which is nonacidic. Classifying NSAIDs on the basis of plasma half-life can be problematic, given the fact that these drugs tend to accumulate in synovial fluid, where the concentration of the drug may remain more stable than in the plasma. Short–half-life NSAIDs potentially could be given less frequently than indicated by their plasma half-life. NSAIDs exhibiting longer half-lives require more time to reach steady-state plasma levels. Drugs with a half-life greater than 12 hours can be given once or twice a day. Plasma levels increase for a few days to several weeks (depending on the specific half-life) but then tend to remain constant between doses. NSAIDs with longer half-lives also enable drug concentrations to equilibrate between the plasma and the synovial fluid, although total bound and unbound drug levels are usually lower in synovial fluid because less albumin is present in synovial fluid than in plasma. However, NSAIDs with longer half-lives or extended-release formulation may be associated with an increased propensity to cause adverse effects.[43] COX-isozyme selectivity is likely to be a critically important factor in determining relative GI and cardiovascular risk, which also should be considered in addition to other pharmacologic properties for each NSAID.[33]

## NSAID Metabolism

Almost all NSAIDs are more than 90% bound to plasma proteins. If total drug concentrations are increased beyond the point at which the binding sites on albumin are saturated, biologically active free drug concentrations increase disproportionately to the increasing total drug concentration. The clearance of NSAIDs is usually by hepatic metabolism, with production of inactive metabolites that are excreted in the bile and urine. Most NSAIDs are metabolized through the microsomal cytochrome P450-containing mixed-function oxidase system. NSAIDs are most often metabolized by CYP3A, CYP2C9, or both. However, some NSAIDs are metabolized by other cytosolic hepatic enzymes.

## Salicylate Metabolism and Aspirin Resistance

Salicylates are acetylated (e.g., aspirin) or nonacetylated (e.g., sodium salicylate, choline salicylate, choline magnesium trisalicylate, and salicylsalicylic acid).[41] Although the nonacetylated salicylates are only weak inhibitors of COX in vitro, they are able to reduce inflammation in vivo. Aspirin is rapidly deacetylated to salicylate, both spontaneously and enzymatically. Differences in the formulation of these agents affect the absorption properties, but not bioavailability. Buffered aspirin tablets contain antacids that increase the pH of the microenvironment, whereas enteric coating slows absorption. The bioavailability of rectal aspirin suppositories increases with retention time. Salicylates primarily bind to albumin and rapidly diffuse into most body fluids. Salicylate is metabolized principally by the liver and excreted primarily by the kidney. In the kidney, salicylate and its metabolites are freely filtered by the glomerulus, then reabsorbed and secreted by the tubules. Salicylate serum levels usually do not correlate well with dosage, however, and small increases in dosage may result in disproportionate increases in serum levels. The drug clearance rate is a function of serum concentration. The primary factors regulating serum salicylate levels are urinary pH and metabolic enzyme activity.

The term "aspirin resistance" is broadly used to describe the failure of aspirin to prevent a thrombotic event, whether as a result of pharmacologic resistance to the anti-platelet effects of aspirin or the inability of aspirin to overcome thrombophilia in a given clinical setting.[44] Factors such as sex, genetic polymorphisms, and clinical factors, including smoking, obesity, and diabetes, may alter aspirin effects on platelet function. Lack of adherence and drug interactions also may play a role in aspirin resistance.

## Pharmacologic Variability

Different patients can respond to the same NSAID in a variety of ways, and the basis for this individual variability remains unclear. Several pharmacologic factors related to NSAIDs may influence this variability, such as dose response, plasma half-life, enantiomeric conversion, urinary excretion, and pharmacodynamic variation.[45] Other important drug factors include protein binding, the metabolic profile of the drug, and the percentage of the drug that is available as the active *(S)* enantiomer. Some NSAIDs exist as two enantiomers; these NSAIDs include the propionic acid derivatives ibuprofen, ketoprofen, and flurbiprofen, which exist as mixtures of inactive *(R)* and active *(S)* enantiomers. Naproxen is composed of the active *(S)* enantiomer. Conversion of the propionic acid NSAIDs from the inactive *(R)* enantiomer to the active *(S)* enantiomer occurs in vivo to various degrees, providing some basis for the variability in patient response. Genetic variability also exists in the cytochrome P450 metabolic enzymes, and consequently some people or ethnic groups metabolize drugs more slowly. For example, Asians frequently experience slow metabolism through the CYP2C9 pathway. A number of single-nucleotide polymorphisms in the genetic sequence of *CYP2C9* have been described, and the product of one of these altered genes, CYP2C9*2, is associated with reduced metabolism of celecoxib and an accompanying increase in plasma concentration of the drug.[42] Finally, the pharmacokinetics of some NSAIDs are affected by hepatic disease, renal disease, or old age.

## Routes of Drug Delivery

NSAIDs are produced in a variety of dosage forms, including intravenous, slow-release, and sustained-release oral preparations, topical preparations in various forms, including gels and patches, and suppositories. Given the desire to reduce NSAID toxicity while preserving drug delivery to a specific site, efforts continue to alter drug formulation and delivery systems. Nanoparticles, liposomes, and microspheres are under investigation to allow dose reduction and

specific targeting. Intra-articular delivery is under consideration, but because joints have efficient lymphatic clearance systems, the utility of this form of targeting remains to be proved.

Topical NSAID formulations were developed to reduce systemic exposure while preserving efficacy. Diclofenac, for example, is available as a solution, gel, or patch. The systemic effects are directly proportional to the surface area, and this method of delivery results in a relatively stable systemic level of diclofenac compared with oral administration.[46]

### Combination Drugs

NSAIDs have also been combined with agents that have gastroprotective effects in the form of "polypills" that are currently available on the market. This strategy may increase compliance with effective protective agents, thereby reducing adverse effects in clinical practice. Combining diclofenac with the synthetic $PGE_1$ analogue misoprostol reduces the risk of NSAID-related peptic ulcerations and mucosal injury, but utility of the combination is often limited by misoprostol-induced cramping and diarrhea.[47] In population-based studies, the combination pill was more effective than diclofenac and misoprostol co-prescription in preventing hospitalization for peptic ulcer disease or GI hemorrhage.[48] The combination of enteric-coated naproxen and the proton pump inhibitor (PPI) esomeprazole into a single pill has been approved by the FDA. This agent reduces endoscopically detected gastric ulcers.[49]

A different strategy is NO-releasing NSAIDs (NO-NSAIDs), which are synthesized by the ester linkage of an NO-releasing moiety to conventional NSAIDs, including aspirin, flurbiprofen, diclofenac, sulindac, and others.[50] The NO moiety is slowly released by enzymatic activity in vivo, likely by esterases, resulting in slow accumulation of the parent NSAID. The lower rate of GI ulceration associated with these drugs is likely related to NO-associated vasodilation and the relatively lower concentration of the parent NSAID.

## THERAPEUTIC EFFECTS

### Anti-inflammatory Effects

NSAIDs are frequently used as first-line agents for the symptomatic relief of many different inflammatory conditions. In double-blind, randomized clinical trials of inflammatory arthritis, NSAIDs have been compared with placebo, aspirin, and each other. Clinical trials of NSAID efficacy in RA (and osteoarthritis) usually employ a design whereby the current NSAID is discontinued and the patient must have an increase in symptoms or flare to enter the study. Although primary outcome measures have some variation, most include parameters that make up the American College of Rheumatology (ACR)20. Efficacy superior to that of placebo is easily demonstrated for NSAIDs within 1 to 2 weeks in patients with active RA who are not receiving corticosteroids or other anti-inflammatory medications.[51] Comparisons of adequate doses of traditional NSAIDs or COX-2–selective NSAIDs with one another almost always show comparable efficacy. Despite improvement in pain and stiffness with NSAIDs, these agents do not usually reduce acute phase reactants, nor do they modify radiographic progression. The anti-inflammatory effects of NSAIDs have also been demonstrated in rheumatic fever, juvenile RA, ankylosing spondylitis, gout, osteoarthritis, and systemic lupus erythematosus (SLE). Although not as rigorously proven, their efficacy is also accepted in treatment of reactive arthritis, psoriatic arthritis, acute and chronic bursitis, and tendinitis.

### Analgesic Effects

Virtually all NSAIDs relieve pain when used in doses substantially lower than those required to suppress inflammation. The analgesic action of NSAIDs is due to inhibition of PG production in peripheral tissues and in the central nervous system (CNS). In the periphery, PGs do not induce pain per se but rather sensitize peripheral nociceptors to the effects of mediators such as bradykinin or histamine.[52] PGs released during inflammation or other trauma lower the activation threshold of tetrodotoxin-resistant sodium channels on sensory neurons. In the CNS, where NSAIDs and acetaminophen exert analgesic effects, PGs also play an important role in neuronal sensitization. COX-2 is constitutively expressed in the dorsal horn of the spinal cord, and its expression is increased during inflammation.[53] Centrally generated $PGE_2$ activates spinal neurons and also microglia that contribute to neuropathic pain.[54] Both COX-1 and COX-2 play a role in nociception as demonstrated by reductions in experimental pain in mice deficient in either COX-1 or COX-2.[55]

### Antipyretic Effects

The NSAIDs and acetaminophen effectively suppress fever in humans and in animals included in experiments. Fever results from the production of PG, primarily $PGE_2$, from vascular endothelial cells via COX-2 and mPGES-1.[56] These PGs generate neuronal signals that activate the thermoregulatory center in the preoptic area of the anterior hypothalamus. $PGE_2$ synthesis is stimulated by endogenous (e.g., IL-1) or exogenous (e.g., LPS) pyrogens. Mice with a targeted deletion of either the COX-2 or mPGES-1 genes fail to develop fever in response to inflammatory stimuli.[57]

Little evidence suggests that any NSAID has superior efficacy as an antipyretic agent. However, in fever associated with viral illnesses, aspirin should be avoided because of the association with hepatocellular failure (Reye's syndrome).[58]

### Other Therapeutic Effects

#### Anti-platelet Effects

Aspirin and traditional NSAIDs inhibit platelet COX-1 to variable degrees. Except for aspirin, inhibition of platelet aggregation is reversible and depends on the concentration of drug in the platelet. Aspirin acetylates platelet COX-1, which cannot be resynthesized. The antiaggregation effect of as little as 80 mg of aspirin can last for up to 4 to 6 days, until the bone marrow can synthesize new platelets.[59]

On the basis of accumulated data showing its benefits, the FDA has approved ASA for use in the secondary prevention of cardiovascular disease. Major trials have shown that meaningful decreases in nonfatal myocardial infarction (MI), nonfatal stroke, and death can be realized by daily administration of ASA of 75 to 325 mg. Major vascular events can be reduced by 10 to 20 events for every 1000 patients treated, at a cost of one to two major GI bleeds.[60]

In the Nurses Health Study of primary prevention of major vascular events, no reduction in rates of MI was observed with the use of ASA, 100 mg every other day, whereas rates of GI bleeding were increased. However, stroke rates were significantly reduced with use of this regimen.[61] The U.S. Preventative Health Task Force has updated its recommendations to encourage use of low-dose ASA in men aged 45 to 79 years and in women aged 55 to 79 years.[60] ASA used for primary prevention of cardiovascular events appears to lower the risk for MI in men and for stroke in women.[62]

### Cancer Chemoprevention

A large body of epidemiologic and animal studies provides evidence that a high-fat diet can be associated with a risk for cancer. AA, one of the major ingredients of animal fats, and the eicosanoids derived from AA are important contributors to the development of cancer.[10] Large-scale epidemiologic studies have long indicated that long-term NSAID use reduces the incidence of a variety of cancers, including colon, intestinal, gastric, breast, and bladder, by 40% to 50%.[10] Given the ability of NSAIDs to inhibit COX and PG production, the COX pathway immediately becomes implicated as playing an important role in the pathogenic process. It is well recognized that growth factors, tumor promoters, and oncogenes stimulate PG production via the induction of COX-2 and that human tumorigenic tissues exhibit increased COX activity compared with their normal, nontumorigenic counterparts. COX-2 is overexpressed in 80% of colorectal cancer tissues. Among the PGs, $PGE_2$ is most abundant in human neoplasms. The inducible mPGES-1 enzyme is highly expressed in tumors, and its absence suppresses intestinal tumorigenesis in animal models. Furthermore, the enzyme that metabolizes intracellular $PGE_2$, 15-hydroxyprostaglandin dehydrogenase, is ubiquitously lacking in tumors, and mice with a genetic deletion of this enzyme have accelerated tumorigenesis.[10] Many natural products, including resveratrol (red wine), catechins (green tea), and curcumin (saffron), also inhibit COX, which may be an important mechanism underlying their putative cancer-preventing effects.[63]

A retrospective cohort study shows that aspirin and traditional NSAIDs specifically reduce cancer risk in the subgroup of patients whose colon tumors express higher levels of COX-2.[64] In a meta-analysis of the effects of ASA (75 mg daily and upward without dose dependence) on cancer, allocation to receive aspirin reduced death due to cancer by more than 20%.[65] Upon analysis of individual patient data, a cancer death benefit was apparent only after 5 years of follow-up, and the benefit increased with scheduled duration of trial treatment. ASA effects appear to be greater on adenomatous cancer than on other cancer types. Other studies demonstrated a reduction in both the incidence of colorectal cancer and death from colorectal cancer, particularly for cancers of the proximal colon.[66] Long-term low-dose ASA use also appears to reduce the risk of prostate cancer.[67]

Clinical trials also demonstrated that traditional and COX-2–selective NSAIDs could cause regression of polyps in patients with familial adenomatous polyposis (FAP).[68] Celecoxib was subsequently approved by the FDA for reduction of polyps in patients with FAP. Although NSAIDs are still among the most promising chemopreventative agents for cancer, cardiovascular and GI adverse effects have dampened enthusiasm.[10] Alternative strategies for blocking effects of $PGE_2$ and other eicosanoids need to be evaluated to determine their effectiveness as cancer prevention and adjunct therapy.

## ADVERSE EFFECTS

NSAIDs share a common spectrum of clinical toxicities, although the frequency of particular adverse effects varies with the compound (Table 59-2). The hazard of individual NSAIDs is related to their pharmacologic characteristics, such as bioavailability and half-life, as well as their potency for inhibition of COX-1 and COX-2.[33,43,69]

### Gastrointestinal Tract Effects

NSAIDs cause GI injury through both topical and systemic effects. After a mucosal injury occurs, NSAIDs inhibit the

**TABLE 59-2** Shared Toxicities of NSAIDs

| Organ System | Toxicity |
|---|---|
| Gastrointestinal | Dyspepsia<br>Esophagitis<br>Gastroduodenal ulcers<br>Ulcer complications (bleeding, perforation, obstruction)<br>Small bowel erosions and strictures<br>Colitis |
| Renal | Sodium retention<br>Weight gain and edema<br>Hypertension<br>Type IV renal tubular acidosis and hyperkalemia<br>Acute renal failure<br>Papillary necrosis<br>Acute interstitial nephritis<br>Accelerated chronic kidney disease |
| Cardiovascular | Heart failure<br>Myocardial infarction<br>Stroke<br>Cardiovascular death |
| Hepatic | Elevated transaminases<br>Reye's syndrome (aspirin only) |
| Asthma/allergic | Aspirin-exacerbated respiratory disease* (susceptible patients)<br>Rash |
| Hematologic | Cytopenias |
| Neurologic | Dizziness, confusion, drowsiness<br>Seizures<br>Aseptic meningitis |
| Bone | Delayed healing |

*Reduced risk in cyclooxygenase-2–selective NSAIDs.

early events necessary to repair superficial injury, as well as later events of cell proliferation and angiogenesis, leading to delayed ulcer healing.[70] Topical injury initiates the initial mucosal erosions by disrupting the gastric epithelial cell barrier, but PG depletion is essential for the development of clinically significant gastric and duodenal ulcers. The propensity of specific NSAIDs to cause acute topical injury depends on the pKa, with most NSAIDs being weak organic acids. Aspirin is particularly prone to cause mucosal injury when administered orally.[24] Nonacidic NSAIDs such as nabumetone, etodolac, and celecoxib do not cause acute mucosal lesions. Esterification of acidic NSAIDs, such as the case for NO-NSAIDs, suppresses mucosal injury.[24]

Acidic NSAIDs accumulate in gastric epithelial cells and lead to generation of reactive oxygen species. NSAIDs also may cause apoptosis, which appears independent of COX inhibition. They also associate with extra-cellular phospholipids, resulting in attenuation of the hydrophobic surface barrier of the stomach.[71] Phospholipids associated with cell membranes are disrupted, leading to increased permeability and acid or bile back diffusion.

The integrity of mucosal defense depends on generation of $PGE_2$ and $PGI_2$ from COX enzymes. COX-1 is abundantly expressed under basal conditions in gastric mucosa, whereas COX-2 is almost undetectable. However, both COX-1 and COX-2 are rapidly upregulated after injury or when pre-existing ulcers are present,[72] which may explain the observation that concurrent *Helicobacter pylori* infection increases the risk of developing peptic ulcers and increases bleeding in people who use NSAIDs.[73]

It appears that concurrent COX-1 and COX-2 inhibition is associated with the highest propensity to develop gastric ulcers.[43] This clinical observation is consistent with animal studies whereby mice deficient for a single COX enzyme or treated with drugs that specifically inhibit either COX-1 or COX-2 do not develop ulceration. Severe gastric lesions are seen when both enzymes are simultaneously blocked. However, when the gastric mucosa is damaged, inhibition of either COX-1 or COX-2 is associated with the development of ulcers.[70] Traditional and COX-2–selective NSAIDs delay ulcer healing, with nonselective NSAIDs doing so to a greater degree.[74] It also should be noted that GI bleeding may be related to the combination of injury and inhibition of platelet aggregation, which is an additional factor in the propensity of aspirin and other nonselective NSAIDs to cause clinically apparent ulcers.[24,70]

## Dyspepsia

Nonulcer dyspepsia is the most common adverse event (10% to 20%) associated with use of NSAIDs and may account for poor tolerability.[75] Dyspepsia is more often reported in younger than in older patients.[76] Although they are expected to reduce dyspepsia, COX-2–selective NSAIDs are also associated with a substantial level of adverse GI symptoms.[75] PPIs reduce dyspepsia in controlled trials.[74] Studies have shown that histamine-2 receptor antagonists (H2RAs) are also effective for reducing dyspepsia.[77] Crucially from a clinical perspective, subjective symptoms of dyspepsia, fecal blood loss, and endoscopic findings correlate poorly. Furthermore, only a minority of patients with serious GI events report antecedent dyspepsia.[78]

## Gastritis and Gastroduodenal Ulcer

Up to 25% of long-term NSAID users will experience ulcer disease, and 2% to 4% of the ulcers will bleed or perforate. These GI events result in more than 100,000 hospital admissions annually in the United States and between 7000 and 10,000 deaths, especially in patients with the highest risk.[47] The risk for ulcer complications appears highest within the first 3 months of use but remains present with longer-term therapy. A recent meta-analysis of observational studies on NSAIDs and upper GI bleeding or perforation published between 2000 and 2008 demonstrated a relative risk of 4.50 (95% confidence interval [95% CI], 3.82 to 5.31) for traditional NSAIDs and 1.88 (95% CI, 0.96 to 3.71) for selective COX-2 inhibitors.[43] For traditional NSAIDs, low and medium doses were associated with a lower risk than were higher doses. Several NSAIDs had a far higher than average risk, including ketorolac, with a risk of 14.54 (95% CI, 5.87 to 36.04), and piroxicam, with a risk of 9.94 (95% CI, 5.99 to 16.50). The risks for celecoxib (1.42; 95% CI, 0.85 to 2.37) and ibuprofen (2.69; 95% CI, 2.17 to 3.33) were lower than for NSAIDs overall. Relative risk for other commonly used NSAIDs, including naproxen, indomethacin, meloxicam, and diclofenac, ranged from 3.98 to 5.63. Drugs with a long half-life or slow-release formulation were associated with higher risk, even accounting for dose.[43] Profound and coincident inhibition of both COX-1 and COX-2 using whole blood assay, as seen for ketorolac, piroxicam, naproxen, ketoprofen, and indomethacin, was associated with a relative risk of greater than 5 for GI bleeding and perforation.[43] The use of low-dose aspirin, even in the absence of other risk factors, increases risk for bleeding and death. Many patients taking low-dose aspirin may do so without the knowledge of their physician, and thus it is essential to query patients specifically on this point.

Other patient-specific factors influence the overall risk for GI ulcers and ulcer complications (Figure 59-5).[47,79] A previous history of ulcer or ulcer complications is an important risk, especially if combined with other risks. Infection with *H. pylori* is likely to be associated with additive effect.[73] It remains unclear if eradication of *H. pylori* would be useful in the primary prevention of NSAID-induced ulcers, but it may be advantageous in patients requiring long-term use of NSAIDs.[47] Eradication alone is insufficient as a single strategy for secondary prevention of ulcer complications. This strategy appears to be most effective in reducing the bleeding risk of patients taking low-dose aspirin but is less useful than use of PPIs in patients taking NSAIDs.[47]

Table 59-3 provides recommendations for patients who need NSAIDs and have GI risks.[47] Misoprostol is as effective in the reduction of gastroduodenal ulcers. Meta-analysis showed a reduction of 74% in gastric ulcers and 53% in duodenal ulcers when compared with placebo.[80] The effectiveness of misoprostol is comparable with the PPI lansoprazole.[81] However, the high prevalence of abdominal cramping and diarrhea limit misoprostol use at full doses. For people who do not tolerate full doses (200 μg four times

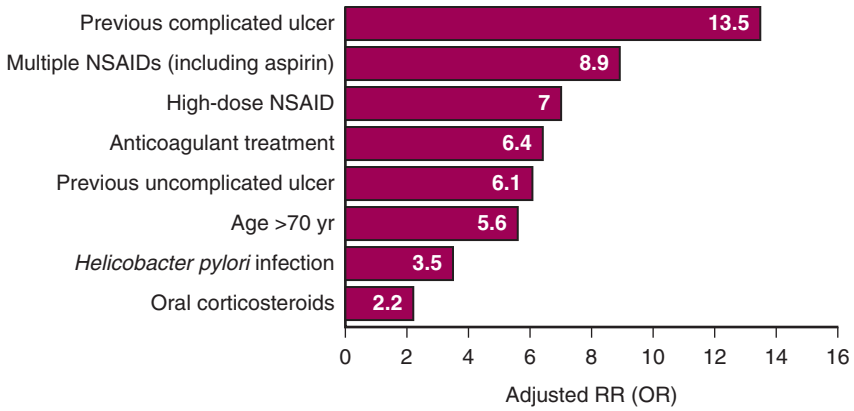



**Figure 59-5** Established risk factors for upper gastrointestinal bleeding associated with NSAID use. OR, Odds ratio; RR, relative risk. *(Modified from Gutthann SP, García-Rodríguez LA, Raiford DS: Individual nonsteroidal anti-inflammatory drugs and other risk factors for upper gastrointestinal bleeding and perforation.* Epidemiology *8:18–24, 1997; Huang JQ, Sridhar S, Hunt RH: Role of* Helicobacter pylori *infection and non-steroidal anti-inflammatory drugs in peptic ulcer disease: a meta-analysis.* Lancet *359:14–22, 2002; and Lanas A, García-Rodríguez LA, Arroyo MT, et al: Risk of upper gastrointestinal ulcer bleeding associated with selective cyclooxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations.* Gut *55:1731–1738, 2006.)*

**TABLE 59-3** Strategies for Gastrointestinal Risk Reduction[47]

| Gastrointestinal Risk | Potential Strategies |
|---|---|
| Low risk | Intermittent NSAID use<br>Low-dose NSAID |
| Moderate risk (1-2 risk factors)<br>Age >65 yr<br>High-dose NSAID<br>Previous history of uncomplicated ulcer<br>Concurrent use of aspirin, corticosteroids, or anti-coagulants | Intermittent NSAID use<br>NSAID + PPI<br>NSAID + misoprostol<br>NSAID + high-dose H2RA* |
| High risk<br>>2 Risk factors<br>History of previous complicated ulcer, especially recent | Alternative treatment<br>COX-2–selective NSAID + PPI<br>COX-2–selective NSAID + misoprostol |
| *Helicobacter pylori* positive | Consider eradication in moderate- to high-risk patients |

*Less effective than PPI or misoprostol.

COX-2, Cyclooxygenase-2; H2RA, histamine-2 receptor antagonist; PPI, proton pump inhibitor.

daily), lower doses of 400 to 600 μg/day may be useful and comparable with PPIs.

PPIs have been used extensively for prevention of NSAID-induced ulcers and are also used for ulcer healing. Their excellent tolerability and availability over the counter have led to their dominance as pharmacologic agents for preventing NSAID-induced gastroduodenal ulcers. Studies have shown a reduction in the endoscopic ulcer rate from 17% in patients taking traditional or COX-2–selective NSAIDs plus placebo to 5.2% and 4.6% in patients taking NSAIDs plus esomeprazole 20 mg or 40 mg, respectively.[82] As noted previously, a combination pill containing naproxen and esomeprazole has been approved for use. It may reduce noncompliance but will be associated with higher cost.

High-dose, twice-daily doses of H2RA reduce the risk of NSAID-induced endoscopic ulcers and are the least costly alternative. However, these agents are inferior to PPIs and, as with PPIs, no randomized clinical outcome trials have been performed to evaluate the efficacy of H2RAs in long-term users of NSAIDs.[24]

### Esophageal Injury

Aspirin and NSAIDs are associated with esophagitis as a result of mechanisms similar to those in the gastric mucosa.[83,84] Esophageal emptying may be slowed in elderly persons, resulting in a prolonged exposure of the mucosa to the irritant action of aspirin and NSAIDs. Gastroesophageal reflux may be an aggravating factor and may lead to stricture formation. Bleeding also may complicate esophagitis. NSAIDs should be prescribed with caution in the presence of gastroesophageal reflux disease.

### Small Bowel Injury

The availability of video capsule endoscopy (VCE) and balloon enteroscopy has advanced the ability to detect small intestinal lesions in patients taking NSAIDs. NSAIDs can cause a concentric "diaphragm-like" stricture in the small bowel, in addition to causing mucosal injury and bleeding. Two recent studies of patients taking NSAIDs for at least 3 months using VCE demonstrated a prevalence for small bowel injuries of 70% to 80%.[85] Furthermore, NSAID-induced small bowel injury is likely a common cause of obscure GI bleeding. NSAIDs that undergo enterohepatic circulation are likely to be associated with higher risk. Small bowel injury may be detected by anemia or symptoms of obstruction related to a stricture.[85] Strategies effective for gastroduodenal ulcers such as misoprostol or certain PPIs also may reduce the risk for small bowel mucosal injury. Strictures may require balloon endoscopy or surgical intervention.[85]

### Colitis

NSAIDs cause erosions, ulcers, hemorrhage, perforations, strictures, and complications of diverticulosis in the large bowel.[86] NSAID-induced injury is more common in the right colon (80%) but can occur in the transverse and left

colon. Suppositories containing NSAIDs can cause erosions, ulcers, and stenoses in the rectum. NSAID colonopathy is in the differential diagnosis of inflammatory bowel disease. Patients with NSAID-induced colonopathy are typically older, and the erosions are more likely to be transverse or circular.[87] There is also a concern that treatment with traditional and COX-2–selective NSAIDs may exacerbate inflammatory bowel disease.[88] NSAIDs are also implicated in the development of collagenous colitis.[89]

## Renal Effects

PGs play a vital role in solute and renovascular homeostasis.[90] It is becoming quite clear that PGs are produced by both COX-1 and COX-2, generally in different locations within the kidney, and that these PGs may play different physiologic roles in renal function.[91,92] COX-1 is highly expressed in the renal vasculature, glomerular mesangial cells, and collecting duct. COX-2 expression is restricted to the vasculature, cortical thick ascending limb (specifically in cells associated with the macula densa), and medullary interstitial cells. COX-2 expression in the macula densa increases in high-renin states (e.g., salt restriction, angiotensin-converting enzyme inhibition, and renovascular hypertension), and selective COX-2 inhibitors significantly decrease plasma renin levels and renal renin activity. COX-2 expression in the macula densa is reduced by angiotensin II and mineralocorticoids. Dehydration or hypertonicity appears to regulate COX-2 expression in the medullary interstitium. COX-2 is also necessary for normal renal development.

### Sodium Excretion

PGs are known to regulate renal sodium resorption by their ability to inhibit active transport of sodium in both the thick ascending limb and the collecting duct and to increase renal water excretion by blunting the actions of vasopressin.[93] The cellular source of COX-2–derived prostanoids that promote natriuresis remains uncertain, but it is possible that they may in large part be derived from the medullary interstitial cells. Sodium retention has been reported to occur in up to 25% of patients treated with NSAIDs and may be particularly apparent in patients who have an existing avidity for sodium, such as those with mild heart failure or liver disease.[93] Decreased sodium excretion in patients treated with NSAIDs can lead to weight gain and peripheral edema. This effect may be sufficiently important to cause clinically important exacerbations of congestive heart failure.

### Hypertension

NSAIDs may cause altered blood pressure, with average increases of mean arterial pressure of between 5 and 10 mm Hg. In addition, use of NSAIDs may increase the risk of initiating anti-hypertensive therapy in older patients, with the magnitude of increased risk being proportional to the NSAID dose.[94] Furthermore, in a large ($n$ = 51,630) prospective cohort of women aged 44 to 69 years without hypertension in 1990, incident hypertension during the following 8 years was significantly more likely in frequent users of aspirin, acetaminophen, and NSAIDs.[95] NSAIDs can attenuate the effects of anti-hypertensive agents, including diuretics, angiotensin-converting enzyme inhibitors, and β-blockers, thus interfering with control of blood pressure.

PGs stimulate renin release, which, in turn, increases secretion of aldosterone and, subsequently, potassium secretion by the distal nephron. For this reason, hyporeninemic hypoaldosteronism that manifests as type IV renal tubular acidosis and hyperkalemia may develop in patients treated with NSAIDs.[93] The degree of hyperkalemia is generally mild; however, patients with renal insufficiency or those who may otherwise be prone to hyperkalemia (e.g., patients with diabetes mellitus and those taking angiotensin-converting enzyme inhibitors or potassium-sparing diuretics) may be at greater risk.

### Acute Renal Failure and Papillary Necrosis

Acute renal failure is an uncommon consequence of NSAID treatment. Acute renal failure occurs because of the vasoconstrictive effects of NSAIDs, and it is reversible. In most cases, renal failure occurs in patients who have a depleted actual or effective intravascular volume (e.g., congestive heart failure, cirrhosis, or renal insufficiency).[93]

A marked reduction in medullary blood flow may result in papillary necrosis that may arise from apoptosis of medullary interstitial cells. Inhibition of COX-2 may be a predisposing factor.[92,96]

### Interstitial Nephritis

Another adverse renal effect resulting from NSAIDs involves an idiosyncratic reaction accompanied by massive proteinuria and acute interstitial nephritis. Hypersensitivity phenomena such as fever, rash, and eosinophilia may occur. This syndrome has been observed with most NSAIDs.

### Chronic Kidney Disease

Use of analgesics, particularly acetaminophen and aspirin, has been associated with nephropathy that leads to chronic renal failure. In one large case-control study, the regular use of aspirin or acetaminophen was associated with a risk of chronic renal failure 2.5 times as high as that for nonuse, and the risk increased significantly with an increasing cumulative lifetime dose.[97] In subjects regularly using both acetaminophen and aspirin, the risk was also significantly increased compared with users of either agent alone. No association between the use of nonaspirin NSAIDs and chronic renal failure could be detected after adjusting for acetaminophen and aspirin use. Pre-existing renal or systemic disease was a necessary precursor to analgesic-associated renal failure, and persons without pre-existing renal disease had only a small risk of end-stage renal disease.[97,98]

## Cardiovascular Effects

The risk of adverse cardiovascular effects associated with NSAID use was not widely appreciated until COX-2–selective NSAIDs were introduced into clinical practice.

Rofecoxib, a potent, highly specific COX-2 inhibitor with a long half-life, has a substantially increased risk of MI and stroke and was removed from the market because of this adverse effect.[7,69] The mechanisms for cardiovascular risks associated with all NSAIDs are likely related to an imbalance between complete inhibition of COX-1 and COX-2 across the dosing interval. The COX-1 isoform is responsible for the generation of platelet thromboxane $A_2$, which facilitates platelet aggregation and thrombus formation. To inhibit this activity, COX-1 must be inhibited by 95% or greater.[99] Anti-thrombotic $PGI_2$ synthesized by endothelial COX-2 is inhibited almost completely by both traditional and COX-2–selective NSAIDs. It is proposed that the relationship between excess cardiovascular risk for all NSAIDs, not only COX-2–selective NSAIDs, is related to the degree of COX-2 inhibition absent complete inhibition of COX-1.[100] Investigators have shown that drugs that inhibit COX-2 less than 90% at therapeutic concentrations in the whole blood assay present a relative risk for MI of 1.18 (95% CI, 1.02 to 1.38), whereas drugs that inhibit COX-2 to a greater degree present a relative risk of 1.60 (95% CI, 1.41 to 1.81).[100]

Relative inhibition of the COX isoforms is not the only mechanism that contributes to cardiovascular hazard. Other actions of NSAIDs, including effects on blood pressure, endothelial function, and NO production, as well as other renal effects, may play a role in cardiovascular risks.[69,101,102] Multiple analyses have demonstrated that the risk for cardiovascular hazard is significantly higher in persons with pre-existing coronary artery disease. Some NSAIDs, notably ibuprofen and naproxen, may interfere with the irreversible inhibition of platelet COX-1 by aspirin, thereby increasing the cardiovascular hazard in aspirin users.[100]

A number of large-scale randomized controlled trials comparing NSAIDs with placebo or with each other have been performed and analyzed to determine the risk of MI, stroke, cardiovascular death, death from any cause, and Anti-platelet Trialists' Collaboration (APTC) composite outcomes.[69] Because event rates in most of these studies were low, uncertainty regarding absolute and relative risk remains. A large network meta-analysis of 31 trials with 116,429 patients and more than 115,000 patient-years of follow-up was reported. The authors concluded that there is little evidence that any NSAID is safe in cardiovascular terms, although naproxen was potentially the least harmful.[103] It appears from analyses of these aggregated clinical trials that all traditional and COX-2–selective NSAIDs except naproxen carry an excess risk of more than 30% compared with placebo.[69] Pairwise comparisons of the most commonly used traditional and COX-2–selective NSAIDs studied in clinical trials also suggest that naproxen may have lower cardiovascular risk.[69] Estimation of absolute risk was performed in another comprehensive meta-analysis of clinical trials.[104] Compared with placebo, allocation to a COX-2–selective NSAID (with celecoxib grouped together with rofecoxib and others) or diclofenac caused approximately three additional major vascular events per 1000 participants per year. One meta-analysis explored the effects of dose and dosing regimen in a pooled analysis of six randomized placebo-controlled trials of celecoxib.[105] Lower doses and once-daily regimens were associated with lower relative risks for the APTC outcomes. This finding confirms data from other studies that suggest that avoiding continuous interference with PG biosynthesis is associated with lower cardiovascular risk.[100]

Because clinical trials have been underpowered to specifically address the relative cardiovascular risk of NSAIDs, investigators have turned to observational data sets. Using a large observational database with 8852 cases of nonfatal MI, a recent case-control study also identified a 35% increase in the risk of MI while using NSAIDs.[100] In a nationwide cohort of patients after MI, an increased risk of death or recurrent MI of approximately 50% for patients using NSAIDs was present at the beginning of treatment and persisted throughout the observation period.[106] The largest meta-analysis of observational studies available to date also clearly demonstrates that higher doses of NSAIDs, with the exception of naproxen, increased the risk of serious cardiovascular events.[107] The effect of dose and slow-release formulation demonstrated that risk was a direct consequence of prolonged drug exposure. It appears that the risk associated with these pharmacologic factors may be even more important than COX-2 specificity for most NSAIDs.[69,100]

A number of strategies have been suggested to mitigate cardiovascular risks associated with NSAID use (Table 59-4).[108] These recommendations take into account a patient's underlying risk, aspirin use, and the interaction between NSAIDs. In addition, the specific choice of NSAID should consider its pharmacologic properties.[69,100]

### Heart Failure

NSAIDs are associated with reduced sodium excretion, volume expansion, increased preload, and hypertension. As a result of these properties, patients with pre-existing heart failure are at risk of decompensation with a relative risk of 3.8 (95% CI, 1.1 to 12.7). After adjusting for age, sex, and concomitant medication, the relative risk was 9.9 (95% CI, 1.7 to 57.0).[109] Studies disagree as to whether NSAIDs are a risk for new episodes of heart failure, although elderly persons may be at particular risk.[109,110] A recent study examining patients who survived first hospitalization for heart failure demonstrated increased risk of death with all NSAIDs at high doses and again demonstrated that lower doses reduced risk for all agents.[111]

### Closure of the Ductus Arteriosus

The maintenance of an open ductus arteriosus and its closure during the postnatal period are regulated by PG.

**TABLE 59-4** Strategies for Reducing Cardiovascular Risk[69,100,108]

| |
|---|
| If using aspirin, take aspirin dose ≥2 hr before NSAID dose.* |
| Do not use NSAIDs within 3-6 mo of an acute cardiovascular event or procedure. |
| Carefully monitor and control blood pressure. |
| Use low-dose, short–half-life NSAIDs and avoid extended-release formulations. |

*Especially ibuprofen. Celecoxib does not appear to interfere with aspirin actions.

COX-1, COX-2, and $EP_4$-deficient mice die from neonatal circulatory failure because the ductus arteriosus remains open. It is inadvisable for pregnant women to take NSAIDs during the last trimester of pregnancy because of the risk of a persistently patent ductus arteriosus.

## Hepatic Effects

Small elevations of one or more liver tests may occur in up to 15% of patients taking NSAIDs, and notable elevations of alanine aminotransferase or aspartate aminotransferase (approximately three or more times the upper limit of normal) have been reported in approximately 1% of patients in clinical trials of NSAIDs. Patients usually have no symptoms, and discontinuation or dose reduction generally results in normalization of the transaminase values, although rare, fatal outcomes have been reported with almost all NSAIDs. The NSAIDs that appear most likely to be associated with hepatic adverse events are diclofenac and sulindac.

In clinical trial reports to the FDA, 5.4% of patients with RA who were treated with aspirin experienced persistent elevations of results in more than one liver function test. In children with viral illnesses, hepatocellular failure and fatty degeneration (Reye's syndrome) are associated with aspirin ingestion.[58]

## Asthma and Allergic Reactions

### Asthma

Up to 10% to 20% of the general asthmatic population, especially those with the triad of vasomotor rhinitis, nasal polyposis, and asthma, are hypersensitive to aspirin. In these patients, ingestion of aspirin and nonspecific NSAIDs leads to severe exacerbations of asthma with naso-ocular reactions. These patients, who were formerly said to have aspirin-sensitive asthma, are now characterized as having aspirin-exacerbated respiratory disease (AERD) because they have chronic upper and lower respiratory mucosal inflammation, sinusitis, nasal polyposis, and asthma independent of their hypersensitivity reactions. It is now thought that production of protective PGs in the setting of AERD is derived from a COX-1. A number of studies have been reported demonstrating that the COX-2–specific NSAIDs, rofecoxib and celecoxib, fail to trigger asthma exacerbation or naso-ocular symptoms in patients with AERD.[112,113] Nevertheless, these studies were performed as challenge tests rather than long-term placebo-controlled trials, and caution is advised. The fact that specific COX-2 inhibitors appear safe in persons with AERD does not imply that other hypersensitivity reactions do not occur.

### Allergic Reactions

A wide variety of cutaneous reactions have been associated with NSAIDs. Almost all the NSAIDs have been associated with cutaneous vasculitis, erythema multiforme, Stevens-Johnson syndrome, or toxic epidermal necrolysis. NSAIDs are also associated with urticaria/angioedema and anaphylactoid or anaphylactic reactions. It should be especially noted that celecoxib and valdecoxib contain a sulfonamide group and should not be given to patients who report allergy to drugs containing sulfa.

## Hematologic Effects

Aplastic anemia, agranulocytosis, and thrombocytopenia are rarely associated with NSAIDs, but they are prominent among the causes of deaths attributed to these drugs. Because of the risk of hematologic effects, phenylbutazone is no longer recommended for use in any condition in the United States and has been taken off the market.[114]

## Effects on the Immune System

Virtually all cell types comprising the immune system produce and respond to PGs. $PGE_2$ is a potent inhibitor of chemotaxis, aggregation, superoxide production, lysosomal enzyme release, and $LTB_4$ generation of activated neutrophils.[115] Antigen-presenting cells play a pivotal role in the immune response, bridging local innate immune responses and the developing acquired immune response, which takes place in more specialized lymphoid organs. Bone marrow cultured in the presence of indomethacin yields increased numbers of dendritic cells, whereas bone marrow cultured in the presence of exogenous $PGE_2$ yields lower numbers of dendritic cells.[116] Dendritic cell cytokines are one of the many important factors that polarize the T cell response toward T helper (Th)1, Th2, or Th17 cytokine profiles. Failure of dendritic cell maturation and/or their secretion of inhibitory cytokines leads to development of T regulatory cells that modulate Th activity and suppress some of their functions.[117] Eicosanoids are likely to be one of the tissue factors that determine dendritic cell phenotype during interaction with specific antigens/pathogens via pattern recognition receptors.[117] Exogenous $PGE_2$ was reported to induce IL-23 production, which is important for generation of Th17 cells.[118,119] A recent study using human peripheral blood mononuclear cells also supports a role for $PGE_2$ in promoting a Th17 response.[120] Although a Th17 immune response is associated with development of autoimmune diseases, the clinical relevance of these observations with respect to NSAID use remains unclear.

$PGE_2$ also plays a role in B and T lymphocytes. $PGE_2$, through increased cAMP, inhibits many T cell functions.[121] Treatment with COX-2–selective NSAIDs severely diminishes proliferation and expression of IL-2, TNF, and IFN-γ. B lymphocytes express COX-2 and produce $PGE_2$.[122] Treatment with traditional and COX-2–selective NSAIDs profoundly reduced production of IgG and IgM after stimulation with minimal effects on proliferation. COX-2 null mice had 64% less IgM and 35% less IgG than normal littermates after in vitro treatment with LPS. COX-2–deficient mice also produce markedly reduced IgG and 10-fold reduced neutralizing antibody to HPV-like particles compared with wild-type mice without evidence of differences in B cell precursors.[123] Similar findings of deficient humoral immune responses have been demonstrated for mice deficient in mPGES-1.[124] Human memory B cell antibody production was also diminished in the presence of a specific COX-2 inhibitor.[123] Again, the clinical relevance of these observations remains unclear with respect to NSAID use.

## CNS Effects

Elderly patients may be particularly susceptible to developing cognitive dysfunction and other CNS effects, including headache, dizziness, depression, hallucination, and seizures, that are related to NSAIDs. Acute aseptic meningitis has been reported in patients with SLE or mixed connective tissue disease who were treated with ibuprofen, sulindac, tolmetin, or naproxen.

## Effects on Bone

The complex effects of prostanoids on bone formation and remodeling have been appreciated for many years. It is now clear that COX-2 is required for many functions of both osteoblasts and osteoclasts.[27] COX-2 is rapidly inducible and highly expressed and regulated in osteoblasts. Parathyroid hormone (PTH) is a strong inducer of COX-2. The production of PG by osteoblasts is an important mechanism for the regulation of bone turnover.[27] The major effect of $PGE_2$ is considered to occur indirectly via upregulation of receptor activator of NF-κB ligand (RANKL) expression and by inhibition of osteoprotegerin (OPG) expression in osteoblastic cells, which facilitates osteoclastogenesis. Genetic deletion of *PTGS2* or COX-2–selective NSAIDs partially block the PTH- or 1,25-OH vitamin D–induced formation of osteoclasts in organ cultures. Recently, a familial disorder, primary idiopathic hypertrophic osteoarthropathy, was found to be associated with a mutation in the enzyme 15-hydroxyprostaglandin dehydrogenase, the enzyme that inactivates $PGE_2$.[125] These patients have chronically elevated $PGE_2$ levels and digital clubbing with evidence of increased bone formation and resorption in the phalanges.

The role of endogenous PG and NSAIDs in skeletal disease remains complex. LPS-induced bone loss can be ameliorated in mice lacking mPGES-1.[126] Inflammatory bone loss likely reflects increased bone resorption and decreased bone formation.[27] It has long been appreciated that NSAIDs can inhibit experimental fracture healing and reduce formation of heterotopic bone in patients.[127] Furthermore, fracture healing is impaired in rats treated with specific COX-2 inhibitors and in mice with genetic deletion of the *COX-2* gene *(PTGS2)*.[128] Given the effectiveness of NSAIDs as analgesics, it is important to understand the clinical concern regarding impaired fracture healing and NSAIDs. A recent meta-analysis found a pooled odds ratio of 3 for nonunion with NSAID exposure (95% CI, 1.6 to 5.6).[129] However, there was a significant association between lower-quality studies, and higher-reported odds ratios for nonunion were observed. When only higher-quality studies were considered, no statistically significant association between NSAID exposure and nonunion was identified.

The impact of NSAIDs on bone mineral density (BMD) also remains unclear.[27] In older men, daily use of COX-2–selective NSAIDs was associated with lower hip and spine BMD compared with nonusers, but in post-menopausal women not taking hormone replacement therapy, a higher BMD was found.[130] It is speculated that the beneficial effects of mechanical loading may be reduced by COX-2 inhibition but that the pro-inflammatory state and increased bone turnover associated with estrogen withdrawal may be suppressed by COX-2 inhibition. Still, a causal role for endogenous PGs in bone loss resulting from estrogen deficiency has not been confirmed.

## Effects on Ovarian and Uterine Function

PGs derived from COX-2 have been implicated as mediators in multiple stages of the female reproductive cycle. Induction of COX-2 immediately after the luteinizing hormone surge was the first observation involving the isoenzyme during a normal physiologic event. It has been suggested that COX-2–derived PGs may signal the time of ovulation in mammals.[131,132] Studies using COX-2 null mice show reproductive failure at ovulation, fertilization, implantation, and decidualization.[133] COX-2–dependent prostanoid production probably leads to the generation of proteolytic enzymes that rupture the follicles. After fertilization, COX-2 also plays a role in embryo implantation in the myometrium.[133] PGs are important for inducing uterine contractions during labor. Murine studies have shown that the mechanism of uterine contraction involves fetal release of $PGF_{2\alpha}$, a compound that induces luteolysis. This pathway leads to reduced maternal progesterone levels, induction of oxytocin receptors in the myometrium, and parturition.

Based on these observations in animals, one could hypothesize that NSAIDs may have an influence on fertility. In fact, studies suggest that luteinized unruptured follicle syndrome as a cause of reversible infertility can be related to the ingestion of NSAIDs.[134] For this reason, although it remains unproved in controlled or observational studies, women should be cautioned that chronic NSAID use may impair fertility.

## Salicylate Intoxication and NSAID Overdose

The new appearance of tachypnea, confusion, ataxia, oliguria, or a rising blood urea nitrogen/creatinine level in a patient, particularly an elderly patient, who takes aspirin or salicylates should suggest the possibility of salicylate intoxication. In adults, metabolic acidosis is masked by hyperventilation because of the stimulation of respiratory centers, which is a direct effect of salicylates. Sudden increases in salicylate levels can occur even if there is no change in dose. This increase is particularly common in patients who experience acidosis from any cause, experience dehydration, or ingest other drugs that displace salicylate from protein-binding sites. Therapy consists of removing residual drug from the GI tract, forced diuresis while maintaining the urinary pH in the alkaline range and with potassium replacement, or hemodialysis if diuresis is unsatisfactory. Vitamin K is recommended because large doses of salicylate may interfere with the synthesis of the vitamin K–dependent clotting factors.

Acute overdoses of NSAIDs are much less toxic than are overdoses of aspirin or salicylates. This subject has been most carefully evaluated for ibuprofen, prompted by its approval for over-the-counter sale to the general public. Symptoms with overdoses ranging up to 40 g include CNS depression, seizures, apnea, nystagmus, blurred vision, diplopia, headache, tinnitus, bradycardia, hypotension, abdominal pain, nausea, vomiting, hematuria, abnormal

renal function, coma, and cardiac arrest. Treatment includes prompt evacuation of the stomach contents, observation, and administration of fluids.

### Adverse Effects of Acetaminophen

Acetaminophen is used widely as the first-line treatment of pain, chiefly because it is viewed as effective and safer than NSAIDs. When used in doses of less than 2 g daily, there is little evidence of toxicity.[135] Acetaminophen-induced acute liver failure is a result of direct injury from the toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine, a highly reactive electrophilic compound that depletes glutathione and subsequently accumulates in hepatocytes.[136] Acetaminophen is a highly predictable hepatotoxin with a threshold dose of 10 to 15 g in adults and 150 mg/kg in children (lower dosages have also been associated with hepatic injury). In the United States, acetaminophen overdoses are usually unintentional, with most persons taking acetaminophen preparations for chronic pain. Intentional self-poisoning with acetaminophen also remains an important problem. Treatment of acetaminophen overdose includes gastric lavage, activated charcoal, or induction of vomiting within the first 3 hours of injection. In addition, intensive support measures and early treatment with *N*-acetylcysteine, which replenishes glutathione, have reduced the mortality associated with acute acetaminophen toxicity. With high doses of acetaminophen, other toxicities may occur, including GI ulcers and bleeding.[137,138] Regular use of acetaminophen has also been associated with an increased risk for chronic renal failure.[97]

## EFFECTS OF CONCOMITANT DRUGS, DISEASES, AND AGING

Because of the widespread use of prescription and nonprescription NSAIDs, ample opportunities exist for interaction with other drugs and for interactions with patient-specific factors.[139] Specific drug interactions are listed on the package inserts of individual agents.

### Drug-Drug Interactions

Because most NSAIDs are extensively bound to plasma proteins, they may displace other drugs from binding sites or may themselves be displaced by other agents. Aspirin and other NSAIDs may increase the activity or toxicity of sulfonylurea, hypoglycemic agents, oral anti-coagulants, phenytoin, sulfonamides, and methotrexate by displacing these drugs from their protein-binding sites and increasing the free fraction of the drug in plasma.[139] NSAIDs may blunt the anti-hypertensive effects of β-blockers, angiotensin-converting enzyme inhibitors, and thiazides, leading to destabilization of blood pressure control.[140] An increased risk of GI toxicity is present when NSAIDs and selective serotonin reuptake inhibitors are taken concomitantly compared with taking either agent alone, and more than an additive risk occurs.[141]

Interactions between aspirin and NSAIDs, particularly ibuprofen, are related to blocking of the ability of aspirin to access the COX active site. This effect may be important when aspirin is used for the prevention of cardiovascular disease. It is prudent to recommend that aspirin be taken 2 hours before ingestion of ibuprofen.[108,142]

### Drug-Disease Interactions

RA and other diseases (e.g., hepatic and renal disease) that decrease serum albumin concentrations are associated with increased concentrations of free NSAIDs. Hepatic and renal diseases also may impair drug metabolism or excretion and thereby increase the toxicity of a given dose of an NSAID to an individual patient. Renal insufficiency may be accompanied by accumulated endogenous organic acids that may displace NSAIDs from protein-binding sites.

### Drug Reactions in Elderly Persons

Aging is accompanied by changes in physiology, resulting in altered pharmacokinetics and pharmacodynamics. Decreased drug clearance may be the consequence of reductions in hepatic mass, enzymatic activity, blood flow, renal plasma flow, glomerular filtration rate, and tubular function associated with aging. Elderly persons are more likely to experience adverse GI and renal effects related to NSAIDs. The increased risk of cardiovascular disease in elderly patients raises concerns of accelerated MI or stroke. The use of aspirin for prevention of cardiovascular disease increases the toxicity of NSAIDs, and conversely the concomitant use of NSAIDs may increase aspirin resistance. Use of a PPI for gastroprotection may interfere with the efficacy of antiplatelet agents such as clopidogrel.[142] Elderly persons have more illnesses than younger patients and therefore take more medications, increasing the possibility of drug-drug interactions. Older patients also may be more likely to self-medicate or make errors in drug dosing. For these reasons, frequent monitoring for compliance and toxicity should accompany the use of NSAIDs in this population.

### Colchicine

Colchicine is frequently used in the context of NSAID replacement therapy when NSAIDs are contraindicated. Colchicine exhibits excellent anti-inflammatory activity in acute gouty arthritis. A recent study has clarified that a low-dose colchicine regimen of 1.2 mg followed in 1 hour by 0.6 mg provides equal efficacy to higher-dose regimens, with a marked reduction in adverse events.[143] Colchicine is also used to prevent acute gouty attacks. Prophylactic treatment appears to reduce the frequency of attacks by 75% to 85% and mitigates the severity of attacks that occur.[144] However, there is concern that prophylactic therapy should be initiated only if hyperuricemia is controlled because tophi may develop without the usual warning signs of acute gouty attacks. Albeit with less efficacy, colchicine treatment also may benefit patients with acute episodes of pseudogout and arthritis resulting from other crystals.

Daily use of colchicine (1.2 to 1.8 mg) is the mainstay of treatment for familial Mediterranean fever (FMF). It is effective in preventing acute attacks and amyloidosis. Colchicine has been used empirically in other rheumatologic disorders in which neutrophils play an important role, such as Behçet's disease, recurring pericarditis, and cutaneous neutrophilic vasculitis.[145]

### Mechanism of Colchicine Action

Colchicine appears to interfere with steps of the inflammatory response in which neutrophils play a central role by interfering with the organization of the fibrillar microtubules involved in cell structure and movement. This interference leads to disaggregation of microtubules and to decreased neutrophil motility and chemotaxis. Furthermore, colchicine inhibits release of chemotactic factors (e.g., leukotriene $B_4$), formation of digestive vacuoles, and lysosomal degranulation, resulting in an inhibition of neutrophil migration into an area of inflammation and a reduction of the metabolic and phagocytic activity of the neutrophils already present. Clinically, this effect results in an interruption in the inflammatory process of gout and other neutrophil-dominated acute inflammatory diseases.[143]

### Adverse Effects of Colchicine

Because the mechanism of colchicine actions is different from that of NSAIDs, the adverse event profile is also different. More than 80% of patients who take a traditional high-dose oral therapeutic dose of colchicine for an acute gouty attack experience cramping, abdominal pain, diarrhea, nausea, or vomiting, and these symptoms usually limit the dose. For this reason, and because of equal efficacy, high-dose colchicine is not recommended for acute gout flares.[143]

Bone marrow depression, hair loss, amenorrhea, dysmenorrhea, oligospermia, and azoospermia have been reported with chronic colchicine treatment.[138] An increase in trisomy 21 may occur in the offspring of patients with FMF who take colchicine at the time of conception. Colchicine can cause subacute-onset muscle and peripheral nerve toxicity, particularly in patients with chronic renal failure. For this reason, colchicine should be used cautiously in patients with chronic renal failure, and dose adjustment should be considered. Patients with colchicine-induced neuromyopathy present with proximal muscle weakness, elevated serum creatine kinase levels, and neuropathy and/or myopathy on electromyography.[146]

Death has occurred with as little as 8 mg of colchicine but is inevitable after the ingestion of more than 40 mg. Treatment includes aspiration of the stomach, intensive support measures, and hemodialysis, although no specific evidence exists that colchicine can be removed by dialysis.

## CHOOSING ANTI-INFLAMMATORY ANALGESIC THERAPY

In choosing an NSAID for a particular patient, the clinician must consider efficacy, potential toxicity related to concomitant drugs and patient factors, and cost. Furthermore, patient preference for factors such as dosing regimen may be taken into account. In addition to choices from the perspective of the individual patient and physician, it may be important to take a broader view. Choice of anti-inflammatory analgesic therapy can also be considered from the perspective of health care institutions and payers. The symptoms and conditions for which NSAIDs are used are extraordinarily common. Consequently, the cost of NSAIDs as a proportion of total drug costs can be high when drugs are expensive. The increased cost of branded NSAIDs has an important pharmacoeconomic impact. On the other hand, adverse events can have important economic consequences, and improved safety may be cost-effective.

Choosing anti-inflammatory analgesic therapy has become increasingly complex with the increased understanding of associated toxicities. Prospectively considering the presence of GI and cardiovascular risk factors is essential when considering treatment options (Table 59-5). GI risks

**TABLE 59-5** Choosing Anti-inflammatory Analgesic Therapy

| Risk Category | Treatment Recommendations |
|---|---|
| **Low** | |
| <65 yr<br>No cardiovascular risk factors<br>No requirement for high-dose or chronic therapy<br>No concomitant aspirin, corticosteroids, or anti-coagulants | Traditional NSAID<br>Shortest duration and lowest dose possible |
| **Intermediate** | |
| ≥65 yr<br>No history of previous complicated GI ulceration<br>Low cardiovascular risk, may be using aspirin for primary prevention<br>Requirement for chronic therapy and/or high-dose therapy | Traditional NSAID + PPI, misoprostol, or high-dose H2RA<br>Once-daily celecoxib + PPI, misoprostol, or high-dose H2RA if taking aspirin<br>If using aspirin, take a low dose (75-81 mg)<br>If using aspirin, take a traditional NSAID ≥2 hr before aspirin dose |
| **High** | |
| Elderly, especially if frail or if hypertension, renal disease, or liver disease is present<br>History of previous complicated ulcer or multiple GI risk factors<br>History of cardiovascular disease and taking aspirin or another anti-platelet agent for secondary prevention<br>History of heart failure | Use acetaminophen, <2 g/day<br>Avoid chronic use of NSAIDs if at all possible:<br>Use intermittent NSAID dosing<br>Use low-dose, short–half-life NSAIDs<br>Do not use an extended-release NSAID formulation<br>If chronic NSAID use is required, consider:<br>Once-daily celecoxib + PPI/misoprostol (GI > CV risk)<br>Naproxen + PPI/misoprostol (CV > GI risk)<br>Avoid use of a PPI if using an anti-platelet agent such as clopidogrel<br>Monitor and treat blood pressure<br>Monitor creatinine and electrolytes |

CV, Cardiovascular; GI, gastrointestinal; H2RA, histamine-2-receptor antagonist; PPI, proton pump inhibitor.

are well known, and strategies to prevent ulceration and bleeding are available. Many questions regarding the risk for cardiovascular events in patients using NSAIDs exist. In general, the data suggest that physicians should be cautious in using NSAIDs in patients with known cardiovascular disease. In patients with risks for NSAID toxicity, avoiding potent drugs with a long half-life or extended-release formulations is prudent. Intermittent dosing rather than continuous daily use reduces toxicity.

Absence of anti-inflammatory activity reduces the effectiveness of acetaminophen for diseases accompanied by a significant component of inflammation (e.g., RA and gout). However, acetaminophen is a safe and effective alternative for milder pain conditions, including osteoarthritis. With respect to patient preference, a survey study demonstrated that only 14% of a large group of patients with rheumatic disease ($n = 1799$) who had RA, osteoarthritis, or fibromyalgia preferred acetaminophen rather than NSAIDs, whereas 60% preferred NSAIDs.[147] In a head-to-head clinical trial of acetaminophen versus diclofenac plus misoprostol, patients in the diclofenac group had significantly greater improvement in pain scores. This finding was magnified in patients with more severe disease at baseline.[148]

Acetaminophen can be tried as the initial therapy in patients with mild to moderate pain for reasons of safety and cost. However, a recent meta-analysis of the safety and efficacy of acetaminophen for spinal pain and osteoarthritis found that this agent is ineffective in the treatment of low back pain and provides minimal short-term benefit for people with osteoarthritis.[149] Furthermore, patients taking acetaminophen were nearly four times more likely to have abnormal results on liver function tests. If patients have moderate to severe symptoms or if evidence of inflammation is present, moving to treatment with NSAIDs may provide more rapid and effective relief.[150]

## CONCLUSION

The strategy of blocking PG production by inhibiting the COX enzymes has provided relief from pain and inflammation for centuries. Given the proven importance of PG in this pathway and the advances in understanding the molecules involved, pharmacologic targeting of enzymes involved in biosynthesis, transport, or degradation may provide therapeutic efficacy. Similar to COX-2, mPGES-1 is induced during inflammation and in other pathologic states. Strategies to inhibit mPGES-1 have been proposed as potential alternatives to inhibiting COX.[151] Drug development has been somewhat hampered because of the species specificity of mPGES-1 binding. However, this strategy may be particularly appealing because pre-clinical data suggest that inhibition of mPGES-1 is associated with a reduced propensity to develop hypertension, thrombosis, atherosclerotic plaques, aortic aneurysm, and neointimal hyperplasia after vascular injury.[152-154] Additionally, receptor antagonists also could be useful. Indeed, a number of $EP_4$ receptor antagonists have proved useful in animal models of RA, osteoarthritis, and pain.[18]



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 60



# Glucocorticoid Therapy

Johannes W.G. Jacobs • Johannes W.J. Bijlsma

**KEY POINTS**

- The mode of action of glucocorticoids is due to interactions between the glucocorticoid receptor and genomic DNA and, in high dosages, might operate through non-genomic mechanisms.
- Glucocorticoids differ considerably in potency and biologic half-life.
- Cortisone and prednisone are biologically inactive and are converted in the liver into biologically active cortisol and prednisolone, respectively.
- Glucocorticoids have disease-modifying and joint-sparing properties in the early phase of rheumatoid arthritis.
- The risk of adverse effects of a glucocorticoid is dependent on the disease, comorbidities, patient, dose, and duration of therapy.
- The risk of adverse effects of low-dose glucocorticoids is generally overestimated.
- After local injection of a glucocorticoid, the risk of local bacterial infection is very low.
- Low to moderate doses of prednisolone during pregnancy appear to be safe.

Even though biologic drugs are increasingly being used, glucocorticoids are still anchor drugs in treatment strategies for patients with rheumatic disease. The first glucocorticoid to be isolated, in 1935, was the endogenous glucocorticoid hormone cortisone. It was synthesized in 1944 and subsequently became available for clinical use. In 1948, cortisone (at that time called compound E) was administered by the American physician Philip S. Hench to a 29-year-old woman with active rheumatoid arthritis (RA) of more than 4 years' duration. This nearly bedridden patient was able to walk after 3 days of treatment. Hench published this case of dramatic improvement in 1949[1] and, along with two colleagues, won the 1950 Nobel Prize in Physiology or Medicine. Later, chemical modification of endogenous steroids enabled production of synthetic glucocorticoids, some of which have proved to be effective anti-inflammatory and immunosuppressive substances with rapid effects.

When the wide array of potentially serious adverse effects became apparent in patients treated with supraphysiologic glucocorticoid dosages, enthusiasm for and use of glucocorticoids decreased. Nevertheless, they continued to be the cornerstone of therapy of many rheumatic disorders, including systemic lupus erythematosus (SLE), vasculitis, polymyalgia rheumatica, and myositis. In addition, the use of glucocorticoids in therapeutic strategies for patients with RA also has become generally accepted.[2] The estimated prevalence of the use of glucocorticoids in the general adult population in the United States for all medical indications is 1.2%; this percentage predominantly reflects chronic glucocorticoid use.[3]

Although knowledge about glucocorticoids has increased during the past several decades, much remains to be learned about the modes of actions of these drugs in rheumatic autoimmune disorders. It is hoped that the unraveling of these mechanisms eventually may lead to new applications of glucocorticoids or novel classes of therapy with fewer adverse effects, as well as to personalized medicine.[4]

## CHARACTERISTICS OF GLUCOCORTICOIDS

### Structure

The precursor molecule of all steroid hormones is cholesterol, which is also a building block for vitamin D, cell membranes, and cell organelles (Figure 60-1). Steroid hormones and cholesterol are characterized by a sterol skeleton consisting of three six-carbon hexane rings and one five-carbon pentane ring. The carbon atoms of this sterol nucleus are numbered in a specific sequence; the term *steroid* refers to this basic sterol nucleus (Figure 60-2). No *qualitative* differences have been noted between the glucocorticoid effects of endogenous cortisol and exogenously applied synthetic glucocorticoids because these effects are, except for higher doses, predominantly genomic (i.e., mediated through the glucocorticoid receptor).[5] However, *quantitative* differences have been identified. The potency and other biologic characteristics of the glucocorticoids depend on structural differences in the steroid configuration. In the 1950s, when more potent synthetic steroid hormones were developed by chemical modification of endogenous steroids, research revealed numerous structural features essential for specific biologic activities. For instance, the 17-hydroxy, 21-carbon steroid configuration (see Figure 60-2) is required for glucocorticoid activity through binding to the glucocorticoid receptor. The introduction of a double bond between the 1 and 2 positions of cortisol yields prednisolone, which has about four times more glucocorticoid activity than cortisol (Table 60-1). Addition of a six-methyl group to prednisolone yields methylprednisolone, which is about five times more potent than cortisol. All these glucocorticoids also have more or less a mineralocorticoid effect. However, the synthetic glucocorticoids triamcinolone and dexamethasone have negligible mineralocorticoid activity.

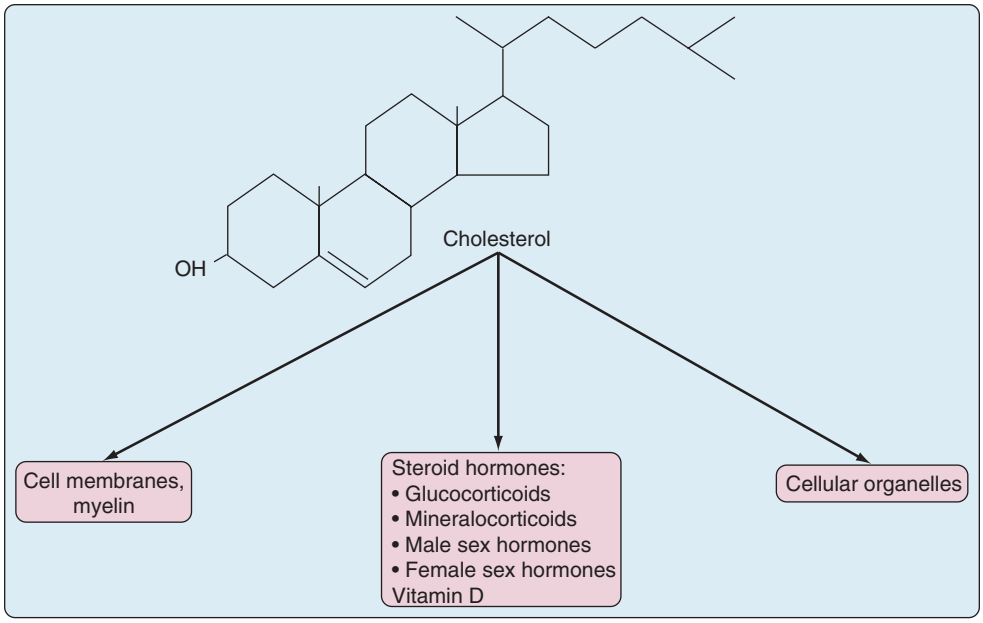



**Figure 60-1** Cholesterol as a building block for steroid hormones, vitamin D, and cell membranes and organelles.

**TABLE 60-1** Pharmacodynamics of Glucocorticoids Used in Rheumatology

| | Equivalent Glucocorticoid Dose (mg) | Relative Glucocorticoid Activity | Relative Mineralocorticoid Activity | Protein Binding | Half-Life in Plasma (hr) | Biologic Half-Life (hr) |
|---|---|---|---|---|---|---|
| **Short-Acting** | | | | | | |
| Cortisone | 25 | 0.8 | 0.8 | – | 0.5 | 8-12 |
| Cortisol | 20 | 1 | 1 | ++++ | 1.5-2 | 8-12 |
| **Intermediate-Acting** | | | | | | |
| Methylprednisolone | 4 | 5 | 0.5 | – | >3.5 | 18-36 |
| Prednisolone | 5 | 4 | 0.6 | ++ | 2.1-3.5 | 18-36 |
| Prednisone | 5 | 4 | 0.6 | +++ | 3.4-3.8 | 18-36 |
| Triamcinolone | 4 | 5 | 0 | ++ | 2->5 | 18-36 |
| **Long-Acting** | | | | | | |
| Dexamethasone | 0.75 | 20-30 | 0 | ++ | 3-4.5 | 36-54 |
| Betamethasone | 0.6 | 20-30 | 0 | ++ | 3-5 | 36-54 |

*Clinically; sodium and water retention, potassium depletion.
–, None; ++, high; +++, high to very high; ++++, very high.

## Classification

Steroid hormones can be classified on the basis of their main function into sex hormones (male and female), mineralocorticoids, and glucocorticoids (see Figure 60-1). Sex hormones are synthesized mainly in the gonads but also in the adrenal cortex. Mineralocorticoids and glucocorticoids are synthesized only in the adrenal cortex; the terms *corticosteroid* and *corticoid* for these hormones refer to the adrenal cortex. Some glucocorticoids also have a mineralocorticoid effect and vice versa. The main endogenous mineralocorticoid is aldosterone, and the main endogenous glucocorticoid is cortisol (hydrocortisone). Although the classification of corticoids into mineralocorticoids and glucocorticoids is not absolute (see later), it is more precise to use the term *glucocorticoid* than the term *corticosteroid* when referring to one of the glucocorticoid compounds.[6]

## Activation

Glucocorticoids with an 11-keto group, such as cortisone and prednisone, are prohormones that must be reduced in the liver to their 11-hydroxy configurations—cortisol and prednisolone, respectively—to become biologically active. For patients with severe liver disease, it is thus rational to prescribe prednisolone instead of prednisone. This formation of biologically active glucocorticoids from their inactive forms is promoted by the reductase action of the intra-cellular enzyme 11β-hydroxysteroid dehydroge-

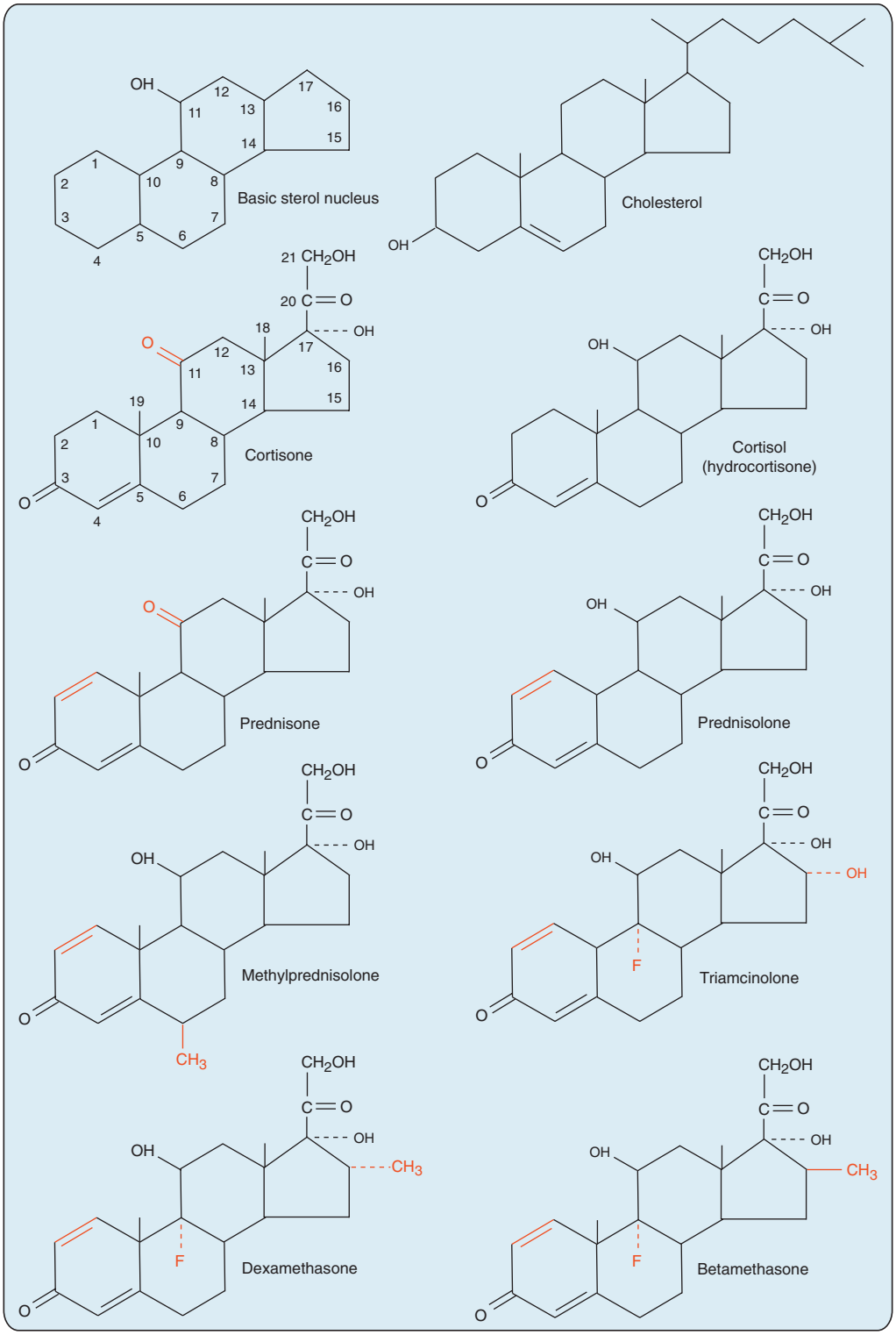



**Figure 60-2** Basic steroid configuration and structure of cholesterol and of natural and some synthetic glucocorticoids. Structural differences compared with cortisol, the endogenous active glucocorticoid, are shown in *red*.

nase (11β-HSD) type 1. The same enzyme can also promote the reverse reaction by dehydrogenation, leading to inactivation of active glucocorticoids. In contrast, 11β-HSD type 2 only has dehydrogenase activity, so it only catalyzes the conversion of active glucocorticoids to their inactive forms. In different tissues, local balance between the intra-cellular enzymes 11β-HSD type 1 and type 2 might modulate intracellular glucocorticoid concentrations and thus tissue sensitivity for glucocorticoids.[7] Synovial tissue metabolizes glucocorticoids via the two 11β-HSD enzymes, with the net effect being glucocorticoid activation. This endogenous glucocorticoid production in the joint increases with joint

inflammation and, vice versa, has an impact on local inflammation and on bone of the joint.[8]

## GENOMIC AND NONGENOMIC MODES OF ACTION

Glucocorticoids at any therapeutically relevant dosage exhibit pharmacologic effects via classic genomic mechanisms. The lipophilic glucocorticoid passes across the cell membrane, attaches to the cytosolic glucocorticoid receptor and heat shock protein. These glucocorticoid complexes then bind to glucocorticoid-responsive elements of genomic DNA, or interact with nuclear transcription factors. This process takes time. When acting through genomic mechanisms, it takes at least 30 minutes before the clinical effect of a glucocorticoid begins to show.[9] Only when high doses are given, such as in pulse therapy, do nongenomic mechanisms also occur, by which glucocorticoids act within minutes. The response to high-dose pulse methylprednisolone therapy may be biphasic, consisting of an early, rapid, nongenomic effect and a more delayed and more sustained genomic effect.[10] Clinically, however, genomic and nongenomic effects cannot be separated.

### Genomic Mechanisms

Most of the effects of glucocorticoids are exerted via genomic mechanisms by binding to the glucocorticoid receptor located in the cytoplasm of the target cells. Glucocorticoids are lipophilic and have a low molecular mass, and thus they can pass through the cell membrane easily. Next to the tissue-specific intra-cellular density of glucocorticoid receptors, the balance of intra-cellular 11β–HSDs (described earlier) probably determines the sensitivity of specific tissues for glucocorticoids.[7] Of the isoforms α and β of the glucocorticoid receptor, only the α isoform, commonly present in all target tissues, binds to glucocorticoids. This is a 94 kDa protein to which several heat shock proteins (chaperones) are bound. Binding of the glucocorticoid to this complex causes shedding of the chaperones. The resulting activated glucocorticoid receptor–glucocorticoid complex is rapidly translocated into the nucleus, where it binds as a dimer to specific consensus sites in the DNA (glucocorticoid-responsive elements) regulating (i.e., stimulating or suppressing) the transcription of a large variety of target genes. This process is termed *transactivation*. Activated glucocorticoid receptor–glucocorticoid complexes also, as monomers, interact with transcriptional factors (such as activator protein-1, interferon regulatory factor-3, and nuclear factor-κB [NF-κB]), leading to inhibition of binding of these transcriptional factors to their consensus sites in the DNA.[11] This process, resulting in downregulation of predominantly pro-inflammatory protein synthesis, is called *transrepression* (Figure 60-4).

The nature and availability of transcription factors may be pivotal in determining the differential sensitivity of different tissues to glucocorticoids because these factors play a crucial role in regulating the expression of a wide variety of pro-inflammatory genes induced by cytokines. The inhibited binding of transcriptional factors to DNA by glucocorticoids results in depressed expression of these genes and inhibition of their amplifying role in inflammation.

The hypothesis has been proposed that adverse effects of glucocorticoids may be based predominantly on transactivation, whereas the anti-inflammatory effects may be mostly due to transrepression. Understanding of these molecular mechanisms could lead to the development of novel glucocorticoids, such as selective glucocorticoid receptor agonists, with a more favorable balance of transactivation and transrepression and, clinically, to a more favorable balance of metabolic and endocrine adverse effects and therapeutic effects.[11] However, although many immunosuppressive effects are based on transrepression, some effects are based on transactivation, such as glucocorticoid-induced gene transcription and protein synthesis of NF-κB inhibitor[12] and of lipocortin-1. Some immunosuppressive effects of glucocorticoids are not based on either transrepression or transactivation. Post-transcriptional mRNA destabilization resulting in decreased protein synthesis may also be an important anti-inflammatory mechanism of glucocorticoids. This mechanism has been proposed to mediate glucocorticoid-induced inhibition of the synthesis of interleukin (IL)-1, IL-6, granulocyte-macrophage colony-stimulating factor, and inducible cyclooxygenase (COX)-2.[13] On the other hand, not all adverse effects are related to transactivation; increased risk of infection is associated with immunosuppression, primarily based on transrepression, which is also the mechanism of suppression of the hypothalamic-pituitary-adrenal (HPA) axis. Moreover, a study in a mouse strain with a deficiency to form dimer glucocorticoid receptor–glucocorticoid complexes and thus with a transactivation deficiency showed, along with a failure of glucocorticoids to exert a full anti-inflammatory response, classic adverse effects in these mice, such as osteoporosis.[14] These data challenge the concept of selective glucocorticoid receptor agonists[15]; furthermore, in an asthma trial, the effect of a selective glucocorticoid receptor agonist was disappointing.[16]

### Nongenomic Mechanisms

Compared with genomic effects, nongenomic effects at high doses of glucocorticoids occur more rapidly—within minutes. One mechanism involves membrane-bound glucocorticoid receptors. Dexamethasone targets these receptors on T lymphocytes, which rapidly impairs T lymphocyte receptor signaling and immune response.[17] Nongenomic actions without involvement of glucocorticoid receptors occur via physicochemical interactions with biologic membranes, altering cell function. For instance, the resulting inhibition of calcium and sodium cycling across the plasma membrane of immune cells contributes to rapid immunosuppression and reduced inflammation.[5]

## GLUCOCORTICOID EFFECTS ON HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

### Hypothalamic-Pituitary-Adrenal Axis and Inflammation

Pro-inflammatory cytokines (such as IL-1 and IL-6), eicosanoids (such as prostaglandin $E_2$), and endotoxins all activate corticotropin-releasing hormone (CRH) at the

hypothalamic level and adrenocorticotropic hormone (ACTH) at the pituitary level. CRH also activates ACTH, and this activation stimulates the secretion of glucocorticoids by the adrenal glands, which is also stimulated by the inflammatory mediators previously mentioned (Figure 60-5). In otherwise healthy persons with severe infections or other major physical stress, cortisol production may increase to six times the normal amount.[18] In patients with chronic inflammatory diseases, such as active RA, this increase of cortisol driven by elevated cytokine levels might be inappropriately low,[19] meaning that cortisol levels, although normal or elevated in the absolute sense, are insufficient to control the inflammatory response. This is the concept of relative adrenal insufficiency.[19-22] Endogenous and exogenous glucocorticoids exert negative feedback control on the HPA axis *directly* by suppressing secretion of ACTH and CRH and also *indirectly* in inflammatory diseases by suppressing the release from inflammatory tissues of pro-inflammatory cytokines that stimulate secretion of ACTH and CRH (see Figure 60-5). Sensitivity of the HPA axis for pro-inflammatory cytokines is probably decreased in persons with RA.[23]

ACTH is secreted in brief, episodic bursts, resulting in relatively sharp increases in plasma concentrations of ACTH and cortisol, followed by slower declines in cortisol levels—the normal diurnal rhythm in cortisol secretion. Secretory ACTH episodic bursts increase in amplitude but not in frequency after 3 to 5 hours of sleep, reach a maximum in the hours before and the hour after awakening, decline throughout the morning, and are minimal in the evening. Cortisol levels are highest at about the time of awakening in the morning, are low in the late afternoon and evening, and reach their lowest level some hours after falling asleep (see Figure 60-5). Glucocorticoids are not stored in the adrenal glands in great quantities; continuing synthesis and release are required to maintain basal secretion or to increase blood levels during stress. The total daily basal or physiologic secretion of cortisol in humans has been estimated to range from 5.7 to 10 mg/m$^2$/day.[24,25] Although during periods of physiologic stress the replacement dose should be higher (discussed later), this need would be covered in persons with primary adrenal insufficiency by oral administration of 15 to 25 mg of cortisol,[24] equivalent to about 4 to 6 mg of prednisolone. This low daily cortisol production rate may explain the cushingoid symptoms and other adverse effects that are sometimes observed in patients with adrenal insufficiency who use glucocorticoids at doses previously regarded to be replacement doses (based on estimates of physiologic secretion of cortisol of 12 to 15 mg/m$^2$/day) but that are in fact supraphysiologic doses.

## Tertiary Adrenal Insufficiency

Chronic suppression of the HPA axis by administration of exogenous glucocorticoids, as a result of negative feedback loops on CRH and ACTH (see Figure 60-5), leads to failure of pituitary ACTH release and thus to partial functional adrenal atrophy with loss of cortisol secretory capability. Although this adrenal insufficiency is a result of the use of glucocorticoids (thus is secondary to glucocorticoids), and although ACTH release is also directly inhibited by glucocorticoids, this adrenal insufficiency is often referred to as tertiary adrenal insufficiency, referring to the inhibition of CRH release.[26] The inner adrenal cortical zone (i.e., the fasciculate-reticularis zone) is the site of cortisol and adrenal androgen synthesis and is dependent on ACTH for structure and function. The outer cortical zone (glomerulosa zone) is involved in biosynthesis of mineralocorticoids (aldosterone) and is functionally independent of ACTH. It stays functionally intact. Patients experience a failure of pituitary CRH and ACTH release and adrenal responsiveness to ACTH. Serum cortisol, ACTH levels, and adrenal responsiveness to ACTH are low, but other pituitary axes function normally, in contrast to the situation in most primary pituitary disorders. Tertiary adrenal insufficiency generally has a less dramatic presentation than primary adrenal insufficiency because aldosterone levels, which are controlled predominantly by the renin-angiotensin system, are preserved; thus mineralocorticoid therapy is not necessary.

The duration of glucocorticoid therapy leading to suppression of the HPA axis depends on the dosage and the serum half-life of the glucocorticoid used, but it also varies among patients, probably because of individual differences in glucocorticoid sensitivity and rates of glucocorticoid metabolism. Prediction with certainty of chronic suppression of the hypothalamic-pituitary-adrenal axis and adrenal insufficiency on the patient level is not possible. This risk may be increased when glucocorticoids are used concomitantly with other steroid drugs such as megestrol acetate and medroxyprogesterone, also inhibiting the HPA axis.[27]

The duration of anti-inflammatory effect of one dose of a glucocorticoid approximates the duration of HPA suppression. After a single oral dose of 250 mg of hydrocortisone or cortisone, 50 mg of prednisone or prednisolone, or 40 mg of methylprednisolone, suppression for 1.25 to 1.5 days has been described. Duration of suppression after 40 mg of triamcinolone or 5 mg of dexamethasone was 2.25 and 2.75 days, respectively.[28] After intramuscular administration of a single dose of 40 to 80 mg of triamcinolone acetonide, the duration of HPA suppression is 2 to 4 weeks, and after administration of 40 to 80 mg of methylprednisolone, suppression lasts 4 to 8 days.[28]

After 5 to 30 days of taking at least 25 mg of prednisolone or its equivalent daily, suppression of adrenal response (measured by a low-dose corticotropin test) was present in 34 of 75 patients studied (45%).[29] In these patients, a basal plasma cortisol concentration less than 100 nmol/L was highly suggestive of adrenal suppression, whereas levels of basal cortisol greater than 220 nmol/L predicted a normal adrenal response in most, but not all, patients.

The risk of clinical (symptomatic) adrenal insufficiency is not negligible in patients on long-term low-dose or medium-dose therapy—for example, those who have had less than 10 mg of prednisolone or its equivalent per day in one dose in the morning. Investigators who performed a review of adrenal insufficiency stated that if the daily dose is 7.5 mg or more of prednisolone or its equivalent for at least 3 weeks, adrenal hypofunctioning should be anticipated, and acute cessation of glucocorticoid therapy in this situation could lead to problems.[18] In 21 patients with RA who were undergoing long-term glucocorticoid therapy (a

mean daily dose of 6.7 mg prednisone equivalents), 52% had a normal (≥5 μg/dL) increase of serum cortisol after an intravenous 30-second bolus injection of 100 μg of human CRH, 33% had a subnormal response of cortisol, and 14% had no response at all.[30] Even patients who have received glucocorticoids for less than 3 weeks or have been treated with alternate-day prednisolone therapy do not have zero risk of suppression of the HPA axis, depending on the dose,[31,32] but the risk is low.

Thus adrenal suppression is difficult to predict, and it probably occurs frequently in daily practice.[33] It seems prudent to consider each patient who is undergoing chronic glucocorticoid therapy as being at risk for tertiary adrenal insufficiency. However, after slow tapering, glucocorticoid therapy often can be stopped (if there is no indication for this therapy any longer); generally, adrenal function gradually recovers during the slow tapering period.

## GLUCOCORTICOID EFFECTS ON THE IMMUNE SYSTEM

Glucocorticoids reduce activation, proliferation, differentiation, and survival of a variety of inflammatory cells, including macrophages and T lymphocytes, and promote apoptosis, especially in immature and activated T cells (Figure 60-6). This activity is mediated mainly by changes in cytokine production and secretion. In contrast, B lymphocytes and neutrophils are less sensitive to glucocorticoids, and their survival may be increased by glucocorticoid treatment. The main effect of glucocorticoids on neutrophils seems to be inhibition of adhesion to endothelial cells. Glucocorticoids inhibit not only the expression of adhesion molecules but also the secretion of complement pathway proteins and prostaglandins. At supraphysiologic concentrations, glucocorticoids suppress fibroblast proliferation and IL-1 and tumor necrosis factor (TNF)-induced metalloproteinase synthesis. By these effects, glucocorticoids may retard bone and cartilage destruction in inflamed joints of patients with early phase RA.[34,35]

### Leukocytes and Fibroblasts

Administration of glucocorticoids leads to an increase in the total leukocyte count because of an increase of circulating neutrophil granulocytes in the blood, although the numbers of other leukocyte subsets in blood, such as eosinophil and basophil granulocytes, monocytes/macrophages (decreased myelopoiesis and bone marrow release), and T cells (redistribution effect), are decreased. Table 60-2 summarizes the effects of glucocorticoids on leukocyte subsets. The redistribution of lymphocytes, which is maximal 4 to 6 hours after administration of a single high dose of prednisone and returns to normal within 24 hours, has no clinical consequences. B cell function and immunoglobulin production are hardly affected. The effects of glucocorticoids on monocytes and macrophages, including decreased expression of class II major histocompatibility complex (MHC) molecules and Fc receptors, may increase susceptibility to infection, however.[36] Effects of glucocorticoids on fibroblasts include decreased proliferation and decreased production of fibronectin and prostaglandins.

**TABLE 60-2** Anti-inflammatory Effects of Glucocorticoids on Immune Cells

| Cell Type | Effects |
|---|---|
| Neutrophils | Increased blood count, decreased trafficking, relatively unaltered functioning |
| Macrophages and monocytes | Decreased blood count, decreased trafficking, decreased phagocytosis and bactericidal effects, inhibited antigen presentation, decreased cytokine and eicosanoid release |
| Lymphocytes | Decreased blood count, decreased trafficking, decreased cytokine production, decreased proliferation and impaired activation, little effect on immunoglobulin synthesis |
| Eosinophils | Decreased blood count, increased apoptosis |
| Basophils | Decreased blood count, decreased release of mediators of inflammation |

### Cytokines

The influence of glucocorticoids on cytokine production and action represents one of the major mechanisms of glucocorticoid action in chronic inflammatory diseases. Glucocorticoids exert potent inhibitory effects on the transcription and action of a large variety of cytokines. Most T helper (Th) type 1 pro-inflammatory cytokines are inhibited by glucocorticoids, including IL-1β, IL-2, IL-3, IL-6, TNF, and interferon-γ, along with IL-17 (associated with Th17 cells), and granulocyte-macrophage colony-stimulating factor (see Figure 60-6). In persons with RA, these cytokines are considered to be responsible for synovitis, cartilage degradation, and bone erosion. Conversely, the production of Th2 cytokines, such as IL-4, IL-10, and IL-13, may be stimulated or not affected by glucocorticoids (see Figure 60-6).[37] These cytokines have been related to the extra-articular features of erosive RA associated with B cell overactivity, such as immune complex formation and vasculitis. Activation of Th2 cells can suppress rheumatoid synovitis and joint destruction through release of the anti-inflammatory cytokines IL-4 and IL-10, which inhibit Th1 activity and downregulate monocyte and macrophage functions.[38]

### Inflammatory Enzymes

An important part of the inflammatory cascade is arachidonic acid metabolism, which leads to the production of prostaglandins and leukotrienes, most of which are strongly pro-inflammatory. Through the induction of lipocortin (an inhibitor of phospholipase A2), glucocorticoids reduce the formation of arachidonic acid metabolites. Glucocorticoids also inhibit the production of COX-2 and phospholipase A2 induced by cytokines in monocytes/macrophages, fibroblasts, and endothelial cells. In addition, glucocorticoids are potent inhibitors of the production of metalloproteinases in vitro and in vivo, especially collagenase and stromelysin, which are the main effectors of cartilage degradation induced by IL-1 and TNF.[39]

### Adhesion Molecules and Permeability Factors

Pharmacologic doses of glucocorticoids markedly inhibit exudation of plasma and migration of leukocytes into

inflammatory sites. Adhesion molecules play a central role in chronic inflammatory diseases by controlling the trafficking of inflammatory cells into sites of inflammation. Glucocorticoids reduce the expression of adhesion molecules through the inhibition of pro-inflammatory cytokines and by direct inhibitory effects on the expression of adhesion molecules, such as intercellular adhesion molecule-1 and E-selectin.[40] Chemotactic cytokines attracting immune cells to the inflammatory site, such as IL-8 and macrophage chemoattractant proteins, also are inhibited by glucocorticoids. Nitric oxide production in inflammatory sites is increased by pro-inflammatory cytokines, resulting in increased blood flow, exudation, and probably amplification of the inflammatory response. The inducible form of nitric oxide synthase by cytokines is potently inhibited by glucocorticoids.[41]

## PHARMACOLOGY AND CLINICAL CONSIDERATIONS

### Pharmacokinetics

Apart from the steroid configuration, biologic characteristics of glucocorticoids also depend on whether they are in free form (as alcohol) or are chemically bound (as ester or salt). In their free form, glucocorticoids are virtually insoluble in water, so they can be used in tablets but not in parenteral preparations. For this reason, synthetic glucocorticoids are formulated as either organic esters or as salts. Esters, such as (di)acetate and (hex)acetonide, are lipid soluble but have limited water solubility and are suitable for oral use and intramuscular, intralesional, and intra-articular injection. Salts, such as sodium phosphate and sodium succinate, are generally more water soluble and thus are also suitable for intravenous use. Dexamethasone sodium phosphate can be used intravenously, whereas dexamethasone acetate cannot. When given intramuscularly, dexamethasone sodium phosphate is absorbed much faster from the injection site than dexamethasone acetate. If an immediate effect is required, dexamethasone sodium phosphate should be administered intravenously because it has a more rapid effect than the same preparation given intramuscularly. Intramuscular administration of dexamethasone acetate has the least rapid effect. For local use, less solubility means longer duration of the local effect, which generally is beneficial.

Water insolubility does not impair absorption from the digestive tract. Most orally administered glucocorticoids, whether in free form or as an ester or salt, are absorbed readily, probably within about 30 minutes. The bioavailability of prednisone and prednisolone is high. Commercially available oral and rectal prednisone and prednisolone preparations are considered approximately bioequivalent.

The affinity of the different glucocorticoids for various plasma proteins varies (see Table 60-1). Of cortisol in plasma, 90% to 95% is bound to plasma proteins, primarily transcortin (also called corticosteroid-binding globulin) and, to a lesser degree, albumin. Protein-bound cortisol is not biologically active, but the remaining 5% to 10% of free cortisol is biologically active. In contrast to methylprednisolone, dexamethasone, and triamcinolone, prednisolone has a high affinity for transcortin and competes with cortisol for this binding protein. Two-thirds of the other synthetic glucocorticoids with little or no affinity for transcortin are (weakly) bound to albumin, so about one-third circulates as free glucocorticoid.

Because only unbound glucocorticoids are pharmacologically active, patients with low levels of plasma proteins, such as albumin (e.g., because of liver diseases or chronic active inflammatory diseases), are more susceptible to effects and adverse effects of glucocorticoids. Dosage adjustment should be considered in these patients. In persons with liver disease, an additional argument for dosage adjustment is reduced clearance of glucocorticoids (discussed later).

Glucocorticoids have biologic half-lives 2 to 36 times longer than their plasma half-lives (see Table 60-1). Because prednisolone has a plasma half-life of about 3 hours, it can be prescribed in a once-daily dose for most diseases. Maximal effects of glucocorticoids lag behind peak serum concentrations. Transcortin binds these compounds more strongly than does albumin. The plasma elimination of glucocorticoids predominantly bound to transcortin is in general slower than that of glucocorticoids predominantly bound to albumin or that of glucocorticoids that do not bind to plasma proteins. Transcortin binding is not a major determinant of *biologic* half-lives of glucocorticoids, but it is a major determinant of distribution to different compartments of the body and of binding to the cytosolic glucocorticoid receptor. Compared with cortisol, synthetic glucocorticoids have a lower affinity for transcortin but a higher affinity for the cytosolic glucocorticoid receptor (discussed later). The affinity of prednisolone and triamcinolone for the glucocorticoid receptor is approximately two times higher, and for dexamethasone it is seven times higher. Before they have been chemically reduced, prednisone and cortisone have negligible glucocorticoid bioactivity because of their very low affinity for the glucocorticoid receptor.

Another important factor determining biologic half-lives of glucocorticoids is the rate of metabolism. Synthetic glucocorticoids are subject to the same reduction, oxidation, hydroxylation, and conjugation reactions as cortisol. Pharmacologically active glucocorticoids are metabolized primarily in the liver into inactive metabolites that are excreted by the kidneys; only small amounts of unmetabolized drug also are excreted in the urine. An inverse correlation has been found between prednisolone clearance and age, which means that a given dose may have a greater effect in older persons.[42] Prednisolone clearance also is slower in African Americans compared with the clearance in Caucasians.[43] The serum half-life of prednisolone is 2.5 to 5 hours, but it is increased in patients with renal disease and liver cirrhosis and in elderly persons. Prednisolone can be removed by hemodialysis, but overall, the amount removed does not require dosage adjustment in patients undergoing hemodialysis. In patients with cirrhosis of the liver, the clearance of unbound steroid is about two-thirds of normal, a difference that should be taken into account with dosing.

### Glucocorticoid Resistance

A small proportion of patients do not react favorably to glucocorticoids or even fail to respond to high doses. Furthermore, the susceptibility to adverse effects of

glucocorticoids varies widely. Several different factors are involved in the variability of glucocorticoid sensitivity in patients with rheumatic diseases,[44-46] and an understanding of the mechanisms involved might eventually permit their modulation.

Hereditary glucocorticoid resistance (which is rare) and increased susceptibility to glucocorticoids have been related to specific polymorphisms of the glucocorticoid receptor gene. The glucocorticoid receptor exists as α and β isoforms, but only the α isoform binds glucocorticoids. The β isoform functions as an endogenous inhibitor of glucocorticoids and is expressed in several tissues. Glucocorticoid resistance has been associated with enhanced expression of this β receptor, but this is unlikely to be an important mechanism for glucocorticoid resistance because in most cells, apart from neutrophilic granulocytes, expression of the β receptor is much less than that of the α receptor.[44] The protein lipocortin-1 (or annexin-1) inhibits eicosanoid synthesis. Glucocorticoids are thought to stimulate lipocortin-1. In patients with RA, autoantibodies to lipocortin-1 have been described. The titers in these patients correlate with the levels of maintenance doses of glucocorticoids, suggesting that these antibodies may lead to glucocorticoid resistance.[47]

Glucocorticoids exert most of their immunosuppressive actions through inhibition of cytokine production; high concentrations of cytokines, especially IL-2, antagonize the suppressive effect of glucocorticoids in a dose-dependent manner.[48] The balance is usually in favor of glucocorticoids, but high local concentrations of cytokines may result in a localized glucocorticoid resistance that cannot be overridden by exogenous glucocorticoids. In addition, macrophage migration inhibitory factor may play a role in glucocorticoid resistance in RA. This pro-inflammatory cytokine is involved in TNF synthesis and T cell activation, suggesting a role in the pathogenesis of RA. Macrophage migration inhibitory factor is suppressed by higher concentrations of glucocorticoids, but it is induced by low concentrations, leading to stimulation of inflammation.[49] Other possible mechanisms of glucocorticoid resistance include activation of mitogen-activated protein kinase pathways by certain cytokines, excessive activation of transcription factor activator protein 1, reduced histone deacetylase-2 expression, and increased P-glycoprotein–mediated drug efflux.[44] Drugs also may play a role in glucocorticoid sensitivity and resistance (see next section, Drug Interactions).

## Drug Interactions

Cytochrome P450 (CYP) is a family of isozymes responsible for the biotransformation of several drugs. Drug interactions can be based on induction or inhibition of these enzymes. Certain drugs (e.g., barbiturates, phenytoin, and rifampin), by inducing CYP isoenzymes (e.g., CYP3A4), increase the metabolism (breakdown) of synthetic and natural glucocorticoids, particularly by enhancing hepatic hydroxylase activity, thus reducing glucocorticoid concentrations (see Figure 60-3). Indeed, rifampin-induced nonresponsiveness to prednisone in inflammatory diseases has been described,[50,51] as has rifampin-induced adrenal crisis in patients receiving glucocorticoid replacement therapy.[52] Clinicians should consider increasing the dosage of glucocorticoids in patients who are concomitantly treated with these medications.

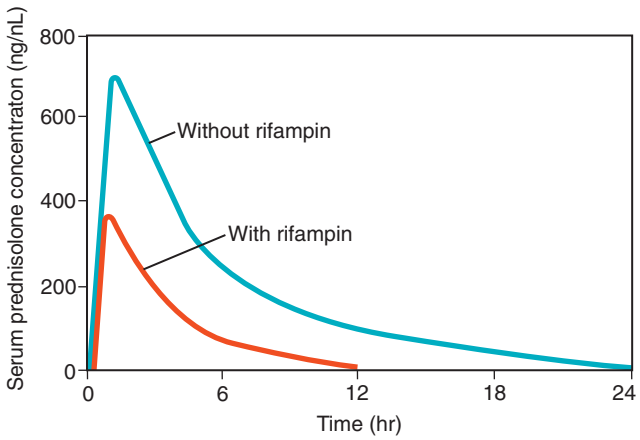



**Figure 60-3** Serum prednisolone concentration in time in one patient, after 0.9 mg/kg oral prednisone daily, in the presence and absence of therapy with rifampin. The *red* curve shows the concentration during a period of continuous administration of both prednisolone and rifampin. The *green* curve shows the concentration after a washout of rifampin of 4 weeks. Rifampin shows a reduced area under the curve, indicating reduced bioavailability of prednisolone during rifampin therapy.

Conversely, concomitant use of glucocorticoids with inhibitors of the drug metabolizing CYP3A4 (e.g., ketoconazole, itraconazole, diltiazem, mibefradil, and grapefruit juice) decreases glucocorticoid clearance and leads to higher concentrations and prolonged biologic half-life of glucocorticoid drugs, thus increasing the risk of adverse effects.[27] On the other hand, antifungal therapies, especially ketoconazole, are known to interfere with endogenous glucocorticoid synthesis and therefore are also used, in doses of 400 to 1200 mg per day, to treat hypercortisolism.[27] Etomidate, a short-acting intravenous anesthetic agent used for the induction of general anesthesia and for sedation, can also lower cortisol levels, which could be clinically relevant in critically ill patients.[27] However, in general, even potent CYP3A4 inhibitors are probably not that important for prednisone and prednisolone metabolism; in addition, the effect of grapefruit juice intake is likely to be of limited clinical significance.[53]

Concomitant administration of prednisolone and cyclosporine may result in increased plasma concentrations of the former drug, whereas concomitant administration of methylprednisolone and cyclosporine may result in increased plasma concentrations of the latter drug. The mechanism involved is probably competitive inhibition of microsomal liver enzymes. Antibiotics such as erythromycin may increase plasma concentrations of glucocorticoids. Synthetic estrogens in oral contraceptives increase the level of transcortin and thus total (the sum of bound and unbound) glucocorticoid levels. Therefore, in women taking oral contraceptives, care is required in the interpretation of cortisol measurements, especially because adrenal insufficiency may be present even if total cortisol levels are within the normal range.[18] Next to glucocorticoids, other steroid drugs such as megestrol acetate and medroxyprogesterone inhibit the HPA axis[27]; this risk may be increased when used concomitantly with glucocorticoids. Sulphasalazine has been reported to increase the sensitivity of immune cells for glucocorticoids,[54] which might be beneficial. Mifepristone is an

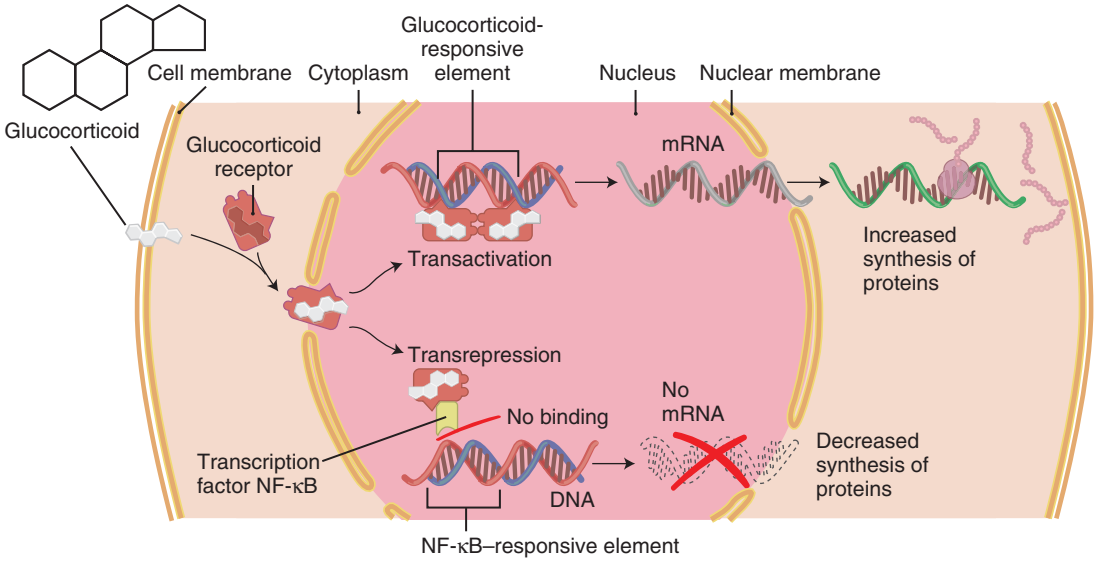



**Figure 60-4** Genomic action of glucocorticoids. Glucocorticoid binds to the glucocorticoid receptor (GCR) in the cytoplasm. This complex migrates into the nucleus. Activation of transcription (transactivation) by binding of GCR-glucocorticoid complex dimers to glucocorticoid-responsive elements of DNA upregulates synthesis of regulatory proteins, thought to be responsible for metabolic effects and also some anti-inflammatory/immunosuppressive effects. Interaction of GCR-glucocorticoid complex monomers with pro-inflammatory transcription factors, such as activator protein-1, interferon regulatory factor-3, and nuclear factor-κB (NF-κB) leads to inhibition of binding of these transcriptional factors to their DNA consensus sites (for NF-κB: NF-κB–responsive elements). Thus the transcription of these pro-inflammatory transcription factors is repressed. This process is called "transrepression" and downregulates synthesis of predominantly inflammatory/immunosuppressive proteins. mRNA, Messenger RNA. *(Modified from Huisman AM, Jacobs JW, Buttgereit F, et al: New developments in glucocorticoid therapy: selective glucocorticoid receptor agonists, nitrosteroids and liposomal glucocorticoids.* Ned Tijdschr Geneeskd *150:476-480, 2006.)*

**TABLE 60-3** Classification of Glucocorticoid Dosages

| | |
|---|---|
| Low dose | ≤7.5 mg prednisone equivalents per day |
| Medium dose | >7.5 mg and ≤30 mg prednisone equivalents per day |
| High dose | >30 mg and ≤100 mg prednisone equivalents per day |
| Very high dose | >100 mg prednisone equivalents per day |
| Pulse therapy | ≥250 mg prednisone equivalents per day for 1 day or a few days |

antiprogesterone drug and glucocorticoid receptor antagonist, and chlorpromazine inhibits glucocorticoid receptor–mediated gene transcription[55]; via these mechanisms, these drugs decrease the effect of glucocorticoids.

## GLUCOCORTICOID THERAPY

Glucocorticoids are widely used in various dosages for several rheumatic diseases. Often it is unclear what is meant by semiquantitative terms used for dosages, such as *low* or *high*. Based on pathophysiologic and pharmacokinetic data, standardization has been proposed to minimize problems in interpretation of these generally used terms (Table 60-3).[6]

### Indications

For each disease, indications for glucocorticoid therapy are discussed in the specific chapters related to the disease. An overview that summarizes only the general use and dosages of glucocorticoids is given here (Table 60-4). Without detailed description, some of the indications and dosages given in Table 60-4 could be considered questionable at first glance. In persons with systemic sclerosis, glucocorticoids, especially in high doses, are contraindicated because of the risk of scleroderma renal crisis, but they may be useful when myositis or interstitial lung disease complicates systemic sclerosis. Glucocorticoids are anchor drugs of the therapeutic strategy in myositis, polymyalgia rheumatica, giant cell arteritis, and systemic vasculitis. For other diseases, glucocorticoids serve as adjunctive therapy or are not used at all. For instance, in persons with RA, glucocorticoids are almost exclusively used as adjunctive therapy in combination with other disease-modifying anti-rheumatic drugs (DMARDs), described later. Glucocorticoids are not given to persons with osteoarthritis with the exception of an intra-articular injection if signs of synovitis of the osteoarthritic joint are present.[56] For generalized soft tissue disorders such as fibromyalgia, glucocorticoids are not indicated, and for localized soft tissue disorders, glucocorticoids should be used only for intralesional injection.[57]

### Glucocorticoid Therapy in Rheumatoid Arthritis

Glucocorticoids are frequently prescribed for persons with RA. Worldwide estimates of the percentages of patients with RA who are treated with glucocorticoids range from 15% to 90%.[58] Aims of this therapy are reduction of signs and symptoms and inhibition of the development of joint damage.

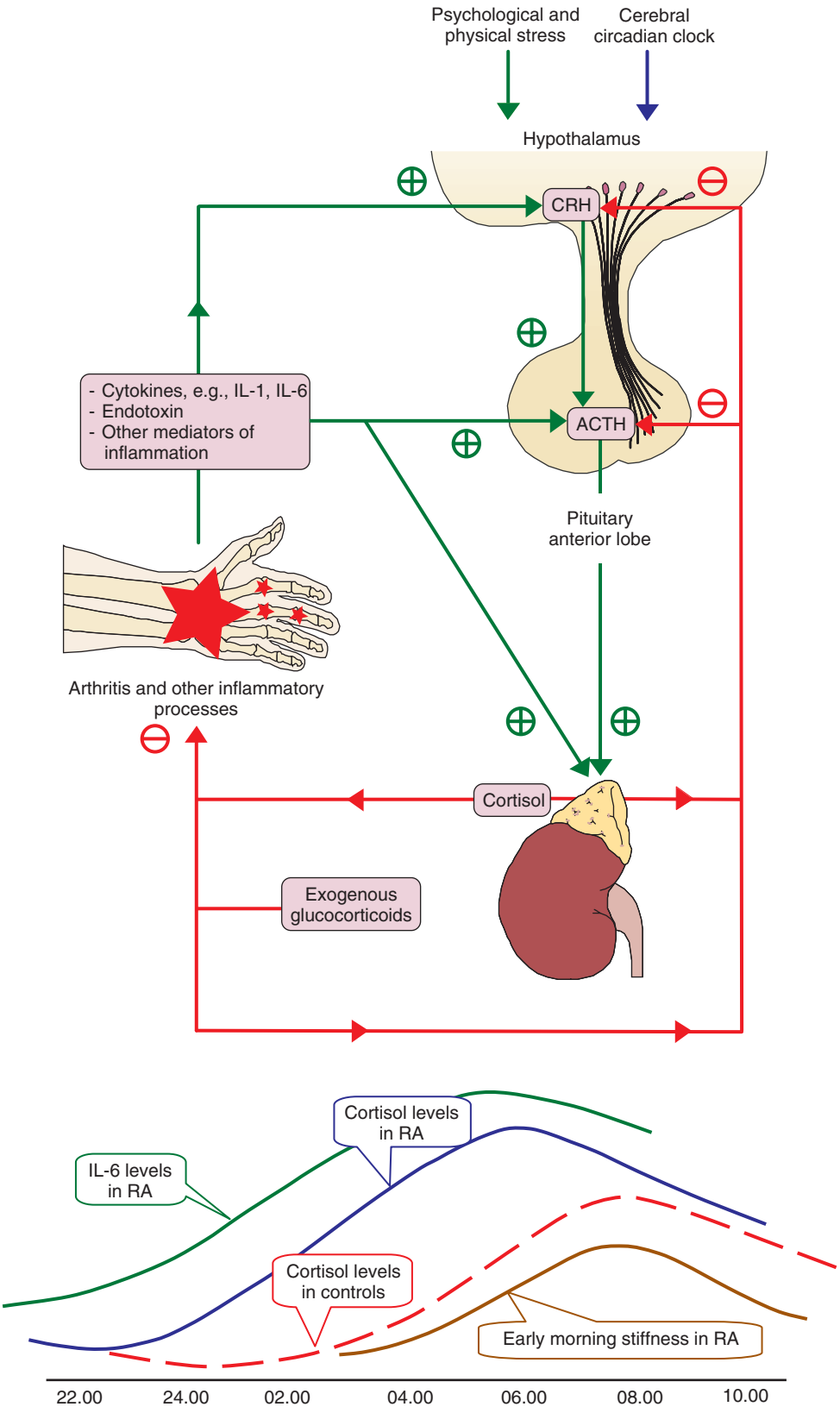



**Figure 60-5** *Upper part,* Stimulation (in *green*, plus signs) and inhibition (in *red*, minus signs) of the hypothalamic-hypopituitary-adrenal axis. *Lower part,* hours on the x-axis; plasma cortisol levels in patients with rheumatoid arthritis (RA) show an earlier and higher circadian rise compared with that in healthy control subjects, possibly caused by the rise in the pro-inflammatory cytokine interleukin (IL)-6; this IL-6 rise is absent in healthy control subjects. IL-6 stimulates the hypothalamus and thus the release of cortisol, but probably also contributes to early morning stiffness and other inflammatory symptoms in (rheumatoid) arthritis. ACTH, Adrenocorticotropic hormone; CRH, corticotropin-releasing hormone.

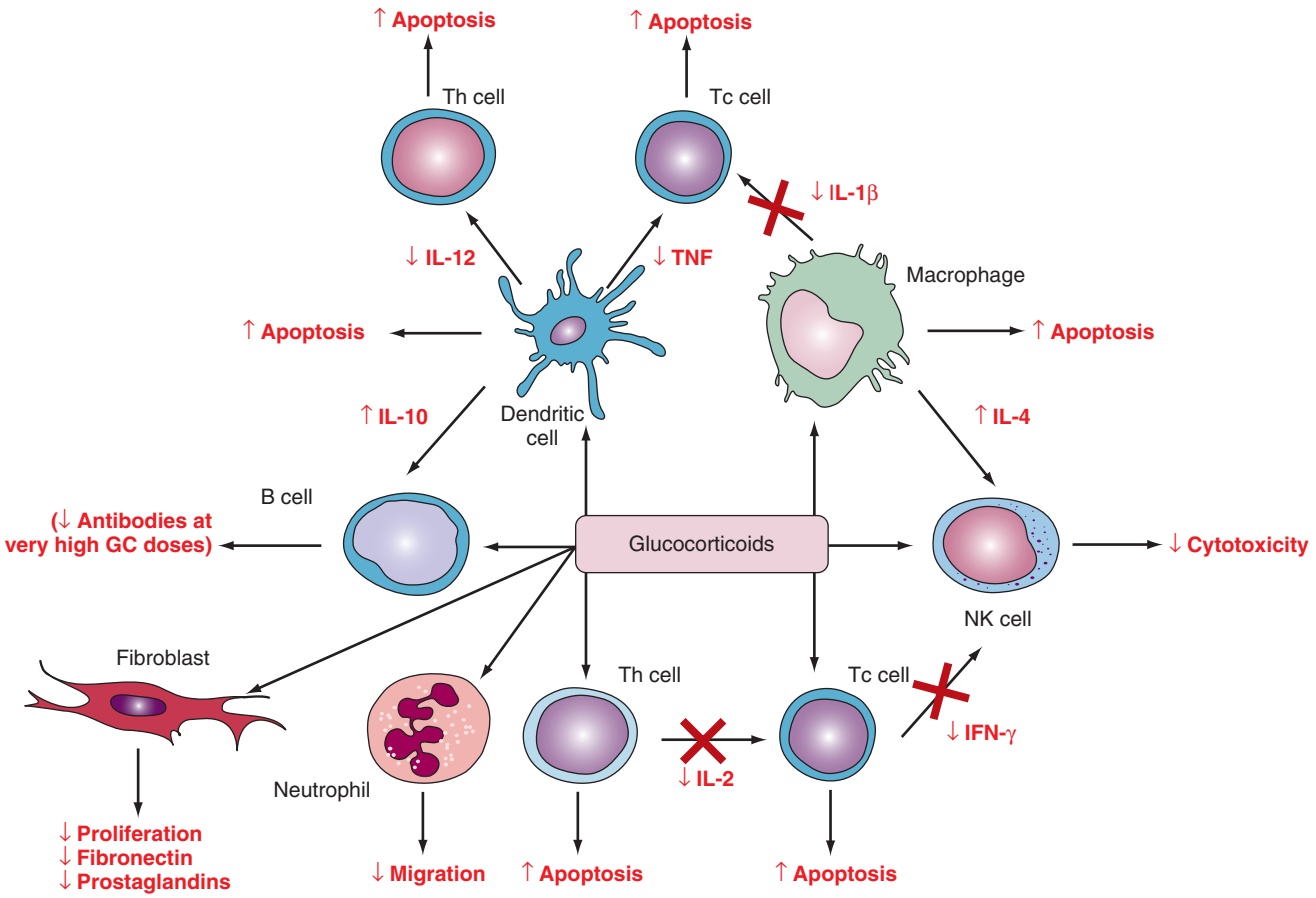



**Figure 60-6** Glucocorticoid (GC) effects (in *red*) on the interplay of inflammatory cells and cytokines. Glucocorticoids act on immune cells both directly and indirectly. The production of pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF), is inhibited and the production of anti-inflammatory cytokines, such as IL-10, by macrophages and dendritic cells is stimulated. Glucocorticoids promote apoptosis of macrophages, dendritic cells, and T cells. All these effects result in inhibition of immune responses. IFN-γ, Interferon-γ; NK cell, natural killer cell; Tc, cytotoxic T cell; Th, T helper cell. *(Modified from Sternberg EM: Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens.* Nat Rev Immunol *6:318-328, 2006.)*

**TABLE 60-4** General Use of Glucocorticoids in Rheumatology, Initial Doses*

| | ORAL | | | Intravenous Very High Dose/Pulse | Intra-articular Injection |
|---|---|---|---|---|---|
| | Low | Medium | High | | |
| **Arthritides** | | | | | |
| Gout, acute | – | 2 | 2 | – | 2 |
| Juvenile idiopathic arthritis | – | 1 | 1 | – | 1 |
| Osteoarthritis | – | – | – | – | 1 |
| Acute CPP crystal arthritis | – | – | – | – | 2 |
| Psoriatic arthritis | – | 1 | – | – | 2 |
| Reactive arthritis | – | – | – | – | 1 |
| Rheumatic fever | – | 1 | 1 | – | – |
| Rheumatoid arthritis | 2 | 2 | 1 | 1 | 2 |
| **Collagen Disorders** | | | | | |
| Dermatomyositis, polymyositis | – | – | 3 | 1 | – |
| Mixed connective tissue disease | – | 1 | – | 1 | 1 |
| Polymyalgia rheumatica | – | 3 | – | 1 | – |
| Sjögren's syndrome, primary | – | – | 1 | – | – |
| Systemic lupus erythematosus | – | 2 | 1 | 1 | – |
| Systemic sclerosis | – | 1 | – | – | – |
| **Systemic Vasculitides, GCA** | – | – | 3 | 1 | – |

*Initial dose is the dose at the start of therapy; it often will be decreased in time depending on disease activity. Doses in prednisone equivalents per day: low, ≤7.5 mg; medium, >7.5 but ≤30 mg; high, >30 but ≤100 mg; very high, >100 mg.

–, Rare use; 1, infrequent use, for therapy-resistant disease, complications, severe flare, major exacerbation, and for bridging the lag time of recently started therapy; 2, frequently added to/used as the basic therapeutic strategy; 3, basic part of the therapeutic strategy.

CCP, Calcium pyrophosphate; GCA, giant cell arteritis.

### Signs and Symptoms

As can be seen in Table 60-4, RA is the only disease in which glucocorticoid therapy is often started and maintained at a low or medium dose as additional therapy. The rationale for this therapy is a probable relative insufficiency of the adrenal gland in patients with active RA.[19] Glucocorticoids are highly effective for relieving symptoms in patients with active RA in doses of less than 10 mg of prednisolone or its equivalent per day. Because of its efficacy, many patients are functionally dependent on this therapy and continue it on a long-term basis.[59] A review of seven studies (including 253 patients) concluded that glucocorticoids, when administered for approximately 6 months, are effective for the treatment of RA.[60] After 6 months of therapy, the beneficial effects of glucocorticoids seem to diminish, but if this therapy is then tapered off and stopped, patients often—especially during the first months—experience aggravation of symptoms, which indicates that the glucocorticoid therapy was effective after all, and which partly can be ascribed to some grade of adrenal insufficiency by persistent suppression of the adrenal gland by the recent glucocorticoid therapy.

### Radiologic Joint Damage: Glucocorticoids as Disease-Modifying Anti-rheumatic Drugs

In 1995, the joint-preserving effects of 7.5 mg of prednisolone daily for 2 years were described in patients with RA of short and intermediate duration who also were treated with DMARDs. The group of patients with RA who participated in this randomized, placebo-controlled trial was heterogeneous, not only with respect to disease duration, but also with respect to stages of the disease and types and dosages of DMARDs.[61] In another trial published in 1997, patients with early phase RA were randomly assigned to step-down therapy with two DMARDs (sulphasalazine and methotrexate) and prednisolone (starting with 60 mg/day, tapered in six weekly steps to 7.5 mg/day, and stopped at 34 weeks) or sulphasalazine alone. For the combined drug strategy, a statistically significant and clinically relevant effect in retarding joint damage was shown compared with the effect of sulfasalazine alone.[62] In an extension of this study, long-term beneficial benefits (up to 11 years) were shown regarding radiologic damage after the combination strategy.[63,64] It has been hypothesized that the superior effect of the combination therapy in this trial can be ascribed to the prednisolone because in three double-blind, randomized trials, the effect of the combination of methotrexate and sulphasalazine was not superior to that of either drug alone.[65-67]

In a German study, 200 patients with early phase RA were treated with methotrexate or intramuscular gold and were randomly assigned for additional treatment with 5 mg of prednisolone or placebo. After 2 years, less progression of radiologic damage occurred in the prednisolone-treated patients than in those treated with placebo.[68] In 2002, the results of the Utrecht study on the effects of 10 mg of prednisolone daily in DMARD-naïve patients with early RA were published. This trial is the only placebo-controlled trial in which prednisolone was applied as DMARD monotherapy as a first step; other DMARDs were added only as rescue medications, which would be considered unethical today. The progression of radiologic joint damage was inhibited by prednisolone in these patients.[69] The Utrecht study reported a 40% decreased need for intra-articular glucocorticoid injections, a 49% decreased need for acetaminophen use, and a 55% decreased need for nonsteroidal anti-inflammatory drugs (NSAIDs) in the prednisolone group compared with the placebo group. These findings indicate that in clinical trials evaluating the clinical effect of DMARDs or glucocorticoids, these additional therapies should be taken into account.[70] In an extension of this study, at 3 years after the end of the 2-year study, after which prednisolone therapy was tapered off and stopped, yearly radiologic progression, albeit substantial, was statistically significantly less in the group that formerly received prednisolone.[71]

In another 2-year study in 250 patients with early phase RA, 7.5 mg/day of prednisolone added to DMARD therapy also retarded joint damage and increased the remission rate compared with placebo added to DMARDs.[72]

Another form of glucocorticoid therapy in RA is the monthly intramuscular application of 120 mg of depot methylprednisolone acetate. However, in contrast to oral glucocorticoids, this treatment showed only a small effect on joint erosion progression at the cost of a significant increase in adverse events.[73]

Studies showing no effect of glucocorticoids on radiologic damage have also been published,[74-76] but in early phase RA, evidence of the joint-sparing effects of glucocorticoids, which persist after therapy is stopped, is convincing, classifying glucocorticoids as DMARDs. A meta-analysis of radiographic outcome analyzed 15 studies of glucocorticoids (two with negative results) that included in total 1414 patients; most studies used a dose of 5 to 10 mg/day of prednisolone or its equivalent for 1 to 2 years. Because different methods had been used in the individual trials, radiographic scores were expressed as a percentage of the maximum possible score for the specific radiographic score method used. The standardized mean difference in progression was 0.40 in favor of strategies using glucocorticoids (95% confidence interval [CI], 0.27 to 0.54). This estimate was considered conservative because the most conservative estimate of the difference in each study had been chosen.[35]

These glucocorticoid studies described above did not have a tight control or treat-to-target design. The primary research question of the second Computer Assisted Management in Early RA clinical trial (CAMERA-II) was whether prednisone would still have disease-modifying properties in early phase RA when added to a highly effective,[77] tightly controlled, methotrexate-based computer-assisted strategy aimed at remission (treat-to-target).[78] Erosive joint damage after 2 years of therapy was limited in both strategy groups, but it was significantly less in the methotrexate-based strategy group that also received prednisone ($n$ = 117), compared with the strategy group that also received placebo ($n$ = 119; Figure 60-7). The strategy with prednisone also yielded more clinical improvement, in the form of earlier suppression of disease activity parameters, earlier sustained remission (after 6 months of therapy compared with after 11 months), and significantly less need to add TNF inhibitor therapy to the strategy to acquire remission. The occurrence of nausea and elevated serum

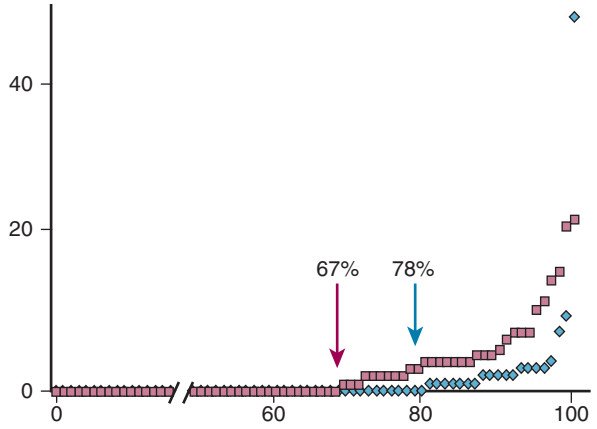



**Figure 60-7** Cumulative probability plots of erosion scores (y-axis: erosion score according to the van der Heijde modification of the Sharp method) at the end of the 2-year second Computer Assisted Management in Early RA clinical trial (CAMERA-II).[76] Each *blue diamond* represents a patient of the methotrexate-based treat-to-target strategy with prednisone, and each *red rectangle* represents a patient of the methotrexate-based treat-to-target strategy with placebo. The plot shows that 78% of patients in the prednisone strategy group versus 67% in the placebo strategy group was still erosion free after 2 years; of those who did have erosions, erosion scores were higher in the placebo strategy group compared with the prednisone strategy group, with exception of the score in one patient, who already had a high score at baseline. The median erosion score was statistically significantly lower in the prednisone strategy group compared with the placebo strategy group.

transaminases was less frequent in the prednisone group compared with the placebo group, probably because doses of methotrexate were significantly lower in the prednisone strategy group, which nevertheless was clinically more effective. The mean (standard deviation [SD]) of maximum dosages of methotrexate (oral or subcutaneous) during the trial was 19.7 (6.1) mg/wk for the methotrexate and prednisone group and 23.4 (4.5) mg/wk for the methotrexate and placebo group ($P$ <0.001). In the prednisone strategy group, mean weight gain after 2 years was 2.9 kg, which was significantly higher than the gain of 1.3 kg in the placebo group. However, in an additional analysis, the extra weight gain in the prednisone group seemed at least partly attributable to a better reduction of weight-loss–inducing disease activity by prednisone, rather than this weight gain representing solely an adverse effect of prednisone.[48] No difference in bone mineral density was found after 2 years between both groups, who received osteoporosis prophylaxis, nor was there a decrease of bone mineral density within each group.[49]

Other studies performed with glucocorticoids in early phase RA also show beneficial results.[79-81] However, because it has not been investigated properly, it is still not known whether glucocorticoids also can inhibit progression of erosions in RA of longer duration than 2 years. A so-called window of opportunity may exist for the treatment of RA.[82] If this window is real, effective treatment of early phase RA with glucocorticoids and other DMARDs results in a positive effect that lasts for a long time and results in disease activity that is easier to control, with less early joint damage. If treatment starts later or is less effective, this opportunity may be lost, resulting in more difficult control of disease activity, because then inflammation is probably also fuelled by joint damage. In addition, because glucocorticoid-induced osteoporosis and peptic ulcer complications (especially if glucocorticoids are combined with NSAIDs) can be prevented effectively, the joint-protective effect of prednisolone in RA during the first 2 years of the disease in a dose of 5 to 10 mg daily is a relevant finding. Long-term adverse effects of glucocorticoids are avoided if prednisolone is tapered and stopped (if possible) after these first 2 years.

The joint-sparing effect of glucocorticoids probably is based on the inhibition of pro-inflammatory cytokines such as IL-1 and TNF,[83] which stimulate osteoblasts and T cells to produce receptor activator of NF-κB (RANK) ligand. This ligand binds to RANK on osteoclast precursor cells and on mature osteoblasts, leading to activation of osteoclasts, which are responsible for bone resorption, periarticular osteopenia, and formation of bone erosions in RA. Furthermore, fibroblast proliferation and IL-1 and TNF-induced metalloproteinase synthesis are inhibited by glucocorticoids, retarding bone and cartilage destruction in inflamed joints of patients with early RA.[34,35,39]

### Prevention of Early Rheumatoid Arthritis Development with Glucocorticoids

Trials with glucocorticoids have been performed to try to prevent arthralgia or early arthritis from progressing to chronic arthritis. In patients who had (very) early phase arthritis or in persons with arthralgia and antibodies to citrullinated proteins or who had a positive rheumatoid factor test, intramuscular glucocorticoid injections did not prevent arthritis development in two placebo-controlled trials,[84,85] but in another placebo-controlled double-blind trial, these injections postponed the need for DMARD therapy and prevented progression into RA in 10% of patients at assessment at 12 months.[86] These results are preliminary and inconclusive, and it is clear that further research is needed.

## Glucocorticoid Pulse Therapy

Glucocorticoid pulse therapy is used in rheumatology, especially for remission induction, treatment of flares of inflammatory rheumatic disorders and vasculitides, and for severe complications of rheumatic diseases, such as visual loss in persons with giant cell arteritis. However, in 144 patients with biopsy-confirmed giant cell arteritis—91 with vision loss upon being seen initially and 53 without vision loss—no evidence was found that intravenous glucocorticoid pulse therapy (usually 150 mg dexamethasone sodium phosphate every 8 hours for 1 to 3 days) was more effective than high and very high doses of oral (80 to 120 mg) prednisone daily in preventing deterioration of vision.[87] In persons with active RA, pulse therapy to induce remission is often applied in the initiation phase of a (new) DMARD strategy to stabilize in the long term the remission induced by the pulse therapy. The beneficial effect of pulse therapy generally lasts about 6 weeks, with a large individual variation in the duration of the effect.[88] For this purpose, pulse therapy with schemes of 1000 mg of methylprednisolone per day intravenously for one or several days has been proven to be effective in many studies. Short-term effects of pulse therapy in patients with *established*, active RA at various dimensions of health status are very similar to long-term effects of effective

**TABLE 60-5** Glucocorticoid Tapering Scheme to Provide to Patients*

| | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday | Sunday |
|---|---|---|---|---|---|---|---|
| Period 1 | High | High | High | High | Low | High | High |
| Period 2 | High | Low | High | High | High | Low | High |
| Period 3 | High | Low | High | Low | High | Low | High |
| Period 4 | Low | High | Low | High | Low | High | Low |
| Period 5 | Low | High | Low | Low | Low | High | Low |
| Period 6 | Low | Low | Low | High | Low | Low | Low |
| Period 7 | Low | Low | Low | Low | Low | Low | Low |
| Duration of each period ____ week(s)† | | | | Low = ____ mg/day† | | High = ____ mg/day† | |

*At each consecutive period, which can last 1 week, 2 weeks, or more weeks, depending on clinical considerations, the number of days per period at which a low dose is taken increases by 1. After completion of period 7, the next step in tapering can be taken; the dose called "low" during the previous seven periods now is "high." In case of aggravation of symptoms, the patient should not diminish the dose and should contact the physician.

†To be filled out by the physician.

conventional DMARD therapy, such as methotrexate, in patients with *early* phase RA.[89]

The risk of adverse effects of pulse therapy is not the same for all rheumatic disorders. Compared with patients with RA, patients with SLE more frequently experience osteonecrosis and psychosis as adverse effects of pulse therapy.[89] Osteonecrosis and psychosis also can be complications of SLE itself, however. Contraindications for pulse therapy are pregnancy and lactation, infections, current peptic ulcer disease, glaucoma, and insufficiently controlled hypertension and diabetes mellitus. In patients with well-controlled hypertension or diabetes mellitus or a family history of glaucoma, pulse therapy can be applied with checks of blood pressure and of blood glucose levels and eye pressure, respectively, before and during high-dose glucocorticoid therapy and pulse therapy.[90]

## Glucocorticoid Tapering Regimens

Because of potential adverse effects, glucocorticoids usually are tapered off as soon as the disease being treated is under control. Tapering must be performed carefully to avoid recurrent activity of the disease and cortisol deficiency resulting from chronic HPA axis suppression. Gradual tapering permits recovery of the adrenal function. There is no best scheme for tapering glucocorticoids based on controlled, comparative studies. Tapering depends on the specific disease being treated; furthermore, it depends on the clinical response and current disease activity and the doses and duration of glucocorticoid therapy, which all are influenced by the individual patient's glucocorticoid sensitivity. Only generic guidelines can be offered. To taper the dose of prednisone, decrements of 5 to 10 mg every 1 to 2 weeks can be applied when the prednisone dose is higher than 40 mg/day, followed by 5 mg decrements every 1 to 2 weeks at doses between 40 and 20 mg/day, and finally 1 to 2.5 mg/day decrements every 2 to 3 weeks at a prednisone dose of less than 20 mg/day. Another scheme is to taper 5 to 10 mg every 1 to 2 weeks down to 30 mg/day of prednisone, and when the dose is less than 20 mg/day, to taper 2.5 to 5 mg every 2 to 4 weeks down to 10 mg/day; thereafter, the dose may be tapered 1 mg each month or 2.5 mg (half a 5 mg tablet of prednisolone) each 7 weeks. For tapering steps over 7 weeks or a multiple number of 7 weeks, a printed schedule can be given to the patient, such as the one shown in Table 60-5, on which the doses and period of tapering should be filled out.

## Stress Regimens and Perioperative Care

Patients taking low-dose glucocorticoid medication on a long-term basis have suppressed adrenal activity and should be advised to double their daily glucocorticoid dose, or increase the dose to 15 mg prednisolone or its equivalent, if they experience fever attributed to infection; they should also seek medical help. In case of major surgery, given the unreliable prediction of adrenal suppression on the basis of the duration and dose of glucocorticoid therapy (see the section in this chapter on the effects of glucocorticoids on the HPA axis), many physicians recommend "stress doses" of glucocorticoids, also for patients with a low risk of adrenal suppression. The scheme of 100 mg of hydrocortisone administered intravenously just before the operation is performed, followed by an additional 100 mg every 6 hours for 3 days, is based on anecdotal information and is not always necessary.[91,92] A scheme with a lower dose, possibly reducing the risk of post-operative bacterial infectious complications, is to continuously infuse 100 mg of hydrocortisone intravenously the first day of surgery, followed by 25 to 50 mg of hydrocortisone every 8 hours for 2 or 3 days. Another option is to administer the usual dose of oral glucocorticoid orally or (the equivalent) parenterally on the day of surgery, followed by 25 to 50 mg of hydrocortisone every 8 hours for 2 or 3 days.

In cases of minor surgery, it is probably sufficient to double the oral dose or to increase the dose to 15 mg of prednisolone or its equivalent for 1 to 3 days. No comparative randomized studies on different perioperative glucocorticoid stress schemes have been published, however. Because aldosterone secretion is preserved in persons with glucocorticoid-induced tertiary adrenal insufficiency, mineralocorticoid therapy is unnecessary, whereas it is necessary in persons with primary adrenal insufficiency.

## Pregnancy and Lactation

In pregnancy, two mechanisms protect the fetus from exogenous glucocorticoids. First, glucocorticoids bound to

transport proteins cannot pass the placenta, in contrast to unbound glucocorticoids. Second, the enzyme 11β-HSD in the placenta, which catalyzes the conversion of active cortisol, corticosterone, and prednisolone into the inactive 11-dehydro-prohormones (cortisone, 11-dehydrocorticosterone, and prednisone), protects the fetus from glucocorticoids in the blood of the mother. The maternal-to-fetal prednisolone blood concentration ratio is about 10:1 as a result of these mechanisms. In contrast, dexamethasone has little or no affinity for transport proteins and is poorly metabolized by 11β-HSD in the placenta; the maternal-to-fetal dexamethasone blood concentration ratio is about 1:1.

If a pregnant woman must be treated with glucocorticoids, then prednisone, prednisolone, and methylprednisolone would be good choices; if the unborn child must be treated—for example, to treat congenital heart block associated with maternal Sjögren's syndrome—then fluorinated glucocorticoids, such as betamethasone or dexamethasone, would be indicated. The risk of adverse effects of antenatal exposure to glucocorticoids such as reduced intrauterine growth and birth weight, neurocognitive adverse effects, and oral cleft seems to be dependent on dose, duration of therapy, and stage of pregnancy. Several studies report conflicting results regarding the occurrence of these adverse effects,[93-95] but doses of glucocorticoid and indications for this therapy differ between studies. Furthermore, it is difficult to discriminate between negative effects and complications of the fetal condition treated and adverse effects of the glucocorticoid therapy. Avoidance of high doses (1 to 2 mg/kg prednisolone-equivalent) in the first trimester of pregnancy is advised,[96,97] whereas low to medium doses of prednisone seem to be safe.[97]

Early postnatal dexamethasone therapy for the prevention or treatment of chronic lung disease has been shown to have negative effects on neuromotor and cognitive function at school age.[98] Prednisolone and prednisone are excreted only in small quantities in breast milk. Breastfeeding is generally considered safe for an infant whose mother is taking these drugs. The exposure of the infant seems further minimized if the infant is breastfed before the mother takes her daily dose, or if breastfeeding is avoided during the first 4 hours after the intake of prednisolone, because curves of milk and serum concentrations of prednisolone are similar in time.[97]

## Intralesional and Intra-articular Glucocorticoid Injections

Injections with glucocorticoids are widely used for arthritis (see Table 60-4), tenosynovitis, bursitis, enthesitis, and compression neuropathies such as carpal tunnel syndrome.[57] Generally, the effect occurs within days; it can be long lasting, but if the underlying disease is active, the effect is of short duration. Administration of a local anesthetic concurrently with intra-articular or soft tissue injection of a glucocorticoid may provide immediate pain relief.

Soluble glucocorticoids (e.g., phosphate salts) have a more rapid onset of action and likely have less risk of subcutaneous tissue atrophy and depigmentation of the skin when given intralesionally. Insoluble glucocorticoids are longer acting and might decrease the soft tissue fibrous matrix to a greater extent than soluble glucocorticoids, so they should be used with caution in places with thin skin, especially in elderly patients and patients with peripheral vascular disease. Administration of insoluble glucocorticoids into deep sites is a safer approach. Short-acting soluble glucocorticoids can be mixed with long-acting insoluble glucocorticoids to combine rapid onset with long-acting effects.

The effect of an intra-articular glucocorticoid injection probably depends on several factors: the underlying disease (e.g., RA or osteoarthritis), characteristics of the treated joint (e.g., size and weight bearing or non–weight bearing), the activity of arthritis, the volume of synovial fluid of the treated joint,[46] the application of arthrocentesis (synovial fluid aspiration) before injection, the choice and dose of the glucocorticoid preparation, the application of rest to the injected joint, and the injection technique. The effect of injections seems to be less favorable in osteoarthritis compared with RA.[99] Arthrocentesis before injecting the glucocorticoid preparation reduces the risk for relapse of arthritis. Triamcinolone hexacetonide, which among the injectable glucocorticoids is the least soluble preparation, demonstrates the longest effect.

Theoretically, rest of the injected joint minimizes leakage of the injected glucocorticoid preparation to the systemic circulation (via the hyperemic, inflamed synovium by enhanced pressure in the joint during activity), minimizes the risk of cartilage damage, and optimizes the condition for repair of inflammatory tissue damage. Advice and procedures for the postinjection period in terms of activity vary from no restrictions, to minimal activity of the injected joint for a couple of days, to bed rest for 24 hours after injection of a knee joint or splinting of injected joints. Based on the literature, no definite evidence-based recommendation can be made, but it seems prudent to rest and not to overuse the injected joint for several days, even if pain is relieved.

It is recommended that intra-articular glucocorticoid injections be repeated no more than once every 3 weeks and be given no more frequently than three times a year in a weight-bearing joint (e.g., the knee) to minimize glucocorticoid-induced joint damage. This recommendation also seems sensible, but there is no definite clinical evidence to support it. As one would expect, accuracy of steroid placement influences the clinical outcome of glucocorticoid injections into the shoulder and probably into other joints as well.[100] This factor is important because it is estimated that a little more than half of shoulder injections are inaccurately placed.[100,101] The reported infection rate of joints after local injections with glucocorticoids is low, ranging from 1 case in 13,900 to 77,300 injections.[102,103] Introduction in the past of disposable needles and syringes has helped to reduce the risk of infection. In a 3-year prospective study in an urban area of 1 million people in the Netherlands, bacterial infections were detected in 214 joints (including 58 joints with a prosthesis or osteosynthetic material) of 186 patients; only 3 of these joint infections were attributed to an intra-articular injection.[104]

Other adverse effects of local glucocorticoid injections are systemic adverse effects of the glucocorticoid, such as disturbance of the menstrual pattern, hot flush–like

symptoms the day of or the day after injection, and hyperglycemia in persons with diabetes mellitus.[57] Local complications include subcutaneous fat tissue atrophy (especially after improper local injection), local depigmentation of the skin, tendon slip and rupture, and lesions to local nerves.[57]

## Improving the Therapeutic Ratio of Glucocorticoids

### Alternate-Day Regimens

For oral, long-term use of glucocorticoid therapy, alternate-day regimens have been devised in an attempt to alleviate adverse effects, such as HPA axis suppression. Alternate-day therapy consists of a single dose administered every other morning, which is usually equivalent to, or somewhat higher than, twice the usual or pre-established daily dose. The rationale for this regimen is that the body, including the HPA, exposed to exogenous glucocorticoid only on alternate days, may recover the other days. This rationale makes sense only for usage of a class and dosage of a glucocorticoid that suppresses the HPA axis activity for less than 36 hours after a single dose. Another prerequisite is that the patient should have a responsive HPA axis that has not been chronically suppressed by previous glucocorticoid regimens. The alternate-day schedule does not work in patients taking medium- or high-dose glucocorticoids on a long-term basis, which suppresses the HPA axis activity for longer than 36 hours.

Alternate-day therapy is unsuccessful in several inflammatory rheumatic diseases. Patients with RA often experience exacerbation of symptoms the day that no glucocorticoid is taken. This experience is in line with the clinical impression that a single dose of glucocorticoids daily is less effective in persons with RA than half that dose, given twice daily. In persons with giant cell arteritis, alternate-day glucocorticoid therapy also is less effective than daily administration.[106,107] Generally, alternate-day regimens are used rarely in rheumatology today, except in patients with juvenile idiopathic arthritis, in whom alternate-day glucocorticoid usage results in less inhibition of body growth than is associated with daily usage.[108] If treatment has been initiated with daily administration, the change to alternate-day therapy preferably should be made after the disease has stabilized.

### Glucocorticoid-Sparing Agents

For several inflammatory rheumatic diseases such as SLE, vasculitis, and myositis, early in the disease, other immunosuppressive drugs such as hydroxychloroquine, methotrexate, and, for systemic vasculitis, cyclophosphamide often are added to therapy with glucocorticoids. For these indications, increasingly, biologic agents also are used.[109] Exceptions are polymyalgia rheumatica and giant cell arteritis, which are managed primarily with glucocorticoids alone. Combination therapy is applied if it is known that the effect of the combination for the specific disease (e.g., systemic vasculitis) is better than that of glucocorticoids alone or if the disease (e.g., inflammatory myositis) seems resistant to the high initial doses of glucocorticoids.

If in a later stage of the disease immunosuppressive drugs are added to therapy with glucocorticoids to enable further reduction of the dose to decrease the risk of adverse effects, these immunosuppressive drugs are termed *glucocorticoid-sparing agents*. Azathioprine and methotrexate are often used for this purpose, although any drug that has an additive or synergistic effect in suppressing the disease activity, thus enabling reduction of the glucocorticoid dose, could be used as a glucocorticoid-sparing agent.

In persons with polymyalgia rheumatica and giant cell arteritis, azathioprine, antimalarial agents, cyclosporine, dapsone, infliximab, adalimumab, leflunomide, and most frequently methotrexate have been tried as glucocorticoid-sparing agents; half of the six randomized trials on methotrexate support its use in this situation.[110-115] A meta-analysis showed a marginal benefit of methotrexate as a glucocorticoid-sparing agent in giant cell arteritis with respect to relapse (relative risk [RR] 0.85; 95% CI, 0.66 to 1.11) and no improved outcome associated with the use of other glucocorticoid-sparing agents.[116]

### Modified-Release Prednisone

The inflammatory process and signs and symptoms of inflammatory rheumatic diseases generally have a diurnal rhythm. Early in the morning, patients experience the most extensive stiffness and other symptoms because of the circadian rhythm of cortisol (see Figure 60-5). In patients with RA who have low or moderate disease activity, serum cortisol maximum and minimum levels shift to earlier times of the day and night, whereas in patients with RA who have high disease activity, the circadian rhythm is markedly reduced or even lost.

The timing of glucocorticoid administration may be important for efficacy and adverse effects. Older data in the literature on this topic were ambiguous.[117,118] More recently, a trial has been performed with a newly developed modified-release prednisone tablet that releases prednisone about 4 hours after ingestion.[119] When it was taken in the evening, thus synchronizing its prednisone release to circadian increases of pro-inflammatory cytokine concentrations, symptoms of RA early in the morning were lessened compared with the symptoms reported when the same dose of prednisone was taken via a normal tablet early in the morning. This 3-month double-blind trial included patients with RA who had a duration of morning stiffness of 45 minutes or longer, a pain score of 30 mm or more on a 100 mm visual analog scale, three or more painful joints, one or more swollen joints, an erythrocyte sedimentation rate (ESR) of 28 mm 1th hour or greater or a C-reactive protein concentration 1.5 times or more the upper limit of normal, and who had been taking glucocorticoids at least 3 months, with a stable daily dose of 2 to 10 mg prednisolone-equivalent for at least 1 month. Patients were randomized in a double-dummy manner to continue their prednisone or to switch to modified-release prednisone. At the end of the trial, the difference in duration of morning stiffness was about 30 minutes in favor of the modified-release prednisone group. No differences were noted in the other clinical variables of disease activity between the two groups. The safety profile did not differ between treatments, and no statistical

difference was found in hypothalamus-pituitary-adrenal axis function.[30,120]

In an open-label observational study among 950 outpatients with RA who were being treated with glucocorticoids and were switching to modified-release prednisone, at assessment 4 months later, improved disease activity was seen.[121] Longer-term benefits and risks of this preparation and application in other inflammatory rheumatic diseases have to be further investigated.[122]

### Other Developments

Deflazacort,[123] an oxazoline derivative of prednisolone introduced in 1969, initially was thought to be as effective as prednisolone while inducing fewer adverse effects, but there is the issue of the real equivalence ratio compared with prednisolone,[124] and deflazacort has not represented a major breakthrough. Knowledge about the mechanisms of glucocorticoids (with the hypothesis that transrepression and transactivation lead, respectively, to predominantly beneficial effects and adverse effects; see earlier) led to the development of selective glucocorticoid receptor agonists.[125] However, the underlying hypothesis was challenged,[15] and the effect of a selective glucocorticoid receptor agonist was disappointing[16]; as yet no such drug has entered the market.

Glucocorticoid preparations releasing nitric oxide, the so-called nitrosteroids, could induce stronger anti-inflammatory effects because nitric oxide also has anti-inflammatory effects.[126] These drugs have yet to be tested in patients.

The drug combination prednisolone and dipyridamole has been reported to boost and extend the net glucocorticoid effect in laboratory models.[127] This synergistic drug, consisting of 3 mg prednisolone and 200 or 400 mg dipyridamole, demonstrated efficacy by statistically reducing pain compared with placebo in persons with osteoarthritis of the hand in a double-blind, placebo-controlled trial and was generally well tolerated.[128] The next required step was to demonstrate an improved therapeutic ratio in patients with other rheumatic diseases in adequate comparative clinical trials by assessing predefined beneficial effects and adverse effects in a standardized manner.[129] After a randomized, double-blind, placebo-controlled, phase IIb clinical trial in RA that showed no meaningful clinical benefit of the synergistic drug compared with prednisolone alone, the development of the synergistic drug has been discontinued.

Liposomes that contain glucocorticoids and are targeted to integrins that are expressed on endothelial cells at sites of inflammation have been studied; these liposomes deliver their glucocorticoids specifically at sites of inflammation.[130] Their selective biodistribution might allow for less frequent and lower dosing, which could result in an improved therapeutic ratio.[131] Clinical studies are needed to see if liposomal glucocorticoids will be effective in clinical rheumatologic practice.

In all, these new glucocorticoid developments, which seemed promising in the past, have not yet lived up to expectations or still must be tested in randomized clinical trials.

Another type of strategy to improve the clinical use of glucocorticoids has been the development of guidelines.[90,132,133]

## ADVERSE EFFECTS AND MONITORING

A toxicity index score for DMARDs based on symptoms, laboratory abnormalities, and hospitalization data of 3000 patients with more than 7300 patient-years from the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) database has been published.[134] Although this score has not been validated and is influenced by confounding-by-indication, it gives an impression of the relative toxicity of glucocorticoids. It is comparable with that of other immunosuppressive medications used in persons with RA, such as methotrexate and azathioprine. A review also showed that the incidence, severity, and impact of adverse effects of low-dose glucocorticoid therapy in RA trials were modest and suggested that many of the well-known adverse effects of glucocorticoids probably are predominantly associated with high-dose treatment.[135]

Because many questions remain to be answered, such as how the effect of glucocorticoids compares with that of high dosages of methotrexate or that of TNF blockers and for how long glucocorticoids should be prescribed and in what dosages, the final role of glucocorticoid therapy in RA has yet to be determined. Presently, guidelines on how to use (low-dose) glucocorticoids and how to monitor this therapy have been developed.[132,133]

Given the diversity of their mechanisms and sites of action, it is not surprising that glucocorticoids can cause a wide array of adverse effects (Table 60-6; Figures 60-8 and 60-9). Most of these adverse effects cannot be avoided. However, the risk of most complications is dosage and time dependent, and minimizing the amount of glucocorticoids minimizes the risk of complications.[132] Dose-related patterns of adverse effects of glucocorticoids have been described.[136] Low-dose glucocorticoid therapy is safer than is commonly thought,[135] and medium- to long-term glucocorticoid therapy in persons with RA is associated with limited toxicity compared with placebo,[137] but sensitivity for

**TABLE 60-6** Common Adverse Effects of Glucocorticoids

| System | Adverse Effect |
|---|---|
| Skeletal | Osteoporosis, osteonecrosis, myopathy |
| Gastrointestinal | Peptic ulcer disease (especially in combination with NSAIDs), pancreatitis, fatty liver |
| Immunologic | Predisposition to infections, suppressed delayed hypersensitivity (tuberculin skin test) |
| Cardiovascular | Fluid retention, hypertension, accelerated arteriosclerosis, arrhythmias |
| Ocular | Glaucoma, cataract |
| Cutaneous | Skin atrophy, striae, ecchymoses, impaired wound healing, acne, buffalo hump, hirsutism |
| Endocrine | Cushingoid appearance, diabetes mellitus, changes in lipid metabolism, enhanced appetite and weight gain, electrolyte abnormalities, HPA axis suppression, suppression of gonadal hormones |
| Behavioral | Insomnia, psychosis, emotional instability, cognitive effects |

HPA, Hypothalamic-pituitary-adrenal; NSAIDs, nonsteroidal anti-inflammatory drugs.

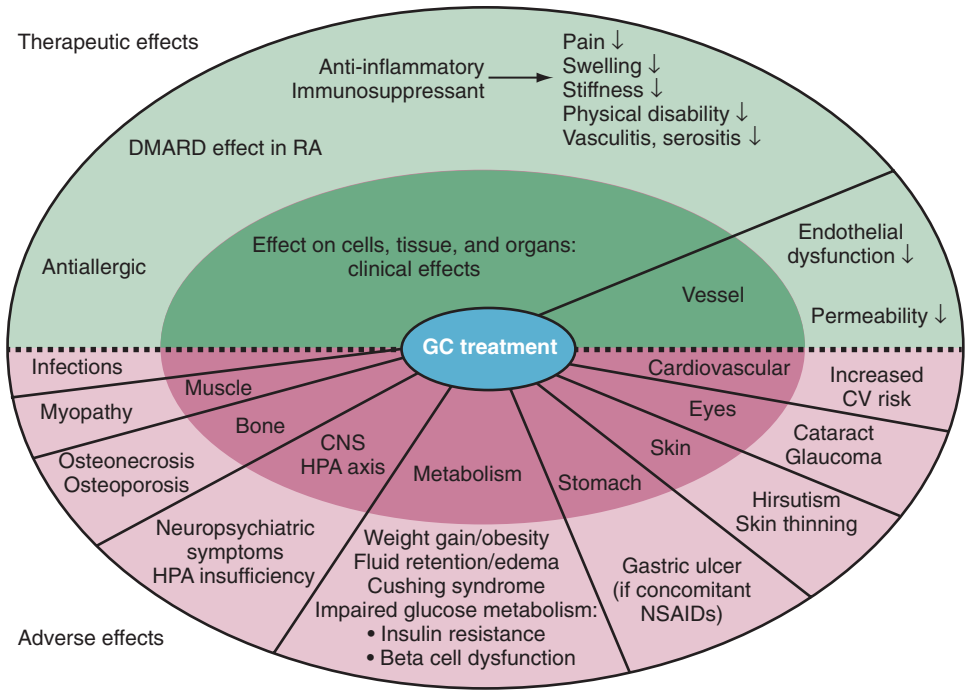



**Figure 60-8** The spectrum of glucocorticoids (GCs) therapy. Beneficial effects are shown in the *green upper part* of the figure, and adverse effects in the *rose-colored lower part*. CNS, Central nervous system; CV, cardiovascular; DMARD, disease-modifying anti-rheumatic drug; HPA, hypothalamic-pituitary-adrenal; NSAIDS, nonsteroidal anti-inflammatory drugs; RA, rheumatoid arthritis. *(Modified from Buttgereit F, Burmester GR, Lipworth BJ: Optimised glucocorticoids therapy: the sharpening of an old spear.* Lancet *365:801-803, 2005.)*

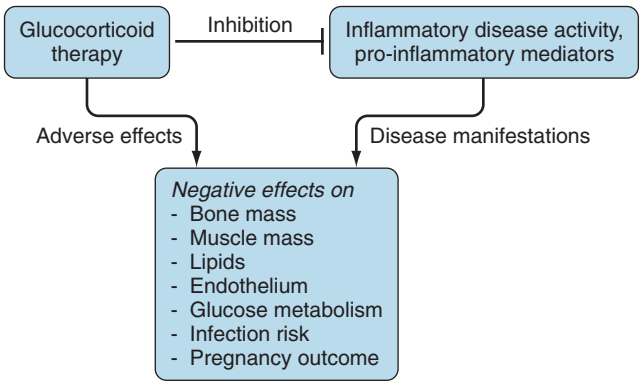



**Figure 60-9** Interplay of glucocorticoid therapy, the inflammatory disease, and negative effects, which can be adverse effects of glucocorticoids and negative effects of the disease itself. Inflammatory diseases have been proven to exert negative effects on bone mass, lipids, endothelium, glucose metabolism and insulin tolerance, infection risk, and pregnancy outcome. These negative effects also are attributed to (especially medium- and high-dose) glucocorticoids. Glucocorticoids suppress the inflammatory disease and thus also these negative disease-related effects.

adverse effects differs for different individuals. Clinical observations indicate some patients experience adverse effects after small doses of glucocorticoids, whereas other patients receive high doses without serious adverse effects. The apparent individual susceptibility to adverse effects does not seem to parallel the individual susceptibility to beneficial effects. Osteoporosis, diabetes, and cardiovascular diseases are ranked among the most worrisome adverse effects of glucocorticoids by both patients and rheumatologists.[138] However, the frequency and severity of glucocorticoid-related adverse effects have seldom been studied systematically. A problem for nonrandomized studies examining glucocorticoid-related adverse effects is bias by indication: Patients with severe disease tend to receive glucocorticoids more frequently than do patients with less severe disease, and both the disease and the glucocorticoids can cause unfavorable signs and symptoms[139]; on the other hand, glucocorticoids decrease disease activity and thus influence the frequency and severity of disease-associated signs and symptoms (see Figure 60-9). Longitudinal data analyses of patients using glucocorticoids at different dosages in time with repeated standardized assessments of disease activity and of other effects would be the best way to disentangle the intricate interaction between glucocorticoids, disease activity, disease-associated signs and symptoms, and glucocorticoid-induced adverse effects.

## Infections

At high doses, glucocorticoids diminish neutrophil phagocytosis and bacterial killing in vitro, whereas in vivo, normal bactericidal and phagocytic activities are found. Monocytes are more susceptible; during treatment with medium to high doses of glucocorticoids, bactericidal and fungicidal activity in vivo and in vitro is reduced. These factors may influence the risk of infection. From epidemiologic studies, treatment with a daily dose of less than 10 mg of prednisolone or its equivalent seems to lead to no risk or only a slightly

increased risk of infection, whereas if doses of 20 to 40 mg daily are used, an increased risk of infection is found (RR 1.3 to 3.6).[140] This risk increases with an increase of the dose and duration of treatment.[141]

In a meta-analysis of 71 trials involving more than 2000 patients with different diseases and who were taking different doses of glucocorticoids, an increased RR of infection of 2 was found. The risk varied according to the type of disease being treated. Five of these trials involved patients with rheumatic diseases and showed no increased risk (RR 1).[140] The same was found in a double-blind, placebo-controlled, 2-year trial in patients with early RA, in which the effect of 10 mg of prednisone daily was compared with that of placebo.[69] In a study, after adjustment for covariates, prednisone use increased the risk of hospitalization for pneumonia in a dose-dependent manner.[141] Another study applying a weighted cumulative-dose model found an elevated risk of serious infections among patients with RA aged 65 years or older, even with chronic low-dose glucocorticoid use; the risk increased with the dosage.[142] In a retrospective RA cohort of patients aged 66 years or older, a clear dose-dependent risk of infections was found, but bias could not be fully excluded, especially because surrogate markers for disease activity had been used as covariates in the analysis.[143] The study suggests, however, that seniors with RA in comparison with younger RA populations may have a higher infection risk related to glucocorticoid use.

Thus in patients treated with glucocorticoids, especially older patients and those who have comorbidities and are taking immunosuppressive co-medications, especially at high doses, clinicians should anticipate infections with usual and unusual organisms because glucocorticoids may blunt classic clinical features and thus delay diagnosis.

## Cardiovascular Adverse Effects

### Mineralocorticoid Effects

Some glucocorticoids also have mineralocorticoid actions (see Table 60-1), including reduced excretion of sodium and chloride and increased excretion of potassium, calcium, and phosphate. This activity may lead to edema, weight gain, increased blood pressure, and heart failure (because of reduced excretion of sodium and chloride); cardiac arrhythmia (because of increased excretion of potassium); or tetany and electrocardiographic changes (as a result of hypocalcemia). Glucocorticoids exhibit no direct effects on kidneys or on renal function.

Low doses of glucocorticoids are not a cause of hypertension, in contrast to higher doses.[144] No formal studies addressing the effects of glucocorticoids in previously hypertensive patients have been reported. Two randomized, controlled studies in patients with myocarditis and idiopathic cardiomyopathy showed no differences between placebo-treated or glucocorticoid-treated groups after 1 year or in survival at 2 and 4 years.[145,146]

### Atherosclerosis and Dyslipidemia

Accelerated atherosclerosis and elevated cardiovascular risk have been reported in patients with SLE, as well as in patients with RA.[147] Glucocorticoids may enhance cardiovascular risk via their potentially deleterious effects on lipids,[148] glucose tolerance, insulin production and resistance, blood pressure, and obesity.[147] Synthetic glucocorticoids have few or no mineralocorticoid effects. In a study, administration of 40 mg prednisone/day did not cause fluid retention, but nevertheless systolic and diastolic blood pressure rose.[149]

However, these risk factors do not seem to be adverse effects of low-dose glucocorticoids. Furthermore, atherosclerosis itself has been recognized as an inflammatory disease of arterial walls,[150] for which glucocorticoids may be beneficial.[151] Glucocorticoids have been found to inhibit macrophage accumulation in injured arterial walls in vitro, possibly resulting in attenuation of the local inflammatory response.[152] Low-dose glucocorticoids might also improve dyslipidemia associated with inflammatory disease.[147,153-155] However, the effects on lipids and other cardiovascular risk factors of low-dose glucocorticoids in inflammatory diseases probably are different from those of medium and high doses of glucocorticoids[148] or of those of glucocorticoid therapy in persons with non-inflammatory diseases. This variation, and the interplay of disease activity, glucocorticoids, and adverse effects (see Figure 60-9), make it difficult to judge the net adverse effects of glucocorticoids on cardiovascular risk and lipids.[156] The finding that a common haplotype of the glucocorticoid receptor gene is associated with heart failure and that this association is partly mediated by low-grade inflammation complicates this issue even more.[157]

In a QUEST-RA study, glucocorticoid use was associated with a decreased risk of cardiovascular morbidity (hazard ratio 0.95; 95% CI, 0.92 to 0.98), but this beneficial effect was smaller than that of other DMARDs.[158] In another study in RA, carotid plaque and arterial incompressibility were associated with glucocorticoid exposure, independently of cardiovascular risk factors and RA clinical manifestations.[159] A literature review showed only a trend of an association in patients with RA between exposure to less than 10 mg/day of prednisone and the risk of major cardiovascular events.[160] In an RA cohort with prospective yearly data assessments, including 779 patients with a total of 7203 patient-years of observation, during which 237 patients died, Cox proportional hazards regression adjusted for potential confounders and for the propensity to receive glucocorticoids was used to assess the hazard ratio of glucocorticoid use for cardiovascular mortality.[161] Compared with no glucocorticoid use, *daily* doses up to 7 mg prednisolone equivalent were not associated with a statistically increased hazard rate; the hazard rate (95% CI) at 8 to 15 mg was 2.3 (1.4 to 3.8), and at 15 mg or more it was 3.2 (1.1 to 9.0). Compared with no glucocorticoid use, *cumulative* doses up to 39 g were not associated with a statistically increased hazard rate; the hazard rate (95% CI) at 40 g or greater was 2.1 (1.3 to 3.3). Compared with no glucocorticoid use, *cumulative* doses *per year* up to 5.07 g were not associated with a statistically increased hazard rate; the hazard rate (95% CI) at 5.08 g or more per year was 2.4 (1.5 to 3.8).

Thus medium and high glucocorticoid doses and long duration of therapy, next to traditional cardiovascular risk factors, including comorbidities such as diabetes mellitus,

inflammatory disease activity, and co-therapies such as COX-2 selective NSAIDs, seem to be the most important cardiovascular risk factors.

## Mortality

In a German biologics register, Cox regression was applied to investigate the impact of time-varying covariates (i.e., disease activity functional disability, treatment with glucocorticoids, and biologic or synthetic DMARDs) on mortality, after adjustment for age, sex, comorbid conditions, and smoking.[162] Disease activity was a clear risk factor for mortality because the risk increased with increasing disease activity. In addition, a dose-dependent risk was seen for glucocorticoid therapy with doses greater than 5 mg prednisone equivalents per day, with an adjusted hazard rate (95% CI) of 1.5 (1.1 to 2.0) for greater than 5 to 10 mg and of 2.0 (1.3 to 3.1) for greater than 10 to 15 mg. Although confounding cannot be fully excluded, similar results have been reported;[163] increased mortality seems to be primarily caused by infections and cardiovascular complications.

## Skeletal Adverse Effects

### Osteoporosis

Osteoporosis is a well-known adverse effect of glucocorticoids; however, the inflammatory disease for which glucocorticoids are given and, especially in inflammatory joint diseases, physical disability and reduced mobility as outcomes of the disease are also risk factors for osteoporosis. Thus glucocorticoids have as adverse-effect, independent of the disease treated, negative effects on bone, but may as therapy for an inflammatory disease have positive effects on bone by suppressing the inflammatory disease and its negative consequences on bone (see Figure 60-10). For instance,

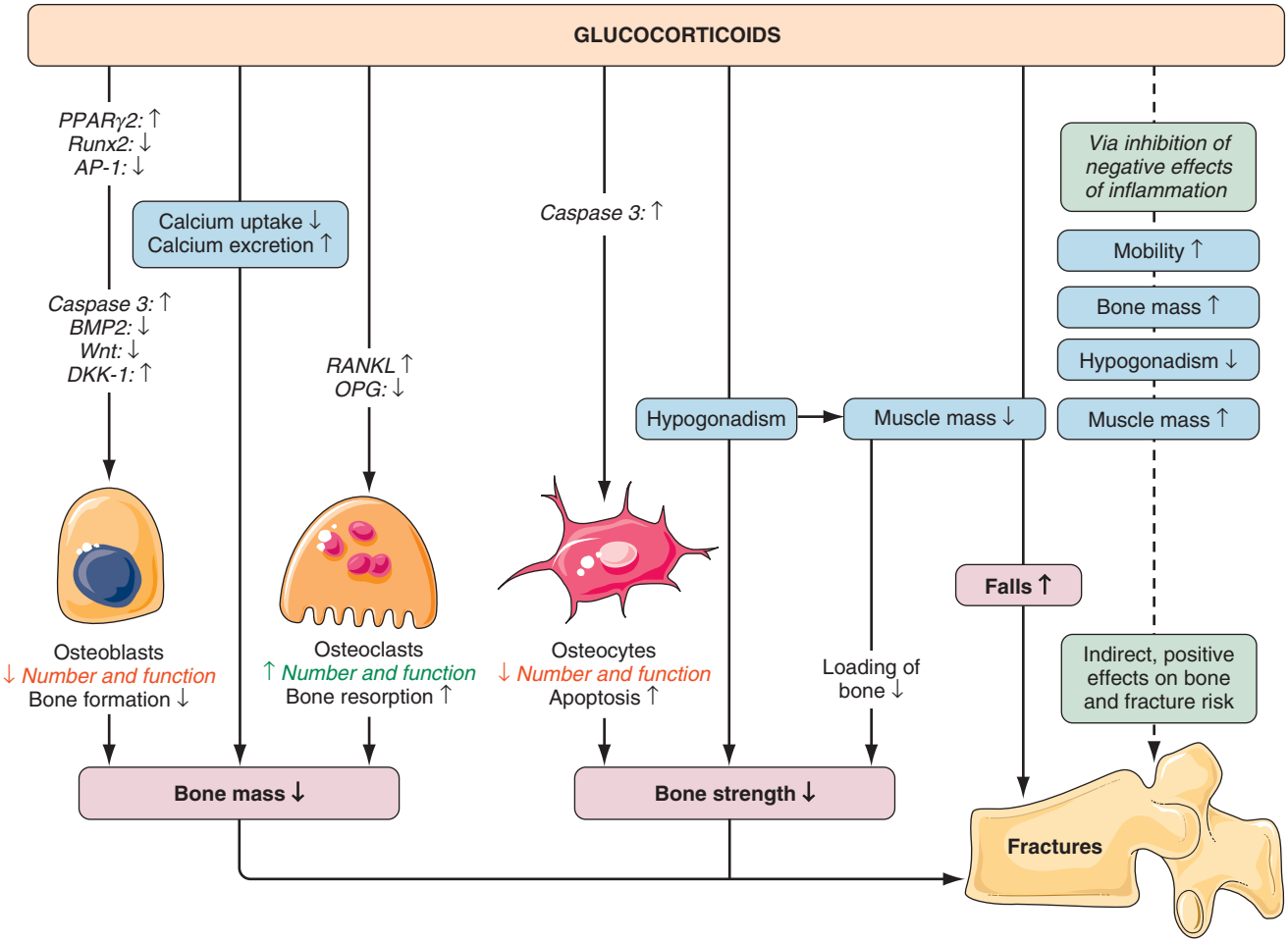



**Figure 60-10** Effects of glucocorticoids on fracture risk: negative effects as adverse effects of this medication and indirect positive effects as therapeutic effects, inhibiting the negative effects of the inflammatory disease.[186-189] AP-1, Activator protein-1 transcription factor complex, including Fos proteins; ↑ increase, stimulation, or upregulation; ↓ decrease, inhibition or downregulation; BMP2, bone morphogenetic protein-2 of the group of BMPs that belong to the transforming growth factor-β superfamily, initiating bone formation; Caspase 3, a critical enzyme for apoptosis and cell survival; DKK-1, dickkopf-1, WNT inhibitor; OPG, osteoprotegerin, the antiosteoclastic decoy receptor for RANKL; PPARγ2, nuclear receptor peroxisome proliferator-activated receptor-γ2 signaling; RANKL, ligand of receptor activator of nuclear factor-κB (RANK), differentiating and activating osteoclasts; Runx2, the Runx2 gene product, stimulating differentiation of mesenchymal cells into osteoblasts; Wnt, Wnt signaling pathway regulating bone homeostasis.

suppressing pro-inflammatory cytokines such as IL-1 and TNF,[83] which stimulate osteoblasts and T cells to produce RANK-ligand, leading to activation of osteoclasts, indirectly has a positive effect on bone mass.

In addition to aromatase inhibitors for breast cancer, androgen deprivation therapy for prostate cancer, and antiepileptic medications,[164] other medications that are associated with a modest increase in fracture risk, especially in elderly persons, and that are not infrequently co-medications of patients taking glucocorticoids, are anti-depressants and proton pump inhibitors (PPIs).[165,166]

The decision whether a patient should be treated depends on fracture risk, effectiveness, safety, and the cost of the treatment. To estimate the risk of fractures for individual patients, several algorithms have been developed, such as the fracture risk in glucocorticoid-induced osteoporosis score (FIGS), which includes the glucocorticoid dosage taken, and fracture risk assessment (FRAX; https://www.shef.ac.uk/FRAX/),[167] for which adjustments also have been suggested for glucocorticoid dosages greater than 7.5 mg prednisone equivalents daily.[168]

To a large degree, osteoporosis can be prevented with calcium and vitamin D supplementation and a biphosphonate if indicated,[169] following national and international guidelines, which are periodically updated.[170,171] Preventive and therapeutic management of glucocorticoid-induced osteoporosis is discussed in another chapter.

### Osteonecrosis

High-dose glucocorticoids for longer periods are implicated as a cause for osteonecrosis, especially in children and patients with SLE. Vascular mechanisms seem to be involved. Ischemia possibly may be caused by microscopic fat emboli or impingement of the sinusoidal vascular bed by increased intraosseous pressure as a result of fat accumulation. An early symptom is diffuse pain, which becomes persistent and increases with activity. Most frequently, hip or knee joints are involved; ankle and shoulder joints are involved less frequently. For early assessment, magnetic resonance imaging is the most sensitive investigation. Radionuclide bone scans give less specific information. Plain radiographs are adequate only for follow-up. Treatment in the early stage includes immobilization and decreased weight bearing. Surgical decompression, joint replacement, or both follow this treatment if needed. There are no preventive measures; awareness is the most important factor in early detection.

### Myopathy

Weakness in proximal muscles, especially of the lower extremities, occurring within weeks to months after the onset of treatment with glucocorticoids or after an increase in the dosage may indicate steroid myopathy. It is often suspected but infrequently found; it occurs almost exclusively in patients treated with high dosages (>30 mg/day prednisolone or its equivalent). Diagnosis is clinical and can be confirmed by a muscle biopsy specimen that reveals atrophy of type II fibers and lack of inflammation; serum muscle enzymes are not elevated. Treatment is withdrawal of the glucocorticoid, if possible, and quite often a prompt decrease in symptoms occurs after withdrawal of the drug. A rare syndrome of rapid-onset, acute myopathy, occurring within days after the start of high-dose glucocorticoids or pulse therapy, has been described; muscle biopsy specimens show atrophy and necrosis of all muscle fibers.

## Gastrointestinal Adverse Effects

### Peptic Ulcer Disease

Data from the literature on upper gastrointestinal safety of oral glucocorticoids are inconclusive. The fact that glucocorticoids inhibit the production of COX-2 without hampering the production of COX-1 supports studies that found no increased risk. In other studies, a RR of serious upper gastrointestinal peptic complications of about 2 was found.[172] When a glucocorticoid is used in combination with an NSAID, the RR of peptic ulcer disease and associated complications is about 4.[173] Thus, in case of co-medication with an NSAID, one should consider co-treatment with a PPI or a switch to a COX-1–sparing NSAID[135] if there is not a high risk of cardiovascular disease (clearly, an inflammatory disease is a moderate risk factor for cardiovascular disease in its own right[150]). In patients treated with glucocorticoids without concomitant use of NSAIDs, there is no indication for gastrointestinal protective agents unless other risk factors for peptic complications are present.

### Other Gastrointestinal Adverse Effects

Although glucocorticoids usually are listed as one of the many potential causes of pancreatitis, evidence for such an association is weak and difficult to separate from the underlying disease, such as vasculitis or SLE.[174] The risk of asymptomatic and symptomatic colonization of the upper gastrointestinal tract with *Candida albicans* is increased in patients treated with glucocorticoids, especially when other risk factors are present, such as advanced age, diabetes mellitus, and concomitant use of other immunosuppressive agents. Glucocorticoids may mask symptoms and signs usually associated with the occurrence of intra-abdominal complications, such as perforation of the intestine and peritonitis, and can lead to a delay in diagnosis with increased morbidity and mortality.

## Ocular Adverse Effects

### Cataract

Glucocorticoids especially tend to stimulate the formation of posterior subcapsular cataract,[175] but the risk of cortical cataract also seems to be increased, with an odds ratio (OR) of 2.6.[176] To some extent, the likelihood or severity of this adverse effect depends on the dose and duration of treatment. In patients treated with glucocorticoids on a long-term basis at a dosage of 15 mg or more of prednisone daily for 1 year, cataract is observed frequently; in patients receiving long-term therapy with less than 10 mg of prednisone daily, the percentage of cataract is less, but cataract may develop at dosages of greater than 5 mg/day of prednisolone equivalent.[136] These cataracts are usually bilateral but

progress slowly. They may cause glare disturbance but usually cause little visual impairment except for end stages.

### Glaucoma

By increasing intraocular pressure, glucocorticoids may cause or aggravate glaucoma. Patients with a family history of open-angle glaucoma are probably prone to the development of this adverse effect, as are patients receiving high doses of glucocorticoids; checks of intraocular pressure are warranted. If ocular pressure is increased, patients need to be treated with medications that reduce this pressure, often for a prolonged period after stopping the glucocorticoid.[177] Topical application of a glucocorticoid in the eye has a more pronounced effect on intraocular pressure compared with systemic glucocorticoid therapy.[178]

## Dermal Adverse Effects

Clinically relevant adverse effects of glucocorticoids on skin include cushingoid appearance, easy bruising, ecchymoses, skin atrophy, striae, disturbed wound healing, acne, perioral dermatitis, hyperpigmentation, facial redness, mild hirsutism, and thinning of scalp hair. Physicians often consider these changes to be of minor clinical importance, but they may be disturbing to patients.[138] No reliable data on the exact frequency of these adverse effects are available, but they are dependent of the duration of therapy and the dose.[136] Many physicians immediately recognize the skin of a patient who has been taking glucocorticoids on a long-term basis.

## Endocrine Adverse Effects

### Glucose Intolerance and Diabetes Mellitus

Glucocorticoids increase hepatic glucose production and induce insulin resistance by inhibiting insulin-stimulated glucose uptake and metabolism by peripheral tissues. Glucocorticoids probably also have a direct effect on the beta cells of the pancreas, resulting in enhanced insulin secretion during glucocorticoid therapy. It may take only a few weeks before glucocorticoid-induced hyperglycemia occurs in persons taking low and medium glucocorticoid doses. In previously nondiabetic subjects, one case-controlled, population-based study suggested an OR of 1.8 for the need to initiate antihyperglycemic drugs during glucocorticoid therapy with doses of 10 mg or less of prednisolone or equivalent per day. This risk increased with higher daily doses of glucocorticoids. The OR was 3 for 10 to 20 mg, 5.8 for 20 to 30 mg, and 10.3 for 30 mg or more of prednisolone or its equivalent per day.[179]

It is likely that the risk is increased further in patients with other risk factors for diabetes mellitus, such as a family history of the disease, advanced age, obesity, and previous gestational diabetes. Postprandial hyperglycemia and only mildly elevated fasting glucose concentrations are characteristic of glucocorticoid-induced diabetes mellitus. Worsening of glycemic control in patients with established glucose intolerance or diabetes mellitus can be expected. Glucocorticoid-induced diabetes usually is reversible when the drug is discontinued, unless the patient had pre-existent clear glucose intolerance.

### Increased Body Weight and Altered Fat Redistribution

Weight gain is an adverse effect of long-term glucocorticoid therapy that is of concern for patients and rheumatologists.[138] Weight gain is due to increased appetite and alterations in fat and glucose metabolism, resulting in an increase of total body and trunk fat. However, weight gain associated with low-dose glucocorticoid therapy for inflammatory diseases seems minor[68,69] and might at least partly also be due to the effectiveness of treatment because active disease has been reported to induce weight loss, possibly as a result of cytokine effects and loss of appetite. In the CAMERA-II trial,[78] the extra weight gain in the prednisone strategy group seemed at least partly attributable to reduction of weight-loss–inducing disease activity, rather than being simply an adverse effect of prednisone.[48] Weight gain in patients with RA who are taking anti-TNF agents seems to be attributable to the same mechanism of efficient suppression of disease activity mechanisms.[180]

One of the most notable effects of long-term endogenous or exogenous glucocorticoid excess is the redistribution of body fat. A centripetal fat accumulation with thin extremities is a characteristic feature of patients exposed to long-term, high-dose glucocorticoids. Potential mechanisms include increased conversion of cortisone to cortisol in visceral adipocytes, hyperinsulinemia, and a change in expression and activity of adipocyte-derived hormones and cytokines, such as leptin and TNF.[181] Protein loss resulting in muscle atrophy also contributes to the change in body appearance.

### Suppression of the Hypothalamic-Pituitary-Adrenal Axis

In the section on effects of glucocorticoids on the HPA axis, mechanisms of chronic suppression of the HPA axis by administration of exogenous glucocorticoids are described. In such a situation, acute discontinuation of glucocorticoid therapy may lead to acute adrenal insufficiency with possible circulatory collapse and death.[27,182] About 10 years after glucocorticoid therapy became available, the first well-documented case of adrenal insufficiency after withdrawal of exogenous glucocorticoid was reported.[183] Acute cessation of glucocorticoid therapy without tapering is indicated for corneal ulceration by herpes virus, which can lead rapidly to perforation of the cornea, and glucocorticoid-induced acute psychosis. In these patients, assessment of the adrenal responsiveness on a corticotropin test seems prudent. Not all patients with a blunted cortisol response have signs or symptoms of adrenal insufficiency, however.

Clinical signs and symptoms of chronic adrenal hypofunctioning are nonspecific and include fatigue and weakness, lethargy, orthostatic hypotension, nausea, loss of appetite, vomiting, diarrhea, arthralgia, and myalgia. These symptoms partially overlap glucocorticoid withdrawal symptoms, such as fatigue, arthralgia, and myalgia. When in doubt, measurements of serum cortisol levels and the corticotropin stimulation test are indicated. The glucocorticoid withdrawal symptoms also are sometimes difficult to discriminate from the symptoms of the primary disease, such as polymyalgia rheumatica. Because mineralocorticoid secretion remains intact via the renin-angiotensin-aldosterone

axis, electrolyte disturbances such as hypokalemia are uncommon.

## Adverse Behavioral Effects

Glucocorticoid treatment is associated with a variety of behavioral symptoms. Although most attention has been directed toward specific dramatic disturbances collectively described under the term *glucocorticoid psychosis*, less florid effects also occur that may cause distress to a patient and warrant medical attention.[138] Minor behavioral manifestations may also occur upon withdrawal of glucocorticoids.

### Steroid Psychosis

Overt psychosis is rare and usually is associated with high-dose glucocorticoids or glucocorticoid pulse therapy, but psychosis also may be a complication of the disease itself, especially SLE. This situation makes it difficult to distinguish whether psychosis in an individual patient with SLE is a complication of the disease, the therapy, or both.

Isolated psychosis represents about 10% of glucocorticoid-related cases, but in most patients, affective disorders are present as well. Around 40% of cases of glucocorticoid-induced psychosis manifest as depression, whereas mania, often dominated by irritability, is predominant in 30% of cases.[184] Psychotic symptoms usually start just after initiation of treatment (60% within the first 2 weeks and 90% within the first 6 weeks), and remission after drug dose reduction or withdrawal follows the same pattern. Although the data are largely anecdotal, persons experiencing steroid psychosis frequently have had prior evidence of some dissociative symptoms. Occasionally, remission occurs without dose reduction.

### Minor Mood Disturbances

Glucocorticoids have been associated with a wide variety of low-grade disturbances, such as depressed or elated mood (euphoria), insomnia, irritability, emotional instability, anxiety, memory failure, and other cognition impairments. Although the symptoms may not become severe enough for a specific diagnosis, they warrant attention—not only because they cause distress to the patient, but also because they may interfere with evaluation and treatment of the underlying disease. Most physicians recognize the occurrence of such symptoms in many patients treated with glucocorticoids; these symptoms may occur in varying degrees in up to 50% of treated patients within the first week. The exact incidence in patients with rheumatic disease who are exposed to the usual doses of glucocorticoids is unknown; most series dedicated to mood disturbances studied high doses.[185] It is important to inform patients about these minor mood disturbances before starting glucocorticoid therapy.[138]

## Monitoring

Glucocorticoid-related adverse effects have seldom been studied systematically. Mostly on the basis of expert and patient opinion, recommendations have been formulated to monitor low-dose glucocorticoid therapy. The conclusion is that, in daily practice, standard care monitoring for serious diseases warranting glucocorticoid therapy need not be extended for patients undergoing low-dose glucocorticoid therapy, except for osteoporosis (for which national guidelines should be followed) and baseline assessments of fasting blood glucose and risk factors for glaucoma, next to a baseline check for ankle edema.[133] Of course, monitoring should be extended for medium and high dosages, not only to monitor for adverse effects of the glucocorticoid therapy, but also for adverse effects of the concomitant medication and complications of the severe disease. For these glucocorticoid dosages, no monitoring guidelines yet exist, but there are recommendations on the management of medium to high-dose glucocorticoid therapy in rheumatic diseases.[90] In these situations, next to good clinical practice monitoring including, for instance, blood pressure measurements, checks of ocular pressure and urine glucose in particular seem to be indicated. When performing clinical trials on glucocorticoids, it is advised that these drugs be monitored and reported in a more comprehensive manner and that more data on the spectrum, incidence, and severity of adverse events of glucocorticoids be sampled.[133] When applied prudently, glucocorticoids are still one of the most relevant therapeutic tools in clinical medicine of the 21st century.

# FUTURE DIRECTIONS

Although biologic therapies are applied frequently in rheumatology, they have not replaced—and in the near future will not replace—glucocorticoids as anchor drugs in therapeutic strategies for autoimmune and inflammatory diseases and vasculitides. In contrast to their established use, there is a paucity of data on the spectrum, incidence, and severity of the adverse effects of glucocorticoids at different dosages and in different diseases, and collection of more data is needed.[133] Additional research into molecular mechanisms and genetic developments might in the future lead to new agents and personalized medicine,[4] but it appears that these developments still have a long way to go. With the exception of modified-release prednisone, new glucocorticoid developments that seemed promising in the past have not yet lived up to expectations or still need to be tested in randomized clinical trials.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 61



# Traditional DMARDs: Methotrexate, Leflunomide, Sulfasalazine, Hydroxychloroquine, and Combination Therapies

Amy C. Cannella • James R. O'Dell

**KEY POINTS**

- Methotrexate is one of the most durable and frequently used disease-modifying anti-rheumatic drugs (DMARDs) in monotherapy as well as the cornerstone of combination therapy for rheumatoid arthritis (RA).
- Leflunomide, sulfasalazine, and hydroxychloroquine are effective therapies in RA and are commonly used in combination therapy.
- Although the precise mechanisms of action of the traditional DMARDs are incompletely understood, most have both anti-inflammatory and immunomodulatory actions.
- The choice of DMARD therapy should be tailored to the individual patient, with attention given to age, fertility plans, concomitant medications, and comorbidities.
- Toxicity from DMARD therapy can cause significant morbidity and rarely mortality; thus appropriate dosing and monitoring for toxicity are essential.
- Combination therapy in RA can be more effective than mono-DMARD therapy in groups of patients with early-stage and established RA.
- The appropriate timing and combinations of DMARD therapy in individual patients is still not defined.

## METHOTREXATE

**KEY POINTS**

- An important mechanism of action for methotrexate (MTX) in addition to blocking dihydrofolate reductase is the increased release of of adenosine, which is a potent inhibitor of inflammation.
- MTX is polyglutamated in cells, and this is responsible for its long therapeutic effect.
- The effects of MTX may be enhanced by splitting the dose (within a 12-hour window) when levels greater than 15 mg/wk are used, or by using a subcutaneous route of administration.
- Combined use of folic acid with MTX abrogates some of the side effects of MTX without decreasing its efficacy.
- The dose of MTX must be adjusted for reduced renal function.
- Although rare, MTX pneumonitis is a serious and potentially fatal complication of therapy.

It would be difficult to overstate the importance of methotrexate (MTX) in the contemporary management of rheumatic disease and, in particular, rheumatoid arthritis (RA). Because of its anti-proliferative effects, MTX was introduced more than 50 years ago to treat cancer. During the last quarter century, it has become the disease-modifying anti-rheumatic drug (DMARD) of choice in the treatment of RA and is used to treat many other rheumatic diseases as well.

### Chemical Structure

MTX is a structural analogue of folic acid and has substitutions in the pteridine group and para-aminobenzoic acid structure (Figure 61-1). The structure of folic acid (pteroylglutamic acid) consists of three elements: a multiring pteridine group, linked to a para-aminobenzoic acid, which is connected to a terminal glutamic acid residue.

### Actions of Methotrexate

Because MTX is a folate analogue, it enters cells via a reduced folate carrier (RFC). Leucovorin competes with MTX for uptake by using the same RFC; however, folic acid enters cells via another group of transmembrane receptors called *folate receptors* (FRs).[1] FRs may be upregulated in cells with increased metabolic activity, including synovial macrophages, and serve as a second conduit for MTX influx.[2,3] MTX efflux occurs via members of the adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters, specifically ABCC1-4 and ABCG2.[4] Genetic polymorphisms may affect MTX transporter proteins (influx and efflux) and can result in a variable MTX response and toxicity profile.[4] Furthermore, multidrug-resistance proteins have been identified that transport MTX, folic acid, and leucovorin out of cells, leading to MTX resistance.[5]

Once inside the cell, naturally occurring folates as well as MTX undergo polyglutamation by the enzyme folylpolyglutamyl synthetase (FPGS). Polyglutamation of MTX (MTX-PG) is essential to prevent efflux of MTX, which easily occurs in the monoglutaminated state. MTX-PG has several key inhibitory effects on intra-cellular enzymes, which result in its postulated anti-inflammatory and anti-proliferative (immunosuppressive) mechanisms: (1) inhibition of aminoimidazole carboxamide ribonucleotide (AICAR) transformylase (ATIC) results in increased intracellular and extra-cellular adenosine, (2) inhibition of thymidylate synthetase (TYMS) results in decreased pyrimidine

synthesis, and (3) inhibition of dihydrofolate reductase (DHFR) results in inhibition of transmethylation reactions essential for cellular functioning (Figure 61-2).

Inhibition of ATIC by MTX-PG leads to accumulation of AICAR and, ultimately, to increased levels of adenosine. Three possible mechanisms are postulated and likely work in combination: (1) AICAR inhibition of adenosine monophosphate (AMP) deaminase leads to excess production of adenosine from AMP, (2) AICAR inhibition of adenosine deaminase (ADA) leads to decreased breakdown of adenosine to inosine, and (3) AICAR stimulation of the ecto-5′-nucleotidase converts extra-cellular AMP to adenosine[6-8] (Figure 61-3).

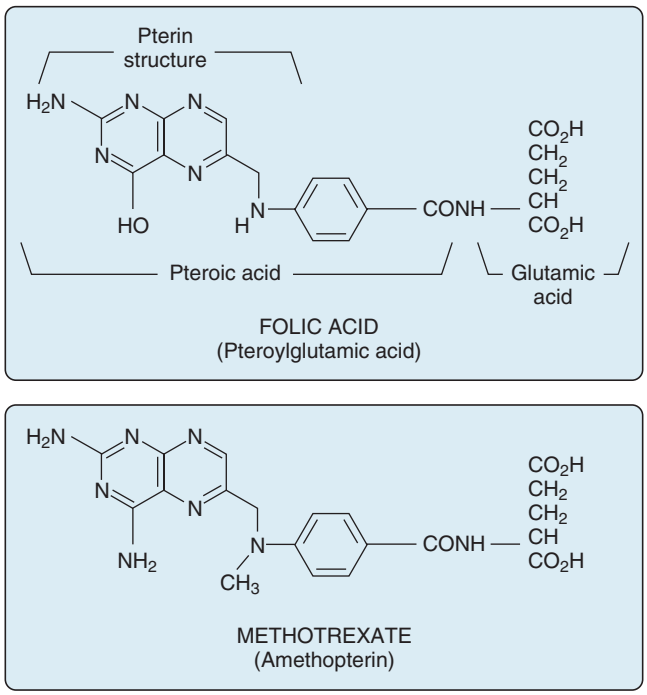



**Figure 61-1** Chemical structure of folic acid and methotrexate.

Adenosine, a purine nucleoside, has been termed a "retaliatory metabolite" because of its tissue protective functions after stressful injurious stimuli.[9] Adenosine, a potent inhibitor of inflammation,[9] induces vasodilation.[10,11] Adenosine's anti-inflammatory effects include regulation of endothelial cell inflammatory functions, including cell trafficking,[10,11] counter-regulation of neutrophils and dendritic cells,[9,12] and cytokine modulation of monocytes and macrophages.[9] Adenosine receptor ligation on monocytes and macrophages suppresses interleukin (IL)-12, a strong pro-inflammatory cytokine.[13] Adenosine also suppresses the pro-inflammatory mediators TNF, IL-6, IL-8, macrophage inflammatory protein (MIP)-1α, leukotriene $(LT)B_4$, and nitric oxide and enhances production of the anti-inflammatory mediators IL-10 and IL-1 receptor antagonist.[14-19] Furthermore, adenosine receptor–mediated processes result in inhibition of the synthesis of collagenase, including tissue inhibitors of metalloproteinases.[20] In sum, adenosine appears to promote a self-limiting, healthy immune response, hastening the transition from neutrophil-mediated inflammation to a more efficient and highly specific dendritic cell–mediated response. Ultimately, adenosine leads to the resolution of inflammation by downregulation of macrophage activation and promotes a shift from a T helper (Th)1 cell to a Th2 cell response.[9]

Evidence that the anti-inflammatory effects of MTX are mediated through adenosine has accumulated in in vitro and in animal studies.[21] However, because of adenosine's short blood half-life of 2 seconds and MTX's long latent period for active metabolites that modulate adenosine, it has been difficult to demonstrate changes in blood adenosine levels directly related to MTX.[22] Recent evidence with use of forearm blood flow as a surrogate marker for

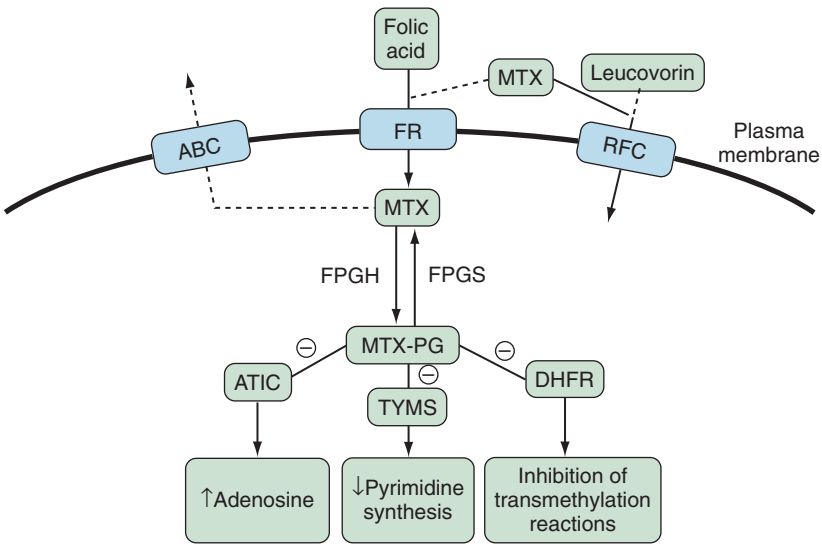



**Figure 61-2** Methotrexate (MTX) enters cells primarily via the reduced folate carrier (RFC) but can use the folate receptor (FR). Once inside the cell, it becomes polyglutamated and can interfere with several cellular enzymes, including 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC), thymidylate synthetase (TYMS), and dihydrofolate reductase (DHFR). ABC, ATP-binding cassette; FPGH, folylpolyglutamate hydrolase; FPGS, folyl-polyglutamyl synthetase; MTX-PG, polyglutamation of MTX.

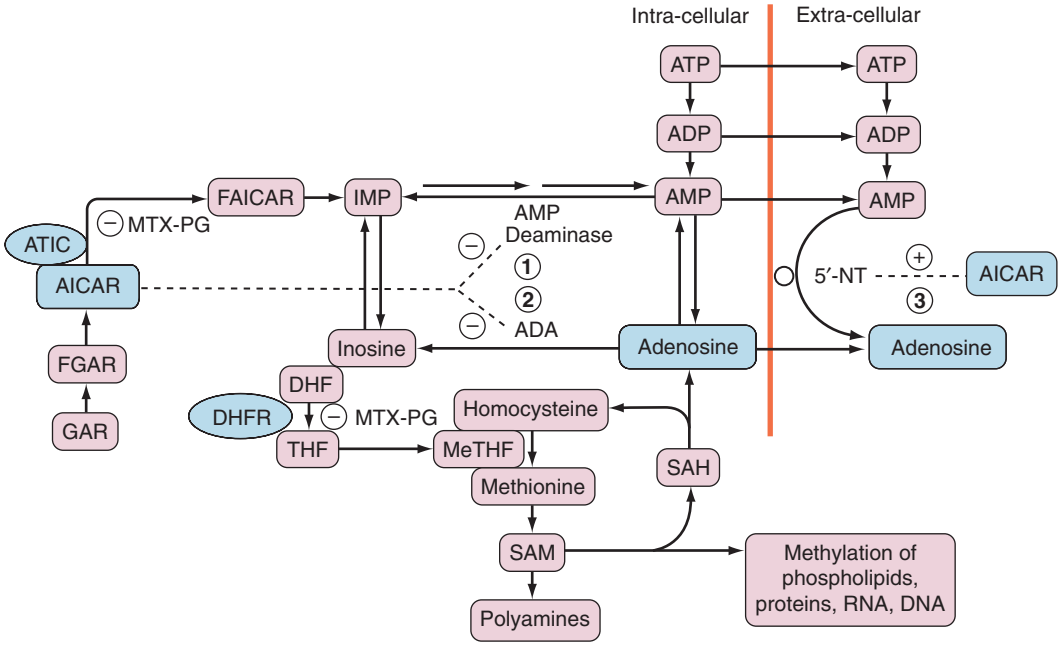



**Figure 61-3** Simplified schema of the effects of polyglutamation of methotrexate (MTX-PG) on intra-cellular and extra-cellular adenosine production and interference with intra-cellular transmethylation reactions. ADA, Adenosine deaminase; ADP, adenosine diphosphate; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMP, adenosine monophosphate; ATIC, aminoimidazole carboxamide ribonucleotide transformylase; ATP, adenosine triphosphate; DHF, dihydrofolate; DHFR, dihydrofolate reductase; FAICAR, formyl-AICAR; FGAR, α-*N*-formylglycinamide ribonucleotide; GAR, β-glycinamide ribonucleotide; IMP, inosine monophosphate; MeTHF, methyltetrahydrofolic acid; 5′NT, 5′-nucleotidase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

adenosine release in patients with RA treated with MTX demonstrated that MTX inhibits deamination of adenosine and potentiates adenosine-induced vasodilation.[23] Demonstration of altered adenosine kinetics in patients treated with MTX coupled with adenosine's known anti-inflammatory effects lends further credence to the hypothesis that MTX increases extra-cellular adenosine, which likely mediates some of the anti-inflammatory effects of MTX.

In addition to vasodilation, adenosine's cardiovascular effects include negative inotropic and chronotropic cardiac effects, inhibition of vascular smooth muscle cell proliferation, pre-synaptic inhibition of sympathetic neurotransmitter release, and inhibition of thrombocyte aggregation.[24] Patients with RA have a higher incidence of cardiovascular disease than the general population.[25] MTX has been suggested to have a preferentially beneficial effect on cardiovascular mortality compared with other DMARDs in RA, and this effect likely occurs via adenosine modulation.[26]

The anti-inflammatory and anti-proliferative effects of MTX may be mediated through its inhibition of transmethylation reactions. Both MTX and MTX-PG inhibit DHFR, resulting in diminution of tetrahydrofolate (THF). THF acts as a proximal methyl donor for several reactions by donating the methyl group for the conversion of homocysteine to methionine. Methionine is then converted to *S*-adenosylmethionine (SAM), which acts as a methyl donor for the following: methylation of RNA, DNA, amino acids, proteins, and phospholipids, and synthesis of the polyamines spermidine and spermine. Upon demethylation of SAM to *S*-adenosylhomocysteine (SAH), SAH is converted to adenosine and homocysteine. Methylation products that are dependent upon SAM, and thus indirectly upon DHFR, to generate THF are required for cellular survival and function, although specific cellular dependence upon each varies[17] (see Figure 61-3).

The role of polyamines deserves further discussion. Spermine and spermidine accumulate in urine,[27] in peripheral blood mononuclear cells,[28] and in synovial fluid and tissue[29] in patients with RA. Metabolism of polyamines by mononuclear cells gives rise to toxic agents, including ammonia and hydrogen peroxide, which may impair lymphocyte function.[30,31] Additionally, accumulation of polyamines in B cells is associated with enhanced production of rheumatoid factor (RF) in vitro, and incubation of these cells with MTX diminishes their ability to secrete both immunoglobulin and RF.[17] These effects are seen with high in vitro concentrations of MTX and may not translate into the in vivo therapeutic effects of MTX in RA.

In addition, MTX inhibits methylation of 2′-deoxyuridylate (dUMP) into 2′-deoxythymidylate (dTMP) by TYMS, resulting in a further mechanism for disruption of DNA synthesis and proliferation of inflammatory cells. This effect has been shown in vitro in human peripheral blood mononuclear cells incubated with low concentrations of MTX.[32] Cell cycle disruption may lead to apoptosis of mononuclear cells via CD95 (APO-1/Fas) ligand-dependent[33] and ligand-independent mechanisms.[34]

Therefore inhibition of transmethylation reactions may lead to MTX efficacy via anti-proliferative and anti-inflammatory mechanisms. Disruption of DNA, RNA,

amino acid, and phospholipid synthesis results in its anti-proliferative effect, which may be mediated via cellular apoptosis. Decreased levels of polyamines may downregulate the production of toxic agents, as well as RF secretion, leading to its anti-inflammatory effect.

In theory, the anti-inflammatory and anti-proliferative properties of MTX already described should make it a potent inhibitor of the immune response that characterizes many rheumatic diseases. Indeed, MTX has become the cornerstone of therapy for RA and is efficacious in multiple other rheumatic diseases. Direct evidence for the immunomodulatory effects of MTX exists, whether studied in in vitro or in vivo systems.

Treatment with MTX modulates monocytic and lymphocytic cytokines and their inhibitors. MTX inhibits pro-inflammatory cytokine IL-1 secretion and to induce the IL-1 receptor antagonist, effectively inhibiting cellular responses to IL-1.[35,36] Soluble TNF receptor (sTNFR p75) synthesis upregulation is also a result of MTX treatment from cultured monoblastic leukemia cells, which results in a diminished TNF inflammatory effect.[37] MTX also inhibits production and secretion of the pro-inflammatory cytokine, IL-6, by cultured human monocytes.[38,39] Reverse transcriptase polymerase chain reaction has been used to study the effects of MTX on gene expression for lymphocytic cytokines.[40,41] MTX increases anti-inflammatory Th2 cytokine (IL-4 and IL-10) gene expression and decreases pro-inflammatory Th1 cytokine (IL-2 and IFN-γ) gene expression in peripheral blood mononuclear cells (PBMCs) of patients with RA.[41]

Prostaglandins (PGs) and LTs are important mediators of joint destruction in RA. MTX modulates the inflammatory enzymes cyclooxygenase (COX) and lipoxygenase (LOX), and their products PG and LT. Thromboxane $B_2$ and prostaglandin $E_2$ activities were reduced in the whole blood of patients with RA treated with MTX when compared with healthy controls.[42] MTX also reduces $LTB_4$ synthesis by neutrophils, resulting in a decrease in total plasma $LTB_4$ levels in patients with RA treated weekly with MTX.[43] In addition to possible direct effects on COX and LOX, MTX exerts an inhibitory effect on neutrophil chemotaxis, which may result in a further reduction of these enzymes in sites of inflammation.[44]

Tissue destruction at sites of inflammation is thought to be related to increased synthesis and activity of proteolytic enzymes released by inflammatory cells, particularly in RA. MTX treatment reduces gene expression of collagenase, metalloproteinase-1, and stromelysin, and to upregulate expression of tissue inhibitor of metalloproteinase (TIMP)-1.[45] MTX may exert direct effects on messenger RNA (mRNA) for certain enzymes, such as collagenase. MTX also likely exerts indirect effects on gene expression via upstream cytokine modulation (IL-1 and IL-6), in the case of matrix metalloproteinase (MMP)-1 and TIMP-1.[46]

## Pharmacology

### Absorption and Bioavailability

At low doses, MTX can be administered either orally or parenterally (subcutaneous [SQ] or IM), and absorption is rapid, peaking at 1 to 2 or 0.1 to 1 hour, respectively. The absorption of low-dose oral MTX (<15 mg/wk) can be variable, and once the oral dose exceeds 15 mg/wk, absorption diminishes by as much as 30%.[47] Absorption is not reduced by concomitant food intake, except for milk, which may be inhibitory,[48] but may be reduced in the setting of intestinal pathology, such as inflammatory bowel disease or malabsorptive conditions.

Orally administered MTX is absorbed via the gastrointestinal (GI) tract and passes through the liver via the portal vein; parenterally administered MTX passes through the liver via the hepatic artery. Parentally administered MTX has higher systemic levels attained compared to orally administered MTX at equal dosing intervals. In addition, the bioavailability of single dose daily oral MTX plateaus at doses of 15 mg/week, whereas parenteral MTX has a linear increase in systemic levels with increasing dosages.[49] Higher systemic levels have not been associated with any increase in toxicity.[49,50] Although not prospectively studied in RA patients receiving long-term MTX treatment, the parenteral route should have diminished potential for hepatotoxicity. This effect has been seen in a retrospective study wherein more elevations in transaminases were noted when oral MTX was administered to the same individuals versus when given parenterally.[51] A recent study at equivalent MTX doses showed improved efficacy in RA clinical endpoints for parenteral versus orally administered MTX.[52]

Forty-one RA patients who received 10 mg/m$^2$ of oral MTX had a mean bioavailability of 70% with a range of 40% to 100%.[53] The mean absorption time was 1.2 hours. Four hours after MTX administration, synovial fluid concentrations equal serum levels.[53] A recent study of high-dose oral MTX (median dose, 30 mg/wk) has shown that mean bioavailability is improved by splitting the dose by 8 hours compared with one single dose (mean bioavailability of 0.9 and 0.76, respectively).[54] The pharmacokinetics of subcutaneous MTX are equivalent to those of intramuscular (IM) MTX; maximum serum concentration is attained within 2 hours of injection by either route.[55] Also, the bioavailability is equivalent between tablets and orally administered parenteral solution.[56]

### Distribution and Half-Life

MTX is 50% to 60% bound to plasma proteins and has a half-life of approximately 6 hours.[53] An increase in free MTX caused by displacement from albumin by more highly protein-bound drugs, such as aspirin, NSAIDs, and sulfonamides, can occur. This is generally of limited clinical significance with low MTX doses because the increase in free MTX is usually only modest.

MTX accumulates in third-space fluids, which can serve as a reservoir for redistribution into the circulation long after the last dose is administered.[57] Caution should be used when administering MTX to patients with pleural effusions or ascites. Furthermore, unexpectedly high levels of MTX have been seen in patients with bladder cancer who have undergone ileal conduit surgery because of enhanced intestinal absorption through the newly fashioned conduit.[58]

The biologically active form of MTX occurs after intracellular polyglutamation (MTX-PG). MTX undergoes up to five polyglutamations, and recent studies have looked at this aspect of MTX pharmacology. Once on a stable dose of

MTX, the median time until 90% of the maximum steady-state concentration of MTX-PG was reached was found to be 27.5 weeks (range, 6.6 to 62.0 weeks).[59]

### Elimination

Most MTX is excreted in the urine within the first 12 hours after administration, except for MTX-PG. MTX undergoes some hepatic metabolism by the enzyme aldehyde oxidase to the 7-hydroxymethotrexate metabolite; this metabolite has unknown significance in RA. MTX and metabolites are excreted by the kidney by glomerular filtration and proximal tubular secretion but also undergo distal tubular reabsorption. The estimated median half-life of elimination of MTX-PG is 3.1 weeks (range, 0.94 to 4.1 weeks), and MTX-PG is undetectable at 15 weeks.[59] $MTX\text{-}PG_3$ is the most common subtype seen (30% of total MTX-PG) and has a median half-life elimination of 4.1 weeks.

### Indications

**Rheumatoid Arthritis.** The efficacy of MTX in RA has been clearly established. Four well-designed, blinded, placebo-controlled trials[60-63] published in 1984 and 1985 had a tremendous impact on the treatment of RA. These trials varied in design and duration: Two of these trials used oral MTX and two used IM MTX, two trials had a crossover and two were parallel, and the duration of treatment varied from 6 to 28 weeks. Although the design and duration of therapy in these trials varied, the conclusions did not, as all showed MTX to be superior to placebo in the short-term treatment of RA. A meta-analysis of these trials by Tugwell and co-workers[64] showed that MTX-treated patients had a 37% greater improvement in swollen joint and tender joint scores, a 39% greater improvement in joint pain, and a 46% greater improvement in morning stiffness. MTX was generally well tolerated in these trials; withdrawal rates ranged from 0 to 32% and were mostly related to minor toxicities (i.e., stomatitis and nausea). Taken together, the results of these trials firmly established MTX as an effective therapy for the treatment of RA, at least in the short term.

Numerous trials have compared MTX with other DMARDs. A meta-analysis done by Felson and co-workers showed that MTX was superior to placebo, auranofin, and probably hydroxychloroquine (HCQ), and was comparable with penicillamine, sulfasalazine (SSZ), and IM gold.[65] No trial has ever suggested that any other synthetic DMARD is superior to MTX.

Accumulating evidence suggests that the short-term benefit of most DMARDs is not sustained, and few patients continue to take these drugs after 3 years.[66,67] MTX appears to have the best durability. Pincus and colleagues have shown that 60% of patients continued MTX at 5 years, compared with less than 25% for penicillamine, gold, HCQ, and azathioprine.[66] Of all the DMARDs, MTX appears to have the best efficacy-to-toxicity ratio.[65] However, despite all the favorable efficacy reports, MTX alone rarely induces remissions of RA, and it has become the cornerstone of combinations of DMARD therapies as discussed later.[68,69]

**Rheumatoid Arthritis–Related Conditions.** MTX has been used successfully in treating Felty's syndrome[70] and the large granular lymphocyte syndrome when it is found in patients with RA.[71] Improvement in neutrophil count occurs within 4 to 8 weeks of MTX initiation in both cases. MTX has been used successfully in adult-onset Still's disease[72] and for the cutaneous vasculitis of RA.[73]

**Juvenile Idiopathic Arthritis.** MTX is efficacious in juvenile idiopathic arthritis. A definitive, randomized, placebo-controlled trial (RCT) demonstrated that MTX at a dose of 10.0 $mg/m^2$ was superior to 5.0 $mg/m^2$ or placebo.[74] Sixty-three percent of children receiving the higher dose (10.0 $mg/m^2$) of MTX improved, compared with 32% in the lower-dose group (5.0 $mg/m^2$) and 36% in the placebo group.

**Psoriatic Arthritis (PsA).** Numerous prospective and retrospective trials have showed a benefit for MTX in PsA.[75] The largest double-blind randomized trial compared weekly oral MTX with placebo and showed statistically significant results only for physician global assessment of arthritis activity and the amount of affected skin surface area; however, this study was small and may have been underpowered to detect differences in joint count, pain, and swelling.[76] Despite the paucity of randomized controlled trial data, MTX remains a commonly used systemic agent in the treatment of PsA.

**Systemic Lupus Erythematosus (SLE).** MTX is efficacious in controlling cutaneous and/or articular manifestations of SLE, particularly in disease resistant to anti-malarials or requiring high doses of systemic steroids.[77] Concomitant folic acid should be administered to abrogate the elevated levels of homocysteine that may be a side effect of MTX therapy and is considered a risk factor for cardiovascular disease in SLE. The role of MTX in treating more severe SLE involvement, including renal, hematologic, or central nervous system (CNS) disease, has not yet been established.[78] Extreme caution should be used in patients with renal disease.

**Vasculitis.** MTX in conjunction with corticosteroids is efficacious in treating early and non–life-threatening granulomatosis with polyangiitis, including upper airway disease and mild renal disease.[79-82] In addition to induction of remission, MTX maintains remission in granulomatosis with polyangiitis, although physician vigilance for patient relapse is warranted.[83] Despite a lack of well-designed studies, MTX has been shown to be efficacious in corticosteroid-resistant Takayasu's arteritis[84] and in relapsing polychondritis.[85]

The use of MTX for polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) has been controversial. Recently, RCTs of MTX in addition to corticosteroids in PMR and GCA have shown conflicting results.[86-89] Thus the routine use of MTX in either PMR or GCA has not been adopted, but some advocate for its use in an effort to more rapidly taper corticosteroid use in patients with intolerable side effects.

**Inflammatory Myopathies.** A review of the published reports of MTX and the inflammatory myopathies polymyositis (PMS) and dermatomyositis (DMS) shows overall positive results.[90] However, despite the frequent use of MTX in the inflammatory myopathies, a recent Cochrane Database Review reveals a paucity of well-designed trials.[91]

**Other Rheumatic Diseases.** MTX has been used in systemic sclerosis. One RCT looking at MTX use in early systemic sclerosis[92] showed a trend in benefit for skin scores

and pulmonary diffusion capacity and a significant benefit for physician global assessment; a second RCT in established systemic sclerosis[93] showed significant benefit for skin scores and total creatinine clearance. In addition, prospective trials have shown that MTX is efficacious in the treatment of corticosteroid-resistant multisystem sarcoidosis,[94,95] and a recent RCT showed that, if initiated early in sarcoidosis, MTX is an effective steroid-sparing agent.[96] MTX is also effective as primary treatment and as a corticosteroid-sparing agent in inflammatory ocular disease.[97,98] Finally, MTX in combination with corticosteroids is effective in the treatment of multicentric reticulohistiocytosis.[99]

## Dose and Drug Administration

MTX is available as 2.5, 5, 7.5, 10, and 15 mg tablets, and as a solution of 25 mg/mL for SQ or IM injection. Recently, the U.S. Food and Drug Administration (FDA) has approved single- and multiple-use, pre-filled autoinjection pens at various dosages.

The starting dose is usually 15 mg given as a single weekly dose, with a range of 5 to 25 mg weekly, and consideration should be given to initiation via the parenteral route for improved efficacy. More frequent administration is associated with a significantly increased risk of liver toxicity.[100] If the oral dose of MTX exceeds 15 mg, consideration should be given to splitting the dose, with each half given 6 to 12 hours apart, for improved absorption and bioavailability. The dosage of MTX can be escalated, usually every 4 to 8 weeks to 25 mg/wk to achieve the desired clinical response. MTX may be administered orally via tablet or parenteral solution; the latter may be less costly. Because its oral bioavailability is variable and decreases at higher doses, parenteral MTX is generally recommended if patients have active disease despite oral doses at approximately 20 mg weekly. In fact, recent data have shown that parenteral MTX is clinically superior to orally administered MTX, and that this may be a preferred route of administration, for initial therapy[101] or if oral MTX is not optimally effective.[52]

Because MTX works by interfering with folate-dependent pathways, a relative folate-deficient state can occur in individuals taking MTX. Concomitant administration of folic acid or folinic acid (<7 mg/week) decreases the frequency of MTX side effects, including GI toxicity (nausea, vomiting, abdominal pain) and stomatitis. Supplementation of folic or folinic acid is protective against elevated transaminase concentrations, results in fewer toxicity-related withdrawals of MTX, and does not interfere with the efficacy of MTX.[102] Folic acid administration also decreases hyperhomocysteinemia in patients on MTX, and this may be important to help decrease the already high cardiovascular risk of patients with RA. There is insufficient evidence to suggest that one form of folate has clinical advantages versus the other, and because folic acid is widely available and less expensive, it has become the preferred agent for most.[102]

Measurement of MTX-PG levels is commercially available ($MTXGlu_n$). It would be valuable to have a marker to predict response and adverse events associated with MTX therapy, but mixed results have been obtained in the search for a trend in the dose-response relationship for $MTXGlu_n$ levels and RA disease activity. A recent study showed no relationship between $MTXGlu_n$ concentration and reduced disease activity in RA.[103] Furthermore, no relationship was identified between $MTXGlu_n$ levels and adverse events. Disease activity was influenced by red blood cell (RBC) folate level, and further study is warranted to determine whether this may serve as a marker for MTX efficacy.

### Geriatric Patients

Patients older than 65 years represent a special subset of patients receiving pharmacotherapy. Pharmacokinetic profiles, including drug distribution, are changed in the elderly as the result of decreases in end-organ blood flow and lean body mass, decreased hepatic drug metabolism, and decreased renal drug excretion. Furthermore, these patients are more likely to have multiple comorbidities, polypharmacy, noncompliance, increased risk for dosage errors, and limited access to medication for financial reasons.[104]

In practice, recommended doses should be reduced when therapy is initiated and should be adjusted for renal function based on creatinine clearance (CrCl).[105] The serum creatinine may be a misleading measure of renal function in older patients because of an overall reduction in lean muscle mass. Dosing recommendations are as follows: initial doses should be approximately 5 to 7.5 mg/wk and should not exceed 20 mg/wk. Dosage adjustments for CrCl are as follows: for a CrCl of 61 to 80 mL/min, reduce the dose by 25%; for a CrCl of 51 to 60 mL/min, reduce the dose by 30%; for a CrCl of 10 to 50 mL/min, reduce the dose by 50% to 80%; and for a CrCl less than 10 mL/min, avoid use[106] (Table 61-1).

### Pediatric Patients

MTX is commonly used in the pediatric population (see Table 61-1). It can be given orally or SQ, with a starting dose of 0.3 to 1 mg/kg/dose once weekly. It is common to start at 0.3 mg/kg/dose and escalate to a dose of 25 mg/wk. It is suggested that at doses greater than 15 mg/wk, parenteral application should be considered because of better bioavailability and tolerability. Guidelines for toxicity monitoring are similar to adult recommendations.

## Toxicity

Despite initial concerns, when given once a week in doses used for rheumatic diseases and monitored correctly, MTX is very well tolerated. Some of the toxicities of MTX (e.g., stomatitis, nausea, and bone marrow depression) are dose dependent, appear to be related to folate deficiency, and respond to folate replacement. Other toxicities (e.g., pneumonitis) appear to be idiosyncratic or allergic and, in most cases, require discontinuation of MTX. Still other toxicities, such as liver fibrosis and cirrhosis, appear to be multifactorial and may depend on the presence of concomitant risk factors, total dose, and frequency of administration.

### Gastrointestinal and Hepatic Side Effects

GI symptoms, including dyspepsia, nausea, and anorexia, are common, occurring in as many as 20% to 70% of patients

**TABLE 61-1** Special Considerations for DMARD Therapy

| | Fertility | Pregnancy | Lactation | Elderly | Pediatrics |
|---|---|---|---|---|---|
| Methotrexate | W: no effect<br>M: reversible sterility<br>Stop 3 mo before conception | Contraindicated<br>FDA category X; abortifacient; teratogenic | Contraindicated; present in breast milk | Lower initial dose (5-7.5 mg/wk); dose based on CrCl | Dosing based on weight |
| Leflunomide | No effect; test levels before conception; may require washout | Contraindicated<br>FDA category X; embryolethal; teratogenic | Contraindicated; unknown concentrations in breast milk | No dosage adjustment required | Dosing based on weight |
| Sulfasalazine | W: no effect<br>M: reversible sterility | Relatively safe;<br>FDA category B, C | Relatively safe; present in breast milk | No dosage adjustment required | Dosing based on weight |
| Hydroxychloroquine | No effect | Relatively safe;<br>FDA category C | Relatively safe; present in breast milk | No dosage adjustment required | Dosing based on weight |

CrCl, Creatinine clearance; FDA, U.S. Food and Drug Administration; M, men; W, women.

within the first year of therapy.[107] These symptoms may be attenuated by adding folic acid or by changing to a parenteral dosing regimen.

The risk of significant liver toxicity appears to be low when MTX is given once weekly to patients who abstain from alcohol and are monitored carefully, and occurs in approximately one patient per 1000 after 5 years of use.[108] However, a recent review of a large North American database of patients with RA and PsA found that elevations in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) greater than 1 times the upper limit of normal (ULN) occurred in 22% and 31% of patients taking MTX or MTX and leflunomide, respectively. Elevations greater than 2 times ULN occurred in 1% to 2% on monotherapy and in greater than 5% on combination therapy. Elevated liver function tests (LFTs) were more likely in patients with PsA.[109] Alcohol consumption, $\alpha_1$–anti-trypsin deficiency, morbid obesity, diabetes, concomitant hepatotoxic drugs, and chronic hepatitis B or C have all been implicated as possible risk factors for MTX toxicity.[110]

### Hematologic Side Effects

Bone marrow toxicity, in most cases, is dose dependent and responds to folic acid administration. Pancytopenia, leukopenia, anemia, and thrombocytopenia can occur, but are rare. In a review by Gutierrez-Urena and associates, clinically significant pancytopenia was found to develop in as many as 1% to 2% of RA patients on MTX therapy.[111] Severe, life-threatening bone marrow toxicity can be treated with folinic acid (leucovorin) and, if necessary, granulocyte-stimulating factor (GSF). Because the elimination of MTX is dependent on the kidney, decreases in renal function may precipitate bone marrow toxicity in patients who have been previously stable. Additional risk factors include hypoalbuminemia, dosing errors, and concomitant use of probenecid or trimethoprim/sulfamethoxazole (TMP/SMX).

### Pulmonary Side Effects

Five clinical pulmonary syndromes have been associated with MTX treatment: acute interstitial pneumonitis (hypersensitivity pneumonitis), interstitial fibrosis, noncardiogenic pulmonary edema (seen in high-dose treatment for malignancy with rare reports in RA), pleuritis and pleural effusions, and pulmonary nodules.[112] Time lapse from initiation of therapy to cumulative dose before the onset of pulmonary toxicity is extremely variable, at 1 to 480 weeks and 7.5 to 3600 mg of MTX, respectively.[112] MTX-induced pulmonary disease is rare and is difficult to quantify, but estimates suggest an incidence of 3.9 cases per 100 patient-years of MTX exposure and a prevalence of 2.1% to 5.5%.[113,114] A recent meta-analysis of MTX and lung disease in RA has shown a modest increased risk of all respiratory events (relative risk [RR], 1.10; 95% confidence interval [CI], 1.02 to 1.19) and respiratory infection (RR, 1.11; 95% CI, 1.02 to 1.21), but not an increased risk for death from lung disease (RR, 1.53; 95% CI, 0.46 to 5.01) or noninfectious respiratory events (RR, 1.02; 95% CI, 0.65 to 1.60), when compared with other DMARDs and biologic agents.[115]

Patients generally are seen with shortness of breath, tachypnea, dry cough, and fever. Chest radiographs most typically show a bilateral interstitial infiltrate (although this finding varies). Infectious causes, including opportunistic organisms, must always be ruled out. If routine evaluations for infection, including sputum studies, and for other medical conditions to explain the pulmonary symptoms are negative, bronchoscopy with bronchoalveolar lavage and transbronchial biopsy is recommended. If MTX pulmonary toxicity is suspected, MTX should be discontinued and supportive treatment initiated with the use of corticosteroids in more severe cases. Some patients with pulmonary toxicity have been successfully restarted on MTX,[116] but clinicians have reported mortality in up to 50% of re-treated patients.[57]

Factors that appear to predispose to MTX lung toxicity include age, blue collar occupation, smoking (in women), diabetes, pleuropulmonary rheumatoid disease, and skin rashes from MTX.[117]

### Mucocutaneous Side Effects

The mucocutaneous toxicities of MTX, which have been reported to occur in up to one-third of patients, are dose dependent and respond to folate replacement. Patients generally report fairly minor oral ulcerations, but severe ulceration of the mouth, esophagus, bowel, and vagina can occur, especially at higher doses.

### Malignancies

The induction of malignancies by MTX is a concern, and several studies have examined this question with conflicting conclusions. Recently, reports of lymphoma in MTX-treated patients with RA have appeared. Because the incidence of lymphoma is already increased in patients with RA,[118] these reports are difficult to interpret. The case for a causative role of MTX has been strengthened, however, because a number of these cases have been B cell lymphomas of the type commonly seen in association with immunosuppression (associated with Epstein-Barr virus) and that may regress after discontinuation of MTX.[119,120] Subsequently, lack of a causal relationship between MTX treatment and the development of lymphoma has been seen in two large series of RA patients—one prospective study[118] and one retrospective study.[121] The potential benefits of MTX for most RA patients thus far outweigh these statistically small risks.[122]

### Miscellaneous

**Methotrexate Flu.** Patients taking MTX may describe flulike symptoms shortly after taking their weekly dose. Nausea, low-grade fevers, myalgias, and chills are the most common signs of the so-called *MTX flu*. These side effects usually respond to supplementation with folic acid, decreasing the dose, switching from oral to parenteral administration, or changing the time of the dose (so that the patient takes MTX right before going to bed).

**Nodulosis.** The development of, or increase in, the number or size of rheumatoid nodules has been reported to occur in patients with RA treated with MTX, with a prevalence of as much as 8%.[123] This may occur in rheumatoid factor–negative patients, and in those in whom the synovitis is under excellent control. The mechanism of this nodule formation has been suggested to be the result of an increase in adenosine, which appears to promote nodule formation.[124] Conversely, nodules have been reported to decrease during MTX therapy.

**Vasculitis.** Despite efficacy in the treatment of the cutaneous vasculitis associated with RA, leukocytoclastic vasculitis has also been attributed to MTX therapy.[125]

### Fertility, Pregnancy, and Lactation

MTX does not seem to adversely affect female fertility but can cause reversible sterility in men.[126] Women and men should discontinue MTX for at least 3 months before attempting to conceive because of its large distribution and long half-life in the liver. Folic acid supplementation is essential before conception. MTX is included in the FDA Pregnancy Category X and is contraindicated during pregnancy. Women of childbearing age who are considered for MTX therapy should receive extensive counseling regarding teratogenic risk and should be placed and maintained on adequate contraception, before therapy is begun. Toxicities include fetal abnormalities such as "aminopterin syndrome" (multiple craniofacial, limb, and CNS abnormalities)[127] and embryonic or fetal loss, and MTX at high doses (1 mg/kg) is an effective abortifacient. MTX is also contraindicated during lactation because small amounts are excreted in breast milk (see Table 61-1).

### Toxicity Monitoring

The American College of Rheumatology (ACR) has recently revised recommendations for the use of DMARDs, and these serve as an excellent resource.[128] Toxicities that require monitoring include myelosuppression, hepatotoxicity, and pulmonary toxicity. Baseline evaluation should include a complete blood count (CBC) with platelets, hepatitis B and C serology in high-risk patients, liver transaminases, and creatinine. Although these guidelines make no recommendations on the need for a baseline chest radiograph, this is a reasonable approach. Liver biopsies are not routinely recommended before MTX is initiated. The rare patients whom one wants to treat with MTX despite abnormalities in screening laboratory or other significant risk factors may require liver biopsy before MTX is initiated. In addition, biopsies are recommended only in those patients who continue to have enzyme abnormalities and for whom continuation of MTX therapy is contemplated.

Monitoring for toxicity should be done every 2 to 12 weeks and is based on the duration of therapy, with more frequent monitoring provided earlier in the course of treatment. Systems review and physical examination should include monitoring for symptoms or signs of myelosuppression (fever, infection, bruising, and bleeding), pulmonary toxicity (shortness of breath, cough, rales), GI intolerance (nausea, vomiting, diarrhea), and lymphadenopathy. Laboratory parameters that should be followed include a CBC with platelets, liver transaminases, and creatinine (Table 61-2).

It is important to consider vaccination status in any patient who is going to use MTX. RA patients have an increased incidence of death from pneumonia,[129] and MTX may reduce the immune response to pneumococcal antigen.[130] Thus any patient in whom MTX is going to be used should first receive the pneumococcal vaccination, with booster as appropriate. Vaccinations for hepatitis B virus for at-risk patients and yearly influenza vaccines are recommended as well. Caution should be exercised when administering live virus vaccinations to patients on MTX.

## Drug Interactions and Contraindications

### Drug Interactions

Drugs that are known hepatotoxins, such as SSZ, leflunomide, and azathioprine, may potentiate liver toxicity when used in combination. Organic acids such as sulfonamides, salicylates, NSAIDs, penicillin G, piperacillin, and probenecid competitively inhibit tubular secretion, and this delays MTX clearance.[131] MTX also undergoes distal tubular reabsorption, which may be enhanced by the addition of hydroxychloroquine (HCQ)[132] and blocked by the addition of folic acid.[131] Drugs that affect renal function should be used with caution because of the renal clearance of MTX and, therefore, the increased risk of MTX toxicity that could occur because of decreased clearance.

**TABLE 61-2** Safety Monitoring

| | Baseline | <3 Months of Drug Therapy | Monitoring Interval 3-6 Months of Drug Therapy | >6 Months of Drug Therapy | Contraindications |
|---|---|---|---|---|---|
| MTX | CBC, LFT, Cr, HBV, HCV; vaccinate: influenza, *Pneumococcus*, HBV | Every 2-4 wk | Every 8-12 wk | Every 8-12 wk | Active infection, symptomatic pulmonary disease, WBC <3000/mm$^3$, Plt <50,000/mL$^3$, CrCl <30 mL/min, history of myelodysplasia or recent lymphoproliferative disorder, LFT >2 × ULN, acute or chronic HBV or HCV, pregnancy, lactation |
| Leflunomide | CBC, LFT, Cr, HBV, HCV; vaccinate: influenza, *Pneumococcus*, HBV | Every 2-4 wk | Every 8-12 wk | Every 8-12 wk | Active infection WBC <3000/mm$^3$, Plt <50,000/mL$^3$, history of myelodysplasia or recent lymphoproliferative disorder, LFT >2 × ULN, acute or chronic HBV or HCV, pregnancy, lactation |
| Sulfasalazine | CBC, LFT, Cr; vaccinate: influenza, *Pneumococcus* | Every 2-4 wk | Every 8-12 wk | Every 8-12 wk | Sulfa allergy, Plt <50,000/mL$^3$, LFT >2 × ULN, acute HBV/HCV, some classes of chronic HBV/HCV |
| Hydroxychloroquine | CBC, LFT, Cr; complete ophthalmologic examination within 1 year | None | None | None | History of vision changes attributed to 4-aminoquinolone derivatives, some classes of untreated HBV/HCV |

*Monitoring at <3, 3-6, and >6 mo need only include CBC, LFT, and Cr.

CBC, Complete blood count; Cr, creatinine; CrCl, creatinine clearance; HBV, hepatitis B; HCV, hepatitis C; LFT, liver function test; Plt, platelets; ULN, upper limit of normal; WBC, white blood cell count.

From Saag K, Geng G, Patkar N: American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying anti-rheumatic drugs in rheumatoid arthritis. *Arthritis Rheum* 59:762–784, 2008.

Several of the aforementioned drugs deserve special mention. TMP-SMX should be avoided or used with extreme caution because of possible hematologic toxicity with MTX. Mechanisms for this toxicity include an additive anti-folate effect from TMP, decreased MTX clearance because of inhibition of tubular secretion by SMX, and altered MTX plasma protein binding. NSAIDs are commonly used in patients with RA as adjunctive therapy. NSAIDs may increase MTX levels by displacing MTX from plasma proteins and limiting tubular secretion. Despite lack of a significant pharmacokinetic or clinical interaction between low-dose MTX and a variety of NSAIDs,[133] vigilance for MTX toxicity should increase whenever NSAID dosages are changed in patients on stable weekly doses of MTX. Low doses of aspirin used for cardiovascular prophylaxis are not likely to be of concern. Furthermore, probenecid should be avoided because it inhibits tubular secretion of MTX.

### Contraindications

MTX should not be used in patients with severe renal, pulmonary, or hepatic impairment, pre-existing bone marrow suppression, alcoholic liver disease, and pregnancy or breastfeeding. Ongoing or active infection is also a contraindication. In most cases, patients who desire to continue drinking alcohol should not be treated with MTX. Mild to moderate renal insufficiency is a relative contraindication, and use of MTX in these patients may require more vigilant toxicity monitoring (Table 61-3).

## LEFLUNOMIDE

**KEY POINTS**

- **Leflunomide reversibly inhibits dihydroorotate dehydrogenase.**
- **Loading doses often are not used in clinical practice because of gastrointestinal toxicity.**
- **Because of enterohepatic recirculation, leflunomide has a very long half-life.**
- **Leflunomide is absolutely contraindicated in pregnancy, and levels must be checked with a washout protocol if needed before conception.**
- **Vigilance must be used for hepatotoxicity.**

Leflunomide, an isoxazole derivative, is a synthetic DMARD approved for the treatment of RA. It emerged from a specific anti-inflammatory drug development program and has potent immunomodulatory effects.

### Chemical Structure

Leflunomide is a low-molecular-weight isoxazole compound and is chemically unrelated to any previous immunosuppressant. Leflunomide is a pro-drug and is rapidly and completely converted to its active metabolite, the malononitriloamide A77 1726. A77 1726 is also known as *teriflunomide* (Figure 61-4).

**TABLE 61-3** Methotrexate, Leflunomide, Sulfasalazine, and Anti-malarials: Summary of Mechanism of Action, Efficacy, and Toxicity

| | Proposed Mechanism of Action | Efficacy | Toxicity |
|---|---|---|---|
| Methotrexate | Inhibition of ATIC→↑ Adenosine<br>Inhibition of TYMS→↓ Pyrimidine synthesis<br>Inhibition of DHFR→↓ Transmethylation reactions | RA<br>LGL/Felty's syndrome<br>JIA<br>PsA<br>SLE<br>Vasculitis | Nausea<br>Hepatotoxicity<br>Bone marrow suppression<br>Pneumonitis<br>MTXflu |
| Leflunomide | Inhibition of DHODH→↓ Pyrimidine synthesis<br>Inhibition of tyrosine kinase→↓ Cell signal transduction | RA<br>SLE<br>PsA | Hepatotoxicity<br>Diarrhea<br>Weight loss |
| Sulfasalazine | Inhibition of arachidonic acid cascade<br>Inhibition of ATIC→↑ Adenosine<br>Multiple cellular effects<br>Systemic effects via MALT | RA<br>JIA<br>Ankylosing spondylitis<br>PsA<br>Reactive arthritis | Nausea<br>Headache<br>Leukopenia<br>Rash |
| Anti-malarials<br>Hydroxychloroquine<br>Chloroquine | ↑ pH of subcellular vesicles→Interference with Ag processing and cell-mediated cytotoxicity | RA<br>SLE<br>Discoid lupus<br>APS<br>Sjögren's syndrome | Nausea<br>Rash<br>Neuromyopathy<br>Retinopathy |

Ag, Antigen; APS, anti-phospholipid syndrome; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase; DHFR, dihydrofolate reductase; DHODH, dihydroorotate dehydrogenase; JIA, juvenile idiopathic arthritis; LGL, large granular lymphocyte; MALT, mucosa-associated lymphoid tissue; MTX, methotrexate; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TYMS, thymidylate synthetase; ↑, increased; ↓, decreased; →, indicates the result.

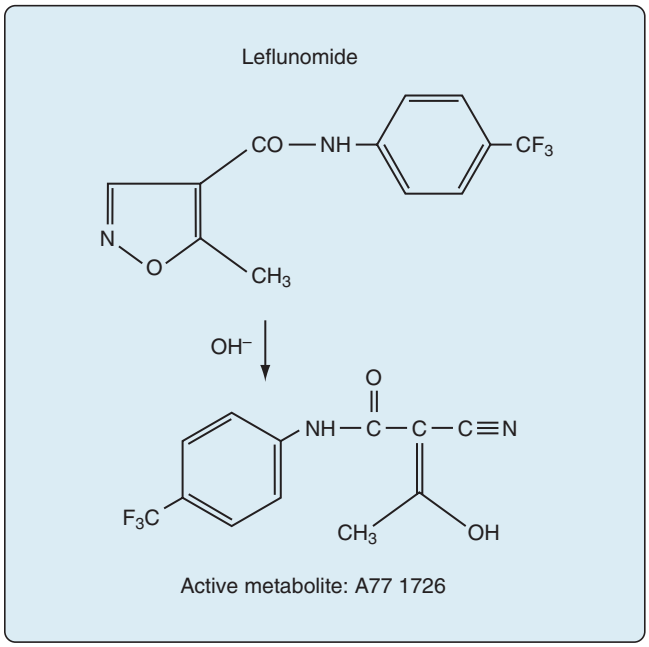



**Figure 61-4** Leflunomide is rapidly and completely metabolized to its active metabolite, A77 1726.

## Actions of Leflunomide

As with MTX, the precise mechanism of action responsible for the effects of leflunomide in rheumatic disease is not completely understood.[134] Leflunomide is immunomodulatory, with the net effect being a reduction in activated T lymphocytes. Its two in vitro mechanisms of action vary depending on concentration: (1) At the concentration of the active metabolite (A77 1726) achieved in patients, its major effect appears to be reversible inhibition of the enzyme dihydroorotate dehydrogenase (DHODH), which results in inhibition of pyrimidine synthesis; (2) at higher concentrations, A77 1726 also inhibits tyrosine kinases, interfering with cell signal transduction.[135]

Activation of T cells results in progression from the resting phase ($G_0$) to the $G_1$ phase, where ribonucleotides are synthesized, and then to the S phase, where cellular DNA is replicated in preparation for mitosis. T cell activation requires significant increases in de novo pyrimidine and purine biosynthesis. Sensors such as proto-oncogenes *(p53)* and checkpoints (cyclins C and D) in this pathway monitor the level of nucleotide pools and prevent damaged cells from replicating.[135]

Ribonucleotide uridine monophosphate (rUMP) is a precursor for the formation of pyrimidine nucleotides and thus is essential for both RNA and DNA synthesis. The steps in de novo rUMP synthesis are seen in Figure 61-5. A critical step in this pathway is the generation of dihydro-orotate in the cytoplasm with subsequent diffusion into the mitochondria, where the enzyme DHODH is located. DHODH converts dihydro-orotate to orotate, and the latter diffuses back into the cytoplasm and is subsequently converted to rUMP and, ultimately, to RNA and DNA.

The first postulated mechanism of action of leflunomide consists of inhibition of DHODH by A77 1726, which lowers orotate levels and leads to a decrease in rUMP and subsequent nucleotide synthesis, resulting in T cell cycle arrest. This mechanism of action has been substantiated by experimental evidence. In vitro mitogen-stimulated activation of T cells is blocked by levels of A77 1726 that inhibit DHODH, and this inhibition can be reversed by the addition of uridine, suggesting that A77 1726 works by disruption of pyrimidine biosynthesis.[136,137] Further, the only

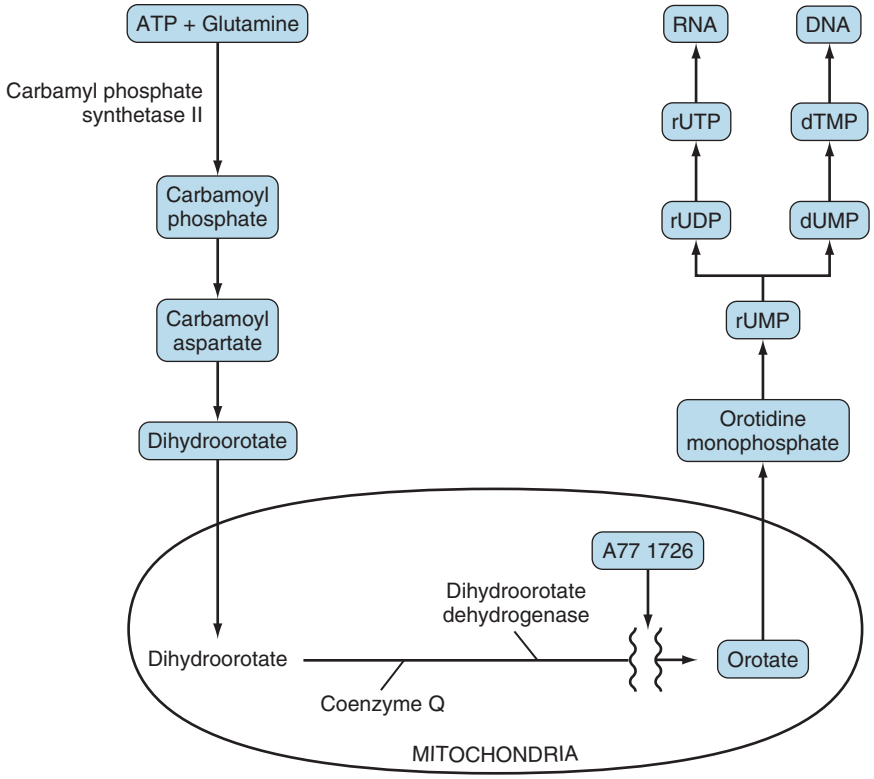



**Figure 61-5** The active metabolite of leflunomide, A77 1726, blocks the conversion of dihydroorotate to orotate within the mitochondria. ATP, Adenosine triphosphate; dTMP, 2-deoxythymidylate; dUMP, 2-deoxyuridylate; rUDP, ribonucleotide diphosphate; rUMP, ribonucleotide uridine monophosphate; rUTP, ribonucleotide triphosphate.

enzyme inhibited by A77 1726 in this pathway, at concentration obtained in vivo, is DHODH.[138]

Evidence also exists to support that inhibition of DHODH produces an arrest of lymphocytes in the $G_1$ phase of the cell cycle.[139] If the level of ribonucleotides, including rUMP, falls below a critical point, cytoplasmic p53 activation occurs, and p53 will translocate to the nucleus and initiate cellular arrest by ultimately preventing transcription of cyclins D and E. In cultures of human T cells, A77 1726 depletes rUMP pools and results in an accumulation of nuclear p53 with resultant cell cycle arrest.[140] In comparison, treatment of cell lines lacking p53 with A77 1726 does not cause a $G_1$ phase arrest.[141]

Resting lymphocytes maintain ribonucleotide requirements largely through salvage pathways and are essentially unaffected by leflunomide.[142] Active, or autoimmune, lymphocytes rely on the de novo pathway and are affected by leflunomide. During the course of treatment with this slow-acting agent, autoimmune lymphocytes should be removed progressively.[135]

At higher concentrations, A77 1726 inhibits phosphorylation of tyrosine kinases that are critical for cell growth and differentiation of activated cells.[143,144] This inhibition has been proposed to partially or completely explain the antiproliferative effects of leflunomide; however, it is unclear whether concentrations sufficient to achieve this effect are obtained in vivo.

Several other additional anti-inflammatory properties of leflunomide have been noted. Leflunomide has the ability to block the activation of nuclear factor-κB (NF-κB),[145] which regulates the expression of genes important in inflammatory processes, including those seen in inflammatory arthritis.[146] Ex vivo and in vitro studies in humans have shown that both leflunomide and MTX inhibit neutrophil chemotaxis, which may decrease the recruitment of inflammatory cells into the joints.[44] Leflunomide also decreases the ratio of MMP-1 to TIMP-1.[44] Finally, leflunomide alters the synthesis of cytokines by augmenting the immunosuppressive cytokine-transforming growth factor-β1 and suppressing the immunostimulatory cytokine IL-2.[147]

## Pharmacology

### Absorption and Bioavailability

The GI tract and the liver rapidly and completely convert ingested leflunomide into A77 1726. Food does not interfere with absorption. Circulating A77 1726 is highly bound (>99%) to plasma proteins, predominantly albumin. Its plasma concentration is linearly correlated with a single oral dose over a range of 5 to 25 mg; steady state is reached in 7 weeks after daily dosing.[148]

### Distribution and Half-Life

A77 1726 has a half-life of approximately 2 weeks (mean, 15.5 days),[148] with a low apparent volume of distribution. A77 1726 undergoes enterohepatic recirculation. In healthy individuals, 90% of leflunomide is excreted by 28 days,[148] but some may be present for much longer periods.

### Elimination

In healthy individuals, the proportions excreted by the kidney and the gut are nearly equal. Because detectable A77 1726 may be present in the body months or years later, the ability to rapidly and effectively eliminate A77 1726 with cholestyramine is important. Oral administration of cholestyramine, 8 g three times daily for 11 days, can lower the apparent half-life of A77 1726 to 1 to 2 days.[149] Furthermore, activated charcoal, 50 g every 6 hours, can reduce plasma levels by 50% within 24 hours.[149]

### Indications

**Rheumatoid Arthritis.** Leflunomide was first shown to be safe and effective in treating RA in a placebo-controlled, dose-ranging, 6-month trial.[148] Two pivotal trials, one in Europe and one in the United States, have compared leflunomide with SSZ and MTX. The European trial had three treatment arms: leflunomide (20 mg/day after a loading dose), SSZ (increased to 2 g/day), and placebo.[150] In this trial, both leflunomide and SSZ were superior to placebo in terms of swollen and tender joint counts, as well as physicians' and patients' overall assessments. It is important to note that both the leflunomide and SSZ groups reported significant effects on slowing of radiographic progression of disease compared with placebo. The U.S. trial compared patients treated with leflunomide (20 mg/day after a loading dose), MTX (7.5 to 15 mg/wk), or placebo.[151] Again, both active drugs were found to be superior to placebo but not different from each other. Both leflunomide and MTX also slowed radiographic progression of disease compared with the placebo group. Another trial compared leflunomide (20 mg/day with loading) with MTX (10 to 15 mg/wk) in a 1-year trial with a 1-year extension.[152] In this trial, MTX was shown to be statistically superior to leflunomide for the clinical outcomes measured, as well as the rate of radiographic progression after 2 years.

**Other Rheumatic Diseases.** Leflunomide has been reported to be effective in treating systemic lupus erythematosus. In a randomized, controlled trial, leflunomide was more effective than placebo in improving markers of lupus disease activity, and was safe and well tolerated.[153] A subsequent small, prospective, open-label trial of patients with lupus nephritis unresponsive to conventional therapy showed that leflunomide was efficacious and well tolerated.[154]

Leflunomide has been shown to be effective in treating PsA and psoriasis when compared with placebo.[155] In an open-label trial of AS, leflunomide was shown to be effective in treating peripheral arthritis, but axial symptoms did not improve.[156]

Both an open-label trial[157] and a randomized, controlled clinical trial[158] have shown the efficacy of leflunomide in maintaining remission in granulomatosis with polyangiitis after successful induction with cyclophosphamide. In the latter trial, leflunomide was superior to MTX in preventing relapse.

Leflunomide is also safe and effective in patients with juvenile idiopathic arthritis (JIA) who did not respond to or could not tolerate MTX.[159]

## Dose and Drug Administration

Leflunomide is available in oral tablets at doses of 10, 20, and 100 mg. Oral leflunomide is rapidly metabolized to A77 1726, which has a very long half-life; therefore the standard recommendation is to start therapy with a loading dose of 100 mg daily for 3 days, then switch to the standard maintenance dose of 20 mg daily. Despite this recommendation, many clinicians no longer prescribe a loading dose because it is believed to increase the drug's GI toxicity.[160] Also, it is common practice to decrease the dose to 10 mg daily if toxicity occurs, or if complete control of the disease can be maintained. Because of its long half-life, some clinicians give leflunomide less often (three to five times per week).

### Geriatric Patients

No pharmacokinetic studies specific to geriatric patients have been done for leflunomide; hence dosing recommendations are the same as for the general population. No clinical experience in patients with renal insufficiency has been reported, so those patients should be monitored carefully. See Table 61-1.

### Pediatric Patients

Although not approved in the United States to treat patients with JIA, leflunomide is used off-label for this condition at a dose of 10 to 20 mg/day. This dosing is often based on a patient's body weight. A recently published example of dosing by body weight is as follows: A patient weighing less than 20 kg receives 10 mg every other day, a patient weighing more than 20 kg but less than 40 kg receives 10 mg/day, and a patient weighing more than 40 kg receives 20 mg/day.[161] See Table 61-1.

## Toxicity

For major controlled trials that have used leflunomide at a dose of 20 mg/day, the incidence of adverse events that resulted in trial withdrawal are shown in Table 61-4. Leflunomide-associated withdrawals (19%) were more frequent than those associated with MTX (14%), were similar in frequency to those associated with SSZ (19%), but were more frequent than those associated with placebo treatment (8%).

### Gastrointestinal and Hepatic Side Effects

The most common side effect that limits the use of leflunomide is diarrhea, which responds to dose reduction and may

**TABLE 61-4** Leflunomide Trial Withdrawals for Adverse Events

| | No. of Patients | Withdrawals |
|---|---|---|
| Leflunomide | 816 | 154 (19%) |
| Methotrexate | 680 | 94 (14%) |
| Sulfasalazine | 133 | 25 (19%) |
| Placebo | 210 | 16 (8%) |

be less common if the loading dose is not used. Abdominal pain, dyspepsia, and nausea from leflunomide appear to be slightly increased versus placebo rates.

Liver toxicity can occur in association with leflunomide administration. Data from a large U.S. cohort of RA and PsA patients show that elevations in ALT/AST levels greater than 1 × ULN occurred in 17% and elevations greater than 2 × ULN occurred in 1% to 2% of patients taking leflunomide. Leflunomide given in combination with MTX resulted in ALT/AST elevations greater than 1 × ULN in 31% and greater than 2 × ULN in 5% of patients. Furthermore, this change in transaminases was more commonly seen in patients with PsA.[109] The European Agency for Evaluation of Medicinal Products (EMEA) reported 296 patients with hepatic abnormalities and 15 patients with liver failure and death while taking leflunomide.[162,163] The FDA reviewed adverse event reports between August 2002 and May 2009, and found 49 cases of severe liver injury, 14 of which resulted in death.[164] Most patients with hepatotoxicity have risk factors, including concomitant administration of another hepatotoxic agent or underlying liver disease.

### Cardiovascular Side Effects

Hypertension has consistently been reported to occur more frequently in leflunomide-treated compared with placebo-treated patients.[150,151] Additionally, elevation of cholesterol levels has been reported in association with leflunomide use.[165] Both of these effects should be monitored because of the excess cardiovascular mortality reported in patients with RA.

### Miscellaneous

**Dermatologic.** Skin rashes have been reported, most commonly occurring between the second and fifth months of treatment and necessitating discontinuation of the drug. Severe skin reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis require leflunomide washout with cholestryramine.

An increased incidence of alopecia has been reported in clinical trials in association with leflunomide treatment.

**Pulmonary.** Recent reports have suggested that leflunomide can cause interstitial lung disease (ILD), usually within 3 months of starting therapy. Patients with preexisting ILD are at highest risk for this potentially fatal complication.[166,167]

**Hematologic.** Rare cases of pancytopenia have been reported in post-marketing surveillance, primarily in patients with known risk factors for blood dyscrasias. An increased risk of lymphoproliferative disorders has not been associated with leflunomide.

**Weight Loss.** Significant weight loss has also been reported to occur in patients taking leflunomide.[168]

### Fertility, Pregnancy, and Lactation

Leflunomide is rated Pregnancy Category X. Animal studies have demonstrated substantial teratogenic and embryolethal effects with small doses of leflunomide. Therefore women of childbearing potential should be strongly counseled about this, and leflunomide should not be prescribed for women who are not practicing reliable birth control methods. A pregnancy test should be considered before therapy is initiated. Leflunomide excretion in milk is unknown; therefore nursing mothers should not receive leflunomide.

It is critically important to note that the active metabolite of leflunomide (A77 1726), largely because of its enterohepatic circulation, may remain in the body for years. Therefore, if a woman who has previously received leflunomide wishes to become pregnant, A77 1726 levels should be measured. Active elimination of leflunomide from the body should be considered for levels above 0.02 mg/L. This can be achieved by the oral administration of cholestyramine for 11 days (8 g, three times daily).[169] Before pregnancy is attempted, verification of levels below 0.02 mg/L should be confirmed on two separate occasions, at least 14 days apart, and the woman should then wait an additional three full menstrual cycles.[170] Patients may require more than one course of cholestyramine to achieve this level. Although no data exist, men wishing to father children should undergo the same washout procedure as women and should wait an additional 3 months after the second drug plasma level is verified to be below 0.02 mg/L (see Table 61-1).

### Toxicity Monitoring

Similar to MTX, the ACR has published guidelines on the initiation and monitoring of leflunomide treatment.[128] Patients taking leflunomide should have a baseline CBC and liver enzyme monitoring, including AST, ALT, and albumin. Serum creatinine measurement is important because leflunomide is partially eliminated by the kidney. The frequency of monitoring depends on the duration of therapy (see Table 61-2). More frequent monitoring may be warranted if concomitant immunosuppressive agents, such as MTX, are given. If patients experience significant toxicity, a washout procedure is indicated to more rapidly eliminate the drug. Caution should be exercised when live virus vaccinations are administered to patients on leflunomide.

## Drug Interactions and Contraindications

### Drug Interactions

Cholestyramine interferes with enterohepatic recycling of leflunomide, resulting in lower serum concentrations. Concomitant use with hepatotoxic agents, including MTX, increases the risk of liver toxicity, and leflunomide must be used with caution and monitored judiciously. Rifampin may increase the serum concentration of A77 1726. Leflunomide may potentiate warfarin therapy.

### Contraindications

Leflunomide should not be used in patients with impaired liver function, severe renal impairment, bone marrow dysplasia, severe immunodeficiency, severe hypoproteinemia, or known hypersensitivity to the drug. The liver is involved in enterohepatic recirculation and biliary excretion; thus

leflunomide use in liver disease is contraindicated. In renal insufficiency, the levels of circulating A77 1726 do not appear to be increased, but the component of free A77 1726 is increased. Leflunomide is contraindicated in the setting of serious infection and should be discontinued in patients with new or worsening pulmonary symptoms or rash. Leflunomide is absolutely contraindicated in pregnancy and breastfeeding (see Table 61-1).

## SULFASALAZINE

**KEY POINTS**

- **Sulfasalazine (SSZ) has anti-microbial and anti-inflammatory properties, but the exact mechanism of action is unknown.**
- **SSZ is commonly used as part of combination therapy for RA.**
- **Gastrointestinal intolerance is a common side effect.**
- **Although rare, surveillance for leukopenia is important early in the course of the treatment.**

SSZ was the first agent to be synthesized specifically for rheumatoid arthritis in 1938, by Professor Nanna Svartz of Stockholm, in collaboration with the Swedish pharmaceutical company Pharmacia. The prevailing notion at that time was that RA was caused by infection, and SSZ was designed with both anti-inflammatory and anti-bacterial properties.

### Chemical Structure

Salicylazosulfapyridine (SASP), now known as *sulfasalazine*, is a conjugate of the anti-inflammatory 5-aminosalicylic acid (5-ASA or *mesalamine*) and the anti-bacterial sulfapyridine joined by an azo bond (Figure 61-6). The abbreviation SASP is still in use as an alternative to SSZ.

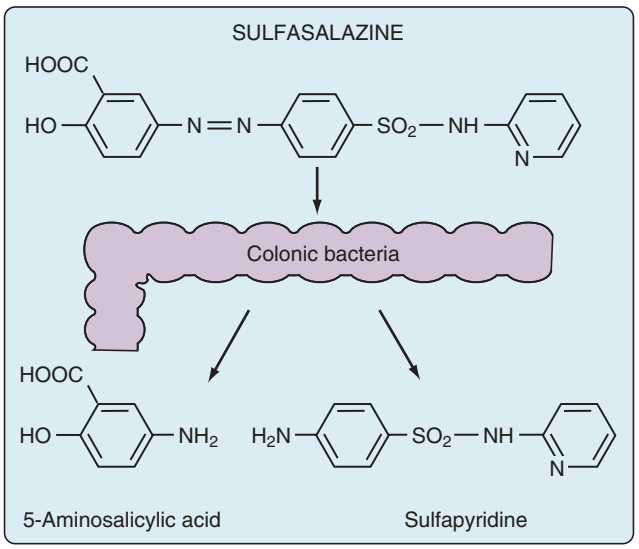



**Figure 61-6** Sulfasalazine and its major metabolites.

### Actions of Sulfasalazine

Despite more than 7 decades of use, the mechanism of action of SSZ in rheumatic disease still is not fully elucidated. When SSZ was designed, its anti-microbial properties were thought to be fundamentally important in the successful treatment of RA, which was postulated to be an enteropathic arthropathy. Through alteration of gut flora, SSZ may downregulate the immune response leading to inflammatory arthritis. Although this hypothesis has never been disproved, this mechanism of action has fallen out of favor for several reasons. To date, no conclusive evidence has been found for an infectious cause of RA, other sulfonamides have failed to result in clinical improvement, and a relationship between gut flora and clinical response to SSZ is lacking.[171] Currently, SSZ is presumed to work via anti-inflammatory and immunomodulatory effects.

SSZ has been demonstrated in vitro to possess multiple anti-inflammatory properties. First, SSZ weakly inhibits the pro-inflammatory effects of the arachidonic acid cascade, with a slight inhibitory effect on prostaglandin $E_2$ synthetase activity,[172] as well as lipoxygenase products.[173] SSZ also downregulates neutrophil chemotaxis, migration, and proteolytic enzyme production and degranulation,[174,175] and inhibits neutrophil activation by decreasing the flux of second messengers involved in intra-cellular signal transduction.[176] Neutrophil migration to sites of inflammation can be downregulated by adenosine. SSZ inhibits folate-dependent enzymes, including AICAR transformylase (ATIC) and DHFR, resulting in increased adenosine release into the extra-cellular milieu.[177] Gadangi and colleagues confirmed that SSZ, similar to MTX, modulates inflammation via increased adenosine release.[178] This effect appears to be the result solely of SSZ because sulfapyradine and 5-ASA are inactive. SSZ is a more potent inhibitor of ATIC than MTX.

SSZ also possesses multiple immunomodulatory properties. In vitro, SSZ inhibits T cell proliferation, natural killer cell activity, and B cell activation, with resultant declines in immunoglobulin synthesis and RF production.[171] In all of these systems, sulfapyridine and 5-ASA are less active than SSZ. Cytokine profiles are also altered by SSZ, resulting in inhibition of the T cell cytokines IL-2[179] and IFN-γ,[180] and the monocyte/macrophage cytokines IL-1, TNF, and IL-6.[181,182] NF-κB is a key transcription factor that when active mediates the transcription of key cytokines, adhesion molecules, and chemokines essential to mounting an immune response. In vitro, SSZ, but not sulfapyridine or 5-ASA, inhibits NF-κB translocation to the nucleus.[183]

SSZ, more so than sulfapyridine, inhibits endothelial cell proliferation and angiogenesis, which likely contributes to the synovitis of RA.[184] The synovial fibroblast also plays a key role in the pathogenesis of RA, and SSZ inhibits fibroblast proliferation and metalloproteinase synthesis.[185] Finally, SSZ inhibits the formation of osteoclasts and may be anti-resorptive in RA.[186]

The active moiety of SSZ is controversial. The parent compound, SSZ, appears to have the most biologic activity when compared with sulfapyridine and 5-ASA. However, the SSZ levels needed in vitro to see the anti-inflammatory and immunomodulatory effects are far greater than those obtained in vivo. No demonstrable relationship has been

shown between plasma SSZ, sulfapyridine, and 5-ASA levels that correlate with clinical efficacy, so serum levels may be irrelevant.[171] In an open, nonrandomized study comparing two groups of patients who received either sulfapyridine or 5-ASA in doses that would represent the molar equivalent of 2 g of SSZ, the group receiving sulfapyridine showed significant improvement in erythrocyte sedimentation rate (ESR) and in some clinical parameters, including grip strength and joint circumference.[187] Taken together, it would appear that both SSZ and sulfapyridine have a role in therapeutic efficacy for RA.

One potential site of action for SSZ that may explain its systemic action despite low serum levels is the mucosa-associated lymphoid tissue (MALT) in the small bowel.[171] In the gut lumen, the therapeutic concentration of SSZ is at least two times greater than in the serum, and drug concentration in the surrounding mucosal tissue is likely also high. The gut immune system is extensive, and active communication with the rest of the body occurs via migration and recirculation of activated lymphocytes.[171] Furthermore, a link between MALT and the joints has been suggested.[188] Evidence that some of the efficacy of SSZ may be mediated via MALT is described as follows. Treatment with SSZ decreases circulating immunoglobulin (Ig)A-producing cells and serum levels of IgA, correlating with disease improvement[189]; SSZ reduces gut mucosa lymphocytes in treated patients[190]; and SSZ has been shown to modulate an immune response elicited by an oral antigen in mice and healthy volunteers.[191]

## Pharmacology

### Absorption and Bioavailability

Less than 30% of SSZ is absorbed by the small bowel; most undergoes enterohepatic circulation and is secreted unchanged in the bile, with a resulting bioavailability of 10%.[171] The steady-state serum concentration of SSZ is 5 μg/mL after an oral dose of 2 g/day. Most SSZ reaches the colon, where intestinal bacteria reduce the azo bond and release the two active components: sulfapyridine and 5-ASA.[192] Most of the sulfapyridine is absorbed from the colon (>90%) and appears in plasma 4 to 6 hours after an oral dose (steady-state serum concentration of 30 μg/mL after 2 g/day dose). Most of the 5-ASA (80% to 90%) remains in the bowel.[171] Sulfapyridine and SSZ are likely the active components in rheumatic disease, and 5-ASA is the active component in ulcerative colitis.[193]

The bioavailability of standard SSZ is similar to that of enteric-coated SSZ.[194] Administration of SSZ with food decreases the blood concentration of both SSZ and sulfapyridine.[195]

### Distribution and Half-Life

Both SSZ and sulfapyridine are widely distributed in the body with more than 99% and 50% to 70% plasma protein binding, respectively.[171,195] Serum and synovial concentrations are comparable.[196] The half-life of SSZ is 6 to 17 hours, with upper-range values characteristic of older patients, and the half-life of sulfapyridine is 8 to 21 hours, with upper-range values characteristic of slow acetylators.[197]

### Elimination

Sulfapyridine is extensively metabolized in the liver by *N*-acetylation and ring hydroxylation with subsequent glucuronidations,[198] and because of genetic variations in acetylator phenotype, wide variability is seen among individuals.[197] Slow acetylators have a reduced clearance rate and higher serum sulfapyridine concentrations. Sulfapyridine is excreted in the urine, and 5-ASA is eliminated primarily in the feces. The small portion of 5-ASA that is absorbed is excreted in the urine as *N*-acetylmesalamine.[199]

### Indications

**Rheumatoid Arthritis.** Multiple published trials since the early 1980s have showed significant benefit in both clinical and laboratory parameters for SSZ (2 to 3 g daily) versus placebo in the treatment of RA.[200] A meta-analysis of randomized controlled clinical trials of SSZ for the treatment of RA was published in 1999.[201] In this analysis, SSZ was compared with placebo and other single DMARD therapies, including HCQ, D-penicillamine (D-Pen), and gold sodium thiomalate or aurothioglucose. In trials of SSZ versus placebo, SSZ was shown to be superior to placebo in patient improvement in multiple clinical parameters. The withdrawal rate because of lack of efficacy was significantly greater in the placebo group than in the SSZ group; however, more patients in the SSZ group than in the placebo group withdrew because of adverse effects ($P$ <0.0001). No single DMARD emerged as clinically superior in this meta-analysis. Subsequent studies have demonstrated equivalent clinical efficacy with SSZ and MTX,[202,203] as well as with SSZ and leflunomide,[150] although a 2-year extension trial did show that beneficial effects are sustained to a greater extent with leflunomide than with SSZ.[204]

**Spondyloarthropathies**

Psoriatic Arthritis. A recently published systematic review of therapies for psoriatic arthritis looked at six randomized controlled trials comparing SSZ with placebo in PsA.[205] Results show that SSZ is efficacious in treating the peripheral arthritis of PsA, but does not appear to influence the axial manifestations.[206] Few data on the prevention of radiographic progression are available.

Ankylosing Spondylitis. A recent meta-analysis reviewed 11 trials that included 895 patients with AS treated with SSZ or placebo.[207] In all patients with AS, SSZ showed some benefit in reducing ESR and easing spinal stiffness. The authors concluded that SSZ may benefit patients at an early disease stage with a higher ESR and peripheral arthritis. This finding is in agreement with those of Clegg and co-workers, who found a significant benefit for SSZ in peripheral arthritis, but not in axial arthritis, in patients with AS.[206]

Reactive Arthritis. Most cases of ReA resolve spontaneously; others become chronic with peripheral or axial arthritis. In a randomized, controlled trial of 134 male veterans with ReA (predominantly peripheral arthritis) who were unresponsive to NSAIDs, SSZ was more effective than placebo.[208]

Inflammatory Bowel–Associated Arthritis. SSZ has been used effectively to treat ulcerative colitis and distal Crohn's disease. No randomized controlled trials have investigated SSZ in terms of peripheral or axial arthritis manifestations

of these diseases, although SSZ is used in clinical practice for peripheral arthritis.

**Juvenile Inflammatory Arthritis.** SSZ is effective in polyarticular and pauciarticular juvenile inflammatory arthritis. A literature review by Brooks in 2001 found reports of 550 patients with juvenile inflammatory arthritis (half with polyarticular and one-third with pauciarticular disease) treated with SSZ.[209] Results showed at least some drug-associated benefit in all subtypes, with the best response noted in late-onset pauciarticular disease and the least benefit observed in systemic-onset disease. Toxicity and intolerance were similar to those noted with adult use of SSZ, except for a substantial incidence of serum sickness in patients with systemic-onset disease.

## Dosing

SSZ is available in regular and enteric-coated tablets of 500 mg and in a suspension of 50 mg/mL. To minimize side effects, most clinicians will prescribe 500 mg daily and will escalate the dose by 500 mg/day every week to the standard dose of 1500 to 3000 mg, divided daily. Dose reduction may ameliorate side effects. Because SSZ can inhibit enzymes in the folate-dependent pathway, concomitant administration of folic acid may be beneficial.

### Geriatric Patients

Dosing recommendations for SSZ in geriatric patients are the same as for the general adult population.[106] Studies of pharmacokinetics in the elderly have shown that although the elimination half-life is longer, it is primarily dependent on acetylator phenotype; however, the dose should be reduced for renal insufficiency.[104] See Table 61-1.

### Pediatric Patients

In pediatric patients, SSZ is dosed initially at 10 to 12.5 mg/kg/day, with a weekly dose increase of 50 mg/kg/day in two divided doses, until a maintenance dose of 2 g/day is achieved. This can be increased to 3 g/day if no response is seen with lower doses. See Table 61-1.

## Toxicity

In general, a majority of adverse effects from SSZ occur within the first several months of treatment and decrease with continued use.[210] The most common early adverse effects include GI effects, headache, dizziness, and rash. They tend to decrease with continued use.[195]

### Gastrointestinal and Hepatic

Nausea and upper abdominal discomfort are the most common adverse effects with SSZ. Nausea frequently occurs with CNS effects, including dizziness and headache. In one large cohort of 1382 RA patients, GI/CNS effects were reported as transient in 8% of patients; SSZ was continued and in another 18% led to discontinuation of therapy.[211] Nausea is more common in patients who achieve higher sulfapyridine levels and in slow acetylators.[212] Diarrhea can occur, usually in the first several months. GI effects may be decreased by administration of enteric-coated preparations. Elevations in liver transaminases may occur and are usually transient. However, they may be accompanied by fever, rash, hepatomegaly and, possibly, eosinophilia.[210]

### Hematologic

Hematologic disturbances are rare, occurring in less than 3% of patients; they usually occur within the first 3 months.[195] The most common abnormalities include leukopenia that usually reverses upon cessation of the drug, although some cases of fatal agranulocytosis have been reported, warranting continued surveillance.[213] Macrocytosis and hemolysis have been reported with SSZ; its use should be avoided in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Folic acid supplementation may be reasonable because of SSZ effects on folate metabolism. Thrombocytopenia is rare.

### Dermatologic

Rashes occur in less than 5% of patients, usually in the first 3 months of therapy.[211] Rashes are usually maculopapular, pruritic, and generalized, although urticaria develops in some patients. Desensitization to SSZ has been reported.[214] Anecdotally, erythema multiforme, toxic epidermal necrolysis, and Stevens-Johnson syndrome have been rarely reported, but were not seen in the major clinical trials. Photosensitivity has also been reported. Patients in whom a rash develops while on SSZ should be cautioned to avoid other sulfonamide-containing agents, such as thiazide diuretics, celecoxib, and antibiotics.

### Pulmonary

Pulmonary toxicity from SSZ is rare and manifests as reversible infiltrates with peripheral eosinophilia, cough, dyspnea, fever, and weight loss.[215] Pathology reveals an eosinophilic pneumonia with interstitial infiltrates with or without fibrosis. Most cases resolve with discontinuation of SSZ with or without corticosteroids.

### Miscellaneous

Minor reactions, such as irritability, anxiety, headache, and difficulty sleeping, may occur.[210,211] Rare cases of drug-induced lupus,[216] hypogammaglobulinemia,[216] and aseptic meningitis have been reported.[217] Advise patients that they may experience orange discoloration of their urine, sweat, and tears.

### Fertility, Pregnancy, and Lactation

No reports have described diminished fertility in women taking SSZ; however, men can have oligospermia, impaired sperm motility, and abnormal sperm morphology[218] that returns to normal 2 to 3 months after cessation of the drug. SSZ is considered FDA Category B, C for pregnancy. SSZ and sulfapyridine cross the placenta, and fetal concentrations are equivalent to maternal concentrations; however, SSZ does not seem to cause or increase fetal abnormalities or spontaneous abortions, and may be one of the DMARDs

of first choice for treating rheumatic disease in women of childbearing age who are or wish to become pregnant.[126] Sulfapyridine is excreted into breast milk, and one report described a child in whom bloody diarrhea developed, which led the American Academy of Pediatrics to classify SSZ as a drug that must be given with caution to nursing women[126] (see Table 61-1).

### Toxicity Monitoring

Most side effects from SSZ occur early in the course of treatment. The ACR guidelines recommend a baseline CBC with platelets, liver enzyme monitoring (including AST, ALT, and albumin), creatinine, and consideration for G6PD.[128] The frequency of monitoring depends on the duration of therapy (see Table 61-2). Vaccination with *Pneumococcus* should be given if appropriate at the initiation of therapy, and yearly influenza vaccination is recommended (see Table 61-2).

## Drug Interactions and Contraindications

### Drug Interactions

Few SSZ-drug interactions are known. SSZ may impair absorption of digoxin and decrease its bioavailability. Rarely, SSZ can increase the effects of oral hypoglycemics and the anti-coagulant effects of warfarin. Broad-spectrum antibiotics may alter gut flora and decrease the bioavailability of sulfapyridine and 5-ASA through reduced cleavage of the azo bond.

### Contraindications

Patients with hypersensitivity to any component of SSZ, or with a sulfonamide or salicylate allergy, should not be prescribed SSZ. Caution should be used in patients with porphyria or GI or genitourinary obstruction. SSZ should not be used in thrombocytopenia, severe liver disease, and active viral hepatitis (see Table 61-2).

# ANTI-MALARIALS

**KEY POINTS**

- **Hydroxychloroquine (HCQ) is a well-tolerated DMARD that is commonly used in combination therapy regimens for RA.**
- **HCQ is more commonly used than chloroquine.**
- **HCQ has a very long half-life, attributed to its affinity for melanin-containing cells in the skin.**
- **Doses of HCQ should not exceed 6.5 mg/kg in chronic therapy to minimize the risk of retinal toxicity.**
- **Although routine laboratory monitoring is not required, ophthalmologic screening is an essential component of toxicity monitoring.**
- **Diabetic patients initiating HCQ should be instructed to follow blood sugars closely because of the hypoglycemic effects of the drug.**
- **HCQ is considered safe in pregnancy; it is recommended that most pregnant patients with SLE remain on the drug to improve pregnancy outcomes.**

The aminoquinolones, including quinine, were first derived from the bark of the Peruvian cinchona tree, and were originally used to treat malaria. To reduce toxicity, the 4-aminoquinolines, chloroquine (CQ), and HCQ were developed. CQ and HCQ are the most common anti-malarials prescribed, although quinacrine is used occasionally.

## Chemical Structure

HCQ and CQ are very similar in their chemical structure, differing only by the substitution of a hydroxyethyl group for an ethyl group on the tertiary amino nitrogen of the side chain of CQ. Quinacrine includes the CQ structure, although it is not a 4-aminoquinolone derivative (Figure 61-7).

## Actions of Hydroxychloroquine

Anti-malarial agents have both immunomodulatory and anti-inflammatory properties, although their precise mechanism of action in rheumatic disease is unknown. Because HCQ and CQ are weak bases, they can pass through cytoplasmic membranes into cytoplasmic vesicles and accumulate, thereby increasing the vesicle pH from approximately

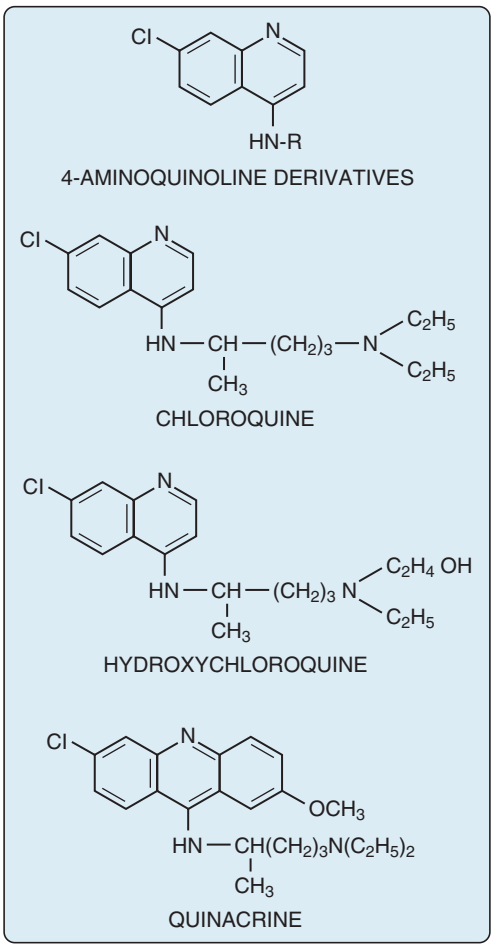



**Figure 61-7** Chemical structures of anti-malarial drugs used to treat rheumatic disease and the basic structure of 4-aminoquinolones. *R* represents the side chain.

4.0 to 6.0 and interfering with acid-dependent subcellular functions. This increased pH has several postulated immunoregulatory effects, including stabilization of lysosomal membranes, attenuation of antigen processing and presentation, and inhibition of cell-mediated cytotoxicity.[219,220] Macrophages and monocytes require precise pH concentrations for protein digestion and antigen processing, which is altered with an increased pH.[221] Furthermore, receptor assembly is disrupted, including the class II major histocompatibility complex (MHC) molecules, because a higher pH in the endoplasmic reticulum stabilizes the MHC protein with invariant chains and prevents their displacement by low-affinity autoantigens. This, combined with decreased membrane receptor recycling, leads to downregulation of antigen presentation.[219,221,222] Furthermore, anti-malarial treatment decreases circulating immune complexes.[223]

Anti-malarials also have inhibitory effects on pro-inflammatory cytokines. CQ inhibits IL-1 and IFN-γ production by monocytes and T cells.[224] CQ also inhibits macrophage TNF mRNA transcription and endotoxin-induced secretion of TNF, IL-1, and IL-6.[225] Studies of HCQ have yielded conflicting results in terms of the ability to inhibit TNF, but HCQ blocks IL-1, IL-6, and IFN-γ production by monocytes.[226,227]

Apoptosis or cell death plays an important role in regulation of the immune system, and defects in apoptosis may allow for longevity and persistence of autoreactive lymphocyte clones and perpetuation of autoimmunity. Both CQ and HCQ upregulate apoptosis and may downregulate autoimmunity by elimination of autoreactive lymphocytes.[220] Furthermore, anti-malarials inhibit the proliferative response of human lymphocytes and natural killer cell activity.[228,229]

Anti-inflammatory properties of the anti-malarials include effects on the arachidonic acid cascade caused by downregulation of phospholipases $A_2$ and C, which contribute to the production of pro-inflammatory prostaglandins and lipid peroxidation.[230-232] Lipid peroxidation is thought to play a role in apoptosis, particularly in response to ultraviolet (UV)A and UVB irradiation.[233] Anti-malarial agents also have antioxidant properties and may protect against tissue damage from free radicals.[234]

Anti-malarials have several other beneficial effects relevant to rheumatic disease that warrant further discussion. First, they are photoprotective, and this is likely the result of locally induced anti-inflammatory effects.[220] Second, both HCQ and CQ inhibit platelet adhesion and aggregation, leading to an anti-thrombotic effect.[235,236] Third, HCQ and CQ favorably alter the lipid profile, with reductions in total cholesterol, triglycerides, very-low-density lipoproteins (VLDLs), and low-density lipoproteins (LDLs), particularly in patients on concomitant corticosteroid therapy.[220,237] Finally, HCQ and CQ have been reported to decrease plasma glucose levels through inhibition of insulin degradation in the Golgi apparatus.[220,238]

## Pharmacology

### Absorption and Bioavailability

HCQ and CQ are administered orally and are rapidly and completely absorbed, with the peak plasma concentration for both occurring within 8 hours.[239] Considerable variability in blood concentrations has been reported among patients treated with the same dose, but higher plasma levels do not correlate with a better therapeutic response.[220]

### Distribution and Half-Life

Anti-malarials accrue in different concentrations in various tissue compartments. Relatively small concentrations, similar to those seen in the plasma, are contained in fat, bone, tendon, and brain. Increased concentrations are seen in the kidney, bone marrow, spleen, lungs, adrenal glands, and liver. The highest concentration occurs in melanin-containing cells, such as those in the skin and the retina.[240] In fact, the skin can serve as a long-term reservoir whereby the drug can exert its effect, or toxicity, even after it has been stopped.[220] The half-life of HCQ and CQ is 40 to 50 days, and plasma levels will increase gradually and equilibrate after 3 to 4 months.[241]

### Elimination

Most of the absorbed drug is excreted in the urine unchanged, but some is metabolized to a desethyl derivative. The remainder is excreted in the feces.[242]

### Indications

**Rheumatoid Arthritis.** The efficacy of anti-malarial agents in rheumatoid arthritis has been seen in their ability to control signs and symptoms of the disease[243-245]; however, they do not retard bone erosions.[246] In a meta-analysis of anti-malarials, HCQ was found to be less toxic but also less effective than CQ.[247] In comparison with other DMARDs, symptomatic efficacy is equal to or slightly less than that of other agents, and they have the slowest onset of action.[248] Anti-malarial agents are particularly suited for use in early, mild RA and in combination therapy.

**Systemic Lupus Erythematosus.** Although anti-malarials are not appropriate as monotherapy for severe manifestations of SLE, they are used frequently to control constitutional symptoms, arthritis, fever, fatigue, and rash. The most convincing data supporting the efficacy of anti-malarials in SLE come from studies in which the medication was discontinued in successfully treated patients. In one double-blind, placebo-controlled drug discontinuation study of 47 SLE patients in remission on HCQ, the risk of disease flare was increased by a factor of 2.5 in the placebo group.[249] Anti-malarials are particularly suited to treat SLE because of their photoprotective effects, and they are especially useful in dermatologic manifestations of SLE.

**Discoid Lupus.** Anti-malarials are effective in discoid lesions, with remission or major improvement reported in 60% to 90% of treated patients.[229] When HCQ or CQ treatment alone is unsuccessful, the addition of quinacrine may be helpful, but long-term use may be limited by the development of yellowish skin pigmentation.[250]

**Anti-phospholipid Antibody Syndrome.** HCQ use is associated with a decreased incidence of thrombosis in patients with anti-phospholipid (aPL) antibodies.[251] HCQ

also diminishes thrombus size and time in mice injected with aPL antibodies, and to reverse aPL antibody–mediated platelet activation.[252,253] Although clinical trials are needed to further establish efficacy, it is reasonable to consider the use of HCQ in patients with aPL antibody syndrome, especially if they are unable to tolerate high levels of anti-coagulation, or if they develop thrombosis despite oral anti-coagulation.[254]

**Sjögren's Syndrome.** HCQ improves local eye and mouth symptoms, arthralgia, and myalgia in a prospective open-label study of patients with Sjögren's syndrome.[255] In addition to causing the immunomodulatory and anti-inflammatory effects discussed earlier, HCQ may inhibit glandular cholinesterase activity and enhance salivary gland secretion.[256]

**Miscellaneous.** Anti-malarials have been reported to be efficacious in small and uncontrolled trials for the treatment of palindromic rheumatism,[257] childhood systemic lupus erythematosus,[258] childhood dermatomyositis,[259] eosinophilic fasciitis,[260] and erosive osteoarthritis.[261] In a controlled trial of 17 patients, the arthritis of calcium pyrophosphate crystal deposition disease improved more in the HCQ than in the placebo group.[262]

## Dosing

HCQ comes in 200 mg tablets, CQ is available in 250 mg and 500 mg tablets, and quinacrine is available from compounding pharmacies. To prevent ocular toxicity, dosing should be adjusted for weight. HCQ should be maintained at a dose of 6.5 mg/kg or less for ideal body weight, or 5.0 mg/kg or less for actual body weight, and the dose of CQ should be 3 mg/kg or less.[263,264] Ideal body weight can be calculated using a simplified formula: for women, 100 lb for the first 5 feet of height, plus 5 lb for every inch of height over 5 ft (45 kg for the first 1.5 m of height, plus 2.3 kg for each 2.5 cm of height over 1.5 m); for men, 110 lb for the first 5 feet of height, plus 5 lb for every inch of height over 5 feet (50 kg for the first 1.5 m of height, plus 2.3 kg for each 2.5 cm of height over 1.5 m).[265] In practice, doses of HCQ rarely exceed 400 mg daily, in divided doses, and doses of CQ rarely exceed 250, dosed daily; HCQ and CQ are both cleared by the kidney and the liver. Diseases of either organ may lead to increased serum drug levels. To reduce the risk of retinal toxicity, dose adjustment is needed for a glomerular flow rate less than 60 mL/min (stage 3 or higher chronic kidney disease).[264]

### Geriatric Patients

No specific pharmacokinetic studies have explored the use of anti-malarials in the elderly; thus dosing recommendations are the same as for the general population.[106] Elderly patients should be screened at baseline for pre-existing ocular disease. See Table 61-1.

### Pediatric Patients

HCQ is used at a dose of 3 to 5 mg/kg/day, typically to a maximum of 400 mg/day. To achieve this dosing in younger children and use the 200 mg tablets, the drug can be dosed every other day. Alternatively, HCQ can be compounded in a 25 mg/mL suspension. It is generally recommended that ophthalmologic screening occur every 6 months; however, some suggest that screening every 12 months is sufficient. See Table 61-1.

## Toxicity

### Ophthalmologic

Early eye symptoms can include defects in accommodation or conversion or blurred vision, which resolves. Retinal toxicity is the most feared side effect. A recent retrospective case-control study found the prevlence of retinal toxicity to be 7.5% with the following risk factors: dosage level too high for weight (OR, 5.67; 95% CI, 4.14 to 7.7 for >5.0 mg/kg actual body weight); duration of use (OR, 3.22; 95% CI, 2.20 to 4.70 for >10 years); kidney disease (OR, 2.08; 95% CI, 1.44 to 3.01 for stage 3 or higher chronic kidney disease); and concurrent tamoxifen use (OR, 4.59; 95% CI, 2.05 to 10.27).[264] CQ has a higher risk of retinal toxicity than HCQ.[266] Within 10 years of use, the prevalence of retinal toxicity remained low (<2%) but rose to nearly 20% after 20 years of use.[264]

HCQ or CQ retinopathy is described as bilateral bull's eye maculopathy, with retinal pigment epithelial (RPE) cell depigmentation in the central macula and sparing of a small foveal island. Testing of the paracentral visual field can usually show toxicity before RPE changes are visible. When advanced, visual loss may be irreversible and may continue despite cessation of the drug because of its long half-life in the retina; so early detection is essential. CQ and HCQ can also cause corneal deposits, which can be associated with halos around lights and are benign and reversible.

### Dermatologic

Rash is a common side effect leading to discontinuation of therapy. HCQ may also cause photosensitivity, alopecia, and depigmentation of hair.[247]

### Neuromuscular

Common neuromuscular symptoms include headache, insomnia, nightmares, and irritability, which are mild and reversible with lowering of the daily dose. Tinnitus and deafness can occur. Neuromyotoxicity has been reported and presents as proximal weakness of insidious onset with a normal creatine phosphokinase (CPK), which may be associated with peripheral neuropathy and cardiac myotoxicity. Muscle biopsy shows curvilinear bodies and muscle fiber atrophy with vacuolar changes.[267]

### Cardiovascular

Rarely, conduction disturbances and cardiomyopathy have been reported.[268]

### Gastrointestinal

Anorexia, nausea, vomiting, diarrhea, and abdominal cramping have been reported.[247]

### Metabolic

HCQ can reduce blood glucose levels and hemoglobin $A_{1C}$, so diabetic patients need to be cautioned to watch their blood sugars closely with initiation of HCQ and may need adjustments in their diabetic medications.[269]

### Fertility, Pregnancy, and Lactation

No reports have described adverse effects on fertility. HCQ and CQ are considered FDA Pregnancy Category C.[126] Because of the long half-life of HCQ, discontinuation of the drug at the time of pregnancy does not avoid fetal exposure. HCQ does cross the placenta, but there have been no reports of adverse outcomes or teratogenic effects in women who continue HCQ during pregnancy.[126] CQ also crosses the placenta and binds more tightly in tissue than HCQ; fetal anomalies in women who took CQ during pregnancy have been reported.[270] Quinacrine should not be used in pregnancy because it is mutagenic. Current recommendations are that HCQ may be continued throughout pregnancy, particularly in SLE, where discontinuation could precipitate a flare, which would be dangerous to both mother and baby.[170] HCQ is also found in low concentration in breast milk, but the American Academy of Pediatrics classifies it as compatible with breastfeeding[126] (see Table 61-1).

### Toxicity Monitoring

The ACR recommends baseline screening with CBC, liver transaminases, and creatinine. Subsequently, no routine laboratory monitoring is recommended.[128] In 2002, the American Academy of Ophthalmology (AAO) released an information statement on screening recommendations for CQ and HCQ retinopathy.[263] Revised guidelines were published in 2011.[271] With new data showing the risk of retinal toxicity increasing toward 1% after 5 to 7 years of use, or a cumulative dose of 1000 g of HCQ, a baseline examination is advised to rule out underlying ocular pathology, including maculopathy, which may render subsequent toxicity screening more difficult to interpret. Annual eye examinations should commence at 5 years or sooner depending on other risk factors.[271] At a minimum, this should include a dilated eye examination, visual field testing with Humphrey 10-2 fields, and screening for color blindness in male patients. In addition, newer objective tests should be used that are more sensitive than visual fields (multifocal electroretinogram, spectral domain optical coherence tomography, and fundus autofluorescence). Despite these recommendations, many rheumatologists would favor annual screening or, at a minimum, screening every 2 years. Vaccination with a yearly influenza vaccine is recommended (see Table 61-2).

## Drug Interactions and Contraindications

### Drug Interactions

HCQ should be used with caution in diabetic patients on hypoglycemic agents. HCQ increases digoxin levels. CQ may increase cyclosporine levels and reduce MTX levels. CQ can interfere with cytochrome P450 enzymes and should be used with caution when combined with other agents metabolized by this pathway. Concomittant use with tamoxifen produces synergism for the risk of retinal toxicity.[264]

### Contraindications

Hypersensitivity to or previous retinal or visual field changes attributable to 4-aminoquinoline agents and severe liver disease caused by viral hepatitis are contraindications to further use (see Table 61-2).

# COMBINATION DMARD THERAPY IN RHEUMATOID ARTHRITIS

**KEY POINTS**

- Monotherapy with MTX infrequently induces remission in RA.
- Combinations of DMARDs can have superior efficacy without increased toxicity.
- MTX is the cornerstone of combination therapy.
- Combinations of traditional DMARDs are as effective as combinations of MTX plus biologic therapy
- Current research is focused on the best strategies for introduction, timing, and patient selection for combination therapy.

In the early 1990s, the use of combinations of DMARDs to treat RA was rare; now this strategy is used by essentially all rheumatologists to treat many of their patients.[272] Currently, the timing and make-up of combinations selected to treat RA is one of the most important decisions that clinicians face.

Monotherapy with MTX is considered by most as the initial treatment of choice for early RA. Four major studies have demonstrated the superiority of combinations of DMARDs versus monotherapy in head-to-head comparisons.[68,273-275] Trials with multiple conventional DMARDs and biologics have shown them to be more effective than placebo when added to the baseline MTX in patients who have active disease despite MTX therapy. With very few exceptions, successful combination trials have included MTX, and it remains the cornerstone of combination therapy.

## History of Combination DMARD Therapy

Early combination DMARD studies were initiated in the late 1970s. The combination of cyclophosphamide, azathioprine, and HCQ produced substantial responses in a small group of patients; however, an unacceptably high number of malignancies were reported.[276] Thus early enthusiasm for combination therapy was limited. During the 1980s, inadequate dosing, the use of DMARDs with marginal efficacy, and problematic trial design contributed to the less-than-exciting results reported with combination therapy. In 1994, the first trial to convincingly show superior efficacy without increased toxicity of combination DMARD therapy in a head-to-head comparison with mono-DMARD therapy was reported,[277] and multiple

trials showing the success of combination therapy have ensued.

## Early Rheumatoid Arthritis

In the late 1990s, three pivotal trials showed the success of combination DMARD therapy in early RA. Researchers in the Netherlands reported success with a step-down approach in the COBRA (Combinatietherapie Bij Reumatoide Artritis) trial.[273] In this trial, patients with early disease were randomly assigned to two groups. A combination of prednisolone, MTX, and SSZ was compared with SSZ alone. Prednisolone was started at 60 mg/day, rapidly tapered, and discontinued by week 28. MTX was given until week 40. The dose of SSZ was the same in the two groups. At 28 weeks, the combination group was significantly better than the SSZ-alone group. While prednisolone and MTX were tapered, clinical responses became similar in the two groups; however, significant benefits in certain parameters were noted in the combination group.[278] It is important to note that combination therapy was not more toxic. Subsequent data confirm that the radiographic benefits conferred by COBRA in the initial trial continued for at least 5 years.[278]

The second important early RA study is the Finland Rheumatoid Arthritis (Fin-RA) trial.[275] In this open trial, patients were randomly assigned to receive combination DMARD therapy (MTX, SSZ, HCQ, and low-dose prednisolone) or monotherapy with SSZ with optional prednisolone. The major endpoint of this trial was remission at 2 years. Significantly, patients who received combination therapy achieved more frequent remissions. A follow-up of this trial at 5 years demonstrated that patients treated initially with the combination were less likely to have evidence of C1-C2 subluxation on cervical spine radiographs.[279]

In a third trial from Turkey, patients with early RA were randomly assigned to receive single DMARD therapy (MTX, SSZ, or HCQ), two-drug therapy (MTX and SSZ or MTX and HCQ), or three-drug therapy (MTX, SSZ, and HCQ), with remission at 2 years as the major outcome.[274] For all endpoints measured, two drugs were statistically superior to monotherapy, and three drugs were statistically superior to the two-drug regimens.

The TEAR (Treatment of Early Aggressive RA) trial (see Chapter 71) enrolled patients with early RA and compared initial combination therapy with MTX, SSZ, and HCQ (triple) or MTX and etanercept with initial MTX monotherapy with step-up to combination therapy at 6 months for patients with a Disease Activity Score (DAS) 28-ESR of 3.2 or greater. Results show that either initial combination therapy is superior to MTX monotherapy at 6 months, with a reduction in DAS28-ESR for combined groups of 4.2 versus 3.6 ($P < 0.0001$). There were no differences in DAS28-ESR seen between either of the initial combination regimens. Between weeks 48 and 102, there were no significant differences in DAS28-ESR between combination groups, whether started as initial or step-up therapy. By week 102, there was a statistically significant change from the baseline modified Sharp/van der Heijde score between the group receiving initial MTX plus etanercept versus triple (0.64 vs. 1.69; $P = 0.047$).[280]

In sum, combination therapy, whether step-up or initial therapy, has been shown in multiple trials to be superior to MTX alone in early rheumatoid arthritis. Predictors of who may respond to MTX monotherapy alone and who needs early combination therapy are still lacking.

## Patients with Active Disease Despite Methotrexate

Combination DMARD therapy was first studied in groups of patients with active disease despite MTX therapy, or suboptimal MTX responders. The first study to show the advantage of combination therapy with MTX and another DMARD compared with continued therapy with MTX alone in this group of patients was the cyclosporine-MTX trial[281] (Figure 61-8). Therapy with the combination of MTX, SSZ, and HCQ, so-called triple therapy, is well tolerated[68,274,275,282] and more effective than MTX monotherapy[68,274,280] or SSZ monotherapy,[275] and, in an open trial of patients with early disease, more effective than the double combination of MTX and SSZ or MTX and HCQ.[274]

In 1996, a 2-year, randomized, double-blind, parallel study of 102 established patients with RA was done to compare triple-drug therapy (MTX, SSZ, and HCQ) versus double therapy (HCQ and SSZ) versus monotherapy with MTX.[68] Significantly more patients receiving triple-drug therapy achieved a modified Paulus 50% response,[283] compared with those given double therapy. Triple therapy was well tolerated, and numerically fewer withdrawals occurred in the combination group compared with the other two groups. This therapy is durable, with 62% of patients remaining on triple therapy for 5 years continuing to maintain a 50% efficacy response.[284]

In follow-up, a 2-year, double-blind trial on patients with moderately advanced disease was done in 2002, in which the triple combination group was compared with two double-combination groups (MTX plus SSZ and MTX plus HCQ) in a head-to-head comparison.[282] Patients were stratified for previous MTX use, and previous users had to have active disease despite receiving 17.5 mg/wk. The triple-therapy group and both double-therapy groups tolerated their treatments well, with only 8% withdrawing for toxicities, which were mostly minor. Triple therapy is superior to either of the double combinations.

A double-blind, placebo-controlled trial (see Figure 61-8) compared the addition of leflunomide or placebo to baseline MTX in suboptimal MTX responders.[285] Leflunomide or placebo was added to MTX. The combination group was statistically superior to the MTX-placebo group in terms of the American College of Rheumatology 20 (ACR20) set criteria for clinical response. The combination was reasonably well tolerated, but side effects including diarrhea, nausea, and dizziness were increased in the combination arm. Elevated ALT levels (>1.2 times normal) occurred more frequently in patients on combination therapy than in those on MTX alone, with increases leading to withdrawal in 2.3% of patients who received the combination. The Rheumatoid Arthritis: Comparison of Active Therapies (RACAT) trial, was a 48 week double-blind, noninferiority trial of 353 established patients with RA with active disease despite MTX therapy (see Chapter 71). Groups were randomly assigned to receive triple DMARD therapy (MTX,

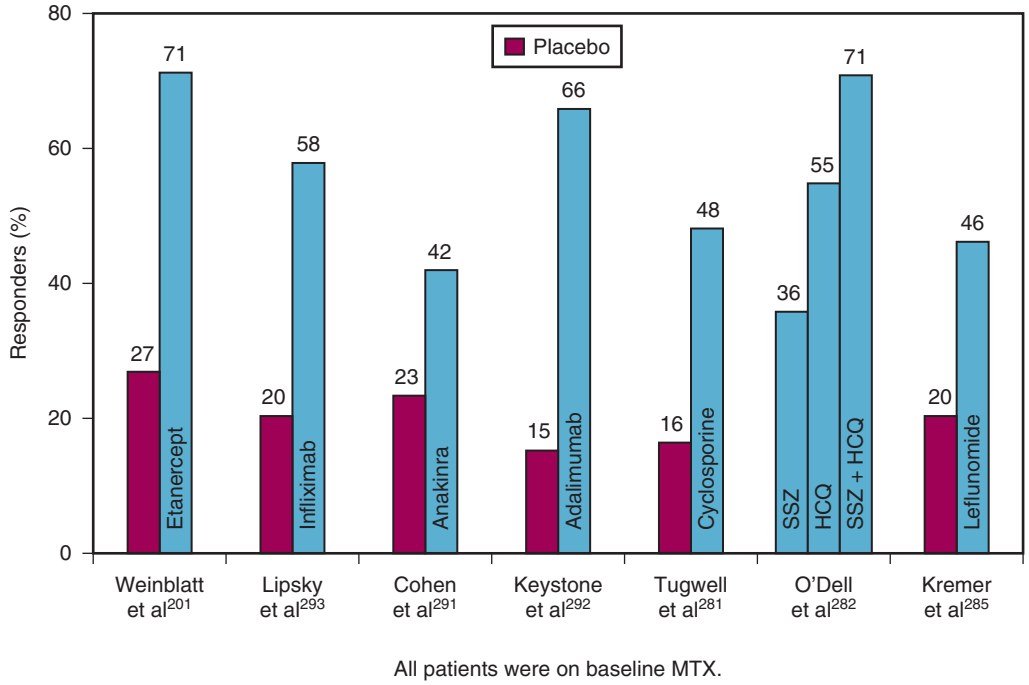



**Figure 61-8** Summary data taken from seven different clinical trials of therapies in patients with active disease despite methotrexate therapy. HCQ, Hydroxychloroquine; MTX, methotrexate; SSZ, sulfasalazine.

SSZ, HCQ) versus MTX and etanercept.[286] Patients who did not respond by week 24 were switched in a blinded fashion to the other therapy. The primary outcome was improvement in the DAS28. Both groups had significant improvement in the first 24 weeks, with only 27% in each group requiring a switch in therapy. Improvement was seen in both of the groups who switched therapy ($P$ <0.001) and the response after switching did not differ significantly between the two groups ($P$ = 0.08). The change in DAS28 (baseline to 48 weeks) was –2.1 with triple therapy and –2.3 with MTX plus etanercept. Triple therapy is noninferior to MTX plus etanercept ($P$ = 0.002). Cost-effectiveness analyses have revealed that the strategy of first using triple therapy before adding etanercept is cost-effective. In this analysis, the cost for one quality adjusted life-year is approximately $1 million (unpublished data).

## Corticosteroids in DMARD Combinations

Corticosteroids have not traditionally been considered DMARDs. However, they clearly fulfill all of the criteria for DMARDs, including halting radiographic progression.[287] Few clinicians who care for patients with RA dispute their efficacy. Indeed, they have been used as baseline therapy for more than half of the patients included in the combination trials discussed previously.

Prednisolone undoubtedly was a critical component for the success of the combination therapy for early rheumatoid arthritis (COBRA) protocol[273] and may have played a role in the success of the combination group in the Fin-RA trial.[275] Kirwan and colleagues' report of the ability of prednisolone to significantly retard radiographic progression of RA compared with placebo is testament to the efficacy of steroids when used in combination with other DMARDs.[287,288] Corticosteroids clearly deserve further formal investigation as a component of combination therapy. The COBRA trial and the Kirwan data have raised another interesting question: Should/could short courses of high-dose steroids be used as a form of induction therapy?[289]

## Biologic Agents in DMARD Combinations

Biologic agents that block TNF (etanercept, infliximab, adalimumab, certolizumab, and golimumab) and IL-1 (anakinra) have been studied in patients with early-stage and established RA in combination with MTX[290-298] (see Figure 61-8). These trials have shown superior improvements in clinical and radiographic endpoints in the combination groups.[295,296] Other biologic agents—rituximab, an anti-CD20 monoclonal antibody; abatacept, a T cell co-stimulatory inhibitor; tocilizumab, an IL-6 receptor antagonist; and tofacitinab, an oral Janus kinase inhibitor—have been studied in combination with MTX.[299-302] In the REFLEX (Randomized Evaluation of Long-Term Efficacy of Rituximab in RA) trial, rituximab versus placebo was added to baseline MTX in patients with RA who were suboptimal responders to MTX with failure of one or more TNF inhibitors.[299] Results showed that the combination group had significant improvements in ACR-N responses. Abatacept versus placebo in addition to background MTX was studied in patients with RA who had a suboptimal response to MTX, and results at 1 year showed significant improvements in clinical and radiographic endpoints.[300] The LITHE (Tocilizumab Safety and the Prevention of Structural Joint Damage) study compared the addition of tocilizumab versus placebo in MTX nonresponders and showed an improvement in structural outcomes at 1 year.[301] Concerns do exist regarding the risks of infection and

infusion reaction in combination therapy that includes biologic agents.

## Selecting the Right Patients for the Right Combination Therapy

Factors that predict a poor prognosis for patients with RA are well accepted and include rheumatoid factor, elevated ESR and CRP, the number of joints involved, erosions, and the presence of certain genetic markers. However, unless these factors can be shown to predict response to certain therapies in a differential fashion, they are of limited therapeutic use. Patient characteristics recommending one therapeutic regimen versus another remain to be fully elucidated. Genetic differences have been suggested to influence outcomes in a differential fashion. Until this observation can be corroborated and factors that predict response to other therapies elucidated, choices will remain largely empiric.

Treatment of patients with RA by using MTX combinations should be the gold standard against which future therapies are compared. Available data demonstrate that a variety of combinations are more effective than MTX alone. Until recently, no direct head-to-head trials comparing combinations of traditional DMARDs to MTX plus biologic agents existed. The TEAR and RACAT trials have shown that the use of traditional DMARD combinations (MTX, SSZ, HCQ) is as effective as combinations that include biologic agents (MTX plus etanercept) as initial therapy and, importantly, in MTX nonresponders. Recent guidelines published by the ACR[303] and the European League against Rheumatism (EULAR)[304] recommendations for the use of DMARDs and biologic agents in RA support this premise.

Although more information is available every day, many questions remain to be answered regarding the appropriate timing of combination therapy and the optimal combinations for specific patients and for specific clinical situations. Future research is needed to clarify the role of corticosteroids and, particularly, biologic response modifiers as components of and alternatives to MTX combination regimens.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 62

# Immunosuppressive Drugs



Jacob M. Van Laar

**KEY POINTS**

- Immunosuppressive drugs are effective and indispensable as remission-inducing and maintenance agents in the management of inflammatory rheumatic conditions. They constitute a heterogeneous group of compounds, each with a unique mode of action and toxicity profile.
- The long-term use of immunosuppressive drugs is associated with an increased risk of bacterial, viral, and fungal infection, as well as a reduced response to vaccinations.
- Cyclophosphamide is a commonly used drug for remission induction in persons with severe systemic lupus erythematosus (SLE) and necrotizing vasculitis. Its toxicities include myelosuppression, infection, ovarian failure, hemorrhagic cystitis, and malignancy, including bladder cancer, especially with high cumulative doses.
- Azathioprine can be effective as a glucocorticoid-sparing agent in remission maintenance therapy, particularly in persons with SLE and necrotizing vasculitis. It can induce severe myelosuppression in patients with low or absent thiopurine methyltransferase (TPMT) activity, which is affected by a polymorphism that can be identified by genetic screening. Severe myelosuppression also can occur in patients with normal TPMT activity, and regular monitoring of white blood counts is recommended.
- The interaction of azathioprine and allopurinol can lead to fatal myelosuppression and should be avoided.
- Cyclosporine can be effective in refractory rheumatoid arthritis, psoriatic arthritis, SLE, and inflammatory eye disease. It affects renal function and blood pressure, and dose reduction may be necessary. Drug interactions between cyclosporine and other drugs can result in clinically relevant changes in plasma concentrations of cyclosporine and/or concomitant medication.
- Mycophenolate mofetil can be used as a remission induction agent in persons with lupus nephritis and is now commonly used for remission maintenance treatment of SLE and necrotizing vasculitis. It is generally well tolerated, although diarrhea and leukopenia may necessitate its discontinuation.
- Tacrolimus is used only rarely for rheumatologic indications, usually in the event that conventional therapies fail.
- Cytostatic agents should be avoided in pregnancy and lactation, and referral to a fertility clinic should be considered for all fertile male and female patients. Other immunosuppressive drugs should only be used during pregnancy if the potential benefits outweigh the potential risks.

Immunosuppressive drugs comprise different classes of drugs that dampen the immune system—notably T and B lymphocytes—functionally and/or numerically (Table 62-1) but do not permanently correct the fundamental imbalance of immune regulation in autoimmune disease. As such, they do not have curative potential, yet they can be effective in remission induction and control of specific rheumatic disease manifestations, and they remain cornerstone drugs in the management of rheumatic conditions. Many immunosuppressive drugs have withstood the test of time, as attested by their ongoing use in transplantation medicine, nephrology, gastroenterology, ophthalmology, dermatology, and rheumatology. Consequently, their therapeutic potential and toxicity profiles hold few surprises. Apart from drug-specific toxicities, the main risk of immunosuppressive treatment is infection. In the absence of validated biomarkers of infection, sound clinical judgment and experience remain indispensable in monitoring patients who use immunosuppressive drugs, often for long periods. The use of live vaccines is contraindicated, and although other vaccinations are generally less effective, annual influenza vaccination is recommended in patients being treated with immunosuppressive medication. Pneumococcal vaccination also should be considered in select patients in accordance with (inter)national guidelines.

This chapter outlines the clinical pharmacology and therapeutic use of immunosuppressive drugs used in rheumatology. These drugs include cytostatic agents that affect bone marrow progenitor cells (cyclophosphamide and azathioprine) and drugs such as cyclosporine, tacrolimus, and mycophenolate mofetil (MMF) that target lymphocytes by inhibiting specific intra-cellular signaling pathways and/or proliferation. Their effects on the immune system overlap with those of traditional disease-modifying antirheumatic drugs such as methotrexate, glucocorticoids, and biologics. The most commonly used immunosuppressive drugs—cyclophosphamide, azathioprine, and MMF—are discussed in more detail. Glucocorticoids, traditional disease-modifying anti-rheumatic drugs (e.g., methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine), biologics, and novel intra-cellular targeting agents are discussed elsewhere. The role of other immunosuppressive drugs (e.g., thalidomide, chlorambucil, sirolimus, and everolimus) in routine rheumatologic clinical practice has not been sufficiently established to merit discussion.

## CYCLOPHOSPHAMIDE

Cyclophosphamide was introduced as a cytotoxic agent in 1958 and is still one of the most potent immunosuppressant

drugs available. In combination with glucocorticoids, cyclophosphamide is particular effective as a remission induction agent in severe systemic lupus erythematosus (SLE) and necrotizing vasculitis.

## Structure

Cyclophosphamide is an oxazaphosphorine-substituted nitrogen mustard and inactive prodrug that requires enzymatic bioactivation (Figure 62-1). Cyclophosphamide belongs to the class of alkylating agents that substitute alkyl radicals into DNA, resulting in cell death. Cyclophosphamide is the alkylating agent of choice for most rheumatic disease requiring such therapy.

## Mechanisms of Action

The DNA-alkylating effects of cyclophosphamide are mediated predominantly through phosphoramide mustard and, to a lesser extent, other active metabolites. These positively charged, reactive intermediates alkylate nucleophilic bases, resulting in the cross-linking of DNA and of DNA proteins, breaks in DNA, and consequently decreased DNA synthesis and apoptosis.[1] The cytotoxicity of alkylating agents correlates with the amount of DNA cross-linking, but the relationship between cytotoxicity and immunosuppressive effects is unclear. The effects of cyclophosphamide are not exclusively limited to proliferating cells or particular cell types. Sensitivity varies among cell populations, however; for example, hematopoietic progenitor cells are relatively resistant to even high doses of cyclophosphamide. The immunosuppressive effects of cyclophosphamide include decreased numbers of T lymphocytes and B lymphocytes, decreased lymphocyte proliferation, decreased antibody production, and suppression of delayed hypersensitivity to new antigens with relative preservation of established delayed hypersensitivity.[2]

**TABLE 62-1** Mechanisms of Action of Immunosuppressive Drugs

| Drugs | Class | Mechanism of Action |
|---|---|---|
| Cyclophosphamide | Alkylating cytotoxics | Active metabolites alkylate deoxyribonucleic acid |
| Azathioprine | Purine analogue cytotoxics | Inhibit purine synthesis |
| Cyclosporine, tacrolimus (FK506) | Calcineurin inhibitors | Inhibit calcium-dependent T cell activation and interleukin-2 production |
| Mycophenolate mofetil | Purine synthesis inhibitor | Mycophenolic acid inhibits inosine monophosphate dehydrogenase |

## Pharmacology

### Absorption and Distribution

Oral and intravenous (IV) administration of cyclophosphamide results in similar plasma concentrations.[3] Peak plasma concentrations of cyclophosphamide occur 1 hour after oral administration. Protein binding of cyclophosphamide is low (20%), and it is widely distributed.[1]

### Metabolism and Elimination

Cyclophosphamide is rapidly metabolized, largely by the liver, to active and inactive metabolites. The formation of the active 4-hydroxycyclophosphamide is mediated by various cytochrome P450 (CYP) enzymes, and genetic variations in the enzymes in patients with lupus nephritis affect responses to cyclophosphamide.[4] 4-Hydroxycyclophosphamide, which is not cytotoxic at physiologic pH, readily diffuses into cells and spontaneously decomposes into the active phosphoramide mustard. The elimination half-life of cyclophosphamide is 5 to 9 hours, and alkylating activity is undetectable in the plasma of most patients 24 hours after a dose of 12 mg/kg.[1] Plasma concentrations of cyclophosphamide are not clinically useful predictors of either efficacy or toxicity. Between 30% and 60% of the total cyclophosphamide is eliminated in the urine, mostly as inactive metabolites, although some cyclophosphamide and active metabolites such as phosphoramide mustard and acrolein also can be detected in urine.[1]

### Pharmacokinetic Considerations in Special Circumstances

**Liver Disease.** Although the half-life of cyclophosphamide is increased to 12 hours in patients with liver failure

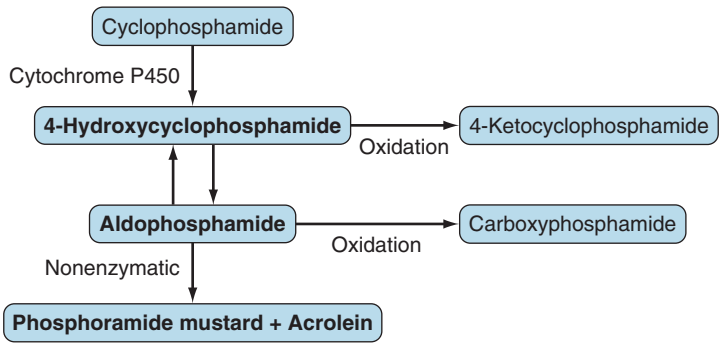



**Figure 62-1** The metabolism of cyclophosphamide. Cyclophosphamide is converted to 4-hydroxycyclophosphamide, in equilibrium with its tautomer aldophosphamide, by cytochrome P450 enzymes. Subsequent nonenzymatic processes lead to the formation of phosphoramide mustard and acrolein. Oxidation of 4-hydroxycyclophosphamide and aldophosphamide through enzymes including aldehyde dehydrogenase results in inactive metabolites. Cytotoxic metabolites are shown in bold type.

compared with 8 hours in control subjects, toxicity is not increased, suggesting that exposure to cytotoxic metabolites is not increased and dose modification in liver disease is generally not required.[1]

**Renal Impairment.** Some studies have shown little alteration in drug disposition with no increased toxicity in patients with impaired renal function.[1] In patients with autoimmune disease and a creatinine clearance of 25 to 50 mL/min and 10 to 25 mL/min, exposure to cyclophosphamide increased approximately 40% and 70%, respectively.[5] In clinical practice, initial cyclophosphamide doses are therefore decreased by approximately 30% in patients with moderate to severe renal impairment, and subsequent doses are titrated according to clinical response and effects on the leukocyte (white blood cell) count. Cyclophosphamide is removed by dialysis and is administered after dialysis, or, alternatively, dialysis can be initiated the day after cyclophosphamide administration.[5]

## Clinical Indications

Cyclophosphamide is most commonly used as a remission induction agent for patients with systemic necrotizing vasculitis or Goodpasture's syndrome, for many patients with organ-threatening SLE, and for some patients with autoimmune disease–associated interstitial lung disease and inflammatory eye disease. In SLE, a remission induction course with IV cyclophosphamide followed by maintenance with azathioprine or mycophenolate to minimize cyclophosphamide toxicity is still commonly used to treat severe organ involvement, including lupus nephritis, although remission induction regimens with MMF (discussed in subsequent text) and the B cell depleting biologic agent rituximab have been propagated as effective and safe alternatives to cyclophosphamide.[6] The original National Institutes of Health (NIH) protocol entailed 6 monthly IV infusions with cyclophosphamide, 1 g/m$^2$, and then one infusion every 3 months for at least 24 additional months,[7] whereas the Euro-Lupus protocol used in Europe entailed administration of six IV infusions of 500 mg of cyclophosphamide every 2 weeks followed by azathioprine maintenance (Table 62-2). A comparison with 6 monthly IV infusions of cyclophosphamide, 500 mg/m$^2$, followed by two further infusions of slightly higher doses 3 and 6 months later, along with azathioprine maintenance therapy, resulted in similar rates of the endpoints of end-stage renal disease or doubling of creatinine concentration with up to 10 years of follow-up.[8]

Cyclophosphamide administered as either IV pulse therapy or orally can also be effective in patients with other serious complications of SLE, including central nervous system involvement and thrombocytopenia and interstitial lung disease associated with systemic sclerosis and other autoimmune diseases.[9-11] Several trials have investigated whether IV cyclophosphamide is as effective as oral cyclophosphamide as remission induction therapy for granulomatosus with polyangiitis (GPA). Although early trial results suggested superiority of oral dosing, more recent clinical trial data pointed to equal efficacy but slightly less hematologic toxicity with IV therapy.[12-15] As in lupus nephritis, shorter induction courses of cyclophosphamide have been reported to be effective in GPA and microscopic polyangiitis.[16] Cyclophosphamide has a steep dose-response curve, making it an ideal compound for dose escalation. High doses of cyclophosphamide, with or without stem cell rescue and lymphoablative antibodies or total body irradiation, have been used for severe juvenile idiopathic arthritis (JIA), rheumatoid arthritis (RA), systemic sclerosis, and SLE.[17] With the introduction of effective biologics and new treatment paradigms for RA and JIA, the clinical need for immunoablative treatment in these diseases has waned. Although large series have shown promising results of immunoablative therapy and stem cell rescue in patients with severe SLE, a recent randomized trial showed that standard-dose IV cyclophosphamide was not inferior to high-dose cyclophosphamide *without* stem cell rescue or lymphoablative antibodies.[18] A small phase 2 trial and a larger prospective, randomized phase 3 trial in 156 patients with systemic sclerosis to compare the safety and efficacy of IV pulse cyclophosphamide and immunoablative therapy *with* stem cell rescue, however, showed superior efficacy of the latter on a range of outcome measures including modified Rodnan skin score, functional ability, quality of life, and survival, albeit at the expense of greater toxicity in the first year as a result of infections and cardiopulmonary events.[19,20]

**TABLE 62-2** Lupus Nephritis Treatment Protocols

| |
|---|
| **National Institutes of Health Protocol** |
| Cyclophosphamide: 6× monthly IV 500-750 mg/m$^2$, then maintenance doses every 3 mo until 1 y after remission, or consider alternative remission maintenance treatment with azathioprine or mycophenolate mofetil; dose adjustments on the basis of nadir leukocyte counts and glomerular filtration rate |
| All patients to receive prednisone, 0.5-1 mg/kg/day for 4 wk, decreasing the every-other-day dose each week, if possible, by 5 mg to achieve a prednisone dose of 0.25 mg/kg on alternate days |
| **Euro-Lupus Protocols** |
| *Low-dose* cyclophosphamide: 6× biweekly IV 500 mg |
| *High-dose* cyclophosphamide: 6× monthly IV 500 mg/m$^2$ of body surface area, followed by 2 quarterly pulses with higher dose (+250 mg depending on leukocyte nadir, maximum 1500 mg) |
| All patients to receive: Glucocorticoids: 3× daily IV methylprednisolone, 750 mg, followed by oral 0.5 mg/kg/day of prednisolone (or equivalent) for 4 wk; after 4 wk, tapering of glucocorticoid by 2.5 mg prednisolone every 2 wk; low-dose glucocorticoid therapy (5-7.5 mg prednisolone/day) was maintained at least until mo 30 after inclusion; dose at discretion of treating physician thereafter. Azathioprine: oral, 2 mg/kg daily, starting 2 wk after last cyclophosphamide infusion until mo 30 after inclusion; choice of immunosuppressant at discretion of treating physician thereafter |

IV, Intravenous.

## Dosage and Route of Administration

Typical dosage regimens are presented in Table 62-2. Dosages for IV pulse therapy with cyclophosphamide range from 0.5 to 1 g/m$^2$, and the dosage for oral therapy is 2 mg/kg. The bioavailability of oral cyclophosphamide is excellent.

## Toxicity

### Hematologic

Reversible myelosuppression manifesting as leukopenia and neutropenia is common and dose dependent. Generally,

platelet counts are not affected with IV pulse doses of less than 50 mg/kg, but with long-term oral use, a mild decrease in the platelet count is common. After a single IV dose of cyclophosphamide, the approximate times to nadir and recovery of leukocyte counts are 8 to 14 days and 21 days, respectively.[21] The white blood cell nadir is about 3000 cells/mm$^3$ after a dose of 1 g/m$^2$ (~25 mg/kg) and 1500 cells/mm$^3$ after a dose of 1.5 g/m$^2$. With long-term use, sensitivity to the myelosuppressive effects of cyclophosphamide is increased, and doses usually need to be decreased over time.

### Infection

Infection with a range of common and opportunistic pathogens is a frequent complication. In 100 patients with SLE, infection occurred in 45 patients during treatment with a cyclophosphamide-based regimen and was the primary cause of death in 7 patients.[22] In this study, infection was equally common in patients receiving oral or IV cyclophosphamide and was associated with a white blood cell nadir at some point in treatment of less than 3000/mm$^3$ (55% infection rate vs. 36%). At the time of infection, the average white blood cell count was normal, however.[22] A higher maximal corticosteroid dose was also associated with increased risk of infection. Half of the infections occurred at prednisone doses of less than 40 mg/day, and a quarter of the infections occurred at doses less than 25 mg/day. Lower rates of infection (25% to 30%) have been reported in patients with SLE who received cyclophosphamide in NIH protocols.[23] Oral cyclophosphamide regimens generally pose a greater risk of infection than IV pulse regimens. Serious infections occurred in 41% and 70% of patients with GPA who were treated with pulse IV and daily oral cyclophosphamide, respectively.[12] These rates of infection are higher than rates reported in long-term NIH protocols, in which 48% of 158 patients experienced 140 infections requiring hospitalization.[24] The reported frequency of cyclophosphamide-associated infection varies, probably as a function of the stage and severity of the underlying disease, the degree of cyclophosphamide-induced immunosuppression, and variations in concomitant glucocorticoid regimens. *Pneumocystis jiroveci* pneumonia has been recognized as a preventable, serious opportunistic infection that complicates treatment of systemic vasculitis with regimens using cyclophosphamide and methotrexate. The risk is highest during the remission induction phase and is greater with oral than IV cyclophosphamide regimens.[25] Surprisingly, in two placebo-controlled, randomized clinical trials in persons with scleroderma lung disease, active treatment for 1 year with either oral cyclophosphamide or sequential treatment with prednisolone plus IV cyclophosphamide followed by azathioprine was not associated with more toxicity, suggesting disease-specific differences in toxicity.[10,11]

### Urologic

The bladder toxicities of cyclophosphamide, hemorrhagic cystitis, and bladder cancer are related to route of administration, duration of therapy, and cumulative cyclophosphamide dose. Bladder toxicity, a particular problem with long-term oral cyclophosphamide, is largely due to acrolein, a metabolite of cyclophosphamide. It is commonly accepted that bladder toxicity can be minimized in patients receiving pulse doses of IV cyclophosphamide by administering mesna, a sulfhydryl compound that binds acrolein in the urine and inactivates it.[26] Direct evidence for the effectiveness of mesna in preventing cystitis, however, comes from its use with ifosfamide in patients with cancer and data from animal models. The data from rheumatology series are consistent with a protective effect but are inadequate to come to firm conclusions, which explains differences between national guidelines.[27] The short half-life of mesna renders it suboptimal for the prevention of bladder toxicity in patients receiving daily oral cyclophosphamide, but oral mesna administered three times a day with daily oral cyclophosphamide was associated with a relatively low incidence of bladder toxicity of 12%.[28]

Nonglomerular hematuria, which may range from minor, microscopic blood loss to severe, macroscopic bleeding, is the most common manifestation of cyclophosphamide-induced cystitis.[29] Nonglomerular hematuria occurred at some time in 50% of 145 patients treated with oral cyclophosphamide and was related to the duration of therapy and cumulative cyclophosphamide dose.[29] The risk of bladder cancer was increased 31-fold (95% confidence interval [CI], 13-fold to 65-fold), and bladder cancer had developed in 7 patients (5%) any time between 7 months and 15 years after the initiation of therapy. The cancer was preceded by nonglomerular hematuria in all patients. Six of the seven patients had a cumulative dose of more than 100 g of cyclophosphamide and a duration of therapy of more than 2.7 years. Smokers were at increased risk of hemorrhagic cystitis and bladder cancer.

### Malignancy

Cyclophosphamide increases the risk of malignancies (other than bladder cancer) twofold to fourfold. In the largest study, 119 patients with RA who had been treated with oral cyclophosphamide were followed up for 20 years.[29] In the cyclophosphamide group, 50 cancers occurred in 37 patients, compared with 26 cancers that occurred in 25 of 119 control subjects with RA. Bladder, skin, myeloproliferative, and oropharyngeal malignancies occurred more commonly in the cyclophosphamide group. The risk of malignancies increased with the cumulative dose of cyclophosphamide, and a malignancy developed in 53% of patients who received more than 80 g of cyclophosphamide. Few malignancies have been reported in patients treated with pulse IV cyclophosphamide regimens. Current data do not allow quantification of the long-term risk of malignancy associated with pulse IV cyclophosphamide treatment, but it is likely to be substantially smaller than that associated with oral regimens.

### Reproductive

Cyclophosphamide, when used to treat autoimmune disease, results in significant gonadal toxicity. The risk of sustained amenorrhea after cyclophosphamide therapy has ranged from 11% to 59%.[30] The risk of ovarian failure depends more on the age of the patient and the cumulative dose of

cyclophosphamide than on the route of administration.[30] Patients younger than 25 years receiving six pulses of IV cyclophosphamide had a low frequency of ovarian failure (none of four patients), whereas patients older than 31 years receiving 15 to 24 pulses all had ovarian failure (four of four patients). The use of alkylating agents in male patients leads to azoospermia, and, if the clinical situation allows, referral to a fertility clinic for banking of sperm (or ova in female patients) should be considered before cyclophosphamide treatment. The offspring of adults who underwent cancer chemotherapy in childhood had no increase in genetic disease.[31]

### Pulmonary

Cyclophosphamide-induced pulmonary toxicity occurs in less than 1% of patients. Early-onset pneumonitis 1 to 6 months after exposure to cyclophosphamide may respond to withdrawal of the drug and treatment with corticosteroids. A more insidious, irreversible, late-onset pneumonitis and fibrosis with radiographic findings of diffuse reticular or reticulonodular infiltrates may occur after treatment with oral cyclophosphamide for 1 to 13 years.[32]

### Miscellaneous

A varying degree of reversible alopecia can occur with daily oral and monthly pulse cyclophosphamide. Cardiotoxicity, a dose-limiting adverse effect in oncology, and water intoxication, as a result of inappropriate anti-diuretic hormone secretion, are rare at standard doses.[33] Unusual hypersensitivity reactions include urticaria and anaphylaxis, although the bladder protectant mesna is a more likely cause of allergic responses in patients receiving both drugs.[34,35]

### Strategies to Minimize Toxicity

Strategies to minimize toxicity include adjusting the dose of cyclophosphamide to avoid a significant degree of leukopenia (white blood cell count <3000/mm$^3$ for daily oral therapy or a nadir of <2000/mm$^3$ for pulse IV therapy) and granulocytopenia.[36] The blood count is monitored initially at 1- to 2-week intervals and monthly thereafter in patients taking stable oral doses. To decrease the risk of infection added by concomitant high-dose corticosteroids, the dose of corticosteroids should be reduced after a clinical response has been obtained. Alternate-day administration of glucocorticoids can be considered in the maintenance phase. Oral cyclophosphamide is best administered as a single dose in the morning with the patient drinking plenty of fluids and emptying the bladder frequently to dilute the urinary concentration of acrolein and to minimize the amount of time the bladder is exposed to it. Prophylaxis against *P. jiroveci* pneumonia is often prescribed, particularly during the induction phase when doses of cyclophosphamide and corticosteroids are higher. The use of mesna to prevent bladder toxicity was described earlier. Urinalysis should be performed monthly, and nonglomerular hematuria should be evaluated by a urologist. All patients who receive cyclophosphamide, particularly patients in whom hemorrhagic cystitis develops, are at increased risk of developing bladder cancer, and lifelong surveillance is required with urinalysis, urine cytology, and, if indicated, cystoscopy.[37] Lastly, drugs that are less toxic than cyclophosphamide such as methotrexate, MMF, and rituximab are an option for inducing remission or for remission maintenance in patients with GPA or lupus nephritis.[38,39]

## Pregnancy and Lactation

Cyclophosphamide is a U.S. Food and Drug Administration (FDA) Pregnancy Category D drug. Cyclophosphamide is teratogenic, particularly in the first trimester, and should be avoided during pregnancy and lactation.[40,41] If a patient becomes pregnant while taking (receiving) this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

## Drug Interactions

Cimetidine inhibits the activity of several hepatic enzymes. It has resulted in increased exposure to cyclophosphamide metabolites in a rabbit model.[42] Ranitidine and presumably other H2 receptor antagonists that have little effect on hepatic drug metabolism are not associated with increased cyclophosphamide toxicity.[43] Allopurinol increases the half-life of cyclophosphamide and the frequency of leukopenia.[44] Cyclophosphamide decreases plasma pseudocholinesterase activity and can potentiate the effect of succinylcholine.[45]

# AZATHIOPRINE

## Structure

Azathioprine is a prodrug that is converted to 6-mercaptopurine (6-MP), which involves the removal of an imidazole group.[46] 6-MP is a purine analogue that acts as a cycle-specific antimetabolite chemotherapeutic agent and interferes with the synthesis of nucleotides, thereby inhibiting proliferation of lymphocytes. Azathioprine has a better therapeutic index than 6-MP and has replaced it in the treatment of rheumatic autoimmune disease.

## Mechanisms of Action

The exact mechanism of action of the active thiopurine metabolites of azathioprine in autoimmune disease is unknown. Thiopurine metabolites such as thioguanine nucleotides decrease the de novo synthesis of purine nucleotides by inhibiting amidotransferase enzymes and purine ribonucleotide interconversion and are incorporated into DNA and RNA.[46] The incorporation of thioguanine nucleotides into the nucleic acids of cells is thought to mediate the cytotoxicity of azathioprine, whereas inhibition of purine synthesis may be more important in decreasing cellular proliferation. Leukopenia is unnecessary for immunosuppression. Azathioprine decreases the circulating lymphocyte count, suppresses lymphocyte proliferation, inhibits antibody production, inhibits monocyte production, suppresses natural killer cell activity, and inhibits cell-mediated and humoral immunity.

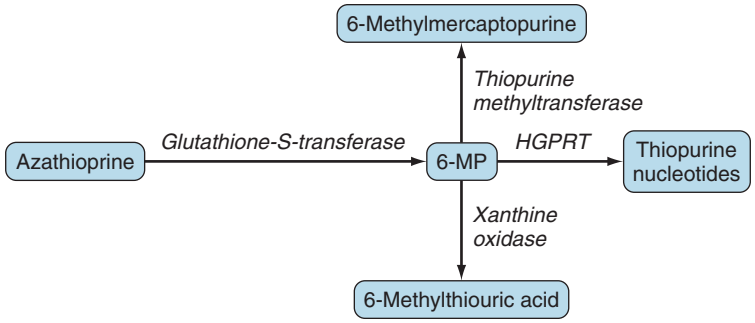



**Figure 62-2** Azathioprine is converted to mercaptopurine (6-MP) enzymatically by glutathione-*S*-transferase and nonenzymatic mechanisms. Xanthine oxidase and thiopurine methyltransferase metabolize 6-MP to the inactive metabolites 6-methylthiouric acid and 6-methylmercaptopurine. Hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) metabolizes 6-MP to active, cytotoxic thiopurine nucleotides.

## Pharmacology

### Absorption and Distribution

Oral azathioprine is well absorbed and rapidly converted to 6-MP, which is further metabolized to several compounds, including 6-thiourate (Figure 62-2), that are excreted in urine. The plasma half-life of azathioprine is less than 15 minutes, but it is 1 to 3 hours for the active derivative, 6-MP.[47] The bioavailability of azathioprine, measured as the concentrations of mercaptopurine achieved after oral administration, varies. In healthy volunteers, bioavailability ranged from 27% to 83%, with an average of 47%.[47] Mercaptopurine is widely distributed with a volume of distribution of 4 to 8 L/kg.[46]

### Metabolism and Elimination

The metabolism of azathioprine is complex[46,48] and has been simplified in Figure 62-2. Two enzymes, xanthine oxidase and thiopurine S-methyltransferase (TPMT), shunt mercaptopurine metabolites to relatively inactive compounds, whereas other enzymes such as hypoxanthine-guanine-phosphoribosyl-transferase lead to the formation of cytotoxic thiopurine nucleotides. Low TPMT activity or inhibition of xanthine oxidase by drugs such as allopurinol leads to decreased detoxification and increased formation of cytotoxic metabolites after the administration of azathioprine or mercaptopurine. Maximal concentrations of mercaptopurine occur 1 to 3 hours after administration of azathioprine, and the half-life of mercaptopurine is 1 to 2 hours.[47] The half-life of the intra-cellular, active 6-thioguanine nucleotides is estimated to be 1 to 2 weeks, however, and concentrations do not change over the 24-hour dose period in patients receiving daily azathioprine.[49] At conventional rheumatologic doses, approximately 1% of mercaptopurine is excreted unchanged in the urine.[49] Increased toxicity can occur with renal impairment (creatinine clearance <25 mL/min), and a modest dose reduction is usually necessary. The substantial interindividual variability in azathioprine disposition and TPMT activity are more important determinants of sensitivity to azathioprine than renal function.[50] Azathioprine is only slightly dialyzable (10%) through conventional hemodialysis membranes.

### Dosage

Azathioprine is often started at a dose of 1 mg/kg daily, and if this dose is tolerated, it is increased to 2 to 2.5 mg/kg after 2 to 4 weeks. A gradual increase in dose is often better tolerated. The onset of immunosuppressive effects is relatively slow, over several weeks, presumably because the active thioguanine metabolites slowly accumulate intra-cellularly.

## Clinical Indications

In current practice, azathioprine is mainly used in the treatment of connective tissue disease rather than inflammatory joint disease. Azathioprine is less effective than methotrexate in RA and has a slow mode of onset when compared with other disease-modifying anti-rheumatic drugs and biologics. Nevertheless, it remains a treatment option for patients with RA who have refractory disease or as a corticosteroid-sparing agent in persons with organ involvement. Azathioprine is used to treat some patients with lupus nephritis, and although it is more effective than corticosteroids alone, it is not as effective as IV pulse cyclophosphamide to induce remission.[51] It is effective in maintenance therapy, however,[52] even after low-dose cyclophosphamide induction. For other manifestations of SLE, including cutaneous disease, azathioprine is widely used as a glucocorticoid-sparing agent.[53]

Azathioprine in combination with corticosteroids is useful in the treatment of a range of other autoimmune diseases including inflammatory muscle disease, inflammatory eye disease, including Behçet's disease,[54] psoriatic arthritis,[55] reactive arthritis, and various forms of vasculitis. In systemic vasculitis, azathioprine is less effective as a remission-induction agent than cyclophosphamide[16] but is safer as a remission-maintenance and glucocorticoid-sparing drug.[40] However, a small study has shown the efficacy of high-dose (1200 to 1800 mg infusions) monthly azathioprine as initial treatment of GPA and lupus nephritis.[56] Azathioprine is frequently used in persons with systemic sclerosis and overlap syndromes, especially in persons with interstitial lung disease or joint involvement.[11,57]

## Toxicity

### Hematologic

Reversible myelosuppression is dose related but varies among individuals. Low-dose azathioprine (1 to 2 mg/kg/day) rarely results in leukopenia or thrombocytopenia. Pure red cell aplasia is also rare. Severe myelosuppression is uncommon and is caused by low or absent TPMT activity. Decreased TPMT activity leads to a decreased ability to detoxify mercaptopurine and results in increased formation of cytotoxic thioguanine metabolites and clinical toxicity.[58] TPMT activity is polymorphic with a trimodal distribution. Approximately 90% of subjects show high activity, 10% show intermediate activity, and 0.3% (the subjects homozygous for the poorly functional polymorphisms) show very low activity.[59,60] The median TPMT activity in African Americans is approximately 17% lower than in white Americans.[60] The 1 in 300 subjects with low or absent TPMT activity are at great risk of severe azathioprine-induced myelosuppression, which has a delayed but sudden onset, most commonly 4 to 10 weeks after azathioprine has been started.[61] In more than half of all cases of leukopenia in patients receiving azathioprine, the patients have a normal TPMT genotype and phenotype, however.

### Gastrointestinal

Liver test abnormalities occur in 34% of patients but are seldom serious. Serious liver toxicity, severe cholestasis, hepatic veno-occlusive disease, and nodular regenerative hyperplasia and pancreatitis are rare.

### Malignancy

Data regarding the risk of malignancy in patients treated with azathioprine for rheumatologic disease are conflicting. Some studies found an increased risk, particularly of lymphoproliferative malignancies, whereas others did not find an increased risk.[62] A 24-year retrospective study of 358 patients with SLE found no difference in malignancy rates between patients who had and had not received azathioprine, and no lymphomas occurred in the azathioprine group.[58]

### Hypersensitivity

Acute hypersensitivity syndromes, usually occurring within 2 weeks of starting therapy, are rare, with a range of manifestations including shock, fever, rash, pancreatitis, renal failure, and hepatitis.[63]

### Other Toxicities

Infection is less common with azathioprine than with alkylating agents; however, infections with a range of bacterial and nonbacterial pathogens, including herpes zoster and cytomegalovirus, may occur. The rate of infection when azathioprine is administered alone or with low doses of glucocorticoids is approximately 2.5 per 100 person-years of exposure. Maculopapular or urticarial rashes can occur. Eosinophilia and drug fever are rare.

### Strategies to Minimize Toxicity

TPMT activity testing is the most commonly used method to identify patients at risk of serious toxicity. More than 23 variants in the *TPMT* gene, associated with decreased TPMT activity, have been identified, with the TPMT*2, TPMT*3A, and TPMT*3C alleles accounting for most of the intermediate- or low-activity cases. The concordance between TPMT genetics and phenotypes is slightly less than 100%. TPMT activity (phenotype) can be measured directly in red blood cell membranes. Alternatively (e.g., in patients who have undergone blood transfusions), genetic polymorphisms can be identified by polymerase chain reaction. Guidelines for TPMT activity testing vary among different specialties, but it is generally recommended that TPMT status be tested before starting azathioprine therapy. Whether testing is cost-effective and clinically useful when compared with traditional monitoring of white blood cell counts is still a matter of debate.[64-67] In the absence of TPMT testing, administration of a low initial dose and careful monitoring of the white blood cell count in patients starting azathioprine is required. Some authors suggest weekly monitoring during the first 15 weeks of azathioprine treatment.[60] When patients are taking a stable dose of azathioprine, blood counts are monitored monthly and liver function tests are monitored every 3 to 4 months.

## Drug Interactions

One of the most important and potentially fatal drug interactions in rheumatology is the ability of allopurinol to dramatically increase the cytotoxic effects of azathioprine through inhibition of xanthine oxidase–mediated inactivation of mercaptopurine.[68] Various strategies have been used to treat hyperuricemia and gout in patients receiving azathioprine, which is a common clinical problem after transplantation. Reduction of the dose of azathioprine by at least two thirds in patients who also are receiving allopurinol is advocated. However, because myelosuppression can still occur after a 75% reduction in dose, careful monitoring is required.[68] Alternatively, uricosurics such as benzbromarone have been effective and safe,[68] and MMF has been substituted for azathioprine as an alternative immunosuppressant.[69] The combination of azathioprine with several other drugs, including sulfasalazine, ganciclovir, angiotensin-converting enzyme inhibitors, carbamazepine, co-trimoxazole, and clozapine, may also increase the risk of myelosuppression. Azathioprine has been associated with resistance to warfarin in case reports.[70]

## Pregnancy and Lactation

Azathioprine is an FDA Pregnancy Category D drug. Azathioprine crosses the placenta, but drug and metabolite concentrations are lower in the fetal circulation, suggesting placental metabolism.[41,71] Data relating to rheumatologic diseases are limited, and although azathioprine is being used during pregnancy, it is better avoided during pregnancy and lactation if possible.[72] In a prospective observational study in 189 pregnant women treated with azathioprine for various autoimmune conditions, the rate of major malformation was

3.5%, which was similar to that of the general population.[73] Its use was associated with prematurity, however. Azathioprine may be considered in cases where the benefits of disease control in the mother give the best chances of term pregnancy and fetal survival.

# CYCLOSPORINE

## Structure

Cyclosporine is a lipophilic endecapeptide derived from a fungus; it is effective in refractory RA, psoriatic arthritis, SLE, and autoimmune eye disease.

## Mechanism of Action

Cyclosporine impairs production of interleukin (IL)-2 and other cytokines, reducing lymphocyte proliferation. Cyclosporine complexes with cyclophilin, one of a group of cytosolic-binding proteins known as *immunophilins*. This complex binds to and inhibits calcineurin, a serine/threonine phosphatase. Inhibition of calcineurin phosphatase activity prevents the translocation of cytosolic nuclear factor of activated T cells to the nucleus, a translocation that is required for the transcription of genes for cytokines such as IL-2 and for T cell activation (Figure 62-3).[74]

## Pharmacology

Two formulations of cyclosporine are available for oral use: (1) the older, oil-based Sandimmune formulation and (2) the newer, microemulsion Neoral formulation. The active drug, cyclosporine, is identical in both formulations, but the microemulsion formulation results in better bioavailability and less intersubject and intrasubject variability. Neoral or equivalent generic formulations are replacing the Sandimmune formulation of cyclosporine, which is no longer universally available.

### Absorption and Distribution

Cyclosporine is poorly and variably absorbed from the gut, with a bioavailability of approximately 30%. Consumption of a high-fat meal increases absorption. Substantial interindividual (threefold) and intraindividual (twofold) variability exists in cyclosporine disposition.[75] The time to peak concentration (1 to 8 hours) and the elimination half-life (3 to 20 hours) vary. Cyclosporine is lipophilic and widely distributed in body tissues, particularly in the lean body mass.[75] Higher concentrations of cyclosporine are found in red blood cells than in plasma, and whole-blood concentrations are used for monitoring, although monitoring is seldom required in persons with autoimmune diseases. The time to maximal concentration is approximately 25%

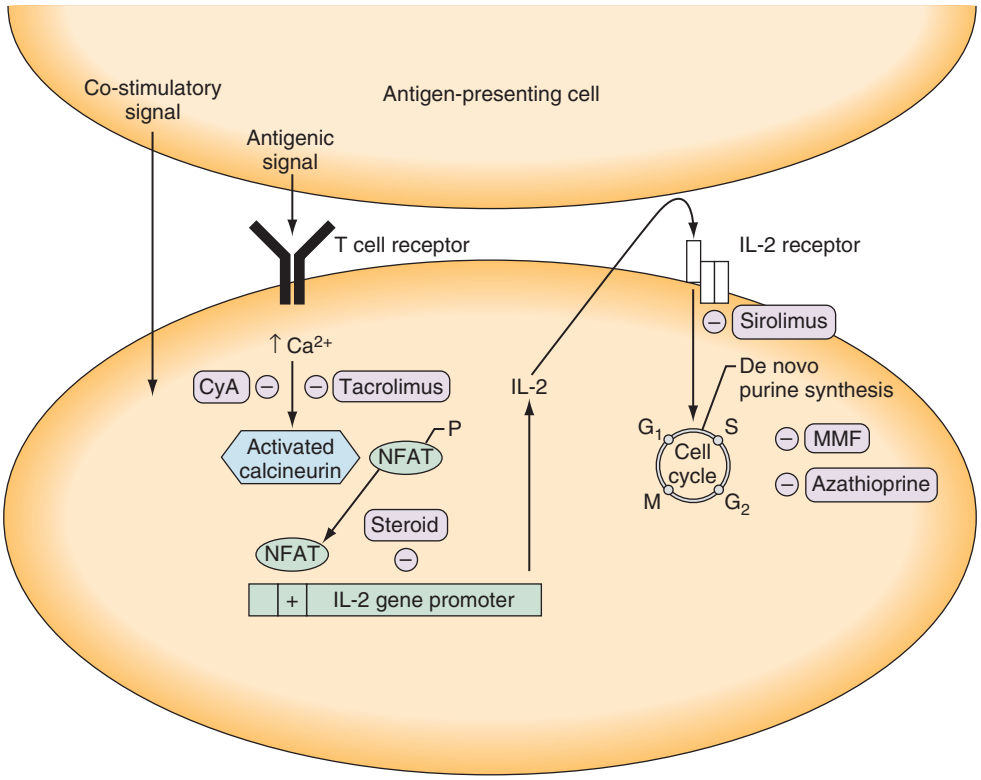



**Figure 62-3** Stages of T cell activation. Multiple targets for immunosuppressive agents. Stimulation of the T cell receptor results in calcineurin activation, a process inhibited by cyclosporine (CyA) and tacrolimus. Calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), enabling it to enter the nucleus and bind to interleukin (IL)-2 promoter. Corticosteroids inhibit cytokine gene transcription in lymphocytes and antigen-presenting cells by several mechanisms. Co-stimulatory signals are necessary to optimize T cell IL-2 gene transcription, prevent T cell anergy, and inhibit T cell apoptosis. IL-2 receptor stimulation induces the cell to enter the cell cycle and proliferate. Signal 3 may be blocked by IL-2 receptor antibodies or by sirolimus, which inhibits second messenger signals induced by IL-2 receptor ligation. After progression into the cell cycle, azathioprine and mycophenolate mofetil (MMF) interrupt deoxyribonucleic acid replication by inhibiting purine synthesis. *(From Denton MD, Magee CC, Sayegh MH: Immunosuppressive strategies in transplantation,* Lancet *353:1083–1091, 1999.)*

shorter, and the maximal cyclosporine concentration and area under the concentration (AUC) curve are increased by approximately 50% with the microemulsion formulation.[76,77] In addition, interindividual and intraindividual variability in cyclosporine disposition are decreased by approximately 50% with the microemulsion formulation.[76,77]

### Metabolism and Elimination

Cyclosporine disposition has two major determinants. First, P-glycoprotein (Pgp), a drug efflux pump, pumps substrates such as cyclosporine out of cells. Pgp, the product of the multidrug resistance gene, is expressed on intestinal epithelial cells and in the liver. Second, cyclosporine is extensively metabolized by the CYP3A enzyme system, which is active not only in the liver but also in intestinal epithelium. Pgp, by limiting drug uptake, and CYP3A4, by assisting drug metabolism in the gut and liver, act to limit the bioavailability of cyclosporine and determine its disposition.[78] Cyclosporine is extensively metabolized to more than 20 metabolites. The rate-limiting step in the elimination of cyclosporine is the formation of metabolites, not their clearance. Cyclosporine elimination is not altered in renal failure; however, because of its nephrotoxicity, cyclosporine is avoided in patients with impaired renal function. Liver disease impairs the excretion of cyclosporine metabolites.

### Dosage

Effective use of cyclosporine requires that appropriate patients be selected for treatment and monitored carefully (Table 62-3). The starting dosage of cyclosporine is 2.5 mg/kg/day, usually administered in divided doses. In obese patients, dosage is based on the approximate ideal body weight. Clinical response is slow, occurring over 4 to 8 weeks, and may be maximal only after 12 weeks or more of treatment. To improve efficacy, the dosage can be increased by 0.5 mg/kg/day at 4- to 8-week intervals to a maximal dosage of 4 mg/kg/day of the microemulsion formulation. If no clinical response occurs in 4 to 6 months, cyclosporine should be discontinued. In patients whose disease is well controlled, the dosage of cyclosporine can be decreased by 0.5 mg/kg/day at 4- to 8-week intervals to determine the minimal effective dose for the individual patient. In patients receiving the older Sandimmune formulation of cyclosporine who convert to the microemulsion formulation, a 1:1 dose conversion is generally used. Because of the greater and more predictable bioavailability of the microemulsion formulation, however, a greater exposure to cyclosporine is likely. Blood pressure (BP) and creatinine should be monitored initially at 2-week intervals after the conversion, and the dose of cyclosporine should be decreased if required.

**TABLE 62-3** Clinical Use of Cyclosporine in Rheumatic Disease

| |
|---|
| Select appropriate patients |
| Contraindications:<br>Current or past malignancy other than basal cell carcinoma, renal impairment, uncontrolled hypertension, or hepatic dysfunction |
| Cautions:<br>Elderly age, obesity, controlled hypertension, premalignant lesions, drugs that interact with cyclosporine, pregnancy |
| Obtain ≥2 creatinine concentrations before starting cyclosporine, and average these to provide baseline creatinine value |
| Start low—cyclosporine 2.5 mg/kg/day in divided doses |
| Stay low—maximum 4 mg/kg/day (microemulsion formulation) |
| Monitor blood pressure and creatinine initially every 2 wk for 3 mo, then monthly if stable |
| If serum creatinine increases >30% above patient's baseline, reduce dosage of cyclosporine by 1 mg/kg/day; recheck serum creatinine in 1-2 wk, and temporarily discontinue cyclosporine if creatinine remains >30% above baseline |
| When creatinine level returns to within 15% of baseline, cyclosporine can be restarted at a lower dosage |

## Clinical Indications

Cyclosporine is effective in the treatment of RA as a single agent and in combination with methotrexate[79] or hydroxychloroquine, but it is used less commonly now because of the availability of more effective and safer treatment options early in the course of RA.[80] Nevertheless, it can still be a useful drug in refractory RA.[81] Cyclosporine can increase mean peak plasma methotrexate levels and AUC by about 20%,[82] which may contribute to the efficacy of the combination. Data comparing the efficacy and safety of cyclosporine with other disease-modifying anti-rheumatic drugs over long periods and in a large number of patients are limited. Cyclosporine is effective for the skin and joint manifestations of psoriasis.[83] Fewer data are available regarding the use of cyclosporine in other rheumatic diseases. In uncontrolled, small studies in persons with SLE,[84] cyclosporine has been reported to improve disease activity, have a glucocorticoid-sparing effect, and improve proteinuria, thrombocytopenia, and leukopenia. The efficacy of cyclosporine as a steroid-sparing drug was confirmed in a randomized clinical trial in patients with severe SLE, but it was not found to be more effective or safer when compared with azathioprine.[85] Cyclosporine has also been reported to be effective in small series of cases in many other autoimmune conditions including pyoderma gangrenosum, Behçet's disease, maintenance therapy of anti-neutrophil cytoplasmic antibody–associated vasculitis, and macrophage-activation syndrome in juvenile RA.[86]

## Toxicity

### Hypertension

Hypertension occurs in approximately 20% of patients with autoimmune disease who receive treatment with cyclosporine. The magnitude of increase in BP is usually mild but clinically significant because it increases the risk of stroke, myocardial infarction, heart failure, and other adverse cardiovascular events associated with elevated BP.[87] The hypertension should be controlled by reducing the dose of cyclosporine or by anti-hypertensive drug therapy.[88]

### Nephrotoxicity

Virtually all patients who take cyclosporine have a small but measurable decrease in renal function that is reversible after

cyclosporine is discontinued. Serum creatinine concentrations have increased approximately 20% in 6- to 12-month clinical trials, but few patients have had to withdraw because of this outcome.[89] Long-term data regarding renal function in patients with RA who are treated with cyclosporine are limited. In one 12-month study, an increase in serum creatinine of more than 30% occurred in 50% of patients; half of these patients responded to cyclosporine dose reduction and half did not, requiring discontinuation of the drug.[88] The small increase in serum creatinine observed in most studies occurs mainly during the first 2 to 3 months of treatment, and then creatinine remains relatively stable over 12 months.[88,89] However, other data suggest that over periods of treatment longer than 1 year, many patients, who over the first year had a stable, acceptable increase in creatinine concentration, subsequently have an increase in creatinine to more than 30% of baseline that is not controlled by cyclosporine dose reduction; such patients must discontinue treatment.[90] Preventable risk factors for cyclosporine-induced nephrotoxicity are a high dosage of cyclosporine (>5 mg/kg/day) and an increase in serum creatinine concentration of more than 50% of the baseline value. The risk of cyclosporine nephropathy is low in patients treated according to the clinical guidelines (see Table 62-3).[91] Renal biopsy specimens in 11 patients with RA who received cyclosporine (average dosage, 3.3 mg/kg/day) for 26 months and had an average increase in serum creatinine of 31% showed no significant cyclosporine-induced renal changes.[92]

### Gastrointestinal

Gastrointestinal upset is common but usually mild and transient. A few patients discontinue cyclosporine therapy for this reason, however.

### Malignancy

In transplant recipients, cyclosporine use has been associated with an increased risk of skin cancer and lymphoma. In 208 patients with RA who were treated with cyclosporine for an average of 1.6 years, the incidence of malignancy and mortality was similar to that of control subjects with RA,[93] but a recent meta-analysis on the risk of immunomodulatory drugs in persons with RA, psoriasis, and psoriatic arthritis did find an increased risk of nonmelanoma skin cancer in patients treated with cyclosporine.[94] Epstein-Barr virus–induced B cell lymphoma, which may be reversible when cyclosporine is discontinued, has been reported in a few patients receiving cyclosporine for a variety of indications.

### Other Toxicities

Other adverse effects that are common but usually of minor significance include hypertrichosis, gingival hyperplasia, tremor, paresthesia, breast tenderness, hyperkalemia, hypomagnesemia, and an increase in serum uric acid.[88] Cyclosporine may result in a clinically insignificant increase in alkaline phosphatase concentrations but does not increase the frequency of abnormal transaminase concentrations in patients also receiving methotrexate.[95]

### Strategies to Minimize Toxicity

Because cyclosporine may increase liver enzymes, as well as potassium, uric acid, and lipid concentrations, and decrease magnesium concentrations, it is prudent to measure these enzymes and concentrations before, and occasionally after, initiating therapy. At least two, and preferably more, recent normal BP and serum creatinine determinations should be obtained before starting treatment.

Many patients with RA have low serum creatinine concentrations, and it is important not to overlook significant cyclosporine-induced elevations in serum creatinine, which may remain within the normal laboratory reference range. If a patient has a baseline creatinine level of 0.6 mg/dL and, after administration of cyclosporine, the level increases to 0.9 mg/dL (which is still in the normal range), this change represents a 50% increase above baseline and requires dose reduction. Cyclosporine concentrations are not useful predictors of efficacy or toxicity in rheumatic diseases and are not routinely performed. Cyclosporine trough concentrations, which are measured approximately 12 hours after the last dose, can be useful if concerns exist about compliance or unusual drug disposition in individual patients.

## Pregnancy and Lactation

Cyclosporine is an FDA Pregnancy Category C drug. Pregnancy outcomes in transplant recipients receiving cyclosporine-based and noncyclosporine-based regimens are similar. Cyclosporine use in pregnancy is not recommended, however, unless the potential benefit exceeds the potential risk to the fetus. Breastfeeding should be avoided.

## Drug Interactions

Cyclosporine and tacrolimus, because of the influence of Pgp and CYP3A4 enzyme activity on their disposition, have many clinically important drug interactions (Table 62-4).[96,97]

**TABLE 62-4** Clinically Important Drug Interactions with Cyclosporine*

| |
|---|
| **Increased Cyclosporine Concentrations** |
| Erythromycin, clarithromycin |
| Azole antifungals: ketoconazole, fluconazole, itraconazole |
| Calcium channel antagonists: diltiazem, verapamil, amlodipine† |
| Grapefruit juice |
| Others: amiodarone, danazol, allopurinol, colchicine |
| **Decreased Cyclosporine Concentrations** |
| Inducers of hepatic enzymes: rifampicin, phenytoin, phenobarbitone, nafcillin, St John's wort |
| **Increased Cyclosporine Toxicity** |
| Increased renal toxicity with aminoglycosides, quinolone antibiotics, amphotericin B, (?) nonsteroidal anti-inflammatory drugs, (?) angiotensin-converting enzyme inhibitors |
| **Cyclosporine Increasing Toxicity of Another Drug** |
| Increased risk of myopathy and rhabdomyolysis with lovastatin and other statins |
| Increased risk of colchicine neuromyopathy and toxicity |
| Increased digoxin concentrations |
| Increased risk of hyperkalemia with $K^+$-sparing diuretics and $K^+$ supplements |

*Most interactions with cyclosporine are also likely to apply to tacrolimus.
†Data are conflicting about whether amlodipine does or does not increase cyclosporine concentrations.

Many drugs such as erythromycin, azole antifungal drugs, and some calcium channel antagonists that inhibit CYP3A4 (inhibiting the metabolism of cyclosporine) also inhibit Pgp. Drug interactions mediated by these dual mechanisms may result in a twofold to fivefold increase in cyclosporine concentrations. Azithromycin, in contrast to erythromycin and clarithromycin, seems unlikely to alter cyclosporine levels. The plasma concentrations and clinical toxicity of several statin lipid-lowering agents are increased substantially by cyclosporine, but the pharmacokinetics of fluvastatin and pravastatin, because they are not metabolized primarily by CYP3A4, are altered less by cyclosporine.[98] Nevertheless, the pravastatin AUC curve, a measure of drug exposure, was five times higher in patients also receiving cyclosporine.[99] Of the calcium channel antagonists, diltiazem, nicardipine, and verapamil increase cyclosporine concentrations; nifedipine and amlodipine have variable effects; and isradipine and nitrendipine do not generally affect concentrations.[100] It is controversial whether nonsteroidal anti-inflammatory drugs (NSAIDs) increase cyclosporine nephrotoxicity. In many clinical studies, cyclosporine and NSAIDs have been safely co-administered[79,101]; however, increased cyclosporine-associated nephrotoxicity with NSAIDs has been reported. Currently, many patients starting cyclosporine also take an NSAID. If the creatinine increases, in addition to decreasing the dose of cyclosporine, discontinuation of the NSAID may be tried. Grapefruit juice increases plasma concentrations of cyclosporine, so patients should be warned to avoid consuming grapefruit.

## TACROLIMUS (FK506)

### Structure

Tacrolimus, previously known as FK506, is a macrolide derived from an actinomycete and is widely used in organ transplantation as an alternative to cyclosporine. Studies in rheumatic autoimmune disease are less advanced.

### Mechanisms of Action

Tacrolimus is about 100 times more potent than cyclosporine and, although structurally different, is also a calcineurin inhibitor. Tacrolimus binds to an intra-cellular binding protein (FK binding protein), and this drug-immunophilin complex, in association with calcineurin, suppresses transcription of cytokines such as IL-2, inhibiting the early steps of T lymphocyte activation (see Figure 62-3).[74]

### Pharmacology

Absorption of tacrolimus after oral administration is poor and highly variable (range, 4% to 93%; average, 25%).[102] Tacrolimus is lipophilic, is widely distributed in tissues, and is almost completely metabolized, with an elimination half-life of 5 to 16 hours.[103] As with cyclosporine, Pgp and CYP3A4 in the liver and gut are important determinants of the metabolism and disposition of tacrolimus. Drugs that inhibit CYP3A4 or Pgp can increase tacrolimus concentrations (see Table 62-4).[102] Impaired hepatic function, but not impaired renal function, increases tacrolimus concentrations.[103]

### Dosage

Tacrolimus is not routinely used for rheumatologic conditions. Different dosages have been used in clinical studies (see the next paragraph).

### Clinical Indications

Tacrolimus has been studied in a number of rheumatic conditions with variable success. In RA, tacrolimus showed only modest efficacy, and with the availability of a plethora of effective conventional and biologic disease-modifying anti-rheumatic drugs, the need for a novel immunosuppressant such as tacrolimus has waned.[104,105] Topical 1% tacrolimus has been used with moderate success in patients with resistant skin disease resulting from SLE, subacute cutaneous lupus erythematosus, and discoid lupus erythematosus.[106] A large open-label, randomized, controlled trial in 150 patients with biopsy-confirmed active lupus nephritis showed that remission induction with tacrolimus (0.6 mg/kg/day) was not inferior to MMF (2-3 g/day) when given with prednisolone (0.6 mg/kg/day) for 6 weeks and followed by azathioprine maintenance, with major infectious episodes occurring in 5.4% versus 9.2% of patients, respectively.[107] In a retrospective study in patients who had interstitial lung disease associated with polymyositis or dermatomyositis, patients treated with tacrolimus in addition to conventional therapy had a significantly improved survival compared with patients who were treated with conventional therapy alone.[108]

### Toxicity

The adverse effects of tacrolimus are dose related and include nephrotoxicity, hypertension, hyperkalemia, hyperuricemia, tremor, hyperglycemia, and gastrointestinal intolerance.[102] Among patients with RA who took 3 mg of tacrolimus daily for more than 1 year, 59% experienced an adverse effect, probably or possibly due to the drug, including diarrhea (15%); nausea (10%); tremor (9%); headache (9%); abdominal pain (8%); increased creatinine (7%); hypertension (5%); and pneumonia, pancreatitis, hyperglycemia, and diabetes mellitus (all <1%). A creatinine increase of greater than 30% from baseline to the end of the study was found in 40%.[109]

## MYCOPHENOLATE MOFETIL

### Structure

MMF, a prodrug, is the inactive 2-morpholinoester of mycophenolic acid, which is hydrolyzed to the active mycophenolic acid (MPA), an antibiotic with immunosuppressive effects.[110]

### Mechanism of Action

Two pathways exist for the synthesis of guanine nucleotides: the de novo pathway and the salvage pathway. MPA reversibly inhibits inosine monophosphate dehydrogenase, a crucial enzyme for the de novo synthesis of guanosine purines.[110,111] Lymphocytes, in contrast to many other cells,

are critically dependent on the de novo purine synthesis pathway and are a relatively selective target for MPA, accounting for the ability of the drug to inhibit reversibly B cell and T cell proliferation without myelotoxicity.[112] MPA results in decreased guanine synthesis and decreased DNA synthesis, decreased lymphocyte proliferation, altered microRNA expression, and decreased antibody production.[111-114] MPA also inhibits proliferation of fibroblasts, endothelial cells, and arterial smooth muscle cells and prevents deposition and contraction of collagen, extra-cellular matrix proteins, and smooth muscle actin.[115]

## Pharmacology

MMF is rapidly and completely absorbed and de-esterified to the active MPA, which is highly (98%) protein bound. Most MPA (>99%) is found in plasma, with little in cells; most is glucuronidated to the poorly active, stable phenolic glucuronide, which is eliminated in the urine (Figure 62-4).[116] Minor metabolites, some of which may be active, have also been described. Peak levels of MPA occur 1 to 2 hours after administration, and secondary peaks, thought to be due to enterohepatic circulation, can be seen. The half-life of MPA is 16 hours.[116] MPA concentrations may vary fivefold to tenfold in persons receiving the same dose.[117] A small amount of this variability may be due to genetic variation in uridine-glucuronosyltransferase enzymes.[118] Renal disease and liver disease have relatively minor effects on the disposition of the active drug, MPA. Generally, dosage adjustments are not required,[116] but because free MPA concentrations are approximately doubled in patients with severe renal impairment (creatinine clearance <20 to 30 mL/min),[119] they may be necessary sometimes. A retrospective study in 16 patients with lupus nephritis showed that concentration-controlled dose adjustments with a target MPA-AUC (from intake until 12 hours after ingestion) of 60 to 90 mg*h/L was associated with optimized MPA exposure and excellent renal outcome at 12 months, although adverse effects were reported in one third of patients, resulting in a switch to azathioprine in two patients.[120] Another observational study also pointed to the potential benefits of therapeutic monitoring in 34 patients with lupus nephritis when dosing was adjusted to achieve an AUC of 30 to 60 mg*h/L.[121] In this study MPA exposure was not associated with adverse events, and patients with an AUC of 30 mg*h/L or greater had a greater renal response at 1 year. The major glucuronide metabolite of MPA accumulates in patients with impaired renal function and may cause increased gastrointestinal adverse effects. Because MPA is highly protein bound, it is not cleared by hemodialysis.[122]

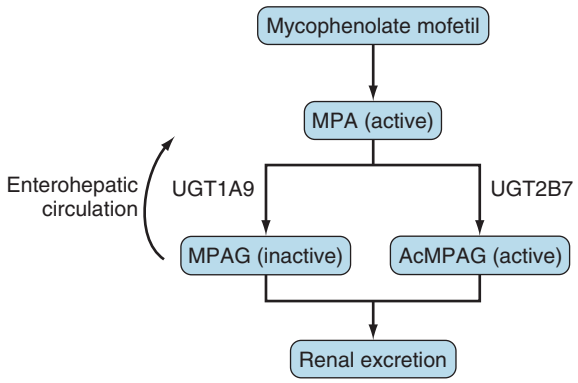



**Figure 62-4** Mycophenolate mofetil is converted to mycophenolic acid (MPA) and is subsequently metabolized to its glucuronide (Glu) conjugates, MPAG and AcMPAG, by different isoforms of UDP glucuronidyl transferases (UGT) in the liver. Pathways of enterohepatic circulation of MPA via the glucuronide conjugate metabolites are shown. The majority of MPAG is excreted in urine.

## Toxicity

MMF is generally well tolerated. The most common adverse effects are gastrointestinal, such as diarrhea, nausea, abdominal pain, and vomiting. Occasional infections, leukopenia, lymphocytopenia, and elevated liver enzymes can occur. Of 54 patients with SLE treated with MMF over a 3-year period, 16% withdrew because of adverse events, with 73% continuing treatment at 12 months.[123] In patients with lupus nephritis, diarrhea was more common and serious infections were less common with MMF than with cyclophosphamide.[41] Enteric-coated mycophenolate sodium and MMF have similar rates of adverse effects.[124] Opportunistic infections, including one that was fatal, occurred in 3 of 10 patients with idiopathic dermatomyositis who were treated with glucocorticoids and MMF.[125]

## Dosage

Effective daily dosages of MMF range from 0.5 to 1.5 g twice a day. In 71 patients with lupus nephritis, the initial dose was 1 g/day with a target of 3 g/day. The mean maximal dose was 2680 mg/day, and 63% of patients tolerated 3 g/day.[39]

## Clinical Indications

MMF is used as a safer alternative to cytostatic agents in the treatment of several rheumatic diseases, notably SLE,[126] systemic sclerosis,[127] vasculitis,[128] and inflammatory muscle disease.[125,129] In a 24-week study in persons with lupus nephritis, mycophenolate was more effective than monthly pulse cyclophosphamide, with a failure rate (without complete or partial remission at 24 weeks, plus those who stopped treatment for any reason) of 34 of 71 (47.9%) compared with 48 of 69 in the cyclophosphamide group (69.6%; $P = 0.01$).[39] In a systematic review of four trials involving 618 patients, MMF was not superior to cyclophosphamide for renal remission, and there was no significant difference for adverse events (infections, leukopenia, gastrointestinal symptoms, herpes zoster, end-stage renal disease, and death) except for a lower incidence of alopecia and amenorrhea with the use of MMF compared with cyclophosphamide.[130] Notwithstanding its appeal as a safer alternative to cyclophosphamide for remission induction therapy of lupus nephritis, MMF is most commonly used for maintenance therapy of lupus nephritis, although systematic reviews comparing its efficacy and safety with azathioprine have not yielded consistent results.[131,132] A study on repeat kidney biopsies in 30 patients with lupus nephritis failed

to detect differences between azathioprine and MMF as maintenance therapy after a short course of IV cyclophosphamide.[133] MMF is an alternative remission induction agent for patients with anti-neutrophil cytoplasmic antibody–associated vasculitis and evidence of low disease activity and who are not at risk of experiencing organ damage.[134] MMF was slightly less effective than azathioprine in maintaining remission, however.[134a] In seven patients with myositis, six had a good clinical and biochemical response to mycophenolate,[129] an observation that was confirmed in another study in six patients who had refractory myositis.[135] In addition, in three studies in patients who had interstitial lung disease associated with dermatomyositis ($n$ = 4) or other connective tissue diseases, including RA ($n$ = 10) and systemic sclerosis ($n$ = 13), MMF was effective in improving signs and symptoms of lung disease.[136-138] MMF may be useful as an alternative immunosuppressant to azathioprine, particularly in patients with gout who require therapy with allopurinol because, in contrast to azathioprine, it does not seem to interact significantly with allopurinol.[72,112] Mycophenolate, 1 g twice daily, was not more effective than placebo in two clinical trials involving 443 patients with refractory RA[139] as assessed with ACR20 responses, and a larger mycophenolate-cyclosporine trial was stopped prematurely. Treatment-related adverse events were experienced by 51.6%, 73.1%, and 36.1% of patients receiving MMF, cyclosporine, and placebo, respectively. Hypertension, increased serum creatinine, muscle cramps, hirsutism, and hypertrichosis were more than twice as common with cyclosporine as with MMF. In all three trials the incidence of serious adverse events with MMF was 12.1% (compared with 11.3% and 7.5% for cyclosporine and placebo, respectively). Although mycophenolate is used in psoriasis as an effective alternative to methotrexate, there are only anecdotal reports of its utility in persons with psoriatic arthritis.

### Pregnancy and Lactation

MMF is an FDA Pregnancy Category C drug. Mycophenolic acid is associated with miscarriage and congenital malformations when used during pregnancy, and therefore it should be avoided whenever possible by women trying to conceive. Switching from MMF to azathioprine in 19 patients with SLE did not result in deterioration of global disease activity, and another study in 54 patients with SLE who had quiescent lupus nephritis showed that replacing MMF with azathioprine rarely leads to renal flares, while pregnancy outcomes were favorable.[140,141] MMF is transferred into the mother's milk, and extreme caution should be used in women with childbearing potential and lactating mothers.

### Drug Interactions

Because MPA is glucuronidated and is not metabolized by CYP oxidation, there are few clinically significant drug interactions. Antacids reduce bioavailability by approximately 15%, and cholestyramine reduces bioavailability by approximately 40%.[142] Rifampin treatment reduced MPA concentrations twofold to threefold.[143] Co-administration with azathioprine is not recommended.

## CONCLUSION

Immunosuppressive drugs are key therapeutic tools in the management of many rheumatic diseases. They include alkylating agents such as cyclophosphamide and purine analogue cytotoxic drugs such as azathioprine with a long history of clinical use in rheumatology, as well as noncytotoxic immunosuppressants such as MMF. In contrast to extra-cellular, exquisitely targeted therapeutics represented by biologics, our understanding of the in vivo mechanism of action of immunosuppressive drugs is still limited. In contrast, their potential efficacy and safety profiles are generally well known, and serious toxicities can usually be prevented by careful monitoring of laboratory tests for white blood cell counts, liver and renal function, and electrolytes. Nevertheless, vigilance is required, because the risk of (opportunistic) infection is increased.[144,145] As a general rule, combination therapy of the different immunosuppressants discussed earlier should be avoided. The individual response to immunosuppressive therapy can be highly variable, and decisions to continue a chosen immunosuppressant should be revisited on a regular basis, weighing the benefits and adverse effects.



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CHAPTER 63

# Anti-cytokine Therapies



Zuhre Tutuncu • Arthur Kavanaugh

**KEY POINTS**

- Inhibiting a single key cytokine can be effective in treatment of autoimmune and inflammatory diseases.
- Most patients with rheumatoid arthritis (RA) have at least a partial response to treatment with tumor necrosis factor (TNF) inhibitors, with some patients achieving quite significant improvements in the signs and symptoms of disease.
- Maintaining clinical efficacy with TNF inhibitors usually requires continued therapy. However, there might be a window of opportunity in the early stage of RA or for patients achieving very low levels of disease activity for inducing long-term remission.
- Treatment with TNF inhibitors significantly decreases radiographic damage, improves quality of life, and helps preserve functional status.
- Combination of a TNF inhibitor with methotrexate achieves additive benefits.
- TNF inhibitors have also proven highly effective in treating patients with ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis, and juvenile idiopathic arthritis.
- TNF inhibitors have been ineffective in the treatment of patients with vasculitis (granulomatosis with polyangiitis and giant cell arteritis).
- Interleukin (IL)-6 inhibition is an effective therapy in RA, with similar improvement in signs and symptoms to treatment with TNF inhibitors as well as in disease progression.
- Although IL-1 inhibition is generally less effective in RA than TNF inhibition, this approach can be highly effective in certain autoinflammatory conditions (e.g., periodic fever syndromes).
- Combination biologic therapy appears to increase the risk of side effects, such as infection, without additional benefits.

In recent years, discoveries that show the immunopathophysiologic basis of rheumatic diseases, combined with biopharmaceutical developments, have introduced biologic therapeutics. These agents target specific components of the immune response that are dysregulated and are thought to be central to the cause and sustenance of the disease process. In the rheumatoid synovium, for example, substantial evidence has been found of upregulation of key proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and others.[1,2] Agents that target these key mediators, such as TNF, have considerable efficacy in the treatment of patients with rheumatoid arthritis (RA) and other systemic inflammatory disorders. The perception of both physicians and patients toward antirheumatic treatment has been altered by the ability of TNF and other cytokine inhibitors to not only to improve the signs and symptoms of disease, but also to preserve functional status and quality of life, and to inhibit disease progression. Moreover, their success has led to research into the targeting of other cytokines relevant to the pathogenesis of autoimmune disorders. In this chapter, we focus on therapeutic agents that target TNF, IL-1, and IL-6.

## TUMOR NECROSIS FACTOR INHIBITORS

TNF plays a central role in the pathogenesis of RA and other inflammatory disorders. Although it can be produced by numerous cell types, in inflammatory conditions such as RA, TNF is produced largely by activated macrophages. Human TNF is synthesized and expressed as a 26 kDa transmembrane protein on the plasma membrane and is cleaved by a specific metalloproteinase (TNF-converting enzyme). After proteolytic cleavage, TNF is converted to a 17 kDa soluble protein, which oligomerizes to form the active homotrimer. The actions of TNF are mediated through two structurally distinct receptors: TNF-RI (55 kDa; CD120a) and TNF-RII (75 kDa; CD120b).[3] The two receptors differ in their binding affinities, signaling properties, and primary functions.[3,4] The binding of TNF to its receptor initiates several signaling pathways. Signaling cascades include the activation of transcription factors (e.g., nuclear factor-κB [NF-κB]), protein kinases (intra-cellular enzymes that mediate cellular responses to inflammatory stimuli, such as c-Jun N-terminal kinase [JNK] and p38 mitogen-activated protein [MAP] kinase), and proteases (enzymes that cleave peptide bonds, such as caspases).

TNF may contribute to the pathogenesis of RA through myriad mechanisms, including induction of other proinflammatory cytokines (e.g., IL-1, IL-6) and chemokines (e.g., IL-8); enhancement of leukocyte migration by increasing endothelial layer permeability and adhesion molecule expression and function; activation of numerous cell types; and induction of the synthesis of acute phase reactants and other proteins, including tissue-degrading enzymes (matrix metalloproteinase enzymes) produced by synoviocytes or chondrocytes. The pivotal role of TNF in mediating such diverse inflammatory activities provided the rationale for targeting this cytokine in systemic inflammatory diseases.[5]

Initially, animal studies proved that inhibition of TNF with monoclonal antibodies or soluble TNF-R constructs ameliorated the signs of inflammation and prevented joint destruction.[6] Subsequently, studies in humans confirmed the substantial efficacy of these compounds.

Currently, five anti-TNF agents are potentially available for clinical use: infliximab, a chimeric anti-TNF monoclonal antibody; etanercept, a soluble dimeric p75-TNF-R/Fc fusion construct; adalimumab, a human anti-TNF monoclonal antibody; golimumab, a human anti-TNF monoclonal antibody; and certolizumab pegol, a Fab fragment of a recombinant, humanized anti-TNF monoclonal antibody linked to a 40 kDa polyethylene glycol (PEG) moiety. Additional agents, including biosimilars, will potentially be available in the near future. For each agent, initial assessment in open-label studies of patients with RA was followed by double-blind, placebo-controlled, randomized clinical trials. Typically, early studies included patients with very active disease that was relatively chronic and refractory. Driven by success in the most difficult populations, studies of all TNF inhibitors have also been performed in patients with early RA. Most studies included patients whose disease remained active despite concurrent use of methotrexate (MTX); some studies assessed the efficacy of a drug as monotherapy. Building on the efficacy achieved in RA, TNF inhibitors have been tested in other inflammatory arthritides, including psoriatic arthritis and ankylosing spondylitis. Moreover, these agents have been studied in other autoimmune conditions, such as Crohn's disease, ulcerative colitis, psoriasis, uveitis, and others.

Although all five available agents are macromolecule TNF inhibitors, some pharmacologic and pharmacodynamic differences among them have been noted.[7] The monoclonal antibodies infliximab, adalimumab, golimumab, and certolizumab pegol are specific for TNF, whereas etanercept binds both TNF and lymphotoxin-α (LT-α; previously referred to as *lymphotoxin*). Given intravenously, infliximab has a high peak concentration (Cmax) followed by steady-state elimination, whereas etanercept, adalimumab, golimumab, and certolizumab pegol, because they are given subcutaneously, have "flatter" pharmacokinetic profiles. With the exception of certolizumab pegol, these agents are capable of affecting Fc-mediated functions, such as complement-dependent cytolysis and antibody-dependent cell-mediated cytotoxicity, and all bind to both soluble and membrane forms of TNF, although some relative differences in affinity may be noted. Other differences, such as effects on cytokine secretion, have been observed in some in vitro studies.[8] Data on apoptosis have been somewhat discrepant. In patients with RA, both the anti-TNF monoclonal antibody infliximab and the soluble receptor construct etanercept can induce apoptosis in synovial macrophages.[9] However, in patients with Crohn's disease, etanercept was not clinically effective at the doses studied and did not induce apoptosis. In contrast, the anti-TNF monoclonal antibodies infliximab and adalimumab were clinically effective and induced apoptosis in highly activated lymphocytes.[10] However, certolizumab pegol is effective in Crohn's disease and does not induce apoptosis. The extent to which these potential differences among TNF inhibitors correlate with any specific aspects of efficacy or toxicity remains to be established.

## Infliximab

### Structure

Infliximab is a chimeric mouse-human monoclonal antibody composed of constant regions of human immunoglobulin (Ig)G1κ coupled to the variable regions of a high-affinity neutralizing murine anti–human TNF antibody. The resulting construct is approximately 70% human (Figure 63-1).

### Pharmacokinetics

Clinical pharmacology studies demonstrate that infliximab has a dose-dependent pharmacokinetic profile following infusions of 1 to 20 mg/kg. In combination therapy with MTX (7.5 mg once a week), serum infliximab concentrations tend to be slightly higher than when administered alone.[11] Infliximab behaves in a consistent manner across different demographic groups (including pediatric vs. adult patients) and among patients with different diseases of varied severity. The half-life of infliximab is approximately 8 to 9.5 days at the 3 mg/kg dose, although longer values have been reported for higher doses. The volume distribution of infliximab at steady state (3 to 5 L) is independent of dose, suggesting a predominantly intravascular distribution. Concomitant use of MTX results in an increase in the area under the curve of infliximab of approximately 25% to 30%.

### Drug Dose

The typical initial dose of infliximab in RA is 3 mg/kg given as an intravenous (IV) infusion in combination with MTX, followed by doses 2 and 6 weeks after the first infusion, then every 8 weeks thereafter. Some RA patients have received infliximab in combination with disease-modifying antirheumatic drugs (DMARDs) other than MTX or as monotherapy. For patients who have an incomplete response, dosing may be increased as high as 10 mg/kg, or the drug may be administered as often as every 4 weeks. In the clinic, increasing the dose of infliximab or decreasing the interval of administration is not an uncommon practice; however, it is not clear to what extent such changes achieve clinical improvements. For patients with psoriatic arthritis and ankylosing spondylitis, the recommended dose is 5 mg/kg, with or without MTX, at 0, 2, and 6 weeks, then every 8 weeks.

## Etanercept

### Structure

Etanercept is formed by the linkage of two soluble p75 TNF-R extra-cellular domains to the Fc portion of human $IgG_1$ (see Figure 63-1). The resultant molecule binds both TNF and LT-α with high affinity and specificity. The TNF-R domains in etanercept bind to two of the three receptor binding sites on the TNF trimer, thus blocking the ability of TNF to interact with cell-bound TNF-R—a prerequisite for signal transduction (see Figure 63-1).

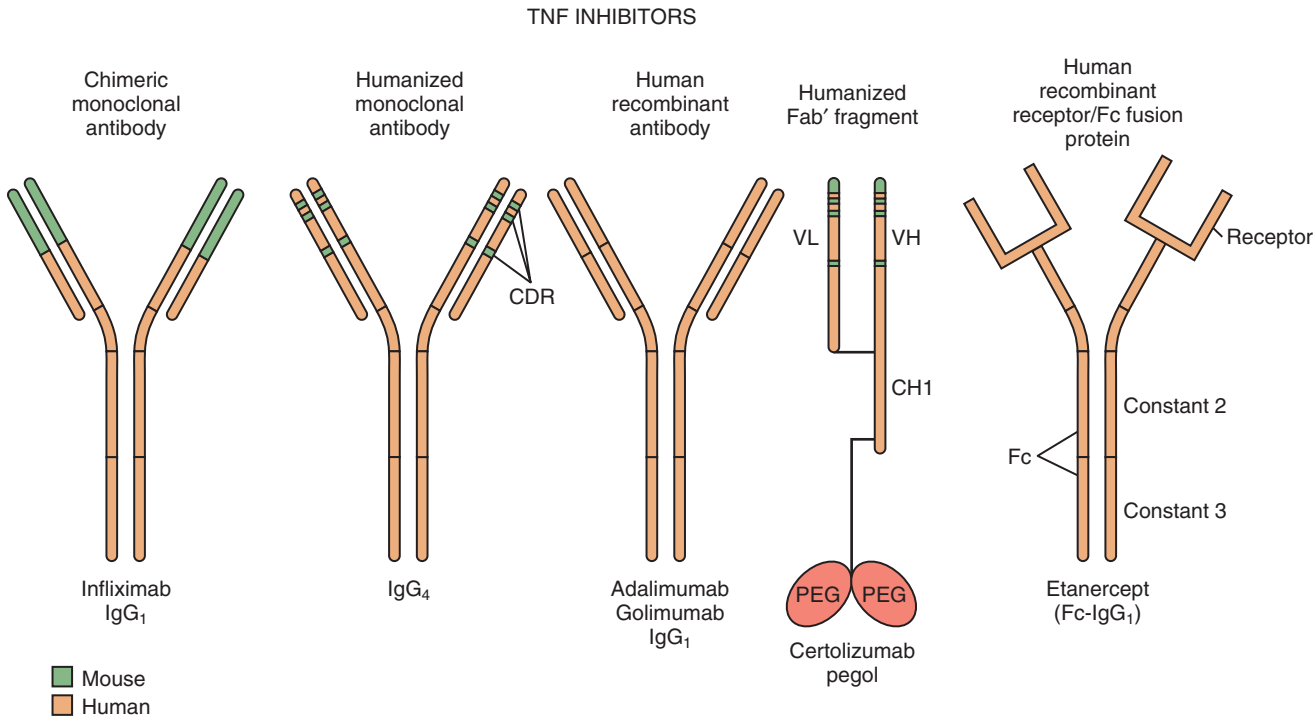



**Figure 63-1** Structures of infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol. CDR, Complementarity-determining region; CH1, complement fixation; Fc, fragment crystallizable; FDA, U.S. Food and Drug Administration; PEG, polyethylene glycol; TNF, tumor necrosis factor; VH, variable heavy; VL, variable light.

### Pharmacokinetics

When administered subcutaneously, etanercept is absorbed slowly, reaching a mean Cmax approximately 50 hours after a single 25 mg dose. The Ig structure affords a half-life of 3 to 4.8 days. The volume of distribution suggests predominantly intravascular distribution.[12] The route of clearance from the circulation is unclear, although it is presumed to be mediated through Fc binding by the reticuloendothelial system. Different from what has been seen with anti-TNF monoclonal antibodies, concomitant use of MTX does not appear to alter the pK of etanercept.

### Drug Dose

Etanercept is administered by subcutaneous injection in doses of 25 mg twice weekly or 50 mg once weekly in RA, psoriatic arthritis (PsA), and ankylosing spondylitis (AS). Etanercept is approved for use as monotherapy or in combination with MTX. In skin psoriasis, a higher dose (50 mg twice weekly) is commonly used for the first 12 weeks of therapy. As is true of all available TNF inhibitors, in the clinic, etanercept has been used in combination with DMARDs other than MTX, for example, leflunomide, sulfasalazine, and others.

## Adalimumab

### Structure

Adalimumab is a human anti-TNF $IgG_1\kappa$ monoclonal antibody generated through repertoire cloning. Adalimumab neutralizes the biologic activity of TNF by binding with high affinity to the soluble and transmembrane forms of TNF and inhibiting the binding of TNF with its receptors (see Figure 63-1).

### Pharmacokinetics

The peak serum adalimumab concentration and the area under the curve increase linearly with doses in the range of 0.5 to 10 mg/kg. Adalimumab appears to have a low clearance and distributes mainly in the vascular compartment. Its elimination half-life is comparable with that of native $IgG_1$ (10 to 13.6 days). Concomitant use of MTX results in an approximate increase in the area under the curve of adalimumab of 25% to 30%.

### Drug Dose

The recommended dosing for adalimumab in RA, PsA, and ankylosing spondylitis is 40 mg subcutaneously every other week. In patients who do not achieve an optimal response, it is possible to increase the dosing frequency to weekly. Adalimumab is approved for use as monotherapy or in combination with MTX. In the clinic, it has been used in combination with a number of DMARDs other than MTX.

## Golimumab

### Structure

Golimumab is a human $IgG_1\kappa$ monoclonal antibody specific for human TNF. Golimumab was created by using

genetically engineered mice immunized with human TNF, resulting in an antibody with human-derived antibody variable and constant regions (see Figure 63-1).

### Pharmacokinetics

Following subcutaneous administration of golimumab to healthy patients and patients with RA, the median time to reach time to peak concentration (Tmax) ranged from 2 to 6 days. Golimumab exhibited dose-proportional pharmacokinetics in patients with active RA over the dose range of 0.1 to 10.0 mg/kg following a single IV dose. The volume distribution for golimumab indicates that golimumab is distributed primarily in the circulatory system with limited extravascular distribution. Median terminal half-life values were estimated to be approximately 2 weeks in healthy patients and patients with RA, PsA, or AS. When golimumab, 50 mg, was administered subcutaneously to patients with RA, PsA, or AS every 4 weeks, serum concentrations appeared to reach steady state by week 12. With concomitant use of MTX, treatment with golimumab every 4 weeks resulted in a mean steady-state trough serum concentration of approximately 0.4 to 0.8 μg/mL in patients with RA, PsA, or AS. Concomitant MTX usage resulted in higher mean steady-state trough concentrations of golimumab in patients with RA, PsA, or AS (52%, 36%, and 21%, respectively), compared with those treated with golimumab alone.[13]

### Drug Dose

Golimumab is administered by subcutaneous injection, 50 mg once a month, in patients with RA, PsA, and AS.

## Certolizumab Pegol

### Structure

Certolizumab pegol is a Fab fragment of a recombinant, humanized anti-TNF monoclonal antibody that has been fused to a 40 kDa PEG moiety. The Fab fragment is composed of a light chain with 214 amino acids and a heavy chain with 229 amino acids. Given this structure, certolizumab is not able to cause Fc-mediated activities complement-dependent or antibody-dependent cytotoxicity as seen with full monoclonal antibodies (see Figure 63-1).

### Pharmacokinetics

Single IV and subcutaneous doses of certolizumab pegol have predictable dose-related plasma concentrations with a linear relationship between the dose administered and the Cmax. A mean Cmax of approximately 43 to 49 μg/mL occurred at week 5 in patients with RA at the recommended dose regimen. Following subcutaneous administration, peak plasma concentrations of certolizumab pegol were attained between 54 and 171 hours postinjection. PEGylation, attachment of polyethylene glycol to Fab, delays the metabolism and elimination of these entities from the circulation by a variety of mechanisms, including renal clearance, proteolysis, and immunogenicity. The terminal elimination phase half-life was approximately 14 days for all doses tested.

### Drug Dose

The recommended dose of certolizumab pegol is 400 mg initially and at weeks 2 and 4, followed by 200 mg every other week. For maintenance dosing, certolizumab pegol 400 mg every 4 weeks can be considered. A 400 mg dose is given as two subcutaneous injections of 200 mg at separate sites in the thigh or abdomen.

### Efficacy of Tumor Necrosis Factor Inhibitors

**Rheumatoid Arthritis.** Infliximab, etanercept, and adalimumab were the first TNF inhibitors approved in many countries worldwide to treat RA. Initial studies demonstrated the efficacy and tolerability of various doses of TNF inhibitors and also established the optimal doses.[14–21] Concurrent therapy with MTX, even at a relatively low dose of 7.5 mg/wk, enhances the clinical response to all agents, and for infliximab and adalimumab, concomitant MTX decreases immunogenicity.[11] Almost all subsequent studies with TNF inhibitor drugs in RA have used such combination therapy.

Earlier trials demonstrated the efficacy and tolerability of infliximab, etanercept, and adalimumab in patients with long-standing, refractory, active disease despite MTX. All three agents have undergone extensive clinical trials that have shown superior efficacy compared with MTX alone as assessed by American College of Rheumatology (ACR) criteria.[19–25] In addition to achieving substantial efficacy, the use of TNF inhibitors was associated with significant improvement in patient's functional status and quality of life.[12,22,25] Perhaps most remarkably, patients receiving TNF inhibitors had a dramatic reduction in the progression of joint damage as assessed by radiographic change scores.[17,22,26]

Following the success achieved in patients with long-standing RA, this therapy was tested in patients with early RA (<3 years' duration). In the Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE) trial, significant improvement in disease activity was noted in patients treated with infliximab plus MTX in comparison with MTX alone, at 54 weeks. In addition, although a significant increase in radiologic destruction was observed in patients treated only with MTX, a reduction in disease progression was observed in the infliximab plus MTX groups.[27] A detailed subanalysis of data from the Anti-TNF Therapy in RA with Concomitant Therapy (ATTRACT) study demonstrated that a significant radiographic benefit was achieved with infliximab plus MTX treatment even among patients who experienced no improvement in signs and symptoms of disease. This finding suggests that there may be an uncoupling among various outcomes, and that treatment with TNF inhibitors might lead to disease-modifying activity even in the absence of a clinical response.[28] Similar to infliximab, etanercept and adalimumab were effective in decreasing signs and symptoms of patients with early-stage RA and decreasing disease activity and the rate of radiographic progression. In long-term, open-label follow-up studies of patients from the clinical trials, responses to etanercept therapy have been sustained during a number of years, with some patients having received more than a

decade of treatment.[19] In the PREMIER trial, significantly less radiographic progression was observed among patients in the adalimumab plus MTX group at both 1 year and 2 years in comparison with the adalimumab or MTX-alone groups, although progression was less with adalimumab monotherapy than with MTX monotherapy.[29] Because this study was the first to include all three possible treatment arms (TNF inhibitor, MTX, and TNF inhibitor plus MTX), the study definitively established that combination therapy with MTX and TNF inhibitor achieves the best outcomes in patients with early-stage RA. Although TNF inhibitor monotherapy was superior to MTX alone in terms of radiographic progression, clinical efficacy was comparable; however, each of these was substantially less effective than the combination.

In the years after these studies, golimumab and certolizumab were approved for treatment of RA. In the GO-FORWARD study, patients with active RA despite treatment with a stable dose of MTX who had not been previously treated with a biologic TNF inhibitor were randomly assigned to receive golimumab, 50 mg, plus MTX; golimumab, 100 mg, plus MTX; or golimumab, 100 mg, monotherapy every 4 weeks. All golimumab regimens were significantly more effective at reducing signs and symptoms and in improving physical function.[30] Patients who were previously treated with one or more doses of TNF inhibitor without a serious adverse reaction were enrolled in the GO-AFTER study. Greater efficacy measured by ACR20 and Disease Activity Score (DAS) 28 was observed in patients who received golimumab 50 mg plus MTX with patients who received MTX alone.[31] Patients with active RA who were MTX naïve and had not been previously treated with TNF inhibitor were randomly assigned to treatment with MTX alone; golimumab, 50 mg, plus MTX; golimumab, 100 mg, plus MTX; or golimumab, 100 mg, alone in the GO-BEFORE study. Golimumab plus MTX led to significant improvement in ACR responses.[32] An MRI substudy of the GO-BEFORE and GO-FORWARD trials demonstrated that patients who received golimumab plus MTX had improvements in inflammation (synovitis and osteitis) greater than patients with received MTX only, as early as week 12 and continuing through week 24.[33]

In the Rheumatoid Arthritis Prevention of Structural Damage (RAPID)-I and RAPID-II studies, lyophilized liquid preparations of certolizumab were evaluated. Patients were randomly assigned to receive loading doses of 400 mg of certolizumab pegol or placebo at weeks 0, 2, and 4, followed by 200 mg of certolizumab pegol, 400 mg certolizumab pegol, or placebo every other week in combination with MTX. Certolizumab pegol–treated groups showed rapid and sustained improvement in clinical disease activity, starting from week 1 and continuing through week 52. Mean radiologic progression was significantly reduced in the certolizumab-treated groups versus MTX alone.[34–36] Sustainability of improvements in RA signs and symptoms and inhibition of joint damage progression, as well as tolerability of certolizumab pegol plus MTX, were evaluated in the RAPID-II open-label extension study.[37] In a broader group of RA patients with inadequate response to more than one DMARD, including prior TNF inhibitor exposure, addition of certolizumab pegol to the regimen resulted in rapid clinical response measured with ACR20 response rates and DAS28 scores, and improved function measured with Health Assessment Questionnaire (HAQ) scores.[38]

**Psoriatic Arthritis (PsA).** The impressive efficacy of TNF inhibitors in RA led to studies of these agents in patients with PsA. Similar to RA trials, multiple randomized clinical trials with five TNF inhibitors demonstrated significant improvement in disease activity, quality of life, function, and slowing of disease progress as evidenced by radiographic changes. In addition, disease-specific clinical findings such as skin and nail psoriasis, enthesitis, and dactylitis have also been shown to improve significantly.

In the Infliximab Multinational Psoriatic Arthritis Controlled Trial 1 (IMPACT 1), the response to infliximab therapy defined by ACR20 and Psoriatic Arthritis Response Criteria was evident as early as week 2, and improvement continued through week 50. Significant improvements were also seen in skin psoriasis and in dactylitis and enthesitis assessments.[39] In the phase III (IMPACT 2) trial, clinical improvement was seen very early in the infliximab-treated groups, and the response was maintained with continued treatment through the end of the study. In the same group of patients, infliximab inhibited radiographic progression and quality of life.[40,41]

In the initial double-blind, placebo-controlled clinical trial with etanercept, substantial improvements were observed in PsA and ACR20 response criteria.[42] In a subsequent study, 59% of etanercept-treated patients met the ACR20 criteria, compared with 15% of the placebo group. During the open-label extension, patients continuing with etanercept treatment maintained or improved their clinical responses and showed inhibition of radiographic progression.[43]

Similar success was achieved in clinical trials with adalimumab. A significantly higher percentage of patients from the adalimumab-treated group reached ACR20, ACR50, and ACR70 response criteria compared with the placebo group.[44] Moreover, the rate of radiographic damage progression was significantly attenuated with adalimumab. As was true in studies of the other TNF inhibitors in PsA, MTX was permitted but not required for enrollment in the study. With this design, there seemed to be no difference in efficacy or toxicity between the combination of MTX plus adalimumab and adalimumab monotherapy. In addition to improvements in various articular manifestations, dramatic improvements were noted in skin psoriasis with adalimumab treatment.

In the GO-REVEAL study, patients with active PsA despite nonsteroidal anti-inflammatory drug (NSAID) or DMARD therapy who had not been previously treated with TNF inhibitor were randomly assigned to receive golimumab, 50 mg or 100 mg, with or without MTX every 4 weeks. Fifty-one percent of patients who received golimumab reached ACR20 response at week 14 in comparison with 9% of patients who received only MTX. Significant improvements in enthesitis and HAQ scores were seen in patients treated with golimumab.[45] Long-term radiologic outcome was assessed by using the van der Heijde–Sharp (vdH-S) method modified for PsA and showed minimal evidence of radiographic disease progression through week 104.[46]

In the RAPID-PsA study, patients with and without prior TNF inhibitor exposure were included. Active and

progressive PsA patients received a loading dose of certolizumab 400 mg, followed by either 200 mg every other week, 400 mg every 4 weeks, or placebo every other week. ACR20 results showed that certolizumab rapidly improved signs and symptoms, with patient response observed as early as the first week of treatment for some patients. ACR20, -50, -70 response rates were higher for each certolizumab group relative to the placebo group. Patients treated with certolizumab 200 mg every other week demonstrated greater reduction in modified total Sharp score (mTSS) compared with placebo-treated patients.[47] Statistically significant improvement in physical function from baseline measured by Health Assessment Questionnaire Disability Index (HAQ-DI) was reported in comparison with the placebo group. Sustained improvements were observed in enthesitis and dactylitis as well.

**Ankylosing Spondylitis.** Five TNF inhibitors have been widely studied in AS; all showed remarkable similarity in their efficacy in relieving symptoms of AS. Studies have shown that TNF inhibitors improve health-related quality of life, patient-reported outcomes, anemia, C-reactive protein (CRP) levels, and sleep quality in patients with AS. TNF inhibitors control inflammation in the spine, as measured by various MRI sequences. Interestingly, despite all of these notable effects, TNF inhibitor therapy does not attenuate radiographic progression in AS. There are several potential reasons for this. Unlike RA, radiographic outcomes in AS measure reflect mainly osteoblastic activity with syndesmophyte formation; it is possible this is affected less by TNF inhibition. Also, the study durations may not have been long enough to show an effect. In that regard, longer-term treatment, that is longer than 4 years, with TNF inhibitors might reduce the rate of new bone formation in patients with AS.[48]

In the initial double-blind, placebo-controlled, randomized trial with infliximab, disease activity in patients taking infliximab measured by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), functional ability measured by the Bath Ankylosing Spondylitis Functional Index (BASFI), and mobility of the spine measured by the Bath Ankylosing Spondylitis Metrology Index (BASMI) significantly improved compared with the placebo group.[49] A significantly higher percentage achieved 50% or greater improvement in the BASDAI, and 25% of patients who completed the trial were in partial remission, according to the Assessment in Ankylosing Spondylitis (ASAS) criteria among patients who continued to receive infliximab every 6 weeks until week 102.[50] In addition, the incidence of enthesitis and, importantly, anterior uveitis significantly decreased during the third year of treatment in comparison with baseline.[51] To analyze the time to relapse after discontinuation of anti-TNF therapy, infliximab was stopped. Interestingly, most patients had a flare up, with a mean time to relapse of 17.5 weeks after cessation of infliximab infusions. Importantly, re-treatment with infliximab was safe and resulted in clinical improvement in all patients to a state similar to that achieved previously.[52] In another trial, 2 years of continuous infliximab therapy resulted in persistent improvement in spinal inflammation determined by both T1-weighted gadolinium-enhanced and by short tau inversion recovery MRI sequences in all AS patients.[53] Of note, disease activity parameters did not directly correlate with MRI results.

Similarly positive results were observed with etanercept therapy in patients with AS. In a double-blind, placebo-controlled, randomized clinical trial, significant improvement in clinical response (defined by ASAS20) was demonstrated in etanercept-treated patients throughout the 24-week study.[54] Sustained clinical response was maintained in the open-label extension of this study throughout 96 weeks of treatment.[55] Health-related quality-of-life assessments also significantly improved with etanercept.[56] The extent of clinical response was similar in subsequent clinical trials. Improvement measured by ASAS20 was noted as early as 2 weeks after etanercept therapy was started, and the response was maintained throughout the trial. In a subsequent multicenter trial, significant improvements in BASDAI, BASFI, and BASMI scores were achieved initially in the etanercept-treated group, and later in all patients after the placebo group received etanercept as well. Relapses occurred at a mean of 6 weeks after cessation of etanercept.[57] Re-administration of etanercept led to similar results in the same group of patients. Most patients were able to completely discontinue NSAIDs. Spinal inflammation on MRI decreased by 54% in the etanercept group but worsened by 13% in the placebo group after 12 weeks of therapy. After switching to etanercept, placebo patients experienced a similar improvement in spinal inflammation.[58] Finally, it was demonstrated that continuous treatment with etanercept for 24 weeks reduced active spinal changes by 69%, as measured by different MRI sequences.[59]

In an adalimumab trial that evaluated long-term efficacy and safety in AS (known as the *ATLAS* trial), patients received either 40 mg of adalimumab or placebo every other week for 24 weeks. The number of participants who met ASAS partial remission criteria was significantly higher in the adalimumab group at weeks 12 and 24 in comparison with the placebo group. The number of participants who met ASAS 5/6 criteria (20% improvement in five of six domains, without 20% worsening in the sixth domain) at weeks 12 and 24 was also significantly higher in the adalimumab-treated group.[60] Significant improvement in health-related quality of life was reported in this group of patients.[61] The efficacy of adalimumab was demonstrated not only by improved clinical indices but also by sacroiliac and spinal inflammation assessed by MRI.[62]

The efficacy of golimumab in AS was evaluated in the GO-RAISE study.[63] Patients with active AS, according to modified New York criteria, were randomly assigned to receive placebo or golimumab every 4 weeks. Patients were allowed to be on stable doses of sulfasalazine, hydroxychloroquine, low-dose corticosteroids, or NSAIDs during the trial. The use of other DMARDs was prohibited. At weeks 14 and 24, patients taking golimumab 50 mg, once a month demonstrated significant ASAS20 and ASAS40 responses (56% and 44%, respectively) versus the placebo group (23% and 15%, respectively).[63] Golimumab significantly reduced MRI-detected spinal inflammation of AS; improvements were correlated with improvement in ASDAS and CRP levels.

Following the new classification of AS in 2011, certolizumab was evaluated in patients with axial spondyloarthritis

(axSpA), including patients with AS and nonradiographic axSpA (nraxSpA). In the RAPID-axSpA study, certolizumab reduced the signs and symptoms of axSpA. ASA20 response rates were significantly higher in patients treated with certolizumab 200 mg every 2 weeks and 400 mg every 4 weeks. The combined certolizumab arms of the study showed significant differences in change from baseline versus placebo in BASFI, BASDAI, and BASMI. Improvement was observed as early as week 1. Similar improvements were reported with certolizumab versus placebo in both AS and nraxSpA subpopulations. In addition, 12 weeks of treatment with certolizumab reduced inflammation in sacroiliac joints and spine in patients with axSpA.[64]

## Mechanism of Action of TNF Inhibitors

Several potential mechanisms of action might explain the efficacy of TNF inhibitors in RA and other conditions (Table 63-1). However, the exact relationship between any particular mechanism and specific aspects of clinical efficacy remains to be demonstrated. Downregulation of local and systemic pro-inflammatory cytokine production and reduction of lymphocyte activation and migration into the joint may be the most relevant mechanisms; for example, serum levels of IL-6 and IL-1 are significantly reduced after administration of anti-TNF monoclonal antibody.[65] The reduction in TNF and the consequent reduction in IL-1 would be expected to reduce the synthesis of matrix metalloproteinase (MMP) and the production of other degradative enzymes. Serial studies have shown that there is in fact a marked reduction in pro–MMP-3 and pro–MMP-1 after anti-TNF therapy.[13,66,67]

As noted, anti-TNF therapy is also associated with a reduction of lymphocyte migration into the joints of patients with RA. With radiolabeled granulocytes, it has been demonstrated that anti-TNF monoclonal antibody significantly reduces cell movement into the affected joints.[68] In addition, post-treatment synovial biopsies show reduced cellular infiltrates, with fewer T cells and macrophages present.[69]

**TABLE 63-1** Potential Mechanisms of Action of Tumor Necrosis Factor Inhibitors

| |
|---|
| **Decrease Production of Other Inflammatory Mediators** |
| Cytokines (e.g., IL-1, IL-6, GM-CSF) |
| Chemokines (e.g., IL-8) |
| Degradative enzymes (e.g., MMPs) |
| Other mediators (e.g., C-reactive protein) |
| **Alter Vascular Function, Leukocyte Traffic and Activation** |
| Decreased adhesion molecule expression and function |
| Angiogenesis inhibition |
| **Modulate the Function of Immunocompetent Cells** |
| T Cells |
| Normalize activation threshold for CD3–T cell receptor signaling |
| Alter Th1/Th2 phenotype, cytokine secretion |
| Increase regulatory T cell number and function |
| Induce apoptosis (?) |
| **Monocytes and Macrophages** |
| Modulate HLA-DR expression |
| Increase apoptosis (?) |

HLA-DR, Human leukocyte antigen DR; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; MMPs, matrix metalloproteinases; Th, T helper.

These effects are thought to be secondary to a reduction in the expression of endothelial adhesion molecules in the synovial tissue. Treatment with anti-TNF monoclonal antibody results in a dose-dependent decrease in soluble forms of intercellular adhesion molecule-1 (ICAM-1) and E-selectin (CD62E).[68] Changes in soluble E-selectin, soluble ICAM-1, and circulating lymphocytes with anti-TNF therapy correlate with clinical outcomes. Vascular endothelial growth factor (VEGF) is a potent endothelial cell–specific angiogenic factor. It is produced in the synovium and is an important regulator of neovascularization in the pannus. After anti-TNF therapy, VEGF serum levels are reduced in patients with RA. This decrease correlates significantly with the clinical benefit observed in these patients.[70] Because angiogenesis is a prominent feature of rheumatoid synovium, the relationship between inflammation and angiogenesis has been investigated. Computerized image analysis of endothelium for multiple markers of endothelium (e.g., von Willebrand factor, CD31) and neovasculature ($\alpha v\beta 3$) has shown reduced vascularity after anti-TNF therapy. A number of other potential mechanisms of action are suggested to be operative for TNF inhibitors (see Table 63-1), although debate on these aspects is ongoing.

## Other Considerations

### Treatment in Other Autoimmune Conditions

The role of TNF inhibitors in the treatment of Crohn's disease, juvenile idiopathic arthritis, and psoriasis has been clearly defined. Based on promising results in various immune conditions, these agents have been used in a variety of other disorders, including idiopathic and spondyloarthropathy-related anterior uveitis, sarcoidosis, Sjögren's syndrome, Behçet's disease, inflammatory myopathies, and various types of vasculitis. Although a number of case reports or small, uncontrolled clinical trials have reported on these conditions, there is a paucity of conclusive data from controlled trials. Perhaps the most notable clinical response has been observed in the treatment of anterior uveitis, especially with anti-TNF monoclonal antibody constructs.[71] On occasion, promising results in uncontrolled trials have been disproved in controlled trials. This was observed in a placebo-controlled trial of etanercept given in addition to standard therapy for remission induction and maintenance in patients with granulomatosis with polyangiitis. Despite promising anecdotal evidence, the addition of the TNF inhibitor not only failed to cause any clinical improvement in the patient but also resulted in greater risk of solid malignancies beyond that observed with cyclophosphamide alone.[72]

Despite evidence that anti-TNF agents can result in the development of certain autoantibodies and even lupus-like syndromes, the safety and efficacy of anti-TNF agents have been assessed in a small group of patients with systemic lupus erythematosus (SLE). Patients with joint involvement experienced remission of arthritis, and a significant reduction in the level of proteinuria occurred with infliximab. In this small study, TNF inhibitor therapy did not lead to adverse events suggestive of an increase in SLE activity; however, as might have been expected, autoantibodies to double-stranded DNA and cardiolipin did increase.[73]

### Cardiovascular Risk and Lipid Profile

Cardiovascular morbidity and mortality appear to be increased in autoimmune conditions. This may be related both to increased prevalence of traditional risk factors for cardiovascular disease and to uncontrolled systemic inflammation, which appears to predispose independently to accelerated progression of atherosclerosis. Treatment with TNF inhibitors appears to be associated with overall improvement in cardiovascular disease risk, related to beneficial effects on lipid parameters and to control of systemic inflammation.[74] Long-term investigations are needed to define the possible beneficial effects of TNF inhibitors on overall and cardiovascular survival in patients with autoimmune disease.

### Monitoring

All patients must be evaluated for active and inactive (latent) tuberculosis infection before initiation of therapy. Appropriate screening tests (e.g., tuberculin skin test, ex vivo testing for tuberculosis, chest radiograph) should be performed on all patients. Use of TNF inhibitor therapy may increase the risk of reactivation of hepatitis B virus (HBV) among patients who are chronic carriers of HBV. Therefore evaluating patients for HBV before starting TNF inhibitor therapy is recommended. No other specific laboratory monitoring is currently required by regulatory agencies during therapy with TNF inhibitors. Nevertheless, because of the rare occurrence of myelosuppression and concern about the risk of infection, clinicians typically assess the complete blood count intermittently during therapy. Assiduous monitoring of patients for any sign or symptom of infection, demyelinating disease, and malignancy is requisite during treatment with all TNF inhibitors. Repeat testing at regular intervals (e.g., annually) for exposure to tuberculosis has been recommended by some regulatory authorities.

### Pregnancy and Breastfeeding

Developmental toxicity studies in rats, rabbits, and mice have not revealed any maternal toxicity, embryo toxicity, or teratogenicity associated with TNF inhibition. Minimal human pregnancy information has been published for these medications, and most data consist of isolated case reports, retrospective surveys, and uncontrolled studies. As the number of patients treated with TNF inhibitors increases, a growing number of pregnancies will be reported among them.[75] Outcome data based on anecdotal observations of small numbers of pregnant women treated with infliximab, etanercept, and adalimumab reveal that the relative rates of live births, miscarriages, and therapeutic terminations were comparable with rates in a national cohort of age-matched healthy women. TNF inhibitors are classified as U.S. Food and Drug Administration (FDA) Pregnancy Category B (animal reproduction studies have failed to demonstrate a risk to the fetus, and no adequate and well-controlled studies have been performed in pregnant women). The use of anti-TNF agents in pregnancy is recommended only if such treatment is clearly needed. If TNF inhibitors are used during pregnancy, transfer to the fetus is possible, and monitoring might be considered on that basis. Because it is not currently known whether TNF blockers are excreted in human milk, or whether they are absorbed systemically after ingestion, it is recommended that TNF blockers not be used by nursing mothers.

### Vaccinations

It is preferred to have all recommended vaccinations brought up to date in patients before initiating treatment with TNF inhibitors. Because data on the response to live vaccinations or the secondary transmission of infection by live vaccinations in patients receiving TNF inhibitors are insufficient, concurrent administration of live vaccines with TNF inhibitors is not recommended.

### Toxicity

In clinical trials, etanercept, infliximab, adalimumab, golimumab, and certolizumab pegol have generally been well tolerated.[21,22,24–32,36] Longer-term follow-up of patients initially enrolled in clinical trials has provided additional safety data for these agents. However, TNF plays a key role not only in the pathogenesis of autoimmune disease but also in normal immune homeostasis. Therefore a number of safety considerations, including the potential risk of infection and malignancy, are germane to the optimal clinical use of these agents.[76]

Additional information concerning adverse effects associated with these agents has been obtained through pharmacovigilance. Adverse events related to the use of TNF inhibitors can be grouped into those that are agent related and those that are target related (Table 63-2)[77] Injection site and infusion reactions and immunogenicity and their sequelae vary, depending on the particular agent. A potentially increased predisposition to infection, development of malignancy, and induction of autoimmune disorders and an association with demyelinating disorders, myelosuppression, and worse outcomes with congestive heart failure might be

**TABLE 63-2** Adverse Effects Potentially Associated with Tumor Necrosis Factor Inhibitors

| |
|---|
| **Target-Related** |
| Infections (including serious infections) |
| Opportunistic infections (e.g., tuberculosis) |
| Malignancies (skin cancer, lymphoma [?]) |
| Demyelinating conditions |
| Hematologic abnormalities |
| Congestive heart failure |
| Autoantibodies (anti-nuclear antibody, anti–double-stranded DNA) |
| Hepatotoxicity |
| Dermatologic reactions |
| Lupus-like syndromes |
| **Agent-Related** |
| Administration reactions |
| Immunogenicity |

considered target-related adverse events. Thus any clinically effective TNF inhibitor might be expected to be associated with such adverse events, although the relative risk among different agents may vary, depending on dose and other factors.

**Infusion and Injection Site Reactions.** Infliximab has been associated with infusion reactions, the most common of which are headache (20%) and nausea (15%). These are rarely severe, are usually transient, and typically can be controlled by slowing the rate of infusion or by treating with acetaminophen or antihistamines.[22] With etanercept, adalimumab, golimumab, and certolizumab pegol, cutaneous reactions at injection sites represent the most frequent administration-related side effect; however, they rarely lead to discontinuation of therapy[25,31] Injection site reactions typically consist of erythematous or urticarial lesions. Although they can arise at sites of previous injections, these reactions seem to be limited to the skin and are not associated with other or systemic features of immediate hypersensitivity. Reactions typically occur close to treatment initiation and abate over time, even with continued dosing.

**Antigenicity.** As is true for any therapeutic agent (especially large protein molecules, some of which contain foreign sequences), antibodies to anti-TNF agents can develop. Although the clinical relevance of these antibodies is presently unclear, they can diminish the half-life of the therapeutic agent and consequently decrease its efficacy. Antibodies to the drug develop in approximately 3% of etanercept-treated patients. In an early study, it was noted that antibodies to infliximab developed in 53%, 21%, and 7% of patients who were receiving 10, 3, and 1 mg/kg infliximab, respectively,[12] RA trials of infliximab with or without concomitant MTX treatment revealed that immunogenicity was decreased by concomitant MTX, perhaps in part because of the increase in the half-life of infliximab associated with MTX use. A multicenter trial of infliximab therapy in Crohn's disease demonstrated that induction of these anti-infliximab antibodies might contribute to hypersensitivity reactions in some patients. Antibodies to adalimumab, golimumab, and certolizumab pegol developed in approximately 4% to 12% of patients; this rate was reduced to 1% with concurrent MTX treatment.[31–33,46,63] Although it is believed that there is a trend toward higher clearance of TNF inhibitors in the presence of antibodies to the construct, routine testing for antibodies to TNF inhibitors is not widely available, nor is it currently recommended.

**Infection.** Given that TNF is a key mediator of inflammation, a major concern surrounding the use of TNF inhibitors is their potential to increase the risk of infection. Although inhibition of TNF in animals does not appear to increase their risk for infection with most pathogens, it does interfere with the ability to mount an inflammatory response against intra-cellular organisms. In experimental models, TNF blockade impaired resistance to infection with mycobacteria, *Pneumocystis carinii*, fungi, *Listeria monocytogenes*, and *Legionella*. In patients with RA, infection with these types of opportunistic organisms has been observed. However, confounding the attribution of infection to any therapeutic agent is the fact that infections occur more frequently and are important contributors to the accelerated morbidity of RA patients compared with the normal population. It is difficult to determine how much of this susceptibility relates to the disease itself and how much it is caused by the effects of immunomodulatory drugs (e.g., steroids and DMARDs). The subset of RA patients with great susceptibility to infection (i.e., those with severe, active disease) is also the subset most commonly enrolled in trials of TNF inhibitors. This is the group of patients for whom these agents have the greatest clinical utility.

In RA trials with TNF inhibitors, a number of infections have occurred. In general, the most frequent infections have been those that occur most commonly among all persons, such as upper and lower respiratory tract infections and urinary tract infections. In most studies, a slightly greater propensity to infection was seen in patients receiving TNF inhibitors; however, this trend is common in most studies of effective therapies for RA. The incidence of serious infection, defined as infection requiring hospitalization or treatment with parenteral antibiotics, among RA patients treated with TNF inhibitors was similar to that of the control groups in individual studies. The incidence also approximated that noted among RA patients before the anti-TNF era[78] In certain subgroups, such as patients with early RA, the overall incidence of infection was less than in patients with more long-standing disease, and infections and serious infections were comparable among TNF inhibitor–treated patients and controls.

It is worth noting, however, that several characteristics of clinical trials might affect investigators' ability to extrapolate their safety data to the clinic. In general, patients enrolled in clinical trials tend to be healthier and therefore less likely to develop adverse effects such as infection, compared with the general population of RA patients in the clinic. Therefore post-marketing data provide an important complement to safety data obtained from clinical trials. Also, clinical trials are powered to assess efficacy and therefore may not include sufficient numbers of patients to ascertain real but small differences in uncommon side effects. A systematic analysis that combined the results from nine clinical trials of TNF inhibitors has been performed.[79] This analysis found an increased risk of serious infection among patients receiving TNF inhibitors compared with controls (3.6% vs. 1.7%). However, a nonstandard definition of serious infection was used, and no attempt was made to control for the time of exposure, which was nearly always longer for patients receiving TNF inhibitors. In this same analysis, a nonsignificant trend toward a greater incidence of serious infection was observed with higher doses of TNF inhibitor. In one of the only clinical trials that had a primary outcome of safety, use of a high-dose TNF inhibitor was also associated with a greater incidence of serious infection compared with a lower dose; the lower dose was no different from placebo in this regard.

In post-marketing surveillance data, also known as *pharmacovigilance*, serious infections have certainly been observed among patients receiving TNF inhibitors[76] The relative impact of potentially confounding factors, such as comorbidities and concomitant medications on the rate of serious infection, remains incompletely defined. This important question has also been addressed by using registries of RA patients.[80,81] In a German registry, rates of infection and serious infection among 858 RA patients receiving treatment with TNF inhibitors were compared with those among

601 patients receiving only DMARDs.[81] The relative risk (RR) for infection (3.3 to 4.1) as well as serious infection (2.7 to 2.8) was significantly higher among patients receiving TNF inhibitors. However, these patients also had more severe and more active RA, thereby placing them at greater risk of infection. When the investigators used propensity scoring methods to control for severity of disease as a confounder, the RRs were reduced. The risk of infection decreased to 2.3 to 3.0, and that for serious infection became a nonsignificant trend of 2.1. In data from a British registry, 7644 RA patients treated with TNF inhibitors were compared with 1354 RA patients on DMARDs alone.[78] In this analysis, the crude rate of serious infection was higher among TNF inhibitor–treated patients (1.28; 95% confidence interval [CI], 0.94 to 1.76), although this did not reach statistical significance. Further, when the rates were adjusted for age, sex, severity of RA, use of corticosteroids, and comorbidity, no differences between the groups were noted (RR, 1.03; 95% CI, 0.68 to 1.57).

In summary, although treatment with TNF inhibitors can result in increased risk of infection and serious infection, other factors such as the severity of RA, the use of other medications such as corticosteroids, and the presence of comorbidities are important contributors to these outcomes. Clinicians must monitor patients closely for signs and symptoms of infection, and it is worth noting that TNF inhibitor therapy itself can mask the initial signs and symptoms of infection.

Opportunistic infections, particularly disseminated *Mycobacterium tuberculosis*, are of concern with the use of TNF inhibitors. Of note, more patients treated with TNF inhibitors have extrapulmonary and disseminated tuberculosis (TB), highlighting the specific role of TNF in controlling this infection. Rates of TB associated with the use of TNF antagonists are higher in geographic regions where TB is more prevalent in the general population. Most cases of TB observed in the early years after the introduction of TNF inhibitors arose within the first few months after initiation of therapy and were probably related to the reactivation of latent TB. Very few cases of TB were observed during clinical trials of the TNF inhibitors, highlighting the important role of pharmacovigilance in identifying safety signals with new therapies. For etanercept, no cases of TB occurred in clinical trials, but 38 cases of etanercept-associated TB were reported worldwide among an estimated 150,000 patients exposed through December 2002. For infliximab, 441 cases of TB were reported among approximately 500,000 initially exposed patients; only 6 cases of infliximab-related TB were reported from clinical trials. Ninety-seven percent of infliximab-related cases occurred within 7 months of treatment initiation, with a median time of onset of 12 weeks. The incidence of TB in clinical trials with adalimumab was greater in earlier clinical trials; this was related to lack of screening, the locations of the studies, and the higher doses used in early trials. The incidence dropped to 1% after adalimumab was reduced to its current dose, and after screening for latent TB infection was instituted before therapy (21 cases in 2400 patients).[82] The incidence of TB was even lower in golimumab and certolizumab pegol trials. The incidence of TB was 0.23 in golimumab clinical trials, and most cases occurred in countries with a high incidence rate of TB.[31–63] In studies with certolizumab pegol, 36 cases of TB occurred among 2367 exposed patients; these cases also occurred in countries with endemic rates of TB. This highlights the benefit of screening for and treating latent TB among patients being considered for TNF inhibitor therapy.[34–36] However, because treated patients may acquire new cases of TB, and because cases of latent TB may be missed because of false-negative screening tests, constant vigilance for TB is required during therapy with TNF inhibitors.

The impact of screening for latent TB in patients receiving anti-TNF agents has been assessed in a Spanish registry; the rate of development of active TB among RA patients treated with anti-TNF agents dropped by 83% with use of the recommended guideline. Current U.S. guidelines recommend purified protein derivative (PPD) skin testing and/or ex vivo testing for tuberculosis, as well as a chest radiograph, before anti-TNF therapy is initiated. If the PPD test is positive without evidence of active infection, treatment for latent TB with isoniazid is recommended. The recommended duration of therapy is 9 months. Recommendations concerning the timing of TNF inhibitor therapy and isoniazid prophylaxis for latent TB vary; however, concomitant initiation appears feasible.[20] During anti-TB treatment, alanine aminotransferase (ALT) monitoring is recommended, especially for those who chronically consume alcohol and/or who take potentially hepatotoxic drugs. Treatment should be adjusted according to local guidelines.

**Malignancy.** Anti-TNF drugs can theoretically affect the host defense against malignancy. To date, the occurrence of malignancies in clinical trials and long-term follow-up of RA patients from clinical trials do not appear to significantly exceed the rate that would be expected in this population. The overall rate of most malignancies in patients with RA is the same as in the normal population. However, risks of certain cancers, such as lymphoma and lung cancer appear to be increased in patients with RA. Although the actual reason is not known, the severity and activity as well as duration of the disease, and the use of immunomodulatory agents such as MTX, seem to play a role in the increased risk of lymphoma in RA patients.[83] Postmarketing analysis of the association between anti-TNF agents and lymphoma is inconclusive. In one population-based analysis, standardized incidence ratios of lymphoma among patients receiving anti-TNF therapies were somewhat higher than those among RA controls; however, this analysis did not adjust for baseline differences between patients.[83]

In a more recent analysis, in which adjustments were made for age, sex, and disease duration, no increased risk of lymphoma was identified among RA patients treated with anti-TNF agents compared with patients treated with other therapies. In clinical trials of anti-TNF monoclonal antibodies, malignancies, including lymphomas and skin cancers, were noted with treatment, but the extra duration of exposure to TNF inhibitors was not accounted for. Overall, TNF inhibitor therapy does not appear to be associated with a major increase in the risk of cancer. Still, this requires consideration for each individual, especially in certain situations, such as RA patients with a history of malignancy. No data from clinical trials address this because patients with cancers are excluded. Data from several

registries have suggested that no increase in risk of recurrent cancer may exist among such patients treated with TNF inhibitors, but more data are required.[84]

Although anti-TNF agents are used frequently in children, a possible increased risk of malignancies in children with autoimmune disorders, particularly in those receiving TNF inhibitors, has been pointed out by the Adverse Event Reporting System of the FDA. Forty-eight reports of malignancy were identified: 31 following infliximab use, 15 following etanercept use, and 2 following adalimumab use. Half of the malignancies were lymphomas, and most cases involved concomitant use of other immunosuppressants.[85] Given these uncertainties, caution is indicated when the use of anti-TNF agents is considered for patients with a history of malignancy or for those at high risk of malignancy for other reasons. Longer-term follow-up of larger numbers of patients will provide clinicians with a better idea of the safety of these agents in this regard.

**Autoimmune Disorders.** Antibodies to double-stranded DNA develop in approximately 10% to 15% of patients treated with any TNF inhibitor.[76] However, symptoms consistent with drug-induced lupus develop in few patients (0.2% to 0.4%). The mechanism and the significance of the development of antibodies are uncertain, although this adverse effect seems relatively specific for TNF inhibitors and is not noted with other biologic agents. Of note, life-threatening lupus involvement (e.g., nephritis, central nervous system lupus) generally does not develop in patients with TNF inhibitor–related lupus, and the diversity of other autoantibodies characteristic of idiopathic SLE (e.g., anti-Sm/RNP, anti-Ro/La, anti-Scl70) rarely develops in these patients. Anti-cardiolipin antibodies have reportedly developed in a few patients, but they are mostly asymptomatic. Among those few patients in whom lupus-like symptoms developed while they were on TNF inhibition therapy, improvement was seen upon discontinuation of therapy. Although the rare occurrence of autoimmune disorders has not dissuaded most clinicians from using TNF inhibitors in patients with RA, some remain cautious about using these drugs in patients with a history of SLE.

**Demyelinating Syndromes.** Several cases of multiple sclerosis (MS) or peripheral demyelinating disease have been reported with anti-TNF therapy in patients with RA, PsA, and Crohn's disease. In addition, two studies of TNF inhibitors in MS patients showed worsening of MS-related symptoms and exacerbations in the treated group.[86] Although some evidence suggests that the incidence of MS may be increased in patients with RA, the association between anti-TNF therapy and MS remains unclear. The risk for a demyelinating disease is very small; however, many clinicians withhold anti-TNF therapy in patients with a history of demyelinating diseases and in those who show signs and symptoms of such disease during anti-TNF therapy.

**Congestive Heart Failure.** Data suggest that TNF may play a role in the pathogenesis of congestive heart failure (CHF), and inhibition of TNF was highly effective in animal models of ischemic cardiomyopathy. However, in trials of TNF inhibitors in patients with stable but severe (class III or IV) CHF, no clinical benefit was observed, and in some treatment arms, higher incidences of mortality and hospitalization for worsening of CHF were reported. Thus TNF inhibition has been largely abandoned as a therapeutic approach in patients with CHF. In patients with RA, treatment with TNF inhibitors does not appear to result in an increased incidence of CHF.[87] In fact, TNF inhibitor therapy may actually improve mortality associated with heart disease and overall mortality in RA patients.

## INTERLEUKIN-1

Members of the IL-1 family include IL-1α, IL-1β, and the naturally occurring IL-1 receptor antagonist (IL-1Ra). Specific cellular proteases process IL-1α and IL-1β to their 17 kDa mature forms. Pro–IL-1α precursor is active intracellularly. However, pro–IL-1β is not active before cleavage with IL-1β–converting enzyme. After cleavage, it is secreted and is fully functional. IL-1Ra is a naturally occurring antagonist protein with amino acid sequence homology to IL-1α and IL-1β. Multiple forms of this protein exist. One is secreted and functions as a competitive inhibitor of IL-1α and IL-1β, binding to the same counter-receptor but transducing no signal. The IL-1 polypeptides bind to two cell surface receptors: type I (IL-1RI) and type II (IL-1RII). IL-1RI is found on most cell types, whereas IL-1RII occurs mainly on the surface of neutrophils, monocytes, B cells, and bone marrow progenitor cells. When IL-1 binds to IL-1RI, the signal transduction is mediated through the association of a second receptor unit, IL-1R accessory protein. The three members of the IL-1 family bind to IL-1RI with similar affinities. Binding of IL-1 to IL-1RII does not lead to signal transduction. IL-1RII acts like a decoy receptor and a competitive inhibitor. Soluble forms of IL-1RII inhibit IL-1 activity by competing with IL-1RI for IL-1 binding. IL-18 is another member of the IL-1 family of inflammatory cytokines. It is now recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases. IL-18 likely plays a role in RA, and strategies to block IL-18 activity are underway in clinical trials.

As with TNF, IL-1 is one of the key mediators of the inflammatory response. Studies in animal models of arthritis have demonstrated the therapeutic potential of IL-1 blockade. IL-1β gene knockout mice show markedly reduced levels of inflammation following immunization with type II collagen. The use of genetically modified mice has helped to confirm the physiologic significance of IL-1Ra. Deletion of this gene in mice results in the spontaneous development of arthritis.

### Anakinra

#### Structure and Mechanism of Action

Anakinra is a recombinant, nonglycosylated homolog of IL-1R that differs from native human IL-1R by the addition of a single methionine residue at its amino terminus. Anakinra blocks the activity of IL-1 by competitively inhibiting IL-1 binding to the IL-1RI receptor (Figure 63-2). Levels of the naturally occurring IL-1R, which are elevated in the synovium and synovial fluid from RA patients, are insufficient for the excess amount of locally produced IL-1.

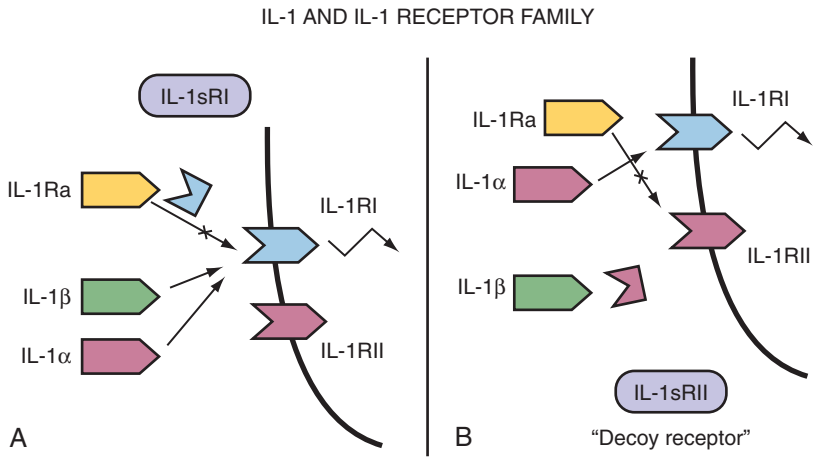



**Figure 63-2** **A** and **B,** Anakinra. Structure and mechanism of action: anakinra is a recombinant, nonglycosylated homologue of interleukin-1. It blocks the activity of IL-1 by competitively inhibiting IL-1 binding to the IL-1RI receptor (see Chapter 26). In addition, IL-1Ra can also bind to the soluble IL-1 receptors.

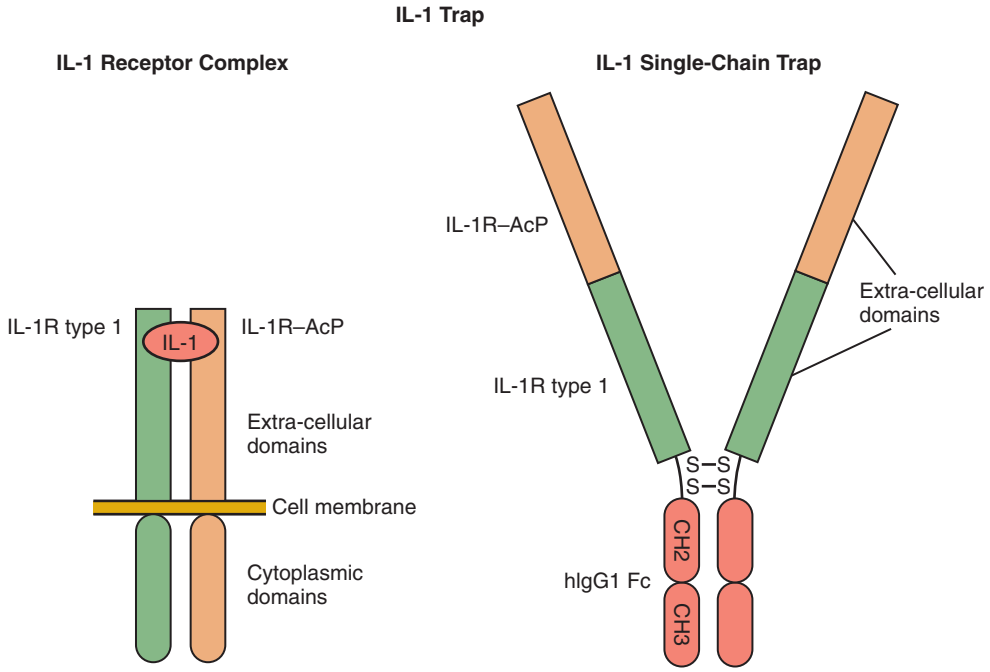



**Figure 63-3** Rilonacept. Structure: Rilonacept is a fusion protein consisting of the human interleukin (IL)-1 receptor extra-cellular domains and the Fc portion of human immunoglobulin G1. CH2, CH3, Constant domains of human immunoglobulin G1; hIgG, human immunoglobulin G; IL-1R–AcP, interleukin-1 receptor accessory protein.

## Pharmacokinetics

In patients with RA, maximum plasma concentrations of anakinra occur 3 to 7 hours after the subcutaneous administration of clinically relevant doses (1 to 2 mg/kg). The terminal half-life ranges from 4 to 6 hours. In RA patients, no unexpected accumulation of anakinra is observed after daily subcutaneous doses for as long as 24 weeks. Estimated anakinra clearance increases with increasing creatinine clearance and body weight.

## Drug Dose

The recommended dose of anakinra for the treatment of patients with moderately to severely active RA is 100 mg/day administered by subcutaneous injection. Anakinra can be used alone or in combination with MTX. Because of the potential for increased risk of infection, it is not recommended for use in conjunction with TNF inhibitors.

**Rilonacept.** Rilonacept, previously known as *IL-1 Trap*, is a fusion protein consisting of the human IL-1 receptor extra-cellular domains and the Fc portion of human IgG1. It incorporates in a single molecule the extra-cellular domains of both receptor components required for IL-1 signaling: IL-1RI and the IL-1R accessory protein (Figure 63-3). Rilonacept has a very high binding affinity for IL-1 (dissociation constant ~1 pM), and it is specific for IL-1β and IL-1α. It is administered subcutaneously with a loading dose of 320, followed by 160 mg once weekly.

Canakinumab. Canakinumab is a human monoclonal antibody that selectively targets IL-1β. It has no cross-reactivity with other members of the IL-1 family, including IL-1α. It is given subcutaneously 150 mg every 8 weeks to patients weighing greater than 40 kg. For patients whose body weight is between 15 and 40 kg, the recommended dose is 2 mg/kg.

### Efficacy

**Rheumatoid Arthritis.** Preliminary clinical studies indicated that anakinra could be safely administered by subcutaneous injection. The efficacy of anakinra in the treatment of active RA was confirmed in a 24-week, phase II, placebo-controlled study with three different doses of anakinra (30, 75, or 150 mg).[88] Improvements were observed in patients in all individual clinical parameters, including swollen and tender joint counts, pain score, duration of early morning stiffness, and patient and physician assessment of disease activity. More patients on the higher dose of anakinra achieved improvement in ACR20 criteria compared with the placebo group. However, the overall magnitude of the reduction in clinical symptoms and signs (20% to 30%) was relatively modest compared with that seen with TNF-blocking agents (60% to 70%). Patients were allowed to go on to a non–placebo-controlled extension study with three different doses of anakinra after completing the 24-week double-blind study. On completion of the extension study, 55% of patients who had previously received placebo achieved an ACR20 response. Of the patients who continued receiving the same dose of anakinra, 49% maintained an ACR20 response at 48 weeks. Analysis of hand radiographs by two different methods after 24 weeks of treatment showed a statistically significant decrease in the rate of progressive joint damage compared with placebo.[89] Improvements in functional status and quality of life were also observed.[90]

Anakinra is a competitive inhibitor of IL-1 that must be continuously present in great excess to be effective, and it must be administered daily. It was hypothesized that the relatively modest clinical results seen with anakinra in patients with RA (compared with those achieved by TNF inhibitors) might be related to the agent rather than to the target. Two lines of evidence appear to refute this hypothesis: the efficacy of anakinra in other inflammatory diseases, and the comparable clinical efficacy of other IL-1 inhibitors in RA.

Based on early studies, the subcutaneous administration of rilonacept in subjects with RA provided evidence of its clinical and biologic activity. However, a double-blind, placebo-controlled clinical trial in patients with moderate to severe RA who were randomly assigned to receive weekly injections of placebo or several doses of rilonacept for 12 weeks showed only modest efficacy.[91]

Canakinumab reduced disease activity in patients with new-onset RA, including patients who failed to benefit from TNF-blocking therapies. However, the long-term benefits and radiologic changes remain unstudied.

**Autoinflammatory Diseases.** Systemic autoinflammatory diseases are rare syndromes that have common systemic manifestations, such as fever, neutrophilia, arthralgias, myalgias, and severe fatigue, that are generally periodic rather than progressive. Familial Mediterranean fever is perhaps the most well-known autoinflammatory disease. Patients with familial cold autoinflammatory syndrome (FCAS) might be seen with a spectrum of disease activity: neonatal-onset multisystem inflammatory disease (NOMID; also known as *chronic infantile neurologic, cutaneous, articular syndrome* [CINCA]), Muckle-Wells syndrome (MWS), and familial cold urticaria. These conditions, share some clinical features, and are associated with various mutations in the *NALP3/CIAS1/PYPAF1* gene, which encodes the protein cryopyrin. Cryopyrin is a key component of the inflammasome; thus the autoinflammatory syndromes may be related to abnormalities in IL-1 regulation. This was proven by the remarkable responses to anakinra reported in these syndromes. In addition, significant and rapid responses have been achieved when anakinra was used to treat patients with adult-onset Still's disease. Improvement in various hematologic, biochemical, and other markers suggests that IL-1 plays a key role in this disease as well. Often, on blocking IL-1β, patients with cryoprin-associated periodic syndromes (CAPS) and other autoinflammatory diseases experience a rapid and sustained cessation of symptoms as well as reductions in biochemical, hematologic, and functional markers of their disease. Because treatment with rilonacept and cakinumab are equally effective in treating autoinflammatory diseases, the culprit in these disease is in IL-1β and not IL-1α.

Rilonacept and canakinununumab are approved for CAPS disorders: FCAS and MWS.[92,93] Canakinumab is also approved for systemic juvenile idiopathic arthritis. Anakinra is the first and only drug that is approved for the treatment of children and adults with NOMID, the most severe form of CAPS.

### Gout

Some patients with recurrent attacks of gouty arthritis who are resistant to colchicine and NSAIDs require steroids to control the disease flares. When treated with anakinra, rilonacept, and canakinumab, a rapid, sustained, and remarkable reduction in inflammation and pain has been observed, and in fact this effect seems to be superior to steroids.[94] IL-1 blockers' effect on controlling gout attacks is currently being investigated in clinical trials.

### Other Disorders

IL-1 blockers are under investigation in various disorders, including stroke, indolent myeloma, type 1 and 2 diabetes, osteoarthritis, and graft-versus-host disease (GVHD).

## Other Considerations

### Toxicity

Anakinra is generally well tolerated. Injection site reactions are the most frequently reported adverse event. In a randomized clinical trial, injection site reactions were reported in 25% of patients given placebo and in 50%, 73%, and 81% of patients given anakinra in doses of 30, 75, and 150 mg/day, respectively.[95] These reactions were generally mild and transient. Infections were uncommon and occurred

at a similar rate in the placebo and treatment groups. Infections that required antibiotic therapy occurred in 12% of the placebo-treated group and in 15% to 17% of the treatment group. These infections consisted primarily of bacterial events such as cellulitis and pneumonia. The incidence of pulmonary infection appeared to be higher among patients with underlying asthma. In placebo-controlled studies, as many as 8% of patients receiving anakinra showed a reduction in the neutrophil count, compared with 2% of patients in the placebo group. Other adverse events reported were headache, nausea, diarrhea, sinusitis, influenza-like syndrome, and abdominal pain. Malignancy rate and incidences were similar to those expected for the populations studied. Long-term follow-up of patients on anakinra has proved the overall tolerability of therapy during several years.

In animal studies, the combination of TNF inhibition and IL-1 inhibition achieved synergistic efficacy in arthritis models. However, when this approach was tested in RA patients, the combination did not achieve any additional clinical benefit but did result in greater toxicity—specifically, an increased incidence of infection and serious infection.[96] Therefore the combination of biologic therapies targeting TNF and IL-1 is currently not recommended.

### Monitoring

Patients should be closely monitored for signs and symptoms of infection. Administration of anakinra must be discontinued if serious infection develops in a patient. Neutrophil counts should be assessed before anakinra treatment is initiated, as well as monthly during anakinra therapy for 3 months, and then every 4 months for as long as 1 year.

### Pregnancy and Breastfeeding

Reproductive studies have been performed only on rats and rabbits, and they have not revealed any evidence of harm to the fetus. However, no well-controlled studies have been conducted in pregnant women. Therefore anakinra should be used during pregnancy only if it is clearly needed. It is not known whether anakinra is secreted in human milk, so it should be discontinued in nursing mothers.

## INTERLEUKIN-6

IL-6 and other members of the IL-6 cytokine family play a critical role in inflammatory and immune responses. IL-6 is a small polypeptide characterized by a four–α-helix bundle structure that is stabilized by intramolecular disulfide bridges. IL-6 is secreted by various cell types, including monocytes, T and B lymphocytes, and fibroblasts. It is detectable at elevated levels in the serum and synovial tissue in inflammatory arthritides, including RA and PsA. IL-6 exerts its activity by binding its receptor component, IL-6R, which exists in soluble and membrane-bound forms, and the accessory protein, glycoprotein 130 (gp130). The IL-6R is constitutively expressed on several cell types, including lymphocytes and hepatocytes. However, soluble forms of IL-6R can productively interact with the 130 kDa signal transducing component gp130, which is expressed on a wide range of cell types (Figure 63-4). IL-6 has multiple effects on various aspects of the immune system to initiate inflammation. IL-6 stimulates the production of T helper (Th)17 cells. These pathogenic cells secrete IL-17 and are involved in the induction of autoimmune injury. IL-6 also plays a role in B cell activation and differentiation. Effects of IL-6 on osteoclast differentiation and activation, including receptor activator of NF-κB (RANK) ligand–dependent mechanisms, have been clearly demonstrated.[97] Recruitment of neutrophils to the inflammatory sites and stimulation of VEGF synergistically with TNF and IL-1β contribute to pannus formation.[98] Levels of IL-6 are directly proportionate with levels of CRP and disease severity. IL-6 knockout mice are resistant to CIA and show reduced levels of serum TNF. All these functions make IL-6 blockade an

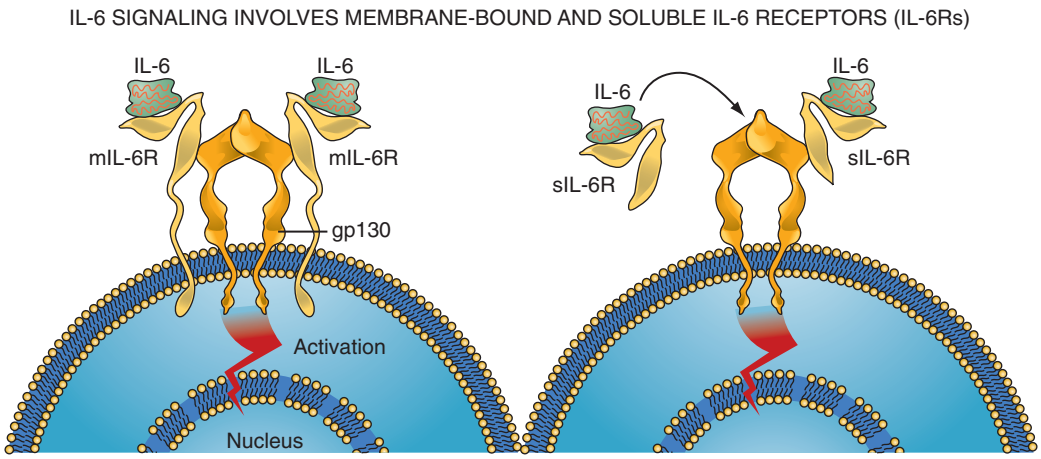



**Figure 63-4** Interleukin (IL)-6. Mechanism of action: IL-6 binds first to the membrane-bound IL-6 receptor (mIL-6R). The IL-6/mIL-6R complex then associates with the signal-transducing membrane protein, glycoprotein 130. IL-6 can also bind to sIL-6Rs which, in turn, can interact with gp130 and activate signaling *(right)*.

attractive biologic target therapy for the treatment of RA and other autoimmune diseases.

## Tocilizumab

### Structure and Mechanism of Action

Previously referred to as *myeloma receptor antibody* (MRA), tocilizumab is a humanized IgG1 monoclonal antibody that binds with high affinity to soluble and membrane-bound forms of the 80 kDa component of the IL-6R. Tocilizumab is a recombinant humanized anti–human IL-6 receptor monoclonal antibody of the immunoglobulin IgG1κ (gamma 1, kappa) subclass with a typical $H_2L_2$ polypeptide structure. Each light chain and heavy chain consists of 214 and 448 amino acids, respectively. The four polypeptide chains are linked intramolecularly and intermolecularly by disulfide bonds. Tocilizumab has a molecular weight of approximately 148 kDa. Treatment with this monoclonal antibody effectively inhibits IL-6–mediated interactions on cells constitutively expressing the IL-6R. In addition, as noted, soluble forms of the IL-6R can productively interact with the 130 kDa signal-transducing component gp130, which is expressed on a wide range of cell types; thus treatment with tocilizumab effectively inhibits a broad array of IL-6–driven processes.

### Pharmacokinetics

Tocilizumab has a nonlinear pharmacokinetic profile.[99] The maximum concentration increases in approximate proportion to increases in dosage, whereas the area under the concentration-time curve increases disproportionately. As the dosage increases, clearance and the apparent elimination rate constant decrease, and terminal half-life and mean residence times are prolonged. MTX therapy, alcohol consumption, age, and race do not affect the pharmacokinetics of tocilizumab. Tocilizumab binds to soluble IL-6R in a dose-dependent manner and saturates the receptor at approximately 0.1 μg/mL. Tocilizumab also competitively inhibits IL-6 binding to soluble IL-6R; complete inhibition is seen at approximately 4 μg/mL.

After IV dosing, tocilizumab undergoes biphasic elimination from the circulation. In patients with RA, the central volume of distribution was 3.5 L, and the peripheral volume of distribution was 2.9 L, resulting in a volume distribution at steady state of 6.4 L.

### Drug Dose

Indications for the use of tocilizumab vary across the globe. In the United States, initial approval for tocilizumab in late 2009 was for use as a single agent or with MTX or other DMARDs to treat adult patients with rheumatoid arthritis in whom one or more anti-TNF agents had failed. The recommended starting dose is 4 mg/kg, followed by an increase to 8 mg/kg based on clinical response. In other countries, it is recommended to start therapy at 8 mg/kg, with the possibility of reducing to 4 mg/kg, for example, in the case of tolerability concerns. It is administered once every 4 weeks as a 60-minute single intravenous infusion. Doses exceeding 800 mg per infusion are not recommended. Development of a preparation of tocilizumab for subcutaneous administration is under way.

### Efficacy

An early double-blind, placebo-controlled, randomized clinical trial revealed statistically significant ACR20 responses with 10 mg/kg and 5 mg/kg single infusions of tocilizumab when given to patients with active RA with no concomitant DMARDs.[99,100] Substantial decreases in measures of the acute phase response, including the erythrocyte sedimentation rate (ESR) and CRP concentration, were seen with 5 and 10 mg/kg as early as 1 week and were largely sustained through week 4. Larger phase II studies tested tocilizumab monotherapy, given at a dose of 4 mg/kg or 8 mg/kg intravenously every 4 weeks during 3 months, to patients with relatively refractory and active RA. Most individual measures of arthritis activity improved to a statistically significant level by week 4, and the extent of response continued to improve through 12 weeks. Sustained clinical responses were maintained for a considerable number of patients during 5 years of an open-label follow-up period.[101] In a large European study known as Chugai Humanized Anti–Human Recombinant IL-6 Monoclonal Antibody (CHARISMA) study, patients with active RA despite receiving MTX treatment for at least 6 months were randomly assigned to receive tocilizumab 2, 4, or 8 mg/kg every 4 weeks alone or in combination with their existing MTX. Another group received MTX alone. Patients on tocilizumab alone at doses of 4 and 8 mg/kg had improvement in ACR20; however, patients remaining on concomitant MTX with tocilizumab achieved statistically significant improvement at 16 weeks compared with those given MTX alone.[102] The ability of treatment with tocilizumab to alter the progression of joint damage was first assessed in patients with relatively early-stage RA in the Study of Active Controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor (SAMURAI).[103] Therapy with tocilizumab was shown to have a beneficial effect on the progression of radiographic joint damage defined by the total Sharp score, in addition to improvements in clinical and functional status.

Tocilizumab has been studied in series of multinational phase III clinical trials involving more than 4000 patients. In the OPTION trial, patients with moderate to severe RA who were MTX-inadequate responders received 4 mg/kg or 8 mg/kg tocilizumab or placebo every 4 weeks. Fifty-nine percent and 48% of patients who received tocilizumab 8 mg/kg and 4 mg/kg plus MTX, respectively, achieved ACR20 at week 24, compared with 27% of patients who received placebo plus MTX. Patients in the placebo and 4 mg/kg groups switched to the 8 mg/kg group (rescue therapy), and by week 24, all patients receiving tocilizumab had significantly better responses in all core set values, including DAS28, HAQ, pain visual analogue scale (VAS), and global VAS.[104] In the TOWARD trial, patients with active disease, despite being on stable doses of DMARDs (the most common being MTX) were randomized to receive monthly 8 mg/kg tocilizumab or placebo infusions. At 24 weeks, the tocilizumab group showed significantly improved ACR20 response rates and DAS28 scores, as well as HAQ and fatigue scores.[105] The AMBITION trial included

patients who were methotrexate naïve or who had discontinued MTX for reasons other than lack of efficacy or toxicity. Seventy percent of patients who received tocilizumab 8 mg/kg achieved ACR20 at week 24, compared with 53% of patients receiving MTX alone. ACR50 and -70 response rates, HAQ scores, and DAS28 remission were also superior in comparison with the MTX group.[106]

The efficacy of tocilizumab for the treatment of patients who had an inadequate clinical response or were intolerant to one or more anti-TNF agents was evaluated in a rheumatoid arthritis study in anti-TNF failures (RADIATE). Fifty percent and 30% of patients who received tocilizumab 8 mg/kg or 4 mg/kg plus MTX, respectively, achieved ACR20 at week 24, compared with 10% of patients who received placebo plus MTX. Of note, the number and type of TNF antagonists previously failed did not appear to affect the ACR20 response rate.[107] The Tocilizumab Safety and the Prevention of Structural Joint Damage (LITHE) trial is a long-term trial designed to evaluate the efficacy of tocilizumab for preventing structural damage in patients who had an inadequate response to prior MTX therapy. At week 24, 56% and 51% of patients who received tocilizumab 8 mg/kg or 4 mg/kg plus MTX, respectively, achieved ACR20 compared with 27% of patients who received placebo plus MTX. Mean joint erosion, joint space narrowing, and total Genant-modified Sharp scores indicated that both tocilizumab doses were associated with significant inhibition of radiographic progression from baseline compared with that of placebo.[108] Clinical, functional, and structural remission in patients with advanced RA was demonstrated in a 52-week trial. Forty-two percent of patients with remarkably severe RA with long disease duration and progressive joint damage achieved clinical remission.[109] Long-term treatment with tocilizumab as long as 4.2 years revealed increasing numbers of patients achieving ACR50 and -70 scores, DAS28 remission, and high retention rates.[79]

## Safety

A number of safety concerns are associated with blocking a major regulatory cytokine such as IL-6. These can be grouped as general immunomodulatory effects (e.g., infection), IL-6–related effects (e.g., abnormalities in liver enzymes, lipid profiles), and finally agent-specific effects (e.g., infusion reactions) (Table 63-3).

Infections are a concern, as they are with all immunomodulatory therapies for RA. In clinical trials of tocilizumab, the occurrence of infection appears compatible with that of other approved biologic agents. Although the overall infection rate with tocilizumab monotherapy was comparable with rates in MTX monotherapy groups, the incidence of overall infection was slightly higher with concomitant DMARD therapy. The most commonly reported infections (5% to 8%) were upper respiratory infections and nasopharyngitis. A similar incidence of serious adverse events (5%) was generally observed across study groups. Serious infections occurred more often in higher-dose groups as compared with the placebo group. Cellulitis, pneumonia, diverticulitis, gastroenteritis, and herpes zoster were the most common infections noted. Only rare cases of opportunistic infection have been reported.[102-104]

**TABLE 63-3** Adverse Effects Potentially Associated with Tocilizumab

| |
|---|
| **Immunomodulatory Effects** |
| Infections/serious infections |
| **Mechanism-based Effects** |
| Increased liver enzymes |
| Abnormalities in lipid profiles |
| Neutropenia |
| Low platelet count |
| Malignancies |
| Demyelinating conditions |
| Gastrointestinal perforations |
| **Agent-Related Effects** |
| Administration reactions |
| Hypersensitivity reactions |

Transient elevations of ALT and aspartate aminotransferase (AST) levels were commonly observed in tocilizumab-treated patients. Elevations were more apparent immediately following infusions, potentially reflecting blockade of the anti-apoptotic properties of IL-6 in hepatocytes. An increase in ALT levels to three or more times the upper limit of normal was noted in 5% to 6.5% of patients who received tocilizumab and concomitant DMARD therapy. These rates were 1.5% and 2.1% in patients who received only DMARD or only tocilizumab, respectively.[102,104-106] To date, elevated transaminases have not been associated with reduced liver function or serious adverse events.[98,102,105]

Lipid profiles were altered in the tocilizumab group compared with placebo. Tocilizumab was associated with increases in all lipid levels, including total cholesterol and its fractions, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. Increases in these parameters were noted by the first assessment at 6 weeks and remained elevated through the clinical trials. Despite elevations in these parameters, clinical cardiovascular events have not increased in clinical trials. Mean increases in the 8 mg/kg tocilizumab plus DMARD group were 30.9 mg/dL (total cholesterol), 21.7 mg/dL (LDL cholesterol), 4.3 mg/dL (HDL cholesterol), and 30.1 mg/dL (triglycerides).[104,105]

A higher proportion of patients receiving tocilizumab had a decrease in neutrophil counts (29%) in comparison with patients who were not receiving this drug (4%). The drop in neutrophil counts was generally mild (grade 1, according to the common toxicity criteria, i.e., 1500 to 2000 cells/$mm^3$ or more) to moderate (grade 2, 1000 to 1500 cells/$mm^3$) in severity and reversed with discontinuation of treatment. To date, no clear association between low neutrophil counts and infection-related adverse events has been noted.[99,104,105]

Treatment with tocilizumab was associated with a reduction in platelet counts. The decrease in platelet counts was below the lower limit of normal and was reported in 8% to 9% of patients receiving tocilizumab with or without MTX. However, no serious bleeding incidents occurred. Only

isolated cases of epistaxis and hemoptysis were reported in patients with moderate to severe thrombocytopenia.

Rare events of gastrointestinal perforation have been reported in clinical trials, primarily as complications of diverticulitis. The overall rate of gastrointestinal perforation was 0.26 event per 100 patient-years. Most patients in whom gastrointestinal perforations developed were taking concomitant NSAIDs, corticosteroids, or MTX. Patients presenting with new-onset abdominal symptoms therefore should be evaluated promptly for early identification of gastrointestinal perforation.

Because tocilizumab is a humanized antibody, infusion-related adverse events might be expected. Adverse reactions associated with the infusion were reported to be 8% and 7% in patients receiving 4 mg/kg and 8 mg/kg tocilizumab together with MTX. The most commonly reported adverse events were hypertension during the infusion and headache and skin reactions within 24 hours after the infusion. These reactions did not result in termination of treatment. Antibodies to tocilizumab were detected in a small group of patients, and very few were associated with medically significant hypersensitivity reactions that led to withdrawal. The development of antibodies and of infusion reactions may be greater among patients receiving the lower dose of tocilizumab.

Rates of malignancy were similar in the tocilizumab- and DMARD-treated groups. Rare cases of demyelinating disorders were reported in clinical trials. Long-term experience with tocilizumab will determine the actual risk for these entities; however, caution needs to be exercised in patients with any risk factors for malignancy or demyelinating disorders.

A systematic literature search on six published randomized controlled trials that assessed the risk of adverse events with tocilizumab revealed that tocilizumab in combination with MTX as a treatment for RA is associated with a small but significantly increased risk of adverse events, which is comparable with that of other biologics. The risk of infection was significantly higher in the 8 mg/kg combination group compared with controls (odds ratio, 1.30; 95% CI, 1.07 to 1.58). No increased incidence of malignancy, tuberculosis reactivation, or hepatitis was seen.[110]

### Drug Interactions

IL-6 inhibition might affect cytochrome P450 substrates. In vivo studies showed that omeprazole and simvastation levels decreased by 28% and 57%, respectively, 1 week after tocilizumab infusion. Upon initiation or discontinuation of tocilizumab, drugs with a narrow therapeutic margin, such as warfarin or drug concentrations of certain drugs such as cyclosporine, should be closely monitored.

Tocilizumab has not been studied, and its use should be avoided in combination with biologic DMARDs, such as TNF blockers, IL-1 blockers, anti-CD20 monoclonal antibodies, and co-stimulation blockers.

It is recommended that live vaccines should not be given concurrently with tocilizumab because clinical safety has not been established. It would be desirable for patients to be brought up to date on all recommended vaccinations before treatment with tocilizumab is initiated.

### Monitoring

Patients should be evaluated for tuberculosis and other infections before treatment with tocilizumab is begun. Antituberculosis therapy should be initiated before tocilizumab is started in patients with active tuberculosis.

It is not recommended to initiate tocilizumab treatment in patients who have ALT and AST levels greater than 1.5 times the upper level of normal or any evidence of liver disease. For all patients, liver function tests should be repeated initially every 4 to 8 weeks during tocilizumab treatment. When the ALT or AST level is between one and three times the upper level of normal, the dose of tocilizumab or concomitant DMARD should be adjusted. For persistent increases in this range, the tocilizumab dose should be modified. If the ALT or AST level is greater than three to five times the upper level of normal, tocilizumab should be interrupted until the level falls below three times the upper level of normal. For ALT or AST elevations greater than five times the upper level of normal, the drug should be discontinued.

Patients receiving tocilizumab should have lipid levels monitored with a goal toward maintaining levels within the target ranges of National Cholesterol Education Program Adult Treatment Panel III or local guidelines. Patients should be managed with lipid-lowering agents if appropriate.

Caution should be exercised when tocilizumab is initiated in patients with a very low neutrophil count at baseline, and all patients should have their absolute neutrophil count (ANC) monitored 4 to 8 weeks after the first infusion. It has been recommended that tocilizumab should not be administered to patients with ANC values less than 2000 cells/mm$^3$. If the ANC falls to between 500 cells/mm$^3$ and 1000 cells/mm$^3$, drug therapy should be discontinued until the ANC reaches above 1000 cells/mm$^3$.

It is not recommended to initiate tocilizumab treatment in patients with a platelet count below 100,000/mm$^3$. Treatment with tocilizumab should be interrupted if the platelet count falls to below 50,000/mm$^3$. Platelets should be monitored every 3 to 8 weeks.

### Pregnancy and Breastfeeding

No adequate and well-controlled studies have been conducted in pregnant women. Therefore tocilizumab is a Pregnancy Category C drug and should be used only if potential benefits justify potential risk to the fetus. It is not known whether tocilizumab is excreted in human milk or is absorbed after oral ingestion.

### Other IL-6 Blockers

**Sarilumab.** Sarilumab is human monoclonal antibody directed against the IL-6 receptor. It showed efficacy in two different doses (150 mg and 200 mg) given subcutaneously every 2 weeks in the MOBILITY studies.[111]

**Sirukumab.** A human monoclonal anti–IL-6 monoclonal antibody, sirukumab has also shown improvements in signs/symptoms of RA when given 100 mg subcutaneously every 2 weeks.[112]

### Emerging Therapies

**IL-17 Blockers.** Rheumatoid synovial tissue produces 1L-17A, which causes cartilage and bone degradation in synovial and bone explants. Serum IL-17 levels and synovial fluid levels are elevated in many patients with RA. Several IL-17A blockers, including the anti–IL-17 monoclonal antibodies secukinumab and ixekizumab, and the IL-17 receptor subunit A monoclonal antibody brodalimumab have been evaluated in RA.[113] Despite the sound immunopathologic rationale in RA, clinical efficacy with IL-17 inhibitors has been modest at best. This result stands in distinction to the dramatic efficacy of IL-17 inhibition in psoriasis. By 2016, secukinumab was approved for psoriatic arthritis and anklyosing spondylitis. The relative position of this and other IL-17 blocking strategies in the therapeutic armamentarium will require additional study.

**B cell—Targeted Therapies.** Following the success with B cell depletion with rituximab, alternative targets are being studied. B lymphocyte stimulator (BLyS) and proliferation-inducing ligand (APRIL) are two TNF family cytokines which play an important role in regulation of B cell maturation, proliferation, function, and survival. TACI, BCMA, and BAFF are the receptors that have unique binding properties for BLyS and APRIL. Belimumab, atacicept and tabalumab are BAFF/BLyS inhibitors (and APRIL, in the case of atacicept) that had been investigated in RA. However, due to low efficacy and potential toxicity, the development plans for these agents in RA were suspended. Belimumab has demonstrated modest efficacy in SLE and has been approved for use in the United States (see Chapter 81).[114]

## CONCLUSION

Treatment of patients with RA with inhibitors of key proinflammatory cytokines TNF, IL-1, and IL-6 is a compelling example of effective targeted biologic therapy. Treatment with these agents, particularly TNF inhibitors, has substantially improved the signs and symptoms of disease. For many patients, quality of life has been improved, the progression of joint damage has been inhibited, and disability has been averted. The success of this approach has "raised the bar" for the goals of treating this pernicious disease and has reinvigorated research aimed at further refinements of therapy. It has also stimulated research into the potential utility of inhibitors of additional cytokines, such as IL-6, IL-15, and IL-18, and other components of the immune system relevant to autoimmune disease.

A number of questions remain regarding the optimal use of these drugs. Longer-term safety data will allow clinicians to more fully assess the risk-benefit ratio for individual patients. Given uncertainties regarding the long-term safety of these drugs and the heterogeneity of clinical responses, research defining the populations of patients expected to derive the greatest benefit with the least toxicity is critical. For example, ongoing research assessing genetic polymorphisms or proteomic differences among treated patients could optimize efficacy while minimizing toxicity. This is also relevant from a cost standpoint. Although the acquisition costs of these agents are relatively high, data supporting their cost-effectiveness, including gains in employment and reduced hospitalizations, are emerging.[115]

The success observed with these biologic therapies has raised additional clinical questions. For example, can very early treatment with highly effective therapy, such as the combination of TNF inhibitor and MTX, truly alter the disease course? What might the optimal treatment paradigms be for various rheumatic diseases? The success of the TNF inhibitors has also generated substantial interest in targeting this cytokine by alternative approaches, including inhibition of key regulatory factors, such as p38 mitogen-activated protein kinase and NF-κB. Advances in biopharmaceuticals could generate agents that possess desirable characteristics in terms of pharmacokinetics, immunogenicity, adverse effects, ease of administration, and cost. These developments should eventually allow clinicians to maximize the use of these novel therapies and achieve clinical benefits that previously were considered unattainable.


The references for this chapter can be found on ExpertConsult.com.

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CHAPTER 64



# Cell-Targeted Biologics and Emerging Targets: Rituximab, Abatacept, and Other Biologics

Peter C. Taylor

**KEY POINTS**

- Rituximab is an effective biologic therapy across the spectrum of rheumatoid arthritis (RA) patient populations but with greatest benefit in seropositive patients. Clinical trials in persons with active, established RA confirm that a single cycle of rituximab given as two infusions of 1 g each, together with once-weekly oral methotrexate, produces an enduring clinical response comparable to that observed with tumor necrosis factor (TNF) blockade. A treatment cycle of two infusions of 500 mg is also efficacious but may result in a lower proportion of patients demonstrating more robust clinical responses and is less likely to inhibit radiographic progression.
- Current data suggest that the most appropriate interval between rituximab courses is 6 to 12 months. Repeat treatment produces American College of Rheumatology (ACR) responses that equal or exceed those from the first course of treatment, with a comparable duration of effect.
- Rituximab has an acceptable safety record in RA trials, but infusion reactions of variable severity can occur; most are mild to moderate. Frequency and severity are reduced by the administration of intravenous methylprednisolone before rituximab infusions.
- Clinical trials in patients with active, established RA confirm that abatacept can result in a meaningful clinical response within 16 weeks.
- Abatacept represents an effective biologic therapy with acceptable safety across the spectrum of RA patient populations. Sustained clinical responses may be incremental for up to 2 years of treatment.
- The benefit-risk profile of abatacept may be most optimal when it is introduced earlier in the RA treatment paradigm. Both rituximab and abatacept slow radiographic progression in patients with RA.
- Current uncertainties include the lack of reliable biomarkers to inform the rational choice of a biologic agent.

As appreciation of the gravity of the social and economic burden imposed by rheumatoid arthritis (RA) has grown, so has the recognition that more favorable clinical outcomes are achieved when synovitis is optimally suppressed. The evidence is particularly compelling early in the course of RA, when intervention with disease-modifying combination therapy results in improved remission rates and increased clinical and radiographic benefits.[1-3] The armamentarium of potential therapeutics has also grown with the identification of relevant disease molecules. Of these, biologic therapeutics targeting tumor necrosis factor (TNF), particularly when used in combination with oral methotrexate, have had notable success in suppressing inflammation and markedly inhibiting the progression of structural damage previously thought to be an unavoidable characteristic of RA.[4,5] However, despite the unprecedented clinical and commercial successes of TNF inhibitors, their availability is restricted by high costs. In addition, a substantial proportion of patients with RA do not demonstrate significant clinical responses.

An entirely different treatment approach to the blockade of pro-inflammatory cytokines is the targeting of cells implicated in the persistence of RA. It is believed that immune responses drive the disease process in RA, and because chronicity is a hallmark of the RA phenotype, it presumably reflects the persistence of immunologic memory, which is induced and maintained by the adaptive immune system. In particular, T and B cells develop highly specific receptors and, after stimulation, expand enormously in number and then persist for long periods. If this is true of aberrant immune responses that lead to disease, then T and B cells represent rational targets for immune intervention. The focus of this chapter is on biologic agents with specificity for cellular targets, namely cell surface molecules associated with B cell subsets, most notably CD20, and co-stimulation molecules expressed on antigen-presenting cells that recognize cognate ligands on T cells. Two drugs that have become an accepted part of the pharmacologic armamentarium for RA treatment will be emphasized: rituximab and abatacept. Rituximab (Rituxan, Genentech, South San Francisco, Calif., and Biogen Idec, Cambridge, Mass.; MabThera, F. Hoffman-LaRoche AG, Basel, Switzerland) is an antibody that selectively depletes a B cell subset that expresses the CD20 antigen. Other biologic agents targeting this antigen that have been in clinical trials are the humanized monoclonal antibody ocrelizumab and fully human monoclonal antibody ofatumumab. Abatacept (Orencia, Bristol-Myers Squibb, New York, N.Y.) is a fusion protein that selectively modulates a co-stimulatory signal necessary for T cell activation. Rituximab is approved in the United States and Europe for use in combination with methotrexate to reduce the signs and symptoms of RA in adult patients who have moderately to severely active disease and have not responded to one or more anti-TNF drugs. Abatacept is the first selective co-stimulation modulator to be approved in the United States and Europe for the treatment of patients with RA

who have an inadequate response to other nonbiologic or biologic disease-modifying anti-rheumatic drugs.

## TARGETING B CELLS

**KEY POINT**

**B cells are major contributors to RA pathogenesis, but their precise roles in the induction and maintenance of abnormal immune activation remain poorly understood.**

The role of B cells in the pathogenesis of RA is not fully understood. Nonetheless, there are a number of known B cell functions of likely relevance, including their role in antigen presentation, secretion of pro-inflammatory cytokines, production of rheumatoid factor and thus immune complex formation, and co-stimulation of T cells. Of note, immune complexes are an important trigger to the production of TNF and other pro-inflammatory cytokines. B cells are also implicated in the process of ectopic lymphoid organogenesis in the rheumatoid synovium.

B cells arise from stem cells in the bone marrow, where they acquire an antibody receptor bearing a unique variable region. A number of maturation and activation steps take place as the B cells migrate from the marrow compartment, through blood, and to perifollicular germinal centers and memory compartments in lymphoid tissue before returning to the marrow as mature plasma cells.[6] Successful maturation and survival of cells are tightly regulated and dependent on a number of trophic signals delivered via cell surface ligands, such as vascular cell adhesion molecule-1 (VCAM-1), and soluble factors, such as B lymphocyte stimulator (BLyS).[7,8]

In the late 1990s, Edwards and colleagues[9,10] suggested that the (assumed) underlying autoreactive response in RA might be driven by self-perpetuating B cells and that the initiation of inflammation results from ligation of the low-affinity immunoglobulin G (IgG) receptor FcRγIIIa by immune complexes. An attractive feature of this hypothesis, particularly in seropositive patients, is that it might account for the tissue tropisms of disease expression in the RA syndrome complex, because FcRγIIIa is expressed in high levels in synovium and other extra-articular tissues that may be involved in RA. Rheumatoid factor–producing cells can capture antibodies bound to antigen before antigen internalization by endocytosis and subsequent presentation of peptide fragments to a T cell, with provision of T cell help to the B cell. Edwards and Cambridge[11] also proposed that such rheumatoid factor–producing B cells might become self-perpetuating by an amplification signal arising from co-ligation of the B cell receptor and small immune complexes formed by IgG rheumatoid factor bound to the complement component C3d, providing a survival signal. In contrast, co-ligation of certain other B cell surface receptors with the B cell receptor may provide a negative survival signal. In the rare event that self-perpetuating, autoreactive B cells arise, having escaped normal regulatory mechanisms, this theory predicts that a B cell depletion strategy would remove the autoreactive B cell clones and their antibody products. Because CD20 is not internalized and is highly expressed on a range of B lineage cells, including pre–B cells, immature B cells, activated cells, and memory cells, but is not found on stem, dendritic, or plasma cells (Figure 64-1), it is an ideal target for B cell depletion by monoclonal antibodies. The hypothesis that B cells represent a therapeutic target in RA is also being tested in the clinic using other strategies for B cell inhibition, as discussed later.

The CD20 antigen is located in the B cell membrane, with 44 amino acids exposed to the extra-cellular space. Its function is unknown, although it may have a role in cell signaling or in calcium mobilization.[12] Interestingly, CD20 knockout mice do not have a clear-cut phenotype or obvious B cell defect.[13] $CD20^+$ B cells represent a prominent population in the rheumatoid synovial tissue in most patients.

## RITUXIMAB AND RHEUMATOID ARTHRITIS

**KEY POINT**

**Rituximab is a depleting chimeric monoclonal antibody lytic for a population of B cells expressing CD20.**

Rituximab is a chimeric mouse-human monoclonal antibody directed against the extra-cellular domain of the CD20 antigen. It initiates complement-mediated B cell lysis and may permit antibody-dependent, cell-mediated cytotoxicity when the Fc portion of the antibody is recognized by corresponding receptors on cytotoxic cells. Rituximab may also initiate apoptosis[14] and influence the ability of B cells to respond to antigen or other stimuli.[15] Rituximab initially

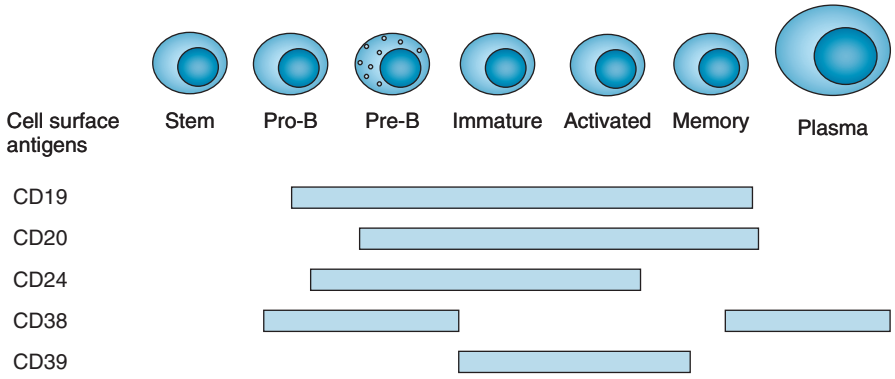



**Figure 64-1** Expression of the CD20 antigen on B lineage cells.

found a role in the clinic as a single-agent treatment for relapsed or refractory low-grade or follicular CD20+ B cell non-Hodgkin's lymphoma, for which it was approved. For this reason, there was wide experience with rituximab in hematologic oncology before initiation of clinical trials in RA and the approval of rituximab in the United States and Europe for the treatment of TNF inhibitor–refractory patients with RA who have active disease.

After rituximab administration, rapid B cell depletion takes place in peripheral blood, and conventional methods for measurement of peripheral blood B cells by means of CD19 expression detect no cells at all in most cases. Investigation of synovial tissue from patients with RA who are treated with rituximab reveal a decrease in synovial B cells and plasma cells in most though not all patients.[16,17] These findings raise the possibility that the inflamed synovium might contain as yet poorly understood rescue mechanisms that provide survival niches or, alternatively, that some B cells may have an inherent resistance against depletion.

Analysis of peripheral blood memory B cells before depletion, during depletion, and after reconstitution seems to be predictive of clinical outcome, with patients who show early relapses having substantially higher IgD+ and IgD-CD27+ memory B cell numbers and proportion during B cell recovery.[18] In addition to these cellular biomarkers, serologic parameters have been analyzed.

Decreases in rheumatoid factor (RF) or anti-citrullinated protein antibody (ACPA) serum levels are reported to be associated with B cell depletion,[19] but further studies are needed to determine the relationships between these serologic changes and clinical response.

## Clinical Studies

**KEY POINTS**

**Rituximab is an effective biologic therapy across the spectrum of RA patient populations.**

**Rituximab appears to have greatest benefit in seropositive patients.**

**Clinical trials in patients with active, established RA confirm that a single cycle of rituximab given as two infusions of 1 g each, together with once-weekly oral methotrexate, produces an enduring clinical response. A treatment cycle of two infusions of 500 mg is also efficacious but may result in a lower proportion of patients demonstrating more robust clinical responses.**

The findings of early clinical studies of B cell depletion therapy in patients with active RA using rituximab in a number of different treatment regimens suggested an encouraging benefit with an acceptable safety profile and pointed to a possible therapeutic role for rituximab in persons with RA.[20-22] However, confirmation of benefit required a randomized, double-blind, controlled study. In a phase IIa study, the efficacy of rituximab in persons with active RA was tested in 161 patients who had failed to respond adequately to methotrexate at a dose of at least 10 mg a week for a minimum of 16 weeks.[23] Patients were assigned to one of four treatment regimens: a 1-g infusion of intravenous rituximab alone on days 1 and 15, methotrexate alone as a comparison arm, intravenous rituximab with cyclophosphamide infusions at a dose of 750 mg on days 3 and 17, or rituximab and methotrexate. All patients received 100 mg of methylprednisolone just before each treatment, in addition to prednisolone, 60 mg daily, on days 2, 4, 5, 6, and 7, and 30 mg daily on days 8 to 14. The primary endpoint was the proportion of patients achieving an American College of Rheumatology (ACR)50 response at week 24, and exploratory analyses were undertaken at week 48. At week 24, a significantly greater proportion of patients achieved an ACR50 in the rituximab and methotrexate combination group (43%; $P = 0.005$) and in the rituximab and cyclophosphamide combination group (41%; $P = 0.005$) than in the group receiving methotrexate as monotherapy (13%) (Table 64-1). Thirty-three percent of the patients receiving rituximab alone achieved an ACR50 response, but this outcome failed to reach statistical significance compared with methotrexate alone ($P = 0.059$). In all the rituximab groups, the mean change from baseline in disease activity score was significant compared with methotrexate alone.

At 48 weeks, exploratory analyses indicated ACR50 and ACR70 responses in 35% and 15%, respectively, of patients in the rituximab and methotrexate group, which was significantly greater than the 5% and 0% responding at the corresponding levels in the methotrexate group. In the rituximab and cyclophosphamide treatment arm, 27% of patients achieved an ACR50 response.

Rituximab treatment was associated with near-complete peripheral blood B cell depletion that persisted throughout the 24-week period of the primary analysis. Patients in the rituximab groups had a substantial and rapid reduction in the concentration of rheumatoid factor in serum, but despite peripheral B cell depletion, immunoglobulin levels did not change substantially.[23] The overall incidence of infection was similar in the control and rituximab groups at 24 and 48 weeks. By week 24, four patients in the rituximab groups and one patient in the control group had acquired a serious infection. Two additional serious infections were reported during the extended 48-week period in the rituximab groups, one of which was fatal. Infusion reactions of any type were reported in 36% of patients receiving rituximab and 30% of patients receiving placebo, although most were

**TABLE 64-1** Percentage of Patients Achieving Responses at 24 Weeks in the DANCER and REFLEX Studies

| Study | Drug Regimen | ACR20 | ACR50 | ACR70 |
|---|---|---|---|---|
| Phase IIa Thurlings et al[19] | 1 g rituximab × 2 + methotrexate | 73 | 43 | 23 |
| | Methotrexate | 38 | 13 | 5 |
| Phase IIb DANCER[20] | 1 g rituximab × 2 + methotrexate | 54 | 34 | 20 |
| | Methotrexate | 28 | 13 | 5 |
| Phase III REFLEX[21] | 1 g rituximab × 2 + methotrexate | 51 | 27 | 12 |
| | Methotrexate | 18 | 5 | 1 |

ACR, American College of Rheumatology; DANCER, Dose-ranging Assessment: International Clinical Evaluation of Rituximab in Rheumatoid Arthritis; REFLEX, Randomized Evaluation of Long-term Efficacy of Rituximab in Rheumatoid Arthritis.

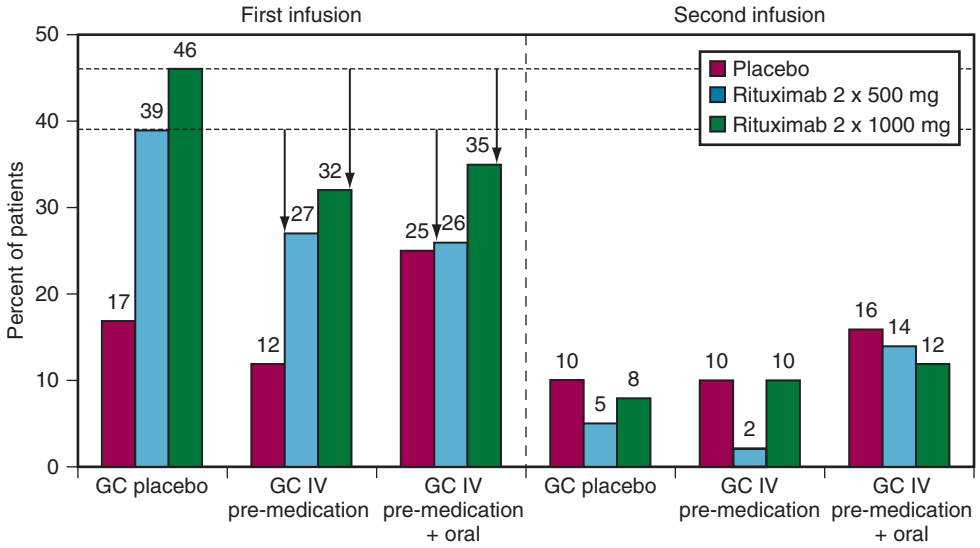



**Figure 64-2** The occurrence of infusion reactions in the DANCER (Dose-ranging Assessment: International Clinical Evaluation of Rituximab in RA) trial, a phase IIb study undertaken to examine the efficacy and safety of rituximab at two doses, with or without glucocorticoids (GCs), in patients with active rheumatoid arthritis (RA) resistant to disease-modifying anti-rheumatic drugs (DMARDs), including biologic agents. Patients were randomized to receive either placebo infusions or rituximab at a dose of 500 mg or 1 g on days 1 and 15, together with one of three glucocorticoid options: glucocorticoid placebo, 100 mg intravenous methylprednisolone before each rituximab infusion, or 100 mg methylprednisolone before each infusion in addition to an oral corticosteroid. Pre-treatment with methylprednisolone before rituximab infusion reduced the incidence and severity of reactions by about one-third. IV, Intravenous. *(Data from Emery P, Fleischmann R, Filipowicz-Sosnowska A; DANCER study group , et al: The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging study.* Arthritis Rheum *54:1390–1400, 2006.)*

characterized as mild or moderate. The reactions included hypotension, hypertension, flushing, pruritus, and rash. In the rare case of severe reactions, it has been suggested that a cytokine release syndrome associated with marked cell lysis after rituximab might be a contributing factor.

In summary, the findings of the phase IIa study indicated that a single course of treatment with rituximab, particularly in combination with methotrexate, produces an enduring response in patients with severe, seropositive, active RA. Further, treatment with rituximab was well tolerated, with a favorable safety profile over 48 weeks of follow-up.

To follow up the phase IIa study, a phase IIb study was undertaken to examine the efficacy and safety of rituximab at different doses, with or without glucocorticoids, in patients with active RA who were resistant to disease-modifying anti-rheumatic drugs (DMARDs), including biologic agents. The findings of this phase IIb study, known as the DANCER (Dose-ranging Assessment: International Clinical Evaluation of Rituximab in RA) trial, have been reported.[24] A total of 465 persons with active disease were recruited. To be included in the study, they had to have (1) failed to respond to at least one DMARD other than methotrexate, but no more than five, or failed to respond to biologic response modifiers, and (2) been treated with methotrexate as a single DMARD for at least 12 weeks, with 4 weeks of stable therapy at a dose of at least 10 mg a week. All other DMARDs were withdrawn at least 4 weeks before randomization—8 weeks for infliximab, adalimumab, and leflunomide. Patients were randomized to receive either placebo infusions or rituximab at a dose of 500 mg or 1 g on days 1 and 15, together with one of three glucocorticoid options: glucocorticoid placebo, 100 mg of intravenous methylprednisolone before each rituximab infusion, or 100 mg of methylprednisolone before each infusion in addition to an oral corticosteroid.

The results at 24 weeks confirmed the significant efficacy of a single course of rituximab in active RA when combined with methotrexate. This benefit was independent of glucocorticoids, although administration of methylprednisolone on day 1 reduced the incidence and severity of first rituximab infusion reactions by about one-third (Figure 64-2). Both rituximab doses were efficacious. At the lower dose, 55% of recipients achieved ACR20 responses, as did 54% of those at the higher rituximab dose—in both cases, significantly greater than the 28% of the subjects who received placebo infusions. Similarly, significantly higher proportions of subjects achieved ACR50, ACR70 (see Table 64-1), and European League against Rheumatism (EULAR) good responses at 24 weeks at both rituximab doses compared with subjects who received placebo infusions (Figure 64-3). At the most stringent ACR70 response level, the difference in the percentage of responders in the placebo, lower-dose rituximab, and higher-dose rituximab groups was most marked at the higher rituximab dose of 1 g 2 weeks apart (5%, 13%, and 20%, respectively; $P < 0.05$). Adverse events reported up to 24 weeks were largely infusion related, particularly at the time of the first infusion.

A trial known as REFLEX (Randomized Evaluation of Long-term Efficacy of Rituximab in RA) was designed to determine the efficacy and safety of rituximab when used in combination with methotrexate in patients with active RA who have had an inadequate response to one or more anti-TNF therapies because of either lack of efficacy (90% of patients recruited) or toxicity (10% of patients recruited); in addition, all patients had radiographic evidence of at least one joint with definite erosion attributable to RA

(Figure 64-4). The recruited cohort consisted of 520 patients with a mean disease duration of 12 years with a background regimen of methotrexate, 10 to 25 mg once a week. After a washout period during which other DMARDs and anti-TNF drugs were withdrawn, patients were randomized to receive a single course of 1 g of rituximab or placebo infusions on days 1 and 15. All patients were given 100 mg of intravenous methylprednisolone before each infusion and a brief course of oral prednisolone between the two doses: 60 mg daily from days 2 to 7, and 30 mg daily from days 8 to 14.[25]

Of the patients assigned to receive rituximab, 82% completed 6 months, compared with only 54% of the patients assigned to receive a placebo. The major reason for study withdrawal was lack of response, reported in 40% of the placebo group and 12% of the rituximab group. At 6 months, significantly more patients receiving rituximab achieved ACR20, ACR50, and ACR70 responses than did those receiving placebo: 51%, 27%, and 12%, respectively, of subjects receiving rituximab, versus 18%, 5%, and 1%, respectively, of those receiving placebo (see Table 64-1). In terms of change in Disease Activity Scale (DAS28), intention-to-treat analyses showed that in patients who received placebo infusions, the reduction from baseline was 0.34, which was less than the 0.6-point reduction considered to be clinically meaningful; in contrast, the reduction was 1.83 in the rituximab group.[25]

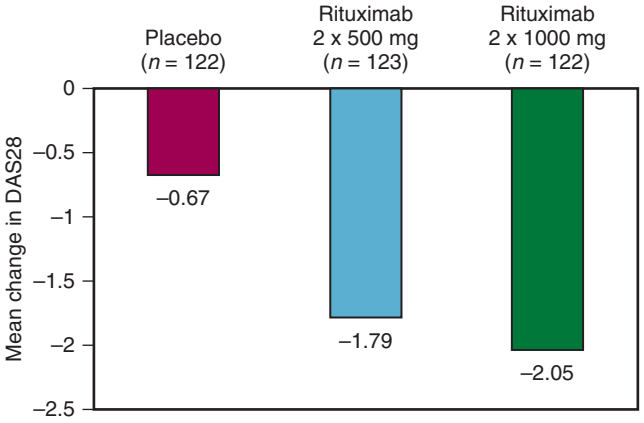



**Figure 64-3** DANCER (Dose-ranging Assessment: International Clinical Evaluation of Rituximab in RA trial) changes in the Disease Activity Scale (DAS)28 at 6 months. Mean changes in DAS28 from baseline in the phase IIb DANCER study were significantly greater in patients treated with two rituximab infusions 2 weeks apart at either 500 mg or 1 g each, compared with placebo infusions ($P < 0.0001$). *(Data from Emery P, Fleischmann R, Filipowicz-Sosnowska A; DANCER study group, et al: The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging study.* Arthritis Rheum *54:1390–1400, 2006.)*

The ACR response evaluates RA treatment based on a 20%, 50%, or 70% improvement in five of seven core components. However, from the patient's perspective, determining the actual benefit of an ACR20 improvement is not straightforward. In the REFLEX study, the rituximab group had significantly greater improvements in all components of the ACR core measures. Rituximab demonstrated a clinically meaningful benefit for patients with RA in physical function as evaluated by a Health Assessment Questionnaire (HAQ) in all non-overlapping ACR response categories. In the active treatment arm, both clinical and subjective parameters of the ACR core components contributed to the assignment of an ACR20 response, whereas in the placebo group, the subjective parameters dominated.[25]

In the REFLEX study, after a single treatment course, the maximal clinical response to rituximab plus methotrexate was observed at 24 weeks. After this time, patients were eligible to exit the study and receive further rituximab treatment based on clinical need. Of the patients in the rituximab plus methotrexate group, 37% (114 of 308) remained in the study over 48 weeks, indicating continued clinical benefit after the single initial treatment course. The majority of patients who withdrew did so to receive further courses of rituximab between weeks 24 and 48 of the study. In contrast, 89% of the placebo plus methotrexate group (185 of 209) withdrew before week 48.[26]

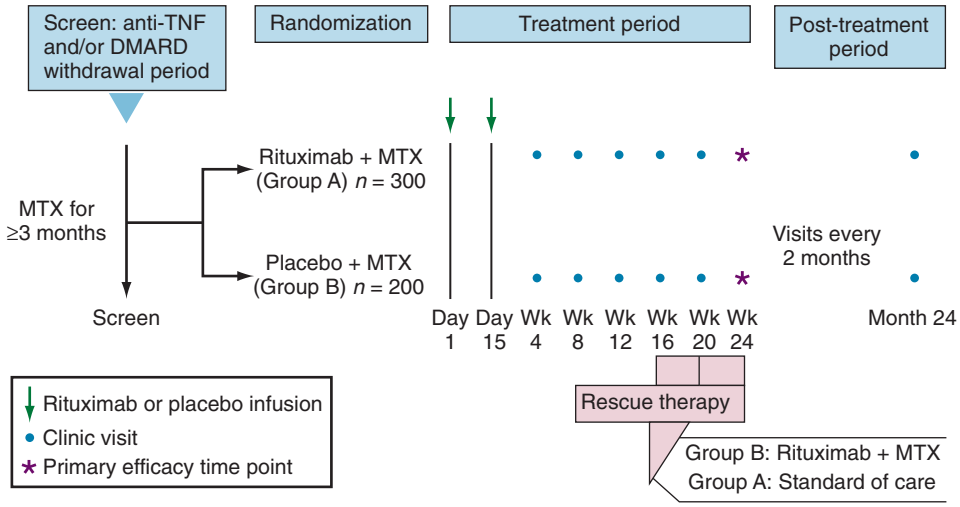



**Figure 64-4** The design of the phase III REFLEX (Randomized Evaluation of Long-term Efficacy of Rituximab in RA) study to determine the efficacy and safety of rituximab when used in combination with methotrexate (MTX) in patients with active rheumatoid arthritis (RA) who have an inadequate response to one or more anti–tumor necrosis factor (TNF) therapies. DMARD, Disease-modifying anti-rheumatic drug.

Another phase III study, known as SERENE (Study Evaluating Rituximab's Efficacy in methotrexate iNadequate rEsponders), confirmed the benefits of rituximab in patients with RA receiving concomitant methotrexate who had active disease at baseline despite methotrexate therapy and who had not received prior biologic therapy.[27] Patients were randomized to receive either placebo or rituximab at one of two doses, 2 × 500 mg or 2 × 1000. From week 24, in an open-label extension, the patients in the rituximab arms who did not achieve remission as assessed by DAS28 received a second course of rituximab, and all patients who had received placebo were treated with the lower rituximab dose. At week 24, a significantly greater proportion of patients receiving rituximab, 2 × 500 mg or 2 × 1000 mg plus methotrexate, achieved the primary endpoint of an ACR20 response versus patients receiving placebo plus methotrexate (54.5% and 50.6% vs. 23.3%, respectively; $P < 0.0001$). By week 48, approximately 90% of patients in all treatment groups had received a second course of treatment. The majority of these repeat treatments (82% to 88%) were given by week 30.

In patients treated with rituximab, efficacy outcomes at week 48 were comparable with those at week 24; additionally, improvement was observed for several clinically important endpoints, including an approximate doubling in the proportion of patients who achieved low disease activity (DAS28 < 3.2) in the rituximab (2 × 1000 mg) plus methotrexate–dose group from week 24 to week 48. The safety profile of rituximab in the SERENE study was comparable with that observed in earlier trials, with the most common adverse event being infusion-related reactions, most of which were not serious, at the time of first infusion. Reductions in immunoglobulin levels were observed, predominantly IgM, but with mean levels remaining within normal limits. No relationships between infectious complications and reduced immunoglobulin levels were observed—in fact, the rate of infection observed in patients receiving rituximab plus methotrexate was low and comparable with that of patients receiving methotrexate alone over the placebo-controlled 24-week period. This low rate of serious infection continued throughout the full 48-week period, with no obvious difference between the rituximab doses.

Results from the IMAGE (International Study in Methotrexate-naïve Subjects Investigating Rituximab's Efficacy) study, which included 748 patients without prior use of either biologic drugs or methotrexate, have been reported. In this group, high-risk patients who had baseline high DAS28 scores or high C-reactive protein (CRP) levels were found to have greater DAS28 improvement at week 52 if they received rituximab in addition to methotrexate.[28]

## Disease Modification

**KEY POINT**

**Rituximab given as two infusions of 1 g each slows radiographic progression in patients with RA.**

The extraordinary success of TNF blockade in inhibiting structural damage to joints in patients with RA has set a new standard to which all new biologic agents must aspire. Inhibition of structural joint damage by rituximab in persons with RA and a previous inadequate response to TNF inhibitors from the REFLEX study was first described over a 1-year period,[29] with a subsequent demonstration that the initial effects of rituximab are maintained over an extended interval of 2 years, with all measures of joint damage significantly improved compared with placebo plus methotrexate.[30] At week 56,[29] the mean change in Genant-modified Sharp score in the placebo plus methotrexate arm was 2.31, compared with 1.0 in the rituximab plus methotrexate group ($P = 0.0043$). Significant differences were also reported for joint space narrowing and bone erosions. At week 104, significantly lower changes in total Genant-modified Sharp score (1.14 vs. 2.81; $P < 0.0001$), erosion score (0.72 vs. 1.80; $P < 0.0001$), and joint space narrowing scores (0.42 vs. 1.00; $P < 0.0009$) were observed with rituximab plus methotrexate versus placebo plus methotrexate.[30] Importantly, within the rituximab group, 87% who had no progression of joint damage at 1 year remained nonprogressive at 2 years. Thus these data confirm that treatment with rituximab plus methotrexate has the benefit of sustained inhibition of joint damage progression in patients with RA who had a previously inadequate response to TNF inhibitors.

Radiographic outcomes were reported for a phase III study designed to determine the efficacy of rituximab in the prevention of joint damage and its safety in combination with methotrexate in the context of patients initiating treatment with methotrexate.[28] This study, known as the IMAGE trial, was a randomized, controlled, double-blind trial involving 748 methotrexate-naïve patients assigned to receive rituximab at doses of either 2 × 1000 mg or 2 × 500 mg every 24 weeks in combination with methotrexate or methotrexate alone. The primary endpoint was radiographic progression measured by modified total Sharp score (mTSS) at week 52. In subjects treated with 2 × 1000 mg rituximab and methotrexate, a significantly smaller change (0.359) in the mTSS was observed compared with subjects taking methotrexate alone (1.079; $P < 0.001$). Furthermore, a significantly higher proportion of subjects treated with MabThera and methotrexate had no progression in their joint damage over 1 year (64% vs. 53%; $P = 0.0309$). By week 52, 65% of these subjects experienced a 50% improvement in symptoms (ACR50) and 47% experienced a 70% improvement (ACR70), compared with 42% and 25%, respectively, for subjects taking methotrexate alone ($P < 0.0001$ for both ACR50 and ACR70 comparisons).

Recent evidence suggests that rituximab inhibits joint damage independently of its effects on disease activity.[31] A random 90% sample of patient data from two arms of the IMAGE trial were divided into low, moderate, or high disease activity at 1 year of treatment by simplified disease activity index, or by swollen joint count or CRP tertiles. Progression of damage by the Genant mTSS was compared between therapies (Kruskal-Wallis and Wilcoxon tests) for each of these subgroups. In subjects treated with methotrexate, 1-year radiographic progression in low, moderate, and high disease activity was 0.40 ± 0.88, 1.04 ± 1.73, and 1.31 ± 3.02, respectively. In contrast, in subjects receiving rituximab plus methotrexate, radiographic progression was 0.38 ± 1.07, 0.39 ± 1.28 ($P = 0.003$ by comparison with methotrexate), and –0.05 ± 0.44 ($P = 0.05$ by comparison with

methotrexate), respectively. These data suggest that, as in the case of biologic TNF and interleukin-6 receptor (IL-6R) inhibition, anti-CD20 antibody conveys profound antidestructive effects and dissociates the link between disease activity and joint damage.

## Safety Issues

**KEY POINTS**

**Rituximab has an acceptable safety record in RA trials, but infusion reactions of variable severity can occur; most are mild to moderate. The frequency and severity of infusion reactions are reduced by the administration of intravenous methylprednisolone before rituximab infusions.**

**A very rare complication in patients with RA who are treated with rituximab is progressive multifocal leukoencephalopathy. Because of the increase in relative risk, patients should be counseled appropriately.**

The rapidity and magnitude of peripheral blood B cell depletion after anti-CD20 therapy raises concerns about potential adverse sequelae. The peripheral compartment recovers after many months, but repopulation occurs predominantly with an immature and naïve subset of B cells. However, it must also be remembered that the circulation contains less than 2% of total B cells.[32] A common concern regarding all therapies directed at B cells is the potential for toxicity related to modulation of humoral immunity. Unlike other newly introduced biologic therapies for RA, rituximab has the considerable advantage of an oncology safety database based on more than 350,000 treatments of patients with non-Hodgkin's lymphoma since 1997.[33] The overall safety conclusions are that serious adverse events are infrequent and often associated with well-defined risk factors such as cardiopulmonary disease or a high number of circulating cancer cells. Of note, in the lymphoma population, prolonged peripheral B cell depletion has not been associated with cumulative toxicity or increased occurrence of opportunist infections.[34-36] However, it cannot be assumed that the toxicity profile will be identical in distinct disease phenotypes with differing pathogenic processes.

In RA open-label,[37] phase II,[23,24] and phase III[21] studies, although decreases in total serum immunoglobulin levels were observed in patients receiving rituximab, concentrations remained within normal limits. Of note, existing antibody titers against tetanus toxoid appear to be unaffected by a single course of rituximab treatment.[38] However, some anecdotal evidence indicates that total serum immunoglobulin concentrations fall below the normal range in patients receiving multiple cycles of rituximab treatment over a number of years in open-label studies.[11] It is unclear whether this outcome results in an increased risk of infection. In phase II studies, the majority of adverse events, including headache, nausea, and rigors, were mild to moderate and were associated with infusions. In a meta-analysis of randomized clinical trial data from three studies that reported adverse events arising after a single cycle of rituximab treatment in a total of 938 patients with RA who were refractory to nonbiologic DMARDs or biologic anti-TNFs,[23-25] it was calculated that the incidence of patients experiencing adverse events of all systems was not higher in the patients treated with rituximab than in the placebo groups (relative risk [RR], 1.062; 95% confidence interval [CI], 0.912 to 1.236; $P = 0.438$).[39]

In the DANCER trial, adverse events associated with rituximab were largely associated with the first infusion; these adverse events occurred in 39% of subjects treated with 500 mg of rituximab (without use of a steroid) and in 46% receiving 1 g, compared with 17% in subjects who received placebo infusions.[24] The corresponding incidence with the second infusion decreased to 5%, 8%, and 10%, respectively. Two serious infusion reactions, hypersensitivity and generalized edema, occurred on day 1. Pre-treatment with methylprednisolone reduced the incidence and severity of reactions by about one-third (see Figure 64-2). Infectious adverse events (largely upper respiratory tract infections) were reported in 28% of subjects who received a placebo and in 35% of subjects who received rituximab. Six serious infections occurred: two in the placebo group, four in patients receiving 1000 mg of rituximab, and none in patients receiving 500 mg of rituximab. No opportunistic infections or tuberculosis reactivations were reported.

Although the overall safety record based on trial data has been favorable, with wider clinical use of biologic B cell depletion, rarer serious complications have come to light. A potential association has been reported between the biologic therapies efalizumab, natalizumab, and rituximab and the rare, progressive, and usually fatal condition termed progressive multifocal leukoencephalopathy (PML), a rare brain disease caused by reactivation of the John Cunningham (JC) virus.[40] PML has been reported in patients receiving rituximab for hematologic conditions and systemic lupus erythematosus (SLE)[41] and, more recently, in patients with RA.[40] The cumulative incidence rate of PML in the RA population has been estimated at 1/100,000 RA admissions in an analysis limited to hospitalized patients with SLE and other rheumatic diseases (including 25 patients with RA), a majority of whom had concomitant risk factors, including HIV, malignancy, or transplantation of bone marrow or another organ.[42] The cumulative reporting rate of 2.2 cases of PML per 100,000 patients with RA treated with rituximab is more than double the estimated frequency in RA (95% CI, 0.3 to 8.0).[40] Although the absolute risk is small, the RR is such that it emphasizes the importance of providing the prospective patient being considered for B cell–depleting therapy, with thoughtful and balanced information about likely benefits, as well as more common through very rare complications.

The profound and enduring peripheral B cell depletion that accompanies use of rituximab raises a potential safety concern for patients in clinical practice who fail to derive adequate symptomatic benefit and may then be exposed to biologic DMARDs of an alternative mechanism of action at a time point before repopulation of circulating B cells can take place. Relatively few data regarding this circumstance are available to date, but preliminary information is available in 185 of 2578 patients with RA who went on to receive a biologic agent with an alternative mechanism of action as documented in a safety follow-up period after participation in trials in which they had previously received rituximab.[43] Of the 185 patients, 89% remained depleted of peripheral B cells at the point of beginning treatment with

a biologic agent with a new mechanism of action. The rate of serious infectious events reported after treatment with rituximab but prior to the second biologic exposure was 6.99 per 100 patient-years, which is comparable with the reported rate of 5.49 per 100 patient-years after exposure to a second biologic agent, the majority of which were TNF inhibitors. No fatal or opportunistic infections were observed, with the nature and course of infectious complications being within expectations for patients with RA receiving biologic therapy. In a population of methotrexate-naïve patients entering the IMAGE study,[28] safety data were consistent with results from previous rituximab clinical trials and further enhance the robust safety profile. Rates of serious adverse events and serious infections were similar between the two MabThera groups and the methotrexate-only group.

Persons with RA, particularly those treated with immunosuppressants, are at an increased risk of infection, and for this reason, vaccination is an important aspect of RA clinical management. The question of whether B cell–depleting therapy could adversely affect immunization responses by suppressing the antibody response from new vaccination or reducing pre-formed antibody from prior vaccination has been investigated, and the findings have been reported.[44-46] These studies indicate that vaccine responses to some, but not all, vaccinations may be diminished in patients treated with rituximab, most strikingly in the first 4 to 8 weeks after administration of rituximab.[44] Although prior vaccination and timing of vaccine administration after rituximab infusion may influence the ability to mount a response, no straightforward relationship exists between peripheral B cell reconstitution and response to immunization. Therefore, when vaccination is indicated, ideally it should be performed prior to rituximab treatment and avoided immediately after B cell depletion with a delay of several months. Of course, this guideline may not be practical for vaccines with seasonal availability (such as those for influenza variants), and it must be recognized that responses to vaccination do not necessarily correlate with the risk of infection. In general, the timing of rituximab administration must be determined according to clinical need without the requirement to delay until after supplies of a seasonally variable vaccine become available and the patient has been immunized.

In a recent meta-analysis of 6 studies enrolling 2728 patients, nonmelanotic skin carcinoma occurred more commonly than other carcinomas in the rituximab treatment groups (0.8% in the rituximab group vs. none reported in the control group). Moreover, the overall incidence of malignancies was higher in the rituximab group (2.1%) compared with the control group (0.6%).[47]

The safety and efficacy profile of rituximab in the treatment of RA discussed thus far is based on reports from randomized placebo-controlled trials of 6 to 12 months' duration. Open-label extension studies have analyzed safety and efficacy results over multiple courses of rituximab.[48] In safety analyses based on 5013 patient-years of rituximab exposure, in a total of 2578 patients with RA who received at least one course of rituximab, infusion-related reactions were the most common adverse event occurring in a quarter of patients during the first infusion of the initial treatment cycle, although only 1% of infusion reactions in total were considered to be of a serious nature. Importantly and reassuringly, the rates of both adverse events and serious adverse events were stable over infusion cycles, with the latter reported as 17.85 events/100 patient-years (95% CI, 16.72 to 19.06). Infections and serious infections over time remained stable across five treatment cycles at four to six events/100 patient-years. No cases of tuberculosis, disseminated fungal infections, or other serious opportunistic infections were identified during the analysis period.

Viral reactivation is a potential concern in immunosuppressed patients. The rate of herpes zoster infections in this large series was 0.98 events per 100 patient-years, similar to that reported for other RA populations. The much more serious and rare opportunistic infection PML occurred in a single patient who also received cancer chemotherapy and radiation, with the event occurring about 18 months after the last dose of rituximab and 9 months after receiving chemotherapy and radiation. The causal relationship to rituximab, if any, is thus not entirely clear. There was no increased risk of malignancy by comparison with reference patients with RA and with the general population in the United States. Myocardial infarction was one of the most common serious adverse events reported in the longer-term analyses at a rate of 0.56 per 100 patient-years, but this is consistent with rates reported in epidemiologic studies of patients with RA.

## Duration of Benefit

**KEY POINT**

**Repeat treatment with rituximab produces clinical responses that equal or exceed those from the first course of treatment, with a comparable duration of effect.**

Among patients with RA who experience clinical responses to rituximab treatment, the time to clinical relapse is heterogeneous. In some patients, relapse is closely correlated to the reappearance of peripheral blood B cells, but in other patients, it may be delayed by years.[49] Clinical relapse is more closely associated with increases in autoantibody levels, but better biomarkers are needed to reliably inform optimal management strategies on an individual basis. All B cell populations are depleted after rituximab therapy. Of residual B lineage cells, more than 80% exhibit a memory or plasma cell precursor phenotype.[50] B cell repopulation occurs at a mean of 8 months after rituximab therapy and depends on the formation of naïve B cells of an immature phenotype resembling those found in umbilical cord blood. Peripheral B cell depletion is accompanied by substantial increases in blood BLyS concentrations, which tend to fall with B cell repopulation.[51] BLyS is a naturally occurring protein required for the development of B lymphocytes into mature plasma cells. Elevated levels of BLyS in persons with RA are believed to contribute to the production of autoantibodies. However, in cases of prolonged clinical responses to rituximab, more gradual reductions in BLyS concentrations have been observed, extending beyond the period of B cell depletion. Thus BLyS may contribute to the survival or regeneration of pathogenic, autoreactive B cells. This hypothesis predicts a potential therapeutic role for BLyS blockade in addition to B cell depletion.

Information concerning the efficacy and safety of repeated cycles of rituximab treatment has emerged from experience in clinical practice and from randomized trials. The recently reported phase III MIRROR (Methotrexate Inadequate Responders Randomised study Of Rituximab) trial was a randomized, double-blind, international study to evaluate the efficacy and safety of three dosing regimens of rituximab in combination with methotrexate in 375 patients with active RA and an inadequate response to methotrexate.[52] Patients were randomized to three groups with two courses of rituximab treatment at varying doses as follows: group A: 500 mg rituximab (all courses) on days 1 and 15, with repeat treatment at 24 weeks; group B: first course 500 mg rituximab on days 1 and 15, a second course of 1000 mg of rituximab, and repeat treatment at 24 weeks; and group C: 1000 mg of rituximab (all courses) on days 1 and 15, with repeat treatment at 24 weeks. The primary endpoint was the proportion of patients achieving ACR20 at week 48. Secondary endpoints included ACR50, ACR70, and EULAR responses. There was a trend toward better efficacy results with the regular 2 × 1000-mg dose compared with the low dose of 2 × 500 mg, and this reached statistical significance for EULAR good/moderate response (2 × 1000 mg = 88% vs. 2 × 500 mg = 72%, $P < 0.05$). Other endpoints, although numerically superior at 48 weeks, did not show any statistically significant difference between the three dosing regimens.

## Current Role

**KEY POINT**

**Rituximab is generally considered to be an effective biologic option in patients with RA, particularly seropositive patients who have inadequate responses to TNF inhibitors.**

Recent advances in our understanding of the pathogenesis of RA emphasize the critical role of B cells in self-sustaining chronic inflammatory processes. Rituximab is an important addition to the therapeutic armamentarium for the treatment of RA. In current clinical practice, the major use for rituximab in the treatment of RA is confined to the TNF inhibitor–refractory population. SWITCH-RA is a global, observational study comparing the effectiveness of rituximab versus an alternative TNF inhibitor in patients with RA who had an inadequate response to one previous anti-TNF agent.[53] In this large cohort, 604 patients received rituximab and 507 received an alternative anti-TNF as a second biologic therapy. Reasons for discontinuing the first anti-TNF were inefficacy, intolerance, and other. Least squares mean (SE) change in DAS28-3-erythrocyte sedimentation rate (ESR) at 6 months was significantly greater in patients who received rituximab than in patients who received an anti-TNF agent: −1.5 versus −1.1; $P = 0.007$. The difference remained significant among patients who discontinued the initial anti-TNF agent because of inefficacy (−1.7 vs. −1.3; $P = 0.017$) but not because of intolerance (−0.7 vs. −0.7; $P = 0.894$). Seropositive patients showed significantly greater improvements in DAS28-3-ESR with rituximab than with an anti-TNF agent (−1.6 vs. −1.2; $P = 0.011$), particularly those who switched because of inefficacy (−1.9 vs. −1.5; $P = 0.021$). The overall incidence of adverse events was similar between the rituximab and TNF inhibitor groups. These real-life data suggest that particularly in seropositive patients and in patients who switched because of inefficacy after discontinuation of an initial TNF inhibitor, switching to rituximab is associated with significantly improved clinical effectiveness compared with switching to a second anti-TNF agent.

Data from a number of clinical trials (IMAGE, MIRROR, SERENE, REFLEX, and DANCER)[24,27,28,52,54] suggest that seropositive patients with RA (RF and/or ACPA) show a higher likelihood of response to B cell–depleting therapy compared with seronegative patients, in particular for improving signs and symptoms and inhibition of radiographic changes. However, it is nevertheless the case in both trials and clinical experience that a proportion of seronegative patients with RA show good clinical responses, although this proportion is less than in the case of seropositive patients.[55] In pooled analyses of data from the MIRROR and SERENE studies, at week 48, odds ratios for seropositive patients versus seronegative patients of achieving ACR20, ACR50, and ACR70 responses were 2.23 (95% CI, 1.38 to 3.58), 2.72 (95% CI, 1.58 to 4.70), and 3.29 (95% CI, 1.40 to 7.82), respectively.[56] These observations generate the hypothesis that other mechanisms may account for lower levels of response in seronegative patients, such as antigen presentation, co-stimulation, and cytokine drive, whereas high levels of response to rituximab therapy may be mediated primarily by the suppression of pathogenic antibodies. In contrast to RF, the type I IFN signature has been shown to be associated with a negative response to rituximab therapy in patients with RA.[57]

The optimal and most cost-effective dosing regimen for rituximab remains a matter of debate. The phase III SERENE study showed equal clinical efficacy for the 500 mg × 2 and 1000 mg × 2 rituximab doses,[27] but the phase III MIRROR trial[52] had differences in some outcomes favoring the higher dosage. Methotrexate-naïve patients (not an approved patient population for rituximab) were studied in the IMAGE study with clinical results that were equivalent, but with a radiographic result that favored the higher dosage.[28] Thus a summary of the current randomized controlled trial (RCT) data on rituximab dosing is that 1000 mg × 2 works well in a clinically meaningful proportion of patients, but not in all patients. The 500 mg × 2 rituximab dose achieves broadly similar results in the relevant patient populations overall and has the advantages of lower cost and possibly a lower rate of serious adverse events but perhaps with lower probability of high-impact clinical responses and inhibition of structural damage. Based on the DANCER study findings, it is recommended that each cycle of 1000 mg × 2 rituximab be given in combination with once-weekly methotrexate, usually at doses of at least 15 mg/week, to optimize efficacy. Further, administration of 100 mg intravenous methylprednisolone is recommended before each rituximab infusion to reduce the frequency and severity of infusion reactions.

Rituximab may also have a role in patients for whom TNF blockade is relatively contraindicated, such as those with connective tissue disease overlap syndromes. At present, uncertainties exist about the implications of long-term peripheral B cell depletion and the timing and need for redosing with rituximab in patients who respond.

Current research suggests that restoration of peripheral B cell numbers takes about 8 months after depletion treatment, although retreatment may be needed earlier. Results have been presented for an open-label study to evaluate the response to repeated courses of rituximab in patients with active RA who are participating in one of several phase II or III studies and to determine the optimal frequency for repeated treatment.[58] In a series of 155 patients with prior exposure to TNF inhibitors, ACR20, ACR50, and ACR70 scores were 65%, 33%, and 12%, respectively, after the first course, and they were 72%, 42%, and 21%, respectively, for the second treatment course, relative to the original baseline. In 82 of these patients who received a third course of rituximab, the median interval between the first and second courses was very similar to that between the second and third courses: 30 to 31 weeks.[58] Further studies are needed to identify the optimal regimens for maintenance therapy that will provide efficacy and limit toxicity.[59] Development of biomarkers informative of management decisions that would optimize response is a highly desirable goal, but as yet no biomarkers are in routine use. The magnitude of clinical response appears to be related to the completeness of peripheral B cell depletion, and this holds true whether the lower 500 mg × 2 dose schedule or the higher 1000 mg × 2 schedule is administered. By means of sensitive measurements permitting detection of very low numbers of preplasma B cells in the circulation, a recent study reported that patients with RA whose disease did not respond to an initial cycle of rituximab have higher circulating preplasma cell numbers at baseline and incomplete depletion. Furthermore, an additional cycle of rituximab administered prior to total B cell repopulation enhances B cell depletion and clinical responses.[60]

Although the available safety data for rituximab in persons with RA are reassuring, these data need to be interpreted with caution until larger numbers of patients have been treated and long-term safety and retreatment data become available. Further, a substantial body of safety data for rituximab in the treatment of non-Hodgkin's lymphoma is available, with similarly low infection rates reported. In oncology, some of the associated adverse events are related to circulating tumor loads. Overall, these data are reassuring with regard to the RA population, although close monitoring of immunocompetence and for the possibility of rare opportunistic infections is advisable.

## Future Directions and Other Approaches to B Cell–Targeted Therapy

**KEY POINT**

**Additional biologic approaches that target B cells are under investigation.**

Rituximab is currently indicated for the treatment of patients with moderate to severe RA who show no response, experience a loss of response with time, or have adverse effects to anti-TNF agents.[61] The findings of recently reported studies indicate that rituximab is also efficacious in a proportion of both patients with RA who are treatment naïve and patients with RA who take methotrexate, particularly if they are seropositive.[27,28] Therefore, in the face of competitive health economic data compared with TNF inhibitors, there has been interest in the potential of rituximab as a first-line biologic agent. However, questions remain about the safety of repeated treatment cycles, although encouraging data are emerging. Although the U.S. Food and Drug Administration (FDA) has received reports of patients who experienced fatal PML after rituximab treatment for SLE and RA, this event appears to be rare. A key issue that determines the future place of B cell depletion therapy will be defining the most effective strategy in early stages of RA to induce a remission and potentially even biologic-free remission, whether this can be achieved safely and effectively with rituximab, and whether any biomarkers can be developed that will reliably inform biologic treatment strategy on an individual patient basis.

Clinical trials and safety data for other antibodies targeting CD20, such as ocrelizumab, a humanized version of rituximab, and ofatumumab, a fully human anti-CD20, have also been reported. In phase I/II trials in persons with RA, ocrelizumab, in combination with methotrexate, was found to be safe and effective at doses consisting of two infusions of 200 mg or higher given 2 weeks apart.[62] Two ocrelizumab dose levels were studied in three phase III RA studies across various patient populations; full findings have yet to be reported. However, in spring 2010, a decision was announced to discontinue development of ocrelizumab for the RA indication after a detailed analysis of the efficacy and safety data from the RA program found that the overall benefit-risk profile of ocrelizumab was not favorable, taking into account other currently available treatment options. This decision was based on an infection-related safety signal that included serious infections, some of which were fatal, and opportunistic infections.

Ofatumumab is a human IgG1κ lytic monoclonal antibody with specificity for human CD20 antigen. It recognizes a unique membrane-proximal epitope on the human CD20 molecule that is distinct from the epitope recognized by rituximab and ocrelizumab.[63] The membrane proximity of this epitope is likely to account for the high efficiency of B cell killing observed with ofatumumab in both in vitro and in vivo pre-clinical studies. A phase I/II study of ofatumumab, administered as two intravenous infusions of 300, 700, or 1000 mg 2 weeks apart in patients with active RA who had an inadequate response to DMARDs, demonstrated significant clinical benefit and reasonable tolerability (which improved after implementation of pre-medication) at all doses investigated when compared with placebo, with the 700-mg dose considered optimal.[63] Despite these positive results in RA studies, further work on ofatumumab by intravenous delivery in autoimmune conditions was discontinued, and focus on a subcutaneous delivery program began. Plans for a study in persons with multiple sclerosis are under way, but further development in RA remains under review, although this situation has not been prompted by the observation of unexpected opportunist infections as was the case for ocrelizumab.

Many other approaches to B cell–targeted therapy are in clinical testing, although it is unlikely that any of these will have a significant impact on the rituximab niche in the near future. Alternative strategies to target the B cell compartment include the use of BLyS antibodies. Belimumab is a

human anti-BLyS monoclonal antibody recently investigated in clinical trials for the treatment of RA and other rheumatic indications. An alternative approach to BLyS inhibition that is still in the early stages of clinical development is to block signaling through BLyS receptors using a soluble receptor, such as transmembrane activator and calcium modulator and cyclophilin ligand interactor immunoglobulin. Preliminary results of a phase II double-blind, placebo-controlled study of belimumab in active RA have been presented.[64] Patients were randomized to receive intravenous belimumab at a dose of 1, 4, or 10 mg/kg or placebo infusions on days 0, 14, and 28, then every 28 days through 24 weeks. The ACR20 response at week 24 in the combined belimumab groups was 29%, compared with 16% in the placebo group; no dose response was observed. The antibody was well tolerated. These preliminary findings with a functional inhibitor of B cells are surprising, given the effectiveness of rituximab; however, it may simply represent a pharmacokinetic problem, indicating that the dose of belimumab was too low. The benefit-risk ratio of belimumab has come under close scrutiny by the FDA, and it seems unlikely that it will progress in clinical development with RA as an indication.

## RITUXIMAB IN OTHER RHEUMATIC CONDITIONS

**KEY POINT**

**Rituximab has demonstrated benefits in studies of RA and anti-neutrophil cytoplasmic antibody–associated vasculitis. Clinical trials in SLE have not shown clinical benefit.**

Rituximab has also been used to treat a number of other rheumatic diseases.[11] Theoretical considerations and preliminary data suggested that B cell depletion using rituximab might have efficacy for immune thrombocytopenia, anti-neutrophil cytoplasmic antibody (ANCA)–associated vasculitis, and SLE. The rationale for the use of rituximab in the treatment of patients with ANCA-associated vasculitis is that elimination of CD20 B cell precursors could lead to transient removal of pathogenic antibodies and remission, assuming that ANCA are produced by short-lived B lineage cells rather than long-living plasma cells. Furthermore, in ANCA-associated vasculitis, the number of activated, circulating B lymphocytes correlates with disease activity and tissue involvement. Therefore the hypothesis that rituximab might induce disease remission in patients with severe ANCA-associated vasculitis has been tested in a phase II/III multicenter, randomized, double-blind, placebo-controlled trial known as RAVE (Rituximab for ANCA-associated Vasculitis). Findings have been reported comparing rituximab (375 mg/m$^2$ administered intravenously once weekly for 4 weeks) with cyclophosphamide (2 mg/kg/day administered orally).[65] Sixty-three patients in the rituximab group (64%) reached the primary endpoint, compared with 52 patients in the control group (53%), a result that met the criterion for noninferiority ($P < 0.001$). The rituximab-based regimen was more efficacious than the cyclophosphamide-based regimen for inducing remission of relapsing disease; 34 of 51 patients in the rituximab group (67%) compared with 21 of 50 patients in the control group (42%) reached the primary endpoint ($P = 0.01$). Rituximab was also as effective as cyclophosphamide in the treatment of patients with major renal disease or alveolar hemorrhage. No significant differences were found between the treatment groups with respect to rates of adverse events. Despite these encouraging data, the true positive effect of rituximab in ANCA-associated vasculitis is difficult to determine because of the simultaneous administration of high-dose glucocorticoids, which may contribute to a substantial decrease in ANCA titers and the observed remission rates.

Given the large body of evidence implicating abnormalities in the B cell compartment in SLE, a recent therapeutic focus has been to develop interventions that target the B cell compartment by multiple mechanisms, and rituximab has been studied most extensively. The best evidence in support of using rituximab for the treatment of patients with ANCA-associated vasculitis or SLE comes from clinical experience, retrospective case series, and small prospective uncontrolled studies, mainly in patients with refractory or frequently relapsing disease.[66,67] However, very recently, two moderately sized phase III randomized placebo-controlled trials of rituximab for the treatment of moderately active nonrenal SLE (EXPLORER) or class III/IV lupus nephritis (LUNAR) have failed to demonstrate superiority of this B cell–depleting agent compared with placebo when added to standard of care, that is, conventional immunosuppressive therapy. Both trials had a relatively short follow-up period. Preliminary data from LUNAR (a study to evaluate the efficacy and safety of rituximab in subjects with International Society of Nephrology/Renal Pathology Society [ISN/RPS] class III or IV lupus nephritis) have been reported.[68] This multicenter, randomized, double-blind, placebo-controlled trial included 144 patients with lupus nephritis (67% of patients had class IV disease) and compared the efficacy and safety of rituximab with placebo. Patients with class III and IV disease and a urine protein to creatinine ratio greater than 1 were randomly assigned to receive either 1000 mg of rituximab or placebo on days 1, 15, 168, and 182, in conjunction with mycophenolate mofetil and corticosteroids. No significant differences were observed in complete or partial renal response or clinical benefit to therapy at week 52, although rituximab administration was associated with significantly reduced titers of antibodies to double-stranded DNA and increased levels of C3 complement component. Serious adverse events, such as infection, were similar between the two patient groups.

The EXPLORER trial (a study to evaluate the efficacy and safety of rituximab in patients with severe SLE) randomly assigned patients with SLE who had moderate to severe disease activity despite treatment with immunosuppressive agents and corticosteroids to receive either placebo or rituximab infusions.[69] Patients with active glomerulonephritis were excluded. The British Isles lupus assessment group index was used to score treatment response four times per week for 52 weeks after the first infusion. Responses were recorded in 66% of patients in the placebo group and 75.1% of patients treated with rituximab. The time to the first moderate or severe flare did not differ between groups, but a trend for a prolonged time to the first "a" score flare in the rituximab group was observed. Annual rates of severe

and moderate disease activity flares were similar, but the mean annual rate of "a" score flares was significantly lower in the rituximab group than in the placebo group (0.86 vs. 1.41). The number of adverse events and overall infections were comparable between groups at 78 weeks, although serious infections were more numerous in the placebo group.

It is unclear why the LUNAR and EXPLORER studies failed to prove the superiority of rituximab to placebo in patients with SLE, although design flaws seem likely. Overuse of concomitant steroids and continued immunosuppressive treatment could help mask the possible benefits of rituximab. Moreover, consensus is lacking regarding the optimal dose and administration regimen of rituximab in patients with SLE and adjustments for the organ or system involved. EXPLORER included patients with very active disease who were treated aggressively with moderate-to high-dose glucocorticoids, which made the short-term detection of treatment benefits difficult.[70] Other potential shortcomings for lupus clinical studies in general, including EXPLORER and LUNAR, is that the length of follow-up may have been too short to demonstrate separation between the different treatments. The unexpected failure to show overall clinical benefit of rituximab in SLE trials may also reflect the inadequacy of the clinical outcome instruments employed. This factor is less likely to be relevant in the LUNAR nephritis study, for which the outcome measurements were more unequivocal. It is noteworthy that in both the EXPLORER and LUNAR studies, outcomes appeared to be more favorable in African-American and Hispanic subjects.[71]

Rituximab has also been used in primary Sjögren's syndrome; granulomatosis with polyangiitis; hepatitis C–associated cryoglobulinemia; ANCA-associated vasculitides other than granulomatosis with polyangiitis, such as polyarteritis nodosa, dermatomyositis, and polymyositis[72]; antiphospholipid syndrome; and scleroderma.[73]

## TARGETING CO-STIMULATORY MOLECULES

**KEY POINT**

**Activated T cells are implicated in the pathogenesis of RA, and co-stimulation is essential in induction of adaptive immune responses.**

Co-stimulation is an essential step in the induction of adaptive immune responses. Although the role of T cells in the perpetuation of RA has been debated and remains poorly understood, it has long been believed that T cell activation is a key event in the pathogenesis. Successful T cell activation requires multiple signals. One signal is provided by presentation of an antigen bound to cell surface major histocompatibility complex (MHC) molecules on antigen-presenting cells to a specific T cell receptor (TCR). In the absence of further signals, T cells become unresponsive and may ultimately be eliminated through apoptosis. An important co-stimulatory signal is provided by an interaction between members of the B7 family (either CD80 or CD86) on antigen-presenting cells and CD28 on T cells (Figure 64-5). Other key interactions between antigen-presenting cells and T cells are mediated by the binding of intercellular adhesion molecule-1 (ICAM-1) to leukocyte function-associated antigen-1 (LFA-1), CD40 to CD40 ligand, LFA-3 to CD2, and so on. After activation, T cells express cytolytic T lymphocyte–associated protein 4 (CTLA-4), which interferes with the B7-CD28 interaction and helps return the cells to the quiescent state.

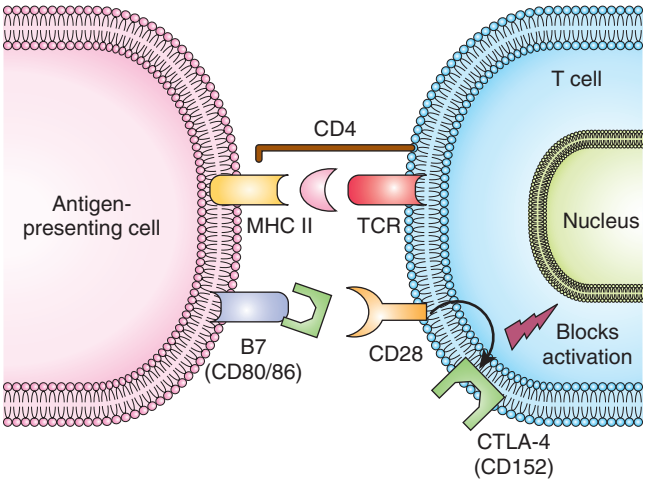



**Figure 64-5** Interactions between antigen-presenting cells and T cells. Successful T cell activation requires multiple signals. One signal is provided by the presentation of an antigen bound to cell surface major histocompatibility complex (MHC) molecules on antigen-presenting cells to a specific T cell receptor (TCR). In the absence of further signals, T cells become unresponsive and may ultimately be eliminated through apoptosis. An important co-stimulatory signal is provided by an interaction between members of the B7 family (either CD80 or CD86) on antigen-presenting cells and CD28 on T cells. After activation, T cells express cytolytic T lymphocyte–associated protein 4 (CTLA-4), which interferes with the B7-CD28 interaction and helps return the cells to the quiescent state.

## ABATACEPT AND RHEUMATOID ARTHRITIS

**KEY POINT**

**Abatacept is a human fusion protein comprising the extra-cellular portion of CTLA-4 and the Fc fragment of IgG-1.**

Abatacept is a novel, fully human fusion protein consisting of the extra-cellular portion of CTLA-4 and the Fc fragment of human IgG-1 (CTLA-4Ig). In December 2005, abatacept (Orencia) became the first co-stimulatory blocker to be approved by the FDA for the treatment of patients with RA who have had an inadequate response to other drugs. Abatacept binds to CD80 and CD86 on antigen-presenting cells, thus preventing these molecules from binding their ligand, CD28, on T cells, with the consequent inhibition of optimal T cell activation. In vitro, abatacept decreases T cell proliferation and inhibits the production of TNF, IFN-γ, and IL-2. CTLA-4Ig showed promising activity in rodent collagen-induced arthritis models, prompting its evaluation in several clinical trials in persons with RA.[74,75]

## Clinical Studies

**KEY POINT**

**Abatacept is an effective therapy across the spectrum of RA patient populations. For a majority of patients achieving clinical responses in the first 6 months, sustained clinical responses follow that may be incremental for up to 2 years of treatment.**

Abatacept has been evaluated in several double-blind, placebo-controlled trials in a number of clinical scenarios in adults with active RA. These scenarios include an inadequate response to conventional DMARDs such as methotrexate or to TNF inhibitors and, more recently, methotrexate-naïve patients in the early phase of disease. In addition, data have been reported in the context of an exploratory phase II study designed to assess the effect of co-stimulation blockade on progression of undifferentiated, early inflammatory arthritis to fulfill classification criteria for RA.

An initial 3-month, phase IIa, double-blind, randomized, placebo-controlled pilot study demonstrated the efficacy of B7 blockade in treating the signs and symptoms in patients with active RA despite treatment with at least one conventional DMARD.[76] In this pilot study, the effect of one of two different biologic co-stimulatory modulators was compared with that of placebo infusions. The two biologic agents used were CTLA-4Ig, which binds approximately fourfold less avidly to CD86 than to CD80, and belatacept, a second-generation CTLA-4Ig with two mutated amino acid residues conferring an increased avidity for CD86 over that of the parent molecule. The proportion of patients achieving ACR20 responses on day 85 was dose dependent, suggesting clinical efficacy for both co-stimulatory blocking molecules.

The findings were confirmed in a multicenter phase IIb study of abatacept plus methotrexate in 339 patients who had active RA despite methotrexate treatment.[77] In this study, patients were randomized to receive infusions of placebo; abatacept, 2 mg/kg; or abatacept, 10 mg/kg at baseline, at 2 weeks, 4 weeks, and then monthly through 6 months. ACR20 responses were achieved in 60%, 41.9%, and 35.3% of patients receiving the 10-mg/kg dose of abatacept, the 2-mg/kg dose of abatacept, and placebo, respectively. At the more stringent ACR50 response level, the figures were 36.5%, 22.9%, and 11.8% (Table 64-2). Improvements in the individual components of the ACR response criteria were generally greater in the 10-mg/kg group than in the 2-mg/kg group. No deaths, malignancies, or opportunistic infections were reported for any patient receiving abatacept during the 6 months of therapy. Patients in the phase IIb study continued to receive blinded therapy for an additional 6 months, during which time response to therapy was maintained. For patients receiving 10 mg/kg of abatacept, the ACR70, ACR50, and ASCR20 response rates were 21%, 42%, and 63%, respectively, compared with 8%, 20%, and 36% for patients receiving placebo infusions. Further, at the higher dose, statistically significant improvements in physical function and health-related quality of life were maintained over the 1-year period.[78] In the phase IIb study, from day 90 onward, statistically significant and progressively rising differences in remission rates were observed between the group receiving methotrexate plus abatacept, 10 mg/kg, and the group assigned to methotrexate and placebo infusions. By 1 year of treatment, 34.8% of patients receiving abatacept plus methotrexate achieved a DAS28 remission (<2.6), in contrast to 10.1% of the patients receiving methotrexate plus placebo ($P < 0.001$).[78] Patients completing the double-blind phase over 12 months became eligible to enter a long-term extension phase in which all participants received methotrexate plus abatacept, 10 mg/kg. At year 3, patients treated with abatacept experienced greater than 70% improvement in swollen and tender joint counts and approximately 50% improvement in pain and physical function.[79] Patients who received placebo infusions during the double-blinded phase and then switched to abatacept during the long-term extension rapidly achieved equivalent efficacy to those treated with abatacept throughout.

**TABLE 64-2** Percentage of Patients Achieving Responses at 24 Weeks in the AIM and ATTAIN Studies

| Study | Drug Regimen | ACR20 | ACR50 | ACR70 |
|---|---|---|---|---|
| Phase IIb AIM[42] | Abatacept 10 mg/kg + methotrexate | 60 | 37 | 17 |
| | Methotrexate | 35 | 12 | 2 |
| Phase III AIM[45] | Abatacept 10 mg/kg + methotrexate | 68 | 40 | 20 |
| | Methotrexate | 40 | 17 | 7 |
| Phase III ATTAIN[46] | Abatacept 10 mg/kg + methotrexate | 50 | 20 | 10 |
| | Methotrexate | 20 | 4 | 1 |

ACR, American College of Rheumatology; AIM, Abatacept in Inadequate Responders to Methotrexate; ATTAIN, Abatacept Trial in Treatment of Anti-TNF Inadequate Responders.

The findings of two large phase III studies of abatacept in different RA populations have been reported. A population of methotrexate-refractory patients was studied in the Abatacept in Inadequate Responders to Methotrexate (AIM) trial. This study was designed to further evaluate the safety and clinical efficacy of abatacept plus methotrexate, as well as the effect on radiographic progression.[80] In the other phase III study, the Abatacept Trial in Treatment of Anti-TNF Inadequate Responders (ATTAIN),[81] the objective was to determine whether abatacept is a safe and effective treatment for patients with RA who were unresponsive to previous anti-TNF treatment.

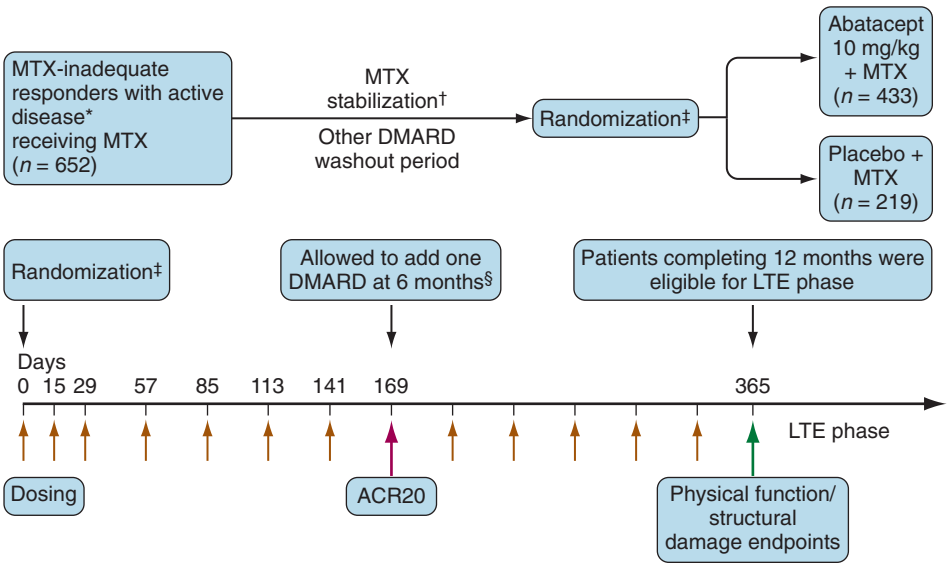
In the AIM study, 652 patients with RA who had an inadequate response to methotrexate were randomly assigned to receive placebo or a fixed dose of abatacept approximating 10 mg/kg on days 1, 15, and 29 and every 4 weeks thereafter for a year; all patients continued to receive background methotrexate therapy.[80] Both patient groups exhibited high disease activity at baseline, with a DAS28 of 6.4. Patients receiving abatacept showed greater improvement in all ACR response criteria at 6 and 12 months than did patients treated with placebo (see Table 64-2). In findings similar to the phase IIb study, abatacept plus methotrexate-induced DAS28 remission (<2.6) in 14.8% of patients at 6 months and in 23.8% at 12 months, compared

with 2.8% and 1.9% of patients receiving methotrexate plus placebo at the corresponding time points ($P < 0.001$). Physical function significantly improved in 63.7% of the abatacept plus methotrexate group, versus 39.3% of the placebo plus methotrexate group ($P < 0.001$). Further, patients taking the abatacept and methotrexate combination had a slower progression of mean structural damage (1.2 mTSS points over 1 year) compared with methotrexate alone (2.3 mTSS points).[80] Of interest, when assessed by conventional clinical outcome measures such as EULAR response, the data suggest that a plateau of clinical efficacy is achieved with abatacept between 4 and 6 months of treatment. However, using more stringent measures for analyses, such as time to a low DAS (DAS28 < 3.2) or time to a sustained low DAS, no plateau of efficacy was observed over the first 12 months, suggesting an ongoing recruitment of clinical benefit with abatacept plus methotrexate.[82]

Patients completing the double-blind phase of the AIM study over 12 months became eligible to enter a long-term extension phase in which all participants received methotrexate plus abatacept at a fixed dose approximating 10 mg/kg every 4 weeks (Figure 64-6). Clinically meaningful reductions in disease activity were maintained through 2 years, accompanied by an improved sense of subjective well-being assessed by patient-reported outcomes.[83] Further, inhibition of structural damage to joints was sustained, as evaluated by plain radiography; the effect after 2 years of abatacept was significantly greater than that at 1 year, with minimal radiographic progression observed over the second year of treatment.[84]

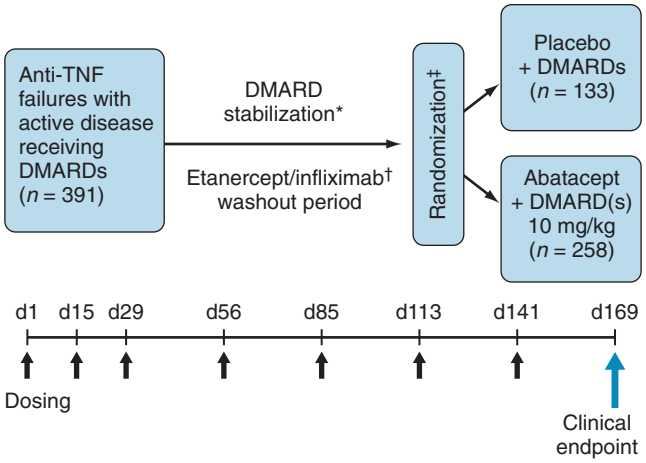
A third trial in methotrexate-inadequate responders provided the opportunity to evaluate two biologic agents in a single study. The placebo- and active comparator-controlled ATTEST (abatacept or infliximab versus placebo, a trial for tolerability, efficacy, and safety in treating RA) study, although not powered to detect superiority, provided information on the relative efficacy and safety profiles of abatacept and infliximab versus placebo in the same population.[85] Patients with an inadequate response to methotrexate were randomized (3:3:2) to abatacept (approved dose), infliximab (3 mg/week) or placebo with background methotrexate. At month 6, patients in the placebo group were switched to abatacept, and infliximab and abatacept groups continued to year 1, with blinding maintained. The primary endpoint of this trial, reduction in DAS28 (ESR) at month 6 for abatacept versus placebo, was met, with mean reductions of −2.53 versus −1.48 ($P < 0.001$), respectively. The proportion of patients achieving low disease activity and DAS28 remission was also greater with abatacept. Improvements in ACR20, ACR50, and ACR70 responses at month 6 were significantly greater versus placebo for both abatacept and infliximab. The onset of ACR20 responses was generally more rapid for infliximab than for abatacept, but responses were similar by month 3. By year 1, DAS28 (ESR) reductions of −2.88 and −2.25 were seen for patients treated with abatacept and infliximab, respectively, and ACR responses were maintained from month 6 with abatacept but not with infliximab.

In the ATTAIN phase III study, abatacept therapy was evaluated in 391 patients with active disease receiving conventional DMARDs or anakinra who failed to respond adequately to at least 3 months of therapy with etanercept, infliximab, or both agents at the approved doses.[81] Anti-TNF therapy was discontinued at the time of enrollment if it had not been stopped previously. After a washout period, patients were randomly assigned in a 2:1 ratio to receive




*≥10 swollen joints (66 joint count), ≥12 tender joints (68-joint count) and CRP ≥ 1.0 mg/dL and ≥3 months on MTX ≥ 15 mg/wk.
†Stable MTX dose for >1 month before enrollment.
‡2:1 randomization to study arm.
§Sulfasalazine, hydroxychloroquine, gold.

**Figure 64-6** Phase III AIM (Abatacept in Inadequate Responders to Methotrexate) study design. ACR, American College of Rheumatology; CRP, C-reactive protein; DMARD, disease-modifying anti-rheumatic drug; LTE, long-term extension; MTX, methotrexate.




* DMARD/anakinra regimen stable for 28 days.
† Washout = 30 days for etanercept; 60 days for infliximab.
‡ 2:1 randomization to study arm.

**Figure 64-7** Phase III ATTAIN (Abatacept Trial in Treatment of Anti-TNF Inadequate Responders) study design. DMARD, Disease-modifying anti-rheumatic drug; TNF, tumor necrosis factor. *(From Genovese MC, Becker JC, Schiff M, et al: Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition.* N Engl J Med *353:1114–1123, 2005.)*

**TABLE 64-3** Comparison of TNF Inhibition Versus Other Biologics Abatacept versus Adalimumab (AMPLE trial)

| Treatment Regimen | Total Number of patients | Number of patients showing MTX-inadequate response | Percentage of patients showing MTX-inadequate response |
|---|---|---|---|
| SC Abatacept + MTX | 318 | 206 | 64.8 |
| Adalimumab + MTX | 328 | 208 | 63.4 |

Estimate of difference (95% CI) between groups was 1.8 (-5.6, 9.2); Intent to treat, confirmed with per protocol population

MTX, Methotrexate.

Modified from Weinblatt ME, et al: Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: findings of a phase IIIb, multinational, prospective, randomized study. *Arthritis Rheum* 65(1):28–38, 2013.

either the same fixed dose of abatacept (approximating 10 mg/kg) or placebo (Figure 64-7). Patients receiving abatacept showed significantly greater improvement in all ACR response criteria through 6 months (see Table 64-2) than did patients treated with placebo (ACR20 response 50.4% vs. 19.5%, $P < 0.001$; ACR50 response 20.3% vs. 3.8%, $P < 0.001$; and ACR70 response 10.2% vs. 1.5%, $P = 0.003$) (Table 64-3). Further, a DAS28 remission was achieved in 10% of patients receiving abatacept versus only 1% of patients receiving placebo infusions plus DMARDs. ACR20 responses were seen irrespective of whether patients had previously been exposed to etanercept, infliximab, or both anti-TNF therapies without an adequate response. Improvement in physical function was also significantly increased in the abatacept group (47% vs. 23%). The incidence of infection was slightly higher in the abatacept group than in the placebo group, although no specific infection was clearly more frequent, and the intensity of infections was similar in the two groups. There were no significant differences in the number of patients discontinuing treatment as a result of infection or in the incidence of serious infection.

All patients who completed the 6-month double-blind phase of the ATTAIN study were eligible to enter a 1-year, long-term extension phase during which all patients received a once-monthly fixed dose of abatacept in addition to at least one conventional DMARD.[86] Of 258 patients randomized to receive abatacept during the double-blind phase, 223 completed 6 months of treatment, and 218 entered the long-term extension. Of these patients, 168 completed 18 months of treatment. The ACR20 responses observed at the end of the double-blind phase were sustained throughout the 1-year extension phase, with the proportion of patients achieving the more stringent ACR50 and ACR70 responses rising to 35% and 18%, respectively, at 18 months. Further, the proportion of patients meeting DAS28 remission criteria doubled to 22.5% by the end of the extension period. Similarly, among all patients initially treated with abatacept and DMARDs who entered the long-term extension phase, the mean reduction in DAS28 from baseline to the end of the double-blind phase was –1.99; by the end of 18 months, the mean reduction from baseline was –2.81. In the double-blind phase, patients assigned to placebo infusions together with DMARDs had a mean reduction in DAS28 of –0.93; at the end of the long-term extension, after crossing over to abatacept infusions, the reduction from baseline was –2.72.[87] These data again emphasize the sustained but relatively slow and incremental clinical responses observed after abatacept therapy.

The second trial conducted in TNF-inadequate responders was a phase IIIb/IV, 6-month, open-label study. The ARRIVE (Abatacept Researched in Rheumatoid arthritis patients with an Inadequate anti-TNF response to Validate Effectiveness) trial was the first to assess the safety of abatacept in patients who switched directly from TNF antagonist therapy without undergoing washout, an approach that may be more clinically relevant for everyday practice.[88] Patients enrolled in this study had high disease activity levels at entry and an inadequate response to up to three TNF antagonists; the inadequate response could have occurred for efficacy, safety, or tolerability reasons. Patients were eligible even if they had a positive purified protein-derivative test result. Abatacept could be administered as monotherapy (for those recruited in the United States only), and patients were not limited to a particular background DMARD. Similar, clinically meaningful improvements were seen in disease activity, physical function, and health-related quality of life, regardless of whether there was a washout period. Post hoc analyses revealed that, numerically, more patients who had previously failed to respond to one TNF antagonist achieved DAS28-defined remission and low disease activity than did those who had failed to respond to two or more TNF inhibitors.

Because T cell activation is believed to be an initiating event in an immunologic cascade observed in RA, co-stimulation blockade might be predicted to have benefits from an early stage of evolution of the syndrome independently of any driving antigen or antigens. The 2-year abatacept study to gauge remission and joint damage progression

in methotrexate-naïve patients with early erosive RA (AGREE) consisted of a 12-month double-blind period, followed by a 12-month open-label period in methotrexate-naïve patients with early RA.[89] Eligibility included short disease duration, poor prognostic factors, including high CRP levels, radiographic evidence of erosions, and seropositivity for RF or ACPA. Patients were randomized 1:1 to receive abatacept plus methotrexate ($n = 256$) or methotrexate alone ($n = 253$) during the first 12-month period.[89] From year 1 onward, all patients received open-label abatacept plus methotrexate. The co-primary endpoints were 28-joint DAS (DAS28)-defined remission and radiographic joint damage progression at year 1.

Significantly more patients treated with abatacept plus methotrexate achieved DAS28 (CRP)-defined remission and ACR50 and ACR70 responses at year 1, and the difference between treatment arms was significant by month 2. Over 1 year, 27.3 versus 11.9% of patients treated with abatacept plus methotrexate versus patients treated with methotrexate alone ($P < 0.001$) experienced a major clinical response (ACR70 maintained for 56 consecutive months).[89] Significant improvements were also seen in physical function at year 1 for patients treated with abatacept plus methotrexate compared with patients treated with methotrexate alone.[89] Improvements in disease activity and ACR responses were sustained or improved over the second year for patients who continued to receive abatacept plus methotrexate therapy, with 55.2% achieving remission at year 2.[90] Changes from baseline to year 1 in Genant-mTSS and erosion score were significantly lower for methotrexate-naïve patients who were randomized to the abatacept plus methotrexate arm.[89] Furthermore, there was an increasing degree of inhibition of progression in year 2 relative to year 1 for patients originally randomized to the abatacept arm.[91] For patients originally receiving methotrexate alone, structural damage progression was reduced over year 2 relative to year 1, following the addition of abatacept.[90] However, overall structural damage progression at year 2 remained greater for these patients compared with patients who received abatacept from baseline.[90]

The potential for early treatment with abatacept to delay the time of progression to fulfillment of classification criteria for RA in persons with ACPA-positive, undifferentiated, early inflammatory arthritis with clinical synovitis of two or more joints was investigated in an exploratory, phase II, 2-year study known as ADJUST (abatacept study to determine the effectiveness in preventing the development of RA in patients with undifferentiated inflammatory arthritis).[92] After 6 months of double-blind, randomized (1:1) treatment with either abatacept at the approved dose of approximately 10 mg/kg ($n = 28$) or placebo ($n = 28$), abatacept treatment was terminated. At year 1, the proportion of patients who developed RA according to ACR 1987 criteria or discontinued because of lack of efficacy was assessed. When treatment with abatacept was stopped after 6 months, 22 and 17 patients treated with abatacept and placebo, respectively, remained in the study inasmuch as they had not fulfilled classification criteria for RA; by year 2, 7 and 4 patients treated with abatacept and placebo, respectively, remained in the study. Numerically, RA developed in more patients treated with placebo than in those treated with abatacept over 1 year (66.7% vs. 46.2%), although CIs overlapped. Radiographic assessments demonstrated an inhibitory effect on structural damage progression at month 6, which was maintained for 6 months after therapy cessation, with similar trends observed for MRI-assessed osteitis, erosion, and synovitis.[92] Abatacept treatment was also associated with reductions in ACPA levels that persisted beyond cessation of the active drug. At enrollment, patients had short symptom duration, and although they did not meet ACR 1987 criteria for RA at the point of recruitment, more than half already had evidence of one or more erosions and were thus likely to have had early RA. The findings must be interpreted in light of this situation.

The popularity among patients of the subcutaneous delivery route out of available choices of parenteral delivery has prompted studies to look at effectiveness and safety of subcutaneously delivered abatacept, which is now available in a subcutaneous formulation, consisting of a fixed dose of 125 mg of the drug, administered once weekly. Six clinical trials have been performed in patients with RA treated with the subcutaneous formulation, including ACQUIRE (a phase II dose-finding study comparing the efficacy of intravenous and subcutaneous formulations),[93] ALLOW (withdrawal-restart study of immunogenicity with subcutaneous abatacept plus methotrexate vs. methotrexate),[94] ACCOMPANY (a study of immunogenicity in subcutaneous abatacept plus methotrexate vs. subcutaneous abatacept),[95] ATTUNE (intravenous to subcutaneous switch study),[96] and AMPLE (head-to-head subcutaneous abatacept plus methotrexate vs. adalimumab plus methotrexate).[97] All of these studies demonstrate an efficacy and a safety profile comparable with that obtained with the classic intravenous administration. In particular, it has been demonstrated that the fixed dose of the drug achieves a serum concentration comparable with that reached with the weight-tiered intravenous regimen, eliciting therapeutic concentrations in more than 90% of patients.

The AMPLE trial (abatacept versus adalimumab comparison in biologic-naïve subjects with RA with background methotrexate) in particular deserves further elaboration. In a noninferiority phase III study design, AMPLE was the first head-to-head study in patients with RA that was powered to compare biologic DMARD agents on a background of methotrexate in persons who were inadequate responders to methotrexate therapy and naïve to biologic DMARD therapy. Methotrexate-refractory persons with active RA who were naïve to biologic therapy were recruited. A total of 318 persons were randomized to weekly subcutaneous 125-mg doses of abatacept without an intravenous loading dose plus methotrexate with once-weekly placebo injections, and 328 persons were randomized to biweekly adalimumab plus methotrexate with once-weekly placebo injections. Because unmarked adalimumab (Humira) syringes could not be obtained, the study was single blinded, using a rigorous protocol, including a blinded assessor at each recruiting site.

The AMPLE trial demonstrated the comparable efficacy of subcutaneous abatacept to adalimumab on background methotrexate with respect to the primary outcome measure, ACR20 at 1 year, with subcutaneous abatacept demonstrating noninferiority to adalimumab (64.8% vs. 63.4%). Furthermore, comparable response and kinetics were seen across all efficacy measures, including ACR 20/50/70 and

DAS28 (CRP) responses, as well as inhibition of radiographic progression. Safety outcomes were balanced with some differences, namely, fewer discontinuations due to adverse events and serious adverse events in the subcutaneous abatacept group. Local injection site reaction complaints were also significantly less frequent in persons receiving subcutaneous abatacept. These findings confirm the clinical impression that among currently available biologic therapies, abatacept is at the better tolerated and safe end of the safety spectrum.

## Safety Issues

**KEY POINTS**

**Abatacept has acceptable safety across the spectrum of RA patient populations.**

**Abatacept is administered either as a 30-minute intravenous infusion that is usually without complications or as a once-weekly, fixed dose, subcutaneous injection that is tolerated with a very low rate of injection site reactions.**

Safety assessments from abatacept clinical trials have in general demonstrated a comparable overall incidence of adverse events and serious adverse events for persons treated with abatacept and placebo. The safety of long-term abatacept treatment is reported to be consistent, with the incidence of overall adverse events and serious adverse events remaining stable up to 7 years.[98]

A safety analysis has been undertaken on pooled data from abatacept clinical trials through December 2007 that included 4150 patients who were exposed to abatacept. This represents 10,365 patient-years of exposure, with an average exposure period of 2.5 years.[98] The incidence of serious infections was generally low, although it was higher for persons treated with abatacept compared with persons treated with placebo over 1 year (serious infections: 3.47 vs. 2.41 events/100 patient-years, respectively). Records of annual incidence rates for serious infections did not appear to show an increase in risk over time. Pneumonia, bronchitis, cellulitis, and urinary tract infection were the most common causes of hospital admission for infections. Opportunistic infections were rarely observed in this pooled cohort, including the following events per 100 patient-years of treatment: *Mycobacterium* tuberculosis, 0.06 events; aspergillosis, 0.02; blastomycosis, 0.01; and systemic *Candida*, 0.01.[98]

The incidence of malignancies (excluding nonmelanoma skin cancer) during the double-blind treatment periods was reported to be 0.59 events/100 patient-years for persons treated with abatacept versus 0.63 events/100 patient-years for persons treated with placebo. This low incidence rate did not rise with increasing exposure. In particular, for lung cancer and lymphoma, the incidence rate was 0.24 lung cancers and 0.06 lymphomas/100 patient-years during the double-blind period and 0.16 lung cancers and 0.07 lymphomas/100 patient-years during the cumulative period. To better interpret malignancy risk data, the incidence rates observed in abatacept studies were compared with those documented in five observational cohorts of patients with RA who were naïve to biologics and were taking nonbiologic DMARDs. Standardized incidence rates for the observational cohorts ranged from 0.4 to 1.06, and for patients treated with abatacept, the risk of lung cancer did not appear to be increased, with standardized incidence rates ranging from 0.65 to 1.84. The lymphoma risk in patients treated with abatacept appeared to be comparable with that in biologic naïve patients with RA, with standardized incidence rates ranging from 0.60 to 1.23.[98] In the pooled trial data from double-treatment periods, autoimmune events were reported in 1.4% of patients treated with abatacept and 0.8% of patients treated with placebo. Most events were mild or moderate in intensity, and the most frequently reported event was psoriasis, with rates of 0.53 and 0.56 events/100 patient-years over the double-blind and cumulative periods, respectively.

In clinical practice it is common to use conventional DMARDs in combination regimens, based on the belief that they provide additive benefits in terms of efficacy without the downside of unacceptable toxicity. Whether these same principles apply to the use of abatacept was addressed in the ASSURE trial (Abatacept Study of Safety in Use with other RA therapies).[99] This multicenter, randomized, double-blind study investigated the safety of adding abatacept or placebo infusions to a background treatment regimen of at least one of the traditional nonbiologic or biologic DMARDs currently approved for RA treatment for at least 3 months. A total of 1456 patients were randomized 2:1 to receive abatacept at a fixed dose approximating 10 mg/kg by weight range or placebo.

A number of interesting observations arose from this study. In the group as a whole, the proportion of serious adverse events occurring in each treatment arm was similar: 13% for abatacept and 12% for placebo. The discontinuations rate due to adverse events was 5% in the abatacept group and 4% in the placebo group. As expected on the basis of prior studies, serious infections occurred more frequently in the abatacept group (2.9%) than in the placebo group (1.9%). Five deaths occurred in the abatacept group and four deaths occurred in the placebo group; it was thought that all but one of the deaths in each group were unlikely to be related to the study drug. All the deaths occurred in patients without concomitant biologic background therapy. However, a subanalysis of the data, based on whether patients were receiving biologic or nonbiologic background therapy, revealed that serious adverse events occurred almost twice as frequently in the subgroup receiving abatacept plus another biologic agent (22.3%) as in the other subgroups (12.5%).

A particularly important observation in this study was the increased number of serious infections observed when abatacept was combined with other biologic therapies (5.8% vs. 1.6% for the subgroup receiving background biologic therapy plus placebo infusions). Further, the clinical benefits of abatacept tended to be less in the patients receiving background biologic therapy than in patients with a background of nonbiologic DMARDs. No cases of lymphoma, demyelinating disorders, or tuberculosis were reported.

The ASSURE trial findings mirrored those of a smaller randomized, placebo-controlled, double-blind pilot study. This phase IIb trial investigated the efficacy and safety of the addition of abatacept infusions at 2 mg/kg over 1 year

in patients with at least 8 of 66 swollen joints and 10 of 68 tender joints despite at least 3 months of treatment with twice-weekly 25-mg subcutaneous etanercept.[100] The biologic combination had limited clinical benefit compared with etanercept and placebo infusions but was associated with an increase in the proportion of patients experiencing serious adverse events (16.5% vs. 2.8%) and serious infections (3.5% vs. 0%). On the basis of these observations, the use of abatacept is not advised in combination with other biologic therapies.

Data are emerging concerning the comparative efficacy, safety, and kinetics of response for the anti-TNF antibody infliximab and abatacept.[101] In a 1-year double-blind study, patients with RA who had an inadequate response to methotrexate (a mean baseline DAS28 of 6.8) and no prior anti-TNF therapy were randomized to receive abatacept at a dose approximating 10 mg/kg every 4 weeks (156 patients); infliximab, 3 mg/kg every 8 weeks (165 patients); or placebo every 4 weeks (110 patients). Patients randomized to receive a placebo were switched to abatacept after 6 months but were not included in the 1-year analyses. At the end of the first 6 months, the frequency of serious adverse events was 5.1%, 11.5%, and 11.8% for abatacept, infliximab, and placebo, respectively. In the same order, the frequency of acute infusion-related adverse events was 5.1%, 18.2%, and 10%. Over the 1-year period, infections reported as serious adverse events were more frequent with infliximab (8.5%) than with abatacept (1.9%). These infections included two cases of tuberculosis, both in patients treated with infliximab. When considered in the light of the clinical response data discussed earlier, this study and others emphasize the relatively slow time to peak clinical response with abatacept in comparison with TNF blockade, with increasing efficacy beyond 6 months. These studies also point to the possibility of a favorable benefit-risk profile over 1 year. It will be critical to see whether these encouraging early safety data are maintained over the longer term.

Of note, the incidence rate of serious infections observed with abatacept is at the lower end of the range reported in patients with RA who are treated with other biologic agents. In summary, the long-term integrated safety data from up to eight abatacept trials, representing more than 10,000 patient-years of exposure, confirm that, overall, abatacept has a favorable safety profile that is consistent with observations from the short-term experience in all RA populations studied, with no new clinically important safety issues identified with long-term exposure. This conclusion is supported by a recent Cochrane review.[102]

## Current Role

**KEY POINT**

**Abatacept is a biologic option in patients with RA who have inadequate responses to TNF inhibitors, although recent evidence suggests that the benefit-risk profile of abatacept may be most optimal when introduced earlier in the treatment paradigm.**

Abatacept may be used as monotherapy or concomitantly with DMARDs other than TNF antagonists. It is not recommended for use concomitantly with IL-1 or TNF antagonists. The encouraging clinical trial data confirm that abatacept, like rituximab, represents a valuable agent in the therapeutic atramentarium for patients with RA who have not responded adequately to TNF blockade. Abatacept has a particular advantage among intravenously delivered biologics for RA in that it is very well tolerated and quick to administer, and infusion-related problems are rare. The more recently introduced subcutaneous formulation adds to the range of options for convenience of delivery depending on patient preference and circumstances. However, the clinical responses and radiographic benefits observed with abatacept appear to be greater in methotrexate-naïve patients compared with patients who have failed to respond to methotrexate or other DMARDs. Patients who previously failed to respond to methotrexate treatment appear to demonstrate higher clinical responses than patients who have failed to respond to TNF antagonists. Furthermore, the findings of the phase III AMPLE trial, which demonstrated noninferiority for efficacy with respect to symptoms and signs of abatacept plus methotrexate compared with adalimumab plus methotrexate, and comparable inhibition of structural damage progression, suggest that the most favorable clinical outcomes with abatacept may be achieved if it is used earlier in the treatment paradigm than was generally the case when it first became available for use in the clinic. Indeed, since the recent availability of the subcutaneous formulation, abatacept is increasingly finding favor as a well-tolerated and first-line choice of biologic therapy with a favorable benefit-risk ratio. The merits of switching a patient from a TNF inhibitor to abatacept or rituximab on the basis of a clinical response that is incomplete are not yet clear-cut with respect to disease modification. Other factors likely to inform the future use and relative positioning of biologics in the clinic include additional long-term safety data, comparative cost-effectiveness analyses, and the perceived convenience of intravenous versus subcutaneous administration.

## Implications for Understanding the Pathogenesis of Rheumatoid Arthritis

**KEY POINTS**

**The clinical efficacy of abatacept in persons with RA validates the importance of co-stimulation in the pathogenesis.**

**Co-stimulation inhibition modulates production of a number of pro-inflammatory cytokines.**

The clinical efficacy of abatacept in a proportion of patients with RA implicates co-stimulatory events in disease pathogenesis. However, abatacept might mediate immunosuppressive effects in RA by a number of different mechanisms. In the RA joint, blocking access of CD28 to CD80 or CD86 might be of little importance because memory T cells, which predominate in inflamed synovium, are much less dependent on this pathway.[103] A more important mechanism of action in the synovium might be the induction of tolerogenic antigen-presenting cells. Binding of CD80 or CD86 on antigen-presenting cells by CTLA-4Ig initiates a "reverse signal," with induction of tryptophan catabolism and inhibition of antigen presentation. Naïve T cells are

located predominantly in lymphoid tissue, and blockade of the CD28-CD80/CD86 interaction in lymph nodes may reduce T cell priming and the production of autoreactive T cells. Interestingly, patients with RA are reported to have higher frequencies of CD28-null T cells.[104] Further, the level of expression of CD28 is significantly reduced on all naïve T cells and memory $CD4^+$, $CD28^+$ T cells, a phenomenon related to overproduction of TNF.[105] A likely consequence of low-density cell surface expression of CD28 is that, for these T cells, abatacept can more readily block the interaction with CD80 and CD86.

The clinical and radiographic benefits of abatacept administration in persons with RA illustrate the importance of the co-stimulation pathway in T cell activation and subsequent amplification of the inflammatory cascade, including pathways that promote tissue destruction.[106] The modulatory effect of co-stimulation blockade on the expression of a range of inflammatory genes in synovial tissue has been demonstrated by quantitative PCR studies and evaluation of synovial biopsies in patients with active RA who received abatacept treatment, having previously failed to respond to TNF inhibitors.[107] Furthermore, a small, largely nonsignificant reduction in cellular content was observed in biopsy samples after abatacept treatment, suggesting that co-stimulation inhibition reduces the inflammatory status of the synovium without disrupting cellular homoeostasis.

## ABATACEPT IN OTHER RHEUMATIC CONDITIONS

**KEY POINTS**

**Abatacept has clinical use for treatment of RA and polyarticular juvenile idiopathic arthritis.**

**Clinical trials of abatacept in persons with psoriatic arthritis have shown benefit.**

In addition to the use of abatacept in the established phase of RA, abatacept in combination with methotrexate is indicated for the treatment of moderate to severe active polyarticular juvenile idiopathic arthritis in pediatric patients 6 years and older who have had an insufficient response to other DMARDs, including at least one TNF inhibitor. Results from a 12-month multicenter clinical trial did not show therapeutic benefit of abatacept over placebo in patients with non–life-threatening SLE. In particular, abatacept failed to prevent new disease flares in patients with SLE who underwent tapering of corticosteroids in an analysis where mild, moderate, and severe disease flares were evaluated together. Serious adverse events were higher in the abatacept group (19.8 vs. 6.8%). Although the primary and secondary endpoints were not met, improvements in certain exploratory measures were found.[108] Studies are ongoing in type 1 diabetes and inflammatory bowel disease.

Abatacept was also used in a 26-week, phase I, open-label, dose-escalation study of psoriasis vulgaris.[109] Sustained improvements of at least 50% in clinical disease activity were reported after four infusions of abatacept in 20 of 43 patients with stable psoriasis vulgaris. Clinical improvement was associated with quantitative reduction in epidermal hyperplasia, which correlated with a quantitative reduction in skin-infiltrating T cells. However, no clear-cut increase in the rate of intralesional T cell apoptosis was identified. It may be that the observed reduction in lesional T cells numbers was due to inhibition of T cell proliferation, T cell recruitment, or apoptosis of antigen-specific T cells at extralesional sites. Altered antibody responses to T cell–dependent neoantigens were observed, but immunologic tolerance to these antigens was not demonstrated. This study illustrates the importance of the CD28-CD152 pathway in the pathogenesis of psoriatic skin disease.

The findings of a 6-month, double-blind, placebo-controlled phase II trial in psoriatic arthritis have been reported.[110] The study included 170 adults meeting the Classification Criteria for Psoriatic Arthritis (CASPAR) with active arthritis (≥3 swollen joints and ≥3 tender joints), active plaque psoriasis (at least one qualifying target lesion ≥2 cm in diameter), and a disease duration of at least 3 months. All participants were required to have an inadequate response to DMARDs including, but not limited to, methotrexate or anti-TNF therapies. Persons who were either intolerant of or had an inadequate response to TNF inhibitors discontinued these therapies at screening and, after a washout period of greater than or equal to 28 days, were assessed for arthritis and psoriasis prior to randomization. Methotrexate was the only DMARD permitted to be continued at a stable dose during the study (in approximately 60% of participants). The participants were randomized to receive placebo or abatacept at a dose of 3 or 10 mg/kg or 30/10 mg/kg (i.e., two initial doses of 30 mg/kg, followed by a 10 mg/kg dose) on days 1, 15, 29 and then every 28 days. The primary endpoint was the ACR20 response at the end of 6 months. Key secondary endpoints included Investigator's Global Assessment (IGA) of psoriasis (ratings ranging from 1 [clear] to 4 [severe]), and the scores for the target lesion (based on the rating of skin erythema, induration, and scaling on a scale of 0 to 4 each), the HAQ Disability Index, and the Short Form (SF)-36 General Health Survey.

A total of 19%, 33%, 48%, and 42% of patients achieved an ACR20 with placebo or doses of abatacept of 3 mg/kg, 10 mg/kg, or 30/10 mg/kg, respectively. Compared with placebo, improvements were statistically significantly higher for the abatacept 10 mg/kg ($P = 0.006$) and 30/10 mg/kg ($P = 0.022$) dose groups, but not for the 3 mg/kg group ($P = 0.121$). An IGA response (i.e., a rating of psoriatic lesions as "clear or almost clear") was seen in 21% of the 30-/10-mg/kg dose group, 25% of the 10-mg/kg group, and 38% of the 3-mg/kg group; the latter was the only one that was statistically significantly different from placebo (26%; no statistical data presented). All doses of abatacept showed improvements in scores on MRI for joint erosion, osteitis, and synovitis; HAQ; and the SF-36. These encouraging early findings suggest that abatacept, at a dose of 10 mg/kg, may be a treatment option for patients with active psoriatic arthritis previously exposed to DMARDs, including anti-TNF therapies. Longer-term follow-up of these patients is ongoing, and further studies are planned.

In a small, open-label study, abatacept treatment was reported to be effective, safe, and well tolerated in patients with early and active primary Sjögren's syndrome, with treatment resulting in improved disease activity, laboratory parameters, fatigue, and health-related quality of life.[111] In

another small open-label study, 31 patients with RA and secondary Sjögren's syndrome completed 6 months of abatacept therapy. Eleven patients with histologic features characteristic of Sjögren's syndrome on a minor salivary gland biopsy had statistically significant improvements in saliva volume and in Schirmer's test for tear volume.[112]

## TARGETING T CELLS

### Clinical Studies

**KEY POINTS**

**A number of approaches to targeting T cells independently from co-stimulation pathways have not shown clear-cut benefit in clinical trials.**

In the early years of investigating the potential of biologic therapies in RA, T cells were among the first targets to be explored. Data from several different pre-clinical animal models of inflammatory arthritis suggested a pathogenic role for $CD4^+$ T cells in response to various arthritogenic antigens presented in the context of class II MHC molecules.[103] These observations led to a number of experimental protocols designed to investigate the effect of depleting and nondepleting antibodies directed at CD4, as well as other T cell–associated molecules. Early randomized, placebo-controlled clinical studies exploring the potential of biologic therapies targeting T cells in the treatment of RA have generally had disappointing results. Some anti–T cell agents were not efficacious; other preliminary trials demonstrating some clinical efficacy were terminated as a result of adverse events, particularly prolonged and profound T cell depletion.[113] However, the primatized monoclonal anti–CD4 antibody keliximab results in a dose-dependent clinical response when administered once weekly over 4 consecutive weeks, and the clinical response correlates with $CD4^+$ T cell coating with keliximab rather than T cell depletion. In two consecutive randomized, double-blind trials with comparable populations, keliximab treatment was associated with $CD4^+$ T cell counts below 250 cells/$mm^3$ in 12% of subjects in one study and in 47% of subjects in the other study.[114]

Examples of biologic therapies targeting other T cell–associated molecules include Campath-1H, a monoclonal antibody directed against CD52; a monoclonal anti–CD5 antibody linked to ricin toxin; and a fusion protein comprising an IL-2 receptor–binding domain coupled to diphtheria toxin ($DAB_{486}$IL-2 fusion toxin). CD52 is a polypeptide expressed on all lymphocytes. Campath-1H was tested as a treatment for refractory RA in two small trials, and although a single intravenous dose of between 1 and 100 mg resulted in significant $CD4^+$ T cell depletion and clinical improvement in more than half of patients, the correlation between biologic action and clinical response was poor.[115,116] Further, therapy was associated with significant acute toxicity, which was presumed to reflect a cytokine release syndrome, including headache, nausea, and hypotension. Arthritis activity returned over time despite prolonged suppression of peripheral blood $CD4^+$ T cell numbers.

CD5 is a transmembrane glycoprotein expressed on 70% of T cells. CD5-1C, a monoclonal antibody linked to ricin, a plant toxin that inhibits protein synthesis, was used to treat RA in a double-blind, placebo-controlled trial.[117] At the doses tested, only modest and transient T cell depletion was observed, and no clinical benefit occurred.

In a strategy designed to selectively deplete activated T cells expressing IL-2 receptor, $DAB_{486}$IL-2 fusion toxin was given by intravenous infusion in open-label and placebo-controlled studies.[118,119] Although a small percentage of patients (18%) exhibited clinical responses in the placebo-controlled study, the incidence of adverse events was significant, including nausea, fever, and raised plasma transaminases. Further, antibodies against diphtheria toxin developed in nearly all patients.

Other approaches to targeting T cells that have been tested include efforts to directly interfere with the trimolecular complex comprising human leukocyte antigen class II, antigenic peptide, and TCR by means of a DR4-DR1 peptide vaccine, TCR Vβ peptide vaccine, collagen, or cartilage glycoprotein 39.[120] Despite a rationale for these approaches based on promising pre-clinical animal model data, all have been abandoned because of borderline or absent clinical benefits in human disease.

## FUTURE DIRECTIONS

**KEY POINT**

**Novel approaches to targeting T cells are being tested, including T cell vaccination.**

The importance of immune regulation in maintenance of the healthy state is perhaps best illustrated by the consequences of immune dysregulation, a phenomenon common to a wide range of chronic inflammatory disease phenotypes. Emerging evidence points to the importance of certain $CD4^+$ T cell subsets in the negative regulation of the adaptive immune system. The best characterized of these subsets are the so-called naturally occurring $CD4^+CD25^+$ regulatory T cells and IL-10–producing Tr1 cells. Recent advances in the understanding of the molecular basis of $CD4^+CD25^+$ regulatory T cell generation include the observation that the X-linked forkhead–winged helix transcription factor Foxp3 is required for $CD4^+CD25^+$ T cell development and function. Although further progress in understanding the pathophysiologic role of regulatory T cells will require the identification of more specific markers for distinct regulatory T cell populations, there is already evidence of the feasibility of enhancing regulatory T cell function in vivo either by TCR modulation, using antibodies to CD3, or by co-stimulatory signals, using a CD28 superagonist.[121]

When a chronic inflammatory disease reflects an antigen-driven process, an attractive goal is to modulate T cell function in such a way as to generate antigen-specific unresponsiveness in the absence of long-term generalized immunosuppression. Some but not all studies report a relative deficiency of regulatory T cells in RA.[29] Of note, antibodies to CD3 and to TNF appear to enhance regulatory T cell function or number in patients with RA.[122-125] This finding raises the possibility that combination treatment with anti-CD3 and anti-TNF might be beneficial in more completely restoring immune regulation in persons with RA. In fact, chronic inflammation and overproduction of TNF perturb

T cell antigen receptor–dependent signaling,[126] suggesting that active inflammation may attenuate tolerogenic signals expected to be induced by a nondepleting anti-CD3 antibody. Thus pre-treatment with TNF blockade might restore tolerogenic signals transduced by the TCR in response to drugs such as anti-CD3. Although the use of anti-CD3 in the clinic has been limited by the occurrence of drug-induced cytokine release syndrome,[127] the effects can be modulated by anti-TNF agents, as demonstrated in patients treated for acute allograft rejection.[128] Such a combination therapy approach has yet to be tested in persons with RA.

A potentially beneficial immunomodulatory response has been demonstrated in a small open pilot study by vaccinating 16 patients with RA with expanded, activated, and irradiated autologous synovial fluid T cells.[129] Vaccination was associated with expansion of $CD4^+$ and $CD8^+$ T cells, many of which expressed the Vβ2 TCR chain. Some were anti-idiotypic, responding specifically to vaccine T cells with the production of IL-10 ($CD4^+$ cells) or granzyme B ($CD8^+$ cells). A broader regulatory response, however, was directed toward activated T cells in general, specifically against peptides derived from the IL-2 receptor α chain. This broader response may be important to generate in a syndrome such as RA, where the precise autoantigen and pathogenic T cell clones are not readily identifiable. This might also explain why earlier attempts at T cell vaccination using TCR-derived peptides have not been pursued.[120] However, the efficacy of such a T cell vaccination approach needs to be validated in further trials and the safety and durability of response must be further investigated before the potential feasibility of such an approach to therapy in routine clinical use can be properly assessed.

Anti–T cell therapies, including anti-CD4, cyclosporine, and CTLA-4Ig, synergize with TNF blockade in mice. Various agonists of immune inhibitory receptors on T cells could be potential adjuncts to anti-TNF, either without methotrexate or used together. The programmed cell death 1 (PD-1) protein, which is among the most potent inhibitory receptors on T cells, is responsible for the "exhausted" phenotype in chronic viral infection. PD-1 superagonists inhibit immune responses and upregulate regulatory T cells. Antibodies targeting PD-1 in persons with cancer have shown encouraging improvements in the context of advanced cancers.[130] It remains to be seen whether agonistic approaches will have a future place in the treatment of RA.

## CONCLUSION

**KEY POINT**

**Rituximab and abatacept both have an established role in the pharmacologic management of RA.**

Despite recent advances in understanding the optimal use of nonbiologic DMARD therapies and the considerable success of biologic therapies targeting TNF, a substantial proportion of patients with RA remain refractory to or intolerant of these therapeutic modalities. The data discussed in this chapter illustrate that there is a role in clinical practice for newer biologic agents with specificity for cellular targets. In particular, depletion of B cells with the monoclonal antibody rituximab results in sustained improvement in the signs and symptoms of RA after just two doses (one treatment cycle), with little evidence of drug-related toxicity despite the profound and lasting depletion of B cells. Similarly, inhibition of T cell co-stimulation by abatacept demonstrates clear clinical efficacy within 16 weeks and, in some cases, additional improvement for 1 year or beyond, with acceptable safety. This observation is in marked contrast to the previously observed unfavorable benefit-risk ratio associated with a T cell–depleting strategy using Campath-1H. The response of patients with RA to this wide spectrum of therapeutic strategies attests to the complexity and heterogeneity of the syndrome and provides further impetus for studies that use these therapies to enhance our understanding of disease pathogenesis.

Although both rituximab and abatacept have been shown to benefit a significant proportion of patients with RA—whether they are naïve to DMARDs or have experience with either biologic DMARDs, nonbiologic DMARDs, or both—to date very little is known about the comparative effects of these treatment approaches on symptoms and signs of disease or on inhibition of structural damage. Furthermore, despite some pointers to biomarkers that might optimize treatment outcomes with a particular therapeutic approach at the cohort level, such as seropositivity in patients with RA who are treated with rituximab, as yet no biomarkers reliably inform the best choice of therapy on an individual basis. Rituximab and abatacept are a welcome addition to the biologic armamentarium for RA, and both agents may also have a role in the pharmacologic management of rheumatic disorders beyond RA alone. It is anticipated that further clinical research and experience in the use of these biologic therapies will help better inform optimal treatment strategies.



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CHAPTER 65



# Novel Intra-cellular Targeting Agents in Rheumatic Disease

Stanley Cohen

**KEY POINTS**

- During the last 25 years, key pathways responsible for signal transduction to the nucleus after ligand binding to cellular receptors on immune cells have been established.
- Protein kinases that phosphorylate intra-cellular proteins are major players in signal transduction.
- Oral small-molecule inhibitors of downstream protein kinases, including p38 MAP kinase and MEK inhibitors, have been investigated, very limited success in rheumatoid arthritis (RA) clinical trials.
- Janus kinase (JAK) inhibitors have been successfully developed as therapies for various malignancies and for RA.
- The efficacy and safety of JAK inhibitors are similar to biologic disease-modifying anti-rheumatic drugs. The observed increase in herpes zoster and, possibly, opportunistic infections, may be unique to these inhibitors.
- Spleen tyrosine kinase (SYK) inhibitors have been associated with modest benefit and significant adverse events.
- Oral small-molecule phosphodiesterase inhibitors are approved for treatment of psoriatic arthritis with modest benefit in skin and joint disease.
- Other signal transduction pathways—BTK and PI3k—are being targeted in clinical trials.

Advances in treatment for rheumatoid arthritis (RA) during the past 2 decades have resulted in dramatic improvements in quality of life for these patients. The development of biologic therapies targeting molecules involved in the pathogenesis of RA has led to significant improvements in the signs and symptoms of disease in as many as 60% to 70% of patients, and resulted in remission in 10% to 50% of patients, depending on the definition of remission and disease duration.[1,2]

Biologic therapies, however, have limitations. More than half of patients continue to have active disease and require either subcutaneous or intravenous administration. These therapies are associated with significant expense and can induce immunogenicity. During the last 25 years, the intracellular signaling pathways involved in signal transduction from the cell surface to the nucleus after ligand-receptor binding to its receptor have been identified. This improved understanding of these pathways has provided opportunities for development of small molecule-therapies that could target these pathways. Interruption of the signaling cascade has been demonstrated in pre-clinical models to reduce pro-inflammatory cytokine production, with improvement in animal models of inflammatory arthritis similar to that seen with biologics.[3,4,5] During the last decade, multiple clinical trials in patients with RA that evaluated small molecules targeting specific kinases have been conducted. The majority of these investigational trials failed because of limited efficacy or significant toxicity. In 2012, tofacitinib, a Janus kinase (JAK) inhibitor, was approved for RA treatment in the United States and Japan, and other JAK inhibitors are under development. This chapter will focus on the various molecules that have been evaluated as potential RA therapies or are presently undergoing active investigation.

## SIGNAL TRANSDUCTION PATHWAYS

Research during the last 25 years has firmly established that reversible protein phosphorylation is a fundamental mechanism of cell signaling.[6,7] Protein kinases are the enzymes that catalyze the transfer of the γ phosphate of a purine nucleotide triphosphate (i.e., adenosine triphosphate [ATP] and guanosine triphosphate [GTP]) to the hydroxyl groups of their protein substrates. A total of 518 kinases have been identified, with the majority selectively phosphorylating serine or threonine peptides. Ninety kinases that phosphorylate tyrosine (protein tyrosine kinases) have been identified that play major roles in signal transduction. These tyrosine kinases exist as receptor tyrosine kinases, such as epidermal growth factor and platelet-derived growth factor, but the majority of receptors interact directly with intra-cellular kinases to facilitate signal transduction. Protein phosphorylation ultimately links membrane events to calcium modulation, cytoskeletal rearrangement, gene transcription, and other canonical features of lymphocyte action.[8]

Many of the major classes of receptors that trigger immune cell activation are linked to protein phosphorylation and are physically associated with protein kinases (Figure 65-1). Multiple signaling pathways after ligand-receptor interaction have made targeting pathways for therapeutic benefit difficult in inflammatory arthritis because of the redundancy of these pathways. Inhibition of one signaling component may be compensated for by increased signaling through an alternative pathway, resulting in incomplete inhibition.

The initial event in T cell receptor (TCR), B cell receptor (BCR), and NK (natural killer) and Fc receptor signaling is phosphorylation of receptor subunits on tyrosine residues.[9,10] Cytokine receptors, especially type I/II cytokine receptors, signal directly by activating kinases, which phosphorylate receptor subunits and thereby initiate signaling in

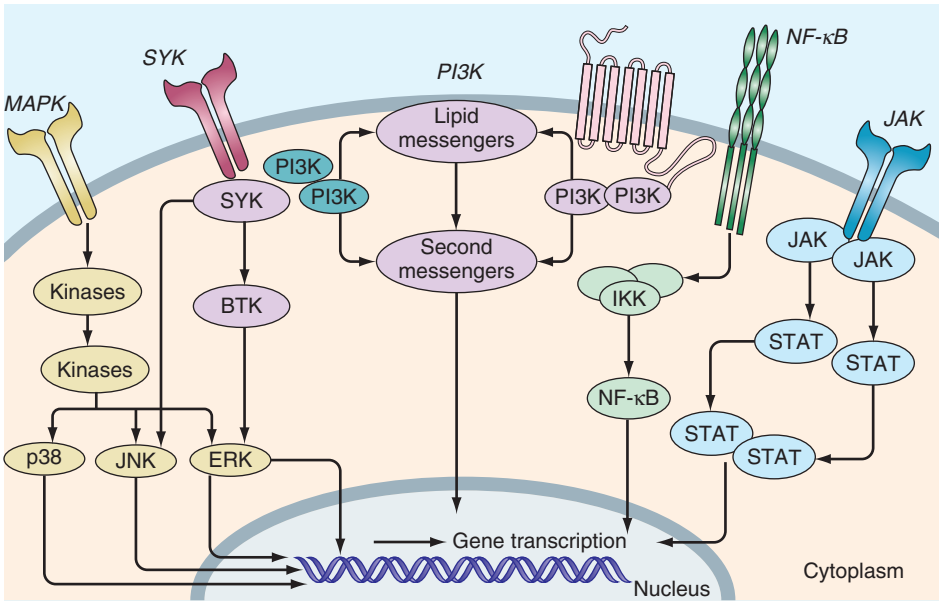



**Figure 65-1** Overview: signal transduction pathways. BTK, Bruton's tyrosine kinase; ERK, extra-cellular signal-regulated kinase; IKK, IκB kinase; JAK, Janus kinase; JNK, c-JUN N-terminal kinase; MAPK, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; STAT, signal transducer and activator of transcription; SYK, spleen tyrosine kinase.

**TABLE 65-1** Protein Tyrosine Kinases Targeted in Rheumatoid Arthritis

| |
|---|
| p38 mitogen-activated protein kinases |
| MEKK |
| Spleen tyrosine kinases |
| Janus kinases |
| Bruton's tyrosine kinase |
| PI3K |

MEKK, Mitogen-activated/extra-cellular response kinase kinase kinase; PI3K, phosphatidylinositol 3-kinase.

T cells and NK cells. Type I and type II cytokine binding activates JAK, which then phosphorylates cytokine receptors, allowing STAT (signal transducer and activator of transcription) DNA-binding proteins to attach to receptors and become phosphorylated. STAT activation leads to their dimerization and translocation to the nucleus, where they regulate gene expression. In B cells, antigen binding leads to activation of three main protein tyrosine kinases (PTKs): the SRC-family kinases, LYN and SYK, and the TEC-family kinase, Bruton's tyrosine kinase (Btk). SYK phosphorylates adaptor protein BLNK and, along with Btk, activates phospholipase Cγ (PLCγ).[11] Activation of PLCγ leads to the release of intra-cellular $Ca^{2+}$ and activation of protein kinase C (PKC), which activate mitogen-activated protein kinases (MAP kinases). The MAP kinase cascade activates transcription factors nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), allowing gene regulation.

Upstream and downstream protein kinases in these pathways have been targeted in oncology as well as inflammatory disorders (Table 65-1). Initial concerns existed about the safety of kinase inhibitors because of the potential lack of specificity to inhibit a specific kinase.[12,13] This lack of specificity has become less of an issue in the clinic with the successful development of multiple and less selective kinase inhibitors, initially in oncology. Imatinib was the first tyrosine kinase inhibitor approved in oncology for chronic myeloid leukemia, and has been highly effective with acceptable toxicity. Subsequently, less selective tyrosine kinase inhibitors have been developed for chronic myelogenous leukemia (CML) treatment.[14,15] Even though off-target kinases are impacted by these inhibitors, safety issues have been acceptable. A total of 13 kinase inhibitors have been approved in oncology, including therapeutics for treatment of renal cell carcinoma and non–small cell lung cancer. The acceptable safety profile has stimulated efforts to develop kinase inhibitors in inflammatory diseases, such as RA, psoriasis, and inflammatory bowel disease.

## P38 MAP KINASE INHIBITORS

The MAP kinases were identified in the 1990s as involved in the production of pro-inflammatory cytokines after cell stimulation by various stressors.[16,17] There are three major families of MAP kinase: extra-cellular signal-regulated kinase (ERK), c-JUN N-terminal kinase (JNK), and p38 (Figure 65-2). The MAPs are intra-cellular enzymes that transmit a signal to the nucleus, resulting in gene transcription.[18,19] Complex parallel and crossover signaling cascades link the three main MAP kinase families. MAP kinase activation is mediated by upstream MAP kinase kinases (MKKs), which are also activated by MKK kinases (MKKK or MAP3K).

p38 MAP kinase is a key regulator of pro-inflammatory cytokine production. Varied cellular stresses, such as inflammatory cytokines, pathogens, and growth factors, activate kinases that regulate the expression of key genes, resulting

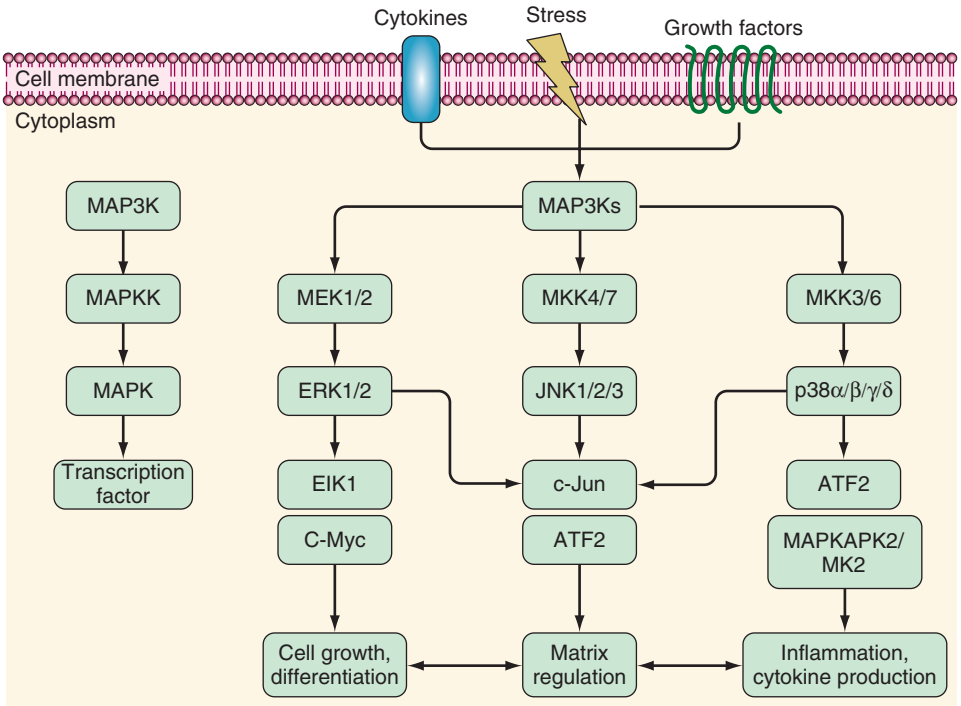



**Figure 65-2** Simplified mitogen-activated protein kinase (MAPK) pathway. ATF, Activating transcription factor; ERK, extra-cellular signal-regulated kinase; JNK, c-JUN N-terminal kinase; MKK, MAPK kinases.

in transcriptional activation of TNF, IL-1 and IL-6, cyclooxygenase (COX-2), and matrix metalloproteinases (MPPs). p38 activation and phosphorylation is regulated by two upstream kinases, MKK3 and MKK6, which are phosphorylated by multiple MAP3Ks. There are four isoforms of p38—α, β, γ, and δ—and p38α is the isoform thought to be the most important regulator of cytokine expression. Phosphorylated activated p38 MAP kinase is also found in the synovial lining and endothelium of vessels in RA synovium.[20] The p38 MAP kinases were the first kinases targeted after the observation that inhibition of this pathway could reduce lipopolysaccharide (LPS)-induced TNF and IL-1 production by monocytes.[21] Several pre-clinical studies have demonstrated that inhibition of p38 MAP kinase suppresses production of inflammatory cytokines, and pre clinical animal models demonstrated reduction in paw swelling and joint damage.[22,23] Several clinical trials have been performed with investigational p38 inhibitors. The p38α isoform has been the primary target in clinical trials. A 24-week double-blind randomized, placebo-controlled clinical trial (RCT) of SCIO-469 monotherapy, a p38α MAP kinase inhibitor, was performed in 302 patients with active RA randomly assigned to receive oral SCIO-469, 30 or 60 mg, three times per day, or 100 mg extended-release daily, or placebo.[24] The primary efficacy endpoint was the ACR20 response rate at 12 weeks. At week 12, no difference was seen between the SCIO-469–treated patients compared with placebo for the ACR20 or ACR50 response. A decrease in acute phase reactants, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), was seen in the SCIO-469–treated patients at week 1, which was not sustained to week 4. This transient reduction in CRP has been reported with other p38 MAP kinase inhibitors. Adverse events (AE) occurring more frequently in SCIO-469–treated patients included rashes, dizziness, constipation and arthralgias, and liver function abnormalities, which were dose related.

Pamapimod, another selective inhibitor of the α isoform of p38 MAP kinase, was studied in a 12-week RCT in 204 patients with active RA. The study compared different once-daily doses of pamapimod to methotrexate (MTX), which was titrated to 20 mg/week by week 8.[25] The primary endpoint was the ACR20 response at week 12. At week 12, pamapimod-treated patients had ACR20 responses numerically lower than patients treated with MTX. Statistically superior results were seen with MTX for ACR50 response and decreases in disease activity score (DAS)28. A transient reduction in CRP levels at week 1, that resolved by week 2, was seen in the 300 mg pamapimod group. AEs increased in a dose-dependent manner and included maculopapular rashes, dizziness, and elevated liver function tests (LFTs). This adverse event profile was similar to that seen with all of the p38 inhibitors evaluated in clinical trials. A companion dose-ranging study that evaluated pamapimod in patients with RA who had active disease despite MTX therapy also failed to show efficacy compared with placebo.[26] Based on the results of these two studies, development efforts of this molecule in RA were terminated.

Two 12-week placebo-controlled RCTs were conducted in Eastern Europe to evaluate VX-702, a highly selective inhibitor of the p38α isoform of MAP kinase.[27] This molecule has little or no central nervous system (CNS) penetration, as was seen with predecessor molecules, and researchers thought it might be associated with less dizziness and headaches. Patients' ACR responses to the dose range investigated were not significantly different from placebo. No new safety issues were reported. Because of VX-702's limited efficacy, development of this therapy was terminated.

Two subsequent reviews of MAP kinase inhibitors proposed theories of why inhibition of p38 MAP kinases in animal models was successful, whereas studies in patients with RA were not.[28,29] Issues raised include dose limitations caused by toxicity, altered biodistribution of newer molecules that prevent CNS penetration, incorrect isoform targeting, and the fact that p38α may have a regulatory role in the induction of anti-inflammatory cytokines.[30] The most plausible explanation, which is supported by the transient acute phase response, is the redundancy of signaling networks, such that blocking a downstream molecule such as p38 would not block upstream kinases that can redirect the signaling flow. Recognition of this concern has stimulated efforts to target more upstream protein kinases, and no further trials in RA with p38 inhibitors are ongoing.

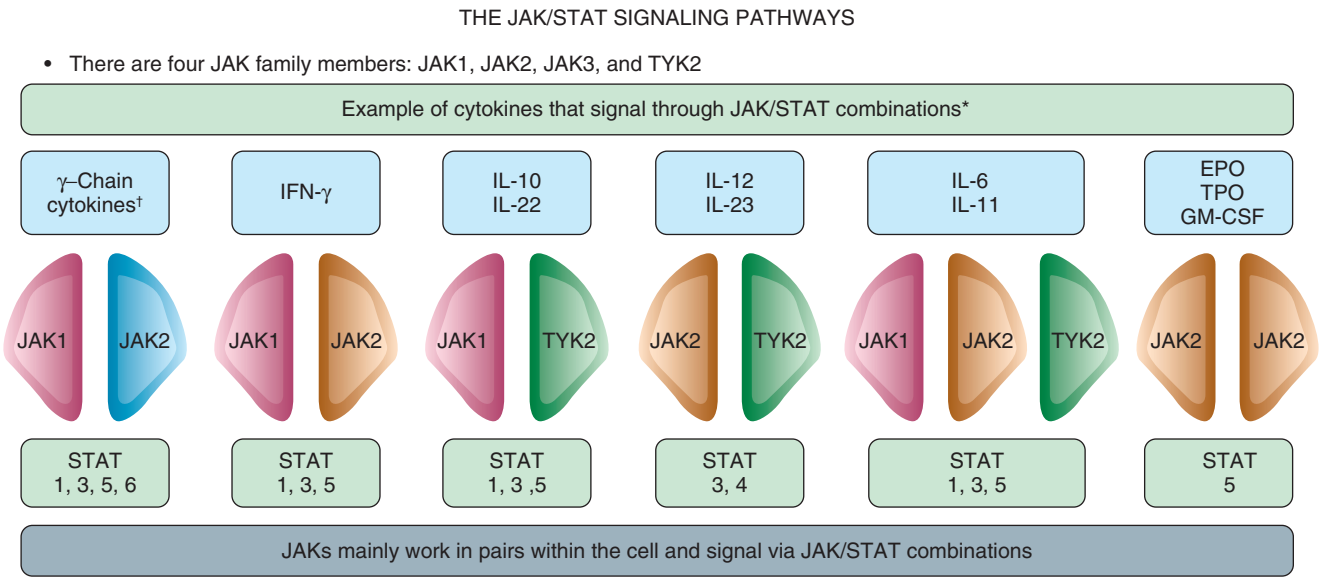
## MEK INHIBITORS

MEK is a MAPKK involved in growth factor signal transduction and cytokine production. Inhibitors of MEK demonstrated efficacy in pre-clinical models of RA.[31] Based on the pre-clinical observations, ARRY-438162, an oral inhibitor of MEK1/MEK2, was evaluated in a double-blind randomized clinical trial. The results were presented in the 2010 European League against Rheumatism (EULAR) meeting, although never published. In this study, 201 patients with RA with active disease despite MTX treatment were randomly assigned to receive various doses or placebo. The primary endpoint was ACR20 response. No difference between the active treatment groups and placebo was demonstrated ($P = 0.459$). The most common active adverse events were skin rashes and diarrhea that were dose related. Serious AEs were rare. No additional trials of molecules targeting MEK in RA are ongoing at the present time.

## SPLEEN TYROSINE KINASE INHIBITORS

SYK inhbitors have been evaluated as a potential treatment for RA. SYK is a nonreceptor protein tyrosine kinase that is a modulator of immune signaling in cells bearing Fcγ-activating receptors, including B cells, mast cells, macrophages, neutrophils, eosinophils, basophils, and synoviocytes.[32,33] SYK binds to the cytoplasmic region of these receptors that contain the immune-receptor tyrosine-based activation motif (ITAM). Receptor binding results in ITAM phosphorylation activating SYK (Figure 65-3). This motif is located in the cytoplasmic portion of FcγR, FcεR, Igα (B cells), CD3ξ (T cells) and integrins.[34] SYK activation activates downstream MAP kinases, PI3K, and PLCγ, with a resultant increase in IL-6 and MMP production.

These observations have led to interest in SYK as a potential target for treatment of RA.[35] SYK has been found to be activated in synoviocytes, and activation of SYK is important for cytokine and metalloproteinase production induced by TNF in fibroblast-like synoviocytes from patients with RA.[36] Pre-clinical models suggest inhibition of SYK could ameliorate inflammatory arthritis.[37-39] An oral agent that inhibits SYK, fostamatinib disodium, a prodrug of the active metabolite R406, was evaluated in pre-clinical models of inflammatory arthritis and found to be effective. Based on this observation, fostamatinib was evaluated in patients with RA in four phase II clinical trials[40-43] and one phase III[44] clinical trial. Doses studied were 100 mg, once per day, 100 mg twice per day, or 150 mg twice per day. Statistically superior improvements in clinical outcomes at 12 to 24 weeks were seen, as measured by ACR response and change in DAS28 score in two of the phase II randomized clinical trials evaluating fostamatinib in combination with MTX in nonbiologic disease-modifying anti-rheumatic drug (DMARD) nonresponders. Clinical response was seen




*O'Sullivan LA, et al: Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol Immunol* 44:2497–2506, 2007.
†Ghoreschi K, et al: Janus kinases in immune cell signaling. *Immunol Rev* 228:273–287, 2009.

**Figure 65-3** Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways. EPO, Erythropoietin; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; TPO, thrombopoietin; TYK, tyrosine kinase.

as early as week 1. In a third phase IIb, 24-week trial, fostamatinib was evaluated as monotherapy compared with placebo and an active comparator toadalimumab.[43] The primary endpoint was the change in DAS28 (CRP) at week 6. Improvement in the fostamatinib-treated patients was seen compared with placebo, but less than that seen with adalimumab.

A 3-month RCT of fostamatinib, 100 mg twice per day or placebo, was conducted in 219 patients in whom biologic agents had previously failed, with the primary endpoint the ACR20 response at month 3.[43] MRI images of patients' wrists and hands were evaluated in a subset of patients. The study failed to meet its primary endpoint at month 3; there was no statistical difference in the ACR20 response between the two groups. Although the study failed, there was a difference in change from baseline between the fostamatinib and placebo groups in MRI synovitis scores.

Because of the efficacy observed in the nonbiologic DMARD-incomplete responder phase II trials, a 52-week phase III multinational RCT was conducted in 918 patients with RA with active disease despite MTX.[44] Patients were randomly assigned to either fostamatinib, 100 mg twice daily; 100 mg twice daily for 4 weeks, then 150 mg daily; or placebo for 24 weeks, then open-label fostamatinib, 100 mg twice daily. The co-primary endpoints were change from baseline in ACR20 response and change in modified Total Sharp van der Heijde score (mTSS) at week 24. Both fostamatinib cohorts achieved a statistically superior ACR20 response compared with placebo-treated patients (44.4% to 49% vs. 34.2%; $P = 0.001$, $P = 0.006$), respectively, but this was a lower response than seen in the phase II program. Additionally, there was no statistical difference in the co-primary endpoint change in mTSS between fostamatinib and placebo.

In all of the phase II/III trials, a dose-dependent increase in AEs, including diarrhea, neutropenia, dizziness, hypertension, and elevated LFTs were observed. Adverse events were more common in the fostamatinib cohorts, including hypertension, diarrhea, neutropenia, and LFT elevations. Serious adverse events occurred in 2.9% to 4.9% fostamatinib-treated patients and 1.6% placebo-treated patients. A small number of malignancies were seen in the fostamatinib cohorts and none in the placebo treated patients. A meta-analysis of the safety profile of fostamatinib in the development program reported the relative risk of the following AEs with fostamatinib: hypertension, 2.80 (confidence interval [CI], 1.02 to 8.43); diarrhea 5.20 (CI, 3.19 to 8.49); neutropenia, 9.24 (CI, 2.22 to 38.42); and LFTs, 2.93 (CI, 1.02-8.43).[45] Based on the failure of fostamatinib to achieve the primary endpoint in the phase III clinical trial and the AE profile, development of the molecule for RA development was terminated.

A second SYK inhibitor has been evaluated in RA patients. MK-8457 is a novel inhibitor of SYK and zeta-chain–associated protein kinase 70 (ZAP70), and was considered to be a more specific SYK inhibitor with less off-target effects. Two small phase II dose-ranging trials utilizing an adaptive design were conducted.[46,47,48]MK8457 was found to be effective in the MTX incomplete responders, similar to fostamatinib, but not in the patients who had not responded to previous TNF inhibitors. Toxicity was significant in both studies, primarily serious infectious episodes, including opportunistic infections. Diarrhea, nausea, and elevations in LFTs were also reported. Hypertension was not seen, possibly because of the limited off-target impact on vascular endothelial growth factor. Because of the significant toxicity noted and failure to see the benefit in TNF in incomplete responders, development of this molecule was terminated.

Both molecules targeting SYK failed as therapies for RA. Significant toxicity was seen with both molecules, including serious infectious episodes, limiting enthusiasm for this kinase as a target in patients with inflammatory arthritis.

## JANUS KINASE INHIBITORS

JAKs are protein tyrosine kinases that bind the cytoplasmic region of transmembrane cytokine receptors and mediate signaling through type 1 and type 2 cytokine receptors.[49] After receptor-ligand interaction, various JAKs are activated, resulting in tyrosine phosphorylation of the receptor and subsequent activation of STATs (signal transducer and activators of transcription), which act as transcription factors. JAK/STAT signaling mediates cellular responses to multiple cytokines and growth factors. These responses include proliferation, differentiation, migration, apoptosis, and cell survival, depending on the signal and cellular context. Activated STATs enter the nucleus and bind to specific enhancer sequences in target genes, impacting their transcription.[50]

JAKs consist of four types: JAK1, JAK2, JAK3, and TYK. The JAKs signal as pairs (see Figure 65-3). JAK3 is primarily expressed in hematopoietic cells and is critical for signal transduction from the common γ-chain of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 on the plasma membrane to the nuclei of immune cells. JAK3 only signals in combination with JAK1. These cytokines are integral to lymphocyte activation, function, and proliferation. JAK3-knockout mice have defects in T and B lymphocytes and NK cells, with no other defects reported. Humans lacking JAK3 develop a severe combined immunodeficiency (SCID), with a deficiency in NK cells and T lymphocytes.[51]

JAK1 and JAK2 were initially not considered as potential therapeutic targets because knocking out these kinases results in germline lethality.[10] However, JAK1/JAK2 inhibitors are under development to treat RA. Ruxolitinib, which has selectivity for JAK1/2, has been approved for myelofibrosis.[52,53] Hormone-like cytokines erythropoietin, thrombopoietin, growth hormone, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5 all signal through JAK2. IL-6, IL-10, IL-11, IL-19, IL-20, IL-22, and IFN-α, IFN-β, and IFN-γ signal through JAK1. Baricitinib, under development for RA, preferentially inhibits JAK1/JAK 2.

TYK2 facilitates signaling for IL-12, IL-23, and type 1 IFNs.[54] TYK2 pairs with either JAK1 or JAK2 to facilitate signaling. At present no specific TYK2 inhibitors are under development for RA.

Additional JAK inhibitors under development have been demonstrated to be potentially more specific JAK1 (GLPG0634) or JAK3 inhibitors (VX-509) with less off-target inhibition. The assays evaluating specificity of the JAK inhibitors vary depending on the substrate utilized,

whether it be biochemical, enzymatic, or cellular, which makes the comparative data difficult to interpret. For example, tofacitinib at the doses used in the clinic demonstrates substantial nanomolar inhibitory potency in enzymatic assays against all JAK family kinases except TYK2, but in cellular assays it shows functional specificity for JAK1 and JAK1/3 versus JAK2.[55] Most now consider tofacitinib to be a pan-JAK inhibitor. A recent preliminary publication demonstrated that clinical profiles of cytokine inhibition for a number of JAK inhibitors in RA are strikingly similar when efficacious doses are considered, suggesting possible limited potential for differentiation of these therapeutics based on their JAK pharmacology.[56]

## Tofacitinib

Tofacitinib is a JAK inhibitor that has been approved by the U.S. Food and Drug Administration (FDA) for patients with RA with active disease, despite MTX treatment at a dose of 5 mg, twice daily, in combination with nonbiologic DMARDs or as monotherapy (Figure 65-4). Tofacitinib has not been approved by the European Medicines Agency (EMA) because of concerns regarding safety. Tofacitinib is also being evaluated for treatment of psoriasis, psoriatic arthritis (PsA), and inflammatory bowel disease. It was under development for treatment of patients undergoing renal transplantation, but this program was discontinued because of toxicities, including serious infections and post-transplantation lymphoproliferative disease.[57] The dose of tofacitinib used in the transplantation program was higher than the dose approved for RA and was used in combination with other potent immunosuppressants.

Tofacitinib has been demonstrated in cellular in vitro assays to reversibly inhibit JAK1, JAK2, JAK3, and, to a lesser extent, TYK2, with functional selectivity for JAK1/3 and JAK1/2 signaling versus JAK2/2. As previously noted, the clinical relevance of this selectivity is unclear.[58] Inhibition of JAK1/3 by tofacitinib will inhibit signaling for cytokines that bind receptors that utilize the common γ-chain receptor, such as IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.[59] Reduction in NK cells has been demonstrated with tofacitinib treatment.[53] It has been postulated that IL-6 inhibition, which signals through JAK1, may play a role in the efficacy of the molecule as well as AEs such as neutropenia and hyperlipidemia, which have been seen with tocilizumab, a monoclonal antibody to the IL-6 receptor. Recent observations suggest that IL-17 production by T helper 17 cells is also inhibited.[60] Tofacitinib does not block IL-1 or TNF signaling.

The pharmacokinetic profile of tofacitinib is characterized by rapid absorption (peak plasma concentrations are reached within 0.5 to 1 hour), rapid elimination (half-life of approximately 3 hours), and dose-proportional increases in systemic exposure.[61] Steady-state concentrations are achieved in 24 to 48 hours with minimal accumulation after twice-daily administration. Tofacitinib is eliminated through both hepatic (70%) and renal clearance (30%). The metabolism is primarily through CYP3A4 and less so by CYP2C19. At the approved dosage, no extrinsic factors or drugs require dose modification based on pharmacokinetic evaluation in pre-clinical studies. The pharmacodynamic effects of tofacitinib have been demonstrated to persist for 4 to 6 weeks after dosing.[62] Tofacitinib is a teratogen, and premenopausal women should utilize contraception and avoid conception for 4 to 6 weeks after the last dose.

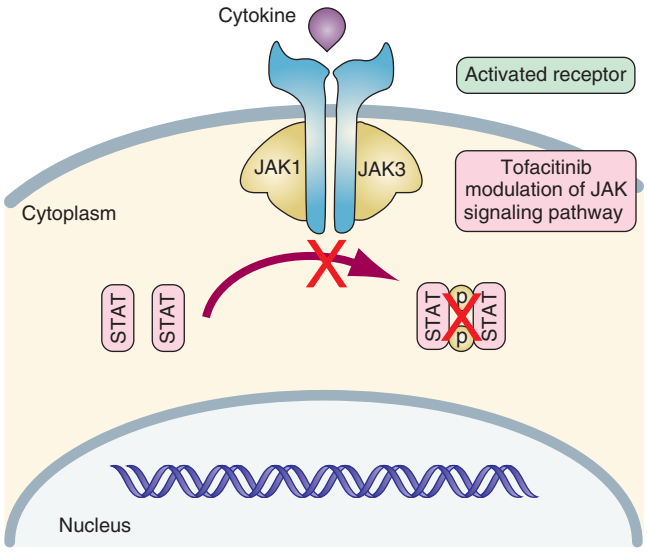



**Figure 65-4** Tofacitinib Janus kinase (JAK) inhibition. STAT, Signal transducer and activator of transcription.

### Efficacy

Tofacitinib was extensively studied before approval. Eight placebo-controlled, randomized, phase II studies were performed in which tofacitinib was given as monotherapy or in combination with background MTX. The phase II program included 1600 patients, and evaluated tofacitinib doses from 1 mg to 30 mg twice daily, for treatment durations ranging between 6 weeks and 6 months. Consistent improvement in clinical outcomes were seen with doses of 5 mg twice daily or greater, but toxicity was greater at higher doses.[63-65] Based on the efficacy and safety profile from the phase II clinical trials, tofacitinib, 5 mg twice daily and 10 mg twice daily doses were progressed to phase III trials.

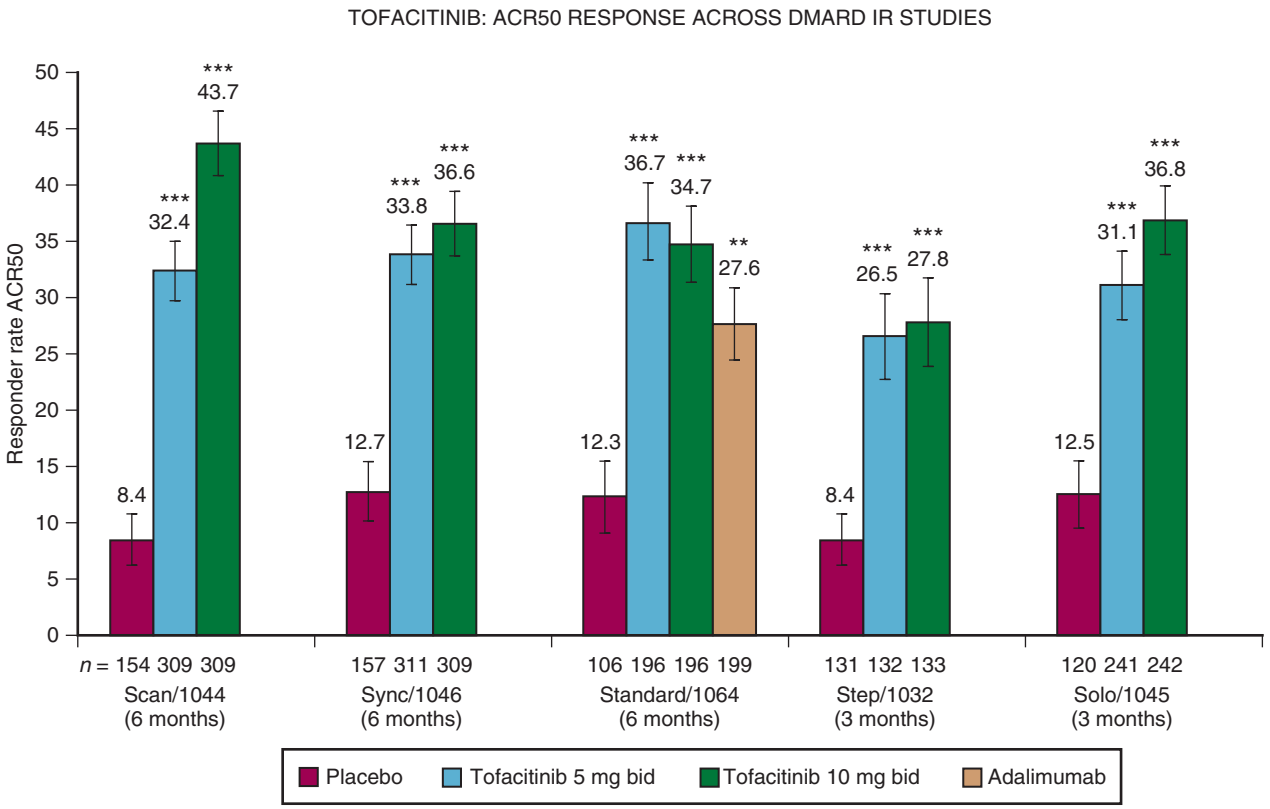
Six phase III RCTs evaluating tofacitinib in RA were conducted in the development program.[66-71] Two of the studies were conducted in patients with RA with active disease despite MTX treatment ("scan, standard"), and one study evaluated patients on MTX or other nonbiologic DMARDs ("sync"). Adalimumab was compared with tofacitinib in the "standard" trial. TNF incomplete responders on background MTX were evaluated in the 6-month "step" study, and tofacitinib monotherapy was evaluated in the 6-month "solo" study (Figure 65-5). The "start" study compared MTX with tofacitinib 5 mg and 10 mg twice daily in MTX-naïve patients with RA during 24 months. Radiographic outcomes were evaluated in the "scan" and "start" studies. A long-term extension (LTE) protocol is ongoing, with more than 12,664 patient-years exposure.[72] At the time of approval of the 5 mg twice daily dose by the FDA, 4800 patients had participated in the clinical trial program.

The clinical trials were conducted globally, with 46% of the patients enrolled outside of North America. In the DMARD incomplete responder population, patients enrolled were 18 to 86 years of age (mean age, 50 to 56

TOFACITINIB: PHASE 3 STUDY DESIGNS-DMARD INCOMPLETE RESPONDERS

| | Duration ≥1 year | | | Duration of 6 months | |
|---|---|---|---|---|---|
| **Study**<br>***n*** | **Study 1044**<br>**"Scan"**<br>***n* = 797** | **Study 1046**<br>**"Sync"**<br>***n* = 792** | **Study 1064**<br>**"Standard"**<br>***n* = 717** | **Study 1032**<br>**"Step"**<br>***n* = 399** | **Study 1045**<br>**"Solo"**<br>***n* = 610** |
| **Population** | MTX IR | DMARD IR | MTX IR | TNF IR | DMARD IR |
| **Background treatment** | MTX | DMARDs | MTX | MTX | None |
| **Distinguishing feature** | Radiograph | Background DMARDs | Active control (adalimumab) | TNF failures | Monotherapy |

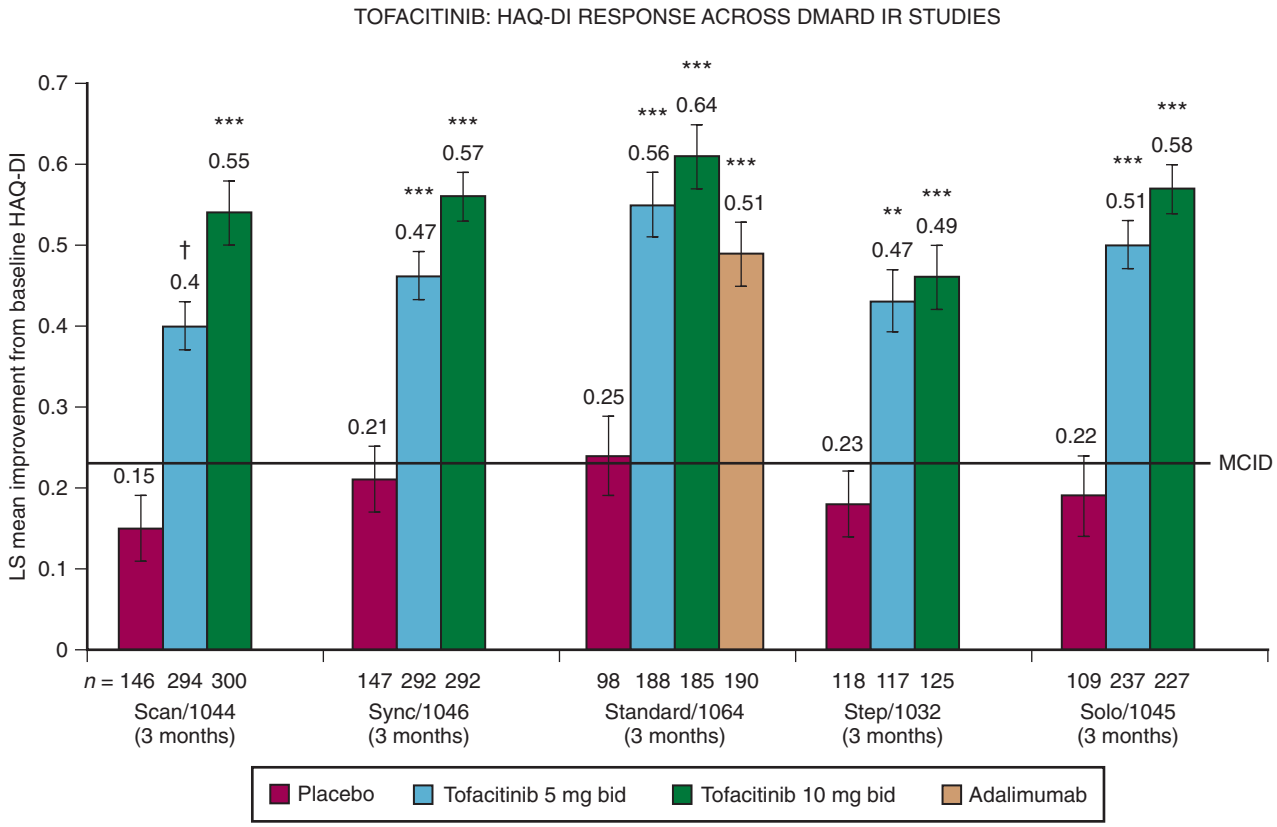
**Figure 65-5** Tofacitinib: phase III study designs. Disease-modifying anti-rheumatic drug (DMARD) incomplete responders (IR). MTX, Methotrexate; TNF, tumor necrosis factor.




*$P \leq 0.05$; **$P < 0.001$; ***$P < 0.0001$ vs. placebo (unadjusted).

**Figure 65-6** Tofacitinib: American College of Rheumatology 50 (ACR50) response across disease-modifying anti-rheumatic drug (DMARD) incomplete responder (IR) studies.

years, 15% ≥65 years), and the majority were women (80% to 87%) with long- standing disease (mean age, 5.7 to 13 years), and high disease activity (DAS-28-4 [ESR] of 5.83 to 6.71). Tofacitinib consistently and significantly improved signs and symptoms of RA, physical functioning, and patient-reported outcomes, such as fatigue, pain, and health-related quality of life (Figures 65-6 and 65-7). Improvements in structural benefit as measured by the mTSS was observed at the 10 mg twice-daily dosage in the MTX nonresponsive responder population, but did not achieve statistical significance at the 5 mg twice-daily dosage. Improvement was demonstrated as early as 2 weeks of treatment. Efficacy with monotherapy was similar to the results with combination therapy with nonbiologic DMARDs. ACR20 responses were in the 50% to 60% range across the studies, with ACR50 rates of 30% to 40% and ACR70 rates of 15% to 20%. ACR responses were comparable to adalimumab in the "standard" study. In the "step" study evaluating TNF inhibitor nonresponders, ACR20 responses were slightly lower, at 41% to 48%, with ACR50 responses of




**$P \leq 0.001$; ***$P < 0.0001$ vs. placebo (unadjusted); LSM, least squares mean; MCID, minimum clinically important difference.
†Statistical significance could not be declared in the Scan study due to the step-down procedure

**Figure 65-7** Tofacitinib: Health Assessment Questionnaire (HAQ)-DI response across disease-modifying anti-rheumatic drug (DMARD) incomplete responder (IR) studies.

27% and ACR70 responses of 10% to 13%. In all of the trials, tofacitinib was statistically superior to placebo for ACR response at study endpoint, as well as improvement in the Health Assessment Questionnaire (HAQ)-DI. Although both doses were efficacious, a dose response in efficacy was suggested because the 10 mg dose was superior to the 5 mg dose for the higher threshhold clinical endpoints, such as ACR70, DAS-28 remission, low disease activity state (LDAS), and HAQ-DI improvement.[73]

The final phase III study was the oral "start" trial that compared monotherapy tofacitinib, 5 mg twice daily and 10 mg twice daily with MTX (mean dose, 18.5 mg) in 985 patients with RA who had not previously received therapeutic doses of MTX.[71] The co-primary endpoints at month 6 were the mean change from baseline in the mTSS score and the proportion of patients with an ACR70 response. The patients enrolled had a mean disease duration of 2.7 to 3.4 years and mean baseline DAS-28 ESR of 6.5 to 6.6. Of the patients, 81% to 86% were seropositive for rheumatoid factor (RF) or cyclic citrullinated protein antibodies. Mean changes in the mTSS from baseline to month 6 were significantly smaller in the tofacitinib groups than in the MTX group (0.2 in the 5 mg tofacitinib group and <0.1 point in the 10 mg tofacitinib group, compared with 0.8 in the MTX group [$P < 0.001$ for both comparisons]). Based on this trial, the FDA allowed the claim for slowing of radiographic progression. Among the patients receiving tofacitinib, 25.5% in the 5 mg group and 37.7% in the 10 mg group had an ACR70 response at month 6, compared with 12.0% of patients in the MTX group ($P < 0.001$ for both comparisons (see Figure 65-7). The 24-month results from this trial have demonstrated persistent improvement in patient-reported outcomes in patients remaining on the study for both doses of tofacitinib and MTX.[74]

## Safety

In the phase II and III studies, the most common adverse events were upper respiratory infections, headache, nasopharyngitis, and diarrhea, which were seen slightly more often in the tofacitinib patients than in the placebo group. The rate of serious adverse events (SAE) with tofacitinib at both doses was similar to placebo and adalimumab (9.76 to 11.8/100 patient-years, tofacitinib; 15.02/100 patient-years, placebo). The most common SAE was pneumonia. Similar rates of SAES were seen in the long-term extension protocol, with pneumonia again the most common reported SAEs. Incidence rate for serious infectious episodes was 2.9/100 patient-years in the phase III trials similar to that seen with biologic therapies. In the long-term extension trials, the rate was 2.3/100 patient-years for the 5 mg dose and 4.06/100 patient-years for the 10 mg dose, which was statistically different because the confidence intervals did not overlap.[75] The rates of adverse events reported in the

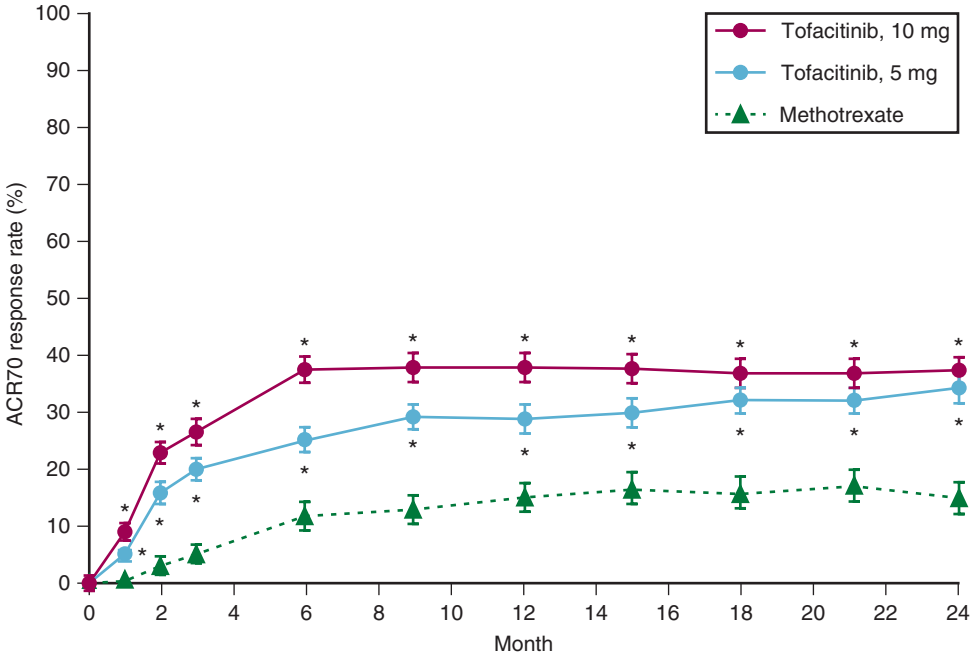



**Figure 65-8** "Start" Study: American College of Rheumatology 70 (ACR70) clinical results over time.

development program were similar to that seen with biologic DMARDs except for an increased incidence of herpes zoster (Figure 65-8).

Nonserious or serious herpes zoster infections were reported in 346 patients in the phase II and phase III clinical trials and the LTE population (incidence rate, 4.27 events/100 patient-years). Incidence rates of herpes zoster infection were 2.81 events/100 patient-years with adalimumab and 1.49 events/100 patient-years with placebo in the phase III population. Rates of herpes zoster infection were similar in patients treated with tofacitinib, 5 mg twice daily and 10 mg twice daily (phase III: 4.39/100 patient-years and 4.23/100 patient-years, respectively; LTE: 4.18 and 4.50/100 patient-years, respectively). A higher rate of herpes zoster was reported in Asian patients (6.75/100 patient-years). This increased rate of herpes zoster has not been previously reported with nonbiologic DMARDs or biologic DMARDs. The overall incidence rates for herpes zoster infections in patients with tofacitinib were stable with time, and the majority of these cases were not considered serious. Because of this observation, if possible, patients with RA should be vaccinated for herpes zoster before initiating tofacitinib.

In phase II and III and the LTE population, tuberculosis (TB) was reported in 16 patients: 4 from the Philippines, 3 from Korea, 2 each from India and China, and 1 each from Japan, Thailand, Mexico, the United States, and Spain. Cases of both pulmonary and extrapulmonary TB were reported. The incidence of TB in the randomized clinical trials was consistent with those reported for TNF inhibitors (0.02 [global] to 2.56 [South Korea]) and nonbiologic DMARDs (0.01 [global] to 0.28 [South Korea]). Screening for latent TB is indicated before initiating tofacitinib.

Excluding cases of TB, 25 patients in the clinical trials and LTE population experienced an opportunistic infection (0.30 events/100 patient-years [95% CI, 0.20 to 0.44]). Two cases of multidermatomal herpes zoster, 8 patients with esophageal candidiasis (several were incidental findings on endoscopies performed for other reasons), 6 patients with cytomegalovirus infection/viremia, 3 patients with cryptococcal infection (2 pneumonia, 1 meningitis), 3 patients with *Pneumocystis* pneumonia, 2 patients with nontuberculous mycobacteria in the lung, and 1 patient with BK virus encephalitis.

The overall mortality rate of tofacitinib, malignancy risk, and lymphoma incidence in the phase II, III, and LTE studies was consistent with the published rates in RA clinical trials for TNF inhibitors and other biologic DMARDs (Figure 65-9). Lymphomas were predominantly non-Hodgkin's B cell lymphomas.

Thirteen gastrointestinal perforations occurred in the development program on tofacitinib, and none in the placebo. These events occurred in patients on background corticosteroids and NSAIDs. The incidence rate reported at the time of approval was 0.10/100 patient-years, similar to what had been reported with TNF and IL-6 inhibitors. Use of this therapy in patients with diverticulitis should probably be avoided, similar to the present standard of care with tocilizumab, which is an IL-6 receptor inhibitor.

Tofacitinib therapy is also associated with changes in laboratory values. Dose-dependent decreases in neutrophils, as seen with TNF and IL-1/IL-6 inhibitors, were infrequently associated with AEs such as neutropenia and not associated with infection risk. Rare significant decreases in hemoglobin, which could be expected with JAK2 inhibition, were seen in the clinical trials. Lymphopenia (<500/uL) was seen in less than 1% of patients in the phase III and LTE trials, but was associated with a dramatic increased risk of serious infections (40%), and monitoring complete blood cell (CBC) and total lymphocyte counts on a regular basis (monthly for 3 months, then every 3 months), is recommended by the regulatory agencies. Discontinuation of

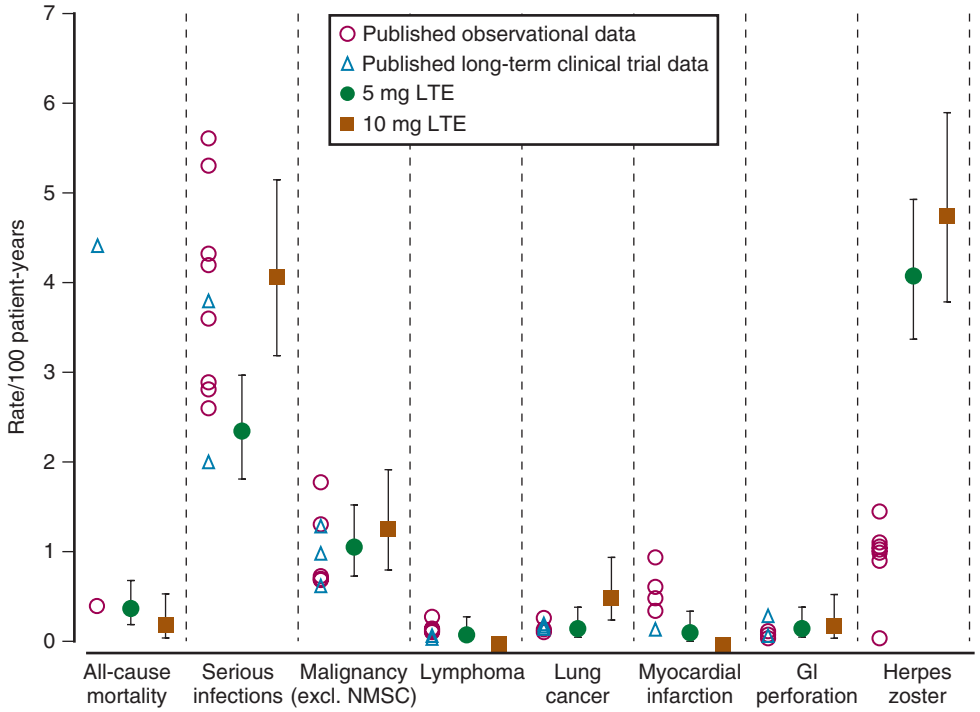



**Figure 65-9** Safety profile of tofacitinib, 5 mg and 10 mg twice daily in the long-term extension (LTE) protocols versus long-term clinical trial and observational data for biologic disease-modifying anti-rheumatic drugs. GI, Gastrointestinal.

treatment is recommended for patients with persistent lymphocyte counts (<500/μL).

Small, clinically insignificant increases in serum creatinine and increased creatinine kinase were noted and have not been associated with significant safety risks for tofacitinib. Increases in tofacitinib-treated patients were 0.02 and 0.04 mg/dL greater than placebo in the development program. The etiology of this minor change is unclear.

Mean 15% to 20% increases in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) that plateaued by 3 months has been observed, similar to that seen with IL-6 inhibitors, and monitoring of lipid levels is indicated after treatment initiation. Increases in rates of cardiovascular AEs have not been shown. Atorvastatin treatment was demonstrated to be effective in patients with elevations in cholesterol on tofacitinib.[76] Monitoring of serum lipids is recommended 8 to 12 weeks after initiating therapy.

Elevations in hepatic transaminases were reported but rarely more than three times the upper limit of normal. Liver function abnormalities were more common in patients on background MTX. Laboratory changes typically normalized upon discontinuation of tofacitinib treatment. Monitoring of LFTs is recommended monthly for 3 months, then once every 3 months.

## Other JAK Inhibitors

Several other JAK inhibitors are under development (Table 65-2). Baricitinib has a selectivity for JAK2/JAK1 and less potency for JAK3, or TYK2.[77] In addition to RA baricitinib is under development to treat psoriasis and diabetic neuropathy. Baricitinib is rapidly absorbed and has a $T_{½}$ of 6 to 8 hours and is dosed once daily.[78]

**TABLE 65-2** Janus Kinase (JAK) Inhibitors in Development

| | |
|---|---|
| Baricitinib | JAK1/2 inhibitor |
| Decernotinib (VX-509) | JAK1/3 inhibitor |
| Fibotinib (GLPG0634) | JAK1 inhibitor |
| Peficitinib (ASP015K) | JAK1/3 inhibitor |
| ABT-494 | JAK1 inhibitor |

A 12-week dose-ranging phase II study in 301 patients with RA was conducted, evaluating 1 mg, 2 mg, 4 mg, and 8 mg of baricitinib as a single daily dose in combination with MTX.[79] The primary endpoint was achieved with a statistically significant difference in the ACR20 response between the combined 4 mg and 8 mg baricitinib groups (76%) compared with placebo (41%) after 12 weeks of treatment ($P < 0.001$). Data at 24 weeks and a subsequent LTE protocol through 52 weeks demonstrated persistence of efficacy at 4 mg and 8 mg of baricitinib.[80,81] Safety signals were similar between the 4 mg and 8 mg doses and were most commonly infections. Rare cases of significant anemia were seen, as would be expected with a JAK2 inhibitor. No significant difference in efficacy between the 4 mg and 8 mg doses were seen in the phase II trials. The 2 mg and 4 mg baricitinib doses were selected for the phase III trials.[82,82a]

### Fibotinib (GLPG0634)

Fibotinib (GLPG0634) is an orally available, selective inhibitor of JAK1 under development for the treatment of RA and Crohn's disease. The molecule displays a JAK1/JAK2 inhibitor profile in biochemical assays, but subsequent

studies in cellular and whole-blood assays revealed a selectivity of approximately 30-fold for JAK1 versus JAK2-dependent signaling.[83] An active metabolite of GLPG0634 has been identified, with a longer half-life than the parent molecule, supporting once-daily dosing.[84] A small, 4-week single-center study, evaluating 36 patients with RA with active disease despite MTX treatment with 100 mg twice daily and 200 mg daily, and placebo, demonstrated that 83% of patients receiving GLPG0634 achieved an ACR20 response, and half of the GLPG0634-treated patients achieved either disease remission or low disease activity. Of the placebo patients, 33% achieved an ACR20 response.[85] A second phase II 4-week, double-blind study of GLPG0634 at 30, 75, 150, and 300 mg once-daily doses, and placebo evaluated 91 biologic-naïve patients with RA who had insufficient response to MTX. GLPG0634 demonstrated efficacy at doses greater than 75 mg daily, with no additional benefit to the 300 mg dose compared with the previous results at 200 mg in the proof-of-concept study.[86] No significant safety issues were reported in these short-duration studies.

Subsequent trials are ongoing evaluating the efficacy and safety of GLPG0634 at 50 mg, 100 mg, and 200 mg dosages as combination therapy or monotherapy with 24 weeks of treatment in 875 patients with moderate to severe RA with active disease despite MTX treatment.[87,87a]

### Decernotinib (VX-509)

Decernotinib (VX-509) is a JAK inhibitor previously in development with selectivity for JAK3 over JAK1/2 and TYK2.[88] In 2011, a phase IIa clinical trial of four doses of VX-509 was reported, and data from the treatment arms of the two highest doses (100 mg and 150 mg) demonstrated statistically significant ACR20, ACR50, ACR70, and DAS-28 responses compared with placebo.[89] The 150 mg treatment arm demonstrated ACR20, ACR50, ACR70, and DAS28 responses of 66%, 49%t, 22% and –3.06%, respectively, whereas the placebo arm showed responses of 29%, 7%, 2%, and –1.25, respectively. A subsequent phase IIb, double-blind, randomized, placebo-controlled 24-week phase IIb study of VX-509 enrolled 358 people with RA with active disease despite methotrexate treatment.[91] Patients were randomly assigned to receive placebo or one of four doses of VX-509. In all VX-509 treatment groups, the proportion of patients achieving ACR20 and ACR50 and the decrease from baseline in DAS-28 were significantly greater than in the placebo group. The three highest dose groups showed ACR20 responses of between 58% and 68%, compared with 18% for placebo, and statistically significant ACR70 responses versus placebo. AEs including infections, SAEs, and hyperlipidemia, were seen more commonly with VX-509. Elevations in transaminase levels and decreases in median neutrophil and lymphocyte counts were observed in the VX-509 groups and were generally mild. Subsequent 24-week results demonstrated similar efficacy and safety results.[92] This molecule is no longer under development for RA.

### Peficitinib (ASP015K)

Peficitinib (ASP015K) is an once-daily oral JAK inhibitor that is in development for RA. ASP015K has shown selectivity of JAK1/JAK3 versus JAK2 in cell-based assays. In a 12-week, double-blind study, patients with active RA not on concomitant DMARD therapy were randomly assigned equally to once-daily ASP015K 25 mg, 50 mg, 100 mg, 150 mg, or placebo.[93] Twenty-five percent of patients had previously received TNF inhibitors. There was a dose response for efficacy with the 100 mg and 150 mg daily doses at week 12, achieving statistically significant ACR20, ACR50, and ACR70 responses compared with placebo. No difference in AEs or SAEs were reported for ASP015K compared with placebo, except for nasopharyngitis, diarrhea, and creatine phosphokinase elevation, which was reported more frequently with ASP015K. Additional trials with this molecule are ongoing.

A subsequent 12-week phase IIb dose-ranging study in MTX incomplete responders failed to achieve a statistically significant difference in the primary endpoint the ACR20 responses from placebo.[94]A statistically significant difference was seen for the change in DAS-28–CRP and DAS-28–ESR. Most common AEs were headache and hyperlipidemia.

### ABT-494

ABT-494 is a second-generation JAK1 inhibitor, which in cellular and in vivo assays, showing 74-fold selectivity over JAK2/3.[95] Clinical trials are ongoing in RA, with preliminary reports indicating response rates similar to other JAK inhibitors.

## BTK INHIBITORS

Oral Btk inhibitors (ibrutinib) have been approved for treatment of patients with mantle cell lymphoma and chronic lymphocytic leukemia who have received at least one previous therapy.[96] Btk inhibitors are in early development as a potential treatment for RA. Btk plays a prominent role in BCR signal transduction and also has a role in Toll-like receptor and FcR signaling in myeloid cells.[97] BTK is a member of the TEC family of cytoplasmic protein tyrosine kinases. In humans, mutations in BTK can result in the development of Bruton's agammaglobulinemia (known also as X-linked agammaglobulinemia), an immunodeficiency characterized by a defect in B cell development and resulting in a significant absence of circulating B cells.[98]

Upon ligand binding to the BCR, Btk is activated by upstream SRC-family kinases LYN, FYN, and SYK (Figure 65-10). Activated Btk drives phosphorylation of PLCγ and subsequent protein kinase C (PKC) activation, which in turn results in calcium flux and the activation of transcription factors, including NF-κB and NFAT, regulating the expression of downstream genes controlling proliferation, survival, and chemokines and cytokines. B cells play multiple roles in the pathogenesis of RA, including antigen presentation, inflammatory cytokine production and RF, and CCP antibody production. Rituximab, which depletes B cells, has demonstrated efficacy as treatment for patients with RA, supporting efforts to develop a small molecule Btk inhibitor that impacts B cell activation.[99]

Animal models of arthritis suggest that Btk inhibition can result in inhibition of B cell receptor–dependent cell proliferation and a reduction of inflammatory cytokine

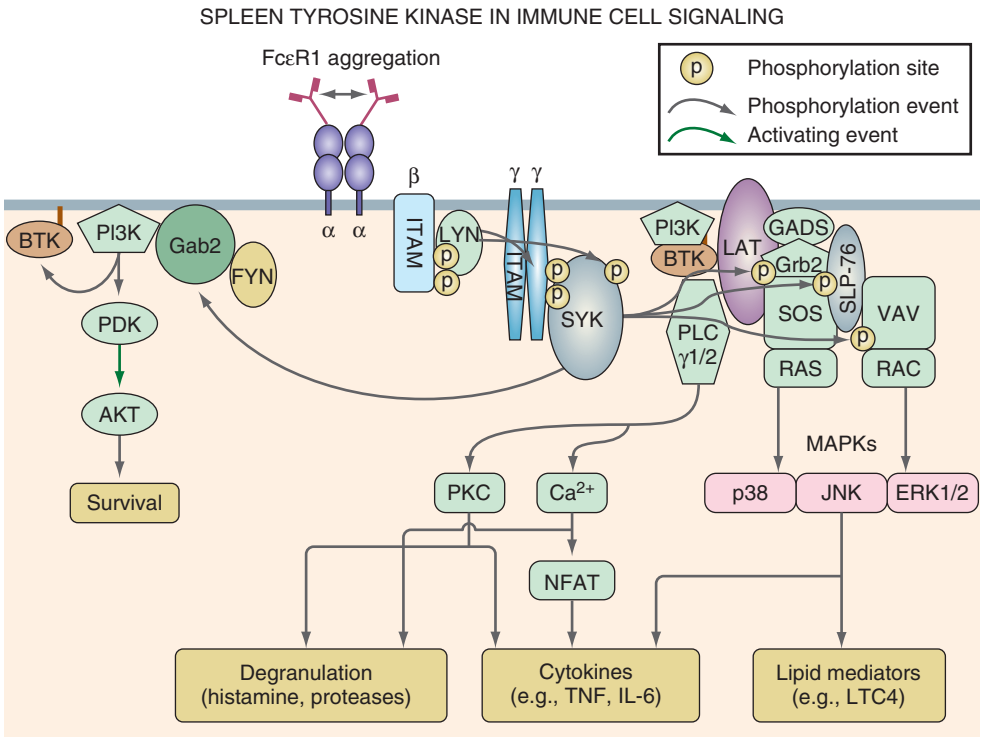



**Figure 65-10** Spleen tyrosine kinase (SYK) in immune cell signal transduction. JNK, c-JUN N-terminal kinase; LTC4, leukotrience C4; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; PDK, phosphoinositide dependent kinase; PKC, protein kinase C.

production from myeloid cells (including TNF, IL-1, and IL-6) by preventing signaling through the FCyRIII receptor.[100,101] Additionally, activated BTK has been demonstrated to be increased in peripheral blood B cells of patients with RA compared with controls.[102]

Early-phase trials with BTK inhibitors in RA are ongoing.[102a,103]

## PI3K INHIBITORS

PI3Ks are lipid kinases that play central role in regulation of cell cycle, apoptosis, DNA repair, senescence, angiogenesis, cellular metabolism, and motility.[104,105] They are intermediate signaling molecules and are most well known for their roles in the PI3K/AKT/mammalian target of rapamycin (mTOR) signaling pathway. PI3Ks transmit signals from the cell surface to the cytoplasm by generating second messengers—phosphorylated phosphatidylinositols—which in turn activate multiple effector kinase pathways, including Btk, AKT, PKC, NF-κB, and JNK/SAPK pathways, and ultimately result in survival and growth of normal cells.

PI3K signaling is mediated by p110 α, β, γ, and δ isoforms. Two isoforms of the catalytic subunits, p110γ and p110δ, are enriched in leukocytes, in which they promote activation, cellular growth, proliferation, differentiation, and survival through the generation of the second messengers phosphatidylinositol biphosphate (PIP2) and phosphatidylinositol triphosphate (PIP3). The functions of PI3K-δ and PI3K-γ in the differentiation, maintenance, and activation of immune cells support an important role for these enzymes in oncology, inflammation, and autoimmunity.[106] PI3K-δ inhibitors are under development for relapsed indolent lymphoma and chronic lymphocytic leukemia.[107]

Nonclinical models utilizing animals either deficient in PI3K-δ and/or PI3K-γ, or treated with inhibitors of these isoforms of PI3K, have established the potential role of PI3K in several inflammatory and autoimmune diseases.[108,109,110] Treatment of mice with inhibitors of PI3K-δ suggests this enzyme is important in the production of antibodies from marginal-zone B cells and peritoneal B1 cells, major sources of autoantibodies in disease. These inhibitors also reduce antigen-specific antibody responses.

Potent inhibitors of PI3K-δ and PI3K-γ enzymes are under development in RA therapy. To date no published data from patients with RA have been published.

## SPHINGOSINE 1 PHOSPHATE MODULATORS

Sphingosine-1-phosphate (S1P) is an abundant, biologically active lysophospholipid. In the immune system, changes in local S1P concentrations and gradients can modify lymphocyte migration patterns, alter inflammatory cell responses, and affect the barrier function of endothelial cells. Interaction of (S1P) with its receptor results in inhibition of lymphocyte egress from primary and secondary lymphoid tissues, resulting in lymphocyte depletion in the peripheral blood. The major and irreversible route of S1P degradation is via S1P lyase (S1PL). Fingolimod, a sphingosine-1-phosphate receptor modulator was approved in 2013 for relapsing-remitting multiple sclerosis.

Pre-clinical studies demonstrated that reduced S1PL activity has a significant effect in reducing the inflammatory response in models of RA.[111,112] LX3305, a small-molecule inhibitor of S1PL, was evaluated as a DMARD. Results of a placebo-controlled, dose-ranging phase II RCT of LX3305

in patients with RA with active disease despite MTX treatment has been reported.[113] In the study, 208 patients received 70 mg, 110 mg, or 150 mg daily, or placebo, for 12 weeks, and the primary endpoint was ACR20 at week 12. Of patients receiving the 150 mg dose, 60% achieved an ACR20 response compared with 49% on placebo, which was not statistically different. Various subanalyses were conducted, suggesting potential benefits, and no significant safety issues were noted. Based on the results of this proof-of-concept study, development in RA was terminated.

## PHOSPHODIESTERASE INHIBITORS

Inhibition of type 4 phosphodiesterase (PDE) has been demonstrated to inhibit TNF production and other pro-inflammatory cytokines.[114,115] Cyclic adenosine monophosphate (cAMP) is the principal second messenger responsible for immune response regulation. PDE4 is the main cAMP-degrading enzyme found in cells of the immune system and keratinocytes. It is the predominant isoenzyme expressed in macrophages, lymphocytes, and neutrophils. Elevation of intra-cellular cAMP inhibits TNF production through the protein kinase A pathway. PDE4 inhibitors are under development for chronic obstructive pulmonary disorder and atopic dermatitis but have not moved forward in development because of gastrointestinal toxicity and possible cardiac toxicity.

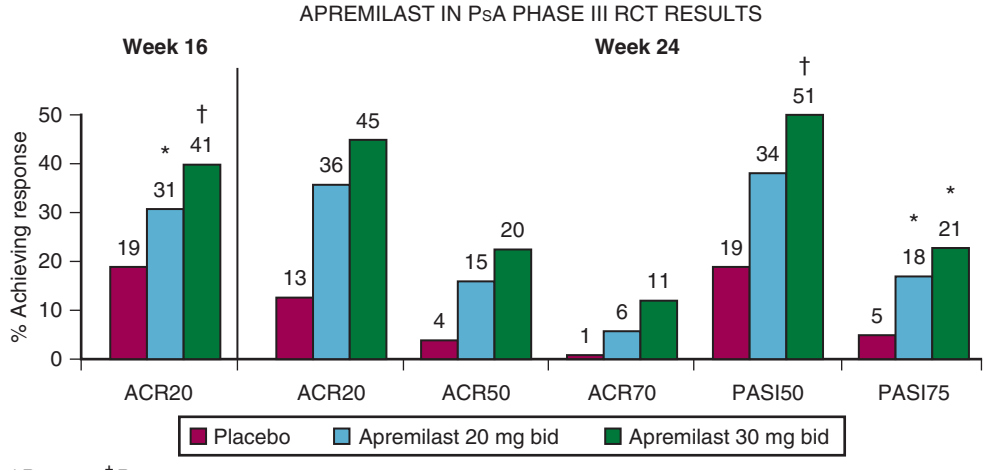
Apremilast inhibits production of the pro-inflammatory cytokines, IFN-γ, TNF, IL-12 and IL-23, and inducible nitric oxide synthase.[116] Apremilast was evaluated in RA but did not meet the primary endpoint, and development was terminated in RA. Data from the RA clinical development program have not been published to date.

Apremilast has been approved for the treatment of psoriasis and PsA. Oral administration of apremilast has an absolute bioavailability of 73%, with peak concentration at 2.5 hours (Tmax). The half-life of apremilast is 6 to 9 hours, necessitating twice-daily dosing. Dosages of apremilast at 20 mg twice daily and 30 mg twice daily was evaluated in three multicenter, randomized, double-blind, placebo-controlled trials of 1493 patients with active PsA.[117,118] Patients ranged in age from 18 to 83 years, with an overall median disease duration of 5 years. Patients were randomly assigned to receive either 20 mg twice daily or 30 mg twice daily. Patients with varied subtypes of PsA were enrolled across the three studies, including symmetric polyarthritis (62.0%), asymmetric oligoarthritis (27.0%), distal interphalangeal (DIP) joint arthritis (6.0%), arthritis mutilans (3.0%), and predominant spondylitis (2.1%). Patients received concomitant therapy with at least one DMARD (65.0%), MTX (55.0%), sulfasalazine (SSZ) (9.0%), leflunamide (LEF) (7.0%) and low-dose oral corticosteroids (14.0%), and NSAIDs (71.0%). Prior treatment with non-biologic DMARDs only was reported in 76.0% of patients, and prior treatment with biologic DMARDs was reported in 22.0% of patients, which includes 9.0% who had not responded to prior biologic DMARD treatment. The primary endpoint in the trials was the percentage of patients achieving an ACR20 response at week 16.

ACR responses with apremilast, 30 mg twice daily, were statistically superior to placebo in all three studies (ACR20 response, apremilast, 32% to 41%; placebo, 18% to 19%; $P < 0.05$). ACR50 and ACR70 responses were numerically greater for apremilast, but did not achieve statistical significance (Figure 65-11). The PASI75 response was 21% for apremilast and 5% for the placebo group. The responses for both skin and arthritis are less robust than the responses generally seen with TNF inhibitors.

Diarrhea, headache, and nausea were the most commonly reported adverse reactions. The most common adverse reactions leading to discontinuation were nausea (1.8%), diarrhea (1.8%), and headache. The initial dose is titrated from 10 mg daily to 30 mg twice daily during a period of 5 days to reduce the likelihood of gastrointestinal AEs. The proportion of patients with PsA who discontinued treatment in the trials because of any adverse reaction was 4.6% for patients taking apremilast, 30 mg twice daily and 1.2% for placebo-treated patients.

Warnings in the package insert include the possibility of weight loss and depression. Weight loss of 5 to 10 lb in the




*$P < 0.02$; †$P < 0.0001$

Data from Kavanaugh A, et al: *Ann Rheum Dis* 73(6):1020-1026, 2014.

**Figure 65-11** Apremilast in psoriatic arthritis. ACR, American College of Rheumatology; AE, adverse event; DMARD, disease-modifying anti-rheumatic drug; PsA, psoriatic arthritis.

PsA clinical trials was reported in 10% of apremilast-treated patients and 3.3% of placebo patients. Depression was seen in a small number of patients, but slightly more frequently than in placebo patients.

## CONCLUSION

The unraveling of the intra-cellular pathways involved in signal transduction, resulting in T/B cell activation and inflammatory cytokine production, has resulted in the development of small-molecule inhibitors to these pathways that can abrogate inflammation in inflammatory diseases. Efficacy similar to biologic DMARDs has been demonstrated with an acceptable short-term safety profile. Long-term follow-up of patients on these signal transduction inhibitors is necessary to determine whether these therapies will be safe as a chronic treatment for patients with lifelong disease. Where these new therapies fall into the treatment algorithm for RA has yet to be determined, but they certainly provide an alternative for patients unwilling to pursue parenteral therapies. Newer molecular inhibitors are under development, and we will learn from the ongoing trials whether inhibiting other signaling pathways such as Btk or PI3k will have any additional value for our patients.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 66

# Urate-Lowering Therapy



Ted R. Mikuls

**KEY POINTS**

**Urate-lowering therapy (ULT) is central to the management of hyperuricemia in gout.**

**The goal of ULT in gout is to reduce the frequency of acute flares and prevent progressive joint destruction and tophaceous deposition. This goal can be achieved by lowering and maintaining serum uric acid (UA) below 5 to 6 mg/dL.**

**Optimal ULT requires careful patient selection with attention to comorbid illness, ongoing education, adoption of a treat-to-target approach, and effective anti-inflammatory prophylaxis with treatment initiation.**

Gout is among the most common forms of inflammatory arthritis, affecting almost 4% of the U.S. population with a prevalence exceeding 12% in individuals older than 80 years.[1] The health burden posed by gout is likely to increase: At least one report demonstrates a 44% increase in prevalence in 2007 to 2008 compared with a similar survey conducted in 1988 to 1994.[1] The rapid growth in gout frequency appears to be largely attributable to a near-endemic increase in hyperuricemia. Hyperuricemia is the serum concentration above which uric acid (UA) precipitates into monosodium urate crystals at a physiologic pH of 7.4, defined as serum urate (sUA) levels greater than or equal to 6.8 mg/dL or 405 μmol/L, resulting from renal "underexcretion" in most cases (~80%), with the remainder caused by "overproduction." UA is the end product of purine degradation, with circulating concentrations reflecting the complex interactions of dietary purine intake, endogenous production, and elimination.

The treatment of chronic gout is based primarily on the use of urate-lowering therapy (ULT). Available ULT classes include (1) xanthine oxidase (XO) inhibitors (allopurinol and febuxostat), considered as first-line agents[2]; (2) uricosurics (probenecid, benzbromarone, and sulfinpyrazone); and (3) uricases (pegloticase) (Table 66-1). This chapter focuses primarily on the most common rheumatic indication for ULT, the treatment of hyperuricemia in gout. The optimal use of ULT in gout, regardless of the specific agent used, requires careful consideration of several factors.

## NONPHARMACOLOGIC TREATMENT OF HYPERURICEMIA

The importance of education and lifestyle advice, including weight loss, select dietary restrictions, and reduced alcohol intake, have been emphasized in recent gout management guidelines.[2,3] Despite a growing list of dietary factors implicated in hyperuricemia and gout, investigations of dietary interventions on health outcomes in gout are lacking. Furthermore, evidence suggests that such interventions in isolation yield only modest results and suffer from a lack of widespread patient acceptance and long-term adherence.[4] In addition to dietary modifications that include reducing the intake of dietary purines (particularly those derived from meats and seafood), fructose, beer, and liquor, weight loss represents an important goal for overweight gout patients. Although even modest amounts of weight loss can be important to overall health, resulting reductions in sUA are typically modest[5] and are alone insufficient in many patients with gout. One recent study has shown that more dramatic weight loss in morbidly obese patients with gout can lead to clinically significant reductions in sUA. Among a small group of morbidly obese patients with gout, bariatric surgery resulted in an approximate 25% reduction in mean sUA accompanying an average postsurgical weight loss of 34 kg.[6]

## Patient Selection and Timing of Treatment Initiation

Hyperuricemia should be treated in patients with gout with recurrent and frequent flares, tophi, and/or radiographic changes consistent with gout.[2,3] Although it has long been suggested that ULT should be initiated only after the acute gouty inflammation has resolved with the rationale that its earlier initiation could amplify the duration and magnitude of symptoms, this dogma has recently been challenged. In a randomized controlled study of 57 men with crystal-proven gout, allopurinol initiation during an acute flare resulted in no differences in self-reported pain or the occurrence of recurrent gout attacks compared with placebo when administered with background anti-inflammatory agents.[7] Conversely, the development of a gout flare that complicates ongoing ULT is not an indication to discontinue or postpone ULT. Results from a cost-effectiveness analysis in nontophaceous gout, involving a hypothetical patient cohort, suggest that the institution of allopurinol is cost effective in patients seen with two or more acute gout flares within a 1-year period.[8] Although there has been substantial speculation regarding the potential role of ULT in cardiovascular disease prevention and the treatment of hypertension, available ULTs are not

**TABLE 66-1** Dosing and Safety Information for Currently Available Urate-Lowering Therapies in the Management of Gout

| | Dose | Route/ Schedule | Half-life | Primary Site of Metabolism/ Elimination | Adverse Effects | Contraindications (C)/Drug Interactions (DI) |
|---|---|---|---|---|---|---|
| **Xanthine Oxidase Inhibitors** | | | | | | |
| Allopurinol | 100-800 mg | PO/daily | 1-2 hr (half-life of active metabolite oxypurinol 15-30 hr) | Met: Hepatic xanthine oxidase and aldehyde oxidase (into oxypurinol)<br>Elim: Renal (renal dosing may be required) | Common: Gout flare; skin rash; nausea; diarrhea; LFT abnormalities<br>Rare: Allopurinol hypersensitivity syndrome (AHS) (more common in HLA-B*5801+); cytopenias | C: Concomitant azathioprine, 6-MP, theophylline, prior hypersensitivity<br>DI: Azathioprine, 6-MP, theophylline, ampicillin/ amoxicillin, uricosurics, thiazides, cyclosporine, warfarin, ACE inhibitors (possible), Dilantin, cyclophosphamide, vidarabine |
| Febuxostat | 40-120 mg | PO/daily | 6-8 hr | Met: Hepatic (glucuronyl conjugation and oxidation via Cyt P450)<br>Elim: Hepatic and renal | Common: Gout flare; skin rash; nausea; arthralgias; LFT abnormalities<br>Rare: Cardiovascular events (unclear association); cytopenias | C: Concomitant azathioprine, 6-MP, theophylline, prior hypersensitivity; severe hepatic impairment<br>DI: Azathioprine, 6-MP, theophylline |
| **Uricosurics** | | | | | * Adverse effects, contraindications, drug interactions similar for available uricosurics—grouped together below | |
| Probenecid | 500-2000 mg | PO/bid | 3-8 hr (500 mg); 6-12 hr (larger doses) | Met: Hepatic (hydroxylation)<br>Elim: Hepatic and renal | Common: Gout flare; nephrolithiasis; rash; flushing; nausea; loss of appetite<br>Rare: Cytopenias, nephrotic syndrome, anaphylaxis, back pain (rare reports of hepatotoxicity with benzbromarone) | C: Prior hypersensitivity; nephrolithiasis; UA overexcretion; concomitantly with other cancer therapies; known blood dyscrasias; active peptic ulcer disease; sulfinpyrazone should be avoided in patients with phenylbutazone/pyrazole allergy<br>DI (more extensive for probenecid/sulfinpyrazone then benzbromarone): allopurinol, NSAIDs, salicylates, penicillins, cephalosporins, fluoroquinolones, imipenem, rifampin, nitrofurantoin, sulfonamides, heparin, dapsone, acyclovir, ganciclovir, zidovudine, alcohol, diazoxide, mecamylamine, pyrazinamide, antineoplastic agents, clofibrate; dyphylline, diuretics, benzodiazepines, methotrexate, riboflavin, thiopental |
| Sulfinpyrazone | 200-800 mg | PO/bid | 3-12 hr | Met: Hepatic (CYP2C9)<br>Elim: Hepatic and renal | | |
| Benzbromarone | 50-200 mg | PO/daily | 3 hr (half-life of active metabolite 6-hydroxybenzbromarone ~30 hr) | Met: Hepatic (CYP2C9)<br>Elim: Hepatic and renal | | |
| **Uricases** | | | | | | |
| Pegloticase | 8 mg* | IV/every 2 wk | Highly variable (days to wk) | Not well defined | Common: Gout flare; allergic reactions; anaphylaxis (~7%); infusion reactions (urticaria, dyspnea, chest discomfort, pruritis, chest discomfort)<br>Rare: CHF exacerbation (unclear association) | C: Allergic reactions to medication or loss of effect (serum UA >6.0 mg/dL indicates development of antipegloticase antibody)<br>DI: Other PEGylated agents (possible) |

*Pre-dosing with fexofenadine 60 mg, acetaminophen 1000 mg, and hydrocortisone 200 mg IV on the day of the infusion.

ACE, Angiotensin-converting enzyme; bid, twice daily; CHF, congestive heart failure; Cyt, cytochrome; Elim, elimination; HLA, human leukocyte antigen; IV, intravenous; NSAIDs, nonsteroidal anti-inflammatory drugs; PEGylated, polyethylene glycosylated; PO, by mouth; tid, three times daily; UA, uric acid .

currently approved for the treatment of hyperuricemia in the absence of gout.

## Duration of Urate-Lowering Therapy

In asymptomatic patients with gout successfully treated with prior ULT, withdrawal of therapy often results in an abrupt increase in sUA, with recurrent attacks occurring in approximately one-third of patients within two years.[9] Similarly, reductions from continuous ULT to an "intermittent" regimen in previously stable patients with gout lead to significantly higher flare rates,[10] whereas ULT discontinuation in the setting of tophaceous gout leads to recurrent gout flares in a vast majority and recurrent tophi in nearly half.[11] Taken together, these reports suggest that ULT should be "lifelong" in patients with gout.

## Target Serum Urate Goals

Lowering and maintaining sUA less than 6.0 mg/dL (<360 μmol/L) leads to improved long-term outcomes in gout, a treatment goal advocated in management guidelines[2,3] with recent recommendations suggesting that an even lower target goal (<5.0 mg/dL or <300 μmol/L) may be needed to control the disease in some patients, including those with tophi.[2] ULT reduces the long-term risk of recurrent flare by approximately 60% for each 1 mg/dL decrease in sUA.[12] Additional evidence suggests that reaching and maintaining sUA concentrations less than 6.0 mg/dL is important for the depletion of total body urate stores.[13] It is important to recognize that these target goals often fall well below the upper-limit normal for sUA in clinical laboratories that define ranges based on population-based distributions.

## Anti-inflammatory Prophylaxis with Urate-Lowering Therapy Administration

Rebound gout flare is the most common complication of ULT regardless of the agent used, rendering anti-inflammatory prophylaxis a key component of successful gout treatment.[2,3] Gout flares complicating ULT are thought to be caused by reduced sUA concentrations that result in the mobilization of monosodium urate crystals from articular and periarticular deposits into joints. The frequency of treatment-related gout flares appears to be higher with more rapid and potent urate-lowering interventions.[14] Both colchicine and nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in reducing gout flares during ULT initiation.[15-17] Although low-dose glucocorticoids (e.g., prednisone or prednisolone equivalent of ≤10 mg daily) are commonly used in this context, data supporting their use are limited. Results from Borstad and co-workers.[16] suggest that anti-inflammatory prophylaxis with low-dose oral colchicine (0.6 mg, twice/day) protects against rebound gout flares and may need to be continued for at least 6 months after ULT initiation. Frequently used in gout flare prevention, the efficacy of low-dose glucocorticoids in this setting has not been well defined. Under active investigation, agents targeting interleukin (IL)-1, including both canakinumab and rilonacept, have demonstrated preliminary efficacy in the prevention of gout flare complicating ULT initiation.[18,19]

### Urate-Lowering Therapy Adherence

Suboptimal treatment compliance poses a major obstacle to effective ULT, with reports consistently demonstrating rates of medication adherence of 50% or less in gout.[20,21] Treatment adherence in gout was recently shown to be the lowest of seven different chronic conditions examined,[20] emphasizing again the importance of patient education in disease management.

# XANTHINE OXIDASE INHIBITION

## Allopurinol

**KEY POINTS**

- **Advantages of allopurinol over the most commonly used uricosurics include its once-daily dosing, effectiveness in both "underexcretors" and "overproducers," and its potential effectiveness in patients with renal insufficiency.**
- **Allopurinol hypersensitivity syndrome (AHS) is a relatively uncommon, albeit potentially serious, adverse event associated with its use.**
- **Allopurinol doses higher than 300 mg/day are frequently required to achieve target sUA goals; evidence and consensus-based gout treatment guidelines recommend the initial use of low-dose allopurinol (≤100 mg/day) with gradual increases in dosing to achieve target sUA goals.**

Available for approximately 50 years, allopurinol accounts for a vast majority of all ULT prescriptions.[22] In addition to its established track record in gout, allopurinol has several advantages: (1) low cost, (2) once-daily oral administration, (3) effectiveness in patients who underexcrete and those who overproduce UA, (4) favorable safety profile, and (5) potential effectiveness in patients with renal impairment. Allopurinol treatment results in significant declines in sUA,[17,23,24] decreased gout flare rates,[17,23-30] and declines in tophus area.[23]

### Role in Rheumatic Disease and Indications

Approved indications for allopurinol include (1) treatment of hyperuricemia in gout, (2) the management of hyperuricemia resulting from the treatment of malignancy (most often leukemia or lymphoma), and (3) the management of nephrolithiasis in the context of increased urinary UA excretion. Although not approved for the treatment of asymptomatic hyperuricemia in the absence of gout, there is evidence suggesting that allopurinol use may have other health benefits. Hyperuricemia is independently associated with cardiovascular morbidity and mortality,[31-34] giving rise to speculation that ULT could be cardioprotective.[35] At least two prospective studies have shown a lower all-cause mortality risk among individuals receiving allopurinol,[35,36] although it was not clear from these investigations whether this benefit was mediated by reductions in cardiovascular risk. In a placebo-controlled study of pediatric essential hypertension, allopurinol use resulted in significant, albeit modest, declines in blood pressure.[37] XO inhibition via allopurinol also has been shown to improve endothelial

function, improving measures of both local and systemic blood flow[38] and has been associated with improvements in renal function in at-risk populations.[39,40]

## Chemical Structure and Mechanism of Action

An antimetabolite in simple organisms, allopurinol inhibits XO (Figure 66-1), a key enzyme in purine catabolism, but does not inhibit the biosynthesis of purines in humans.

## Pharmacology

Pharmacologic characteristics of the ULTs are summarized in Table 66-2. Allopurinol is approximately 90% absorbed from the gastrointestinal (GI) tract and is metabolized into oxypurinol, its active metabolite. Peak serum concentrations for allopurinol and oxypurinol are achieved within approximately 1 to 2 hours and 4 to 5 hours, respectively. The plasma half-life of allopurinol is relatively brief (1 to 2 hours) although the half-life of oxypurinol is substantially longer (~15 hours or longer), allowing for once-daily dosing. Allopurinol is eliminated primarily via glomerular filtration, whereas oxypurinol undergoes some degree of renal tubular reabsorption. With renal mechanisms primarily responsible for drug elimination, the plasma half-life of allopurinol, and to a greater degree oxypurinol, are increased with renal insufficiency.

**TABLE 66-2** Agents Not Approved for Gout Treatment That Display Urate-Lowering Properties

| Agent | Usual Indication |
|---|---|
| Losartan | Hypertension, congestive heart failure |
| Fenofibrate | Hyperlipidemia, hypertriglyceridemia |
| Atorvastatin | Hyperlipidemia |
| Rosuvastatin | Hyperlipidemia |
| Guaifenesin | Upper respiratory airway congestion |
| Leflunomide | Rheumatoid arthritis |
| Salicylates (high dose) | Analgesia, fever, anti-inflammatory |

## Dose and Drug Administration

Allopurinol is available as 100 mg and 300 mg pills for once-daily oral administration (see Table 66-2), with split dosing advocated in some instances for daily doses 600 mg or higher. Used in the treatment and/or prevention of tumor lysis syndrome, allopurinol is available intravenously. Approved at daily doses as high as 800 mg in the United States and 900 mg in Europe, allopurinol is rarely administered at doses exceeding 300 mg/day in everyday clinical practice,[41] although greater doses are often required to appropriately manage the signs and symptoms of gout. It is well established that only a modest proportion of patients achieve a target sUA less than 6.0 mg/dL with allopurinol 300 mg/day, the most commonly used dose. With a target sUA threshold of less than 5.0 mg/dL, investigators have shown that only one-fourth of patients with gout achieve this goal with 300 mg of daily allopurinol, a proportion that increased to 78% with a daily dose of 600 mg.[42] The limitation of "standard" dose allopurinol has been proved in randomized clinical trials that have compared fixed daily doses of 300 mg with febuxostat in various doses. In those studies, approximately 40% of allopurinol-treated patients with gout achieved a final sUA less than 6.0 mg/dL.[17,23] Recent gout treatment guidelines recommend that allopurinol be started in low doses (e.g., 100 mg daily) and increased by 100 mg every 2 to 5 weeks as required to achieve the target.[2,3] Data from two studies suggest that each 100 mg increase in allopurinol leads to an additional decline in sUA approaching 1.0 mg/dL.[43,44] Implementing a "start low and go slow" approach and other key elements of proposed "best

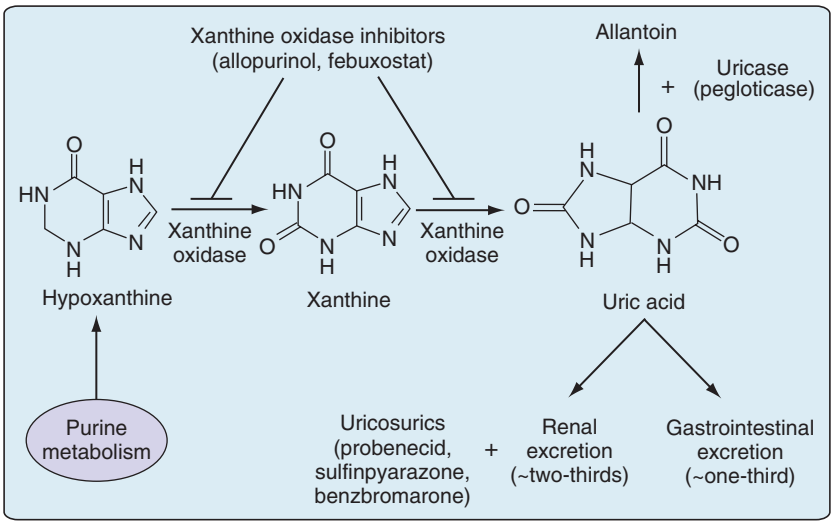



**Figure 66-1** Endogenous synthesis and elimination of uric acid (UA). UA is the end product of purine degradation in humans. Xanthine oxidase (XO), which converts hypoxanthine to xanthine and xanthine to UA, is a rate-limiting enzyme in this process, and is targeted by select urate-lowering therapies in gout treatment, including allopurinol and febuxostat. Although humans do not express a functional form of uricase, other mammalian species are able to catalyze the conversion of UA into allantoin, which is far more soluble. Recombinant forms of uricase, including pegloticase, have been developed for use in treatment failure gout. UA in humans is eliminated primarily in the kidneys. Renal excretion of UA is enhanced through uricosuric administration.

practices."[2,3] investigators recently reported a 92% success in achieving sUA treatment goals in gout.[45] Among those receiving allopurinol, the most commonly used ULT in the study, patients who achieved the target required a median daily dose of 400 mg.[45]

There is general consensus that *initial* allopurinol dosing should be adjusted for diminished renal function,[3,46,47] which prolongs the plasma half-life of oxypurinol. Commonly cited dosing algorithms suggest administering an initial daily dose of 100 mg or less for patients with a glomerular filtration rate (GFR) below 20 mL/min, with even lower doses for those with more severe renal impairment.[48] Whether existing dosing guidelines preclude the use of incremental dosing beyond these recommended renal thresholds has been the subject of debate. The standard recommended renal dosing guidelines[48] are not evidence based and are founded largely on a single retrospective case series showing that patients in whom allopurinol hypersensitivity syndrome (AHS) developed were more likely to have renal insufficiency. Indeed, of patients with chronic kidney disease (CKD) in whom AHS developed, many received "appropriately" dosed allopurinol.[49,50] In a review of 120 patients with gout receiving allopurinol, more than half (57%) required titration of daily doses higher than the "renal threshold" recommended by Hande,[48] a strategy that was well tolerated in the vast majority of patients.[51]

### Toxicity

AHS is an uncommon but potentially fatal treatment complication with approximately 90% of cases occurring within 60 days of allopurinol initiation.[52] AHS is characterized by the presence of an erythematous desquamating rash (similar to Stevens-Johnson syndrome), fever, eosinophilia, and end-organ damage, including hepatitis and renal failure.[48] AHS complicates as many as 0.1% to 0.4% of allopurinol treatment courses, although rates have been estimated to be as low as 1 in 56,000 allopurinol users.[53] In addition to its association with higher initial allopurinol doses,[54] AHS appears to be substantially more common in individuals positive for *HLA-B*5801*. In a small Taiwanese case-control study, all patients in whom AHS developed were positive for *HLA-B*5801*, compared with only 15% of allopurinol-treated patients without AHS.[55] This risk allele is present in approximately 2% to 7% of Caucasians, 7% of Africans, and 8% or more of Asian Indians.[56] With genetic assays commercially available, testing for *HLA-B*5801* has been proposed to be cost effective and even cost saving when implemented in high-risk populations,[57] such as Korean patients with CKD and all individuals of Han Chinese or Thai descent.[2] It has been estimated that the use of appropriately low starting doses, adjusted for renal function, may by itself reduce AHS risk by approximately 10-fold.[54] Given the potential severity of AHS, patients should be educated about the remote possibility of this adverse event and cautioned to discontinue allopurinol if a rash develops, particularly if accompanied by fever or mucocutaneous lesions.

Recognizing the rare occurrence of AHS, allopurinol is generally well tolerated. Estimates suggest that less than 5 to 10% of those exposed are intolerant to the drug.[58] Rebound flares are among the most common complication that accompanies allopurinol and other ULT, an issue that is most prominent in the early phases of drug initiation and can be mitigated by anti-inflammatory prophylaxis. Isolated maculopapular skin rash can occur outside the context of AHS and is estimated to complicate approximately 1% to 3% of allopurinol treatment courses. Other common adverse events associated with allopurinol use are summarized in Table 66-1. Liver function abnormalities can be seen in approximately 6% to 7% of allopurinol users,[24] although rates of severe liver injury appear to be exceedingly low. The role and recommended frequency for laboratory surveillance in toxicity monitoring has not been well defined.

### Fertility, Pregnancy, and Lactation

Recognizing that there have been no human studies of its use in pregnancy,[59] allopurinol is classified as a Pregnancy Category C agent (animal reproduction studies have shown an adverse effect on fetus without adequate human studies). Both allopurinol and oxypurinol are expressed in breast milk, and because drug effects in the developing infant are largely unknown, it should be administered to a nursing mother with caution.

### Drug Interactions and Contraindications

Allopurinol drug interactions have been well characterized. Azathioprine and 6-mercaptopurine (6-MP) are metabolized primarily by XO, hence co-administration of allopurinol leads to marked increases in circulating drug levels that can lead to bone marrow suppression.[60-62] Theophylline is also metabolized by XO, and therefore co-administration of this agent with allopurinol can lead to increased theophylline levels and potentiate toxicity. The co-administration of allopurinol with ampicillin/amoxicillin has been associated with a higher incidence of drug-related rash.[63] Thiazide diuretics may also reduce the renal excretion of allopurinol and oxypurinol with suggestions that this could potentiate drug-related toxicity, including the risk of AHS.[64] Uricosurics increase the renal excretion of oxypurinol, thus offsetting to some degree the urate-lowering effect of allopurinol.[65] Allopurinol co-administration may increase drug levels of cyclosporine and warfarin, mandating close monitoring of drug levels and bleeding parameters, respectively. Other allopurinol-associated drug interactions are summarized in Table 66-1. Although regimens for desensitization have been described, allopurinol should be avoided in patients with known allergies to allopurinol, including AHS.

## FEBUXOSTAT

**KEY POINTS**

**Febuxostat is a potent inhibitor of xanthine oxidase (XO), with a chemical structure that is distinct from that of allopurinol.**

**Febuxostat represents an important alternative to patients for whom allopurinol is not effective, either because of intolerance or lack of efficacy.**

Febuxostat is a potent and selective inhibitor of XO that is structurally distinct from allopurinol.

## Role in Rheumatic Diseases and Indications

Febuxostat is approved for the treatment of hyperuricemia in patients with gout. Similar to other ULTs, febuxostat is not indicated for the treatment of asymptomatic hyperuricemia. Given its unique structure, febuxostat represents an important alternative means of XO inhibition, particularly in patients with gout who are intolerant to allopurinol.[58] The role of febuxostat in gout management was previously addressed by an international panel, providing guidance specific to indications, contraindications, monitoring, and issues requiring future research.[66] Because of its urate-lowering effect, febuxostat is thought to exert potential benefits beyond the treatment of gout, with at least one study demonstrating improvements in vascular function[67] in patients with tophaceous gout. Another planned study will examine the agent's utility in preventing further reductions in renal function in patients with CKD.[68]

### Chemical Structure and Mechanism of Action

In contrast to allopurinol, febuxostat is a nonpurine analog that reduces serum and urinary urate concentrations through selective XO inhibition (see Figure 66-1). In contrast to allopurinol, which may inhibit other enzymes involved in purine and pyrimidine synthesis, febuxostat demonstrates significant enzymatic inhibition only for XO at therapeutic concentrations.[69]

### Pharmacology

After oral administration, febuxostat is rapidly absorbed from the GI tract with approximately 50% absorption, reaching peak plasma concentrations within a few hours[70] with near-complete plasma protein binding (see Table 66-1). Febuxostat displays linear pharmacokinetics that are not time dependent, with drug metabolism occurring primarily in the liver through conjugation via uridine diphosphate glucuronosyltransferase and oxidation via cytochrome P450 enzymes.[70] Peak urate-lowering effects with febuxostat generally occur during the first 5 to 7 days of treatment. Drug elimination occurs via both hepatic and renal pathways. Although active metabolites are produced via oxidation, these are present in much lower plasma concentrations.

### Dose and Drug Administration

Febuxostat is available in 40 mg tablets in the United States, in 80 mg tablets doses in the United States and Europe, and 120 mg tablets doses in Europe for oral administration. The usual dosing ranges from 40 mg to 120 mg daily (see Table 66-1). Febuxostat should be initiated at a lower dose (40 mg to 80 mg daily) and increased to higher doses (80 mg to 120 mg daily) if sUA levels remain higher than 6.0 mg/dL after 2 weeks. In a phase II study, sUA levels less than 6.0 mg/dL were obtained by 56%, 76%, and 94% of patients receiving 40 mg, 80 mg, and 120 mg/day of febuxostat, respectively, compared with 0% for placebo.[14] Mean sUA reductions were greater with higher daily doses, ranging from a 37% reduction in the 40 mg/day group to 59% in the 120 mg/day group. Two subsequent trials lasting 28 weeks ($n = 1067$)[17] and 52 weeks ($n = 762$)[15] compared febuxostat (80 mg to 240 mg/day) with fixed-dose allopurinol (300 mg daily) with both trials by using the primary outcome of a sUA less than 6.0 mg/dL at the last three consecutive monthly observations. The primary endpoint was achieved by 48% to 53%, 62% to 65%, and 69% of patients with gout receiving daily doses of 80 mg, 120 mg, and 240 mg, respectively, compared with 21% to 22% of patients receiving fixed-dose allopurinol. Secondary outcomes in the trial by Becker and co-workers.[15] showed declines in both gout flare rates and an approximately 70% to 80% reduction in gout tophus area during follow-up. These findings were not significantly different from those observed with allopurinol. Studies of febuxostat[15,17,24] to date have used fixed-dose allopurinol (≤300 mg/day) as an active comparator. As detailed previously, current gout treatment guidelines recommend initiating low-dose allopurinol (i.e., 100 mg/day) with incremental dosing as needed to achieve target urate thresholds.[3,46] Because optimal allopurinol dosing strategies were not used in these studies, these results almost certainly overestimate the incremental effectiveness of febuxostat relative to optimally dosed allopurinol.

Metabolized primarily in the liver, febuxostat may not require renal dosing.[71] This receives support from a few small short-term pharmacokinetic studies that included a limited number of patients with renal impairment.[72,73] There are insufficient data from longer-term studies of febuxostat in patients with moderate renal impairment (serum creatinine of ~1.6 to 2.0 mg/dL) and essentially no data from patients with more severe renal dysfunction (serum creatinine >2.0 mg/dL). Available data, albeit limited, suggest that those with mild or moderate renal impairment (estimated creatinine clearance [Cr CL] between 30 and 90 mL/min) experience similar efficacy and similar rates of toxicity compared with those with preserved renal function receiving the same doses of febuxostat.[15,24]

### Toxicity

Similar to other ULTs, rebound gout flares are the most common complication of febuxostat administration, underscoring the importance of anti-inflammatory prophylaxis.[14] Other adverse reactions observed with febuxostat are summarized in Table 66-1, adverse effects that appear to occur at similar rates to those observed with allopurinol.[24,70] In initial studies comparing febuxostat with allopurinol, there was a slightly higher rate of cardiovascular events in patients receiving febuxostat (0.74 events per 100 patient-years; 95% CI, 0.36 to 1.37) compared with those randomly assigned to allopurinol (0.60 events per 100 patient; 95% CI, 0.16-1.53).[70] In the large 6-month CONFIRMS trial, investigators found no differences in the rates of cardiovascular events between patients treated with febuxostat (40 mg and 80 mg/day) and allopurinol (200 mg to 300 mg/day), recognizing the limited power of this study with only six adjudicated events occurring during follow-up.[24] Large studies are currently underway that will compare the cardiovascular safety of febuxostat versus allopurinol during extended follow-up.[74,75]

Because of its unique structure and select XO inhibition, it has been suggested that febuxostat may represent a

rational alternative for patients with gout with a history of allopurinol hypersensitivity. Whether febuxostat can be effectively administered in such patients is not clear, given post-marketing reports of hypersensitivity in patients given febuxostat. In a small retrospective study involving 13 patients with gout with a history of severe allopurinol-related adverse reactions, 12 were subsequently treated safely with febuxostat (10 achieved target sUA goals).[76] A hypersensitivity vasculitis of the skin developed in a single patient.

### Fertility, Pregnancy, and Lactation

The effects of febuxostat on fertility are not known. Likewise, there have been no studies of febuxostat in pregnant women although results from animal studies have not suggested a significant risk of teratogenicity.[70] However, in the absence of appropriate human studies, febuxostat is labeled as Pregnancy Category C. It is not known whether the drug is excreted in human milk and febuxostat should be used only with caution in nursing women, because its effects in developing infants are unknown.

### Drug Interactions and Contraindications

Although formal drug-interaction studies have not been performed, febuxostat should be used with caution with drugs that are metabolized by XO (azathioprine, 6-MP, theophylline) (see Table 66-1). Its use should be avoided in patients with prior hypersensitivity to febuxostat. Because of hepatic metabolism, febuxostat should not be used in patients with moderate to severe liver impairment.[66]

## URICOSURICS

**KEY POINTS**

- **Probenecid, sulfinpyrazone, and benzbromarone are the most common uricosuric agents used in gout treatment worldwide.**
- **Although often requiring twice-per-day to three-times-per day dosing, uricosurics are potentially effective in lowering sUA in patients who underexcrete uric acid, the most common pathologic defect leading to hyperuricemia.**
- **Probenecid and sulfinpyrazone may have limited efficacy in the context of moderate to severe renal insufficiency.**

Uricosurics represent the first class of ULT to be used in gout treatment.[77] A complex system of renal handling of urate has been proposed that sequentially includes four components[78]: (1) near-complete glomerular filtration, (2) proximal reabsorption, (3) tubular urate secretion occurring more distally, and (4) reabsorption for a second time more distally. Available uricosurics diminish the postsecretory reabsorption of UA, therefore promoting its elimination and reducing sUA. Uricosurics address the most common physiologic defect in gout, UA underexcretion. In addition to promoting renal UA excretion, uricosurics may inhibit the tubular secretion of a number of other compounds, including penicillins. Although many agents display uricosuric properties, the most commonly used uricosurics in gout worldwide are probenecid, sulfinpyrazone, and benzbromarone. Probenecid is the most widely used uricosuric in gout treatment. Primarily because of concerns of treatment-related toxicity, sulfinpyrazone and benzbromarone are less widely available, and neither is available in the United States.

Although the focus of this section is on "primary" uricosurics, there are several medications approved for the treatment of nongouty conditions that exert uricosuric effects. "Secondary" uricosurics and their indications are summarized in Table 66-2.[79-84] Salicylates exert a paradoxical effect on renal urate elimination, with the inhibition of active secretion with lower doses (e.g., <1 g/day) and a "uricosuric-like" inhibition of UA reabsorption at higher doses (>4 to 5 g/day). Along with salicylates, losartan has perhaps been most closely scrutinized for its hypouricemic properties, an effect that is mediated via inhibition of URAT1 and appears to be specific to this angiotensin receptor blocker.[80] The magnitude of sUA lowering that can be achieved with these "secondary" hypouricemics is modest. Losartan used at an anti-hypertensive dose of 50 mg/day resulted in mean sUA declines of approaching 9%.[80] It is important to recognize that the urate-lowering effect of losartan appears to be negated when co-administered with hydrochlorothiazide,[85] a combination used in hypertension treatment.

### Role in Rheumatic Disease and Indications

Probenecid, sulfinpyrazone, and benzbromarone are used to treat hyperuricemia associated with gout and are indicated in patients with gout who underexcrete UA (24-hour urine UA, <700 mg). Probenecid is also approved as an adjuvant to penicillin therapy, increasing plasma concentrations and prolonging the terminal half-life of penicillin and other penicillin derivatives. Although potentially effective for a majority of patients with gout, uricosurics are much less commonly used than allopurinol and have been designated as second-line ULTs in gout management guidelines.[2] In a population-based study from the United Kingdom, uricosuric therapy accounted for less than 5% of all ULT prescriptions.[22]

### Mechanism of Action

Uricosurics work primarily through the inhibition of the renal tubular urate-anion exchanger, URAT1 (SLC22A12) in addition to GLUT9 (SLC2A9) (Figure 66-2). Thus uricosurics reduce UA reabsorption, promote renal elimination, and decrease sUA.[86-90]

### Pharmacology

Administered orally, uricosurics undergo GI absorption and are extensively protein bound in the serum. Half-lives of probenecid[91] and sulfinpyrazone[92] are relatively brief, ranging from approximately 3 to 12 hours (see Table 66-1). Although benzbromarone also has a relatively short half-life of approximately 3 hours, its active metabolite 6-hydroxybenzbromarone has a much longer half-life, allowing for effective once-daily administration.[93] Elimination of the uricosurics is primarily via hepatic metabolism,

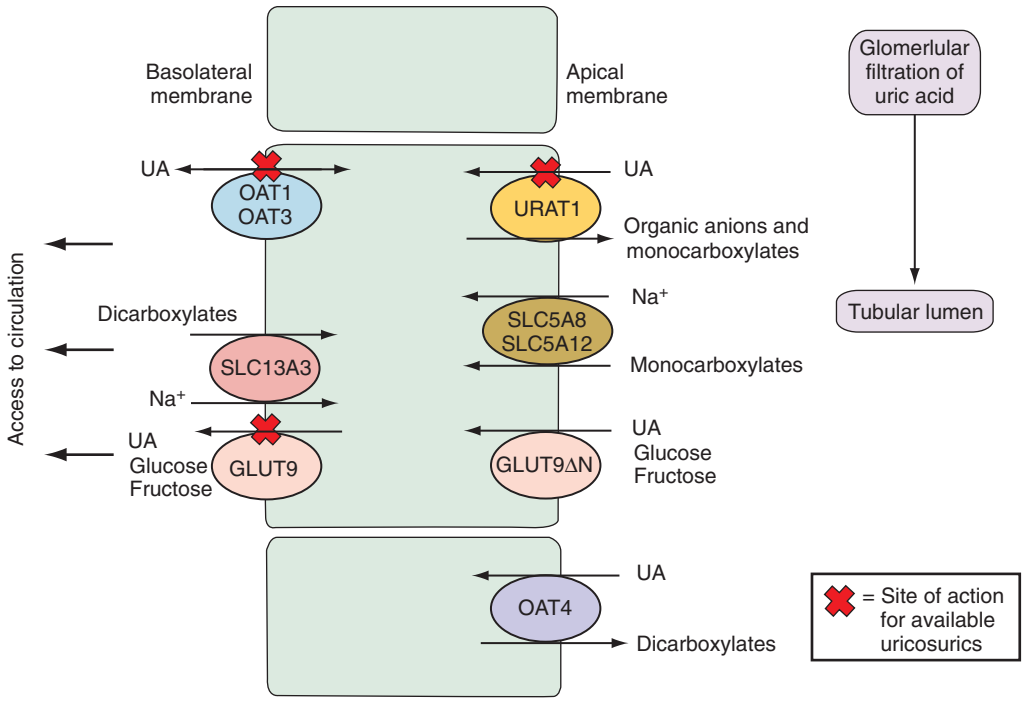



**Figure 66-2** Renal handling of uric acid (UA) and sites of action for available uricosurics, including probenecid, sulfinpyrazone, and benzbromarone in the proximal renal tubule. Serum UA undergoes near complete glomerular filtration. UA reabsorption is then mediated primarily by URAT1 and GLUT9 transporters in the proximal tubule, reabsorption that is inhibited by available uricosurics. Tubular reabsorption of UA requires sodium ($Na^+$)-dependent loading of monocarboxylates and dicarboxylates, which are subsequently exchanged with UA via URAT1 (monocarboxylates) and organic anion transporter (OAT)-4 (dicarboxylates). Monocarboxylates include lactate, pyruvate, acetoacetate, hydroxybutyrate, and acetate. Dicarboxylic acids include oxalic acid, malonic acid, and succinic acid, among others. In addition to facilitating UA exchange, GLUT9 mediates the reabsorption of glucose and fructose. RDEA 594 is an investigational uricosuric that appears to inhibit URAT1 specifically, whereas tranilast (also an investigational agent) inhibits both URAT1 and GLUT9 transporters *(Modified from Dalbeth N, Merriman T: Crystal ball gazing: new therapeutic targets for hyperuricaemia and gout.* Rheumatology [Oxford] *48:222-226, 2009, with permission.)*

followed by variable excretion of metabolites in the urine, bile, and/or feces. The metabolism of probenecid is limited to side-chain hydroxylation, *N*-depropylation, and glucuronic conjugation of its carboxyl group.[94-96] Both sulfinpyrazone and benzbromarone are metabolized via cytochrome P450 (CYP), with CYP2C9 representing a principal mediator.[97,98] Warfarin is also metabolized by primarily via CYP2C9, explaining its drug-drug interactions with both sulfinpyrazone and benzbromarone.

## Dose and Drug Administration

Usual probenecid dosing is 500 to 2000 mg daily in divided doses. Probenecid (500 mg) is also formulated with colchicine (0.5 mg) as a combination tablet (col-probenecid). In a 12-week randomized study involving patients with preserved renal function, probenecid, 1500 mg/day was associated with a 32% reduction in plasma urate concentration,[99] with older studies documenting other qualitative benefits of therapy, including softening of tophi, functional improvement, and improved pain symptoms.[29,100] Sulfinpyrazone is given in divided doses at an initial daily dose of 200 to 400 mg, increasing to 800 mg daily, if necessary to achieve target sUA goals. Although there is no level of renal function below which probenecid and sulfinpyrazone are known to completely lose efficacy,[53] these drugs may lose effectiveness in patients with moderate to severe renal insufficiency.[101] In a retrospective study of 57 patients with gout, probenecid yielded moderate efficacy as a ULT with baseline sUA, but not renal function per se, predicting treatment response.[102] In contrast to common dogma, these results suggest that probenecid can be used effectively in select patients with mild to moderate CKD.

Benzbromarone is administered once daily and may be effective in patients with moderate renal impairment. Benzbromarone treatment in usual doses (50 to 200 mg/day) leads to between 25% and 50% reductions in sUA[103-106] in addition to decreases in gout flare rates and tophi.[107-109] In a randomized controlled trial, benzbromarone maintained its hypouricemic effect even among patients with a Cr CL as low as 20 to 40 mL/min.[106] Among patients intolerant to or experiencing treatment failure with allopurinol 300 mg/day, 92% of those administered benzbromarone (200 mg/day) achieved a sUA less than 5.0 mg/dL compared with 65% of those receiving probenecid (2000 mg/day).[110]

Uricosurics may also serve as effective adjuvants to XO inhibition. Although uricosurics enhance oxypurinol excretion, the active metabolite of allopurinol, the effect on oxypurinol clearance[65] does not appear to negate the additive urate-lowering effect gained by using these medications in combination. Among patients failing allopurinol monotherapy (200 to 300 mg/day), 86% achieved a target sUA of less than 5 mg/dL after the addition of probenecid (1000 mg/day).[111] Likewise, the combination of

benzbromarone and allopurinol has been shown to have a more potent urate-lowering effect than allopurinol alone, and the combination has been shown to yield a more rapid resolution of tophi than allopurinol monotherapy.[109]

### Toxicity

As with other ULTs, rebound gout flares represent a common complication of uricosurics. Because urinary UA acts as a potential nidus for stone formation, uricosuric therapy is associated with an increased risk of nephrolithiasis. In a longitudinal study including more than 780 patient-years of benzbromarone exposure, nephrolithiasis developed in approximately 10% (21 of 216 patients).[112] Of the 21 patients with incident nephrolithiasis, oxalate stones developed in 7 patients, and stones composed of either UA or a combination of UA and calcium developed in 14 patients. In addition to limiting uricosurics to patients who underexcrete UA, the risk of treatment-related nephrolithiasis can be mitigated by optimizing fluid intake and alkalinizing the urine with a goal of maintaining a urine pH higher than 6.0.[113] Other adverse effects observed with uricosurics are summarized in Table 66-1. Rare adverse effects include anaphylaxis, anemia (including aplastic and hemolytic anemia), other cytopenias, fever, nephrotic syndrome, and back pain. Rare reports of severe liver injury led to the withdrawal of benzbromarone by its primary manufacturer in 2003, a decision recently challenged in systematic risk-benefit analysis of the drug.[53] Because of its NSAID-like properties, sulfinpyrazone has been associated with an increased frequency of blood dyscrasia (rare) in addition to upper GI disturbances including peptic ulcer disease.[114]

### Fertility, Pregnancy, and Lactation

There are limited data regarding the impact of uricosurics on fertility, fetal development, and use with nursing infants; thus these agents should be used with caution in such patients only when the potential benefits of treatment outweigh its potential risks.

### Drug Interactions and Contraindications

With the knowledge that tubular secretion plays a central role in the renal clearance of numerous drugs, drug-drug interactions with the use of uricosurics, particularly probenecid, are well-known (see Table 66-1). Inhibition of tubular drug secretion appears to be greater for probenecid and sulfinpyrazone than benzbromarone due to the increased URAT1 "specificity" of the latter agent. Drug interactions observed with uricosurics are summarized in Table 66-1.

As with all ULTs, the uricosurics should not be initiated during an acute gout flare and should not be used in patients with known allergies to these agents. Because of the potential for cross-reactivity, sulfinpyrazone should be avoided in patients with allergies to phenylbutazone or other pyrazole compounds. Except under special circumstances, these agents should be avoided in patients with nephrolithiasis, evidence of UA overexcretion, and during cancer treatment including chemotherapy and/or radiation. Caution should also be used in patients with known blood dyscrasias, active peptic ulcer disease, and/or significant hepatic or renal disease.

## URICASES

### Pegloticase

**KEY POINTS**

- **Pegloticase facilitates the conversion of UA into allantoin; the latter is far more soluble.**
- **The intravenous administration of pegloticase is associated with rapid and marked declines in sUA, which may be particularly important in the rapid depletion of total-body urate levels.**
- **Antigenicity and an immune response directed towards the drug are major limitations in the repeated dosing of pegloticase.**

Pegloticase, a modified mammalian uricase, is a biologic parenterally administered agent and represents the most recently approved ULT in gout. Unlike other mammalian species, humans have lost the ability to synthesize functional uricase that converts UA into allantoin, the latter 5 to 10 times more soluble. The use of alternative uricases (e.g., rasburicase, a recombinant uricase from *Aspergillus flavus*[115]) in the treatment of tumor lysis syndrome has been substantially limited by drug-related antigenicity and prohibitively high rates of anaphylaxis with repeat drug administration. In contrast to older generation uricases, pegloticase appears to be less allergenic and has been administered successfully to many patients as repeated intravenous infusions.

### Role in Rheumatic Disease and Indications

Pegloticase is approved for the treatment of hyperuricemia in patients with treatment refractory gout. Approved with an orphan drug status in the United States, pegloticase is indicated in a small subset of patients with gout. Treatment-refractory disease is characterized by severe disabling gout, often accompanied by marked tophaceous deposition and significant comorbidity, in which conventional ULT is either contraindicated or ineffective.[116] Pegloticase administration is associated with rapid and marked declines in sUA. In clinical trials, pegloticase use resulted in plasma concentrations as low as 0.5 to 1 mg/dL within 24 hours of an initial dose.[117] As a consequence, pegloticase administration has been associated with dramatic regression of tophi[118] and depletion of urate stores, raising speculation that pegloticase or other uricase formulations could play a role as an induction therapy in select patients with severe tophaceous gout and excessive total body urate stores (Figure 66-3).

### Chemical Structure and Mechanism of Action

Pegloticase is a recombinant mammalian uricase linked to polyethylene glycol (PEG). Pegloticase facilitates the conversion of UA into allantoin. The conversion of allantoin from UA generates hydrogen peroxide ($H_2O_2$) that is rapidly scavenged by erythrocytes.[119]

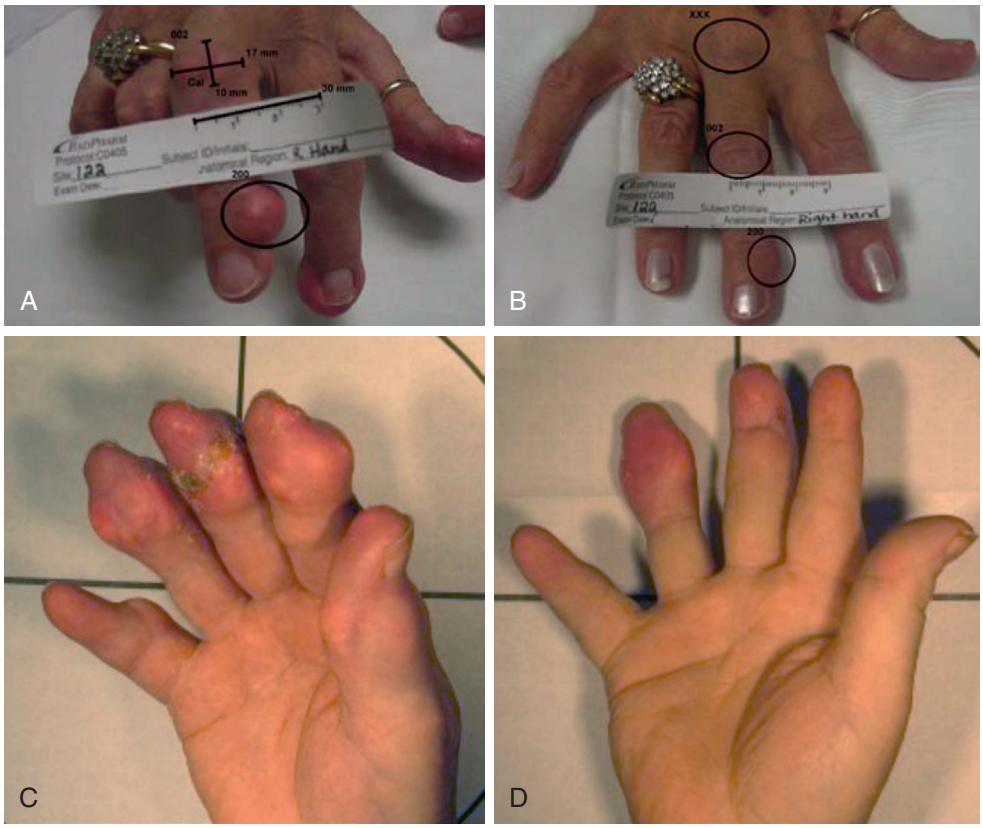



**Figure 66-3** **A**, Baseline tophus in patient 1 involving the medial aspect of the right third distal interphalangeal (DIP) joint. **B**, Same patient with resolution of tophus after 13 weeks of pegloticase therapy. **C**, Baseline tophi in patient 2, and (**D**) same patient after 25 weeks of pegloticase treatment *(From Baraf HS, et al: Tophus burden reduction with pegloticase: results from phase 3 ransomized trials and open-label extension in patients with chronic gout refractory to conventional therapy.* Arthritis Res Ther *15:R137, 2013, with permission.)*

## Pharmacology

The pharmacokinetics of pegloticase follows a one-compartment linear model. Maximum serum concentrations and the magnitude of urate-lowering effect after intravenous pegloticase administration increase in a dose-dependent fashion.[120] Pegloticase pharmacokinetics are not affected by age, sex, weight, or underlying renal function. A single dose of pegloticase (≥2 mg) has been shown to have a highly variable duration of effect, suppressing sUA to less than 7.0 mg/dL for periods ranging from 1 to 8 days.[120] The high degree of variability in drug elimination and treatment durability appears to be related at least in part to the presence and concentration of circulating anti-pegloticase antibody (see Dose and Drug Administration below).

## Dose and Drug Administration

Pegloticase is approved for intravenous infusion administered during a 2-hour period at a dose of 8 mg every 2 weeks (see Table 66-1). Regulatory approval of pegloticase is based on results from two 6-month replicate randomized placebo-controlled studies[117] in addition to open-label follow-up extensions ($n$ = 212). Patients in these clinical trials had pre-treatment sUA exceeding 8.0 mg/dL, had symptomatic gout, and had failed prior allopurinol therapy based on reported intolerance or ineffectiveness at a maximum medically appropriate dose. Patients received prophylaxis against infusion reactions (see Table 66-1) and against rebound gout flares (colchicine, NSAID, or glucocorticoid). The primary outcome for both randomized studies was a sUA of less than 6.0 mg/dL for 80% or more of the sampling period from month 3 to 6 of follow-up. This outcome was met by 47% and 38% of patients who received pegloticase, 8 mg by IV every 2 weeks, in the two randomized studies.[117] In pooled analyses, 40% of patients with baseline tophi who received pegloticase, 8 mg every 2 weeks, (vs. 7% of patients receiving placebo) had complete resolution of tophi by the time of the final study visit.

Although nonresponders generally experienced plasma urate reductions equally as dramatic as responders after their initial pegloticase infusion, nonresponders usually lost the treatment effect within the first 3 months of therapy. Efficacy loss, defined as sUA rising higher than 6.0 mg/dL during treatment, appears to be strongly associated with the formation of antipegloticase antibodies (primarily immunoglobulin (Ig) M and IgG subtypes that bind the PEG portion of the drug). Clinically meaningful manifestations of anti-pegloticase antibody formation, including an increased risk of anaphylaxis and neutralization of drug effect, appear to be most striking at antibody titers exceeding 1:2430. It is recommended that sUA, rather than drug antibody titers, be monitored closely during treatment with discontinuation of pegloticase if preinfusion sUA increases higher than 6.0 mg/dL.[121]

### Toxicity

The most common serious adverse event observed with pegloticase therapy is anaphylaxis, observed in approximately 7% of patients (see Table 66-1). Antipegloticase antibody can be detected in a vast majority of patients (~90%) receiving treatment, although clinically meaningful antibody titers are encountered less often. In clinical studies, anaphylaxis occurred despite prophylaxis that included antihistamine and glucocorticoid therapy with an onset of symptoms typically seen within 2 hours of drug administration. Treatment-related anaphylaxis is far more common among patients with treatment failure; thus close sUA surveillance and discontinuation of pegloticase for patients with sUA higher than 6.0 mg/dL is an essential element in risk mitigation. Post hoc analysis of the replicate studies suggests that nearly all cases of anaphylaxis would have been prevented with the use of this strategy. The concomitant use of other ULT should be avoided, which might otherwise mask antibody-mediated increases in sUA. Recognizing overlap with symptoms of anaphylaxis, infusion reactions occur in as many as 25% of patients receiving pegloticase with manifestations that include urticaria, dyspnea, chest discomfort, pain, or both, erythema, and pruritis. Infusion reactions can occur anytime in the course of therapy, with rare reports of delayed hypersensitivity reactions.

As with other ULTs, rebound gout flare is common after the administration of pegloticase. Other adverse events observed with pegloticase are summarized in Table 66-1. In the clinical studies and in open-label follow-up, exacerbations of congestive heart failure were also more common with pegloticase than placebo although a causal association with active drug therapy has not been established.[121]

### Fertility, Pregnancy, and Lactation

No studies have examined the impact of pegloticase on either fertility or pregnancy in humans, and it is unknown whether pegloticase is excreted in human milk. In the absence of appropriate human studies, pegloticase is classified as Pregnancy Category C and it is not recommended for use in nursing mothers.

### Drug Interactions and Contraindications

Recognizing that antipegloticase antibodies bind the PEG portion of the agent, pegloticase should be used with caution in patients receiving other PEG-containing therapies. Whether the formation of antipegloticase antibodies precludes or impacts future treatment with other PEGylated molecules is unknown. Pegloticase is contraindicated in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency because of an increased risk of hemolysis and methemoglobinemia. Patients with an increased risk should be screened for G6PD deficiency before initiation.[121]

## URATE-LOWERING DRUGS IN DEVELOPMENT

The treatment armamentarium in chronic gout will likely grow in the future, with several novel ULTs under development, including novel uricosurics and agents that inhibit endogenous UA synthesis.[122]

Ulodesine (BCX4208) represents a potentially novel mechanism by inhibiting endogenous UA synthesis via the inhibition of purine nucleoside phosphorylase (PNP). Along with adenosine deaminase, PNP plays a role in purine degradation, acting proximally to XO by metabolizing inosine into hypoxanthine. In a 3-week study of 60 patients with gout (all with baseline sUA >8.0 mg/dL), 31% to 36% of patients given ulodesine (40 mg, 80 mg, and 120 mg daily) achieved a final sUA of less than 6.0 mg/dL.[123] Decreases in sUA ranged from 2.7 to 3.4 mg/dL compared with a decline of just 0.4 mg/dL with placebo. Well tolerated in this short-term study, the potential immunosuppressive effects of ulodesine warrant further study, recognizing that inherited PNP deficiency results in severe combined immunodeficiency.

Lesinurad (RDEA594) is a metabolite of RDEA806, a non-nucleoside reverse transcriptase inhibitor, and was approved for use in gout by the U.S. Food and Drug Administration (FDA) in December, 2015. Lesinurad appears to specifically inhibit URAT1 and OAT4, lacking a significant effects on other organic anion transporters (see Figure 66-2).[124] More selective inhibition may limit drug interactions that complicate probenecid use. In contrast to probenecid,[65] the co-administration of lesinurad does not appear to increase the renal elimination of oxypurinol or febuxostat,[125] suggesting that this may be an ideal uricosuric to use in combination with first-line XO inhibitors. The addition of lesinurad to allopurinol (300 mg/day) led to dose-dependent declines in sUA.[126] After the addition of lesinurad (400 or 600 mg/day), the proportion of patients achieving sUA goal of less than 6.0 mg/dL with febuxostat alone (40 or 80 mg/day) increased from between 56% and 67% to 100%.[127]

In addition to displaying urate-lowering properties, tranilast has been studied for possible therapeutic effects in a number of conditions, including allergy, malignancy, and conditions characterized by excessive tissue fibrosis. Similar to currently available uricosurics, the urate-lowering effects of tranilast are mediated through the inhibition of URAT1 and GLUT9 transporters (see Figure 66-2).[128] In a preliminary study, tranilast administration (300 mg/day) was associated with a 14% decline in sUA with 28% of patients achieving a sUA less than 6.0 mg/dL.[129]

A peroxisome-proliferator–activated gamma modulator, arhalofenate (MBX-102) has been highlighted in recent studies as a novel agent demonstrating dual uricosuric and anti-inflammatory effects.[122] In addition to inhibiting renal UA transporters (URAT1, OAT4, OAT10), in vitro studies have shown that the agent effectively blocks monosodium urate–induced production of IL-1. Large-scale human studies of arhalofenate in the treatment of gout have yet to be undertaken.

Levotofisopam is a benzodiazepine derivative approved in countries outside the United States for the treatment of anxiety and autonomic instability and has recently been demonstrated to yield potentially important urate-lowering effects. In an initial proof-of-concept study involving 13 patients with gout, patients were given levotofisopam 50 mg twice daily, with a mean sUA reduction of 49% after 1 week of treatment.[122] The treatment was well tolerated, and all patients achieved a sUA of less than 6.0 mg/dL.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 67

# Analgesic Agents in Rheumatic Disease



Gregory R. Polston • Mark S. Wallace

**KEY POINTS**

- Numerous analgesic agents can be used to treat pain related to rheumatic disease.
- Acetaminophen, nonsteroidal anti-inflammatory agents, and opioids have intrinsic analgesic properties that are most efficacious for nociceptive and inflammatory pain.
- Anti-depressants, anti-convulsants, and muscle relaxants lack intrinsic analgesic properties but are effective in neuropathic and functional pain and can enhance the effects of other analgesics.
- The use of opioids for chronic pain remains controversial, but with proper patient selection and adherence to universal precautions, risks can be minimized and benefits maximized.
- Many drugs can increase and decrease the metabolism of opioids through interaction of CYP2DG and CYP3A4 systems. This can greatly vary the effects of opioid in different individuals.
- Increased reports of methadone overdose are thought to be the result of coadministration of drugs that inhibit the CYP3A4 system.
- Although the tricyclic anti-depressants are efficacious in a variety of pain syndromes, compliance is an issue because of unacceptable side effects and delayed onset.
- Newer serotonin and norepinephrine reuptake inhibitors (such as duloxetine) are better tolerated and have a faster onset than older tricyclic anti-depressants. Numerous anti-convulsants have been studied with conflicting results. Only gabapentin and pregabalin consistently demonstrate efficacy Muscle relaxants are intended for short-term use only.

Pain is the most common reason for patients to seek medical attention, yet undertreatment of acute and chronic pain persists despite decades of efforts to provide clinicians with information about analgesics.[1] A major consequence of physicians undertreating patients who initially are seen with pain as a result of rheumatic disease is the development of chronic pain. Chronic pain has been demonstrated to have deleterious effects on many aspects of a patient's daily life. These effects include deterioration in physical functioning, the development of psychologic distress and psychiatric disorders, and impairments in interpersonal functioning.[2] In addition to personal suffering, chronic pain imposes a burden on society in increased health care costs, disability, and lost workdays.

## THE PHYSIOLOGY OF PAIN PERCEPTION (THE PAIN EXPERIENCE)

The "pain experience" involves more than just the sensation of pain. Pain activates many areas of the brain that interact, resulting in the pain experience, which will differ among individual patients. The "pain experience" involves more than just the sensation of pain. Pain activates many areas of the brain that interact and result in the pain experience, which will differ among individual patients. The pain experience involves three components: biologic, psychological, and sociological. As described in this chapter, activation of various parts of the nervous system will contribute to each of these components. The three components of the pain experience include (1) sensory/discriminative (biologic), (2) affective/emotional (psychological), and (3) evaluative/cognitive (sociological).[3]

The sensory/discriminative component of pain provides information on the intensity, location, and quality of the pain. These pathways consist of peripheral receptor activation, axon depolarization, and ascending pathways to the cortex for processing.

The ascending pathways that carry impulses from the nociceptor to the sensory cortex also give off fibers to brain stem structures and deep brain structures. Activation of these structures in the brain stem and deep brain will stimulate emotional and sympathetic responses from the individual, leading to the emotional/affective component of pain.

Finally, ascending pain pathways also send projections to the forebrain structures, where the pain is processed on a cognitive and evaluative level. This process explains why patients respond differently to pain on the basis of culture, gender, and past experiences.

The main neurotransmitter in primary afferents is the excitatory amino acid glutamate. Activation of nociceptors causes the release of glutamate from pre-synaptic terminals in the spinal cord dorsal horn. This release acts on the ionotropic glutamate receptor amino-3-hydroxy-5-methylisoxazole-4-proprionic acid postsynaptically to cause rapid depolarization of dorsal horn neurons and, if threshold is reached, action potential discharge.[4]

## PAIN CLASSIFICATION

Pain can be mechanistically divided into types: nociceptive, inflammatory, functional, and neuropathic. Nociceptive pain is transient pain in response to a noxious stimulus that activates high threshold afferents. Nociceptive pain serves a protective function. Inflammatory pain is the spontaneous

hypersensitivity to pain that occurs in response to tissue damage and inflammation (e.g., post-operative pain, traumatic pain, arthritis pain). Functional pain is hypersensitivity to pain resulting from abnormal central processing of normal input (e.g., pathologic irritable bowel syndrome, fibromyalgia). Neuropathic pain is spontaneous pain and hypersensitivity to pain that occurs in association with damage to or lesions of the nervous system (e.g., peripheral neuropathy, postherpetic neuralgia). Nociceptive pain and inflammatory pain are prevalent in rheumatic disease. Functional pain and neuropathic pain are probably less prevalent in rheumatic disease; however, both should always be considered because poorly controlled pain can lead to nervous system dysfunction and functional and neuropathic pain.[5]

## PHARMACOLOGIC TREATMENT OF CHRONIC PAIN

The efficacy of analgesics is dependent on the pain mechanism. Primary analgesics are more efficacious in nociceptive and inflammatory pain, whereas adjuvant agents are more efficacious in neuropathic and functional pain. Each pain classification involves different pain mechanisms, and within each classification are multiple different pain mechanisms.[6]

Pain is mediated through both peripheral and central mechanisms, and often more than one mechanism of pain is active in a given patient. Thus the use of two or more agents with differing mechanisms increases the likelihood of interrupting pain signals and relieving pain.

Pharmacologic agents for the management of chronic pain are divided into primary analgesics, which have intrinsic analgesic properties, and adjunct analgesics, which may have primary analgesic properties in neuropathic pain but usually enhance the analgesic effects of primary analgesics when used in non-neuropathic pain syndromes. Table 67-1 summarizes these agents. Nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX)-2 inhibitors will be discussed elsewhere (see Chapter 59).

## OPIOIDS

Numerous studies have demonstrated the efficacy of opioids in a variety of chronic pain states, including neuropathic and non-neuropathic. However, studies to document long-term efficacy have not been conducted. Long-term opioid therapy to treat chronic pain remains controversial. During the last century, the pendulum has swung back and forth with regard to the use of the opioids to treat pain. In the mid-20th century, opioids were limited because of fears of addiction and diversion. In the late 20th century, the pendulum swung to the other side, and physicians used opioids liberally to treat chronic pain. With the turn of the 21st century, the pendulum is moving back toward the middle with recognition of the importance of opioids in chronic pain management but an understanding of the need to balance these benefits with risks. Recently published guidelines acknowledge that opioid analgesics have an important role in pain management and that underuse of these agents may contribute to suboptimal pain management.[7] However, these guidelines also acknowledge that abuse of prescription opioids has become an epidemic, with dramatic increases seen in the United States. Therefore a set of universal precautions has been developed as a guide to help physicians who prescribe opioids[8] (Table 67-2).

Evaluating patients with chronic noncancer pain can be problematic because the perception of pain is not only dependent on physiologic factors but also the patient's psychological and social background. Thus developing a pain treatment plan that includes opioids can be challenging. Historically, the use of opioids was reserved for short-term treatment or for end-of-life care. In the late 1980s, however, this practice changed. Chronic pain became recognized as a disease, and experts began recommending more liberal prescription practices. Unfortunately, while the use of opioid prescriptions increased, so too did opioid overdoses and prescription drug substance abuse disorders. The increase in abuse and overdoses has become so rampant that we now are in the midst of an opioid epidemic that presents dangers not only for patients but for society as a whole. To combat this problem, a number of strategies have been proposed to identify patients who are more likely to benefit from opioid therapy, thus improving patient selection and outcome. These include specific guidelines to always consider nonopioid therapies first, as well as guidelines for upper-dose daily limits for opioid analgesics.[9] Additional recommendations include taking a full social history at the

**TABLE 67-1** Analgesic Options to Treat Chronic Pain

| |
|---|
| Primary analgesics |
| Acetaminophen |
| Nonsteroidal anti-inflammatory drugs/cyclooxygenase-2 inhibitors |
| Opioids |
| Adjunct analgesics |
| Tricyclic anti-depressants |
| Serotonin-norepinephrine reuptake inhibitors |
| Anti-convulsants |
| Muscle relaxants |
| Topical agents |

**TABLE 67-2** Universal Precautions for Long-Term Opioid Use

| |
|---|
| Diagnosis with appropriate differential |
| Psychological assessment of patient, including risk of addictive disorders |
| Informed consent |
| Treatment agreement |
| Preintervention and postintervention assessment of pain level and function |
| Appropriate trial of opioid therapy with or without adjunctive medication |
| Reassessment of pain score and level of function |
| Regular assessment of the "4 A's" (analgesia, activities of daily living, adverse effects, and aberrant drug-taking behaviors) |
| Periodic review of pain diagnosis and comorbid conditions, including addictive disease |
| Documentation |

From Gourlay DL, Heit HA, Almahrezi A: Universal precautions in pain medicine: a rational approach to the treatment of chronic pain. *Pain Med* 6:107-112, 2005.

**TABLE 67-3** Risk Factors for Poor Patient Outcomes with Chronic Opioid Therapy

| |
|---|
| **High** |
| Patient intake of >120 mg morphine equivalent/day or methadone |
| High abuse risk (current abuse, young patient age, significant psychiatric history, prior aberrant behaviors, initial screening score) |
| Lack of clear reason for pain |
| Comorbidity (renal, hepatic, respiratory, sleep apnea) |
| Use of other sedatives, stimulants, anxiolytics, or sleeping medication |
| Multiple medications to combat side effects |
| Younger patient age |
| Lack of functional improvement |
| Rapid dose escalation after starting medication |
| Poorly treated mental health issues |
| Others in home with high risk for diversion or unstable living conditions |
| **Moderate** |
| Patient intake of 40 to 120 mg morphine equivalent/day |
| Prior patient substance abuse diagnosis in remission for greater than 6 months |
| Moderate risk on initial risk questionnaire |
| Prescriber "uneasy" about providing opioids for the patient |
| **Low** |
| Patient intake <40 mg morphine equivalent/day |
| Compliant with medication and attempting nonopioid therapies |
| Clear indication for pain |
| Low risk on initial risk questionnaire |
| Clear functional improvement |

start of therapy. This history should include alcohol use, illegal drug use, family history of substance abuse, and whether others in the patient's home are at risk for misuse. Using validated patient questionnaires to predict success with chronic opioid therapy such as the Opioid Risk Tool,[10] Diagnosis, Intractability, Risk, Efficacy (DIRE),[11] or the Screener and Opioid Assessment for Patients with Pain[12] is also recommended. Along with a complete physical examination, the patient can be stratified into risk groups (low, medium, high) (Table 67-3), which will offer insight as to how best to monitor individual patients. Other efforts include making every patient sign an opioid agreement, consent to random drug testing, and demonstrate clear functional improvement to continue this therapy. State prescription and monitoring programs have been created to help document compliance and identify "doctor shopping." State and national regulations, including Risk Evaluation and Mitigation Strategies (REMS)[13] for all long-acting and extended-release opioids have also been implemented. The REMS program requires specific education for providers, patients, and caregivers regarding the use of these drugs, along with documentation of any benefit and review of misuse, abuse, or addiction. Another promising method is the development of new formulations of long-acting opioid analgesics with abuse-deterrent and tamper-resistant formulations.

Along with the recommendations above, the physician should first prescribe a short-acting weak opioid such as hydrocodone or codeine. If the patient requires more than three or four short-acting weak opioids per day, consider converting to a long-acting opioid if the pain is continuous. It is generally believed that controlled-release or long-acting opioids provide convenience to patients by reducing the number of daily doses required and also provide a pharmacokinetic profile that results in reduced serum level peaks and troughs. These characteristics result in an improvement in the consistency of effective analgesia and a potential reduction in opioid-related side effects that are often correlated with high peak serum levels. Physicians should prescribe long-acting opioids only for around-the-clock pain and not for intermittent or breakthrough pain. No clinical evidence exists that long-acting opioids decrease the risk of misuse or addiction, and there is concern that the total daily dose of opioids increases simply because of higher-dose increments. When a patient switches to a long-acting opioid, access to a short-acting opioid for breakthrough pain can be continued but should be monitored and controlled. Evaluation by a pain specialist may be considered when morphine equianalgesic dosages exceed 90 mg/day. The benefits of levels higher than 180 mg/day have not been established, and recent evidence suggests a significant increase in morbidity and mortality when doses exceed 100 mg/day.[14]

## Opiate Receptor Classes

Early work by Martin and associates led to the postulation of three opiate receptors: mu, kappa, and sigma.[15] Later studies by Kosterlitz and colleagues led to the identification of the delta opioid receptor.[16] With the exception of the sigma receptor, all of the opiate receptors are responsible for opioid-induced analgesia. Opioid receptors are mediated through a G protein, leading to a cascade of events that typically inhibit neuron activation.[17] Persistent activation of G protein–coupled receptors typically results in progressive loss of effect, known as *tolerance* (discussed later).

## Opiate Receptor Distribution and Mechanisms of Opioid-Induced Analgesia

The main sites of action of the opiates are believed to be located in the brain and spinal cord; however, under some circumstances, peripheral mechanisms are involved. Responses of an individual patient may vary dramatically with different mu-opioid receptor (MOR) agonists. If problems are encountered with one drug, the prescribing physician should try another. Mechanisms underlying variations in individual responses to morphine-like agonists are poorly understood; however, they are thought to be the result of MOR polymorphisms.

Numerous sites in the brain have MORs. Activation of receptors located in the mesencephalic periaqueductal gray[18] appears to be the most important cause of opioid-induced analgesia. MOR agonists block release of the inhibitory transmitter γ-aminobutyric acid (GABA)[19] from tonically active periaqueductal gray (PAG) systems that regulate activity in projections from the PAG to the medulla. This results in an increase in PAG outflow to the medulla, leading to activation of medullospinal projections and release of noradrenaline or serotonin at the level of the spinal dorsal horn. This release can attenuate dorsal horn excitability and analgesia. PAG activation can also increase the excitability of the dorsal raphe and the locus coeruleus, from which ascending serotonergic and noradrenergic projections originate to project to the limbic forebrain. This

process leads to the euphoric effects sometimes experienced with systemic MOR agonists.[20]

In the spinal cord, MOR agonists are limited for the most part to the substantia gelatinosa of the superficial dorsal horn, the region in which small, high-threshold sensory afferents terminate. Most of these opiate receptors are located pre-synaptically and postsynaptically on small peptidergic primary afferent C fibers. Pre-synaptic activation of the MOR prevents the opening of voltage-sensitive calcium ion channels, thereby preventing transmitter release. Postsynaptic activation of the MOR increases potassium conductance, resulting in hyperpolarization and reduced excitation induced by the pre-synaptic release of glutamate.[21] The ability of spinal opiates to reduce the release of excitatory neurotransmitters from C fibers pre-synaptically and to decrease the excitability of dorsal horn neurons postsynaptically is believed to account for powerful and selective effects upon spinal nociceptive processing. In humans, an extensive literature indicates that a variety of opiates delivered spinally (intrathecally or epidurally) can induce a powerful analgesia.[21]

Systemic delivery of the opioids will reduce nociceptive pain through a central mechanism located in the brain and spinal cord, as described previously, whereas the peripheral application has no effect. However, under conditions of inflammation, which result in an exaggerated pain response (hyperalgesia), the peripheral application of the opioids will reduce the hyperalgesia. This action is believed to be mediated by opiate receptors on the peripheral terminals of small primary afferents that become active under inflammatory conditions. Whether the effects are uniquely on the afferent terminal or on inflammatory cells that release products that sensitize the nerve terminal is not known.[22]

## Tolerance

With time, a given dose of an opioid shows less effect, and an increased dose is required to produce the same physiologic response. Tolerance to different effects of opioids occurs at different rates. For example, tolerance to sedation and nausea occurs earlier than analgesic tolerance. Some effects, such as constipation, never show tolerance.

The mechanism of opioid tolerance is controversial. Apparent opioid tolerance may be an indication of disease progression with a resultant increase in pain intensity; this is the first event that should be ruled out before true tolerance is assumed. Many cellular mechanisms can lead to tolerance. First, long-term opioid exposure can lead to receptor internalization, dephosphorylation, and desensitization. Second, exposure to high doses of opioids can lead to an increase in intra-cellular cyclic adenosine monophosphate (cAMP), activation of bulbospinal pathways, and glutamate receptor phosphorylation, producing an excitatory state (opioid-induced hyperalgesia).[23] An incomplete cross-tolerance occurs between the various opioids, and when a patient develops tolerance to one opioid, switching to another opioid can result in an increased effect.

## Physical Dependence

Physical dependence is not addiction (and the terms should not be used interchangeably). Physical dependence is a pharmacologic effect characteristic of a number of different types of medications. Physical dependence is defined as the occurrence of an abstinence syndrome (withdrawal reaction) following abrupt discontinuation of the drug, substantial dose reduction, or administration of an antagonist. Physical dependence is generally assumed to occur with regular opioid use for as brief a period as a few days.

Opioid withdrawal is manifested by significant somatomotor and autonomic outflow (reflected by agitation, hyperalgesia, hyperthermia, hypertension, diarrhea, pupillary dilation, and release of virtually all pituitary and adrenomedullary hormones) and by affective symptoms (dysphoria, anxiety, and depression).[24] Opioid withdrawal can be minimized by slowly tapering the opioid. These phenomena are considered to be highly aversive and motivate the drug recipient to make robust efforts to avoid the withdrawal state. Initially, drug addicts are driven to repeated doses of opioids because of the euphoric effects. However, with time, the euphoric effects are lessened, and addicts are driven to continue use to avoid withdrawal.

## Addiction

Identification of the disease of addiction is important for safe and effective clinical management of pain in individuals with addictive disorders. The disease of addiction affects approximately 10% of the general population, and its prevalence may be higher in subpopulations of patients with pain. Active addiction is a contraindication to the use of the opioids; a past history is a relative contraindication, but opioids can be used successfully with appropriate monitoring. A persistent misunderstanding is prevalent among health care providers, regulators, and the general population regarding the nature and manifestations of addiction, which may result in undertreatment of pain and stigmatization of patients who use opioids for pain control.

Evaluation for addiction in a patient who is prescribed long-term opioids for pain control is often problematic. This risk of opioid addiction and misuse can be assessed with validated questionnaires (Table 67-4).[10] Although the concept of addiction may include the symptoms of physical dependence and tolerance, physical dependence or tolerance alone does not equate with addiction. In the chronic pain patient who is taking long-term opioids, physical dependence and tolerance should be expected, but the maladaptive behavior changes associated with addiction are not expected.[25]

Addiction is a behavioral pattern characterized by compulsive use of a drug and overwhelming involvement with its procurement and use in spite of potential harm.[24,26] For patients with continuous pain, inadequate pain management (e.g., as-needed dosing schedule, use of drugs with inadequate potency, use of dosing intervals that are too long) can lead to behavioral symptoms that mimic those seen with psychological dependence and can be mistaken for addiction termed *pseudoaddiction*. In the case of pseudoaddiction, problem behaviors resolve after sufficient pain relief is established. However, behaviors related to true addiction may also resolve after dose escalation. Thus it can be difficult to distinguish pseudoaddiction from true addiction; careful evaluation and management may be required until the circumstances are sorted out (see Table 67-4).[27]

**TABLE 67-4** Opioid Risk Assessment Tool

| | Male | Female |
|---|---|---|
| Family history (parents and siblings): | | |
| Alcohol abuse | _____(3) | _____(2) |
| Illegal drug use | _____(3) | _____(2) |
| Prescription drug abuse | _____(4) | _____(4) |
| Personal history: | | |
| Alcohol abuse | _____(3) | _____(3) |
| Illegal drug use | _____(4) | _____(4) |
| Prescription drug abuse | _____(2) | _____(2) |
| Mental health: | | |
| Diagnosis of ADD, OCD, bipolar, schizophrenia | _____(2) | _____(2) |
| Diagnosis of depression | _____(2) | _____(2) |
| Other: | | |
| Age between 16 and 45 years | _____(2) | _____(2) |
| History of preadolescent sexual abuse | _____(2) | _____(3) |
| Total | _____ | _____ |

Scoring:
0-3 Low risk: 6% chance of developing problematic behaviors
4-7 Moderate risk: 28% chance of developing problematic behaviors
≥ 8 High risk: >90% chance of developing problematic behaviors

ADD, Attention deficit disorder; OCD, obsessive-compulsive disorder.
Modified from Webster LR, Webster RM: Predicting aberrant behaviors in opioid-treated patients: preliminary validation of the Opioid Risk Tool. *Pain Med* 6:432–442, 2005.

**TABLE 67-5** Opioid Drug Interactions

| |
|---|
| Tramadol, oxycodone, hydrocodone, and codeine are converted to active metabolites by CYP2D6<br>Drugs that inhibit this enzyme will decrease opioid effects: fluoxetine, paroxetine, quinidine, duloxetine, terbinafine, amiodarone, sertraline, and others. |
| Methadone and fentanyl are converted by CYP3A4<br>Drugs that inhibit this enzyme will increase opioid effects: several anti-retrovirals, clarithromycin, itraconazole, ketoconazole, nefazodone, telithromycin, aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil, cimetidine, and others |
| Morphine, hydromorphone, and oxymorphone are not significantly metabolized by CYP450 isoenzymes |

From Daniell HW: Inhibition of opioid analgesia by selective serotonin reuptake inhibitors. *J Clin Oncol* 20:2409, 2002; U.S. Food and Drug Administration: www.fda.gov/cder/drug/drugInteractions.

## Opioid Pharmacology

### Morphine

Morphine is the gold standard against which all other opioids are measured. Morphine can be administered by oral, rectal, subcutaneous, intravenous, intramuscular, and intraspinal routes. Oral bioavailability of morphine is approximately 25% (range, 10% to 40%). Peak plasma concentrations occur 0.5 to 1 hour after ingestion, with a half-life of approximately 3 to 4 hours. To increase the dosing interval of oral morphine, several sustained- and controlled-release preparations have been developed (Table 67-5). The average protein binding of morphine is approximately 35%, but this decreases with renal and hepatic dysfunction. Because of the hydrophilicity of morphine, poor central nervous system (CNS) penetration occurs and little tissue accumulation with repeated dosing. Morphine is metabolized primarily in the liver to two main metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). M3G is the primary metabolite with potent CNS excitatory properties. Because of its polarity, CNS penetration is poor; however, in renal failure, M3G plasma concentrations can be high enough to drive this metabolite into the CNS, leading to excitation. M6G possesses potent MOR agonism, but because of its high polarity, CNS penetration is poor. However, similar to M3G, M6G can accumulate in renal impairment, leading to exaggerated opioid effects. Only approximately 10% of unmetabolized morphine is excreted renally, whereas 90% is excreted renally as morphine glucuronide (70% to 80%) and normorphine (5% to 10%) conjugates.[28]

### Methadone

Methadone is a long-acting MOR agonist with potency similar to that of morphine but with two important differences[19]: (1) It has a long half-life, and (2) it has high oral bioavailability. Routes of administration are oral and intravenous, with oral delivery by far the most common. Intravenous routes have been used as a single loading dose for post-operative pain control. Because of its low hepatic clearance and therefore low first-pass effect, oral bioavailability is approximately 80%. Methadone has higher lipid solubility than morphine, which results in faster CNS penetration and onset of action; however, its lipid solubility leads to a higher volume of distribution and a shorter duration of action with initial dosing. Low hepatic clearance leads to accumulation, a longer duration of action, and possible overdose with repeated dosing.

Because of the long half-life of methadone (average, 15 to 30 hours, with published reports ranging from 8 to 59 hours) with drug accumulation during several days, methadone needs to be administered with caution with long intervals between dose adjustments (5 to 7 days). Deaths have been reported with the use of methadone for chronic pain, and in November 2006, the U.S. Food and Drug Administration (FDA) issued a public health advisory for methadone with a black box warning. Although details are often unclear, many of these deaths may be the result of conversion of patients from other opioids to methadone. Methadone has been associated with QRS prolongation, which can lead to sudden cardiac death. If a physician is unfamiliar with the use of methadone, he or she should seek consultation with a pain specialist, especially when considering prescribing methadone at greater than a low dose (20 to 30 mg/day).[29]

The L-isomer of methadone possesses opioid activity, whereas the D-isomer is weak or inactive as an opioid. Evidence suggests that D-methadone is anti-nociceptive as a result of its *N*-methyl-D-aspartate (NMDA) receptor antagonist activity, making methadone attractive in treating neuropathic pain.[30]

Methadone is metabolized primarily by CYP3A4, secondarily by CYP2D6, and, to a smaller extent, by CYP1A2 and additional enzymes that are under study. CYP3A4, the most abundant metabolic enzyme in the body, can vary

30-fold among individuals in terms of its presence and activity in the liver. In addition, drugs that inhibit this enzyme (several anti-retrovirals, clarithromycin, itraconazole, ketoconazole, nefazodone, telithromycin, aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil, cimetidine, and others) will increase the effect of methadone, possibly leading to overdose. This enzyme is also found in the gastrointestinal tract, so methadone metabolism actually starts before the drug enters the circulatory system. The amount of this enzyme in the intestine can vary as much as 11-fold, partially accounting for the variable breakdown of methadone.[31]

Because the half-life of methadone is so long, it has been used extensively to treat opioid dependence and withdrawal. The use of methadone to treat addiction requires a separate clinic and physician license. However, methadone can be used to treat pain under the routine medical Drug Enforcement Administration[32] license.

### Fentanyl

Fentanyl is a potent opioid with very high lipid solubility. This high lipid solubility leads to fast onset, a short duration of action, high protein binding (80%), and a high volume of distribution. The high volume of distribution accounts for the short duration of action because of the high concentration gradient from the plasma to fat and muscle. However, with repeated dosing, the duration of action increases while fat and muscle stores become saturated with fentanyl. Although the analgesic half-life is approximately 1 to 2 hours, the terminal half-life is approximately 3 to 4 hours.[33]

Routes of delivery of fentanyl include transdermal, transmucosal, intravenous, and intraspinal. Subcutaneous delivery is also used in terminal cancer patients. High potency and lipid solubility make fentanyl ideal for transdermal and transmucosal delivery. Two transdermal fentanyl patches are available on the market: reservoir and matrix. The reservoir patch can be accessed and the fentanyl extracted, whereas the matrix patch is tamperproof. Each system delivers fentanyl during a period of 72 hours; however, some patients will deplete the patch within 48 hours, requiring more frequent patch changes. This variability can result from skin perspiration, fat stores, skin temperature, and muscle bulk. After application, the skin serves as a depot, and systemic levels rise for the next 12 to 24 hours, then remain stable until 72 hours. Peak concentration occurs somewhere between 27 and 36 hours (25 microgram patch, 27 hours; 100 microgram patch, 36 hours). A steady state is reached after several applications. After removal of the system, plasma concentrations fall by 50% during 17 hours.[34]

Three transmucosal products are currently available. The fentanyl oralet contains fentanyl in a base of food starch, confectioners' sugar (2 g vs. the 30 g in a Snickers bar), edible glue, citric acid, and artificial berry flavor. The oralet is placed between the patient's gum and cheek and is allowed to dissolve during a period of 15 minutes, with bioavailability of approximately 50%. Onset is fast, with a peak effect at approximately 35 minutes. The fentanyl buccal tablets use an oravescent delivery system that generates a reaction that releases carbon dioxide when the tablet comes in contact with saliva. Transient pH changes accompanying the reaction optimize the dissolution (at a lower pH) of fentanyl through the buccal mucosa. Onset is fast, with a peak effect at approximately 25 minutes. The BioErodible MucoAdhesive (BEMA; BioDelivery Sciences International, Raleigh, N.C.) fentanyl delivery system is composed of water-soluble polymeric films. This system consists of a bioadhesive layer bonded onto an inactive layer. The active ingredient, fentanyl citrate, is incorporated into the bioadhesive layer, which adheres to the moist buccal mucosa. The amount of fentanyl delivered transmucosally is proportional to the film surface area. It is believed that the inactive layer isolates the bioadhesive layer from the saliva, which may optimize delivery of fentanyl across the buccal mucosa, resulting in higher bioavailability (71%). Onset is fast, with a slightly longer peak effect (60 minutes) compared with the other transmucosal systems; however, there may be a longer duration. Chewing and swallowing any transmucosal fentanyl product results in lower bioavailability and peak effect because swallowed fentanyl is poorly absorbed from the gastrointestinal tract.[35]

Fentanyl is metabolized primarily by CYP3A4 to the inactive metabolite norfentanyl; therefore drugs that inhibit this enzyme will increase the drug effect (see prior discussion of methadone). Only opioid-tolerant patients (patients taking >60 mg/day of oral morphine equivalent) should be started on fentanyl products because of risks of severe respiratory depression.

### Oxycodone and Oxymorphone

Oxycodone is administered by the oral or rectal route. Oral bioavailability is 60% and protein binding, 45%. Onset and duration of action are similar to morphine. Oxycodone is metabolized by the CYP3A4 enzyme primarily to noroxycodone, which has approximately 25% potency of the parent compound but also has neuroexcitatory effects. Minor metabolites include oxymorphone, which is more potent than oxycodone and has a longer half-life, and noroxymorphone, which has no analgesic properties.[36]

Oxycodone is available as an immediate-release tablet or solution and as a controlled-release preparation. The controlled-release preparation has an immediate release effect of as much as 40% of the contents, followed by a sustained 12-hour release component. The immediate release effect results in rapid onset, followed by a sustained effect. This can be a disadvantage because the patient may perceive the duration as short when coming down from the high peak plasma concentration caused by the immediate release.

Oxymorphone is a metabolite of oxycodone and can be delivered via the oral and rectal routes. Both the bioavailability and protein binding are low (10%), but the terminal half-life is long (10 to 12 hours). Most of the oxymorphone is metabolized to oxymorphone-3-glucuronide, which is an inactive metabolite. Oxymorphone is available in immediate-release and sustained-release preparations.[37]

### Hydromorphone

Hydromorphone is administered by the oral, intravenous, intramuscular, and subcutaneous routes. Oral bioavailability

is low (ranging from 25% to 50%), and protein binding is less than 20%. The kinetics are very similar to morphine. Hydromorphone has an extensive first-pass effect, and 95% is metabolized to the inactive hydromorphone-3-glucuronide. A 24-hour-release preparation of hydromorphone has been approved by the FDA. This preparation uses an osmotic piston-driven system in pill form that slowly delivers hydromorphone during 24 hours.[38]

### Meperidine

Meperidine can be delivered by the oral, rectal, intravenous, intramuscular, subcutaneous, and intraspinal routes. Meperidine use has decreased with time because of side effects and risks associated with the parent compound and metabolites (see later). Oral bioavailability is approximately 50%, protein binding is approximately 60%, and peak concentrations in plasma usually are observed in 1 to 2 hours. Onset and duration are similar to morphine. Large doses of meperidine repeated at short intervals may produce an excitatory syndrome that includes hallucinations, tremors, muscle twitches, dilated pupils, hyperactive reflexes, and convulsions. These excitatory symptoms are caused by accumulation of the metabolite, normeperidine, which has a half-life of 15 to 20 hours compared with 3 hours for meperidine. Because normeperidine is eliminated by the kidney and the liver, decreased renal or hepatic function increases the likelihood of such toxicity. As a result of these properties, meperidine is not recommended for the treatment of chronic pain because of concerns over metabolite toxicity. It should not be used for longer than 48 hours or in doses greater than 600 mg/day.[39]

Severe reactions may follow the administration of meperidine to patients being treated with monoamine oxidase (MAO)[40] inhibitors. Two basic types of interactions can be observed. The most prominent is an excitatory reaction ("serotonin syndrome"). This reaction may be the result of the ability of meperidine to block neuronal reuptake of serotonin and resulting serotonergic overactivity. Another type of interaction, a potentiation of opioid effect caused by inhibition of hepatic CYPs, also can be observed in patients taking MAO inhibitors, necessitating a reduction in the doses of opioids.

### Hydrocodone

Hydrocodone is a weak MOR agonist. It is delivered only by the oral route and is mostly prescribed in acetaminophen-containing compounds. It has just been reclassified as a Schedule II opioid because of high abuse rates, numerous overdoses, and unintentional acetaminophen toxicity in the United States. A long-acting hydrocodone that does not contain acetaminophen was approved by the FDA in 2013. Its approval was controversial, and how it should be used in clinical practice is not known. Hydrocodone has an oral bioavailability of approximately 25%, with low protein binding in the rapid-release form. It is metabolized to hydromorphone, but its analgesic activity is not dependent upon metabolism to hydromorphone. However, rapid metabolizers may experience a faster onset of action because of the production of hydromorphone. Onset and duration of effect are similar to morphine.

### Codeine

Codeine has an exceptionally low affinity for the MOR, and the analgesic effect of codeine is caused by its conversion to morphine. The oral bioavailability is approximately 60% and is poorly protein bound. The onset and duration of effect are similar to morphine. It is administered via the oral route.

The conversion of codeine to morphine is effected by CYP2D6. Well-characterized genetic polymorphisms in CYP2D6 lead to the inability to convert codeine to morphine, thus making codeine ineffective as an analgesic for approximately 10% of the white population. Other polymorphisms can lead to enhanced metabolism and thus to increased sensitivity to the effects of codeine.[41] Variation in metabolic efficiency is evident among ethnic groups. For example, Chinese individuals produce less morphine from codeine than whites and are less sensitive to the effects of morphine.[42]

### Tramadol

Tramadol is a synthetic codeine analogue with a dual mechanism of action. Analgesia results through weak MOR agonism and inhibition of uptake of norepinephrine and serotonin.

Tramadol is 68% bioavailable after a single oral dose, with approximately 20% protein binding. Its affinity for the opioid receptor is only equal to that of morphine. However, the primary *O*-demethylated metabolite of tramadol is two to four times as potent as the parent drug and may account for part of the analgesic effect. Tramadol is supplied as a racemic mixture, which is more effective than either enantiomer alone. The (+)-enantiomer binds to the receptor and inhibits serotonin uptake. The (−)-enantiomer inhibits norepinephrine uptake and stimulates $\alpha_2$-adrenergic receptors.[43] The compound undergoes hepatic metabolism and renal excretion, with an elimination half-life of 6 hours for tramadol and 7.5 hours for its active metabolite. Analgesia begins within an hour of oral dosing and peaks within 2 to 3 hours. The duration of analgesia is approximately 6 hours. The maximum recommended daily dose is 400 mg. Tramadol is also available in a 24-hour extended-release preparation.

Physical dependence on and abuse of tramadol have been reported. Although its abuse potential is unclear, tramadol probably should be avoided in patients with a history of addiction. Because of its inhibitory effect on serotonin uptake, tramadol should not be used in patients taking MAO inhibitors and triptans. Seizures have been reported with concomitant use of selective serotonin receptor inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic anti-depressants (TCAs), and neuroleptics.[43]

### Tapentadol

Tapentadol is a strong opioid with a dual mechanism of action, similar to tramadol. It is a strong MOR agonist and a serotonin and norepinephrine reuptake inhibitor. Because of its first-pass metabolism, its bioavailability is low (32%) and protein binding is low (20%). The metabolites

of tapentadol are inactive. The half-life is approximately 4 hours.

Similar to tramadol, tapentadol is contraindicated in patients taking MAO inhibitors, triptans, SSRIs, SNRIs, TCAs, and neuroleptics because of the risk of serotonin syndrome. There does not appear to be a risk of seizures, as is seen with tramadol. Tapentadol is currently available as an oral immediate-release drug; however, an extended-release preparation is in development (Figure 67-1).

## Toxicity

### Respiration

Although effects of opioids on respiration are readily demonstrated, clinically significant respiratory depression rarely occurs with standard analgesic doses in the absence of other contributing comorbidities or concomitant use of sedatives. In addition, the respiratory depressant effect of the opioid is significantly reduced with continued opioid use because of patient tolerance. It should be stressed, however, that respiratory depression represents the primary cause of morbidity secondary to opiate therapy.[44] In humans, death from opiate poisoning is nearly always the result of respiratory arrest or obstruction.[45] For example, in November 2006, the FDA notified health care professionals of reports of death and life-threatening adverse events, such as respiratory depression and cardiac arrhythmias, in patients receiving methadone.[46] Methadone appears to be involved in approximately one-third of all prescription opioid–related deaths, exceeding hydrocodone and oxycodone despite being prescribed one-tenth as often. This has led to revisions in methadone conversion tables.

At therapeutic doses, opiates depress all phases of respiration (rate, minute volume, and tidal exchange). High doses can produce irregular and agonal breathing.[47] A number of factors can increase the risk of opioid-induced respiratory depression, even at therapeutic doses. These include[19] (1) concomitant use of sedatives such as alcohol, benzodiazepines, and tranquilizers; (2) obstructive and central sleep apnea; (3) extremes in age (both newborns and elderly); (4) comorbidities such as pulmonary disease and renal disease; and[19] (5) removal of the painful stimulus. Pain serves as a respiratory stimulant, and removal of pain (e.g., neurolytic block with severe cancer pain) will reduce the ventilatory drive, leading to respiratory depression. Dose dumping is the unintentional rapid release of the opioid from controlled-release medications. This can occur after ingestion of alcohol and is thought be one of the causes of overdoses.

### Sedation

Opiates can produce drowsiness and cognitive impairment, which can increase respiratory depression. These effects most typically are noted following initiation of opiate therapy or after a dose increase but usually resolve with continued opioid use. If the sedation does not resolve, other causes of the sedation should be investigated, such as concomitant use of other sedatives or the presence of sleep apnea. In the absence of these variables, opioid rotation may result in less sedation.[48]

### Neuroendocrine Effect

Regulation of the release of hormones and factors from the pituitary is under complex regulation by opiate receptors in the hypothalamic-pituitary-adrenal (HPA) axis. The opioids block the release of a large number of many HPA hormones, including sex hormones, prolactin, oxytocin, growth hormone, and anti-diuretic hormone.

In males, the opioids inhibit adrenal function, resulting in reduced cortisol production and reduced adrenal androgens. In females, the opioids lower luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release. In both males and females, long-term opioid therapy can result in endocrinopathies, including hypogonadotropic hypogonadism, leading to decreased libido, and females may experience menstrual cycle irregularities. These changes are reversible with removal of the opiate. Mechanisms of neuroendocrine effects of the opioids are thought to be the result of a direct effect on the pituitary and an indirect action on the hypothalamus, blocking the release of gonadotropin-releasing hormone (GnRH) and corticotropin-releasing hormone (CRH). Secretion of thyrotropin is relatively unaffected.

Opioids inhibit the release of dopamine from neurons of the tuberoinfundibulum of the arcuate nucleus. Prolactin release from lactotrope cells in the anterior pituitary is under inhibitory control by dopamine; therefore the reduced dopamine caused by the opioids results in an increase in plasma prolactin. Prolactin counteracts the effects of dopamine, which is responsible for sexual arousal, and high levels of prolactin can result in impotence and loss of libido. Long-term opioid use can increase growth hormone by inhibiting somatostatin release; this regulates GH-releasing hormone secretion.[47]

Anti-diuretic hormone (ADH) and oxytocin are synthesized in the perikarya of the magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus and are released from the posterior pituitary. Kappa opioid receptor agonists inhibit the release of oxytocin and anti-diuretic hormone (and cause prominent diuresis). MOR agonists have minimal effect or tend to produce anti-diuretic effects in humans, and reduce oxytocin secretion.[49] Some of the opioids (i.e., morphine) stimulate histamine release, resulting in hypotension and secondary ADH release. The effects of the opioids on vasopressin and oxytocin release may reflect both a direct effect upon terminal secretion and indirect effects upon dopaminergic and noradrenergic modulatory projections into the paraventricular and supraoptic hypothalamus.[50]

### Miosis

Lumination of the pupil activates a reflex arc, which, through local circuitry in the Edinger-Westphal nucleus, activates parasympathetic outflow through the ciliary ganglion to the pupil, producing constriction. The parasympathetic outflow is locally regulated by GABAergic interneurons, and the opiates are believed to block GABAergic interneuron–mediated inhibition, resulting in miosis.[51] At high doses of agonists, the miosis is marked, and pinpoint pupils suggest opioid intoxication. Although some tolerance to the miotic effect develops with therapeutic

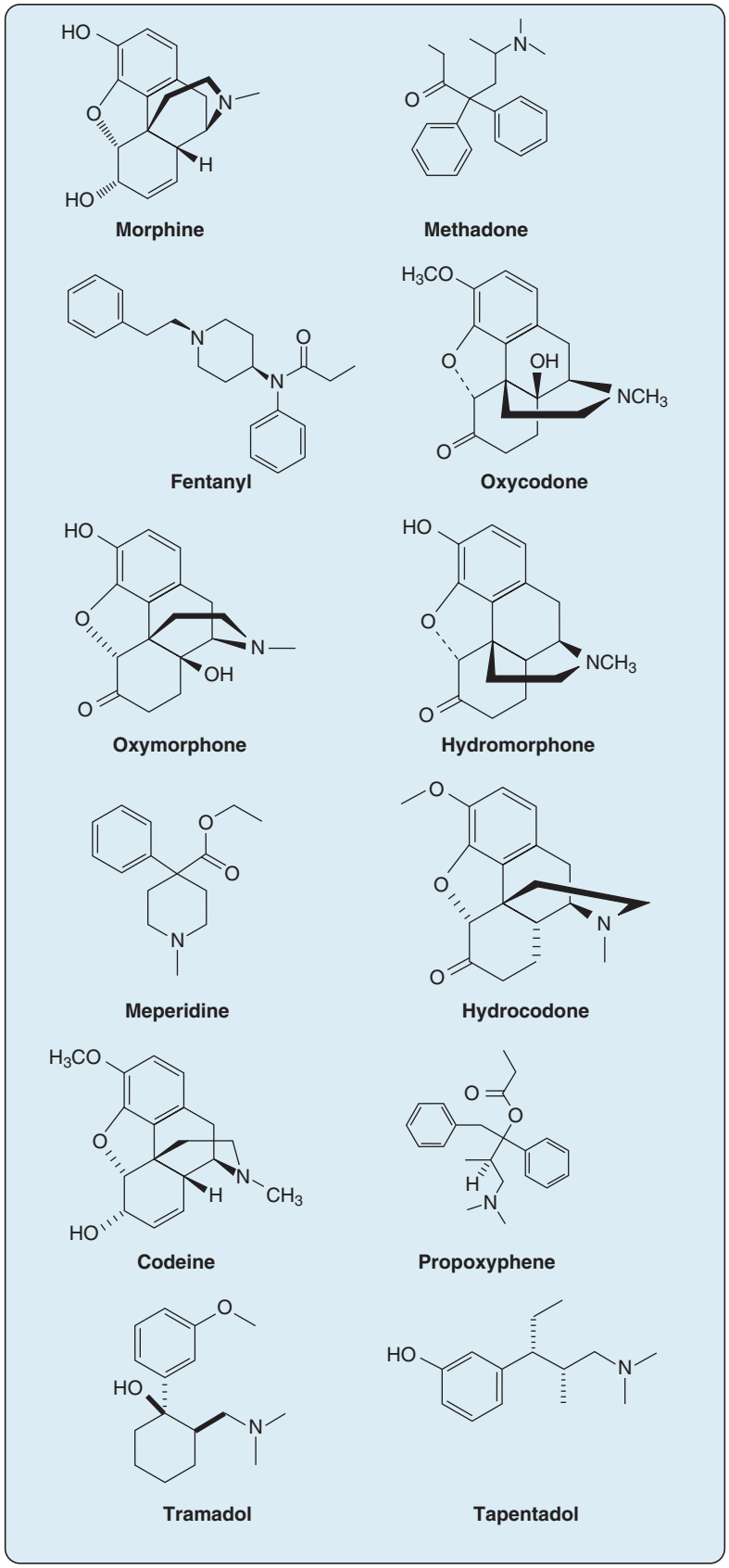



**Figure 67-1** Opioid structures.

doses of the opioids, high circulating concentrations of opioids continue to result in constricted pupils.

### Myoclonus and Seizure

Myoclonus and seizures have been reported in patients receiving high doses of opiates.[49] In addition, seizure-like activity can occur with extremely high doses of the opioids. Seizures may also occur at lower doses of meperidine because of the normeperidine metabolite, which lowers the seizure threshold. Myoclonus and seizure activity is thought to be to the result of inhibition of GABA from inhibitory interneurons in the hippocampal pyramidal cells and dorsal horn cells.[52] The opiates also have a direct stimulatory effect through interaction with G inhibitory and stimulatory-coupled receptors.[53] It has been suggested that, in addition to the normeperidine metabolite, which is seizurogenic, morphine-3-glucuronide (from morphine) may induce myoclonus and seizures at high doses.[54]

### Nausea and Vomiting

The gastrointestinal tract is under the control of the vomiting center located in the brain stem. The vomiting center is activated by the chemoreceptor trigger zone (CRTZ), the vestibular system, and the gastrointestinal tract. The opioids activate the CRTZ, sensitize the vestibular system, and reduce gastric emptying time, all of which will lead to activation of the vomiting center.[55] Drugs that reduce CRTZ activity (antidopaminergics, 5-hydroxytryptamine$_3$ [5-HT$_3$] serotonin receptor antagonists) and vestibular sensitization (anticholinergics, antihistamines) will reduce opioid-induced nausea. Drugs that enhance gastrointestinal motility are also effective (e.g., metoclopramide).[56]

### Constipation

It is estimated that 40% to 95% of patients treated with opioids experience constipation, and that changes in bowel function can be demonstrated even with acute dosing.[57] Opioid receptors are densely distributed in enteric neurons between the myenteric and submucosal plexuses and on a variety of secretory cells.[58] Stimulation of MOR in the intestines reduces propulsatile contractions and diminishes intestinal secretions.[58] The prolonged transit time of the intestinal contents, along with reduced intestinal secretion, leads to increased water absorption, increasing the viscosity of bowel contents and constipation. In addition, anal sphincter tone is increased, and reflex relaxation in response to rectal distension is reduced. All of these effects combine to contribute to morphine-induced constipation.[59] Tolerance to opioid-induced constipation does not occur.

### Biliary Spasm

Relaxation of the sphincter of Oddi is suppressed by the opioids, which can result in an increase in the common bile duct pressure, leading to symptoms of biliary colic. Therefore treatment of the pain of biliary colic with the opioids can lead to an exacerbation of the pain rather than relief.

### Urinary Retention

MOR agonists inhibit the urinary voiding reflex, increase the tone of the external sphincter, and induce bladder relaxation, which can result in urinary retention. This effect is mediated by MOR and delta-opioid receptor activation in the brain and spinal cord. This will result in higher bladder volumes and sometimes requires catheterization.[60]

### Pruritus

Pruritus can occur with all of the MOR agonists. The mechanism is thought to be the result of disinhibition of itch-specific neurons, which have been identified in the spinal dorsal horn.[61] It can occur with both systemic and spinally administered doses but is more common with spinally administered doses.[62] It tends to be focused in the trunk and face.

### Immunosuppression

The effect of the opioids on immune function is controversial. The opioids suppress immune function through a direct effect on immune system cells and indirectly by activating sympathetic outflow and modulation of the HPA axis.[63] A proposed mechanism for the immune suppressive effects of morphine on neutrophils is through nitric oxide–dependent inhibition of nuclear factor-κB (NF-κB) activation.[63] Others have proposed that the induction and activation of mitogen-activated protein (MAP) kinase may play a role.[65] Pain itself is immunosuppressive; therefore a reduction in pain will counteract and probably outweigh the direct immunosuppressive effects of the opioids. In addition, it appears that acute delivery of the opioids is more immunosuppressive than long-term delivery, suggesting tolerance to this effect.

### Sweating

The opioids exert a wide range of effects on thermoregulation, with high doses leading to hyperthermia and low doses leading to hypothermia.[66] Excessive sweating has been reported in as many as 45% of patients taking methadone. The mechanism appears to be related to release of histamine.[67]

## ANTI-DEPRESSANTS

Anti-depressants have long been used to treat chronic pain. In the past, they were more often chosen to improve mood than to treat pain because of coexisting anxiety and depression. After separate studies showed improved pain control in patients without depression, as well as in patients with depression without improvement in mood, it was realized that these medications have independent analgesic actions.[68] Further, improvement in pain is seen earlier and at lower doses than improvements in depression, also demonstrating this analgesic efficacy.[69] A recent meta-analysis of 18 randomized, placebo-controlled studies with multiple anti-depressants concluded that strong evidence exists for the efficacy of anti-depressants for pain relief, fatigue, and sleep disturbance, and in improving health-related quality of life.[70] The FDA has now approved the SNRI duloxetine for

fibromyalgia, diabetic peripheral neuropathy, and chronic musculoskeletal pain, and the SNRI milnacipran for fibromyalgia. Nevertheless, not all anti-depressants are effective pain medications. For example, SSRIs have minimal analgesic properties and therefore have a limited role in the treatment of chronic pain.[71] Trazadone, used mainly for sleep, is another anti-depressant without analgesic actions.

The main site of analgesic action for all of the anti-depressants is thought to be the reuptake inhibition of norepinephrine and serotonin at the level of the spinal cord and higher. This inhibition increases the extra-cellular concentration of these two monoamines, resulting in activation of descending inhibitory pain pathways, and ultimately decreases pain.[72] A peripheral action has also been proposed for the TCAs because topical application produces analgesia in animal models of pain.[72] Current investigation has shown that topical application can result in the inhibition of NMDA receptors and the blockade of sodium and calcium channels, which alone or in combination could explain their peripheral analgesic properties.[72-74] Because TCAs are effective in blocking sodium channels, and the proliferation of sodium channels plays a key role in the pathogenesis of neuropathic pain, this may be one of the main sites of action for these drugs.[75] Finally, TCAs potentiate endogenous opioids (Figure 67-2).[76]

## Tricyclic Anti-depressants

TCAs are considered first-line agents in the treatment of chronic neuropathic pain and fibromyalgia.[77] Two systematic reviews of 17 randomized controlled trials (RCTs) of 10 anti-depressants have shown numbers needed to treat

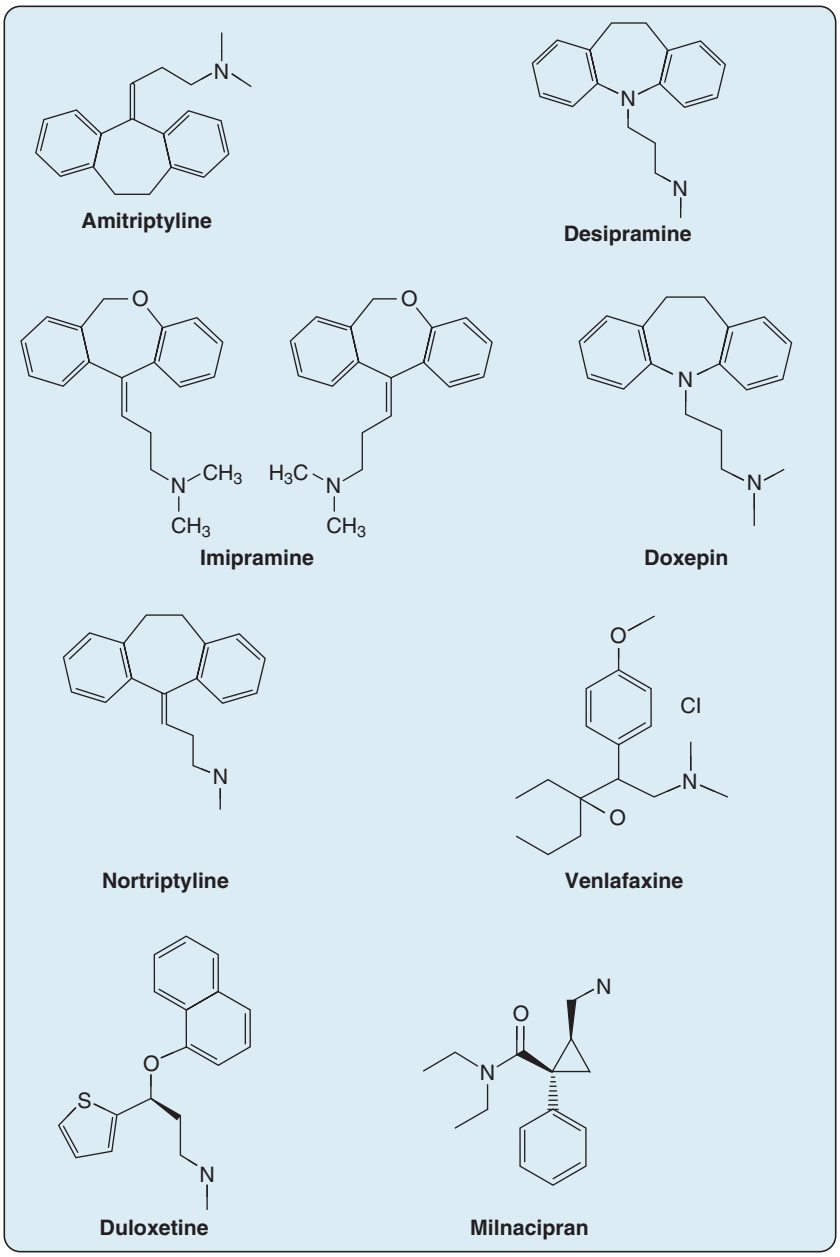



**Figure 67-2** Chemical structures of anti-depressants.

**TABLE 67-6** Analgesic Efficacy of Anti-depressants

| | ANIMAL | | | HUMANS | |
|---|---|---|---|---|---|
| **Anti-depressant** | **Number of Studies** | **Positive Results** | | **Number of Studies** | **Combined NNT** |
| TCAs | 126 | Acute pain tests<br>Chronic pain models | 81%<br>95% | 23 | 3.1 |
| SSRIs[§] | 39 | Acute pain tests<br>Chronic pain models | 44%<br>33% | 3 | 6.8 |
| SNRIs[¶] | 10 | Acute pain tests<br>Chronic pain models | 100%<br>100% | 3 | 5.5 |
| Others[‖] | 7 | Acute pain tests<br>Chronic pain models | 100%<br>100% | 1 | 1.6 (bupropion) |

*Data modified from Eschalier A, Ardid D, Dubray C: Tricyclic and other anti-depressants as analgesics. In Sawynok J, Cowan A, editors: *Novel aspects of pain management: opioids and beyond,* New York, 1999, Wiley-Liss, pp 303–310. Complete up to 2005. Only approximately 10% of the animals were treated by using chronic pain models.

[†]From Finnerup N, Otto M, McQuary H, et al: Algorithm for neuropathic pain treatment: an evidence based proposal, *Pain* 118:289–305, 2005.

[‡]Number of patients treated to improve the health of one patient (at least 50% decrease in pain intensity).

[§]For example, fluoxetine, fluvoxamine, sertraline, paroxetine, and citalopram.

[¶]For example, venlafaxine, milnicipran, and duloxetine.

[‖]For example, mirtazapine and bupropion.

NNT, Number needed to treat; SNRIs, serotonin-norepinephrine reuptake inhibitors; SSRIs, selective serotonin reuptake inhibitors; TCAs, tricyclic anti-depressants.

From Mico J, Ardid D, Berrocoso E, et al: Anti-depressants and pain. *Trends Pharmacol Sci* 27:348–354, 2006.

(NNTs) of approximately 2.5 for neuropathic pain (Table 67-6).[78] All TCAs have shown fairly equal efficacy within the class.

TCAs are usually divided into tertiary amines (amitriptyline, imipramine, and doxepin) and secondary amines (nortriptyline and desipramine). Nortriptyline and desipramine are demethylated in the liver from amitriptyline and imipramine, respectively. The tertiary amines tend to block the reuptake of serotonin more than norepinephrine, and the secondary amines are more selective in their inhibition of norepinephrine uptake.

Tricyclics should be started at the lowest dose possible and titrated slowly. A typical starting dose for amitriptyline, nortriptyline, or desipramine is as low as 5 to 10 mg before bedtime. Doses can be increased by the same amount as the starting dose approximately every 7 days (Table 67-7). Studies have shown improved analgesia with amitriptyline in the range of 25 to 50 mg, but some studies have gone as high as 200 mg. Response should be seen within 3 to 4 weeks (see Table 67-7).

### Side Effects

Unfortunately, side effects with all of the TCAs are common and can limit use, especially in the elderly and in patients with hepatic impairment. Side effects tend to be less with the secondary amines. Sedation is common because of its antihistamine effects but can be beneficial if there are sleep complaints. Anticholinergic side effects, which include dry mouth, constipation, urinary retention, and blurred vision, are also prevalent. Both anticholinergic and antihistamine side effects are dose dependent and can decrease with time; slow titration can improve compliance. Weight gain and sexual dysfunction are frequently reported. Cardiac side effects include orthostatic hypotension and dysrhythmias. Cardiac history should be reviewed, and a baseline electrocardiogram (ECG) should be considered for patients older than 40 years. Seizure thresholds are lowered. Risk of suicidal thinking and behavior is increased in children, adolescents, and young adults as old as 24 years. Further, if these medications are taken intentionally or accidentally in overdose, they are dangerous and can be extremely lethal at relatively low doses. When these medications are stopped, it is recommended that they be reduced by 25% per week to decrease side effects.

## Serotonin-Norepinephrine Reuptake Inhibitors

The SNRIs (venlafaxine, duloxetine, and milnacipran) also have analgesic properties. Together, they benefit in treating painful diabetic peripheral neuropathy, neuropathic pain, fibromyalgia, and, most recently, chronic musculoskeletal pain. Venlafaxine, the first drug in this class, is an inhibitor of serotonin reuptake at low doses, and at higher doses it inhibits norepinephrine reuptake. This means that it acts more like an SSRI at lower doses and does not become an SNRI until higher doses, at which point more side effects occur. Venlafaxine has some effectiveness with polyneuropathy,[79] including painful diabetic neuropathy[80] and fibromyalgia, but all of these indications are off-label.[81] Doses range from 75 to 225 mg/day. Milnacipran has been approved for use in fibromyalgia in the United States but not in Europe. A recent meta-analysis of five studies showed that this drug was superior to placebo, except for the side effect of sleep disturbance, in treating fibromyalgia.[82] Doses ranged from 25 to 200 mg/day. This same paper found four duloxetine studies that showed superiority of duloxetine to placebo in fibromyalgia except for fatigue symptoms.[82] Long-term benefit of 6 months has been shown with single daily doses of 60 or 120 mg per day of duloxetine for fibromyalgia. Starting doses are 20 to 30 mg in the morning. Duloxetine has also been approved to treat diabetic peripheral neuropathy, fibromyalgia, and generalized musculoskeletal pain in the United States. It is superior to placebo at doses between 60 and 120 mg per day with this disorder, and patients typically respond within the first week.[83] Last,

**TABLE 67-7** Tricyclic Anti-depressants: Pharmacodynamics

| Drug | Amitriptyline | Desipramine | Doxepin | Imipramine | Nortriptyline |
|---|---|---|---|---|---|
| Half-life | 9-27 hr | 7-60 hr | 6-8 hr | 6-18 hr | 28-31 hr |
| Metabolism | Hepatic | Hepatic | Hepatic | Hepatic | Hepatic |
| Excretion | Urine | Urine | Urine | Urine | Urine |
| Protein binding | ≥90% | 90%-92% | 80%-85% | 60%-95% | 93%-95% |
| Therapeutic dose/day | 10-150 mg | 10-150 mg | 10-150 mg by mouth | 10-200 mg | 10-150 mg |
| Administration | Bedtime or 2 times/day | Bedtime or 2 times/day | Bedtime or 2-3 times/day | Bedtime or 2 times/day | Bedtime or 2-3 times/day |
| Titration | ≥7 days/dose change | ≥3 days/dose change | ≥7 days/dose change | ≥7 days/dose change | ≥3 days/dose change |
| **Metabolism/Transport Effects** | | | | | |
| Substrate CYP1A2 | Minor | Minor | Minor | Minor | Minor |
| Substrate CYP2C9 | Minor | | | | |
| Substrate CYP2C19 | Minor | | Minor | Major | Minor |
| Substrate CYP2D6 | Major | Major | Major | Major | Major |
| Substrate CYP3A4 | Minor | | Minor | Minor | Minor |
| Substrate CYP2B6 | Minor | | | Minor | |
| Inhibits CYP1A2 | Weak | Weak | | Weak | |
| Inhibits CYP2A6 | | Moderate | | | |
| Inhibits CYP2B6 | | Moderate | | | |
| Inhibits CYP2C9 | Weak | | | | |
| Inhibits CYP2C19 | Weak | | | Weak | |
| Inhibits CYP2D6 | Weak | Moderate | | Moderate | Weak |
| Inhibits CYP2E1 | Weak | Weak | | Weak | Weak |
| Inhibits CYP3A4 | | Moderate | | | |

*Data from Wolters Kluwer Health: www.uptodate.com.

duloxetine is efficacious in treating chronic musculoskeletal pain, including discomfort from osteoarthritis and chronic lower back pain.[84]

### Side Effects

Side effects of all three of these SNRIs include nausea, dry mouth, and constipation. Sexual side effects tend to be less than with SSRIs. Duloxetine should not be used in patients with coexisting hepatic insufficiency. Milnacipran is not recommended for patients with end-stage renal disease but is unique in that it is not metabolized by cytochrome P450 isoenzymes. Patients with serotonin syndrome, caused by iatrogenic overstimulation of central and peripheral serotonin receptors, are seen with neuromuscular hyperactivity, autonomic hyperactivity, and altered mental status. It may occur abruptly and progress rapidly when these drugs are used at high doses or combined with other medications that stimulate serotonin. These drugs can also impair platelet aggregation, particularly if used concomitantly with aspirin or NSAIDs. Dose-related increases in blood pressure have been reported and should be followed. SNRIs carry a black box warning in the United States for increased risk of suicidal thinking and behavior in children, adolescents, and adults younger than 25 years. When these medications are discontinued, withdrawal symptoms have been reported. It is recommended that the dose be decreased by 25% per week to minimize these symptoms (see Tables 67-5 through 67-10).

**TABLE 67-8** Potential Tricyclic Anti-depressant Drug Interactions

| |
|---|
| Monoamine oxidase inhibitors |
| Drugs that inhibit cytochrome P450 isoenzymes |
| Drugs that prolong the QT interval |
| Central nervous system–active drugs |
| Antidopaminergic drugs |
| Alcohol |
| Lithium |
| St. John's wort |
| Tryptophan |

## ANTI-CONVULSANTS

Neuropathic pain (NeP) is chronic pain initiated by nervous system lesions or dysfunction and maintained by a number of mechanisms. Excess stimulation of nociceptive pathways or damage to non-nociceptive pathways alters the balance between painful and nonpainful inputs so that pain results

**TABLE 67-9** Serotonin-Norepinephrine Reuptake Inhibitors: Pharmacodynamics

| | Venlafaxine | Duloxetine | Milnacipran |
|---|---|---|---|
| Half-life | 5-7 hr | 8-17 hr | 6-8 hr |
| Metabolism | Hepatic | Hepatic | Hepatic |
| Excretion | Urine | Urine 70%<br>Feces 20% | Urine |
| Protein binding | 35% | ≥90% | 13% |
| Therapeutic dose/day | 37.5-225 mg/day | 20-60 g/day | 12.5-200 mg/day |
| Administration | 2-3 times | Daily | Twice daily |
| Titration | ≥4-7 days/dose change | ≥7 days/dose change | 1-2 days/dose change |
| **Metabolism/Transport Effects**<br>Substrate CYP1A2 | | Major | |
| Substrate CYP2C19 | Minor | | |
| Substrate CYP2C9 | Minor | | |
| Substrate CYP2D6 | Major | Major | |
| Substrate CYP3A4 | Major | | |
| Inhibits CYP2B6 | Weak | | |
| Inhibits CYP2D6 | Weak | Moderate | |
| Inhibits CYP3A4 | Weak | | |

*Data from Wolters Kluwer Health: www.uptodate.com.

**TABLE 67-10** Potential Serotonin-Norepinephrine Reuptake Inhibitor Drug Interactions

| |
|---|
| **Milnacipran** |
| Monoamine oxidase inhibitors |
| Serotonergic drugs |
| Triptans |
| CNS-active drugs |
| Digoxin |
| Alcohol |
| Drugs that interfere with hemostasis |
| Antidopaminergic drugs |
| St. John's wort |
| Tryptophan |
| **Duloxetine** |
| Monoamine oxidase inhibitors |
| Drugs that inhibit cytochrome P450 isoenzymes |
| CNS-active drugs |
| Triptans |
| Serotonergic drugs |
| Alcohol |
| Drugs that interfere with hemostasis |
| Antidopaminergic drugs |
| St. John's wort |
| Tryptophan |
| **Venlafaxine** |
| Monoamine oxidase inhibitors |
| Drugs that inhibit cytochrome P450 isoenzymes |
| CNS-active drugs |
| Triptans |
| Serotonergic drugs |
| Drugs that interfere with hemostasis |
| Antidopaminergic drugs |
| Alcohol |
| Protease inhibitors |
| St. John's wort |
| Tryptophan |

CNS, Central nervous system.

without nociceptor stimulation. Several cellular and molecular mechanisms operating over different periods of time are thought to be involved in the abnormal peripheral and CNS activity associated with NeP.[85] Many of these mechanisms can be modulated by the anti-convulsants. First, nerve injury is reported to evoke spontaneous discharges from the cell bodies of myelinated fibers at the levels of the dorsal root ganglia. The mechanism of spontaneous activity is thought to be secondary to an increase in concentration of sodium channels in neuromas, dorsal root ganglion cells, and areas of demyelination.[86] Second, spinal inhibitory interneurons modulate the peripheral-to-central transmission of pain signals, thus "gating" ascending sensory information.[87] GABA and glycine and their receptors are abundant in the superficial dorsal horn,[88] but their levels are regulated by primary afferent input and change significantly after nerve injury.[88]

Finally, increased glutamatergic neurotransmission may also contribute to hyperexcitability and NeP through activation of both NMDA receptors and non-NMDA amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA)/kainate-type glutamate receptors. Therefore drugs that modulate sodium channels, increase GABA, reduce glutamate release, or block glutamate effects can potentially reduce neuropathic pain.

The four main mechanisms of action of the anti-convulsants that result in pain reduction include[19] (1) calcium channel modulation, (2) sodium channel blockade, (3) NMDA antagonism, and (4) $GABA_A$ agonism. The net result of the mechanisms of action consists of reduced spontaneous pain and hypersensitivity through membrane stabilization, reduced neurotransmitter release, and reduced postsynaptic cellular activation in the dorsal horn of the spinal cord. Table 67-11 summarizes the mechanisms of action of the various anti-convulsants.

With the exception of pregabalin and gabapentin, studies on the efficacy of the various anti-convulsants to treat pain have been inconsistent. Pregabalin is FDA approved to treat postherpetic neuralgia (PHN), painful diabetic peripheral neuropathy (DPN), fibromyalgia, and pain related to

**TABLE 67-11** Mechanism of Action of Anti-convulsants

| | Sodium Channel Blockade | NMDA Antagonism | $GABA_A$ Agonism |
|---|---|---|---|
| Carbamazepine | X | X | X |
| Clonazepam | | | X |
| Gabapentin | | X | |
| Lamotrigine | X | X | |
| Levetiracetam | | X | |
| Oxcarbazepine | X | X | |
| Phenytoin | X | | |
| Pregabalin | | X | |
| Topiramate | X | X* | X |
| Valproic acid | | | X |

*Through AMPA/kainate antagonism.

AMPA, Amino-3-hydroxy-5-methylisoxazole propionic acid; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate.

**TABLE 67-12** Comparison of Gabapentin and Pregabalin Pharmacology

| | Gabapentin | Pregabalin |
|---|---|---|
| FDA-approved pain indication | Postherpetic neuralgia | Postherpetic neuralgia<br>Diabetic neuropathy<br>Fibromyalgia |
| Mechanism of action | Modulates calcium channel opening by binding to the $\alpha_2$-δ subunit | Modulates calcium channel opening by binding to the $\alpha_2$-δ subunit |
| Pharmacokinetic profile | Nonlinear: plasma concentration is not dose proportionate | Linear: plasma concentration is dose proportionate |
| Oral bioavailability | 60%, 900 mg<br>47%, 1200 mg<br>34%, 2400 mg<br>33%, 3600 mg | 90% at all doses |
| Effective dose | 1800 mg/day<br>No additional benefit with doses higher than 1800 mg/day | 150 mg/day<br>Dose range from 150-600 mg/day |
| Schedule in U.S. | Unscheduled | Schedule V |

FDA, U.S. Food and Drug Administration.

spinal cord injury; gabapentin is FDA approved to treat PHN; carbamazepine is FDA approved to treat trigeminal neuralgia; and valproic acid and topiramate are FDA approved to treat migraine headaches. The discussion in this chapter will focus on pregabalin and gabapentin, which have the most evidence in support of treating chronic neuropathic pain (Table 67-12).

## Mechanism of Action

Pregabalin and gabapentin are synthetic molecules that are structurally related to GABA. Both structures are derived by the addition of a cyclohexyl or branched-chain aliphatic carbohydrate moiety to the GABA backbone. However, the three-dimensional shapes of pregabalin and gabapentin are significantly different from GABA. The amine ($NH_2$) and carboxyl groups ($CO_2H$) are closer to each other than in the native GABA structure. It is proposed that this difference in the three-dimensional structure of pregabalin and gabapentin versus GABA accounts for their different pharmacologic activities.[90,91]

Although the exact mechanism of action of gabapentin and pregabalin is unknown, results from animal models suggest that pregabalin modulates neuronal hyperexcitability, resulting in analgesic and anticonvulsant effects. Reduction of neurotransmitter release occurs because pregabalin selectively binds to the $\alpha_2$-δ subunit of the N and P/Q subtypes of calcium channels of neurons in the brain and spinal cord, thereby modulating calcium influx into presynaptic cells.[92] Therefore there is less postsynaptic activation through the AMPA/kainite, NMDA, and neurokinin receptors. Unlike the opioids, which limit calcium influx via a G protein pathway, no tolerance is associated with pregabalin and gabapentin. Gabapentin and pregabalin do not have any effect on L-type calcium channels (e.g., verapamil); therefore there is no effect on blood pressure. New evidence suggests that gabapentin's site of action, unlike pregabalin, is at the locus coeruleus rather than the spinal dorsal horn. A recent study delivering gabapentin by the spinal route had no effect on pain.[18,93]

## Pharmacology of Gabapentin and Pregabalin

### Gabapentin

Gabapentin has a nonlinear bioavailability, with a reduction in absorption while the dose increases. This is the result of an active and saturable transport mechanism.[94] Gabapentin, 900 mg, has 60% bioavailability, and a 3600-mg dose has only 33% bioavailability. More frequent delivery of smaller doses may improve bioavailability through the saturable transport mechanism. It is less than 3% protein bound, and because of its high lipid solubility, it readily penetrates the CNS. Gabapentin is not metabolized and is excreted unchanged in the urine. Therefore renal impairment will significantly increase the drug half-life. Studies have shown efficacy at doses as high as 1800 mg and 2400 mg; however, doses higher than 1800 mg/day do not appear to be more efficacious. The current maximum FDA-approved dose to treat pain is 1800 mg/day. Because of its nonlinear kinetics, titration of gabapentin to an effective dose can be prolonged with an average onset of 10 to 14 days after initiation.

### Pregabalin

Pregabalin is not dependent on active transport for absorption and therefore has linear kinetics with bioavailability of approximately 90%. Unlike gabapentin, pregabalin absorption is independent of dose. It has negligible protein binding and readily penetrates into the CNS. Pregabalin is not metabolized and is excreted unchanged in the urine; therefore, renal impairment will significantly increase the drug half-life, and dose adjustments are required.[90] The effective dose of pregabalin is between 150 and 300 mg per day. Because of the linear kinetics and high bioavailability, the effective dose can be achieved in 2 to 3 days; therefore onset is faster than with gabapentin.

### Toxicity of Gabapentin and Pregabalin

Neither gabapentin nor pregabalin has any significant drug interactions, and protein binding is minimal. Because gabapentin and pregabalin have similar mechanisms of action, their side-effect profiles are very similar. The most common side effects with at least more than twice the incidence of placebo in controlled trials were dizziness, somnolence, dry mouth, peripheral edema, blurred vision, weight gain, and thinking abnormalities. Dizziness and somnolence usually began shortly after initiation of therapy, and most cases resolved with continued dosing. Dizziness and somnolence are dose related and can be reduced with slow titration. This side effect can also be an advantage when administered at night, in that it will improve sleep. Controlled studies with gabapentin and pregabalin have demonstrated improved patient sleep quality when compared with placebo.[19,95] Xerostomia (dry mouth) is dose dependent; however, it is

usually mild, and few withdrawals of patients from clinical trials have resulted from this side effect. Peripheral edema occurred in almost a third of participants in clinical trials. It does not appear to be dose related, nor is it associated with any cardiovascular, renal, or hepatic abnormalities. It is more common with the concomitant use of a thiazolidinedione antidiabetic agent.[96] Blurred vision is dose related and usually resolves with continued use. It is not associated with any ocular abnormalities. Weight gain has been reported to be as high as 8% of baseline weight but is not associated with any cardiac, renal, or hepatic abnormalities. A mildly increased appetite was reported in epilepsy and fibromyalgia trials. There is no association with baseline body mass index (BMI), gender, or age. Weight gain appears to plateau and is mild. Thinking abnormalities are typical of anti-convulsants and are consistent with the calcium channel modulation mechanism of these agents. Thinking abnormalities tend to be mild but can be bothersome enough in some patients to lead to discontinuation.

Anti-convulsants, including gabapentin and pregabalin, have approximately twice the risk of suicidal ideation versus placebo. This increased risk is not age related, and appears to occur at between 1 and 24 weeks of therapy. Patients should be warned of this side effect and instructed to contact their health care provider immediately if it occurs.

Studies with pregabalin have shown a mild PR interval prolongation that was not associated with increased risk of second- or third-degree atrioventricular block. Pregabalin may result in a mildly decreased platelet count, which is not associated with an increase in bleeding-related adverse events. Pregabalin may also result in mild elevations in creatinine kinase, which are asymptomatic in most patients.

In controlled clinical trials of pregabalin, more patients reported euphoria as compared with placebo. In a follow-up drug likability study in recreational drug users of sedative-hypnotics, pregabalin participants reported a "good drug effect," "a high," and "liking" to a degree that was similar to 30 mg of diazepam. In addition, clinical studies showed withdrawal symptoms of insomnia, nausea, headache, and diarrhea. Therefore the FDA approved the drug with a category V controlled substance schedule. Although pregabalin is Schedule V, it has very low abuse potential. As with any anticonvulsant therapy, pregabalin and gabapentin should be withdrawn slowly during at least a week to avoid withdrawal symptoms (Figure 67-3).

## MUSCLE RELAXANTS

Patients frequently describe muscular pain as "spasms," and clinicians clearly know that loss of range of motion is detrimental to their patients. It makes sense that a medicine that acts as a muscle relaxant would be of great value in treating patients with these complaints. Unfortunately, skeletal muscle relaxants do not have a primary role in the treatment of chronic pain because of limited true effects on muscles, significant side effect profiles, drug interactions, or addiction potential.

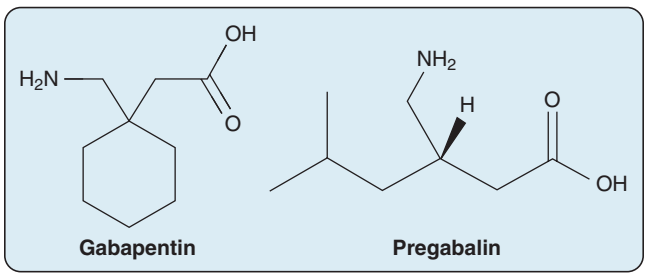



**Figure 67-3** Chemical structures of pregabalin and gabapentin.

The list of medications classified as skeletal muscle relaxants is highly diverse (Figure 67-4). Because each of the drugs in this class has a unique mechanism of action and side effect profile, each medication from this class must be examined separately. Adding to the difficulty of understanding these medications is the lack of consensus as to how or why they provide benefit. The two approved clinical indications are for treatment in upper motor neuron diseases that result in spasticity and in peripheral musculoskeletal disorders that cause pain and spasms. Each of these medications can thus be classified as an antispastic or antispasmodic agent. These medications should not be used as first-line medications and usually should be used in conjunction with other pain medications. In addition, most antispasmodic medications come with restriction of use to 2 to 3 weeks. Despite this, many skeletal muscle relaxants are prescribed on a long-term basis for chronic conditions.[97]

Understanding the reflex arc in muscle is important in understanding where these agents act. The simplest reflex is monosynaptic. A muscle spindle transmits an efferent signal through the Ia afferent neuron; this enters the dorsal horn of the spinal cord and synapses on an alpha motoneuron. This neuron then exits the spinal cord through the ventral root and innervates the extrafusal fibers of the same muscle, causing a contraction in the muscle. The Ia afferent signal is also transmitted polysynaptically through interneurons that inhibit alpha motoneurons of antagonist muscles, causing them to relax. Gamma motoneurons located in the ventral horn adjust the sensitivity of the muscle spindles to stretch. They travel with the alpha motoneurons and receive input from cutaneous receptors and many supraspinal pathways, including the corticospinal and reticulospinal tracts. Polysynaptic spinal reflexes with supraspinal connections are much more abundant than monosynaptic reflexes. These connections are both excitatory and inhibitory, providing improved control and feedback to set and fine-tune motor tone. The main tract that inhibits spinal reflexes is the dorsal reticulospinal tract, and the main excitatory pathway is the bulbopontine tegmentum.[98]

Spasticity occurs in upper motoneuron disorders, such as multiple sclerosis, spinal cord injuries, traumatic brain injuries, strokes, and cerebral palsy. These diseases cause loss of descending inhibition from the brain to the spinal cord, leading to muscular hypertonicity and increased resistance to stretch. This presentation, which is classically described as a "clasp-knife phenomenon," tends to affect the flexors of the upper extremities and the extensors of the lower extremities more than other muscle groups. Medications approved for spasticity include baclofen, dantrolene, tizanidine, and diazepam.

The antispasmodic agents are used to treat muscle pain and spasms when hypertonicity, hyper-reflexia, or other signs of upper motoneuron disorders are not present. This presentation is much more common than spasticity and would include syndromes such as fibromyalgia, tension

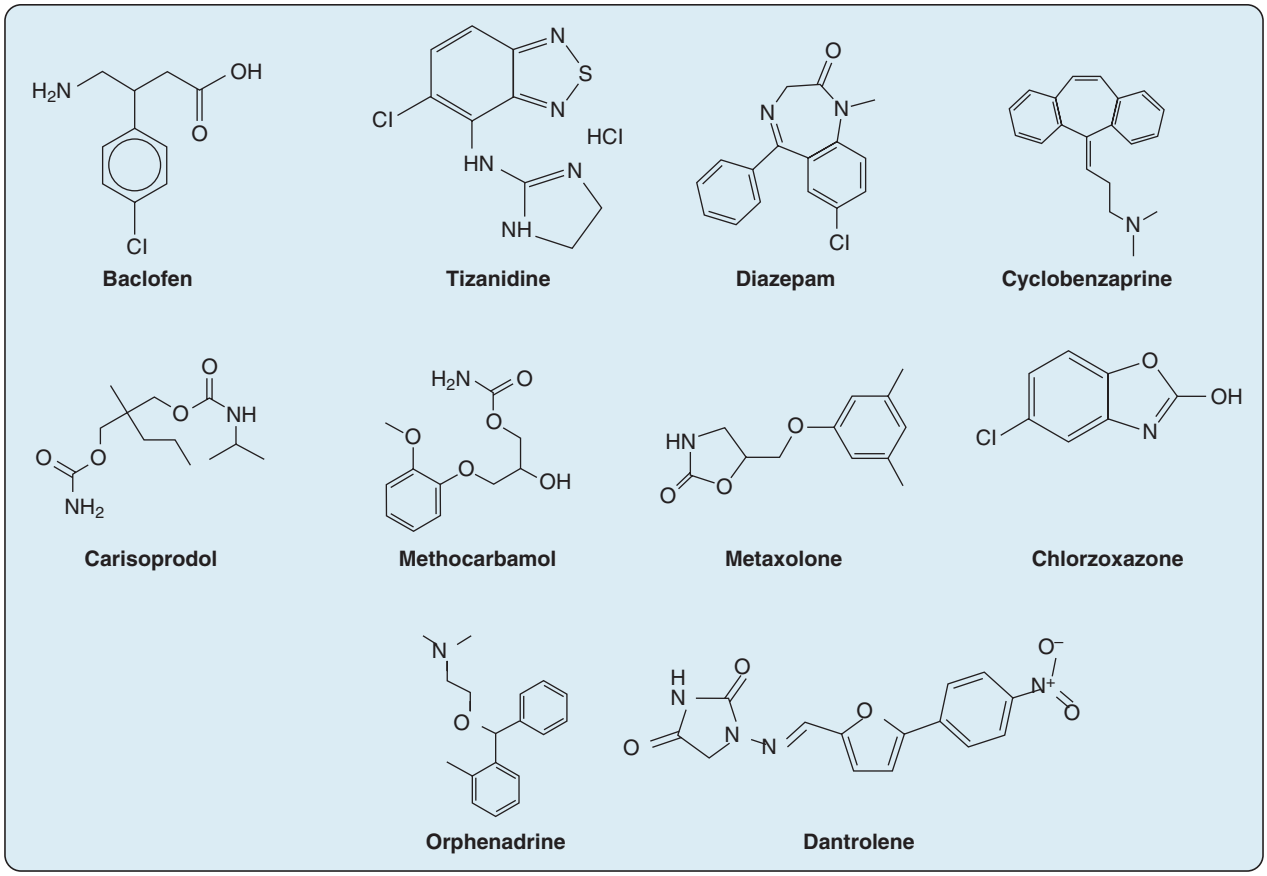



**Figure 67-4** Chemical structures of muscle relaxants.

headaches, myofascial pain disorders, and nonspecific back pain. Medications include cyclobenzaprine, carisoprodol, metaxalone, chlorzoxazone, methocarbamol, and orphenadrine. Tizanidine and benzodiazepines are approved for both spasticity and musculoskeletal disorders. How each of these drugs improves these conditions is not completely clear, but the nonspecific sedative effects may have greater importance than the effects on muscles or spinal reflexes.

## Antispasmodic Medications

### Baclofen

Baclofen blocks pre-synaptic and postsynaptic $GABA_B$ receptors[99] and disrupts polysynaptic and monosynaptic reflexes at the spinal cord level.[99] Oral doses usually start at 5 mg, three times per day, with a maximum dose of 80 mg per day. This medication frequently can be used intrathecally for the same indication with a reduction in clinical side effects. Sedation, weakness, hypotension, nausea, depression, and constipation have been reported. Taper slowly and adjust for renal impairment. Withdrawal symptoms include hallucinations, seizures, and pruritus.

### Dantrolene

Dantrolene acts directly on the muscle by decreasing the release of calcium from skeletal muscle sarcoplasmic reticulum.[101] It has a black box warning about hepatotoxicitiy.[102] Its risk is much greater with patients taking 800 mg per day or more compared with doses of 400 mg per day. It is recommended that the patient stop this medication after 45 days if no benefit is derived.

### Tizanidine

Tizanidine is a centrally acting agonist of the $\alpha_2$ receptor[103] that reduces the release of excitatory amino acids from the pre-synaptic terminal of spinal interneurons.[104] This reduces tonic stretch reflexes and polysynaptic reflex activity.[105] Doses start at 2 to 4 mg at night and increase by 2 to 4 mg three to four times per day with a maximum dose of 36 mg per day. Side effects include hypotension, sedation, and dry mouth. Its major route of elimination is renal, and renal impairment can significantly decrease clearance. It is metabolized via CYP1A2, and concomitant use with ciprofloxacin or fluvoxamine is contraindicated.[106] Elevated liver functions—three times the upper level of normal—have occurred in 5% of patients.[106] Monitoring of liver function is recommended. Acute withdrawal can cause hypertension, tachycardia, and hypertonia. Use with alcohol should be warned against, and oral contraceptives will decrease clearance with doses as low as 4 mg per day.

### Diazepam

Diazepam works by central blockade of $GABA_A$,[101,107] which is an inhibitory neurotransmitter in the CNS. It was the

first drug used to treat spasticity and is often used to compare new agents in clinical trials. Efficacy for spasticity and muscle pain is believed to be similar to that of benzodiazepines as a class.[108] Clinical trials showing efficacy with benzodiazepines are based on tetrazepam, which currently is not available in the United States.[109] It is not considered a first-line agent for spasticity or muscle pain because of its sedative effects, drug interactions, and significant abuse potential.

## Antispasmodic Medications

### Cyclobenzaprine

Cyclobenzaprine is structurally related to tricyclic antidepressants and thus causes sedation through its anticholinergic effects. It acts primarily at the brain stem to reduce tonic somatic motor activity influencing both gamma and alpha motoneurons. Dosing at 5 mg, three times per day, has similar efficacy when compared with 10 mg, three times per day, with reduced side effects.[110] The maximum daily dose is 30 mg. This medication also comes in 15- and 30-mg extended-release formulations. Concomitant use with MAO inhibitors is contraindicated. This drug should not be used during the acute recovery phase of myocardial infarction, or in patients with arrhythmias, heart block, congestive heart failure, or hyperthyroidism. The risk of seizures with tramadol is increased if this medication is added.[111] The risk of serotonin syndrome can be increased in patients taking SSRIs.[112]

### Carisoprodol

Carisoprodol in animal studies has shown muscle relaxation through altered interneuronal activity in the spinal cord and in the descending reticular formation of the brain. It is metabolized in the liver to meprobamate, a Schedule IV medication with abuse potential.[113] Meprobamate binds to $GABA_A$ receptors, which results in further sedation. Doses start at 250 mg, as often as four times per day, with a maximum dose of 1400 mg per day. It is contraindicated in patients with a history of acute intermittent porphyria. CYP1C19 inhibitors such as omeprazole or fluvoxamine may increase carisoprodol levels and decrease meprobamate. CYP1C19 inducers such as rifampin or St. John's wort increase exposure to meprobamate.

### Methocarbamol

Methocarbamol, a carbamate derivative of guaifenesin, is a CNS depressant with no direct action on striated muscle, the motor end plate, or the nerve fiber. It comes in 500- and 750-mg tablets and can be given as many as four times per day, with a maximum dose of 8 g per day. The parenteral form has been associated with skin sloughing and thrombophlebitis. It should be used with caution in patients with myasthenia gravis receiving anti-cholinesterase agents.

### Metaxalone

Metaxalone also has no direct actions on muscles. Its effects occur mainly through generalized CNS depression. It tends to cause much less sedation than other drugs in this class, and has limited drug interactions. It is contraindicated in drug-induced, hemolytic anemias and in significantly impaired renal or hepatic function. The dose most commonly used is 800 mg, three or four times per day.

### Chlorzoxazone

Chlorzoxazone is a centrally acting agent that acts primarily at the level of the spinal cord and subcortical areas of the brain, where it inhibits multisynaptic reflex arcs. Doses are 250 to 750 mg, three or four times per day. Hepatocellular toxicity has been reported with this drug.

### Orphenadrine

Orphenadrine is derived from diphenhydramine. It does not directly relax muscles and possesses greater anticholinergic properties than diphenhydramine. Its dosage recommendation is 100 mg, twice a day. It is contraindicated in patients with glaucoma, pyloric, or duodenal obstruction, stenosing peptic ulcers, prostatic hyperplasia or obstruction of the bladder neck, cardiospasm (megaesophagus), and myasthenia gravis.

### Efficacy

Clinical studies with these drugs are very limited, poorly controlled, and of short duration. One meta-analysis[114] found fair evidence that tizanidine and baclofen are roughly equivalent and are similar to diazepam for the treatment of spasticity. Investigators also state that data on efficacy for muscle relaxants with musculoskeletal conditions are limited, but for treatment compared with placebo, cyclobenzaprine, tizanidine, carisoprodol, and orphenadrine show a consistent trend favoring active treatment. Data demonstrating effectiveness for chlorzoxazone, methocarbamol, baclofen, and dantrolene are limited. Last, data for metaxalone are mixed.

# EMERGING TARGETS

## Nerve Growth Factor Inhibitors

Nerve growth factor (NGF) is a ligand to the tyrosine kinase (TrKA) receptor located on sensory neurons. Inflammation results in increased levels of NGF, which stimulate the TrKA receptor and lead to an increase in inflammatory mediators and increased sensitivity to pain. Overexpression of NGF also leads to a proliferation of sympathetic fibers, which may be important in the production of inflammatory and neuropathic pain. Because NGF is essential for the survival and development of sensory neurons, NGF inhibitors may lead to peripheral nerve dysfunction and neuropathies. Tanezumab, a recombinant humanized monoclonal antibody targeting NGF, is in phase II trials and is under development to treat pain associated with osteoarthritis, low back pain, and metastatic bone cancer.[115] In 2010, the FDA halted all clinical trials for this entire class of drug because of reports that the drugs are associated with osteonecrosis. In 2012, an advisory panel to the FDA voted unanimously to allow the trials to continue but without

concomitant use of NSAIDs, because patients on NSAIDs had greater joint damage. Studies using tanezumab in osteoarthritis of the knee and hip are promising. Tanezumab combined with NSAIDs is better than anezumab monotherapy; however, there are more adverse events with the combination therapy.[116,117]

## Cannabinoid Agonists

Cannabinoid receptors type 1 (CB1) are located at multiple locations in the peripheral and central nervous system, whereas CB2 receptors are located on inflammatory cells (monocytes, B/T cells, mast cells). CB2 activation results in a reduction in inflammatory mediator release, plasma extravasation, and sensory terminal sensitization. Activation of peripheral CB1 receptors results in a reduction in the release of pro-inflammatory terminal peptides and a reduction in terminal sensitivity. Activation of central CB1 receptors leads to reduced dorsal horn excitability and activates descending inhibitory pathways in the brain. The net result is a reduction in both pain and hyperalgesia. Inhaled cannabis has been extensively studied in various pain syndromes with mixed results. More recent well-controlled trials in neuropathic pain have shown promise.[118] Sativex is a sublingual spray containing a mixture of tetrahydrocannabinol and cannabidiol, which failed to reach the primary efficacy endpoint in a phase III cancer pain trial. Studies with cannabis in musculoskeletal pain are limited; however, it has been shown to reduce the pain of rheumatoid arthritis and chronic pain of various other causes.[119]

## AMPA/Kainate Antagonists

Glutamate, an excitatory amino acid neurotransmitter, has been implicated in pain perception. Two types of glutamate receptors are found in the CNS: ionotropic and metabotropic. Ionotropic receptors are further functionally categorized into NMDA and non-NMDA receptor subtypes (2-amino-3-[3-hydroxy-5-methylisoxazol-4-yl]propionic acid [AMPA] and kainate receptors).[120] AMPA and kainate receptors are prevalent within the dorsal horn of the spinal cord, and activation is thought to mediate rapid excitatory neurotransmission, resulting in central sensitization mechanisms. Tezampanel, an intravenously administered antagonist of AMPA and kainate receptors, has been shown to be effective in reducing human experimental pain.[121] NGX426, an oral prodrug of tezampanel, has been shown in experiments to be effective in reducing human pain.[122]

## Angiotensin II Type 2 Receptor Antagonists

Angiotensin II type 2 receptors (AT2) are expressed on small fibers and the dorsal root ganglion. Angiotensin converting enzyme inhibitors have failed to show any effects on pain, and attention has been directed at the more specific type 2 receptors. A phase II study with EMA-401 (an AT2 receptor antagonist) in postherpetic neuralgia in 183 participants resulted in significant reductions in the primary outcome measure of pain intensity. Patients responses on secondary outcome measures of pain relief onset, 30% and 50% responder rate, McGill pain questionnaire, and Patient Global Impression of change were also positive. The drug appears to be safe and well tolerated.[123]



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 68



# Nutrition and Rheumatic Diseases

Lisa K. Stamp • Leslie G. Cleland

**KEY POINTS**

- Nutritional factors can have pro-inflammatory effects, anti-inflammatory effects, or both.
- Nutritional factors have been implicated in the etiology of a variety of rheumatic disorders, including gout and rheumatoid arthritis (RA).
- Many patients with rheumatic disease believe diet plays an important role in their symptoms.
- Dietary supplementation with omega-3 fatty acids can reduce signs and symptoms of inflammation, as well as reduce the need for escalation of treatment with disease-modifying anti-inflammatory drugs and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with RA.
- RA, systemic lupus erythematosus, gout, and NSAID use have been linked to increased risk for thrombotic cardiovascular events. Fish oil supplements have been associated with improvement in multiple aspects of cardiovascular health and reduction in cardiac death.

Nutrition plays a role in the management of most chronic diseases. Physicians provide dietary advice to patients with diabetes, heart disease, and obesity as part of standard clinical care. Although the role of diet and nutrition is well established in the etiology and management of gout, the role of nutrition in other rheumatic diseases, such as rheumatoid arthritis (RA), is less well accepted. In general, dietary advice is not part of standard clinical practice for patients with inflammatory rheumatic diseases. Despite a widespread lack of conviction among physicians about the role of nutrition, many people with arthritis believe food plays an important role in their symptom severity, and approximately 50% will have tried dietary manipulation in an attempt to improve their symptoms.[1]

It is well recognized that inflammatory disease processes can interfere with nutritional status. The impact of nutritional factors on the inflammatory response has also been recognized. Furthermore, diets and lifestyles have changed considerably through industrialization, with significant effects on rates of obesity, food choices, and consumption of different dietary components. In light of this, the role nutrition in the etiology and management of rheumatic diseases is becoming increasingly important.

## NUTRITION AND THE INFLAMMATORY PROCESS

**KEY POINTS**

- Omega-3 fatty acids are immunoregulatory.
- Vitamin D has multiple immunosuppressive effects.
- Antioxidants can be acquired through the diet.
- Adipose tissue is metabolically active and has effects on the inflammatory response.
- Probiotics have anti-inflammatory properties.

### Role of Omega-3 Fatty Acids and the Inflammatory Process

#### Fatty Acid Biochemistry

Fatty acids are divided into three groups on the basis of the number of double bonds they contain. Saturated fatty acids have no double bond, monounsaturated fatty acids have one double bond, and polyunsaturated fatty acids (PUFAs) have two or more double bonds. C18 fatty acids are prominent in the diet and provide the index fatty acids for the above classification. PUFAs are further grouped according to the site of the double bond proximal to the methyl (omega) terminus as n-6 or n-3. Vertebrates do not have the enzymes required to introduce double bonds in the n-3 and n-6 positions; therefore these fatty acids must be obtained from the diet. Thus they are known as *essential fatty acids*.

In general, the Western diet contains more n-6 fats than n-3 fats because of the dominance in processed foods and the visible fats of soybean, safflower, sunflower, and corn oils, which contain the n-6 fat linoleic acid (LA; 18:2n-6). The n-3 homologue of LA, α-linolenic acid (ALA; 18:3n-3), is present in flaxseed oil, which is generally a minor dietary component. LA and ALA may be used in energy metabolism or be converted to the C20 fatty acids arachidonic acid (AA) and eicosapentaenoic acid (EPA), respectively. AA and EPA are then incorporated into cell membranes and tissues. EPA can be further metabolized to the n-3 fatty acid docosahexaenoic (DHA).

The critical process linking fatty acids and inflammation is the metabolism of AA and EPA to eicosanoids, which act

as inflammatory mediators. AA is metabolized via cyclooxygenase (COX) to n-6 eicosanoids (prostaglandin [PG] $E_2$, thromboxane [TX] $A_2$ or via 5-lipoxygenase [5-LOX] to n-6 leukotrienes [LTs]). Through the same pathways, EPA is metabolized to n-3 PGs and LTs, respectively. In comparison to the n-6 eicosanoids produced from AA, EPA is a poor COX substrate such that n-3 PGs are not as readily produced (Figure 68-1). EPA and DHA competitively inhibit production of most n-6 eicosanoids; prostacyclin ($PGI_2$) is an exception. Increased dietary consumption of n-3 fatty acids such as EPA increases the proportion of EPA incorporated in cellular membranes and tissues, partly at the expense of AA incorporation. The net result is an alteration in the balance of n-3/n-6 eicosanoid production (see Figure 68-1). EPA and DHA can also be metabolized through lipid oxygenase pathways to novel specialized pro-resolving lipid mediators (SPMs) of inflammation (see later).

### Pro-inflammatory Actions of Eicosanoids

In general, the n-6 eicosanoids ($PGE_2$ and $TXA_2$) are pro-inflammatory, whereas the n-3 eicosanoids are either less potent in their effects ($TXA_3$) or less abundant ($PGE_2$). $TXA_2$ promotes interleukin (IL)-1β and tumor necrosis factor (TNF) production by mononuclear cells,[2] whereas $PGE_2$ results in vasodilatation, increased vascular permeability, and hyperalgesia (see Figure 68-1). $PGE_3$ is edemagenic, although little is produced. $LTB_5$ is 10 to 30 times less potent than $LTB_4$ as a neutrophil chemotaxin.

### Effect of n-3 Fatty Acids on Pro-inflammatory Cytokine Production

IL-1β and TNF production may be reduced as a consequence of dietary n-3 fatty acid supplementation. Although some of this cytokine inhibition is mediated through effects on eicosanoids, there also appears to be eicosanoid-independent cytokine inhibition. Fatty acids may have direct effects on intra-cellular signaling mechanisms, including nuclear factor-κB (NF-κB) and peroxisome proliferator–activated receptor (PPAR)-γ, thereby affecting cytokine production.[3] There are also effects on the inflammasome, a protein complex that has a central role in control of the immune system. The NLRP3 inflammasome is activated by a variety of stimuli, including pathogenic organisms and monosodium urate crystals, that lead to production of IL-1β and IL-18.[4] n-3 Fatty acids have recently been shown to inhibit NLRP3-dependent caspase activation and IL-1β production.[5]

### Effects of n-3 Fatty Acids on Major Histocompatibility Expression

The number of major histocompatibility (MHC) molecules expressed on antigen-presenting cells (APCs) is an important determinant of T cell response to antigen. Patients with RA have high levels of class II MHC expression on T cells and synovial lining cells.[6] In vitro studies show that EPA and/or DHA reduces monocyte expression of human leukocyte antigen (HLA)-DR and HLA-DP molecules and reduces the ability of monocytes to present antigen to autologous lymphocytes.[7] Thus n-3 fatty acids may have an anti-inflammatory effect via suppression of pathogenic T cell activation through inhibition of APC function. A more recent murine study has shown that fish oil also has effects on MHC molecules on B cells and T cell organization at the immunologic synapse.[8]

### Effect of n-3 Fatty Acids on Adhesion Molecule Expression

Adhesion molecules expressed on endothelial cells and leukocytes mediate the transit of cells from the circulation into tissues. Intercellular adhesion molecule-1 (ICAM-1) and its cognate receptor, leukocyte function-associated antigen (LFA)-1, have been shown to be important in migration of leukocytes into inflamed synovium in animal models.[9] ICAM-1 blockade has also been reported to reduce disease activity in RA.[10] In vitro n-3 fatty acids decrease human monocyte ICAM-1 and LFA-1 expression.[7] In addition, dietary n-3 fatty acid supplementation reduces soluble ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) plasma concentrations.[11]

### Effect of n-3 Fatty Acids on Degradative Enzymes

Proteinases have a pivotal role in cartilage degradation and bone erosion. n-3 Fatty acids added in vitro can suppress proteinases a disintegrin and metalloproteinase (ADAM family) with thrombospondin-1 domains (ADAMTS)-4, ADAMTS-5, and matrix metalloproteinase (MMP)-3 in IL-1α-stimulated bovine chondrocytes.[12] This inhibition of chondrocyte proteases is a mechanism through which n-3 fatty acids may inhibit cartilage degradation and bone erosion.

The receptor activator of NF-κB (RANK)/ligand (RANKL)/osteoprotegerin (OPG) pathway is also important in bone pathophysiology in RA. Increased RANK/RANKL and decreased OPG contribute to bone erosion. Three months of dietary fish oil supplementation has been reported to decrease the RANK/OPG ratio, which may help prevent bone resorption that leads to erosions.[13]

### Importance of the Balance of n-3 and n-6 Fatty Acids in the Inflammatory Process

The balance of AA and EPA can be altered through dietary fatty acid intake. In humans, the conversion of dietary ALA to tissue EPA is inefficient and fish/fish oils are a more effective way to increase EPA and DHA in tissues. Changes in AA/EPA ratios in tissues have downstream effects on eicosanoid production and the resulting pro-inflammatory/anti-inflammatory environment. Dietary supplementation with fish oil in humans results in decreased production of $PGE_2$,[14] $TXA_2$,[14] and $LTB_4$[15] with increased production of $TXA_3$[16] and $LTB_5$.[17] These data provide a mechanistic basis for beneficial effects of dietary n-3 fatty acid supplementation in the control of inflammatory diseases. Dietary fish oil supplements have been shown to increase vascular production of prostacyclin ($PGI_2$).[18] Although the role of $PGI_2$ in inflammation is not well defined, it is a potent vasodilator and inhibits platelet aggregation, as well as disaggregating platelets. These effects favor maintenance of

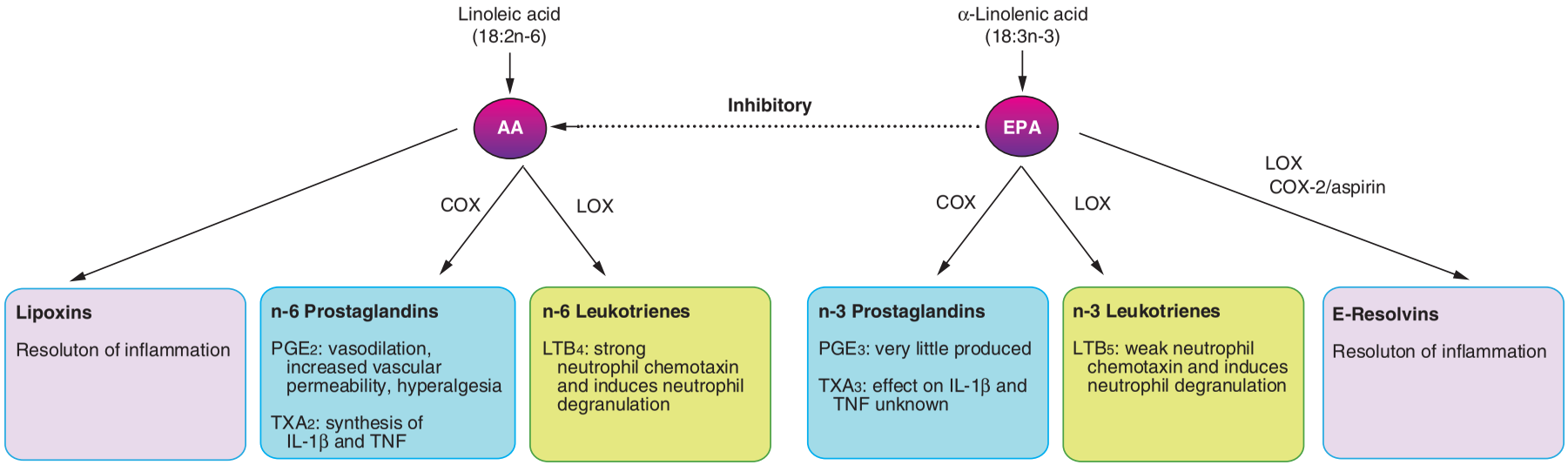



**Figure 68-1** Metabolism of linoleic acid and α-linolenic acid to n-6 and n-3 prostaglandins and leukotrienes. AA, Arachidonic acid; COX, cyclooxygenase; EPA, eicosapentaenoic acid; IL-1β, interleukin-1β; LOX, lipoxygenase; LTB, leukotriene B; PGE, prostaglandin E; TNF, tumor necrosis factor; TXA, thromboxane A.

vascular patency. Importantly, patients with several of the major rheumatic diseases (e.g., RA, systemic lupus erythematosus [SLE] and gout) are at high risk for serious cardiovascular events and mortality, to which nonsteroidal anti-inflammatory drug (NSAID)–associated COX-2 inhibition may contribute.

## SPECIALIZED PRORESOLVING MEDIATORS AND N-3 FATTY ACIDS

It has recently been recognized that resolution of inflammation is an active rather than passive process. SPMs, which include lipoxins, resolvins, protectins, and maresins, synthesized from the n-3 fatty acids EPA and DHA, have an important role in this process (Table 68-1). SPMs are formed through the actions of multiple lipid oxygenases (LO), including 5-LO and COX-2, with the yield of some SPMs increased by acetylation of COX-2 by aspirin (see Figure 68-1). Resolvins derived from EPA are known as *E-resolvins*, whereas those derived from DHA are known as *D-resolvins*. Production of resolvins can be increased by dietary n-3 fatty acid supplementation.[19] The resolvins have a variety of anti-inflammatory actions, including inhibition of TNF and IL-1β production and inhibition of human polymorphonuclear leukocyte transendothelial migration.[20] Resolvins have been shown to reduce pain and joint stiffness but not joint/paw edema in adjuvant-induced arthritis.[21] SPMs have been detected in synovial fluid from patients with RA, suggesting that these mediators may have local anti-inflammatory effects.[22] The presence and role of SPMs in other inflammatory rheumatic diseases has not been investigated to date.

### Vitamin D and the Inflammatory Process

Vitamin D has multiple immunosuppressive effects in addition to its effects on bone and calcium metabolism. The biologically active form of vitamin D (1,25-dihydroxyvitamin $[OH]_2D_3$) interacts with vitamin D receptors, which are expressed on a variety of cells, including osteoblasts, T cells, dendritic cells (DCs), macrophages, and B cells. These cells also have the capacity to convert more abundant $25(OH)D_3$ to $1,25(OH)_2D_3$ and to degrade $1,25(OH)_2D_3$. Accordingly, the molecular machinery is in place for important autocrine and paracrine effects of vitamin D at sites of inflammation.

DCs have a central role in activation of the immune system and in response to self. $1,25(OH)_2D_3$ inhibits the differentiation of monocyte precursors into mature DCs, down regulates expression of class II MHC molecules on DCs, inhibits IL-12 production, and promotes DC apoptosis, thereby inhibiting DC-dependent T cell activation.[23,24] In addition, $1,25(OH)_2D_3$ can promote DC expression of tolerizing functions, which instruct T regulatory (Treg) cells, which in turn may inhibit the development of autoimmunity.[25]

Vitamin D inhibits monocyte/macrophage pro-inflammatory cytokine production, including TNF, IL-6, and IL-1α.[26,27] Vitamin D has direct effects on T cells, in particular inhibition of proliferation and cytokine production by T helper (Th)1 cells, and may enhance Th2 cytokine production.[28] $1,25(OH)_2D_3$ has also been shown to reduce Th17 cell differentiation through its effects on DCs, as well as direct effects on Th17 cells that lead to inhibition of IL-17A, IL-21, IL-22, and IFN-γ production and enhancement of FOXP3, cytotoxic T lymphocyte–associated antigen (CTLA)-4, and IL-10.[25,29] Importantly, the more abundant, weakly active form of vitamin D, $25(OH)D_3$, can be converted by DCs to potently active $1,25(OH)_2D_3$, with consequent alteration in T cell responses toward an anti-inflammatory phenotype, which is characterized by high CTLA-4 and reduced IL-17, IFN-γ, and IL-21 production.[30] Thus the level of free $25(OH)D_3$ available to DCs may have a pivotal role in determining the balance between T cell inflammatory and regulatory responses.[30]

$1,25(OH)_2D_3$ inhibits the proliferation of activated B cells, induces activated B cell apoptosis, and inhibits plasma cell differentiation and immunoglobulin secretion.[31] Thus vitamin D deficiency may have a role in the etiology of B cell–mediated autoimmune disorders, whereas vitamin D supplementation may have beneficial effects in B cell–mediated autoimmune diseases, such as SLE and RA.

The array of relevant effects of vitamin D on the immune system suggests that vitamin D status may be important with regard to the etiology and management of rheumatic diseases more generally.

### Reactive Oxygen Species/Antioxidants and the Inflammatory Process

Production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are part of the

**TABLE 68-1** Effects of Adipokines

| Precursor Family | AA | EPA | DHA | | | |
|---|---|---|---|---|---|---|
| | Lipoxins | E-Resolvins | D-Resolvins | | Protectins | Maresins |
| | LXA4 | RvE1 | RvD1 | RvD2 | PD1 | MaR1 |
| **Actions** | ↓Pain signals<br>↓PMN adhesion<br>↓Angiogenesis and cell proliferation | ↑Macrophage phagocytosis<br>↑Neutrophil apoptosis<br>↓Organ fibrosis<br>inhibits NF-κB | ↑IL-10, ↓LTB4<br>↓Adhesion receptors<br>↓Pro-inflammatory cytokines TNF<br>↓Chemotaxis neutrophils | ↑PMN adhesion to endothelial cells | ↓NF-κB and COX-2 expression<br>↓TNF and IFN-γ<br>↓T cell migration | ↓Pain |

AA, Arachidonic acid; COX, cyclooxygenase; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; IFN, interferon; IL, interleukin; LT, leukotriene; LXA4, lipoxin A4; MaR1, maresin 1; NF-κB, nuclear factor-κB; PD1, protectin D1; PMN, polymorphonuclear neutrophil; RvD1, resolvin D1; TNF, tumor necrosis factor; ↑, increase; ↓, decrease.

normal immune response. Acting through transcription factors such as NF-κB, ROS increase production of pro-inflammatory eicosanoids and cytokines, including $PGE_2$, TNF, and IL-1β. Thus unchecked production of ROS may cause inflammation and tissue damage. Antioxidant enzymes such as superoxide dismutase and glutathione peroxidase remove superoxide, thereby providing protection from oxidative damage. Vitamin C (ascorbic acid), vitamin E (α-tocopherol), and β-carotene are acquired through the diet and can act as ROS scavengers.

## Obesity and the Inflammatory Process

With excess energy intake and reduced energy expenditure, body weight and adiposity increase. Obesity (body mass index [BMI] >30 kg/m$^2$) is a significant health problem globally. The World Health Organization estimates that by 2015, approximately 2.3 billion adults will be overweight, and more than 700 million will be obese.

Adipose tissue was originally thought to be simply a fat store. However, it is now recognized that adipose tissue and adipocytes are metabolically active and contribute to systemic inflammatory responses (Figure 68-2). Adipocytes release the pro-inflammatory cytokines TNF, IL-1β, and IL-6. IL-6 enters the systemic circulation and increases C-reactive protein (CRP) and serum amyloid A production by the liver.

Adipocytes produce adipokines: leptin, resistin, and visfatin (pro-inflammatory), and adiponectin (anti-inflammatory) (see Figure 68-2). Although the primary function of

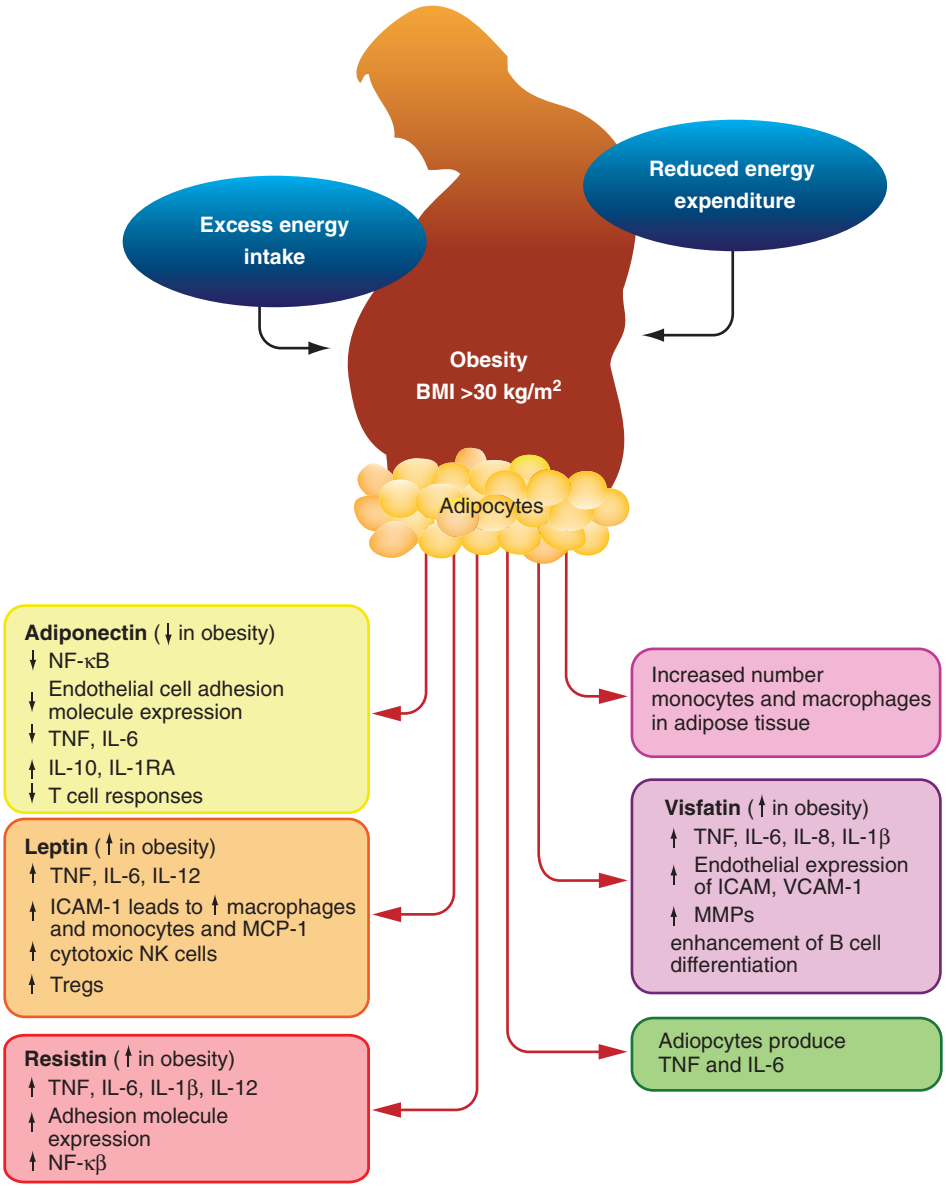



**Figure 68-2** Inflammatory mechanisms of obesity. BMI, Body mass index; ICAM, intercellular adhesion molecule; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MMPs, matrix metalloproteinases; NF-κB, nuclear factor-κB; NK, natural killer; Th, T helper; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.

leptin is appetite control, it also has a number of pro-inflammatory actions. Leptin increases the expression of adhesion molecules such as ICAM-1 and monocyte chemoattractant protein (MCP)-1, thereby favoring recruitment of monocytes/macrophages into adipose tissue. Leptin also increases IL-1β, TNF, and IL-6 production by monocytes/macrophages. Leptin increases proliferation of Th1 cells, and inhibits proliferation of Th2 cells and Treg cells. By contrast, adiponectin has anti-inflammatory effects, which include inhibition of TNF-induced adhesion molecule expression and inhibition of NF-κB, a pivotal intra-cellular factor in activation of inflammatory responses. Adiponectin also reduces production of TNF and IL-6 while promoting production of the anti-inflammatory cytokines IL-10 and IL-1RA. Adiponectin also increases the number of Tregs. Production of adiponectin is inhibited by TNF, which thereby helps sustain the pro-inflammatory alteration of homeostasis found in obesity. Resistin is produced by mononuclear cells and adipocytes. Resistin increases macrophage/monocyte and adipocyte production of TNF, IL-6, and IL-1β, and also increases expression of the adhesion molecules ICAM-1, VCAM-1, and MCP-1.[32] Visfatin, which is produced by lymphocytes and adipocytes, has similar pro-inflammatory effects, including induction of IL-8, IL-6, IL-1β, and TNF; increased endothelial expression of ICAM-1, VCAM-1, and MMPs; and enhancement of B cell differentiation.[32] The net result of obesity is thus an inflammatory state associated with an increase in circulating CRP.

## Probiotics and the Inflammatory Process

Probiotics are defined as live microorganisms, which confer a health benefit to the host. The most common probiotics are lactobacilli and bifidobacteria. These organisms are present in foods, often dairy fermented products such as yogurt, as well as in supplements such as probiotic drinks in lyophilized form. Probiotics have anti-inflammatory properties; the mechanism of anti-inflammatory action varies among different probiotics species and different species strains. Thus, although certain probiotic strains may confer health benefits, these benefits may not be seen with other probiotic species or strains.

Probiotics may exert their putative anti-inflammatory effects by interacting with intestinal epithelial cells. Resultant modulation of intestinal microflora and fortification of intestinal barrier function may lead to altered exposure of the immune system to microbes and direct effects on immune cells within the intestine. Associated effects relevant to anti-inflammatory responses include accumulation of $CD4^+$ Treg cells in inflamed areas[33]; inhibition of DC activation[34]; antagonism of NF-κB, which leads to decreased IL-1β and TNF production; and induction of the inflammation-modulating cytokine TGF-β.

## Summary

Dietary components can have a variety of effects on the inflammatory process and on bone destructive pathways (Table 68-2). Thus alterations in diet may have effects on both the risk and management of rheumatic diseases, as is discussed later in this chapter.

**TABLE 68-2** Effects of Dietary Components on Inflammatory and Bone Destructive Pathways

| |
|---|
| **n-3 Fatty Acids** |
| Decreased production of n-6–derived eicosanoids ($PGE_2$, $TXA_2$, $LTB_4$), which have pro-inflammatory effects |
| Increased production of n-3–derived eicosanoids ($PGE_3$, $TXA_3$, $LTB_5$), which in general are less pro-inflammatory |
| Decreased IL-1β and TNF production |
| Increased production of specialized proresolving lipid mediators (resolvins, protectins, maresins, lipoxins) |
| Decreased major histocompatibility complex II expression by antigen-presenting cells |
| Decreased adhesion molecule expression: ICAM, VCAM, LFA |
| Decreased expression of matrix metalloproteinases |
| Alteration in RANK/OPG ratio |
| **Vitamin D** |
| Inhibition of monocyte differentiation into DCs and promotion of DC apoptosis |
| Induction of tolerogenic Tregs with enhanced suppressive activity |
| Inhibition of monocyte/macrophage IL-1β and TNF production |
| Inhibition of Th1 cell proliferation and cytokine production |
| Enhancement of Th2 cytokine production |
| Reduction in Th17 cell differentiation and IL-17A production |
| Inhibition of proliferation of activated B cells, differentiation of activated B cells and immunoglobulin secretion |
| **Antioxidants** |
| Scavenge reactive oxygen species |
| Reduce pro-inflammatory eicosanoids, TNF, and IL-1β |
| **Probiotics** |
| Generation of $CD4^+$ Tregs in inflamed areas |
| Inhibition of DC activation |
| Decreased IL-1β and TNF production via inhibition of nuclear factor-κB |
| Increased production of transforming growth factor-β |

DC, Dendritic cell; ICAM, intercellular adhesion molecule; IL, interleukin; LFA, leukocyte function-associated antigen; LTB, leukotriene B; PGE, prostaglandin E; RANK/OPG, receptor activator of nuclear factor-κB/osteoprotegerin; Th, T helper; TNF, tumor necrosis factor; TXA, thromboxane A; VCAM , vascular cell adhesion molecule.

# NUTRITION IN THE ETIOLOGY OF RHEUMATIC DISEASES

## Rheumatoid Arthritis

**KEY POINTS**

- **Assessment of dietary intake is difficult, and identification of a single dietary factor that regulates disease might not be possible.**
- **Omega-3 fatty acids may protect against RA.**
- **The association between vitamin D and RA remains unclear.**
- **Obesity might affect disease activity and outcomes in RA.**

A number of epidemiologic studies have examined the role of nutrition in the etiology of RA. Assessment of dietary intake in epidemiologic studies is difficult, and to identify the effects of a single dietary variable and distinguish it from other nutritional and lifestyle factors may not be possible. Notwithstanding, a number of nutritional factors are candidates for an influence on the development of RA.

### Omega-3 Fatty Acid Consumption

The long-chain n-3 fats, EPA and DHA are most abundant in fish and fish oils. A protective effect against RA of fish intake has been reported. For example, the Seattle Women's Health Study showed reduced risk for RA in participants who consumed two or more fish meals per week with an adjusted odds ratio (OR) of 0.57 (95% confidence interval [CI], 0.35 to 0.93) compared with participants who consumed less than one fish meal per week.[35] Another population-based, case-controlled study reported a modest decrease in the risk of RA in subjects who consume oily fish one to seven times per week compared with those who seldom or never consumed fish (OR, 0.8; 95% CI, 0.6 to 1).[36] A subsequent long-term cohort study found that sustained consumption of fish was associated with reduced risk for RA (relative risk [RR], 0.48;95% CI, 0.33 to 0.71).[37]

### Red Meat and Protein Consumption

High consumption of red meat has been associated with an increased risk of inflammatory polyarthritis (OR, 1.9; 95% CI, 0.9 to 4.0).[38] Although one study reported that meat and offal were associated with an increased risk for RA,[39] this was not confirmed in other studies.[40,41] Whether the association between red meat consumption and inflammatory arthritis is causative remains unclear, although the presence of significant amounts of AA in red meat may provide some explanation for the association.

### Tea and Coffee Consumption

Tea and coffee have been identified as potential risk factors for RA. In the Finnish National Health Study, consumption of four or more cups of coffee per day was associated with an increased risk of rheumatoid factor (RF)-positive RA, but not RF-negative RA, after adjustment for potential confounders such as age, smoking, and gender (RR, 2.2; 95% CI, 1.13 to 4.27).[42] The Iowa Women's Health Study found no association between daily caffeine intake and risk for RA. However, women who consumed four or more cups of decaffeinated coffee per day were at increased risk for RA compared with non–coffee drinkers (RR, 2.58; 95% CI, 1.63 to 4.06). Furthermore, women who consumed three or more cups of tea per day had a reduced risk of RA (RR, 0.39; 95% CI, 0.16 to 0.97).[43] More recent studies have not shown an association between tea/coffee and RA.[40,44] A recent meta-analysis stratified by seropositivity reported a positive association between coffee consumption and seropositive RA (RR, 1.34; 95% CI, 1.16 to 1.52) but not seronegative RA (RR, 1.09; 95% CI, 0.88 to 1.35). No association was found between tea consumption and RA.[45]

Although the epidemiologic evidence for an association between coffee/tea and RA is equivocal, interest has been sustained by the presence of metabolically active agents within these beverages. For example, the major catechin in tea inhibits induction of inducible nitric oxide synthase (iNOS) by stimulated macrophages.[46] iNOS generates highly reactive free radical products while di-imidating substrate arginine moieties to citrulline. Citrullinated peptides/proteins are recognized immunogens that provide a focus for autoimmunity in RA. Green tea extract has been shown to increase chemokine receptor expression and reduce chemokine production in RA synovial fibroblasts.[47] Gallic acid, a natural polyphenolic acid found in tea, has been reported to induce apoptosis of RA fibroblast-like synoviocytes (FLS) and to reduce the expression of IL-1 and IL-6 genes in RA FLS.[48]

### Alcohol Consumption

Alcohol consumption may reduce the risk for RA. In a case-control study of 515 patients with RA, alcohol consumption was associated with a reduced risk of anti-citrullinated protein antibody (ACPA)-positive RA.[49] A dose-dependent inverse relationship between alcohol consumption and risk of RA was also demonstrated in two independent case-control studies (the Swedish Epidemiologic Investigation of Rheumatoid Arthritis [EIRA] and the Danish case-control study of rheumatoid arthritis [CACORA] studies).[50] The reduction in risk of RA was more pronounced in patients with the shared epitope compared with those without the shared epitope and most pronounced in smokers with the shared epitope.[50] In the most recent study, with use of data from the Nurses Health Studies I and II involving 1.90 million years of person-time from 1980 to 2008, there was a modest reduction in the risk of RA with long-term moderate alcohol use. The adjusted hazard ratio (HR) for alcohol use of 5 to 9.9 g/day was 0.78 (95% CI, 0.61 to 1.00), with a stronger association observed in those with seropositive disease (HR, 0.69; 95% CI, 0.50 to 0.95).[51]

With regard to candidate mechanisms for this putative reduction in risk for RA, alcohol has been shown to downregulate the production of pro-inflammatory cytokines and upregulate production of the anti-inflammatory cytokine IL-10.[52,53] Furthermore, in a murine model of arthritis, ethanol almost totally prevented the development of collagen-induced arthritis, and in those mice that did develop arthritis, the disease was less severe. These anti-inflammatory effects of ethanol were associated with reduced leukocyte migration, downregulation of NF-κB, and reduced production of the pro-inflammatory cytokines IL-6 and TNF, but not the anti-inflammatory cytokine IL-10.[54]

### Sugar-Sweetened Beverages

Sugar-sweetened beverage consumption has been associated with gout and other inflammatory diseases, including diabetes. In the Nurses' Health Study I and II, women who consume one or more sugar-sweetened beverage per day have an increased risk for seropositive RA (HR, 1.63; 95% CI, 1.15 to 2.30) compared with women who consume no or less than one serving per month. This effect remained significant after adjustment for BMI. No association between sugar-sweetened beverage consumption and seronegative

RA was observed.[55] Sucrose consumption has been implicated in the development of periodontal infection, which has been linked to the development of RA. Soda drinks, which contain high-fructose corn syrup and sucrose, have been associated with higher CRP, IL-6, and TNF receptor-2 concentrations in patients at risk of cardiovascular disease (CVD).[56] However, the exact mechanisms linking sugar-sweetened beverages and RA remain to be determined.

### Vitamin D

As noted earlier, vitamin D has anti-inflammatory actions. Although vitamin D has been implicated in reducing the risk of the autoimmune diseases, diabetes,[57] and multiple sclerosis, its association with the risk of RA is less clear.[58]

The Iowa Women's Health Study reported that a higher intake of vitamin D was associated with a reduced risk of RA (RR, 0.67; 95% CI, 0.44 to 1.00, $P = 0.05$) in women aged 55 to 69 years.[59] However, in a large study of 186,389 women followed up for 22 years, no association was found between dietary intake of vitamin D and the risk of developing RA.[60] Apart from supplements, the main source of vitamin D is de novo synthesis in the skin, thus estimated dietary intake may be a poor predictor of serum vitamin D concentrations. In a study of 79 patients with RA, no association was found between prior serum vitamin D concentrations and subsequent development of RA.[61] However, it is notable that the geometric means for both patients and controls in this study were only half the lower reference level of 60 nmol/L, which has been set subsequently to reflect a level that suppresses secondary hyperparathyroidism resulting from vitamin D insufficiency. A recent meta-analysis revealed a reduced risk of RA with higher vitamin D intake (RR of highest vs. lowest intake groups, 0.76; 95% CI, 0.58 to 0.94).[62] Randomized controlled trials of sufficient size and duration are required to determine whether the observed relationship between vitamin D and RA is causal and to establish the most effective dose, duration, and serum vitamin concentration to prevent RA.

### Antioxidants and Risk of Rheumatoid Arthritis

Oxygen free radicals (e.g., nitric oxide, superoxide, and hydroxyl radical) are implicated in the tissue damage observed in RA.[63] Antioxidants, including vitamin E (α-tocopherol), vitamin C (ascorbic acid), β-carotene, and selenium, may have a protective role against tissue damage caused by oxygen free radicals. This fact, combined with evidence that markers of antioxidant nutritional status are lower in patients with established RA compared with healthy controls,[64] led to the hypothesis that antioxidants may protect against the development of RA. Despite this biologic plausibility, the available data do not provide clear evidence for a protective effect of antioxidants as dietary supplements in relation to the development of RA.

Low serum concentrations of vitamin E, β-carotene, retinol, and selenium have been reported to be weakly associated with an increased risk for RA in some, but not all, studies.[65-67] The strongest association between risk of RA and antioxidants was found for a combined antioxidant index, rather than for any specific antioxidant.[65]

High dietary intake of β-cryptoxanthin (a carotenoid found in fruit and vegetables) and zinc may protect against the development of RA.[68,69] Low vitamin C intake has been associated with an increased risk of inflammatory polyarthritis with an adjusted OR of 3.3 (95% CI, 1.4 to 7.9) for the lowest tertile of vitamin C intake (<55.7 mg/day) compared with the highest intake tertile (>94.9 mg/day).[70] However, another study found no association between intake of vitamin C, vitamin E, zinc, or selenium and the development of RA.[40]

The Women's Health Study, a randomized, double-blind, placebo-controlled trial designed to evaluate the effects of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer, has been used to address the influence of vitamin E supplementation (600 IU on alternate days) on the development of RA. During the 10-year follow-up period, 106 cases of RA occurred with no significant association between vitamin E supplementation and incidence of cases of RA. A statistically insignificant trend toward inverse association between vitamin E and development of seropositive RA was seen on subgroup analysis.[71]

### Obesity and Rheumatoid Arthritis

Studies that examined the association between obesity and the risk of RA have had conflicting results, with findings of both increased risk of RA[72,73,74] and no association between the two.[75,76] The most recent study, a prospective cohort study that followed more than 200,000 women during a 30-year period, reported an increased risk of RA associated with high BMI in patients diagnosed with RA before age 55 years. Furthermore, if women were obese at age 18 years, they had a 35% increased risk for RA, which reached an almost 50% increased risk with regard to seropositive RA.[74]

The association between obesity and a pro-inflammatory state provides a rational link between disease activity and severity in RA. Higher BMI has been associated with less radiographic damage.[77-80] Increased plasma concentrations of the adipokines, leptin, adiponectin, and visfatin have been observed in patients with RA compared with healthy controls.[81] Visfatin and leptin have been associated with increased and reduced radiographic joint damage, respectively,[80] although more recent data suggest that adipokines do not mediate the observed association between BMI and radiographic damage.[78] Although radiographic damage may be less in patients with higher BMI, other indicators of disease severity and comorbidities are adversely affected. For example, higher BMI (≥30 kg/m$^2$) has been associated with higher disease activity, Health Assessment Questionnaire (HAQ) scores, pain scores, and requirement for joint replacements.[82] Higher BMI is also associated with less chance of achieving disease remission or low disease activity.[82,83] Obese patients with RA are also at increased risk for important comorbidities, including hypertension, diabetes, and ischemic heart disease.[82,84] The cause of the apparent disconnect between obesity and disease severity and radiographic damage remains unclear, but one potential explanation is altered body composition in RA, which may create an inaccurate BMI measurement.

The body can be divided into fat mass and fat-free mass (consisting of body cell mass and connective tissue).

Rheumatoid arthritis commonly leads to changes in body composition, with fat-free mass reported to be 13% to 14% lower in patients with RA.[85] This loss of fat-free mass is often associated with increased fat mass with unchanged body weight, a state known as *rheumatoid cachectic obesity*. Approximately one in five RA patients may have rheumatoid cachexia, as defined by fat-free mass index lower than the 25th percentile, and fat mass index higher than the 50th percentile of a reference population.[86] BMI, the commonly used measure of obesity, does not distinguish between fat mass and fat-free mass, thus individuals of similar height and weight, but different body composition, will have a similar BMI. Patients with RA have been shown to have higher body fat and BMI than healthy controls. However, for a given mass of body fat, BMI was lower in patients with RA compared with healthy controls.[87] On the basis of this, the authors suggest that the categories of BMI should be adjusted downward in patients with RA.

## Gout

| KEY POINTS |
|---|
| The link between diet and gout has been recognized for centuries. |
| High intake of meat, seafood, and alcohol is associated with increased risk of gout. |
| Dietary fructose increases serum urate. |
| Higher intake of low-fat dairy products has been associated with a reduced risk of gout. |
| Increased BMI predisposes to gout. |

For centuries gout has been associated with overindulgence in rich food and wine. Gout occurs when serum urate (SU) concentrations reach super saturation concentrations (~6.8 mg/dL at 37° C), resulting in the formation of monosodium urate crystals, which deposit in joints and soft tissues. Urate is the end product in the breakdown of purines, which are a product of cellular turnover or are ingested in the diet. The purines adenosine and guanine are present in nucleic acids and the intra-cellular energy transporters adenosine triphosphate and guanosine triphosphate. Accordingly, foods derived from metabolically active animal tissues may increase dietary purine load.

### Dietary Factors and Gout

Several large clinical studies have identified an association between high intake of meat and seafood (but not total protein intake) and both SU concentrations[88] and gout.[89] In comparison, the risk of gout is reduced with higher intake of low-fat dairy products and long-term coffee consumption.[89,90] More recently, fructose, which is found in corn syrup, sugar-sweetened soft drinks, and fruit juices, has been associated with hyperuricemia and gout.[91,92] Fructose increases SU through increased purine degradation.[93] Furthermore, urate and fructose share a common transporter within the kidney (*SLC2A9*).[94] Recent data shows a gene environment interaction between fructose and the renal urate transporter *SLC2A9*[95] and variations in *SLC2A9* influence the serum urate response to a fructose load.[96] Alcohol consumption other than moderate wine intake is associated with an increased risk of gout, with beer conferring a higher risk than liquor.[97]

Dietary vitamin C supplementation has been shown to reduce the risk of gout (RR of gout with no supplementation vs. 1000 to 1499 mg vitamin C/day, 0.66 [95% CI, 0.49 to 0.88]).[98] Vitamin C (ascorbic acid) is an important vitamin that can only be obtained through dietary intake. Because ascorbic acid is water soluble, it is not stored within the body and thus must be regularly supplemented through the diet to maintain the ascorbic acid pool. Dietary sources of ascorbic acid include fresh fruits and vegetables, in particular citrus fruits and green leafy vegetables such as broccoli.

### Fasting and Gout

Prolonged periods (2 weeks to 8 months) of fasting in obese patients result in a significant increase in SU and in some cases the development of gout.[99] In patients with previously documented gout, a 1-day fast led to an increase in SU of 0.5 to 2.1 mg/dL with a mean rise of 1.1 mg/dL, with refeeding associated with a return in SU to baseline levels after 24 hours.[100] Explanations for the increase in SU during fasting include increased urate production, decreased excretion because of decreased glomerular filtration rate, altered renal tubular transport of uric acid, and competition with ketones for renal tubular excretion.[101]

### Obesity and Gout

High BMI predisposes to gout.[102] In a study comparing obese men (mean BMI, $34 \pm 4$ kg/m$^2$) with healthy controls (BMI, $21 \pm 1$ kg/m$^2$), SU concentrations were raised to a similar degree in the obese patients (~$8.0 \pm 1.6$ mg/dL vs. controls, $5.2 \pm 0.81$ mg/dL), regardless of body fat distribution (predominantly visceral or predominantly subcutaneous). By contrast, 80% of patients with hyperuricemia and accumulation of fat subcutaneously had low 24-hour urinary urate excretion compared with 10% of their counterparts with fat accumulation predominantly in a visceral distribution. These data suggest that the mechanism of hyperuricemia may vary depending on body fat distribution.[103] In a separate study that used computed tomography to determine transverse area of abdominal fat components, visceral fat was shown to correlate strongly with SU, whereas no relationship was seen between SU and BMI or cross-sectional area of subcutaneous fat.[104]

## Osteoarthritis

| KEY POINTS |
|---|
| Obesity is associated with knee osteoarthritis (OA). |
| Direct biomechanical effects of obesity contribute to OA. |
| Increased leptin provides another link between obesity and OA. |

Associations have been established between obesity and onset and progression of knee OA. There is an almost

exponential increase in the risk of knee OA while BMI increases.[105] The evidence for an association between obesity and OA of the hip or hand is less well defined, although a meta-analysis reported a twofold increase in the risk of hand OA in obese individuals compared with those of normal weight.[106] In addition to direct biomechanical effects of obesity on the joint, there appears to be an important role for the adipokines, including leptin, adiponectin, resistin, visfatin, and chimerin.

Expression of leptin was increased in advanced OA cartilage compared with minimally damaged OA cartilage, with a correlation between the leptin mRNA expression in advanced OA cartilage and BMI. Furthermore, leptin was shown to reduce chondrocyte proliferation and increase IL-1β, MMP-9, and MMP-13 expression.[107]

Dietary antioxidants (vitamin E, vitamin C, and β-carotene) have been reported to reduce progression of knee OA but have no effect on the onset of OA.[108,109] The association between serum vitamin D concentrations and OA remains unclear. One study reports no association between vitamin D and risk of joint space narrowing or cartilage loss in knee OA,[110] but another study reports a positive association between knee cartilage volume and serum vitamin D concentrations.[111] A recent meta-analysis concluded that there was moderate evidence for an association between low levels of vitamin D and radiographic progression of knee OA and strong evidence for an inverse association between vitamin D and cartilage loss.[112]

## NUTRITION IN THE MANAGEMENT OF RHEUMATIC DISEASES

The role of nutrition in the management of gout is well accepted. However, its role in other rheumatic diseases such as RA is less routine. Despite a relative lack of interest or emphasis from physicians, many patients consider food may contribute to their arthritis and seek information about or try putative dietary remedies. A convincing, informed approach to nutritional advice is thus an important aspect of management, which can help patients avoid worthless interventions that are expensive, time consuming, in some cases harmful, and can divert patients from more effective measures (summarized in Figure 68-3). Furthermore, positive advice regarding dietary choices may empower the patient at a time when they often experience a sense of loss of control. Well-informed, authoritative dietary advice can also protect patients from poorly grounded advice from relatives, friends, and nonauthoritative Internet sites about dietary measures.

### Rheumatoid Arthritis

**KEY POINTS**

**Omega-3 fatty acids modestly reduce disease activity and NSAID requirements.**

**There is no evidence for the benefit of antioxidants in the management of RA.**

**Fasting, vegetarian/vegan, and elimination diets are difficult to sustain, and it is difficult to predict which patients may respond.**

**The vascular benefits of omega-3 fatty acids are important in RA patients because they have an increased risk of cardiovascular disease.**

**Some dietary variables may interact with methotrexate.**

#### Dietary n-3 Fatty Acids in the Management of Rheumatoid Arthritis

Fish oil is a rich source of the anti-inflammatory long-chain n-3 fatty acids EPA and DHA. The threshold dose of EPA and DHA usually required to obtain an anti-inflammatory effect is 2.7 g/day of EPA plus DHA, which is the equivalent of nine or more standard 1 g fish oil capsules or 10 mL of bottled fish oil per day. This dose is generally more than that with which patients will self-prescribe, although community awareness of required doses varies. Tissue levels of EPA and DHA from fish and fish oils are increased when dietary n-6 fatty acid intake is reduced concomitantly through substitution of n-6 rich visible fats (e.g., with a base of corn oil, soy oil, sunflower oil) with unsaturated products with less n-6 fat (with a base of olive oil, rapeseed/canola oil, or flaxseed oil).[113]

Krill oil has been promoted as a potent form of marine oil that allows an anti-inflammatory dose of omega-3 fats to be taken within a single capsule. There is no substance to these claims as there are no published trials of krill oil treatment in RA, and krill oil has less EPA plus DHA than standard fish oil (approximately 25% for krill oil vs. 30% for fish oil).[114] The EPA and DHA are present within phospholipids and as free fatty acids in krill oil and within triglycerides in fish oil.[115,116] EPA and DHA are moderately better absorbed from krill oil such that equal gravimetric doses of krill oil and fish oil supplements achieve similar blood levels of omega-3 fatty acids.[114-116] Krill oil contains astaxanthin, which gives it a reddish color and has antioxidant properties. As discussed elsewhere in this chapter, there is no evidence for important antiarthritic or anti-inflammatory effects of antioxidants. There have been no studies into the effect of astaxanthin in RA. Because krill oil is substantially more expensive than fish oil and, in contrast to fish oil, has not been subjected to systematic study of anti-inflammatory effects, krill oil is not a suitable substitute for fish oil.

In general, patients with recent-onset or more established RA can be expected to achieve better disease control when anti-inflammatory doses of fish oil are co-administered with appropriately intensive combination disease-modifying anti-rheumatic drug (DMARD) therapy. In a longitudinal cohort study of patients with recent-onset RA (<12 months' duration), response-driven, intensive combination DMARD therapy was combined with either supplemental fish oil or placebo. At 3 years, those patients compliant with fish oil therapy had improved self-reported function in activities of daily living, lower tender joint counts, lower erythrocyte sedimentation rate (ESR), higher remission rates (72% vs. 31%), and less NSAID use than those who did not consume fish oil.[117] The better outcome for patients taking fish oil is consistent with randomized controlled trial data that show reduced symptoms with fish oil compared with placebo treatment. This study also demonstrates the feasibility of long-term fish oil use, in this case

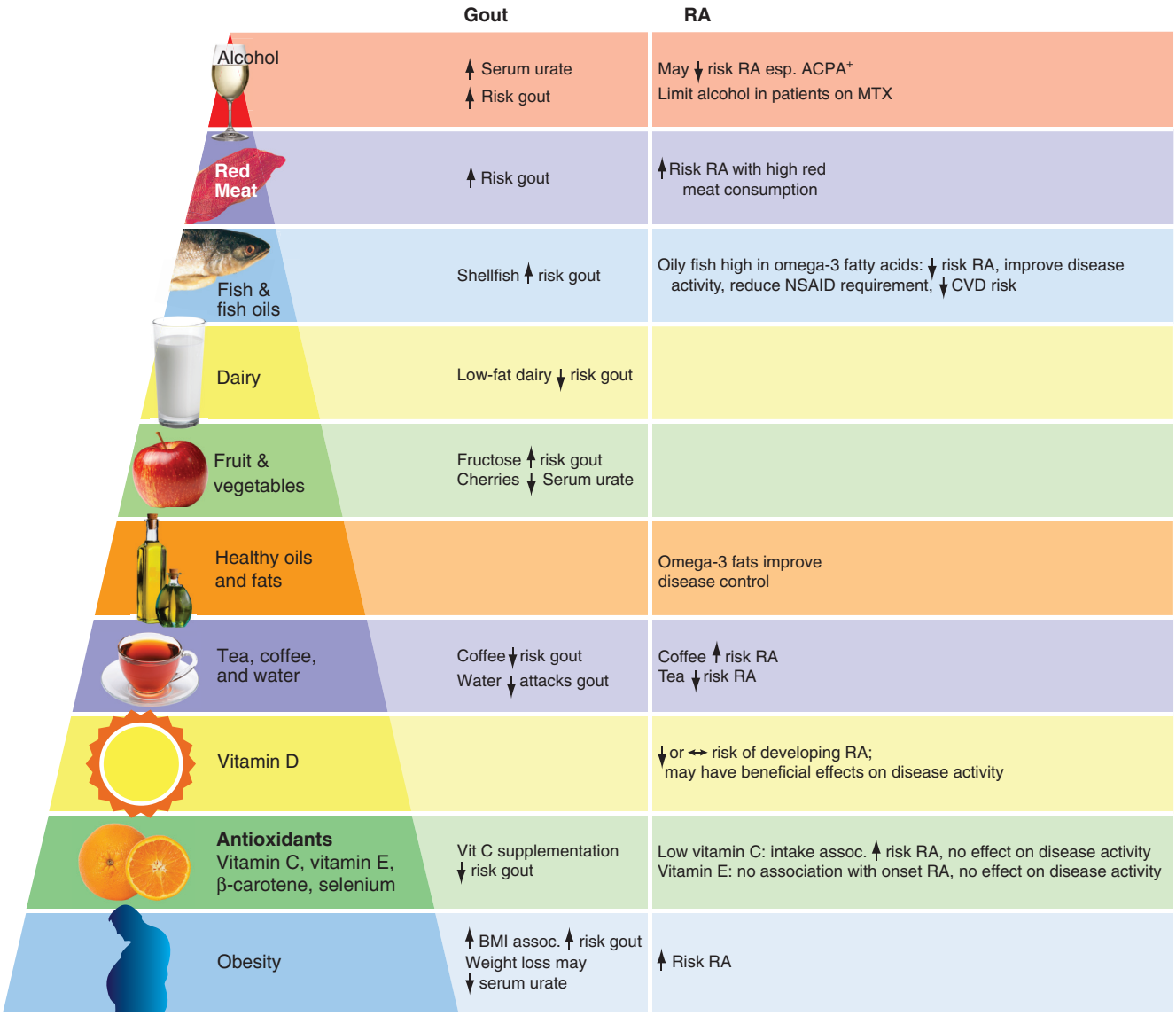



**Figure 68-3** Summary of nutrition in rheumatic diseases. ACPA, Anti-citrullinated protein antibody; BMI, body mass index; CVD, cardiovascular disease; MTX, methotrexate; NSAID, nonsteroidal anti-inflammatory drug; RA, rheumatoid arthritis; Vit, vitamin.

more than 3 years. Although fish oil usage may identify more compliant patients, overall DMARD usage was similar in the two groups. A more recent study compared high-dose fish oil to low-dose fish oil, 5.5 or 0.4 g/day, respectively, of the omega-3 fats, EPA + DHA, in DMARD naïve patients with early RA. Fish oil was used in combination with a treat-to-target predetermined intensive combination DMARD protocol. During 12 months of treatment, patients receiving high-dose fish oil were more likely to achieve ACR remission (HR, 2.17;95% CI, 1.07 to 4.42; $P = 0.03$) and triple therapy with methotrexate, sulfasalazine, and hydroxychloroquine (HR, 0.28; 95% CI, 0.12 to 0.63; $P = 0.002$) was less likely to fail.[118]

Anti-inflammatory doses of fish oil have been shown to reduce NSAID requirements in patients with RA in other studies.[119,120] NSAIDs alter the balance of $PGE_2/TXA_2$ in favor of $TXA_2$, thereby increasing the production of IL-1β and TNF by monocytes.[121] By contrast, fish oil has been shown to reduce the production of these cytokines.[14] Fish oil also lacks many of the adverse effects associated with NSAIDs (Table 68-3). The direct effects of fish oil and their influence on NSAID use may potentially reduce long-term tissue damage associated with release of these cytokines, although this remains to be established.

Important for both patients and physicians to recognize is that, as with most standard DMARDs, there is a latent period of as long as 15 weeks before the symptomatic benefits of anti-inflammatory doses of fish oil are experienced. One small pilot study has shown that this latent period can be reduced with intravenous administration of n-3 fats.[122] Although the inconvenience and cost of intravenous therapy is a barrier to this approach, the benefits of short-term intravenous therapy can be prolonged by a switch to oral n-3 supplementation.[123]

**TABLE 68-3** Comparison between Nonsteroidal Anti-inflammatory Drugs and Anti-inflammatory Doses of Fish Oil

| | NSAIDs | Fish Oil |
|---|---|---|
| COX inhibition | COX-1/COX-2 selectivity varies depending on agents | Nonselective |
| NSAID sparing | No | Yes (↓ prostaglandin $E_2$) |
| Serious cardiovascular events | Increased risk for MI | Reduced cardiac mortality |
| Blood pressure | Increased | Reduced |
| TNF and IL-1β | Increased | Reduced |
| Upper gastrointestinal bleeding | Increased | Not reported |
| Time to effect | Rapid | Delayed (≤3 mo) |

COX, Cyclooxygenase; IL-1β, interleukin-1β; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug; TNF, tumor necrosis factor.

**Adverse Effects of Fish Oils.** The most common adverse effects associated with fish oil in anti-inflammatory doses are a fishy aftertaste, gastrointestinal upset, and nausea. These side effects are clearly neither organ nor life threatening, but they can be dose limiting. Patient preferences vary, but in general, bottled fish oil layered on juice is the most efficient way to take an anti-inflammatory dose (one quick swallow compared with 10 to 15 standard fish oil capsules). Fish oil is best tolerated with food and not on an empty stomach. In general, fish oil is best tolerated when take just before the evening meal or other main meal of the day. The term *fish oil* defines oil prepared from fish bodies to provide a distinction from cod liver oil, which is prepared from fish livers and is rich in the fat-soluble vitamins A and D. Standard fish oil contains more EPA plus DHA (30% w/w) than cod liver oil (EPA plus DHA ~20%) and is the preferred material. Vitamin D can be given separately as needed, and vitamin A supplementation is best avoided because negative effects on bone mineral density and fracture risk have been reported.[124]

Although anti-inflammatory doses of fish oil have not been associated with serious toxicity, concerns may be raised. One consideration is a putative bleeding tendency associated with therapeutic fish oil ingestion. This notion has its origins in studies of Greenland Eskimos consuming their aboriginal diet, in whom myocardial infarction is rare and bleeding times were prolonged relative to those observed in Danes.[125] However, these concerns need to be placed within the context of the high amounts of EPA and DHA in the aboriginal Eskimo diet (more than double the threshold anti-inflammatory dose) and the lower amounts of antagonistic n-6 fatty acids compared with Western diets. Although bleeding episodes have not been a feature of long-term fish oil therapy for RA,[117] and platelet EPA is fourfold lower than found in the Eskimos, surgeons may ask that fish oil be discontinued before elective surgery. Also, pharmacists may advise patients to discontinue fish oil while taking warfarin, even though no increase in bleeding tendency has been seen in patients undergoing cardiac surgery when taking fish oil concomitantly with warfarin, aspirin, or aspirin plus clopidogrel therapy.[126,127] More recent clinical trials also suggest that higher doses of fish oil can be taken for prolonged periods without increased bleeding events or other serious adverse effects.[118,128]

Two recent studies have shown an apparent protective effect of low plasma phospholipid DHA against prostate cancer.[129,130] However, the warning of the authors regarding therapeutic use of fish oil is problematic for several reasons. Neither study was designed to examine the effects of fish oil supplementation on prostate cancer risk, and the observed levels of DHA were low and in the range that is only weakly associated with dietary intake. Furthermore, the influence of intake of red meat and animal fat, which have been associated with increased risk for prostate cancer and are an important dietary sources of EPA and DHA in those who infrequently or rarely eat fish, was not considered as a potential confounder.[131]

Another concern is the possible presence of the environmental contaminants methylmercury, polychlorinated biphenyls (PCBs), and dioxins, which are concentrated in large carnivorous fish. These toxins are excluded from properly prepared fish oil for therapeutic use. The U.S. Food and Drug Administration has accorded "generally regarded as safe" status to intakes of as much as 3 g/day of long-chain n-3 fatty acids from marine sources.

**Role of Omega-3 Supplementation in Rheumatic Diseases with Increased Cardiovascular Disease Risk.** A number of rheumatic diseases, including RA, SLE, gout, and psoriatic arthritis are associated with increased cardiovascular mortality. n-3 Fatty acids have the potential to alter cardiovascular risk through multiple mechanisms, including stabilization of the myocardium, leading to reduction in cardiac arrhythmias, reduced blood pressure, stabilization of atheromatous plaques, reduced triglycerides and increased high-density lipoprotein (HDL), decreased platelet thromboxane release, increased vascular prostacyclin release, and anti-inflammatory effects (Figure 68-4). A recent meta-analysis of trials of n-3 and n-6 fatty acid supplements at various doses and with various cardiac endpoints reported no reduction in overall cardiac events, although a significant association between plasma long-chain n-3 PUFA levels and reduced cardiac events was found.[132] A meta-analysis of randomized trials of omega-3 supplements and particular cardiovascular outcomes showed a modest but significant reduction in cardiac death.[133] The effect size appeared to have reduced in more recent studies, possibly as a result of more intensive management of tractable cardiovascular risk factors.

To date there have been no specific studies of the cardiovascular benefits of n-3 fatty acid supplementation in patients with RA or other rheumatic diseases. However, patients with early RA receiving fish oil have been shown to have lower triglycerides, increased "good" HDL cholesterol, less NSAID use, greater disease suppression, and reduced platelet synthesis of $TXA_2$, all of which can be expected to reduce cardiovascular risk.[117] The blood levels of patients taking EPA plus DHA achieved with anti-inflammatory doses of fish oil[117] are above the threshold associated with reduced risk for sudden cardiac death.[134]

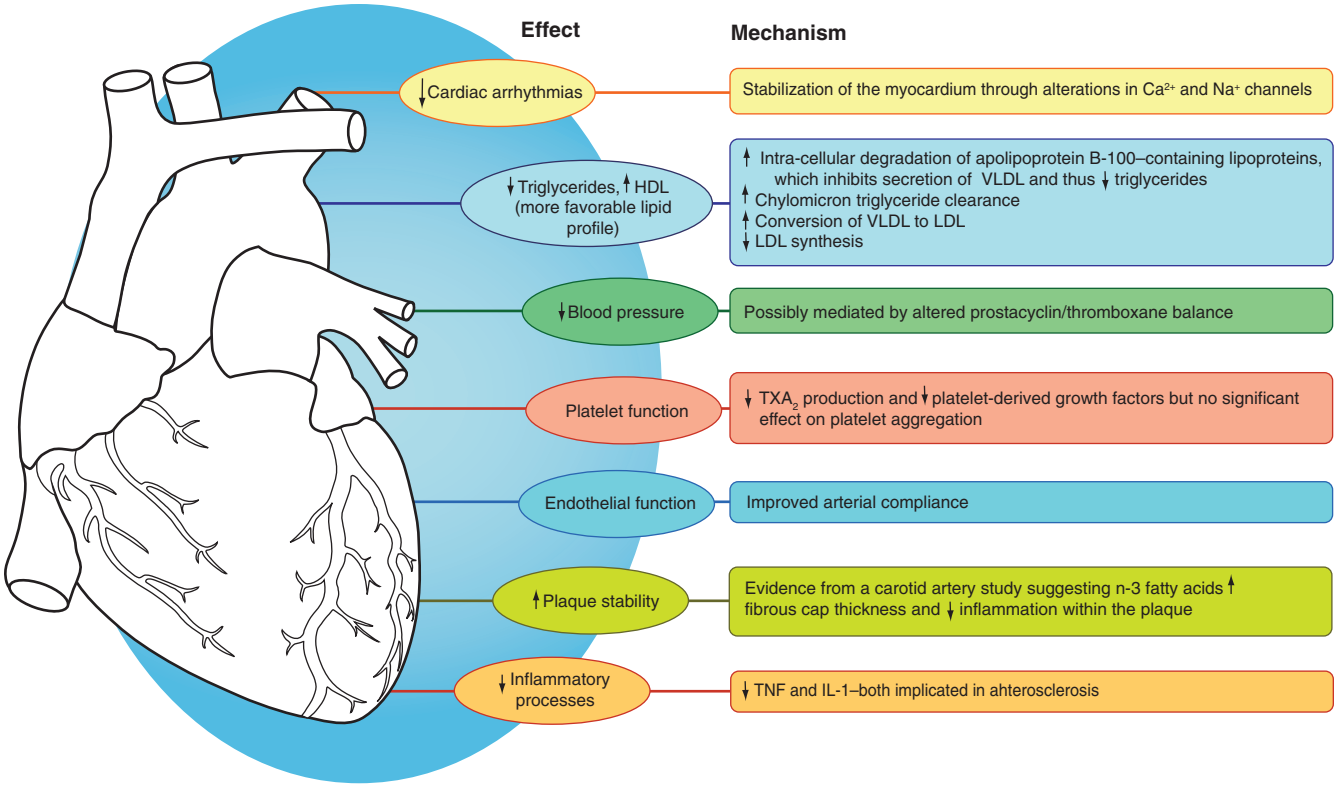



**Figure 68-4** Effects of omega-3 fatty acids on the cardiovascular system. HDL, High-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; TNF, tumor necrosis factor; $TXA_2$, thromboxane $A_2$; VLDL, very-low-density lipoprotein.

## Antioxidants in the Management of Rheumatoid Arthritis

Despite the association between ROS and RA, a recent meta-analysis shows no convincing evidence that antioxidant supplementation improves disease control in patients with RA.[135]

**Vitamin E.** Serum concentrations of vitamin E are similar in patients with RA and healthy controls.[136] In rats with CIA fed 200 mg/kg of vitamin E for 4 weeks, a significant reduction in leptin, TNF, and IL-6 concentrations was seen compared with control-fed mice.[137] A 12-week placebo-controlled study of vitamin E supplementation in patients with RA showed a reduction in pain scores but no effect on joint tenderness score, duration of morning stiffness, swollen joint count, or laboratory parameters.[138]

**Vitamin C.** Although animal models have shown benefits with vitamin C supplementation, human studies have not shown any clinical benefit in patients with RA.[139]

**Selenium.** Although not an antioxidant per se, selenium is found at the active site of the enzyme glutathione peroxidase, an important antioxidant enzyme. Plasma concentrations of selenium are reduced in patients with RA compared with healthy controls,[140] and an inverse association between serum selenium concentrations and active joints has been reported.[141] Notwithstanding, studies of selenium supplementation have not shown clinical benefit in patients with RA, despite increases in serum and red blood cell selenium concentrations.[142,143] However, polymorphonuclear cell selenium concentrations do not increase with dietary supplementation, which may explain the lack of clinical effect.[144]

## Vitamin D and the Management of Rheumatoid Arthritis

Animal models have shown that $1,25(OH)_2D$ can prevent and have therapeutic benefits in experimental arthritis.[145] Studies examining the relationship between serum vitamin D concentrations and disease activity in RA have been conflicting: Both an inverse relationship[146,147] and no relationship[148] have been observed. However, increases in patient global visual analogue scores seen in patients with lower serum vitamin D may confound results of the disease activity scale (DAS)28.[149]

In humans, the vitamin D receptor has been shown to be expressed at sites of cartilage erosion and in chondrocytes and synoviocytes in patients with RA but not healthy control subjects, suggesting that vitamin D may have local effects within the inflamed joint.[150] Supplemental vitamin D is usually prescribed to patients with RA for treatment or prophylaxis of osteoporosis. However, a 12-month trial of vitamin D (calciferol) 100,000 IU/day showed improvement in RA disease activity and a reduction in the requirement for analgesics and NSAIDs.[151] In another small study of 19 patients with RA, oral α-calcitriol 2 μg/day was observed to have a beneficial effect on disease activity compared with placebo.[152] Neither significant side effects nor increases in serum calcium were reported in either of these studies. Vitamin D has been reported to induce TNF production, suggesting that it may alter responses to TNF inhibitor therapy in RA. A recent study showed that $1,25(OH)_2D_3$, but not TNF blockade, suppressed IL-17A and IL-22 production in co-cultures of Th17 cells and RA synovial fibroblasts from patients with early RA.

Furthermore, the combination of $1,25(OH)_2D_3$ and TNF blockade resulted in a significant additive effect with reductions in IL-6, IL-8, MMP-1, and MMP-3.[153] These data suggest there may be a rationale for patients receiving TNF inhibitor therapy to receive supplemental vitamin D.

It is important to recognize that cod liver oil, a rich source of dietary n-3 fatty acids, is also rich in vitamin D. Cod liver oil has been used in the management of RA in sufficient doses to deliver anti-inflammatory doses of long-chain n-3 fatty acids.[154] Although serum vitamin D concentrations rose significantly, the study did not allow for the respective contributions of vitamin D and co-ingested n-3 fatty acids to be evaluated. The relatively high content of vitamin A in cod liver oil militates against its use for delivering anti-inflammatory doses of n-3 fatty acids.

### Dietary Restriction in Rheumatoid Arthritis—Fasting, Vegetarian, and Elimination Diets

A number of studies have examined elimination of different nutrients from the diet in the management of RA. At the extreme, total fasting and subtotal fasting reduced clinical and laboratory parameters of disease activity in some patients within a few days, but deterioration occurred on reintroduction of food.[155] Thus fasting is an impractical management strategy because it should last no longer than 7 days and the positive effects are short-lived, whereas RA is a chronic disease. A number of potential mechanisms for this improvement have been postulated, including a psychological or placebo effect, weight loss, and immunosuppression as a result of reduced caloric intake, alteration in fatty acids and IL-6 concentrations, and alterations in gut flora.

Elemental diets are used with the aim of providing major food groups as simpler components (e.g., protein as free amino acids, fat as medium-chain triglycerides, carbohydrates as small sugars). Minimal general benefit was seen in only a minority of patients, with a lack of benefit observed in objective parameters such as inflammatory markers, and swollen and tender joint counts.[156,157]

Vegetarian and vegan diets have also been studied in RA. Response to these diets has been variable, with poor patient compliance and high dropout rates resulting from both lack of efficacy and adverse effects (mainly nausea and vomiting).[158,159] More recently, a meta-analysis of four clinical trials of fasting, followed by vegetarian diets that lasted at least 3 months, showed that clinically significant long-term benefits in RA can occur.[160] However, the diet required is strict, and no means currently exist to predict which patients will respond.

Elimination diets involve avoidance of foods that are putatively allergenic. Foods are deemed to be "allergenic" if their elimination results in decreased disease activity and subsequent ingestion results in increased disease activity. Although such diets may result in improvement in a proportion of patients, variable responsiveness and compliance can be a significant limitation.[161,162] A systematic review of dietary studies in patients with RA highlighted the uncertainty of response, high risk of bias in existing studies, and the not insignificant risk of adverse effects.[163] Notwithstanding, from a practical perspective, if a patient believes their arthritis is caused by hypersensitivity to a particular food item or group, it may be worth assessing the effects of dietary avoidance in an $n = 1$ study of sequential withdrawal and challenge. Objective clinical and laboratory signs of disease activity should be documented after one or more cycles of withdrawal and reintroduction of the suspected food item. The findings can then be used as a basis to decide whether the food item should be avoided by the individual in the longer term.

### Interactions between Diet, Obesity, and Disease-Modifying Anti-rheumatic Drugs

It is well recognized that certain dietary components may interact with DMARDs. Methotrexate remains the anchor drug in the management of RA and is also used commonly in many other rheumatic diseases. Alcohol may increase the risk of hepatotoxicity in patients receiving methotrexate, and alcohol intake should be moderate at most. Methotrexate has multiple potential actions, many of which can be traced to its action as a dihydrofolate reductase inhibitor. Routine supplementation with folic acid is recommended to reduce potential adverse effects associated with methotrexate.[164] However, higher red blood cell folate concentrations have been associated with higher disease activity in patients with RA receiving methotrexate.[165] In some countries fortification of flour, pasta, rice, and bread with folic acid is a governmental requirement. Not surprisingly, such dietary fortification may lead to increased methotrexate doses in some patients.[166]

Methotrexate treatment increases extra-cellular accumulation of adenosine, which has multiple anti-inflammatory effects on neutrophils and macrophages, including inhibition of IL-1β and TNF synthesis. Caffeine is a methylxanthine and acts as an adenosine receptor antagonist. Caffeine may therefore interfere with the effects of methotrexate. In rats with adjuvant arthritis, caffeine reverses the anti-inflammatory effects of methotrexate.[167] In patients with RA, less intense caffeine intakes have been shown to have no effect on methotrexate efficacy.[168] In patients treated with methotrexate 7.5 mg per week, without folate, responses were reduced in those consuming caffeine in doses greater than 180 mg/day compared with doses less than 120 mg/day.[169]

Obesity may also have an impact on response to therapy. Several studies have reported an association between high BMI (≥25 $kg/m^2$) and poor response to therapy, particularly infliximab in patients with RA.[83,170-172]

## Gout

**KEY POINTS**

- **Dietary interventions in gout should address the association between gout and the metabolic syndrome and increased cardiovascular disease.**
- **Dehydration is a trigger for acute gout.**
- **Weight loss may reduce serum urate.**

Sustained reduction of SU to less than 6 mg/dL, or less than 5mg/dL if tophi are present, is critical for the effective management of gout. This is generally achieved through a

combination of urate-lowering therapy and dietary/lifestyle modifications. Dietary intervention on its own is insufficient to achieve target serum urate concentrations. When considering dietary interventions in the management of gout, it is important to recognize that gout is associated with the metabolic syndrome and with an increased risk of cardiovascular disease and mortality. It is important to note that there are no randomized controlled trials to support dietary and lifestyle interventions in gout.

The diet usually advocated for gout entails restricted intake of foods and drinks that contain higher amounts of purines or that are thought to precipitate acute attacks of gout. Restricted items include meat, seafood, beer/wine, and legumes. Such diets are frequently high in saturated fats and carbohydrates, which may add to the risk of metabolic syndrome.

Dehydration is a potential trigger for acute gout attacks, and increased water intake has been associated with a reduced risk of gouty attacks.[173] In a small short-term study in healthy volunteers, ingestion of cherries reduced serum urate (before urate, 3.6 ± 0.2 mg/dL vs. after urate, 3.1 ± 0.25 mg/dL; $P$ <0.05) and increased urinary urate excretion.[174] It has been suggested that cherries may prevent gout flares. In a study of 633 individuals, cherry intake during a 2-day period was associated with a 35% lower risk of gout attacks compared with no cherry intake (OR, 0.65; 95% CI, 0.5 to 0.85). A similar association was observed with cherry extract. Furthermore, when cherry intake was combined with allopurinol treatment, the risk of gout was 75% lower than during periods without either (OR, 0.25; 95% CI, 0.15 to 0.42).[175] In another small retrospective study of 24 patients with crystal proven gout consuming one tablespoon of tart cherry juice for greater than or equal to 4 months, there was a significant reduction in gout flares from 6.85 ± 1.34 flares/year to 2.0 ± 0.6 flares/year ($P$ = 0.0086).[176] The exact mechanism of action of cherries in reducing gout flares remains unclear and is likely multifactorial. Cherry products contain high levels of anthocyanins that have a number of anti-inflammatory and antioxidant effects, including inhibition of cyclooxygenase and scavenging of nitric oxide radicals.[177-179] Cherry juice has also been shown to inhibit production of IL-1β by human monocytes ex vivo by approximately 60%.[176]

Vitamin C supplementation may reduce SU concentrations.[180,181] In a study of 184 patients without gout randomly assigned to receive to placebo or vitamin C 500 mg/day for 2 months, SU was reduced significantly in the vitamin C group, with a mean reduction of –0.5 mg/dL (95% CI, –0.5 to 0.02 mg/dL) compared with the placebo group ($P$ <0.0001).[182] In the subgroup of 21 patients with baseline SU greater than 7 mg/dL, the mean reduction in serum urate was 1.3 mg/dL. However, in a small study of patients with gout, vitamin C, 500 mg/day for 8 weeks had no clinically significant urate-lowering effect alone or in combination with allopurinol.[183] Whether a larger dose of vitamin C has a more potent urate-lowering effect in patients with gout remains to be determined.

In obese patients, weight loss may reduce SU[184,185] and provide other health benefits. In one study, 13 males with gout lost weight during a 16-week period (prestudy BMI, 30.5 ± 8.1 kg/m$^2$; poststudy BMI, 27.8 ± 7.9 kg/m$^2$). SU decreased in all participants; baseline SU, 9.6 ± 1.7 mg/dL, falling to 7.9 ± 1.5 mg/dL; $P$ = 0.001. A decrease in frequency of gouty attacks was seen from 2.1 ± 0.8 attacks/month to 0.6 ± 0.7 attacks/month ($P$ = 0.002).[186] In a study of patients with type 2 diabetes, bariatric surgery, which resulted in marked weight loss, was associated with a significant reduction in serum urate. Furthermore, in the subgroup of patients with gout, serum urate was below the treatment target of 0.36 mmol/L at 12 months despite cessation of urate-lowering therapy.[187] However, not all studies have shown a reduction in SU with weight loss.[188]

There are currently no data on whether n-3 fatty acid supplementation has a role in the management of gout. However, given the effects of n-3 fatty acids on the inflammasome, they may be useful in the management of acute gout or, potentially, as prophylaxis against acute gout in conjunction with urate lowering therapy. Further clinical studies in these situations are required. Gout is often associated with hypertriglyceridemia, upon which anti-inflammatory doses of fish oil can be expected to have an ameliorating effect.[117,189]

## Osteoarthritis

**KEY POINTS**

**Weight loss is important in the management of OA.**

**From a mechanistic viewpoint, n-3 fatty acids may be beneficial and can be an alternative or adjunct to NSAIDs for long-term analgesia.**

Weight loss is generally considered an important aspect of the management of OA of weight-bearing joints. A meta-analysis of weight loss studies in patients with knee OA reported that moderate weight loss (≥5%) is associated with statistically significant improvement in self-reported disability scores. Although knee pain was also reduced, this did not reach statistical significance.[190] In a study of 44 patients with knee OA, a mean weight loss of 20% 6 months after bariatric surgery was associated with a significant improvement in pain and Western Ontario and McMaster osteoarthritis index (WOMAC) scores. Furthermore, a significant reduction in serum IL-6 and CRP was found, with a significant increase in the N-terminal propeptide of type IIA collagen, a biomarker of cartilage synthesis.[191]

Degradation and loss of articular cartilage and synovial inflammation are all characteristic features of OA. MMPs have an important role in cartilage degradation, and n-3 fatty acids have been shown to reduce IL-1α–induced expression of MMP-3 and MMP-13 in bovine chondrocyte cultures.[12] The anti-inflammatory effects of n-3 fatty acids, as compared with n-6 fatty acids discussed previously, may reduce synovial inflammation.

A high-fat diet rich in n-3 fatty acids has been shown to mitigate the effects of obesity on injury-induced OA in mice and to accelerate wound repair. By contrast, a high-fat diet rich in n-6 fatty acids was found to increase the severity of the OA.[192] OA-prone guinea pigs fed on a n-3 fatty acid–rich diet had amelioration of OA of a severity similar to that observed in an OA-resistant guinea pig strain.[193] An inverse relationship between plasma total n-3 fatty acids and DHA and patellofemoral cartilage loss, but not

tibiofemoral cartilage loss or synovitis, has been reported in patients with or at risk of knee OA.[194] Taken together, these studies suggest that n-3 fatty acid supplementation may be beneficial in reducing/preventing structural progression in OA.

To date there are limited human data on the role of n-3 fatty acid supplementation in OA. A 2-year study of high- versus low-dose fish oil used to treat OA revealed clinically significant improvements in pain and disability scores in both groups, with similar improvement at 12 months and greater improvement at 24 months in those receiving low-dose fish oil.[195] This finding suggests that the lower dose of n-3 fatty acids may be appropriate in OA. Further placebo controlled studies of n-3 fatty acid supplementation in OA are warranted.

On the basis of epidemiologic studies, as well as the role of vitamin D in bone health, two clinical trials examining the role of vitamin D supplementation in osteoarthritis have been undertaken. In patients with symptomatic knee OA, no difference was found between oral vitamin D treatment, at doses sufficient to elevate 25-hydroxyvitamin D plasma to higher than 36 ng/mL, and placebo treatment after 2 years with regard to knee pain or MRI-assessed loss of cartilage volume.[196] In a placebo-controlled study of 107 patients with symptomatic knee OA taking 25(OH) vitamin D at greater than or equal to 50 nmol/L, treatment with cholecalciferol 60,000 IU per day for 10 days, followed by 60,000 IU once a month for 12 months resulted in a small but statistically significant reduction in pain and WOMAC scores.[197] Further studies are required to determine the effects of vitamin D supplementation on progression of OA and to better define patients to whom it should be offered.

### Probiotics in the Management of Rheumatic Diseases

**KEY POINT**

**Insufficient data exist to support routine use of probiotics in inflammatory rheumatic diseases.**

Although most interest has focused on the role of probiotics in inflammatory bowel diseases, there are a number of studies in animal models of arthritis, as well as human studies in RA and spondyloarthritis.

Probiotics have been reported to enhance the antiarthritic effects of methotrexate in adjuvant-induced arthritis in rats,[198,199] whereas in collagen-induced arthritis, probiotics downregulated Th1 effector cells, leading to suppression of joint inflammation and reduction in cartilage destruction.[200]

In studies of patients with rheumatic diseases, results have varied depending on the probiotic administered. In a study of 21 patients with RA, 12 months of supplementation with *Lactobacillus rhamnosus* had no effect on disease activity.[201] By contrast, patients with RA who were treated for 8 weeks with *Lactobacillus casei* had a reduction in serum TNF, IL-6, and IL-12, and a reduction in disease activity.[202,203] In a 60-day study of 45 patients with RA, improvement in pain and patient global scores were seen with *Bacillus coagulans* GBI-30 supplements compared with placebo.[204] In 63 patients with active spondyloarthritis, no difference in outcomes was found after 12 weeks of probiotic supplement containing *Streptococcus salivarius*, *Bifidobacterium lactus*, and *Lactobacillus acidophilus* compared with placebo.[205] Alterations in gut microbial flora may influence the absorption and metabolism of drugs. Of particular importance for patients with rheumatic diseases are potential effects on DMARDs, especially sulfasalazine, which is metabolized to the active metabolites sulfapyridine and 5-aminosalicylic acid by intestinal microbes. Probiotics that alter intestinal microflora may potentially alter efficacy and toxicity of sulfasalazine. Although alterations in sulfasalazine metabolism with probiotic supplements have been demonstrated in an animal model, similar effects were not observed in a human study.[206]

In summary, although theoretic considerations and empiric data provide some support for use of probiotics in RA, the evidence is not sufficient to recommend routine use.

## CONCLUSION

Rheumatic diseases are typically chronic, and, as with any chronic disease, nutritional issues are intrinsic to optimization of long-term health. Although patients as individuals can make their own nutritional choices, it is beneficial for rheumatologists to be adequately informed regarding the plausibility and evidence for frequently considered nutritional supplements, toward which this chapter has been directed. In terms of positive advice for patients and referring physicians, the strongest case can be made for dietary supplementation with fish oil in adequate doses for inflammatory arthritis. For long-term analgesia in rheumatic diseases, fish oil can be recommended in favor of NSAIDs. To the extent that fish oil is taken instead of NSAIDs, the risks for serious upper gastrointestinal complications and increased risk for serious thrombotic cardiovascular events associated with NSAIDs can be avoided because fish oil has not been associated with these risks. Furthermore, fish oil has been shown to reduce cardiovascular risks through mechanisms independent of and additional to its NSAID-sparing effects.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 69

# Etiology and Pathogenesis of Rheumatoid Arthritis



Gary S. Firestein

**KEY POINTS**

- Rheumatoid arthritis (RA) is a complex disease involving numerous cell types, including macrophages, T cells, B cells, fibroblasts, chondrocytes, neutrophils, mast cells, and dendritic cells.
- Several genes are implicated in susceptibility to RA and severity of disease, including class II major histocompatibility complex (MHC) genes, *PTPN22,* peptidylarginine deiminases, and many genes that regulate immune responses.
- Evidence of altered immunity, including high serum levels of potentially pathogenic antibodies that bind to modified proteins (e.g., by citrullination), can be present years before the onset of clinical arthritis and result, in part, from environmental stress or the microbiome of mucosal surfaces.
- Adaptive immune responses directed against citrullinated proteins could account for the class II MHC gene associations because the modified peptides bind with high avidity to the RA-associated MHC proteins and can be efficiently presented to T cells.
- Innate immune responses such as complement activation or Toll-like receptor activation can contribute to the initiation and perpetuation of synovial inflammation.
- Stromal elements, especially fibroblast-like synoviocytes, contribute to the pathogenesis of disease by producing cytokines, proteases, and small molecule mediators of inflammation.
- Cytokine networks involving TNF, IL-6, and many other factors participate in disease perpetuation and can be targeted by therapeutic agents.
- Bone and cartilage destruction are primarily mediated by osteoclasts and fibroblast-like synoviocytes, respectively.

Rheumatoid arthritis (RA) is perhaps the most common inflammatory arthritis, affecting from 0.5% to 1% of the general population worldwide. Although the prevalence is surprisingly constant across the globe, regardless of geographic location and race, there are some exceptions. For instance, the occurrence of RA is somewhat lower in China (~0.3%), whereas it is substantially higher in other groups, such as the Pima Indians in North America (~5%). Because of its prevalence and the ready accessibility of joint samples for laboratory investigation, RA has served as a useful model for the study of all inflammatory and immune-mediated diseases. As such, the information gleaned from these studies provides new and unique insights into the mechanisms of normal immunity.

RA is primarily considered a disease of the joints, but abnormal systemic immune responses are evident and can cause a variety of extra-articular manifestations such as vasculitis, nodules, and accelerated atherosclerosis. These manifestations clearly show that RA has features of a systemic disease that can involve many organs. In some cases, autoantibody production with the formation of immune complexes that fix complement contribute to these extra-articular findings. One of the mysteries of RA is why the synovium is the primary target, although the unique structure of its vascular bed could provide an environment that is ideal for innate and adaptive immune responses.

Although the precise causes of RA remain uncertain, environmental and genetic influences clearly are factors. Clues have been provided by detailed immunogenetic studies and the observation that underlying autoimmunity antedates the onset of arthritis by up to a decade. Progress in understanding the pathogenesis has been even more robust: The roles of small-molecule mediators of inflammation (e.g., arachidonic acid metabolites), autoantibodies, cytokines, growth factors, chemokines, adhesion molecules, epigenetics, aberrant signal transduction, and matrix metalloproteinases (MMPs) have been carefully defined. Synovial cells can exhibit aggressive behavior and can invade and destroy articular cartilage, subchondral bone, tendons, and ligaments. Irreversible loss of articular cartilage and bone begins soon after the onset of RA, and early interventions can probably improve long-term outcomes. Increased appreciation of how comorbidities, especially cardiovascular disease and accelerated atherosclerosis, can affect mortality has also led to attempts to suppress synovial and systemic inflammation.

## HOW RHEUMATOID ARTHRITIS BEGINS

The cause and pathogenesis of RA are complex and multifaceted. A variety of predetermined factors (genes) and stochastic factors (random events and environment) contribute to susceptibility and pathogenesis. As an introduction, a summary of how these mechanisms interact to create

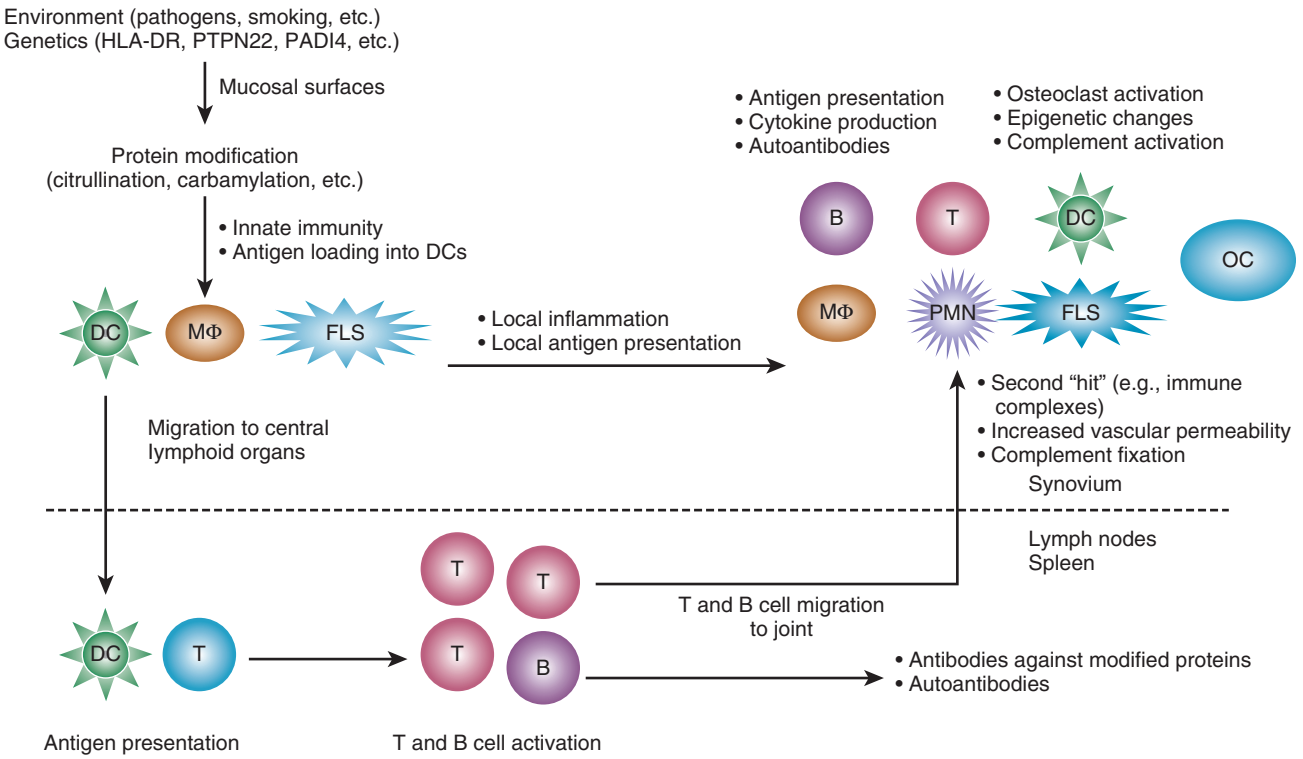



**Figure 69-1** Disease mechanisms that likely occur in rheumatoid arthritis. Innate immunity could activate fibroblast-like synoviocytes (FLS), dendritic cells (DC), and macrophages (MΦ) in the earliest phases in persons with underlying immune hyperreactivity directed toward modified peptides, such as through citrullination with antibody production. The genetic makeup of an individual, including the presence of certain gene polymorphisms in genes that regulate immune responses and environmental exposures, are both required. Chronic inflammation leads to citrullination of proteins in a variety of sites, including mucosal surfaces such as the lungs or the joint. In a genetically susceptible person, these modified peptides are processed by DCs, which can migrate to the central lymphoid organs to present antigen and activate T cells, which can in turn activate B cells. The antibodies can trigger synovial inflammation through a second "hit," perhaps as a result of activation of synovial mast cells by immune complexes. The antibodies can then fix complement in the joint and release chemoattractants that attract lymphocytes to the synovium and enhance adaptive immune responses in the target organ. In addition, repeated activation of innate immunity can directly lead to chronic inflammation and possibly antigen presentation in the synovium. In the latter phases of disease, many cell types activate osteoclasts (OC) through the receptor activator of nuclear factor-κB (NF-κB)/receptor activator of NF-κB ligand (RANK/RANKL) system, although FLS and T cells likely provide the greatest stimulus. Autonomous activation of FLS also might contribute to this process. HLA-DR, Human leukocyte antigen-DR; PADI, peptidyl arginine deiminase; PMN, polymorphonuclear neutrophil.

and perpetuate RA is shown in Figure 69-1. Individual mechanisms are discussed in greater detail throughout the chapter.

The initiation of RA begins years before the onset of clinical symptoms. The onset involves certain specific genes that can lead to the production of pathogenic antibodies that bind to modified proteins, help break tolerance, and lead to autoreactivity. It is likely that the earliest phases are marked by repeated environmental stress, either through toxic exposures or activation of innate immunity (see Figure 69-1).[1] Cigarette smoke, bacterial products, viral components, and other environmental stimuli can contribute to these responses. These stresses induce enzymes that lead to the modification of normal peptides, most commonly through conversion of arginine to citrulline but possibly through other pathways (e.g., the conversion of lysine to homocitrulline).

This circumstance occurs often in healthy persons, although some populations, most notably Asians, have genetic associations that can favor increased citrullination through increased expression of peptidyl arginine deiminase (PADI) 4. In most people the process is self-limited and has no untoward effects. However, in people with class II major histocompatibility complex (MHC) genes implicated in RA, these altered peptides bind very tightly into the antigen-binding groove and are presented to T cells far more efficiently than the native unmodified protein. Because T cells are not exposed to the altered peptide during development, they are not deleted in the thymus and can respond with a normal adaptive immune response directed against these new epitopes.

These exposures most likely occur at mucosal surfaces, such as the lung, gut, and oral cavity, although modification of peptides also can occur in the synovium itself. Altered peptides are loaded into dendritic cells (DCs), and antigen presentation has the potential to occur in the synovial germinal centers or, more commonly, in central lymphoid organs after the loaded DCs migrate via the lymphatics. T cells are activated and can produce pathogenic cytokines such as IL-17 at these sites and help B cells produce antibodies. These antibodies are most commonly directed against citrullinated peptides but could also recognize other altered peptides, such as through carbamylation. The anti-citrullinated protein antibodies (ACPAs) are produced

against a variety of proteins, including fibronectin, vimentin, α-enolase, histones, and many others.

Over a period of years, the titers of ACPAs with broad specificity increase many fold.[2] There is not an individual "RA-associated" ACPA but instead a general rise of the many different antibodies. Findings of synovial biopsies during the pre-RA time, even if individuals have arthralgias, are essentially normal.

The ACPAs themselves are not sufficient to initiate synovitis, and a "second hit" is clearly necessary. The nature of the second signal is not well defined, but in animal models it can occur with the formation of immune complexes. These complexes do not have to be specific for altered peptides but can actually be totally irrelevant, such as to viruses or other xenoantigens. They can engage Fc receptors in the synovium, especially on mast cells, which lead to local release of vasoactive mediators and increase access of ACPAs to the joint. Once in the joint, if they encounter their antigen, they can fix complement and initiate a cascade of events leading to recruitment of innate and adaptive immune cells, production of additional cytokines and chemokines, and activation of local fibroblast-like synoviocytes (FLS) and macrophages. As the cells are recruited and activated, ultimately a tipping point is reached and clinical synovitis ensues.

Additional genetic associations amplify the normal adaptive response to modified proteins, true autoreactivity against the native proteins through epitope spread, or local synovial inflammatory responses. T cell signaling polymorphisms can favor these events (e.g., protein tyrosine phosphatase-22 [PTPN22]). Cytokine promoter polymorphisms, signal transduction gene polymorphisms, and other genetic markers also could provide positive feedback to enhance the response. Epigenetic changes that imprint synovial cells through DNA methylation, microRNA (miRNA) production, or histone modification can enhance local immune dysregulation.

Ultimately, a destructive phase proceeds, which can have antigen-dependent and antigen-independent mechanisms and is mediated by mesenchymal elements such as fibroblasts and synoviocytes. Bone erosions are subsequently caused by activated osteoclasts, whereas cartilage dissolution results from proteolytic enzymes produced by synoviocytes in the pannus or synovial fluid neutrophils. Anti-inflammatory mechanisms such as soluble TNF receptors, suppressive cytokines, cytokine-binding proteins, protease inhibitors, lipoxins, antioxidants, anti-angiogenic factors, and natural cytokine antagonists are not present in sufficient concentrations to truncate the inflammatory and destructive process. Suppressing this response requires therapeutic interventions that either modulate pathogenic cells or neutralize the effector molecules produced by the rheumatoid process, or restore tolerance.

Highly targeted therapeutics to individual cytokines, mediators, or cell lineages demonstrate remarkably diverse clinical responses. Although overlap exists, some patients have cytokine-dependent disease (e.g., TNF or IL-6), some have B cell–dependent disease, and others have T cell–dependent disease. These clinical observations suggest that RA is not a single pathogenic entity but is instead a collection of disorders that varies depending on the genetic makeup and environmental triggers for an individual patient. By understanding the basic mechanisms of disease, we can begin to untangle which RA a particular person might have and how to personalize therapy.

## ETIOLOGY

**KEY POINTS**

- **Genes play a key role in RA susceptibility and severity.**
- **Class II MHC genes, especially those with certain sequences in the antigen-binding groove, are the most prominent genetic associations.**
- **The human leukocyte antigen (HLA)-DR associations could be related to efficient binding and presentation of modified peptides.**
- **Newly defined genetic associations in more than 100 genes including polymorphisms in *PTPN22*, *PADI4*, and genes involved in immune function suggest that the associations in RA are complex and involve many pathways.**
- **The microbiome and environmental stress at mucosal surfaces contribute to the etiology of RA.**

Although the cause of RA remains unknown, the interaction of environmental and genetic factors is almost certainly responsible; either one is necessary but not sufficient for full expression of the disease. The most compelling evidence for a genetic component is in monozygotic twins, in whom the concordance rate is perhaps 12% to 15% when one twin is affected, compared with 1% for the general population. The fact that concordance is not higher provides key evidence that other influences such as the environment, epigenetics, or microchimerism from maternal-fetal transfer might be as important as or even more important than the genetic component. The risk for a fraternal twin of a patient with RA is also high (~2% to 5%) but similar to the rate for other first-degree relatives.

Although the immunogenetics is not fully understood, one of the best-studied and perhaps most influential genetic risk factors is the class II MHC haplotype of an individual. Single nucleotide polymorphisms (SNPs) in *PTPN22* and *PADI4* increase risk in some (but not all) racial and ethnic groups. Genome-wide screens have implicated more than 100 genes, many of which are involved with immune function.[3] However, most genes have a relatively modest contribution, and the susceptibility polymorphism confers only a 1.05- to 1.2-fold increase. Combinations of genes can clearly interact with one another, and a 45-fold increase in risk is conferred by a combination of *HLA-DR*, *PTPN22*, and the *TRAF1-C5*.[4] However, this combination is found in less than 1% of persons with RA, and algorithms of RA other SNPs have not yet identified a critical combination that can account for the disease. Additional progress in understanding the role of genes in RA, including rare variants that might be more important than some common polymorphisms, will require sophisticated bioinformatics to clarify how individual alleles contribute to susceptibility, severity, and response to targeted therapies.

## Role of Human Leukocyte Antigen-DR in the Disease Susceptibility and Severity

The structure of class II MHC molecules on antigen-presenting cells is associated with increased susceptibility and severity of RA and accounts for about 40% of the genetic influence. A genetic link between HLA-DR and RA was initially described in the 1970s with the observation that HLA-DR4 occurred in 70% of patients with RA, compared with about 30% of control subjects, giving a relative risk of having RA to those with HLA-DR4 of approximately 4-fold to 5-fold.

The susceptibility to RA was originally ascribed to an association with the third hypervariable region of DRβ chains, from amino acids 70 through 74. The epitope is glutamine-leucine-arginine-alanine-alanine (QKRAA), a sequence found in DR4 and DR14, in addition to some DR1β chains. The "susceptibility epitope" (SE) on DR4β chains with the greatest association with RA are DRB*0401, DRB*0404, DRB*0101, and DRB*1402 (Table 69-1). Up to 96% of patients with RA exhibit the appropriate HLA-DR locus in some populations.[5] In certain ethnic and racial groups, the association with QKRAA is not as prominent. The QKRAA epitope also predicts increased severity of established RA, with a greater prevalence of extraarticular disease and erosions in patients with two copies. Other HLA genes such as DRB*1301 contain the DERAA sequence and are associated with decreased susceptibility to RA.[6]

One intriguing possibility that could account for some patients who do not fit within this paradigm is microchimerism.[7] Maternal cells expressing the SE have the potential to survive and persist in the circulation throughout adulthood. These noninherited maternal antigens could then confer increased risk of disease in the children of women who express SE.

The region associated with RA (QKRAA) primarily faces away from the antigen-binding cleft of the DR molecule that determines the specificity of peptides presented to $CD4^+$ T helper (Th) cells. Attempts to elute peptides from the binding pocket of RA-associated alleles have not revealed a single antigen that is either unique to or associated with RA. The SE also plays a role in shaping the T cell repertoire in the thymus or altering intra-cellular HLA-DR trafficking and antigen loading, which could contribute to the association. Alternatively, QKRAA could serve as an autoantigen as a result of molecular mimicry in some situations because some xenoproteins such as gp110 from the Epstein-Barr virus (EBV) also include this sequence.

More recent informatics approaches to the role of the SE suggest that the most prominent association is actually with amino acids 11 and 13.[8] These residues lie in the bottom of the antigen-binding groove of HLA-DR. A leucine or valine at amino acid 11 is associated with increased risk of developing disease, whereas serine at amino acid 13 confers decreased risk. These key residues could play a major role selecting "arthritogenic" peptides. In fact, careful studies of antigen-binding affinities show that citrullinated vimentin peptides bind much more avidly to the RA-associated alleles than the unmodified protein. HLA-DR genes that are not associated with RA do not display this disparity. Functional studies show that these same RA-associated alleles present antigen more efficiently to T cells with higher amounts of cytokines such as IL-17 and interferon (IFN)-γ.[9,10]

Based on these observations, the shared epitope might not be considered an independent risk factor for RA but instead a marker for immunoreactivity to altered peptides that lead to ACPAs.[11] In a large series of patients with early undifferentiated inflammatory arthritis, one-third of patients met criteria for RA within 1 year. Progress to RA occurred regardless of HLA-DR genotype if patients were $ACPA^+$. When patients were stratified according to ACPA, the shared epitope did not make an additional contribution to progression from undifferentiated arthritis to RA. In other studies, however, the presence of the shared epitope and ACPAs together is associated with even greater disease severity, indicating that the role of MHC might be more complex.

**TABLE 69-1** Nomenclature for Human Leukocyte Antigen-DR Alleles and Associations with Rheumatoid Arthritis

| Old Nomenclature (HLA-DRB1 Alleles) | Current Nomenclature | Association with RA |
|---|---|---|
| HLA-DR1 | 0101 | + |
| HLA-DR4 Dw4 | 0401 | + |
| HLA-DR4 Dw14 | 0404/0408 | + |
| HLA-DRw14 Dw16 | 1402 | + |
| HLA-DR4 Dw10 | 0402 | – |
| HLA-DR2 | 1501, 1502, 1601, 1602 | – |
| HLA-DR3 | 0301, 0302 | – |
| HLA-DR5 | 1101-1104, 1201, 1202 | – |
| HLA-DR7 | 0701, 0702 | – |
| HLA-DRw8 | 0801, 0803 | – |
| HLA-DR9 | 0901 | – |
| HLA-DRw10 | 1001 | – |
| HLA-DRw13 | 1301-1304 | 1301 associated with protection |
| HLA-DRw14 Dw9 | 1401 | – |

HLA, Human leukocyte antigen; RA, rheumatoid arthritis.

Modified from Weyand CM, Hicok KC, Conn DL, Goronzy JJ: The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. *Ann Intern Med* 117:801, 1992.

## Additional Polymorphisms: Cytokines, Citrullinating Enzymes, *PTPN22*, and Others

The genetic influence on RA has also led to studies evaluating non-MHC genes. SNPs in promoter regions, coding regions, or areas with no known function have been extensively investigated in RA with a variety of methods, including genome-wide association studies. Table 69-2 shows some of the SNPs and microsatellites that have been associated with RA. The relative contribution for most is modest, and variations in technique, stage of disease, and patient populations result in some disagreement among various reports.

Given the importance of cytokines in RA, it is not surprising that many studies have focused on these genes.

**TABLE 69-2** Key Genetic Associations in Rheumatoid Arthritis

| Gene | Odds Ratio for Risk Alleles | Comment |
|---|---|---|
| *HLA-DR* | 4- to 5-fold | |
| *PTPN22* | ~2-fold | Not in Asian populations |
| *PADI4* | ~2-fold | Primarily in Asian populations |
| *TRAF1-C5* | From 1.2-fold to 2-fold | |
| *STAT4* | From 1.2-fold to 2-fold | |
| *TNFAIP3* | From 1.2-fold to 2-fold | |
| *IL2/21* | From 1.2-fold to 2-fold | |

Examples of genes with an odds ratio >1.0 and <1.2: *CTLA4, CD40, CCL21, CD244, IL2Rb, TNFRSF14, PRKCQ, PIP4K2C, IL2RA, AFF3, REL, BLK, TAGAP, CD28, TRAF6, PTPRC, FCGR2A, PRDM1, CD2-CD58, IRF5, CCR6, CCL21, IL6ST,* and *RBPJ.*

The most intriguing evidence relates to TNF. This proinflammatory factor is a major cytokine in the pathogenesis of RA, and the TNF genes are located in the MHC locus on chromosome 6 in humans. Several polymorphisms of the TNF promoter, including positions −238 and −308, can alter gene transcription. Associations among the TNF polymorphisms and RA susceptibility and radiographic progression have been reported, although agreement is not uniform. In addition, certain polymorphisms in cytokines, especially TNF or Fc receptors, have been associated with a differential response to therapy. For instance, substitution of a T for a C at position −857 in the TNF promoter might confer greater responsiveness to TNF inhibitors.[12] IL-6 receptor polymorphisms that affect IL-6 function have also been associated with RA.[13]

Among the many noncytokine and non-MHC genetic linkages described for RA, the ones associated with *PADI* and *PTPN22* have the strongest effect on susceptibility. The *PADI* genes are responsible for the post-translational modification of arginine to citrulline and creating the neoantigens that bind so efficiently to RA-associated MHC proteins. PADIs also have other less well-characterized functions, such as citrullination of histones that can then remodel chromatin. Four isoforms known as *PADI1* through *PADI4* have been identified. An extended *PADI4* haplotype that can lead to increased levels of PADI4 protein as a result of enhanced messenger RNA (mRNA) stability is associated with RA.[14] In a Japanese cohort, a twofold increase in risk of RA was observed with *PADI4* SNPs. Similar studies confirm the association in Asian populations, and more recent data suggest that it is also a risk gene in North America.

PTPN22 associations have been discovered in large-scale screening efforts to identify SNP associations in RA.[15] An association at position 1858 in the *PTPN22* gene confers a twofold increase in risk. The allele containing thymidine leading to an amino acid substitution (R620W) was present in 8.5% of control subjects but was found in nearly 15% of patients with seropositive RA. Other studies have demonstrated a similar association with systemic lupus erythematosus (SLE), type 1 diabetes, and several other autoimmune diseases. *PTPN22* encodes a phosphatase that regulates the phosphorylation status of several kinases important to T cell activation, including Lck and ZAP70. The R620W allele surprisingly results in a gain of function that alters the threshold for T cell receptor (TCR) signaling. Because the *PTPN22* allele is rare in Japan, other genes (e.g., *PADI4*) influence susceptibility in this and other particular ethnic or racial populations.

The genes associated with RA consistently involve immune regulation, and many are commonly shared with other immune-mediated diseases such as SLE, type 1 diabetes, and celiac disease.[16] Cytokine polymorphisms such as for TNF and the IL-1 inhibitor, IL-1Ra, are not surprising. Genes that regulate adaptive immune responses in T cells such as *PTPN22* and the co-stimulation receptor CTLA have also been associated with RA. Other genes associated with B cell function and/or antigen presentation such as *BTLA* (B- and T-lymphocyte attenuator), Fc receptors, and *CD40* are also implicated. Polymorphisms have also been identified in signal transduction pathways that regulate immune function such as TRAF1-C5 and signal transducer and activator of transcription (STAT) 4, cell migration *(ELMO1)*, and fetal development *(LBH)*. The consistent thread in this analysis is that most gene associations for RA cluster to innate immunity, adaptive immunity, matrix regulation, and inflammation. Aside from providing insight into the mechanisms of disease, they could also contribute to responses to targeted therapies.

## Interactions between Genes and Environment

A number of environmental factors clearly contribute to RA susceptibility, and a low level of vitamin D recently has been associated with increased risk.[17] Microchimerism, including male microchimerism in women, also has been associated with increased risk.[18] However, smoking is the best defined environmental risk factor. The reason for its influence on the development of synovitis is not fully defined but could involve the activation of innate immunity and *PADI* in the airway. Smoking induces PADI expression in the airway and increases protein citrullination, which could provide a stimulus for generation of ACPAs in susceptible persons[19] (Figure 69-2). High-resolution computed tomography studies demonstrate associations between ACPA formation and bronchial thickening in the pre-RA stages, suggesting that the local inflammatory response could begin at this mucosal site.[20] Other environmental factors such as oral contraceptives appear to provide modest protection from RA, perhaps as a result of changes in the hormone milieu.[21]

The interaction between HLA-DR and tobacco exposure is perhaps the best example of how genes and the environment conspire to enhance risk. Although smoking and the SE alone modestly increase the likelihood of developing RA, the combination is synergistic.[22] A person with a history of cigarette smoking and two copies of the SE increases the odds of developing RA by up to 40-fold. The mechanism of the interaction probably relates to increased protein citrullination in smokers and the ability of SE-containing HLA-DR molecules to present citrullinated proteins. The extent of smoking is also predictive, with the greatest risk seen with at least 20 pack-years. The risk declines slowly with cessation of smoking, taking more than a decade to begin approaching the risk in nonsmokers.[23] Alcohol consumption can decrease this risk, and exposure

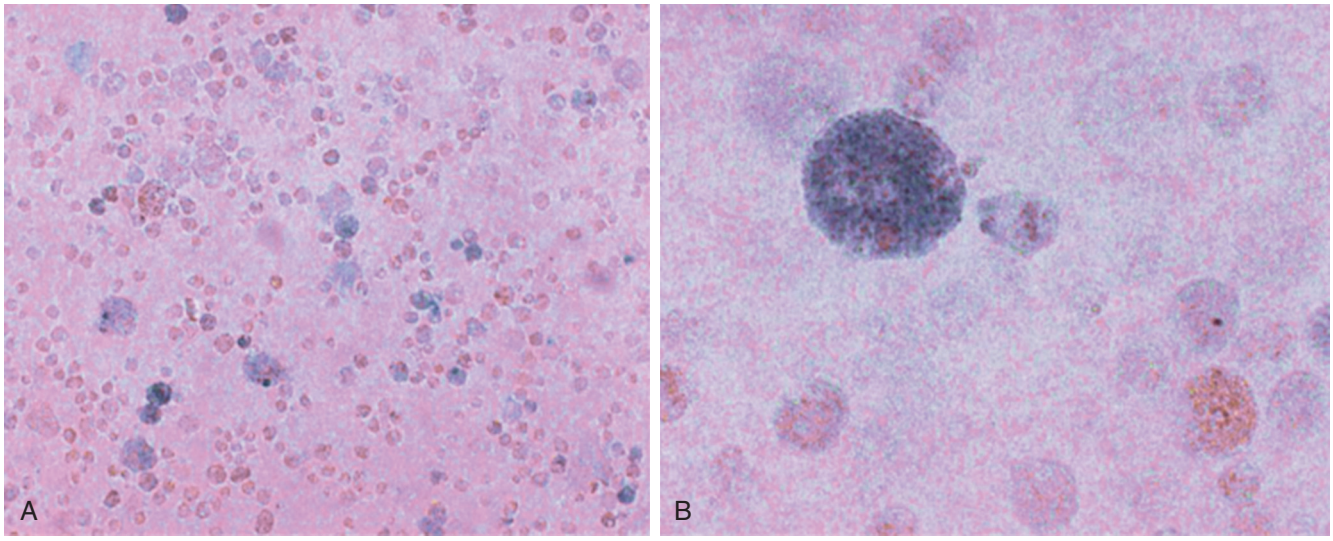



**Figure 69-2** Citrullinated proteins in alveolar macrophages in smokers. Immunostaining of alveolar macrophages from the bronchoalveolar lavage in smokers shows citrullinated proteins *(blue-brown color)*. Nonsmokers do not have significant staining. *(From Klareskog L, Stolt P, Lundberg K, et al: A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR [shared epitope]-restricted immune reactions to autoantigens modified by citrullination.* Arthritis Rheum *54:38–46, 2006.)*

to other inhaled irritants such as silica dust increases risk, demonstrating the complexity of the environment and human behavior on understanding disease susceptibility. Diet is also a potential risk, and obesity could contribute to disease susceptibility through the production of adipokines (for a detailed discussion of nutrition and RA risk see Chapter 68).[24]

## Microbiome

The observation that airway inflammation might contribute to disease risk has led to various studies to evaluate contributions at other mucosal sites. Similar associations are observed in animal models of arthritis, where arthritis susceptibility and severity can be lower in rodents raised in a germ-free or defined bacteria environment. The most prominent association is with periodontitis, commonly caused by *Porphyromonas gingivalis*. This bacterium is one of the few that produces its own PADI, which could lead to local citrullination of proteins such as fibronectin. More recent data suggest that *P. gingivalis* can also act by stimulating Toll-like receptor (TLR)2, which increases IL-1 production and leads to production of pathogenic Th17 cells.[25] In the gut, expansion of *Prevotella copri* species occurs in patients with early-stage RA, with a concomitant decrease in *Bacteroides*.[26] These differences are not seen in long-standing RA, although it is not clear whether that is a result of treatment or other factors that might alter the gut microbiome. Regardless, the interactions between host and bacteria, even nonpathogenic commensals, could play a major role in immune-mediated diseases like RA.

## Sex

RA is one of many chronic autoimmune diseases that predominates in women. The ratio of female-to-male patients is 2 : 1 to 3 : 1, which is not as high as Hashimoto's thyroiditis (25 : 1 to 50 : 1) or SLE (9 : 1). The sex effect is often observed in some animal models of autoimmunity such as the NZB/NZW model of SLE, in which female mice have more severe disease. Estrogens are one obvious explanation, and some data support the concept that these hormones modulate immune function.[27] For example, autoantibody-producing B cells exposed to estradiol are more resistant to apoptosis, suggesting that autoreactive B cell clones might escape tolerance. Estrogen receptors are expressed on FLS and increase production of metalloproteinases. In macrophage cell lines, estrogen can enhance production of TNF. Spontaneous arthritis occurs in mice with a specific mutation in the *ZAP70* gene, which regulates T cell responses. Exogenous administration of estrogen actually ameliorates disease, suggesting that the interactions between sex hormones and disease are complex.[28] Nulliparity has also been suggested as a risk factor in early studies, but more recent reports do not support this notion. Thus the specific mechanisms responsible for the female preponderance of RA are not fully understood.

Pregnancy is often associated with remission of the disease in the last trimester. More than three quarters of pregnant patients with RA improve in the first or second trimester, but 90% of these women experience a flare of disease associated with a rise in rheumatoid factor (RF) titers in the weeks or months after delivery. The mechanism of protection is not defined but might be due to the expression of suppressive cytokines such as IL-10 during pregnancy, production of α-fetoprotein, or alterations in cell-mediated immunity. One intriguing finding is that fetal DNA levels in the maternal peripheral blood correlate with the propensity for improved symptoms in pregnant women with RA. It is not certain whether the DNA itself contributes or whether it is a marker for increased leakage of fetal cells into the maternal circulation.[29] Immune responses directed against paternal HLA antigens can occur and lead to the production of alloantibodies in the maternal circulation. Maternal-fetal disparity in class II HLA phenotypes can correlate with pregnancy-induced remission. More than

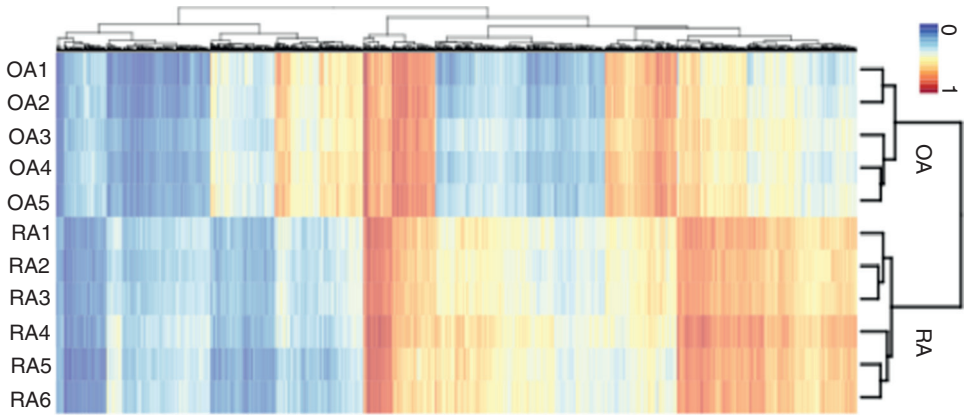



**Figure 69-3** Abnormal DNA methylation in fibroblast-like synoviocytes (FLS). Using chip technology, the methylation pattern of DNA from osteoarthritis (OA) and rheumatoid arthritis (RA) FLS was determined. Note that the cells from the two diseases segregate based solely on the methylation pattern. The methylation signature is stable and involves many genes involved in immune responses. *(From Nakano K, Whitaker JW, Boyle DL, et al: DNA methylome signature in rheumatoid arthritis.* Ann Rheum Dis *72:110–117, 2013.)*

three-fourths of pregnant women with maternal-fetal disparity of HLA-DRB1, DQA, and DQB haplotypes have significant improvement, whereas disparity is only observed in one-fourth of women whose pregnancy is characterized by continuous active arthritis.[30] Therefore, suppression of maternal immune responses to paternal HLA haplotypes might be protective. This question remains unsettled because another study failed to find a correlation between the HLA disparity and clinical improvement during pregnancy.[31]

## Epigenetics

Epigenetics describes phenotypic or gene expression properties caused by mechanisms other than changes in the underlying DNA sequence. Modification of cytosine-phosphatidyl-guanine (CpG) DNA sequences by methylation, for example, can suppress gene expression and plays a role in cell differentiation. Histone acetylation also alters accessibility of DNA for transcription factors and RNA polymerases. miRNAs can bind to DNA and suppress expression of key genes involved with the inflammatory process. Epigenetic information such as DNA methylation can be transferred from one generation to the next, thereby providing an alternative mechanism for rapidly altering disease susceptibility in a population as a result of the environment.

Some data from peripheral blood studies suggest that a modest overall decrease in methylation occurs in RA mononuclear cells. However, the data are difficult to interpret because blood mononuclear cells are a mixture of different cell types, and epigenetic signatures in one lineage could be diluted out by other cells. Most information on epigenetics in RA comes from studies of RA synovium or cultured synoviocytes. Individual candidate genes, such as *IL6*, *CXCL12*, and ephrin-B1 *(EFNB1)* are hypomethylated, whereas others, like *HLA-DRB3*, *EBF2*, and *IRX1*, have an increased number of methylated loci.[32] Unbiased approaches using chip technology suggest that the overall level of methylation in RA FLS is not different from osteoarthritis (OA) or normal cells. However, there are clear patterns of differential methylation in RA, with some loci being hypermethylated, whereas others are hypomethylated (Figure 69-3).[33]

Differential methylation in FLS is stable over many cell passages, and detailed analysis indicates that the differentially methylated genes are enriched in pathways such as cell adhesion, focal adhesion, matrix regulation, cytokine-cytokine receptor interactions, and other functions important to immune function. A portion of this signature could be due to the local cytokine milieu, because IL-1 can suppress expression of DNA methyl transferases. The effect of cytokines is transient in culture, and the cells return to baseline after the cytokine is removed. These data suggest that permanent imprinting of FLS in RA could be due to other factors and could even be inherited.[34] Dietary influences—for example, ingestion of methyl donors such as folate—or even exposure to methyl donors in utero can also profoundly alter DNA methylation and adaptive immune functions.

Few data are available on histone marks in RA. However, histone deacetylases are expressed in RA FLS, and histone deacetylase HDAC1 is overexpressed.[35] The use of small interfering RNA to knock down HDAC1 or HDAC2 increases expression of the p53 tumor suppressor gene and TNF-induced expression of MMP-1.[36] Cytokines such as TNF can increase HDAC1 expression in cultured FLS. SIRT1 also exhibits HDAC activity and is overexpressed in RA FLS and synovium. Blocking SIRT1 in FLS reduces IL-6 and IL-8 production. HDAC inhibitors are also effective in several animal models of arthritis, including adjuvant arthritis in rats and collagen-induced arthritis in mice.[37] These data indicate that histone marks can play a key role in remodeling the chromatin to increase production of inflammatory mediators in RA.

Analysis of synoviocytes shows that several miRNAs such as miRNA-124a are decreased in RA compared with OA cells. This particular miRNA can suppress cell cycling and chemokine genes. Increasing miRNA-124a levels in RA synoviocytes decreased the production of the chemokine macrophage chemoattractant (MCP)-1.[38] Forced expression of miRNA-203 increases metalloproteinase and

IL-6 expression by synoviocytes as well.[39] miRNA-155 is especially interesting because it is overexpressed in RA synovial cells and peripheral blood cells. Knocking down this miRNA reduces MMP and cytokine expression in these cells. In vivo miRNA-155 deficiency has mixed effects in animal models, with decreased severity in collagen-induced arthritis and no effect in the passive K/BxN serum transfer model.[40] The differences in the two models might result from distinct mechanism of disease; the latter is purely a model of innate immunity, whereas adaptive immunity plays a prominent role in the former. Several other miRNAs are differentially expressed in RA, including miRNA 16, 124a, 202, 134a, 23, 346, and 15a.[41] Ultimately, unbiased profiling using technologies will be needed to fully assess how miRNA patterns influence disease pathogenesis in RA.

The role of epigenetics in RA is not completely understood. It is not clear whether these observations occur before the onset of disease, are involved with the transition from asymptomatic autoimmunity to clinical disease, or participate in the destructive phase in established RA. Environmental stress plays a major role in disease susceptibility—perhaps a greater role than DNA polymorphisms. The mechanisms probably involve epigenetic dysregulation of gene expression, leading to decreased thresholds for autoreactivity in the adaptive immune system.

## Changing Epidemiology

The history of RA reveals the surprising observation that it is a relatively new disease in Europe and Northern Africa. Examination of ancient skeletal remains in Europe and Northern Africa fails to reveal convincing evidence of RA, even though other rheumatic diseases such as OA, ankylosing spondylitis, and gout are readily discernable. In contrast, typical marginal erosions and rheumatoid lesions are present in the skeletons of Native Americans found in Tennessee, Alabama, and Central America from thousands of years ago. The first clear descriptions of RA in Europe appeared in the seventeenth century, and the disease was distinguished from gout and rheumatic fever by Garrod in the mid nineteenth century. Although the theory is still controversial, the disease might have migrated from the New World to the Old World coincident with opening of trade and exploration routes. Because genetic admixture was relatively limited, an undefined environmental exposure, in collaboration with risk-associated MHC genes, potentially caused RA in susceptible Europeans. The most obvious explanation, of course, would be that an infectious agent is responsible. However, other environmental influences such as tobacco smoking were also introduced to the Old World at the same time and could play a role. Recent data implicating smoking-induced citrullination of proteins in the airway are especially intriguing clues to the introduction of RA in Europe.

The severity and incidence of RA appeared to decrease in the late twentieth century (Figure 69-4).[42] In certain well-defined populations, including Native Americans, the incidence of RA has gradually declined by as much as 50% during the past half of the twentieth century. However, when this Olmsted County cohort was extended into the early twenty-first century, the trend reversed.[43] The age-adjusted increase occurred in all age groups but mainly occurred in women.[43] Because smoking has decreased in the United States, a change in tobacco use is probably not the explanation. Other influences such as hygiene and other lifestyle modifications related to industrialization might contribute, and an infectious agent might have variable prevalence as a result of these societal changes, as with many other infectious diseases. Currently the lifetime risk of developing RA is remarkably high—namely, 3.6% in women and 1.7% in men.[44] The second most common autoimmune rheumatic disease is polymyalgia rheumatica, with a lifetime risk of 2.4% in women and 1.4% in men.

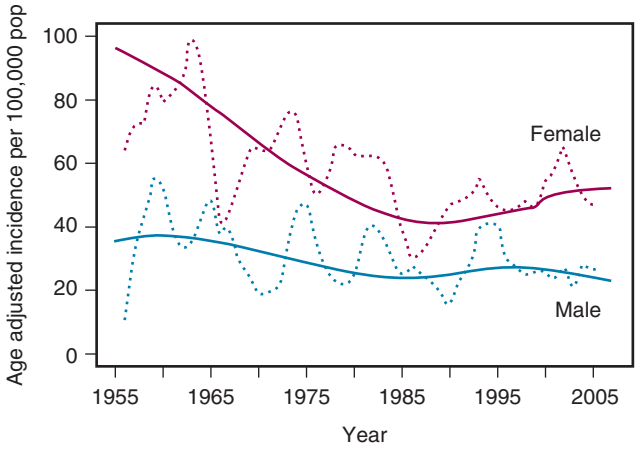



**Figure 69-4** Population studies in Minnesota demonstrated a gradually decreasing incidence of rheumatoid arthritis from 1960 to 1995. However, the trend appears to have reversed from 1996 to 2007 in women. *(From Myasoedova E, Crowson CS, Kremers HM, et al: Is the incidence of rheumatoid arthritis rising? Results from Olmsted County, Minnesota, 1955-2007.* Arthritis Rheum *62:1576–1582, 2010.)*

# PATHOGENIC MECHANISMS

**KEY POINTS**

- **Although an etiologic link has not been established, pathogens such as viruses, retroviruses, bacteria, and mycoplasma have been associated with RA.**
- **A single specific "RA pathogen" is unlikely, although the microbiome at mucosal surfaces plays a key role.**
- **Repeated inflammatory stress, especially through specialized receptors that recognize common molecules produced by pathogens in a genetically susceptible individual, might contribute to altered peptides through citrullination, breakdown of tolerance, and subsequent autoimmunity.**

Considerable effort has been expended to assess the role of infectious agents in RA (Table 69-3). A potential pathogen could initiate disease through a variety of mechanisms, including direct infection of the synovium, activation of innate immunity by pattern-recognition receptors that bind to components of the agent, or through molecular mimicry that induces an autoreactive adaptive immune response.

**TABLE 69-3** Etiology of Rheumatoid Arthritis: Role of Infection

| Infectious Agent | Potential Pathogenic Mechanisms |
|---|---|
| *Mycoplasma* | Direct synovial infection; superantigens |
| Parvovirus B19 | Direct synovial infection |
| Retroviruses | Direct synovial infection |
| Enteric bacteria | Molecular mimicry (QKRAA, e.g., in bacterial heat shock proteins) |
| *Mycobacteria* | Molecular mimicry (proteoglycans, QKRAA), immunostimulatory DNA (Toll-like receptor 9 activation) |
| Epstein-Barr virus | Molecular mimicry (QKRAA in gp110) |
| Bacterial cell walls | Toll-like receptor 2 activation |
| *Porphyromonas gingivalis* | Toll-like receptor 2 activation and protein citrullination |
| *Prevotella* sp. | Altered gut microbiome |

## Infectious Agents: Direct Infection and Innate Immune Responses

As previously noted, bacteria and other pathogens have been indirectly implicated in RA. The interactions between the microbiome and mucosal surfaces in RA indirectly contribute, possibly by modifying proteins or shaping the T cell repertoire. In this section, a more direct role of pathogens in the initiation and propagation of RA is discussed.

### Toll-like Receptors and the Inflammasome in the Joint

Infectious agents could contribute to the initiation or perpetuation of RA through a variety of mechanisms. Some arthrotropic microorganisms could infect the synovium and cause a local inflammatory response. There is increasing awareness that the innate immune system could also directly affect the onset and course of synovitis. Pathogen-associated molecular pattern receptors, especially the TLRs, are expressed by sentinel cells in the host that provide a first line of defense (see Chapter 17). These receptors recognize preserved structures in bacteria and other infectious agents and permit rapid release of inflammatory mediators, activation of antigen-presenting cells, and enhancement of adaptive immune responses.

At least 11 TLRs exist in humans, such as TLR2 (which binds peptidoglycans), TLR3 (which binds double-stranded RNA [dsRNA]), TLR4 (which binds lipopolysaccharide [LPS]), and TLR9 (which binds bacterial DNA containing CpG motifs). Many of these pattern recognition receptors are expressed by rheumatoid synovial tissue and cultured FLS including TLR2, TLR3, TLR4, and TLR9. Exogenous TLR ligands such as bacterial peptidoglycan and DNA, as well as endogenous ligands (e.g., heat shock proteins [HSPs], fibrinogen, and hyaluronan), are present in arthritic joints (see the subsequent discussion). Engagement of these receptors participates in certain animal models of arthritis and can exacerbate synovial inflammation, and their absence can limit or prevent arthritis. TLR3, which recognizes viral dsRNA and activates the anti-viral response, is also expressed by synovial cells in the intimal lining. Necrotic debris containing mRNA from RA synovial fluid cells activates TLR3 signaling and pro-inflammatory gene expression in synovitis.

The role of innate immunity in RA led to the notion that repeated engagement of TLRs in the synovium could help initiate or exacerbate disease. This hypothesis could explain why specific pathogens have been difficult to identify in the joint, and could mainly participate by increasing vascular permeability and ingress of pathogenic antibodies (e.g., ACPAs). In contrast, a genetically susceptible person could potentially break tolerance if the TLRs are repeatedly engaged and permit autoimmune responses against articular antigens. Several animal models of disease require TLR ligands for initiations, such as TLR9 in adjuvant arthritis. TLR2 is required for streptococcal cell wall arthritis, and the chronic T cell–dependent phase of that model requires TLR4. Mice lacking TLR4 have significantly less joint damage induced by IL-1 overexpression, even though synovial inflammation is still robust.[45] These data suggest that endogenous TLR ligands play a key role in matrix regulation independent of inflammatory responses.

A second mechanism that regulates innate immunity involves the inflammasome. This complex includes several proteins involved in recognition of "danger signals" and products of pathogens such as muramyl dipeptides and uric acid. One central component is cryopyrin, also called NALP3, which is linked to caspase 1 (IL-1 convertase) by adapter proteins. When the inflammasome is engaged, caspase 1 is activated and IL-1 is processed and released. Mutations in this pathway, especially in cryopyrin, have been associated with autoinflammatory disorders such as Muckle–Wells syndrome and familial cold autoinflammatory disease. Inflammation induced by uric acid crystals or adenosine triphosphate uses this pathway and can be abrogated by IL-1 inhibitors. Cryopyrin is abundant in RA synovium and is constitutively expressed by FLS and macrophages. Other inflammasome components and related genes are highly expressed in RA peripheral blood cells, including ASC, MEFV, and cryopyrin-NALP3.[46] Expression in cultured FLS is markedly increased by TNF. In addition, a cryopyrin/NALP3 SNP might be associated with increased risk of RA. Because the TNF-induced inflammasome activation can induce cytokine production after exposure to bacterial products and other danger signals, it could participate in synovial IL-1 and IL-18 regulation.

### Bacteria, Mycobacteria, Mycoplasma, and Their Components

Active infection of synovial tissue by pyogenic bacteria is an unlikely cause of RA, and extensive searches for a unique or specific organism in synovial tissue or joint effusions have been negative. Antibodies to certain organisms such as *Proteus* are reportedly elevated in the blood of patients with RA, but this finding could represent nonspecific B cell activation. Most patients with RA and reactive arthritis contain bacterial DNA sequences in their synovium. The bacteria identified are not unique and generally represent a cross-section of skin and mucosal bacteria, including *Acinetobacter* and *Bacillus* spp. The accumulation of bacterial products is probably due to phagocytosis by local macrophages.

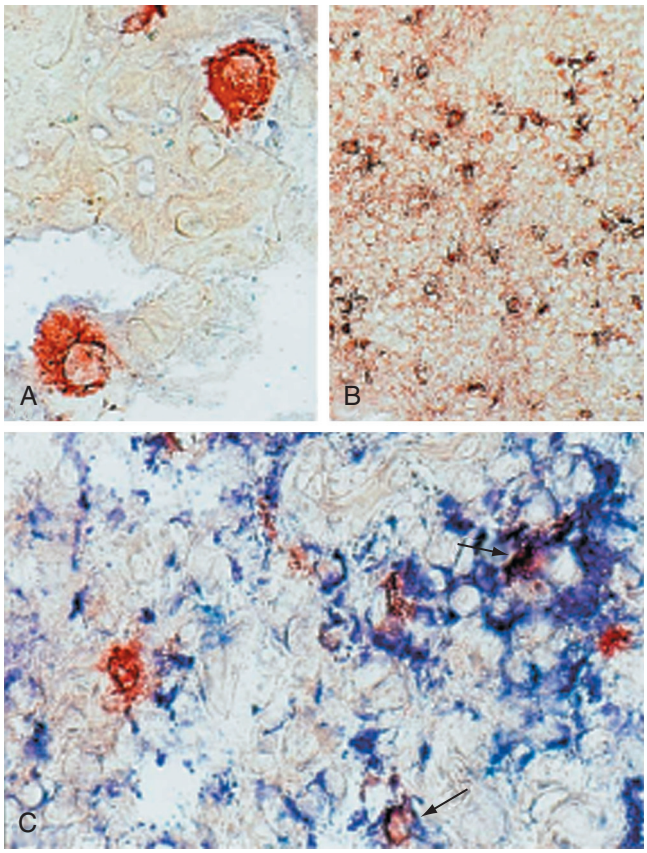



**Figure 69-5** Accumulation of bacterial peptidoglycan in rheumatoid synovium. **A** and **B,** Immunohistochemistry shows synovial cells containing peptidoglycan *(red)*. **C,** Double staining studies show that bacterial peptidoglycan accumulates in synovial macrophages *(arrows)*. These bacterial products can activate Toll-like receptors and stimulate cytokine production. *(From Schrijver IA, Melief MJ, Tak PP, et al: Antigen-presenting cells containing bacterial peptidoglycan in synovial tissues of rheumatoid arthritis patients coexpress costimulatory molecules and cytokines.* Arthritis Rheum *43:2160, 2000.)*

Bacterial peptidoglycans are also abundant in RA synovial tissue (Figure 69-5). Antigen-presenting cells containing these products express TLRs that can be ligated and produce pro-inflammatory cytokines. The peptidoglycans could either activate cells in situ, or phagocytic cells from other sites or the blood could engage the molecules and then migrate to the joint. In either case, it is not difficult to imagine how they can contribute to synovial inflammation.

Several animal models of arthritis are dependent on TLR2, TLR3, TLR4, or TLR9. For instance, rodents injected with streptococcal cell walls (TLR2 ligand) develop severe polyarticular arthritis. The initial phase of disease resolves and is then followed by a chronic T cell–dependent phase that resembles RA. The arthritogenicity of complete Freund's adjuvant in the rat adjuvant arthritis model is dependent on mycobacterial DNA that binds to TLR9 and activates an adaptive immunity. Endogenous TLR4 ligands such as HSPs and fibrinogen also play a role in immune complex models such as passive K/BxN arthritis.[47]

Mycoplasma-derived superantigens such as from *Mycoplasma arthritidis* can directly induce T cell–independent cytokine production by macrophages and exacerbate or trigger arthritis in mice immunized with type II collagen. Despite circumstantial evidence, most efforts to identify *Mycoplasma* and *Chlamydia* organisms or DNA in joint samples have been negative, and no direct evidence exists to support these organisms as etiologic agents.

### Epstein-Barr Virus, dnaJ Proteins, and Molecular Mimicry

EBV is a polyclonal B lymphocyte activator that increases the production of RF. Rheumatoid macrophages and T cells have defective suppression of EBV-induced proliferation of human B cells. Patients with rheumatoid disease have higher levels of EBV shedding in throat washings, an increased number of virus-infected B cells in the circulating blood, higher levels of antibodies to normal and citrullinated EBV antigens, and abnormal EBV-specific cytotoxic T cell responsiveness compared with control subjects. $CD25^+$ B cells from patients with RA respond more briskly to EBV than do those from normal persons, with increased polyclonal IgG and IgM production.[48] Defective elimination of EBV-transformed lymphocytes in RA has fueled speculation that immune dysfunction contributes to initiation of disease.

Additional intriguing data implicating EBV in RA are derived from sequence homology between the susceptibility cassette in HLA-DR proteins and the EBV glycoprotein gp110. Like DRB*0401, gp110 contains the QKRAA motif, and patients with serologic evidence of a previous EBV infection have antibodies against this epitope. Hence T cell recognition of EBV epitopes in some patients with the SE might elicit an immune response directed at innocent bystander cells through "molecular mimicry." This hypothesis could account for disease perpetuation in the absence of active infection in patients with a specific MHC genotype. RA T cells, especially synovial fluid T cells, have increased proliferative responses to gp110, perhaps supporting the molecular-mimicry link between a variety of QKRAA-containing proteins and arthritis. gp110 is only one of many xenoproteins such as the *Escherichia coli* HSP dnaJ containing QKRAA, so this might be a general phenomenon. Of particular interest, responses directed against dnaJ protein also recognize human Hsp40 proteins DnaJA1 and DnaJ2. Therefore, molecular mimicry is not limited to HLA-DR and can involve other cellular proteins.[49]

### Parvovirus

Parvovirus B19 infection has been implicated in some patients with RA based on serologic evidence, including the nonstructural protein NS1. Only about 5% of patients have evidence of recently acquired parvovirus B19 infection at the time of disease onset. Of interest, 75% of RA synovium samples contain B19 DNA compared with about 20% of non-RA control subjects. Immunohistochemical evidence of the B19 protein VP-1 was detected in patients with RA but not in patients with other forms of arthritis. However, evidence of the B19 genome in RA joint samples was not found in other studies.

The mechanisms of B19-induced synovitis, when it does occur, could also involve alterations in FLS function.[50] In a cell-culture model of synoviocyte invasion into cartilage,

infection with the parvovirus significantly increased the migration of cells into the matrix. Mice that are transgenic for the B19 protein NS1 were more susceptible to collagen-induced arthritis and developed high titers of anti–type II collagen antibodies. These data suggest that the B19 genome might not cause arthritis but can enhance an arthritogenic response to other environmental stimuli. This phenomenon could result from B19-induced apoptosis, where NS1 can form adducts with various products in the apoptotic bodies. These alterations create neoepitopes involving DNA, histone H4, and other proteins that can become immunogenic and enhance autoreactivity.[51]

### Other Viruses

Because rubella virus and the rubella vaccine can cause arthritis in humans, the virus has attracted some attention as a possible triggering agent. Live rubella virus can be isolated from the synovial fluid of some patients with chronic inflammatory oligoarthritis or polyarthritis without clinical evidence of rubella. However, most patients with rubella do not have the classic polyarticular involvement and display an oligoarthritis involving large joints. As with B19 infection, it is possible that a small subset of patients with chronic polyarthritis that is called RA actually have direct infection with wild-type or attenuated rubella virus.

Studies of synovial tissue in a variety of inflammatory and non-inflammatory arthropathies have also demonstrated DNA of other viruses such as cytomegalovirus and herpes simplex, but not adenovirus or varicella-zoster. As with bacterial DNA, parvovirus, and EBV, the localization of viral DNA to the inflamed joint is probably related to the migration of inflammatory cells containing the viral genome or other nonspecific mechanisms rather than an active infection.

Retroviral infections could also contribute to the etiology of RA. However, extensive searches for potential agents have not been fruitful. Endogenous retroviruses are abundant in inflamed and normal synovium, and some transcripts are expressed in RA cells. In one study, higher levels of HERV-K10 gag protein from a common endogenous retrovirus were detected more often in RA compared with OA and normal peripheral blood mononuclear cells. Indirect evidence implicates retroviral infection, such as the demonstration of zinc-finger transcription factors in cultured synoviocytes that increase signaling through p38 mitogen-activated protein (MAP) kinase. In addition, the pX domain of one human retrovirus, human T lymphotropic virus-1 (HTLV-1), causes synovitis in transgenic mice, and synoviocytes from patients infected with HTLV-1 have increased cytokine production.

## Autoimmunity

**KEY POINTS**

**Evidence of autoimmunity can be present in RA many years before the onset of clinical arthritis.**

**Antibodies such as ACPAs and RFs are commonly associated with RA.**

**Autoantibodies in RA can recognize either joint antigens such as type II collagen or systemic antigens such as glucose phosphate isomerase.**

**These autoantibodies can potentially contribute to synovial inflammation through several mechanisms, including local activation of complement.**

The idea that aberrant immune responses are directed toward self-antigens in RA was recognized with the discovery of RF in the blood of patients with the disease. Initially described by Waaler and later by Rose, it was not until the mid 1950s that Kunkel and colleagues firmly established that RF is an autoantibody. ACPAs have largely supplanted RFs as the most sensitive and specific antibody associated with RA. They reflect the underlying immune dysregulation in RA and, in addition to being a biomarker of disease, can also play an important role in the pathogenesis of inflammation in the joint and perhaps even in mucosal sites.

### Anti-citrullinated Protein Antibodies

One of the most striking observations related to autoantibodies is the observation that immunoglobulins that bind to citrullinated proteins are produced by patients with RA and have significant prognostic implications. The discovery originated with reports in the 1970s that antibodies directed against keratin were detected in rheumatoid serum and that the primary target antigen was filament-aggregating protein, filaggrin. These antibodies actually bind to epitopes on filaggrin that contain citrulline, which is derived from post-translational modification of arginine by PADI. Humans have four isoforms of PADI. PADI2 and PADI4 are especially abundant in synovium,[52] and, as previously noted, certain SNPs are associated with RA. The function of PADIs in normal immune responses is not certain, citrullination of some chemokines can decrease their chemoattractant properties, and modification of histones can regulate gene expression in stressed cells.

Induction of *PADI* expression and citrullination of peptides are not specific to RA and can occur in many inflammatory settings.[53] Not only are other inflammatory arthropathies marked by citrullinated proteins, but other organs such as the lungs in smokers have significant *PADI* activity. The presence of citrullinated proteins (CPs) in the lungs of smokers or in other mucosal sites could provide the antigen exposure that can contribute to anti-CP antibody production and begin the long road to the development of RA. CPs are also present in most animal models of arthritis. Immunohistochemistry demonstrates CPs in RA synovial tissue infiltrating cells (Figure 69-6), as well as in extracellular deposits that often co-localize with various isoforms of *PADI*, especially *PADI2* and *PADI4*. Moreover, ACPAs are produced by the rheumatoid synovium, especially tissues with lymphoid aggregates.

The specific proteins that are modified vary widely but include many normal constituents such as fibrinogen, vimentin, and fibronectin, as well as xenoproteins such as EBV-derived peptides.[54] The pattern of protein citrullination or the specificity of antibodies to particular proteins does not thus far have predictive value for developing RA or the severity of disease. One interesting subset involves

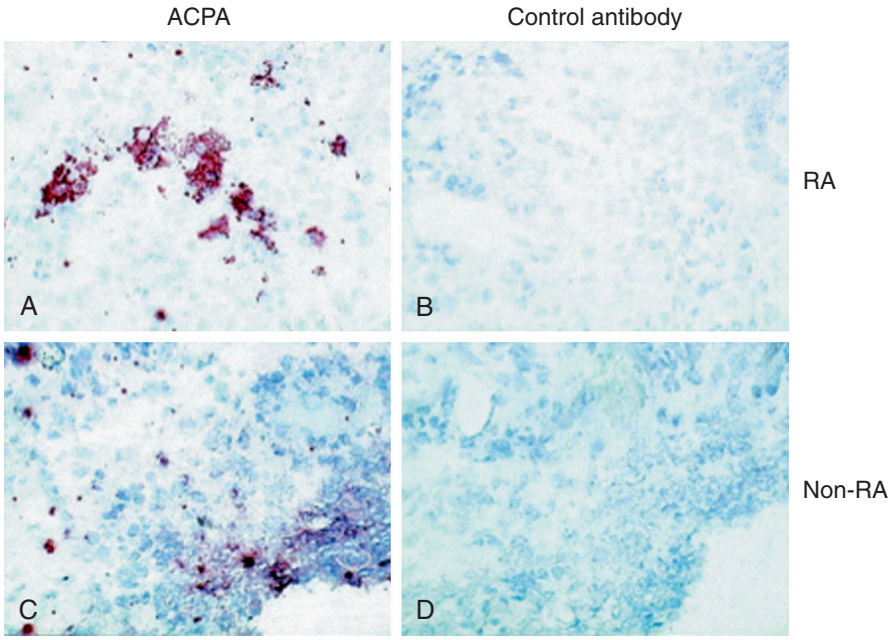



**Figure 69-6** **A** to **D,** Citrullinated proteins in inflamed synovium. Both rheumatoid arthritis (RA) and nonrheumatoid synovium contain citrullinated proteins, detected with an anti-citrullinated protein antibody (ACPA) (*red-brown* in synovium). Control is an irrelevant antibody. Although citrullinated proteins are not specific, the production of ACPAs is more specific to rheumatoid arthritis. *(From Vossenaar ER, Smeets TJ, Kraan MC, et al: The presence of citrullinated proteins is not specific for rheumatoid synovial tissue.* Arthritis Rheum *50:3485, 2004.)*

antibodies that recognize mutant citrullinated vimentin (MCV). These antigens have two modifications that render them more immunogenic: First, there are amino acid changes in the primary sequence such as glycine to arginine. Second, either the new amino acid or another arginine on the protein is citrullinated.[55] The mechanism of amino acid changes has not been defined but could be due to oxidative stress that causes mutations in the genes encoding the protein. These anti-MCV antibodies might be more specific for RA in established disease and more predictive for radiographic progression than other ACPA tests.[56]

ACPAs are present in the serum of 80% to 90% of patients with RA who have established disease using conventional assays. They are also more specific than RF for RA with a specificity approaching 90%. When custom-designed arrays are used, however, as many as 10% of patients negative for ACPA have other ACPA specificities.[57] Perhaps most interesting, ACPA, like RF, can appear long before the onset of clinical arthritis and could be a marker for immune hyperreactivity and subclinical inflammation, leading to protein citrullination in a variety of tissues (Figure 69-7).[58] The more peptides recognized by the repertoire of ACPAs, such as to vimentin, enolase, or various fragments of fibronectin, the greater the likelihood that RA will develop in a person with arthralgias.[59] It is of interest that elevated serum cytokines also predict the development of RA.[60] A genetic contribution to ACPA production was confirmed in the Native American population, where ACPAs are present in nearly 20% of unaffected first-degree relatives and in more than 10% of more distant relatives.[61] ACPAs are also produced by synovial tissue B cells and can be detected in synovial fluid. However, the fact that ACPAs, like RFs, can be detected years before the onset of arthritis and can be found in some normal relatives of patients with RA indicates that the antibodies alone cannot account for initiation of the disease.

ACPAs are predictors of more aggressive disease marked by bone and cartilage destruction. In fact, some data suggest that the HLA-DR associations in RA are actually due to an association between the SE and ACPA production. This association extends to accelerated atherosclerosis in RA, where ACPA positivity is an independent risk factor for ischemic heart disease. In patients with early undifferentiated inflammatory arthritis, ACPAs are also predictive for persons who will progress to RA.[62]

Despite these caveats, ACPAs have pathogenic potential. For instance, ACPAs can activate the classic and the alternative complement pathways.[63] The antibodies have minimal effect when directly injected into mice, and they enhance the arthritogenic potential of anti–type II collagen antibodies in the collagen-induced arthritis model.[64] Mice immunized with citrullinated fibronectin, but not native fibronectin, can develop inflammatory arthritis.[65] Hence the autoreactivity and autoantibodies are not simply a marker of disease but can participate in the disease process. Citrullination can also increase T cell responses to arthritogenic antigens. For instance, citrullination of albumin leads to the formation of antibodies that also cross-react with the unmodified protein. Citrullinated type II collagen and vimentin are more immunogenic than the native protein, most likely because of increased affinity in the binding groove of HLA-DR proteins that contain the SE.[66] ACPAs can also bind to and enhance the activity of osteoclast precursors, suggesting that they can directly contribute to increased bone damage in ACPA$^+$ patients.[67]

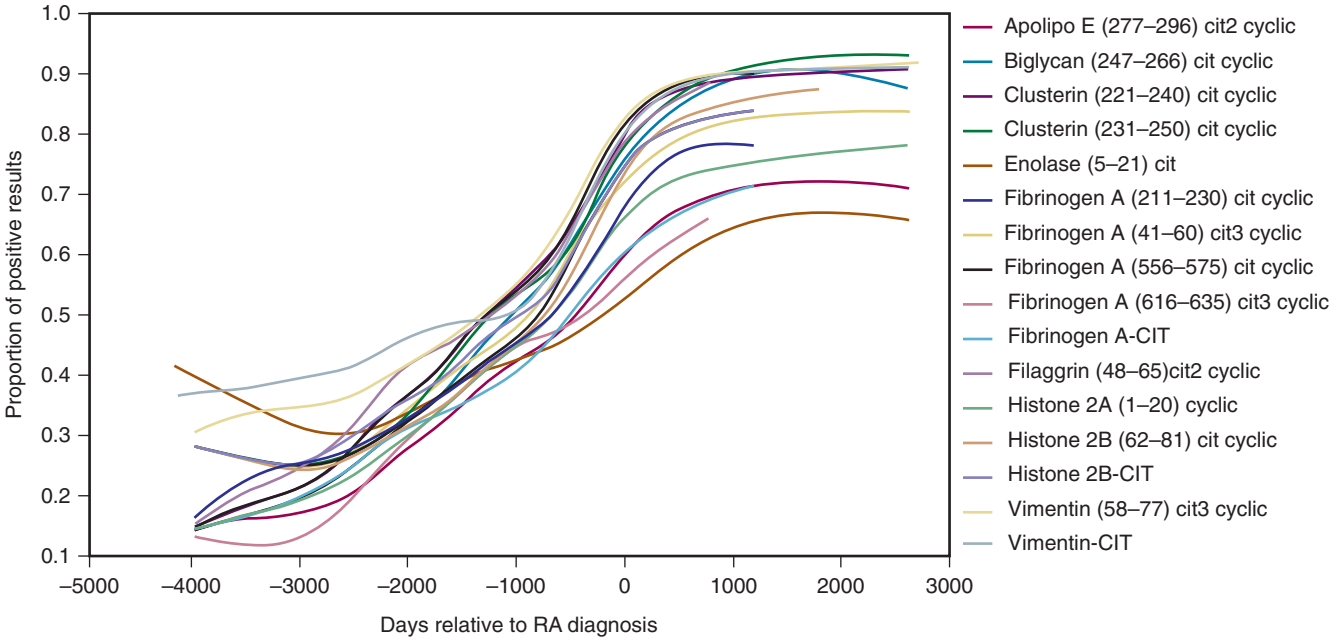



**Figure 69-7** Autoantibody production in rheumatoid arthritis (RA). Anti-citrullinated protein antibodies are detected in the blood long before the onset of clinical arthritis in many patients. Multiple specificities are detected, and all increase at basically the same rate. The levels peak shortly after the onset of symptoms. CIT, Full protein citrullinated in vitro; cit, one citrullinated arginine; cit2, two citrullinated arginines; cit3, three citrullinated arginines; cyclic, peptide is cyclic rather than linear. *(From Sokolove J, Bromberg R, Deane KD, et al: Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis.* PLoS One *7:e35296, 2012.)*

### Antibodies to Other Modified Antigens: Anti-carbamylated Peptide Antibodies

ACPAs are the best characterized antibodies directed against modified antigens in RA. However, other antibody systems are also present and could define additional subsets of disease. For example, some patients have antibodies that recognize carbamylated proteins. In this case, lysine is converted to homocitrulline through a non-enzymatic reaction with isocyanic acid and creates neo-epitopes in much the same manner as conversion of arginine to citrulline. Both ACPAs and anti-carbamylated protein antibodies (ACarPs) are present in a subset of patients with RA. More intriguingly, up to 30% of ACPA$^-$ patients have ACarPs, including IgG and IgA antibodies.[68] Like ACPAs, these novel antibodies, such as to carbamylated fibrinogen, can occur before the onset of clinical disease in nearly 40% of patients with RA.[69] The antibodies can participate in the inflammatory response, and mice immunized with carbamylated peptides can develop erosive arthritis. Like ACPAs, the antibodies alone do not cause synovitis. However, lymphocytes from immunized animals can transfer disease to naïve mice.[70] It is tempting to speculate that carbamylated peptides bind avidly to other HLA-DR molecules and define a specific at-risk population for RA.

### Rheumatoid Factor

The identification and characterization of RF as an autoantibody that binds to the Fc portion of IgG was the first direct evidence that autoimmunity might play a role in RA. Seropositive patients have more severe clinical disease and complications than do seronegative patients, including increased cardiovascular complications, although this outcome might be due to the association of RFs with ACPAs.[71] RF can also activate complement by the classic pathway, and there is clear evidence of local complement production and consumption in the rheumatoid joint. Large quantities of IgG RF and RF-containing complexes are produced by rheumatoid synovial tissue and detected in cartilage. B cell–targeted therapies such as rituximab can deplete peripheral B lymphocytes and modestly decrease titers of RF. This effect does not correlate precisely with clinical responses, and synovial RF production is not significantly changed by B cell depletion. Nevertheless, RF levels often decrease in responders and increase again coincident with clinical relapses.

Three-quarters of patients with RA are seropositive when standard tests for RF are used. First-degree relatives of seropositive patients with RA are frequently seropositive, suggesting a genetic contribution. Although IgG and IgM RFs are the most abundant in RA, IgA and IgE RF have also been detected. IgE RF can potentially complex with aggregated IgG in synovial tissue, and the subsequent complexes could then degranulate synovial mast cells through activation of Fc receptors in the synovium.

The RFs produced in persons with RA differ from those produced by healthy people or from patients with paraproteins. The avidity of RF for the Fc portion of IgG is much greater in RA than in Waldenström's macroglobulinemia or in cryoglobulins. Many RFs expressed by abnormal B cells (such as Waldenström's macroglobulinemia) and by normal B cells in human tonsils are derived from the germline. In contrast, RFs in RA are derived through rearrangements and somatic mutations of the germline genes. RF production can be quite high in rheumatoid synovium; IgM RF represents about 7% of the total IgM and 3% of the total IgG produced by synovial cell cultures.

RFs in RA primarily use the IgG variable heavy 3 *(IGHV3)* gene and a variety of variable light (VL) genes, whereas natural antibodies with RF activity use *IGHV1* or *IGHV4* and the *IGKV3* genes.[72] The κ light chain repertoire expressed in RF-producing cells isolated from one RA patient was enriched for two specific Vκ genes but contained many somatic mutations and non–germline-encoded nucleotides.[73] Therefore, the selection and production of these specific RFs were likely due to antigenic drive rather than derived directly from the germline. Additional RFs have been identified with characteristics similar to an antigen-driven response. A crystal structure of one IgM RF bound to IgG showed a key contact residue of the RF with the Fc portion of IgG containing a somatic mutation, supporting the notion that the mutations are related to affinity maturation.

## Autoimmunity to Cartilage-Specific Antigens

Because synovial tissue inflammation is a hallmark of RA, it is only natural to assume that certain joint-specific antigens might play an etiologic or pathogenic role. The emerging picture of autoimmunity in RA tends to implicate patterns of self-directed responses, rather than a single epitope that encompasses all patients at all times during the disease. It is quite possible that articular autoimmunity could vary with the stage of disease, the clinical manifestations, and treatment.

**Type II Collagen.** The discoveries that immunization with type II collagen can cause arthritis in rodents and that the disease can be passively transferred by IgG fractions containing anti-collagen antibodies or by transfer of lymphocytes from affected animals spawned extensive studies defining the mechanisms of disease, the dependence on class II MHC genes, and the potential relationship with RA. T cells are required for initiation of collagen-induced arthritis, and a major immunogenic and arthritogenic epitope on type II collagen resides in a restricted area of the type II collagen chains. The disease is caused by anti-collagen antibodies that localize to the joint and activate complement.

In contrast, type II collagen antibody generation is probably not the initiating event in RA but can amplify the inflammatory response (Table 69-4). It is very likely that immunoreactivity begins with antibodies that bind to citrullinated type II collagen, with epitope spread to include the unmodified or denatured protein. Of interest, T cell responses to type II collagen, especially a dominant epitope at amino acid 263-270, are much greater if the epitope is glycosylated or if the protein is citrullinated.[74] Sera from patients with RA contain antibody titers to denatured bovine type II collagen that are significantly higher than those found in control sera.[75] Anti-collagen antibodies purified from the sera of patients with RA can activate complement, generating C5a when they bind to cartilage. In addition, isolated synovial tissue B lymphocytes actively secrete anti–type II collagen antibodies in almost all patients with seropositive RA, whereas articular cells from patients who do not have RA do not secrete these antibodies. Synovial fluid T cells also recognize and respond to type II collagen, and 3% to 5% of RA synovial fluid–derived T cell clones are autoreactive to the protein.

**TABLE 69-4** Examples of Autoantigens in Rheumatoid Arthritis

| |
|---|
| Cartilage antigens |
| Type II collagen |
| gp39 |
| Cartilage link protein |
| Proteoglycans |
| Aggrecan |
| Citrullinated proteins |
| Carbamylated proteins |
| Glucose-6-phosphoisomerase |
| HLA-DR (QKRAA) |
| Heat shock proteins |
| Heavy-chain binding protein (BiP) |
| hnRNP-A2 |
| Immunoglobulin G |

BiP, Binding immunoglobulin protein; HLA, human leukocyte antigen; hnRNP, heterogeneous nuclear ribonucleoprotein.

**gp39 and Other Cartilage-Specific Antigens.** Several other cartilage components besides type II collagen also elicit immune responses in RA. Among the most provocative is cartilage glycoprotein gp39. Several gp39 peptides can bind to the HLA-DR*0401 molecule and stimulate proliferation of T cells from patients with RA. BALB/c mice, which are often resistant to experimental arthritis, develop polyarticular inflammatory arthritis after immunization with gp39 and complete Freund's adjuvant. Although anti-gp39 antibodies are detected in only a small percentage of patients, detection of these antibodies is relatively specific for RA.[76] Other examples of potential cartilage autoantigens include proteoglycans, aggrecan, cartilage-link protein, and other types of collagen. Proteomic analysis of RA serum using peptide arrays to detect multiple autoantibodies also identifies anti-gp39 antibodies in patients with early RA, which may be associated with less aggressive disease.[76a]

## Autoimmunity to Nonarticular Antigens

Autoimmune responses in RA can also involve antigens that are broadly expressed beyond the joint.[77] These antigen-antibody systems comprise a pattern of autoimmune responses that can potentially lead to synovial inflammation.

## Glucose-6-Phosphoisomerase

Spontaneous inflammatory arthritis develops in K/BxN mice as a result of antigen-specific immunity against a seemingly irrelevant nonarticular antigen.[78] Autoantibodies to the ubiquitous enzyme glucose-6-phosphate isomerase (GPI) cause the disease, and transient synovitis also develops in normal mice injected with the serum of the affected K/BxN mice. Mice immunized with GPI also develop antibodies and then inflammatory arthritis. The passive arthritis model requires the alternate complement pathway, Fc receptors (especially FcRγIII), and mast cells, but not T or B cells. IL-1 is more important than TNF in this model, and the IL-1 knockout mice are almost completely protected from disease. Notably, this effect can be overcome by

administration of a TLR ligand like LPS, which shares a downstream signaling pathway with IL-1. Other cytokines, such as IL-6, and signaling pathways, such as p38 MAP kinase and upstream kinases such as MKK3 and MKK6, are also required for full expression of the disease.

Immunohistochemical studies show that the target protein, GPI, adheres to the surface of cartilage, which permits local antibody binding and complement fixation. Initiation of synovial inflammation requires mast cells that increase vascular permeability and provide access to the synovium and cartilage.[79] The initial phase involving mast cell and vascular permeability is augmented by antibody-mediated complement fixation when serum proteins have access to GPI-decorated cartilage.

Although the model initially appears to be the result of a ubiquitous antigen, the display of GPI on the surface of cartilage suggests that it has much in common with arthritis models caused by "joint-specific" antigens. Although initial data suggested some specificity for RA, anti-GPI antibodies are detected in a relatively small percentage of patients with RA and are not specific for the disease. Nevertheless, it might, along with several other antibody systems, contribute to local complement fixation and inflammation.

**Heterogeneous Nuclear Ribonucleoprotein-A2 and Heavy-Chain Binding Protein.** Several other autoantigens that are expressed in synovium have been characterized in RA, although they are also produced in many other locations. For instance, antibodies directed against the heterogeneous nuclear ribonucleoprotein-A2 (hnRNP-A2), sometimes called RA33, occur in about one-third of patients with RA, as well as patients with other systemic autoimmune diseases. Anti-RA33 antibodies are also produced in the TNF transgenic mouse model of RA, suggesting that pro-inflammatory cytokines can independently lead to a breakdown of tolerance for this particular protein.[80] Of interest, RA33$^+$ patients with early RA (symptoms for less than 3 months) tended to have less destructive disease.[81] Although not especially sensitive or specific for RA when used in isolation, an algorithm involving anti–RNP-A2, RF, and ACPA can be used to predict which patients with early synovitis will progress to erosive RA.[82]

Autoantibodies that bind to stress-protein Ig heavy-chain binding protein (BiP) have also been observed. About 60% of patients with RA have anti-BiP antibodies, and the specificity is reportedly more than 90%. In addition to humoral responses, RA T cells can proliferate in response to this protein.

**Heat Shock Proteins.** The HSPs are a family of mainly medium-sized (60 to 90 kDa) proteins produced by cells of all species in response to stress. Another smaller HSP (HSP27) can serve as substrates for enzymes such as p38 MAP kinase that regulate stress responses. Immunity against HSPs contributes directly to synovitis and joint destruction in the adjuvant arthritis model in rats in which T lymphocytes recognize an epitope of mycobacterial HSP65 (amino acids 180 through 188). Some of these cells also recognize cartilage proteoglycan epitopes, perhaps explaining the targeting of joints.

Some patients with RA have elevated levels of antibodies to mycobacterial HSPs, especially in synovial fluid. The majority of T cell clones isolated from RA synovial fluid with specificity to mycobacterial components express the γδ–TCR and do not display CD4 or CD8 surface antigens. Freshly isolated synovial fluid T cells from patients with RA briskly proliferate in response to recombinant 65 kDa HSP, whereas proliferation to other recall antigens such as tetanus toxoid is not increased. Synovial fluid mononuclear cells activated by 60 kDa mycobacterial HSP inhibit proteoglycan production by human cartilage explants.

## SYNOVIAL DISEASE AND BIOLOGY

**KEY POINTS**

- The synovium in RA is marked by intimal lining hyperplasia and sublining infiltration with mononuclear cells, especially CD4$^+$ T cells, macrophages, and B cells.
- Intimal lining FLS display unusual aggressive features.
- Macrophages in the intimal lining are highly activated and produce many cytokines.
- Lymphocytes can either diffusely infiltrate the sublining or form lymphoid aggregates with germinal centers.
- Sublining CD4$^+$ T cells mainly display the memory cell phenotype.
- Synovial B cells and plasma cells in RA exhibit evidence of antigen-driven maturation and antibody production.
- DCs can potentially present antigens to T cells in synovial germinal centers.
- Mast cells produce small molecule mediators of inflammation and can participate in disease initiation by increasing vascular permeability.
- Neutrophils are rarely present in RA synovium but are usually abundant in synovial effusions.

The primary inflammatory site in RA is the synovium. Infiltration of synovial tissue with mononuclear cells, especially T cells and macrophages, and synovial intimal lining hyperplasia are hallmarks of the disease (Figure 69-8). In this section, the various cell lineages and histologic patterns of rheumatoid synovium are discussed.

### Synovial Intimal Lining Cells: Type A and Type B Synoviocytes

The synovial intimal lining is a loosely organized collection of cells that form an interface between the synovium and the synovial fluid space. The intimal lining cells lack tight junctions and a definite basement membrane. The increase in cell number in RA can be quite substantial. In the normal joint, the lining is only one to two cell layers deep, whereas in RA it is often 4 to 10 cells deep. Two major cell types are found in the lining: a macrophage-like synoviocyte (also known as a type A synoviocyte) and an FLS (also called a type B synoviocyte). The former are derived from the bone marrow and express macrophage surface markers such as CD68, Fc receptors, and CD14, as well as abundant HLA-DR, whereas the latter express little if any class II MHC antigens, are devoid of macrophage markers, and have a scant endoplasmic reticulum. FLS express some proteins that are unusual for mesenchymal cells, including vascular cell adhesion molecule-1 (VCAM-1), CD55 (decay

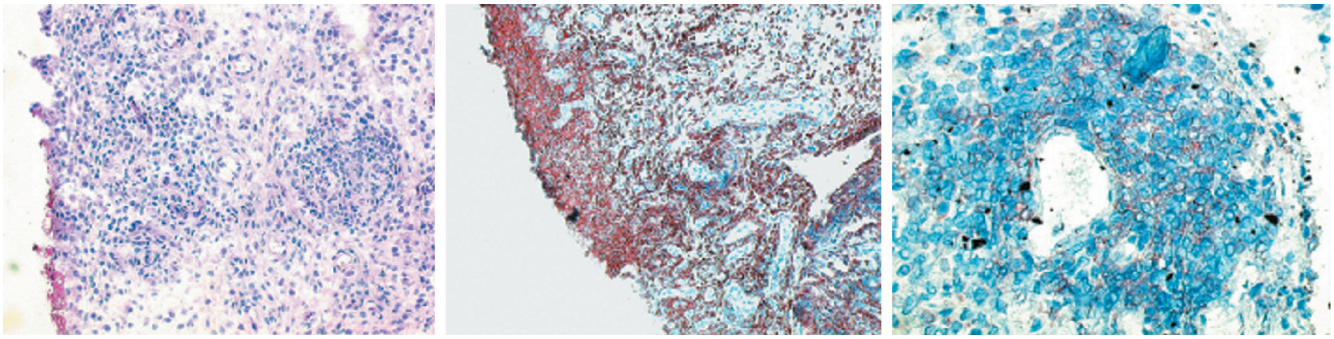


**Figure 69-8** The histopathologic appearance of rheumatoid arthritis synovium. Intimal lining hyperplasia, angiogenesis, and a prominent mononuclear cell infiltrate are present. Panels show standard histology *(left panel)*, as well as immunostaining for macrophages (*brown* in the intimal lining, *middle panel*) and a perivascular T cell aggregate *(right panel)*. *(Courtesy Dr. Paul-Peter Tak.)*

activating factor), cadherin-11, junctional adhesion molecule C (JAM-C), and the proteoglycan-synthesis enzyme, uridine diphosphoglucose dehydrogenase. The relative numbers of type A and B cells are usually similar in normal synovium. There is an absolute increase in both cell types in RA, although the percentage increase in macrophage-like cells is often greater. In addition, the type A synoviocytes tend to accumulate in the more superficial regions of the intimal lining.

Intimal lining synovial macrophages and sublining macrophages are terminally differentiated cells that presumably do not divide in the joint, and the accumulation of cells in RA is likely from the ingress of new bone marrow–derived precursors. Mesenchymally derived FLS can divide locally in response to the growth factors generated by the activated immune response. Platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, TNF, and IL-1 produced by many different cells combine with products of arachidonic acid metabolism to induce proliferation of these cells. In addition, pluripotential mesenchymal stem cells that arise in the bone marrow and circulate through the blood can migrate into the synovium and differentiate into type B synoviocytes.[83] Retention of macrophages in the intimal lining is probably due to expression of adhesion molecules such as VCAM-1 and JAM-C on FLS and is likely directed by chemotactic factors produced by FLS. For example, in micromass cultures composed of laminin and FLS, the FLS migrate to the surface of the micromasses and then can recruit macrophages in the interior to the "lining" (Figure 69-9).[84] Formation of this artificial intimal lining is dependent on the adhesion molecule cadherin-11.

Although local proliferation of cells in the intimal lining can occur, mitotic figures in rheumatoid synovium are rare, and thymidine uptake indicating DNA synthesis occurs in only a small percentage of synovial cells. Using a monoclonal antibody that recognizes dividing cells, an even lower rate of cell division (~0.05%) is apparent.[85] A somewhat higher percentage of cells that express the cell cycle–specific antigen proliferating cell nuclear antigen is present in RA lining compared with OA. This finding correlates with the lining cell expression of the proto-oncogene c-Myc, which is intimately linked with fibroblast proliferation.

The architecture of the synovial intimal lining is distinct from other lining layers in the body. In contrast to serosal surfaces, the intimal lining does not include epithelial cells, it lacks a basement membrane, and it has no tight junctions. Rather than serving as a discrete barrier, it is a loose association of cells that is discontinuous in some locations. Cadherin-11, from a class of adhesion proteins that are ubiquitous in various tissues, serves as the major mediator of homotypic aggregation by FLS.[86] Immunohistochemistry shows abundant expression of this protein in the intimal lining. Its importance in the synovial architecture was confirmed in cadherin-11 knockout mice, in which the intimal lining was virtually nonexistent. As previously noted, cadherin-11 mediates self-aggregation of FLS in vitro. Blocking cadherin-11 with antibodies suppresses arthritis in the passive K/BxN model. These data suggest that FLS not only organize the intimal lining formation, but also, like T cells, B cells, and macrophages, play a critical role in the pathogenesis of inflammatory arthritis.

Two major populations of adherent cells can be readily identified when rheumatoid synovium is enzymatically dispersed and cultured in vitro. One type of cell is macrophage-like, which expresses HLA-DR antigens, Fc receptors, and monocyte lineage–differentiation antigens, and is capable of phagocytosis. The macrophages, which constitute about 20% of the total cell number in the rheumatoid joint, can be derived either from the intimal lining or the sublining region. These cells are highly activated in the synovium and produce large amounts of inflammatory mediators, including cytokines and arachidonic acid metabolites.

A second type is defined by the presence of antigens expressed primarily on fibroblasts and by the absence of phagocytic capability, DR antigens, or antigens of the monocytic lineage. When the enzymatically dispersed cells are cultured for several passages, this latter cell type survives and proliferates, resulting in a relatively homogeneous population of fibroblast-like cells. Most published reports of cultured synoviocytes utilize this population of cells.

Fibroblast-like cells grow slowly, with a doubling time of 5 to 7 days, and can be passaged for several months in vitro. Although it has not been proven that these cells originate solely from the synovial intimal lining, the fact that a significant percentage of cells express VCAM-1 and CD55 suggests that at least some are derived from this region. Synovial fibroblasts from RA have been imprinted in vivo, possibly as a result of epigenetic changes like DNA methylation (see Figure 69-3), and exhibit an aggressive phenotype when cultured in vivo or when implanted into mice.

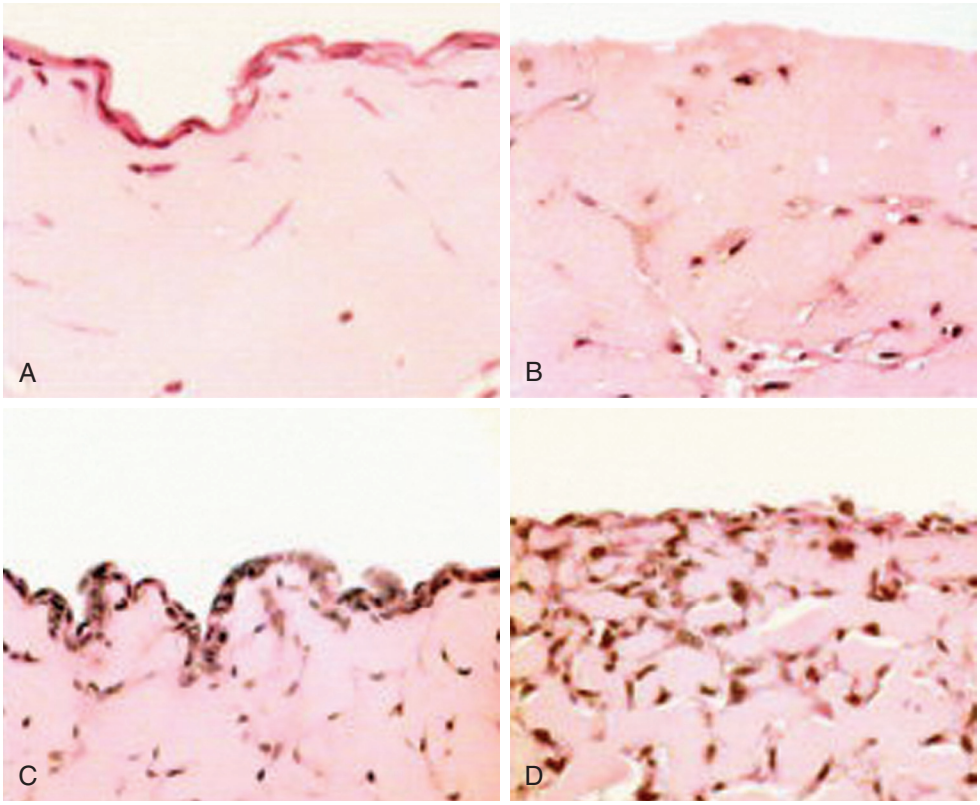



**Figure 69-9** Fibroblast-like synoviocytes (FLS) are programmed to form the synovial intimal lining. Human and mouse FLS spontaneously form lining structures when embedded in a three-dimensional matrix composed of the matrix protein laminin. The cells migrate to the surface of the structure over 3 weeks and resemble an intact synovial lining. The figure shows parallel experiments using human **(A)** and mouse **(C)** fibroblast-like synoviocytes. Of interest, dermal fibroblasts from human **(B)** or mouse **(D)** skin do not migrate to the surface, indicating that the synoviocytes are preprogrammed for this function and that it is not a property of all fibroblasts. *(From Kiener HP, Watts GF, Cui Y, et al: Synovial fibroblasts self-direct multicellular lining architecture and synthetic function in three-dimensional organ culture.* Arthritis Rheum *62:742, 2010.)*

Experiments examining synovial tissue and FLS gene expression profiles suggest that specific patterns correlate with synovial histopathology.[87] FLS from highly inflamed synovium exhibited a TGF-β gene signature that has also been described in cells with features of both smooth muscle cells and fibroblasts that are found in many mucosal surfaces known as *myofibroblasts*. A second pattern observed in cells derived from relatively non-inflammatory RA tissue had increased expression of insulin-like growth factor–regulated genes. Attempts to distinguish RA and OA FLS expression profiles have offered mixed results, but some differences have been observed using microarray technology. Using a systems biology to analyze the transcriptome, a network of invasive processes could be established that includes twist basic helix-loop-helix transcription factor 1 (TWIST1) and periostin (which regulates integrin-mediated cell migration) as key differentially expressed genes.[88] These networks, which have also been explored in FLS epigenetic signatures, can potentially identify unanticipated therapeutic targets.

## Aggressive Behavior of RA Fibroblast-like Synoviocytes

**Tumor-like Properties.** Rheumatoid FLS demonstrate some aggressive properties compared with cells obtained from other synovia or tissues. For instance, adherence to plastic or extra-cellular matrix is generally required for normal fibroblasts to proliferate and survive in culture. Although FLS typically grow and thrive under conditions that permit adherence, RA synoviocytes can also proliferate in an anchorage-independent manner.[89] In addition, cultured RA synoviocytes can exhibit defective contact inhibition and express proto-oncogenes, such as c-Myc, that are typically abundant in tumor cells. Poorly regulated cell growth likely occurs in vivo as well, and studies examining X-linked genes demonstrate oligoclonality in the synoviocyte population from RA, but not OA, synovium.[90] Oligoclonality is especially prominent in cells derived from the invading pannus, which is the most aggressive region of the synovium. Increased telomerase activity, another feature of transformed tissue, is also present in RA synovium and can be observed in fibroblast growth factor (FGF)–stimulated RA synoviocytes. Epigenetic analysis suggests that overexpression of certain miRNAs or differential DNA methylation is associated with increased cytokine production or more aggressive behavior.

**Matrix Invasion.** The most compelling evidence suggesting that RA synoviocytes are permanently altered is derived from studies in a severe combined immunodeficiency (SCID) mouse model in which synoviocytes and cartilage are co-implanted. The rheumatoid cells adhere to and invade into the cartilage matrix, unlike OA and skin fibroblasts[91] (Figure 69-10). Because these synoviocytes are devoid of T cells and macrophages, there is no contribution from an immune response to murine antigens. The invading

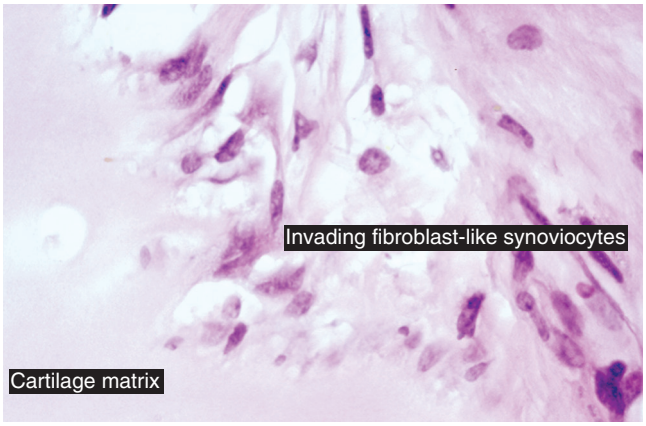



**Figure 69-10** Invasion of rheumatoid arthritis (RA) synoviocytes into cartilage explants in severe combined immunodeficiency syndrome (SCID) mice. RA fibroblast-like synoviocytes were co-implanted with normal human cartilage into the renal capsule of SCID mice. Note that the synoviocytes attached to the cartilage and invaded into the matrix. Several chondrocytes in lacunae are also present. *(Courtesy Dr. S. Gay.)*

cells express VCAM-1, which could facilitate adhesion to cartilage or chondrocytes, as well as proteases that digest the cartilage matrix. If explants are introduced into two locations in the SCID mice, the rheumatoid FLS can migrate from one site to another, presumably via lymphatics and the bloodstream.[92] This finding raises the intriguing possibility that imprinted, aggressive cells can "metastasize" from joint to joint and contribute to the polyarticular nature of RA.

Blocking IL-1 function in the SCID model with IL-1 receptor antagonist (IL-1Ra), a natural antagonist to IL-1, has no effect on synoviocyte invasion but decreases perichondrocyte matrix loss. In contrast, IL-10 or blocking membrane type I MMP, Ras, or c-Myc expression decreases invasion. Surprisingly, overexpression of soluble TNF receptors has little effect in this model. These studies suggest that excessive production of IL-1 and underexpression of IL-10 contribute to the invasive properties of RA synoviocytes. In another study, transfecting normal synoviocytes with the human papillomavirus gene encoding E6, which inactivates p53, induced the rheumatoid phenotype. RA FLS also have prolonged and exaggerated responses to synovial cytokines. For example, TNF induces higher levels of chemokines and MMPs than do control FLS, perhaps because of prolonged activation of transcription factors like nuclear factor-κB (NF-κB).[93] Overall, these studies paint an interesting picture of RA FLS serving as key effector cells that display unique aggressive properties in inflammatory synovitis.

Various cytokines in the joint enhance FLS migration and invasion into matrix, most notably PDGF. The signaling molecules that regulate this process could represent interesting therapeutic targets. Phosphoinositide 3 kinases (PI3Ks) are especially important. FLS express three isoforms (α, β, and δ); α is expressed by many cell lineages, but δ is usually restricted to bone marrow–derived cells.[94] The δ isoform is unexpectedly expressed by FLS and is markedly induced by TNF. Functional studies and the use of kinase inhibitors suggest that PI3Kδ is perhaps the most important isoform that regulates cell movement and matrix invasion.

## Synovial T Lymphocytes

### Immunohistologic Patterns

In chronic RA, the synovium contains a collection of T lymphocytes that can lead to an organizational structure that resembles a lymph node. The distribution of lymphocytes in the tissue varies from discrete lymphoid aggregates to diffuse sheets of mononuclear cells, with the most prominent location for T cells being the perivascular region. These collections consist of small, $CD4^+$ memory T cells ($CD45RO^+$) with scant cytoplasm. Scattered $CD8^+$ T cells can accumulate in the aggregates, and overall, substantially more $CD4^+$ cells are present. Peripheral to these foci is a transitional zone with a heterogeneous mixture of cells including lymphocytes, occasional undifferentiated blast cells, plasma cells, and macrophages (see Figure 69-8). Synovial lymphoid aggregates occur in at least 15% to 20% of patients; although originally considered specific for RA, it is now clear that they are equally prevalent in psoriatic arthritis and are even seen in some people with OA.[95] The presence of aggregates correlated with higher production of ACPAs and RF in synovium, but not with clinical disease activity.

Considerable heterogeneity exists in the histologic patterns from patient to patient and within a single joint. Synovial biopsy studies suggest that at least six sites must be evaluated to decrease the risk of sampling error to 10% to 20% or less. In situations in which the synovial tissue of more than one joint from an individual patient is available, the same general histopathologic patterns are usually apparent in tissue from separate sites.

**Regulation of T Cell Aggregate Formation.** T cells often constitute 30% to 50% of cells in RA synovia, and most are $CD4^+$. About 5% of cells are B lymphocytes or plasma cells, although in some tissues the percentage can be considerably higher. The B cells are located primarily within reactive lymphoid centers, whereas plasma cells and macrophages are often found outside these centers. This arrangement is consistent with T cell–dependent B lymphocyte activation. Plasma cells, the main immunoglobulin producers, migrate away from the germinal centers after differentiation. CD4 cells in RA synovium are in intimate contact with B lymphocytes, macrophages, and DCs.

Aggregate formation is complex and involves numerous signals to orchestrate the organization of individual cell lineages. In some cases, follicular DCs are present and are involved in forming true germinal centers (discussed in a subsequent section). The presence or absence of aggregates and germinal centers is a dynamic process. In one study evaluating synovial biopsies, the presence of lymphoid neogenesis was not restricted to patients with autoantibodies. Even though the cytokine and chemokine profile supports the formation of these structures, progression to fully differentiated follicles was uncommon.[96]

Chemokines play a central role in the organization of tissues into lymphoid structures such as aggregates and germinal centers. CXCL13 and CCL21 are especially important, and their expression in rheumatoid synovium correlates with the presence of this microarchitecture.[97] The former, in particular, is produced by synovial follicular DCs. Similarly, plasma cells expressing the chemokine receptor

CXCR3 are present in the rheumatoid synovium. The CXCR3 ligand, Mig/CXCL9, is highly expressed by intimal lining synoviocytes and sublining cells and recruits these cells to the T cell aggregates.[98]

The architecture of lymphoid structures in rheumatoid synovium is also regulated by members of the TNF superfamily. Lymphotoxin-α and -β (LTα and LTβ, respectively), as well as lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT), form trimeric molecules in various combinations that can bind to distinct cell surfaces. These three cytokines regulate the function and organization of lymphoid tissues. Deletion of either LTα or LTβ seriously impairs lymphoid development, whereas lymph nodes develop normally in LIGHT-deficient mice. In the SCID mouse model using RA tissue explants, depletion of $CD8^+$ T cells led to loss of follicular DCs, depletion of LTα1β2, and disintegration of the lymphoid follicles.[99]

Although LTα is difficult to detect in RA, other TNF superfamily members such as LTβ, TWEAK (TNF-like weak inducer of apoptosis), and LIGHT are present.[100] In addition to regulating lymphoid aggregate and germinal center formation, LT can also directly stimulate FLS to produce chemokines such as CCL2 and CCL5 that attract T cells into the joint. LIGHT also enhances osteoclast differentiation and induces expression of MMP-9, TNF, IL-6, and IL-8 by macrophages. LTβ and LIGHT blockade using soluble receptors decreases the severity of collagen-induced arthritis.[101] However, the inhibitor was not effective in the passive arthritis model in which the pathogenic antibodies were directly administered to mice. Hence the LIGHT axis has a more important role in the early phases of disease when adaptive immunity to joint antigens is developing. TWEAK can also play a role in Th17 differentiation, and its inhibition suppresses disease severity in collagen-induced arthritis.[102]

### Synovial T Cell Phenotype

**Co-stimulatory Molecules.** RA synovial T lymphocytes exhibit an activated surface phenotype, with high expression of HLA-DR, CD69, and CD27. The co-stimulatory molecule CD28 is highly expressed by synovial T cells in RA. Its ligands, CD80 and CD86, are also displayed on antigen-presenting cells in the joint, thereby providing an excellent environment for T cell activation. The importance of the CD80/86-CD28 interaction is supported by the observation that abatacept blocks CD80/86 and is effective in RA. Other co-stimulatory molecule pairs such as CD40L on T cells and CD40 on antigen-presenting cells are also expressed in RA synovium.

**Adhesion Molecules.** Synovial lymphocytes also express adhesion molecules of the very late activation antigen (VLA) and lymphocyte function–associated antigen (LFA) superfamily of integrins, as well as help recruit and retain lymphocytes to the synovium. The cytokine milieu of the joint induces adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and connecting segment-1 (CS-1) fibronectin on vascular endothelium. These, in conjunction with chemokines and other chemoattractants, attract cells that express the correct adhesion molecule counter-receptors on their surface.

**Chemokine Receptors.** Synovial T cells in RA express characteristic receptors to specific chemokines. The chemokine receptor CCR5 is the ligand for macrophage inhibitory proteins 1α and 1β and is highly expressed in the infiltrating RA T cells. This particular receptor, along with CXCR3, is preferentially found on Th1 cells, an observation that might explain accumulation of this phenotype in the rheumatoid synovium. In fact, expression of nonfunctional CCR5 alleles that protect from HIV infection might diminish the risk of RA. The chemotactic factor stromal cell–derived factor-1 (SDF-1) is also produced by synovial tissue, and its specific receptor, CXCR4, is displayed by rheumatoid synovial T cells. Other T cell phenotypes recruited to the rheumatoid synovium include Th17 cells that express CCR2 or CCR6 and produce IL-17 and $CD4^+CD25^+$ regulatory T (Treg) cells that can suppress immune responses (see the subsequent discussion of T cell subsets). Preferential recruitment of $CCR6^+$ Th17 cells into the rheumatoid joint appears to be due to the local release of the chemokine CCL20.[103]

**T Cell Receptor Rearrangements.** In some patients, a pattern emerged suggesting an increased number of T cells expressing Vβ3, Vβ14, and Vβ17, especially in synovial tissue. These Vβ genes are structurally related and are unusually susceptible to activation by superantigens. Most studies have not found evidence for the restricted clonality of T cells in RA synovial fluid, synovial tissue, and blood or identified expansion of different Vβ or Vα genes.

**Determinants of T Cell Phenotype.** The local accumulation of T cells in the joint is not always related to proliferation induced by a particular antigen. Instead, antigen-independent processes related to the expression of chemokines and adhesion molecules on vascular endothelium and circulating lymphocytes help determine the mononuclear cell infiltrate. Although local antigen-specific expansion can occur, it is probably responsible for a relatively small percentage of the T cell infiltrate. Cells that encounter their appropriate antigen in the correct cytokine and antigen-presenting cell environment can potentially activate other local cells through direct cell-cell contact or the elaboration of lymphokines. A high level of telomerase activity is also present in synovial lymphocytes in RA, which is a reflection of their proliferative activity and correlates with the severity of synovial inflammation.[104] An alternative explanation for T cell activation in RA is that antigen presentation mainly occurs in central lymphoid organs. In this scenario, DCs that encounter antigens in the synovium or other sites migrate to lymphatic tissue and then present antigen to naïve T cells. Once activated, these T cells enter the circulation and can migrate to the joint, where they can produce cytokines and activate resident cells.

### Synovial T Cell Immunoreactivity

The histopathologic appearance of RA, with exuberant infiltration of the synovium with T lymphocytes, is characteristic of tissues with chronic antigen-specific responses. However, the synovium can only respond to inflammation in a limited number of ways, and many chronic immune-mediated arthropathies have similar histologic patterns, including lymphoid aggregates in OA. In fact, chronic arthritides that clearly are not mediated by T cells (e.g., chronic tophaceous gout) exhibit many of the same features.

The microheterogeneity of the rheumatoid synovial tissue, with different numbers and proportions of cell lineages in each area, could reflect responses to different antigens in various synovial locations in the synovium. Although synovial T cells in these varied locations display an activated phenotype, proliferative and cytokine responses are usually less than normal peripheral blood cells or even autologous peripheral blood T cells from the same patient. Spontaneous and stimulated cytokine production, including Th1 factors such as IFN-γ and IL-2, are relatively low. Responses directed toward most recall antigens are also deficient, although RA synovial T cells can proliferate briskly to certain HSPs. Th17 cells have been detected in both the blood and synovium, perhaps because of exposure to a cytokine milieu that includes IL-1, IL-6, and/or IL-23 that can promote differentiation toward production of IL-17 family members (especially IL-17A and F).

The mechanisms of decreased responsiveness in the synovial compartment likely include exposure to suppressive factors (e.g., TGF-β or IL-1Ra), abnormal redox potentials that suppress TCR signal transduction, the presence of Tregs, or induction of anergy. Another contributor to local anergy could be the relative lack of the co-stimulatory molecule CD80 on HLA-DR$^+$ FLS, which could induce anergy. This phenomenon has been observed in co-cultures of T cells with synoviocytes.

As noted earlier, modified proteins such as through citrullination provide a rich source of antigenic stimulation, although many different peptides are involved. Animal models also suggest that breakdown of tolerance can be due to a combination of factors early in development. For instance, mutations in ZAP70, which is involved with TCR signaling, can lead to spontaneous T cell–dependent inflammatory arthritis in mice.[105] An abnormal *ZAP70* gene leads to defective positive and negative selection in the thymus and allows autoreactive T cells to escape. As a result, mice produce autoantibodies (e.g., RF and anti-dsDNA) and develop a severe destructive arthritis. The disease can be transferred by thymocytes or peripheral CD4$^+$ T cells into syngeneic mice with a normal *ZAP70* gene. This autoimmune response might be more relevant to antigen spread rather than the initial response to modified proteins that were not present during development.

These studies demonstrate that minor changes in the TCR complex and signaling can alter T cell selection and induce arthritis. The finding is especially relevant in light of the association of a *PTPN22* SNP with RA, because this gene is also involved with TCR signaling. Despite the clear T cell dependence of the model, the cytokines implicated are remarkably similar to autoantibody-dependent models of RA. Therefore the cytokine profile in RA represents a final common pathway for a variety of autoimmune mechanisms. This concept is supported by the diversity of responses to targeted therapies in RA and suggests that RA is not a single disease but instead is a group of mechanistically distinct entities with a shared phenotype.[105a]

## Activation of Synovial Cells by Cell-Cell Contact with T Lymphocytes

Even though T cell activation is relatively modest in rheumatoid synovium, direct cell-cell contact permits these cells to participate in synovial cytokine networks and matrix destruction. Membranes prepared from activated T cells can directly stimulate macrophages and FLS to produce cytokines and MMPs.[106] The membrane constituents that regulate this process vary, depending on the particular culture conditions, but include adhesion molecules such as LFA-1 and membrane-bound TNF. Hence a T cell displaying these proteins can potentially contribute to macrophage and fibroblast activation in an antigen-independent fashion. One of the best-characterized consequences of this pathway is the ability of T cells to enhance synovial macrophage TNF production in a contact-dependent manner after exposure to macrophage-derived IL-15.[107]

Although the traditional paradigm suggests that T cells in the joint drive an antigen-specific response, cell-cell contact influences can be antigen-independent and only require co-localization of memory T cells with synoviocytes or macrophages. Because T cells with a memory phenotype accumulate into the joint because of the release of chemoattractants, there is no requirement for a specific antigen. Instead, activation of innate immunity by nonspecific stimuli permits subsequent ingress of the correct T cell phenotype to engage resident synovial intimal lining cells, sublining fibroblasts, or macrophages. The combination of adaptive immune responses and antigen-independent stimulation could contribute to the diversity of individual responses to targeted therapy and the relative paucity of complete remissions.

### Restoring T Cell Tolerance

Because synovial T cell responses and autoreactivity play a key role in the pathogenesis of RA, one potential therapeutic approach is to restore tolerance. This concept has been somewhat problematic because the underlying defect has not been fully defined and could include abnormal thymic selection, genetic factors that lead to altered immune responses, presentation of abnormal proteins in the context of specific MHC proteins, or inadequate regulatory T cell function. What is clear, however, is that current approaches do not "cure" RA, because cessation of therapy usually leads to a recurrence of disease.

Aggressive treatment of early RA (within the year of clinical disease) with methotrexate and a TNF inhibitor could lead to long-lasting remissions.[108] In some cases, therapy can be withdrawn after 1 year of treatment, and patients do not experience a flare for at least an additional year. The mechanism of prolonged remission despite discontinuing therapy in early disease contrasts with less successful attempts to withdraw therapy in long-standing disease. Additional individualized therapies that enhance Treg function, alter co-stimulation, or delete pathogenic T cells are other ways to restore homeostasis and normal immune function.

## Regulatory T Cells

The role of classically defined inducible Tregs (CD4$^+$CD25$^+$CD127$^+$) in RA remains poorly defined. Deficiency of this subset, which produces IL-10 and TGF-β and regulates other T cells through cell contact, could contribute to autoimmunity. The number of Tregs is generally low

in RA synovium, although some reports suggest that it is similar to OA synovium.[109] Interestingly, the number decreases after intra-articular injection with corticosteroids.[110] Characteristic regulatory cells that express Foxp3 and CD39 can be found in RA lymphoid aggregates, especially adjacent to DCs.[111]

The transcription factor STAT3 can also play a role in defining Treg differentiation in the RA joint. Activated STAT3 is abundant in the rheumatoid synovium and can enhance Th17 differentiation while suppressing production of Tregs, whereas STAT5 appears to have the opposite effect.[112] Thus modulating the balance between STATs in RA T cells, such as through Janus kinase (JAK) inhibition, could change the balance between pathogenic and immunosuppressive lymphocytes. Fms-like tyrosine kinase ligand also appears to regulate Tregs in RA and increase their formation.[113]

Studies of RA peripheral blood generally demonstrate normal numbers of Tregs, although the $CD4^+CD25^+$ subset appears to accumulate in rheumatoid synovial effusions. However, peripheral blood Treg responses in vitro to stimuli such as anti-CD3 and anti-CD28 antibody displayed an anergic phenotype and were unable to suppress T cell or monocyte cytokine production. This abnormality might be reversed when patients are treated with TNF inhbitors.[114]

These data suggest that abnormal Treg function could be secondary to cytokine imbalance in RA rather than a primary event. Treatment can have a significant effect on the number and function of Tregs, which makes evaluation of their role in patient samples difficult to interpret. Nevertheless, enhancing or restoring Treg activity as a therapeutic intervention has the potential to downregulate other T cells and cytokine production.[115]

Enhancing the number or activity of Tregs has been evaluated in animal models of arthritis. Antigen-induced arthritis is exacerbated when $CD4^+CD25^+$ cells are depleted and suppressed when they are passively transferred to affected animals. Treg function is low in some inflammation models.[116] Methotrexate also increases Treg function in collagen-induced arthritis. Administration of neuropeptides such as vasoactive intestinal peptide appears to suppress collagen-induced arthritis by enhancing the Treg function in synovium and lymph nodes. However, it is still difficult to ascribe efficacy of therapeutic agents in RA to increased numbers or function of Treg.

## Synovial B Cells

Synovial B cell and plasma cell hyperreactivity increasingly are viewed as key participants in the perpetuation and initiation phases of RA. This notion has been fueled by the descriptions of novel spontaneous models of arthritis in mice, such as the K/BxN model, in which loss of tolerance leads to autoantibody production, activation of innate immunity, and chronic synovitis. Furthermore, B cell–directed therapies such as anti-CD20 antibody are effective in RA. The rheumatoid synovium contains a relatively small number of B cells (<5% of the total cell population), but they are functionally relevant as producers of ACPAs, RFs, and other antibodies that can form immune complexes and fix complement.

### Cytokine Regulation of Synovial B Cells

Although many rheumatoid synovial tissues exhibit a diffuse infiltration with mononuclear cells, a significant percentage also have discrete lymphoid follicles populated by B cells in the sublining region. Follicular DCs, B cells, plasma cells, and T lymphocytes collect in these aggregates. The germinal centers are highly organized structures in which affinity maturation occurs. B cells are present in the aggregates and express the maturation marker CD20, as well as proliferation antigens such as Ki67. The formation of these structures is dependent on soluble and membrane-bound cytokines, including lymphotoxin. B cells accumulate in lymphoid aggregates in RA synovium under the influence of a variety of chemotactic factors, including CCL21 and CXCL13.

A member of the TNF superfamily of cytokines known as B lymphocyte stimulator (BLyS, also called BAFF) has also been identified as a key molecule that regulates B cell differentiation. BLyS binds to transmembrane activator and CAML interactor (TACI), which is present on both B and T cells. If this system is blocked using recombinant TACI-Ig to absorb the cytokine and a related B cell differentiation factor called "a proliferation-inducing ligand" (APRIL), then the number of B cells is dramatically reduced and antibody production is decreased. The same construct is effective as a therapeutic agent in collagen-induced arthritis, a model that depends on autoantibody production.[117]

BLyS is produced in RA synovium, especially by macrophages. Synovial lining type B synoviocytes also can release BLyS, which can be induced by TNF and IFN-γ in vitro. APRIL is localized mainly to DCs in synovial germinal centers. TACI-Ig, which binds both BLyS and APRIL, disrupts the germinal centers and decreases Ig receptors in SCID mice implanted with rheumatoid synovium.[118] Results of clinical trials neutralizing BLyS have been mixed, suggesting that targeting of this cytokine is not as effective as B cell depletion with anti-CD20 antibody. Although improved benefit might be possible with a biologic agent that blocks both APRIL and BLyS, TACI-Ig markedly decreases serum immunoglobulin concentrations but has limited efficacy in RA.[119]

### Synovial B Cell Maturation

B cells isolated directly from germinal centers of RA synovium demonstrate a heterogeneous pattern of V-gene usage and rearrangement. The majority of VH genes are not mutated, suggesting that they are recent immigrants from the peripheral blood and are activated locally. For RF-producing cells, shared mutations containing an identical sequence throughout the variable domain of immunoglobulins have been identified in synovial tissue.[120] Preferential utilization of a limited number of VH and DH gene segments and marked preference for a DH reading frame encoding particular hydrophilic residues have also been observed, consistent with antigen-related selection and maturation. Analysis of expressed heavy-chain variable domains supports the notion that the B cell response in RA synovium is oligoclonal. Similarly, B cell clones isolated from either RA synovium or bone marrow with "nurselike" cells have limited VH usage.

B cells associated with follicular DCs in the rheumatoid synovium can further differentiate and develop additional mutations, suggesting antigen-driven selection. Plasma cells in other areas of the synovium have distinct rearrangements compared with the B lymphocytes associated with DCs. This finding raises the question of whether the plasma cells arise locally or migrate from the blood. Although plasma cell rearrangements are not always similar to the B cells, groups of plasma cells use similar genes, albeit with distinct mutations. Therefore the plasma cells could derive from synovial B cell clones that mutated and whose progeny proliferate and differentiate.

Maturation and survival of B cells depend on stromal cells and cells with nurselike properties that support lymphocyte maturation in the thymus. The synovium of patients with RA also contains nurselike cells, which can increase expression of CD40 and class II MHC proteins on B cells. B cell survival is supported by this population of cells, whereas autoreactive clones evade deletion and produce autoantibodies. A variety of cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and IL-8 are produced by RA nurselike cells, and direct contact with B cells is critical for maximal proliferation and antibody production.

### Role of B Cells in RA: Clinical Improvement after B Cell Depletion

The diversity of responses to targeted therapies suggests that many parallel mechanisms can contribute to RA. As with TNF inhibitors, anti-CD20 antibody (rituximab) demonstrates efficacy in RA in a subset of patients. Response rates are higher in patients with antibody profiles that include RF and ACPA, perhaps in combination with chemokines like CXCL13 that attract B cells to synovium. Increasing levels of CXCL13 also predict the return of B cells in the peripheral circulation and could be a useful biomarker for stratifying patients or planning additional courses of rituximab.[121] On the other hand, higher numbers of plasmablasts, as determined by IgJ expression, in the blood are associated with poor response to B cell depletion by ocrelizumab and rituximab (in about 20% of patients). The depleting antibodies do not affect plasma cells, which lack CD20, and persistent disease could be related to the presence of plasmablasts in the blood or joint.[122]

The precise function of CD20 is not well defined; it is expressed on mature B cells but not on plasma cells. Rituximab causes a rapid and profound depletion of peripheral blood B cells. In mice, anti-CD20 antibody depletes B cells in blood, spleen, lymph nodes, and bone marrow. The few remaining B cells in the spleen were of the B1 but not the B2 phenotype. Peritoneal B cells are only partially depleted, suggesting that some sites are privileged and protect B cells despite adequate drug penetration.

In RA, serial synovial biopsies demonstrate only partial B cell depletion after rituximab therapy despite the virtual absence of peripheral B cells. The clinical responses do not always correlate closely with the extent of synovial depletion, although some patients with the most impressive responses appear to have marked declines in the number of B cells in the post-treatment synovial specimens. Surprisingly, B cell depletion does not consistently decrease autoantibody production or cytokine production in the synovium, nor does a change in ACPA or RF levels predict a clinical response.[123] Longer term biopsy studies suggest that plasma cells are decreased in synovium 24 weeks after the treatment with rituximab and that this outcome correlates with decreased symptoms.

The simplest explanation for B cell contributions to RA lies in their ability to produce autoantibodies and differentiate into long-lived plasma cells. Although an increase in RF or ACPA titer can presage clinical relapse, autoantibody titers do not correlate well with disease activity, and RA-associated antibodies can be detected in persons even after a dramatic response. The synovial biopsy data on synovial autoantibody production also suggest that B cells play a more complex and nuanced role in the disease.

Perhaps more important, B cells are potent antigen-presenting cells that could serve this function either in the inflamed synovium or in central lymphoid organs. In a SCID mouse model using rheumatoid synovial explants, T cell activation, lymphoneogenesis, and cytokine production depend on B cells.[124] In the same model, treatment with TACI-Ig to inhibit APRIL and BLyS decreases synovial inflammation and IFN-γ production in tissues with germinal centers.

B cells can also make an array of cytokines, including LTα, TNF, and IL-6. Although macrophages and synoviocytes might produce greater amounts of pro-inflammatory factors, production by B cells in strategic locations in the joints and elsewhere might help drive synovitis. An interesting $FcRL4^+$ B cell subset is increased in RA and can produce TNF and RANKL. The presence of another subset, namely $CD79a^+$ B cells, correlates with erosive disease in patients with long-standing RA.[125] Alternatively, regulatory B cells, like Tregs, could decrease inflammatory responses in RA through the production of suppressive cytokines such as IL-10 and TGF-β. It is not clear whether B cell selectively depletes B cell subsets, and it is possible that the regulatory B cells could be relatively enriched by either rituximab or perhaps other targeted therapies.

## Dendritic Cells

DCs are potent antigen-presenting cells that populate synovial tissue and synovial effusions of patients with RA. DCs sample the environment, especially at mucosal and skin surfaces; process antigens; and then migrate to a central lymphatic site, where they present the antigens to T cells. An emerging appreciation of DC function in the etiology of RA led to the concept that the cells could be loaded with local modified or autoantigens and mucosal sites and migrate to central lymphoid tissues, where they can participate in the initiation of disease. Later, synovial DCs could present antigens in the joint to elicit a local immune response. DCs are also potent producers of type I IFNs, which has been implicated in numerous autoimmune diseases such as lupus and RA. Moreover, IFN signatures have been noted in the joint and blood of patients with RA.

Rheumatoid synovium could function like lymphoid tissues, with mature DCs expressing CD86, CD83, and DC-LAMP localized in perivascular lymphocytic infiltrates and aggregates. Ultrastructural analysis of the synovium shows the DCs in contact with lymphocytes, where they

can present antigens. The presence of DCs is not unique to RA and has been identified in other inflammatory arthritides, including gout and spondyloarthropathies.

Like other immunocompetent cells, DCs are recruited to synovium through the generation of chemotactic factors and expression of the appropriate adhesion molecules. The subsequent localization with the synovium and the formation of microarchitecture such as lymphoid aggregates also depends on production of chemokines in the synovial environment. DCs express the chemokine receptor CCR7, which usually permits them to migrate to lymphoid tissue and orchestrate organization into the appropriate germinal centers. Synoviocytes in RA express two CCR7 ligands (CCL19 and CCL21), which provide a signal for the DCs to remain in the peripheral tissue. Immature DCs and plasmacytoid DCs are also scattered throughout the synovial sublining region. B cell–enriched lymphoid follicles, which are usually organized around follicular DCs (FDCs), are also found in some RA joint tissues. Cultured FLS can perform FDC functions in vitro and could contribute in vivo as well. Synoviocytes derived from patients with RA can bind to germinal-center B cells and suppress B cell apoptosis.

FDC-containing ectopic lymphoid structures in rheumatoid synovium also express high levels of activation-induced cytosine deaminase, a gene that participates in Ig class switching. $ACPA^+$ plasma cells surround these aggregates, suggesting that the aggregates play a role in autoantibody production. The architecture and $ACPA^+$ cells persist when tissue is transplanted into SCID mice. Therefore synovial DCs not only help organize the microarchitecture of synovial tissue but also participate in the development of high-affinity IgG autoantibodies.[126] Aside from presenting antigens, DCs produce cytokines that can influence T cell differentiation in the joint, including IL-12 and IL-23, which can enhance the bias toward the Th1 and Th17 phenotypes (addressed in the following text); APRIL, which enhances B cell survival; and, as previously noted, type I IFNs.

Cytokines in the rheumatoid synovium such as GM-CSF influence the proliferation and maturation of DCs. Increased numbers are found in the tissue and synovial effusions, where they constitute up to 5% of mononuclear cells. RA synovial tissue DCs do not always behave normally. For example, IL-10 suppresses DC function, in part by decreasing expression of CD86 and class II MHC molecules.[127] Synovial FDCs, along with macrophages and FLS, also express the receptor for macrophage colony-stimulating factor (M-CSF, also called CSF-1). Blocking this receptor with a monoclonal antibody suppresses adaptive immunity and collagen-induced arthritis but not a passive serum transfer model, suggesting that the benefit could be a result of suppressing antigen presentation by FDCs.[128]

## Mast Cells, Polymorphonuclear Leukocytes, and Natural Killer Cells

Mast cells are present in the synovial membranes of patients with RA and, in some patients, are located at sites of cartilage erosion. Rheumatoid synovial membranes contain more than 10 times as many mast cells than control synovial samples from patients undergoing surgery for meniscectomy. Mast cells and histamine are also found in a majority of synovial fluid specimens from inflammatory synovitis. A strong correlation exists between the number of mast cells in synovial tissue and the degree of lymphocyte infiltration.[129] Mast cells from RA synovium express significantly higher amounts of the C5a receptor compared with OA synovium.

Resident mast cells in synovium respond to cytokines that stimulate mast cell growth and chemotaxis. Extracts of mast cells can induce adherent rheumatoid synovial cells to increase production of prostaglandin $E_2$ ($PGE_2$) and collagenase, and immunostaining of RA synovial mast cells demonstrates tryptase and TNF. Mast cell–derived heparin has significant effects on connective tissue. In particular, it can modulate the effects of hormones on osseous cells and thereby alter the balance of bone synthesis toward degradation. Synovial mast cells also express Fcε receptors, as well as Fcγ receptor type I and II, but not type III. Immune complexes that ligate type I receptors release $PGD_2$ and TNF, whereas type II receptor ligation releases histamine.[130]

Mast cells have been implicated in the initiation of inflammation in the passive K/BxN model.[131] More recent studies, however, suggest that mast cells might not be contribute as much as originally thought.[131a] The role of mast cells in established disease is also uncertain, and the production of leukotriene $B_4$ ($LTB_4$) in the same model requires neutrophils but not mast cells. However, treatment of synovial tissue explants cultures with a c-Kit tyrosine kinase inhibitor that blocks stem cell factor signaling and decreased production of TNF.[132] These data suggest that mast cells might contribute to synovial cytokines in established disease.

More intriguing, pre-formed IL-17A is present in granules of synovial mast cells in RA.[133] The conventional view of IL-17 is that it mainly comes from activated T cells. Cultured mast cells release the IL-17 when they are stimulated with TNF or immune complexes. A correlation also exists between the presence of IgE and FcR1ε and mast cell degranulation in rheumatoid synovium. More direct evidence of mast cell involvement in RA comes from an open-label clinical trial using the c-Kit inhibitor masitinib in RA, where evidence of clinical efficacy was observed. c-Kit is a receptor tyrosine kinase that binds to stem cell factor and facilitates mast cell growth and survival.

Neutrophils are common in RA synovial effusions, but very few infiltrate the synovium. The cells are immunologically active and can produce IL-17B and IL-1Ra. Synovial neutrophils also release extra-cellular traps (NETs), which are composed of extruded DNA. These traps can capture bacteria and contribute to host defense. In RA, synovial, synovial fluid, and peripheral blood neutrophil NETosis is increased and can be further enhanced by RA serum (especially $ACPA^+$ serum), IL-17A, and TNF.[134] The resultant extra-cellular DNA includes PADI4 and citrullinated peptides such as vimentin.[135] The NETs can then induce other cytokines, IL-6, IL-8, and chemokines. This process can serve as a powerful feedback loop that can increase release of cytokines and citrullinated proteins that might drive immune complex formation.

Natural killer (NK) cells also have been identified in RA synovium.[136] Cytotoxic NK cells contain large amounts of granzymes, which are serine proteases. One potentially

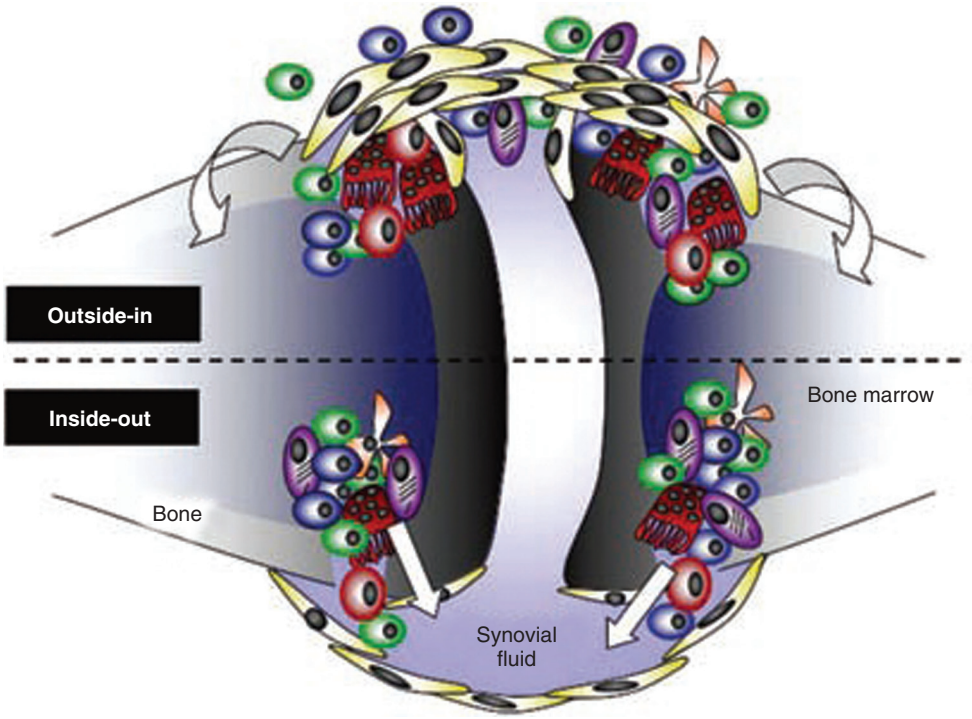



**Figure 69-11** Initial synovial lesion in rheumatoid arthritis (RA). Although traditional paradigms suggest that it begins as a synovial disease, the initial insult could be at a distant site. The figure depicts two possible scenarios: (1) Clinical inflammation in RA begins in the synovium and then extends into the bone and bone marrow, and (2) the initial inflammatory lesion is in the bone marrow, which can extend into the synovium as cells migrate through cortical pores or through the bloodstream. *(From Schett G, Firestein GS: Mr Outside and Mr Inside: classic and alternative views on the pathogenesis of rheumatoid arthritis.* Ann Rheum Dis *69:787, 2010.)*

important immunoregulatory role of NK cells is that they can stimulate B cells to produce RFs. A subset of NK cells that express large amounts of CD56 are unusually abundant in RA synovial tissue and fluid.[137] These cells could potentially produce cytokines or enhance pro-inflammatory cytokine production by T lymphocytes and macrophages. IL-32 exacerbates arthritis and joint damage in collagen-induced arthritis, possibly by increasing the number of synovial NK cells.[138] Little information is available on invariant NK T cells (iNKTs), which respond to lipid antigens. However, blocking CD1d and iNKT function decreases the severity of collagen-induced arthritis in mice.

## Bone Marrow Cells

Although most attention has been directed at the synovium, cartilage, and cortical bone in RA, the subcortical bone and bone marrow also contribute to synovial inflammatory responses. Primitive bone marrow mesenchymal cells can traverse cortical bone through pores in murine collagen-induced arthritis before the onset of clinical disease, take up residence in the synovium, and produce mediators that enhance synovitis.[139] This process is TNF-dependent and is abrogated in mice that lack TNF receptors. $CD34^+$ mesenchymal cells in rheumatoid bone marrow are more highly activated as judged by their NF-κB status and their ability to differentiate into fibroblast-like cells that produce MMPs and proangiogenic factors.

Bone marrow also can contribute other relevant cells, including macrophage lineage cells that migrate to the synovium and nurselike cells that support the survival of B cells. Just as the bone marrow can influence the synovium, the reverse is also true. Invasive pannus can rupture through cortical bone and invade the marrow space in some patients. When this occurs, B cell aggregates are especially prominent in the marrow and occur in an environment rich in B cell chemoattractants such as CCL21 and B cell survival factors such as BLyS.

The bone marrow changes in RA could be either a result of synovitis or a primary event (Figure 69-11).[140] The most common explanation for RA is that synovitis occurs first and that it expands into the marrow through soluble factors and direct extension (the "outside-in" hypothesis). The notion that bone marrow reactivity might be a primary event and that the activated cells migrate directly to the synovium through cortical bone or via the bloodstream has received increasing attention (the "inside-out" hypothesis). The bidirectional interactions between the synovium and the bone marrow suggest that both "inside-out" and "outside-in" mechanisms participate in RA.

## Synovitis in Pre- versus Early-Stage versus Late-Stage Rheumatoid Arthritis

The observation that autoantibody levels are elevated in patients with RA many years before the clinical arthritis manifests also supports the notion that a pre-clinical phase can precede symptomatic synovitis. In addition, profiling of cytokine and chemokine levels in stored serum samples of persons in whom RA ultimately develops shows increased levels of chemokines and cytokines, especially in the $ACPA^+$ population.[141] This finding reinforces the concept that RA

is a continuum that can progress to clinical synovitis. However, the time lines and the environmental stimuli that cause progression are not known.

In murine models, abundant evidence of synovial inflammation and transcription factor activation is present before clinical synovitis is apparent.[142] In contrast, synovial biopsies from "pre-RA" persons with arthralgia but no evidence of arthritis are largely normal, even if they are $ACPA^+$ and/or $RF^+$ and progressed to bona fide RA within 2 years. There was a trend toward higher numbers of $CD8^+$ T cells in the synovium but no other apparent increase in cellular infiltrate or cytokine production.[143]

During the first few weeks of symptomatic RA, the synovium occasionally demonstrates a paucity of lymphocyte infiltration, endothelial cell injury, tissue edema, and neutrophil accumulation; in general, it has a histologic appearance similar to long-standing disease. The extent of lymphoid aggregation, T cell infiltration, and synovial-lining hyperplasia can resemble chronic disease even when symptoms have been present for a short period. The cytokine patterns of these biopsies as determined by immunohistochemical analysis indicated similar levels of T cell factors (such as IFN-γ) and non–T cell factors (such as IL-1 and TNF). The tumor suppressor gene *p53* is also expressed in early-stage RA, most likely as a result of intense oxidative stress in the environment.

Biopsy specimens of asymptomatic joints from patients with early- or late-stage RA also show lymphocyte infiltration, cytokine production, and *p53* expression. Although IFN-γ, IL-1, and TNF levels are increased compared with normal synovium, they are modestly lower than in clinically active joints. Some cytokines such as IL-8 and the number of macrophages are higher in the painful joints. Macrophage and plasma cell infiltration in early RA might predict more erosive or severe disease.

These studies suggest that the transition from being asymptomatic with arthralgia to having synovial inflammation with symptoms in "early" RA, as defined by the duration of symptoms, is rapid. Once symptoms occur, the transition from "early" to "chronic" disease is equally rapid in terms of histology, cytokines, and mechanisms of disease.

## SYNOVIAL FLUID AND THE SYNOVIAL FLUID CARTILAGE INTERFACE

**KEY POINTS**

- **RA synovial effusions contain neutrophils and mononuclear cells, including T lymphocytes and macrophages.**
- **Immune complexes that contain autoantibodies such as RFs or ACPAs can fix complement, leading to the generation of chemoattractants.**
- **Small molecule mediators of inflammation such as prostaglandins and leukotrienes are present in RA synovial fluid.**

Synovial effusions accumulate in the joints of most patients with active RA as a result of a marked increase in fluid influx that cannot be removed despite an increase in lymphatic flow. An inverse relationship exists between the molecular weight of proteins and their concentrations in minimally inflamed synovial fluid. High-molecular-weight serum proteins gain access more easily to synovial fluid in inflamed joints, and the relatively high concentration of IgG in RA synovial fluid is good evidence for local synthesis of immunoglobulins. Markedly increased permeance of proteins in patients with rheumatoid disease is also evidence of the severe microvascular lesion in rheumatoid synovitis. In general, assays for autoantibodies or assessments of exudate versus transudate in synovial fluid have little value; the concentration of serum proteins in effusions is usually about half to two-thirds the level in the blood.

Newer techniques evaluating the synovial fluid proteome in RA using mass spectroscopy might provide new insights into the key mediators. More than 400 proteins in RA effusions were identified as potential biomarkers of disease activity, including C-reactive protein and six members of the S-100 family of calcium granule binding proteins.[144] Additional studies show higher levels of proteins related to complement activation, as well as MMPs and neutrophil-derived proteins. None of these is a particular surprise given the genes expressed by the synovial intimal lining and the abundance of neutrophils in RA fluid. Perhaps more interesting, the "citrullinome" of RA fluids—that is, the pattern of protein citrullination—showed about 3000 different citrullinated peptides, including multiple peptides from vimentin, fibrinogen, and α-enolase.[145] In another study, immune-mediated membranolytic pathways mediated by perforin and the membrane attack complex of complement, both of which are hallmarks of RA effusions, markedly increased citrullination of neutrophils.[146]

### Polymorphonuclear Leukocytes

Large numbers of polymorphonuclear neutrophils (PMNs) accumulate in rheumatoid synovial effusions and enter via postcapillary venules in the synovium. Neutrophils adhere to activate synovial microvasculature as a result of their abundant surface expression of selectins and the β2 integrins. After adherence, chemotactic agents produced by endothelium and resident synovial cells facilitate exit from the intravascular space into the tissue. Thus, considering the survival time of PMNs in synovial fluid, an average rheumatoid effusion containing 25,000 PMNs per $mm^3$ could exceed 1 billion cells each day. The ultimate fate of these cells is usually apoptosis or NETosis with release of DNA and citrullinated peptides. Neutrophil survival requires expression of the forkhead transcription factor gene *FOXO3*.[147] Mice that lack this gene are resistant to inflammatory arthritis because of a shortened PMN life span.

High concentrations of chemotactic agents in the synovial fluid in RA recruit a large number of cells to the intra-articular cavity. Few PMNs are seen in the synovium itself; once in the tissue they move rapidly to the synovial fluid, drawn by agents such as C5a that are generated as a result of complement activation, $LTB_4$, platelet-activating factor, and chemokines. CXC chemokines including ENA-78 and IL-8 are especially abundant in synovial fluid and can attract neutrophils into the intra-articular space. RA PMNs can also release chemokines such as macrophage-inflammatory protein (MIP)-3α into the milieu that promote migration of additional cells into the joint space.

Once in the joint, neutrophils engage immune complexes through Fc receptors, especially FcRγI and FcRγIII,

that activate spleen tyrosine kinase (Syk). This process initiates a signaling cascade that includes the MAP kinases and NF-κB, cytoskeletal reorganization, release of granule content, generation of reactive oxygen and nitrogen species, and enhanced phagocytosis. Many of the cells contain immune complexes within phagosomes that include IgG and IgM along with RF and complement proteins such as C1q, C3, and C4. PMNs from synovial fluid in RA release de novo synthesized proteins including matrix proteins such as fibronectin, neutral proteinases, and IL-1. Neutrophils also secrete IL-1Ra and oncostatin M, which is a member of the IL-6 family. Although the amount of IL-1Ra each neutrophil produces is low compared with macrophages, the sheer number of PMNs allows them to produce large amounts in synovial effusions.

Neutrophil cells also release numerous proteases that can adversely affect the lubricating properties of synovial fluid and the integrity of the cartilage, including elastase and trypsin. Neutrophil collagenase (MMP-8) can digest native collagen in cartilage, which then makes it susceptible to degradation by other MMPs, such as the stromelysins. Synoviocytes are generally considered major producers of the MMPs, but animal models of arthritis suggest that neutrophil-derived proteases also play a significant role in cartilage damage.

Although difficult to assess in humans, animal models demonstrate a role for neutrophils in the inflammatory processes. The passive K/BxN and collagen-induced arthritis models, which depend on autoantibodies to bind to the cartilage and fix complement, require neutrophils for full expression of the disease. Depleting neutrophils with antibodies almost completely prevents synovial inflammation in these models. In the K/BxN model, neutrophils along with mast cells can also initiate the vascular permeability that permits pathogenic antibodies to gain access to the joint space.

## Synovial Fluid Lymphocytes

The lymphocyte subsets in synovial fluid differ from those of peripheral blood and synovial tissue. Even though synovial effusions contain an abundance of T cells, the CD4 to CD8 ratio is actually reversed compared with that of blood or synovial tissue, with an excess of $CD8^+$ cells relative to $CD4^+$ lymphocytes. In addition, synovial tissue is nearly devoid of neutrophils, which often constitute 50% to 75% of synovial fluid cells. The percentage of Tregs ($CD4^+CD25^+$) is also higher in synovial fluid than in peripheral blood.

Synovial fluid contains T cells, which express high levels of surface HLA-DR antigens. Surface activation antigens are increased on synovial fluid lymphocytes, including VLA-1 and CD69. Of $CD4^+$ cells in rheumatoid synovial fluid, most are memory cells and express CD45RO on their surface. Despite the activated phenotype, synovial fluid T cell function is usually deficient compared with peripheral blood cells. For instance, synovial fluid lymphocyte proliferation in response to mitogens or most recall antigens such as tetanus toxoid is significantly less than that of paired blood T lymphocytes. Mycobacterial antigens and the 60 kDa HSP are exceptions because proliferation is actually greater in synovial fluid cells, perhaps because they can also activate TLRs. Cytokine production by synovial fluid T cells in vitro is also low, including mitogen-induced expression of IFN-γ and IL-1.

Defective T cell responses by RA synovial fluid mononuclear cells could be due to anti-inflammatory cytokines such as IL-1Ra and TGF-β.[148] Nonspecific components of joint effusions such as hyaluronic acid can be toxic and indirectly suppress T cell activation. The mechanism of diminished T cell activation also could be due to defective TCR signaling. Articular T cells have diminished tyrosine phosphorylation of proteins after stimulation, especially the key signal transduction pathway p38 MAP kinase. Furthermore, tyrosine phosphorylation of the TCR ζ chain, an early event in TCR signaling, is low compared with peripheral blood T cells. The hyporesponsiveness of synovial fluid T cells correlates with a significant decrease in the levels of the intra-cellular redox-regulating agent glutathione.[149] Restoration of intra-cellular glutathione increases proliferation of RA synovial fluid T cells. Therefore, oxidative stress in the inflamed environment can suppress antigen-specific T cell responses.

## Platelets and Platelet Microparticles

Platelets are a rich source of growth factors and cytokines, including PDGF. They are present in rheumatoid synovial effusions and can form microparticles that contain immune complexes. Some of these particles can display citrullinated peptides such as fibrinogen and vimentin, which can then increase pathogenic innate and adaptive responses.[150] This process is dependent on collagen receptor glycoprotein VI. The microparticles activate cultured synoviocytes and, through an IL-1–dependent mechanism, stimulate cytokine secretion. Platelet depletion also suppresses arthritis severity in animal models.

## Intra-articular Immune Complexes and Complement Fixation

### Synovial Fluid Immune Complexes

Complexes containing Igs are abundant in the blood and synovial fluid of patients with RA, especially those containing IgM and citrullinated proteins. The most prevalent antigens in these aggregates contain IgG bound to RF as a result of their ability to bind the Fc portion of Ig, as well as ACPAs and citrullinated peptide complexes. Use of more sensitive techniques has revealed that circulating immune complexes in RA contain up to 20 polypeptides, including albumin, Ig, complement, type II collagen, fibrinogen, and acute-phase reactants, as well as DNA. These complexes can potentially fix complement, releasing chemotactic peptides and factors that activate neutrophils and other inflammatory cells.

### Immune Complexes Embedded in Cartilage

The aggregates of RF, IgG, ACPAs, and various peptides become embedded into cartilage and other tissues that are in contact with synovial fluids. Electron microscopic studies demonstrate immunoglobulin complexes with damage to the cartilage matrix in the microenvironment. Immune complexes are absent under areas of cartilage invaded

actively by synovial pannus, suggesting that phagocytic cells in the invasive synovium bind to and ingest the immune complexes. Rheumatoid cartilage contains more than 40-fold more IgM and more than 10-fold more IgG than healthy cartilage extracts. IgM RF is found in the majority of RA cartilage extracts but not in OA or healthy control subject extracts. In addition, more than 60% of the RA cartilage extracts are positive for native and denatured type II collagen antibody.

### Synovial Fluid Complement

Biologically active products of complement activation accumulate in synovial fluid during acute inflammation, suggesting a critical role for intra-articular immune complex formation. The liver is the major source of complement synthesis in humans, and passive transfer of serum proteins into effusions accounts for many of the complement proteins found there. However, synovial tissue also actively produces complement proteins. Macrophages and fibroblasts produce complement proteins under the influence of cytokines such as IFN-γ, IL-1, and TNF. Synovial tissue analysis shows that all complement genes from the classic pathway are expressed in RA, as well as in healthy synovium.

Despite the local production of complement components, the activities of C4, C2, and C3 and total hemolytic complement in rheumatoid synovial effusions are lower than in synovial fluids from patients with other joint diseases. Although the most prominent evidence of activation implicates the classic pathway, cleavage products of the alternate pathway, including factor B and properdin, have also been documented in RA. IgM RF appears to be a more important determinant of complement activation than IgG RF in serum and synovial fluid. Accelerated catabolism of C4 in RA and the presence of C4 fragments in the plasma correlate with titers of IgM RF.

The interactions between PMNs and the complement system are considerable. Neutrophil lysosomal lysates contain enzymes that cleave complement proteins and generate chemotactic activity such as C5a from serum. C5a, in addition to being a principal chemotactic factor in inflammatory effusions, mediates lysosomal release from human PMNs and induces cytoskeletal rearrangement. The chemotactic anaphylatoxins C3a and C5a are often present in rheumatoid effusions, as are the terminal complement components that comprise the C5b-C9 membrane attack complex.

### Targeting Complement

Inhibiting complement proteins has clear therapeutic potential in RA. In addition to theoretic considerations and evidence of complement consumption, a polymorphism near the C5 locus has been associated with increased risk of RA. As proof of concept in pre-clinical studies, intra-articular treatment with a soluble complement receptor (sCR1) inhibits joint swelling in rat antigen-induced arthritis. C5-deficient mice have decreased joint inflammation in collagen-induced arthritis and the passive K/BxN model. Absence of C3 or factor B also inhibits collagen-induced arthritis. Unlike the C5 knockout mice, which have normal antibody responses, the C3- and factor B–null animals have lower levels of anti–type II collagen antibodies. The role of C3 convertase has been explored in more detail using transgenic mice that produce the regulatory protein complement receptor 1–related gene/protein y (Crry). These mice have no obvious phenotype and are not more susceptible to infection. However, collagen-induced arthritis is suppressed in Crry transgenic mice.

These data suggest that classic and alternative complement pathways participate in RA. A humanized anti-C5 antibody has been evaluated in a placebo-controlled study. The antibody inhibits C5 activation and function of the C5b-C9 attack complex. Although the monoclonal antibody was well tolerated, only modest evidence of clinical efficacy was found. Similarly, a C5a receptor antagonist did not decrease inflammation in a short-term placebo-controlled synovial biopsy study even though the compound was effective in pre-clinical models and blood levels were in the therapeutic range.[151] C5 alone is not sufficient to sustain inflammation in the rheumatoid joint, and it probably participates in a redundant inflammatory network. It is not clear whether targeting other complement components such as C3 convertase would be more successful.

## Arachidonate Metabolites

### Prostaglandins

Arachidonic acid metabolites are produced in the inflamed joint via oxidation by cyclooxygenases (COX) to prostaglandins and thromboxanes or by lipoxygenases to leukotrienes. COX inhibitors are clearly effective in RA, although the effect is modest and is due to combination of local synovial effects and spinal analgesic action. Animal models of arthritis are variably sensitive to prostaglandin; indomethacin almost completely prevents adjuvant arthritis in rats but has minimal benefit in collagen-induced arthritis in mice. This finding serves as a reminder that results in rodent models do not always correlate with human disease.

Stable prostaglandins, especially $PGE_2$, produce vasodilation, increase vascular permeability, and are involved centrally in fever. They also display some anti-inflammatory activities that could account for limited efficacy. For example, the drug misoprostol, a prostaglandin analogue, has modest but significant anti-inflammatory or immunomodulatory effects. Physiologic concentrations of $PGE_2$ inhibit IFN-γ production by T cells, HLA-DR expression by macrophages, and T cell proliferation.

Production of prostaglandins in RA depends on both COX-1 and COX-2 (see Chapter 24). The former is constitutively expressed and is responsible for the normal endogenous production of prostaglandins in the joint, as well as in other tissues. COX-2, on the other hand, is an inducible enzyme responsible for increased prostaglandin synthesis in inflamed tissue. Cytokines such as IL-1 and TNF induce COX-2 gene expression by cultured synoviocytes and macrophages. COX-2 mRNA and immunoreactive protein are increased in RA synovium.[152] Most nonsteroidal anti-inflammatory drugs, including indomethacin and ibuprofen, inhibit both enzymes. Much of the anti-inflammatory activity and analgesia in RA results from COX-2 inhibition. Targeting prostaglandin receptors such as $E_2$ or $E_4$ is an alternative approach that might have less

deleterious effects on the gastric mucosa and cardiovascular disease. More recent data suggest that PGE receptor 4 (EP4) can also act on DCs and T cells to enhance production of Th1 and Th17 cells.[153]

$PGI_2$, like $PGE_2$, can also contribute to synovial inflammation. For instance, mice lacking the $PGI_2$ receptor have significantly decreased arthritis severity in the collagen-induced arthritis model compared with wild-type mice even though they make similar amounts of anti-collagen antibodies.

### Leukotrienes

LTBs are a potent pro-inflammatory produced by neutrophils and chemotactic for neutrophils, eosinophils, and macrophages. They also promote neutrophil aggregation and adherence to endothelium. Peripheral blood PMNs from patients with rheumatoid disease have an enhanced capacity for $LTB_4$ production compared with normal persons.[154] In murine collagen-induced arthritis, $LTB_4$ antagonists decreased paw swelling and joint destruction, suggesting a pivotal role for this potent chemoattractant. Surprisingly, $LTB_4$ blockade thus far has demonstrated minimal efficacy in RA.

### Anti-inflammatory Arachidonic Acid Metabolites

Certain arachidonic acid metabolites such as 15-deoxy-delta(12,14)-PGJ(2) can bind to peroxisome proliferators, activate peroxisome proliferator-activated receptors (PPARs), and inhibit cytokine production and inflammation in animal models of arthritis. Cyclopentenone prostaglandins can also inhibit NF-κB by blocking one of the key enzymes that activates this pathway, namely IκB kinase-β (IKKβ). Lipoxins and resolvins represent a unique class of lipid mediators that help resolve inflammatory diseases. Lipoxins (LX) have a trihydroxytetraene structure and are produced from arachidonic acid via the lipoxygenase pathways. $LXA_4$ binds with high affinity to a G protein–coupled receptor denoted lipoxin $(LX)A_4$ receptor (ALXR). Activation of ALXR inhibits recruitment of neutrophils by attenuating chemotaxis, adhesion, and transmigration into tissues and by diminishing chemokine and cytokine production. $LXA_4$ significantly decreased cytokine and MMP expression in FLS through an NF-κB–dependent mechanism.

## PERIPHERAL BLOOD LYMPHOCYTES

The number of $CD4^+$ Th cells is mildly increased in the circulation of patients with RA, with a concomitant decrease in $CD8^+$ lymphocytes. The surface phenotype of circulating T cells in RA is highly variable. For instance, an increased percentage of αβ and γδ TCR–bearing cells expressing HLA-DR and the adhesion protein VLA-4 ($\alpha_4\beta_1$ integrin) has been observed. The latter is especially critical in that VLA-4 plays an important role in the recruitment of cells to the synovium through interactions with VCAM-1 on endothelial cells. Other markers of activation are not necessarily elevated on RA T cells in the circulation. Therefore, peripheral-blood T cells express some, but not all, phenotypic characteristics of prior activation. It is not clear whether this process occurs in the peripheral or central lymphoid organs or whether cells are activated in the synovium and re-enter the circulation via the synovial lymphatics.

Immunoregulatory dysfunction has been described in RA peripheral lymphocytes. One early observation was the inadequate control of EBV-infected B lymphocyte growth because of a defect in T cell function in RA. The abnormal T cell response correlates somewhat with disease activity, but the abnormality is present in T cells of some patients with inflammatory arthropathies other than RA.[155] IFN-γ and IL-2 production is significantly suppressed in RA lymphocyte cultures.

T cell diversity and maturation are abnormal in RA. Whereas thymic output normally decreases with age, this process appears to be accelerated in RA.[156] The presence of TCR rearrangement excision circles (TRECs) is a measure of thymic release of mature T cells. Using this parameter, the thymic output in RA may decline prematurely. Similarly, telomere attrition suggests inappropriate "aging" of the T cells. This effect could be due to a primary defect in peripheral T cell homeostasis or impaired thymic function with increased T cell turnover as a result of chronic immune stimulation. This concept is supported by the observation that telomeric length in RA T cells is shorter than in normal control subjects and more closely resembles older populations.

Activated B lymphocytes are also present in the peripheral blood of patients with RA. The number of circulating B cells that spontaneously produce RF and other autoantibodies is significantly higher in persons with RA compared with normal persons. B cells that are enriched in autoantibody production are characterized by a surface determinant CD5. This antigen is normally expressed by T cells but is also displayed by fetal B cells and a small number of immature B cells in adults. Patients with RA who have normal circulating numbers of lymphocytes show an abnormal κ-to-λ–chain analysis compared with control subjects, implying oligoclonal B cell proliferation. As previously noted, plasmablasts are also present in rheumatoid blood, and their presence can be a harbinger of B cell repopulation after treatment with anti-CD20 antibody.

Studies to characterize gene expression profiles in the peripheral blood cells of patients with RA have had mixed results. In some cases, RNA transcripts can potentially distinguish between RA and other forms of inflammatory arthritis and include differential expression of tumor suppressors, MAP kinases, and other pro-inflammatory proteins. Other limited subsets of genes have been proposed as markers for disease activity or to predict response to targeted therapy. However, the patterns are inconsistent, perhaps because blood contains a highly heterogeneous population of cells.

## ROLE OF T CELL CYTOKINES

**KEY POINTS**

**Several T cell subsets have been implicated in the pathogenesis of RA.**

**Relatively low levels of T cell cytokines are present in RA synovium compared with macrophage or fibroblast products.**

**The T cell cytokines that are present, such as IFN-γ and IL-17, can contribute to the pathogenesis of RA and can be produced by Th1 cells or Th17 cells, respectively.**

**Regulatory T cell function, which suppresses activation of other T cells, might be low in RA synovium.**

**T cells can also contribute to synovial inflammation through antigen-independent mechanisms such as direct cell-cell contact with macrophages and synoviocytes.**

The cytokine milieu in RA is rich in factors produced by many cell lineages. Many factors produced by T lymphocytes are surprisingly low in RA, whereas those generated by macrophages and by synovial fibroblasts are markedly increased (Table 69-5). Although low, the T cell cytokines can have profound effects, especially in the synovial microenvironment. The production and contribution of T cell–derived cytokines can be examined on the basis of functional lymphocyte subsets.

## Type 1 T Helper Cell Cytokines

Early investigation into the cytokine profile of RA suggested a bias toward the Th1 phenotype, which produces cytokines such as IL-2 and IFN-γ and expresses the chemokine receptors CXCR3 and CCR5. This process might be driven, in part, by local production of IL-7 by stromal cells and macrophages, which supports the production of Th1 and Th17 cells. Unexpectedly, lower serum IL-7 levels in patients with early-stage inflammatory arthritis are associated with progression to RA.[157] Many T cell clones derived from RA synovial tissue produce a Th1 cytokine pattern. Considerable data have accumulated on the relative abundance and function of the prototypic Th1 cytokine, IFN-γ, which is the most potent inducer of HLA-DR and enhances antigen presentation. IFN-γ also induces adhesion molecules on the surface of endothelial cells and can help recruit inflammatory cell accumulation at sites of injury. One important function of IFN-γ is its capacity to alter the balance of extra-cellular matrix synthesis and degradation by decreasing collagen synthesis and inhibiting MMP production by cytokine-stimulated cultured FLS.[158]

Relatively low concentrations of IFN-γ have been detected in RA joints, which are below the amounts needed to induce HLA-DR expression on monocytes. Immunohistochemical analysis clearly demonstrates IFN-γ in RA synovial T cells, although the percentage is less than in chronically inflamed tonsils. However, the role of IFN-γ is actually quite complex because administration of the cytokine does not exacerbate RA and, if anything, results in improvement in some patients. IFN-γ knockout mice or IFN-γ receptor deficiency can exacerbate collagen-induced arthritis in mice and serve as a reminder that the effects of cytokines can be highly variable depending on the model and timing of expression.

Another major Th1 cytokine, IL-2, is a T cell–derived cytokine that serves as an autocrine or paracrine T cell growth factor. IL-2 is detected in only a small percentage of RA synovial effusions and synovial tissues and, when present, is only found in low concentrations.[159] TNF, GM-CSF, and IL-6 can be expressed by Th1 and Th2 cells and are abundant in synovial fluid and produced by RA

**TABLE 69-5** Partial List of Relative Production of Selected Synovial Cytokines in Rheumatoid Arthritis according to Cellular Source

| Cellular Source | Level of Production in RA Synovium |
|---|---|
| **T Cells** | |
| IL-2 | – |
| IL-3 | – |
| IL-4 | – |
| IL-6 | ± |
| IL-13 | ± |
| IL-17A, F | + |
| IL-21 | + |
| IFN-γ | ± |
| TNF | – |
| Lymphotoxin-α | – |
| RANKL | ++ |
| GM-CSF | – |
| **Macrophages†/Fibroblasts‡** | |
| IL-1 family (e.g., IL-1 and IL-18) | +++ |
| IL-1Ra | + |
| IL-6 family (e.g., IL-6, IL-11 and LIF) | +++ |
| IL-10 family (IL-10 and IL-22) | + |
| IL-12 family (e.g., IL-12 and IL-23) | + |
| IL-15 | ++ |
| IL-16 | + |
| IL-32 | + |
| Alarmins (e.g., IL-33 and HMGB1) | ++ |
| TNF | ++ |
| M-CSF and IL-34 | + |
| GM-CSF | ++ |
| B cell growth factors (e.g., BLyS and APRIL) | ++ |
| TNF superfamily (e.g., LIGHT and TWEAK) | ++ |
| RANKL | + |
| Growth factors (TGF-β, PDGF, FGF) | ++ |
| Chemokines (e.g., IL-8, MCP-1) | +++ |
| Interferons (e.g., IFN-β and IL-29) | ++ |
| **Dendritic Cells** | |
| IFN-α | + |
| IL-12 | + |
| Chemokines (e.g., CXCL13 and CCL21) | + |
| **Mast Cells** | |
| TNF | + |
| IL-17A | + |

*–, Absent or very low concentrations; +, present.
†Tissue macrophages or type A synoviocytes.
‡Tissue fibroblasts or type B synoviocytes.
APRIL, A proliferation-inducing ligand; BLyS, B lymphocyte stimulator; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; LIF, leukemia inhibitory factor; LIGHT, lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells; MCP-1, monocyte chemoattractant; M-CSF, macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; RA, rheumatoid arthritis; RANKL, receptor activator of NF-κB ligand; TWEAK, TNF-like weak inducer of apoptosis.

synovial tissue. However, the primary sources of these cytokines in the rheumatoid joint are macrophages and fibroblasts.

## Type 2 T Helper Cell Cytokines

Th2 cytokine levels are quite low in rheumatoid joints. Using immunoassays, IL-4 and LTα are generally not detected in RA synovial fluid. In situ hybridization also shows little or no IL-4 mRNA in RA synovial tissue. When sensitive nested reverse-transcriptase polymerase chain reaction techniques are used on synovial biopsies, Th2 cytokines IL-4 and IL-13 are absent in RA, whereas both IFN-γ and IL-12 (a cytokine that regulates T cell maturation toward the Th1 and Th17 phenotypes) are usually present. The Th2 cytokine IL-10, which has potent anti-inflammatory activities, is expressed in RA synovium; however, like many other cytokines, macrophages rather than T cells are the major producers in RA. Some data suggest that synovial fluids in early synovitis have high concentrations of Th2 cytokines such as IL-4 and IL-13 and that this pattern distinguishes patients who progress to RA. As the disease evolves in these patients, the Th2 signature diminishes.[160]

## Type 17 T Helper Cytokines

The discovery of Th17 cells and their critical role in animal models of autoimmunity has generated considerable interest. Th17 cells are also abundant in RA synovial effusions. The pro-inflammatory cytokine produced by Th17 cells, IL-17, is encoded by six genes (IL-17A through F). IL-17A and, to a certain extent, IL-17F mimic and synergize with IL-1 and TNF effects on FLS, including induction of collagenase and cytokine production. IL-17A is present in modest but functionally relevant concentrations in RA synovial effusions.[161] IL-17 receptors IL-17RA and IL-17RC are expressed by synoviocytes and, when engaged, activate the transcription factor NF-κB and initiate an inflammatory cascade. In addition to its effect on mesenchymal cells, IL-17 can participate in bone erosion by enhancing osteoclast activation. Bone resorption in an in vitro model using synovial explants and bone shows that blockade of IL-17, IL-1, and TNF is more effective than blocking the individual factors. IL-21 is also produced by RA T cells, which can serve to further enhance Th17 cell numbers and IL-17 production. Similarly, IL-22 can be produced by Th17 cells and is detected in RA synovial effusions. Like IL-17A, IL-22 activates FLS to produce osteoclast-activating factors such as RANKL.

Immunohistochemistry suggests multiple sources of IL-17. Although IL-17 is present in sublining T cells, some is derived from mast cells. In some studies, IL-17 is present in the synovium of a minority of patients with RA. Immunoreactive IL-17 can be detected near the erosive front of pannus, and thus it likely participates in extra-cellular matrix destruction. Animal models of arthritis demonstrate that IL-17 inhibition is anti-inflammatory and protects animals from bone and cartilage destruction.[162] IL-1, IL-23, and/or TGF-β in the joint can potentially enhance Th17 cell differentiation in humans. BLyS also has the potential to enhance Th17 cell differentiation and promote inflammatory arthritis. All of these cytokines are present in RA synovium, thereby providing an excellent milieu to enhance the generation of these cells.

The role of the IL-17 family is clear and impressive in animal models of arthritis, where blocking IL-17A in particular has dramatic effects on inflammation and matrix destruction. The data in humans are less robust, perhaps because the segregation of human T cells into clear subsets is not as well defined. Clinical studies with anti–IL-17A antibodies and soluble IL-17 receptors in RA are encouraging, but the benefit is modest compared with TNF inhibitors.[163]

## T Helper Cell Cytokine Imbalance

The relative abundance of Th1 and Th17 cells suggests that the synovium resembles a Th1-like delayed-type hypersensitivity reaction and/or a Th17 autoimmune environment. Th2 cytokines and cellular responses that normally suppress Th1 activation are nearly absent, thereby raising the possibility that the lack of T cell activation along the Th2 pathway in RA contributes to disease perpetuation. For example, addition of exogenous IL-10 or IL-4 to cultures of synovial tissue cells or synovial tissue explants suppresses synthesis of pro-inflammatory cytokines and MMPs by cultured RA synovial tissue explants.[164] The inhibitory action of IL-4 might be mediated by decreased c-Jun and c-Fos expression, which is required for efficient production of MMPs and cytokines. In addition, IL-10 and IL-4 increase release of other anti-inflammatory cytokines such as IL-1Ra by synovial cells. Although IL-10 protein is present in RA synovial fluid and the gene is expressed by synovial tissue cells, in vitro studies of cultured synovial cells suggest that not enough IL-10 is produced to suppress IFN-γ production.[165]

The notion that Th1 and Th17 cytokines initiate and perpetuate arthritis, in part as a result of the influence of IL-7, IL-21, and IL-22, whereas Th2 cytokines are suppressive, is supported by studies in animal models. For instance, IL-4 and IL-10 were administered individually or in combination in collagen-induced arthritis.[166] The cytokines had modest or no benefit when used separately, but together the effect was impressive. Clinical improvement correlated with decreased synovial IL-1, TNF, and cartilage destruction. The complexity of cytokine networks in inflammatory arthritis is underscored by studies on the role of IL-12 in collagen-induced arthritis. In early-stage arthritis, IL-12 administration increases the incidence of collagen-induced arthritis, whereas anti–IL-12 is beneficial.[167] However, in late-stage disease, IL-12 administration suppresses arthritis and anti–IL-12 causes an exacerbation. As previously noted, administration of anti–IL-17A antibody to block the prototypical Th17 cytokine provides only a modest benefit. Alternative approaches that inhibit both IL-17A and F, such as soluble receptor constructs, have been tested but do not increase efficacy. The role of Th17 cells is clearer in other inflammatory diseases, most notably psoriasis, where IL-17A blockade demonstrates a robust response.[168] An alternative approach, such as ustekinumab to block IL-12 and IL-23 and subsequent Th17 differentiation, has modest efficacy in RA compared with psoriasis. These data suggest that IL-17 and Th17 cells play a role in RA but are a major contributor in a relatively small subset.

Although enhancing Th2 cytokines is attractive, a clinical trial using IL-10 in RA did not demonstrate significant clinical benefit or improvement in histologic evidence of synovial inflammation.[169] It is possible that combinations of Th2 cytokines will be required to coordinate a maximum effect. Inhibitors of T cell cytokine signaling through JAK3 demonstrate clear efficacy in RA. However, many of these compounds also block JAK1 and could suppress signaling of other cytokines.

## ROLE OF MACROPHAGE AND FIBROBLAST CYTOKINES

**KEY POINTS**

- **Macrophage and fibroblast cytokines are abundant in RA synovium.**
- **Cytokine networks involve pro-inflammatory cytokines such as IL-1, TNF, IL-6, IL-15, IL-18, GM-CSF, and IL-33. These and many other factors help perpetuate synovial inflammation.**
- **Chemokines that recruit inflammatory cells into the joint are commonly produced by macrophages and fibroblasts.**
- **Cytokines with anti-inflammatory activity such as IL-1Ra, IFN-β, and IL-10 are produced in rheumatoid synovium, although in amounts insufficient to suppress pro-inflammatory cytokine function or production.**

Virtually every macrophage and fibroblast pro-inflammatory mediator investigated in the RA synovium is abundant. In this section, some of the major cytokines and effectors produced in the joint are described, with an emphasis on the prevalence of macrophage and fibroblast products as major forces that perpetuate RA. Macrophages, in particular, are the most vigorous producers of cytokines, whereas fibroblasts produce most of the proteases. The role of macrophage and fibroblast cytokines in the pathogenesis of RA was initially suggested by studies involving patient samples, as well as pre-clinical experiments in animal models. Studies that profile blood cytokines and chemokines indicate that panels of these factors increase many years before the onset of clinical disease. Surprisingly, similar increases are found in unaffected first-degree relatives and suggest that combinations of common environmental and genetic factors contribute to this observation.[170]

### Pro-inflammatory Macrophage and Fibroblast Cytokines

#### Interleukin-1 Family

The IL-1 family is a ubiquitous group of polypeptides with a wide range of biologic activity; they include IL-1α, IL-1β, IL-18, IL-33, and IL-1Ra, which is a natural inhibitor of IL-1 (a description of IL-1Ra is provided in a subsequent section). Abundant animal data indicate that IL-1 can serve as a key regulatory factor in inflammatory arthritis. For instance, recombinant IL-1β induces the accumulation of PMNs and mononuclear leukocytes in the joint space and the loss of proteoglycan from articular cartilage when injected directly into rabbit knee joints. Transgenic mice that overexpress IL-1 also develop inflammatory arthritis, whereas mice that lack the natural IL-1 antagonist IL-1Ra have increased susceptibility to collagen-induced arthritis. In most cases, IL-1 blockade in animal models modestly decreases synovial inflammation while markedly diminishing bone and cartilage destruction.

**Interleukin-1.** Synovial macrophages are the most prolific source of IL-1 in the rheumatoid joint, and nearly half of all macrophages in the RA synovium express IL-1β.[171] IL-1 protein is especially found in synovial lining macrophages adjacent to type B synoviocytes and in sublining macrophages near blood vessels. The IL-1 in the lining can subsequently activate type B synoviocytes to proliferate and secrete a variety of mediators. A broad range of stimuli induce IL-1 production by macrophages; for example, immunoglobulin Fc fragments and, to a lesser extent, immune complexes can generate IL-1 production by rheumatoid synovial macrophages. Collagen fragments can induce IL-1 production, and type IX collagen, which has been found only in articular cartilage and localized into intersections of collagen fibrils, is a potent inducer of IL-1 by human monocytes.

Within the rheumatoid joint, IL-1 induces fibroblast proliferation, stimulates the biosynthesis of IL-6, IL-8, and GM-CSF by synovial cells, and enhances collagenase and prostaglandin production. It increases glycosaminoglycan release in human synovial fibroblast cultures, although the effect of IL-1 on the production of intact proteoglycan molecules by intact articular cartilage explants can be the opposite. IL-1 induces a number of adhesion molecules on FLS and endothelial cells, including VCAM-1 and ICAM-1, and enhances bone resorption.

IL-1 has been implicated in RA, but inhibition of this mediator using various IL-1–targeted biologic agents has only a modest clinical benefit. Even combinations of IL-1 and TNF-directed therapy do not provide benefit beyond TNF blockade alone despite biologic effects on acute-phase reactants and rates of infection.[172] In contrast, diseases with a well-defined role for IL-1, such as autoinflammatory diseases, have a much better clinical response. These data suggest that IL-1 plays a modest role in the clinical manifestations of RA.

One explanation for the relatively modest benefit of IL-1 in RA relates to its signaling mechanisms. IL-1 activates NF-κB through the kinase MyD88, which is the same pathway as TLRs. When IL-1 signaling is blocked, it is possible that TLR ligands in the synovium, including exogenous ones such as peptidoglycan or endogenous ones such as HSPs, can provide the stimulus required to overcome IL-1 blockade. This concept was tested in the passive K/BxN model in which mice that lacked the IL-1 receptor had markedly decreased arthritis severity. When small amounts of the TLR4 ligand LPS were administered, robust synovitis ensued. If a similar system occurs in RA, then IL-1 blockade would be unable to control synovitis as long as TLR signaling remains intact.

**Interleukin-18.** In addition to IL-1α and IL-1β, a homologous protein in the IL-1 family known as IL-18 has been implicated in RA. This cytokine was originally defined by its ability to bias the immune response toward the Th1 phenotype, especially in the presence of IL-12. In collagen-induced arthritis, IL-18 inhibition significantly attenuates disease.[173] Subsequent studies showed that IL-18 induces GM-CSF, nitric oxide (NO) production, and TNF

expression by synovial macrophages. IL-18 is expressed by RA synovial tissue, especially by synovial fibroblasts and macrophages, and its production is markedly increased by TNF and IL-1β. A natural inhibitor, the IL-18 binding protein has the potential to be used as a therapeutic agent to block both the pro-inflammatory effects and pro-Th1 effects of IL-18. One potential concern for IL-18 as a target for RA is the fact that the IL-1 convertase inhibitor had only a modest benefit. IL-18, like IL-1β, is processed by this enzyme in order to produce biologically active cytokine. However, an IL-1 convertase inhibitor that blocks both IL-1β and IL-18 release by macrophages also had limited efficacy.

**Interleukin-33 and Other Alarmins.** IL-33 is a novel cytokine that signals through the ST2 surface receptor. Like high-mobility-group box 1 (HMGB1), it is an "alarmin" that provides a danger signal as a result of tissue damage and necrosis with release of intra-cellular contents. Blocking IL-33 decreases inflammation in several animal models of arthritis, including collagen-induced arthritis and antigen-induced arthritis. Mast cells are activated by IL-33 and can subsequently release their content. Thus mast cell–dependent models such as passive K/BxN are less severe in $ST2^{-/-}$ mice.[174] More intriguing, the ability of IL-33 to trigger mast cells could play a role in the release of mast cell products such as TNF and IL-17A in RA synovium. IL-33 can also bias T cells toward the Th2 phenotype, so the fact that it is expressed in RA synovium and synovial effusions raises some questions about its precise function in long-standing disease. HMGB1 is also highly expressed in RA joints and can regulate a number of pro-inflammatory pathways. Blocking HMGB1 decreases damage and immune responses in a variety of autoimmune models. It has also been implicated as a key factor that can convert OA FLS to a more aggressive RA-like phenotype.[175]

## Tumor Necrosis Factor and the Tumor Necrosis Factor Superfamily

The TNF superfamily is an extended group of related genes that play a major role in inflammation, immune responses, cell survival, and apoptosis. At least 19 members of the family have been identified, with TNF identified as the eponymous member. Each cytokine has its own preference for cell surface receptor, although there is some promiscuity of receptor binding and functional overlap. The TNF superfamily members exhibit conserved amino acid sequences, suggesting a single ancestral gene. Many TNF superfamily members include type II membrane protein characteristics and can be released from cell surfaces after proteolytic cleavage. A C-terminal conserved domain called the TNF-homology domain is also shared by several superfamily members. The active forms of the proteins are homotrimers, except for LTα and LTβ, which can form either heterotrimers or homotrimers. Several members have been previously discussed in the relevant sections.

TNF is a pleiotropic cytokine that has been implicated as a key pro-inflammatory cytokine in RA and detected in rheumatoid synovial fluid and serum. It is produced as a membrane-bound protein that is released from the cell surface after proteolytic cleavage by TNF convertase, a membrane MMP. IL-1 and TNF have many similar activities, including the ability to enhance cytokine production, adhesion-molecule expression, proliferation, and MMP production by cultured synoviocytes. In some systems, the effects of these two agents are synergistic. Although they share many functions and signal transduction pathways, IL-1 and TNF use distinct surface receptors and intracellular signaling pathways.

The efficacy of TNF inhibitors in RA demonstrates its critical role in the disease; heterogeneity of the rheumatoid process is also apparent because only about one-third of patients have a dramatic response to TNF inhibitors. Efficacy requires continuous therapy because cessation typically leads to a flare of the disease. Perhaps more interesting, early aggressive therapy with TNF inhibitors can induce long-term remissions in a limited number of patients even after therapy is withdrawn. This exciting notion suggests that interventions in the earliest stages of disease could prevent the establishment of chronic synovitis.

TNF, like IL-1, stimulates collagenase and $PGE_2$ production by human synovial cells, induces bone resorption, inhibits bone formation in vitro, and stimulates resorption of proteoglycan and inhibits its biosynthesis in explants of cartilage. In situ hybridization and immunohistochemical studies show that TNF is primarily produced by synovial macrophages in RA.

Animal models have also supported the general role played by TNF in inflammatory arthritis. For instance, overexpression of TNF in transgenic mice leads to an aggressive and destructive synovitis. In fact, arthritis also spontaneously occurs in transgenic mice that express only a membrane-bound form of TNF on T cells.[176] TNF blockade is an effective anti-inflammatory agent in many animal models of arthritis, although the effects on bone and cartilage destruction are less prominent than with IL-1 inhibitors and are likely mediated through downstream inhibition of IL-1 production.[177] TNF inhibition in RA significantly decreases or prevents joint damage.[178]

TNF blockade is not more effective than traditional agents such as methotrexate or many other biologic agents. Patients with an inadequate response to a TNF inhibitor can still respond to either rituximab or abatacept, which supports the notion that multiple independent pathways can contribute to the pathogenesis of RA and the heterogeneous nature of the disease. Some evidence suggests that some patterns of synovial gene expression are associated with a higher likelihood of a good response to a TNF blocker, but the predictive power is relatively minor.[179] Thus far, various algorithms that include autoantibodies, blood cytokine profiles, and other analytes do not provide enough stratification to be clinically useful. Surprisingly, patients with little or no clinical improvement still have significant delay or arrest of joint damage. This observation supports the concept that inflammation and destruction have distinct pathogenic mechanisms.

## Interleukin-6 Family

IL-6 is a complex cytokine produced by many cell types including T cells, monocytes, and FLS. Originally defined by its B cell–stimulating properties, it induces immunoglobulin synthesis in B cell lines, is involved in the differentiation of cytotoxic T lymphocytes, and is a major factor

in the regulation of acute-phase response proteins like C-reactive protein by the liver. The IL-6 receptor includes a common chain (gp130) that is shared with other cytokines and an IL-6–specific chain (IL-6R). IL-6R can be shed from cell surfaces, it can bind to IL-6, and it can be delivered to cells that lack IL-6 receptors by combining with gp130. IL-6 signaling proceeds through the JAKs, especially JAK1, and phosphorylation of STAT3.

A striking correlation between serum IL-6 activity and serum levels of acute-phase reactants such as C-reactive protein, $\alpha_1$–anti-trypsin, fibrinogen, and haptoglobin occurs in patients with RA. Very high levels of IL-6 are present in RA synovial fluid, and synovial cells in culture from diverse inflammatory arthropathies produce IL-6. In situ hybridization of synovial tissue also shows IL-6 mRNA in the intimal lining, and immunohistochemistry studies show IL-6 protein in the lining and sublining regions. Although many synovial macrophages express the IL-6 gene, the majority of IL-6 appears to be produced by type B synoviocytes.

The pivotal role of IL-6 in RA has been demonstrated by clinical trials using monoclonal antibodies that bind to IL-6R or to IL-6 itself. The clinical responses are similar to TNF inhibitors, including protective effects on bone and cartilage damage.[180] IL-6R antibody is also effective in patients who have not responded to TNF blockade. This observation is surprising considering that TNF is considered upstream of IL-6 in the cytokine cascade and that TNF inhibition markedly decreases IL-6 levels in RA. Neutropenia, increased liver enzymes, and altered blood lipids have been observed with IL-6 inhibition, although the relative importance of these observations is still uncertain. JAK inhibitors have also demonstrated efficacy in RA, which might be due in part to the effect on IL-6 signaling.

Cytokines with structural similarity to IL-6 and that share receptor subunits have also been implicated in RA. Several of these cytokines—IL-11, leukemia inhibitory factor (LIF), and oncostatin M—are expressed by rheumatoid synovium and can be detected in synovial effusions. The biologic effects of these factors are complex and can be either protective (e.g., by increasing expression of protease inhibitors such as tissue inhibitors of metalloproteinase [TIMP]) or pro-inflammatory (e.g., by increasing expression of chemokines or MMPs), depending on the culture conditions or the specific model evaluated. This dichotomy among the family members is demonstrated by the fact that IL-11 administration ameliorates collagen-induced arthritis, whereas antibodies to oncostatin M are protective.

### Interleukin-12 Family

The IL-12 family includes a group of cytokines that play a key role in the differentiation of T cells and inflammation. IL-12 is a heterodimeric cytokine with two subunits (e.g., p35 and p40) encoded on separate genes and produced by antigen-presenting cells. IL-23 and IL-27 have similar heterodimeric structures to IL-12, with p29/p40 and p28/EB13 components, respectively. In the context of antigen presentation, IL-12, IL-23, and IL-27 can bias T cell responses toward the Th1 phenotype. IL-23 can also play a pivotal role in the production of Th17 cells, along with IL-1 and TGF-β. The IL-12 family of cytokines are generally produced by macrophages in the rheumatoid joint but are also produced by other antigen-presenting cells such as DCs. Although clinical data defining the role of these cytokines are still lacking, anecdotal case reports indicate that patients with RA who have undergone treatment of malignancy with recombinant IL-12 experienced a flare of the disease. In contrast to psoriasis and psoriatic arthritis, where the IL-23-blocking biologic ustekinumab shows efficacy, this agent has limited efficacy in RA.[180a] This experience is similar to IL-17-blocking strategies in RA and psoriasis. Animal models, including adjuvant arthritis in rats and collagen-induced arthritis in mice, show that disease is partially ameliorated by neutralization of IL-12, IL-23, or IL-27, depending on the timing of treatment. In some situations, however, IL-27 has anti-inflammatory effects.

### Interleukin-15

IL-15 is an IL-2–like cytokine that regulates numerous immunologic functions relevant to RA, including T cell chemotaxis and proliferation, production of immunoglobulins by B cells, and the generation of NK cells. Although IL-15 can serve as an IL-2–independent mechanism for activating T cells, its role in RA may be related to its key role in TNF regulation. Macrophages are the primary source of IL-15 in RA, and the cytokine is able to induce a cell-contact mechanism of macrophage TNF production that requires T cells. Although T lymphocytes are required for this process, macrophages actually produce the TNF. This network provides a potential mechanism whereby local IL-15 production in the synovium can lead to autocrine production of TNF in a T cell–dependent but antigen-independent fashion. IL-15 has been demonstrated in RA synovial macrophage cells. Soluble IL-15 receptors can function as an IL-15 inhibitor, and when used in vivo, they can decrease joint inflammation in collagen-induced arthritis. Clinical trials in RA using an anti–IL-15 antibody had a signal for efficacy, although it was not sufficient to continue developing this particular biologic agent.[181]

### Interleukin-32

IL-32 activates NF-κB and induces the production of several pro-inflammatory cytokines and chemokines, including TNF, IL-1, IL-6, and IL-8. It has been implicated in Crohn's disease by virtue of its ability to markedly enhance caspase 1 activation and IL-1 production in cells that have been exposed to muramyl dipeptides. IL-32 is expressed by RA synovial tissue, especially in synovial-lining macrophage-like cells.[182] The level of IL-32 expression correlates with the presence of other cytokines implicated in RA, including TNF, IL-1, and IL-18. Injection of IL-32 into the joints of naïve mice causes a robust transient synovitis. The synovial response could be partially abrogated by anti-TNF antibodies, suggesting that IL-32 induces this cytokine in vivo. These data suggest that IL-32 might be upstream from several pro-inflammatory mediators in RA and could represent a therapeutic target.

### Colony-Stimulating Factors

GM-CSF supports the differentiation of bone marrow precursor cells to mature granulocytes and macrophages. As

with other major CSFs, GM-CSF also participates in normal immune responses. It is a potent macrophage activator, including the induction of HLA-DR expression, IL-1 secretion, intra-cellular parasite killing, and priming for enhanced release of TNF and $PGE_2$. Neutrophil function is also regulated by GM-CSF, which enhances antibody-dependent cytotoxicity, phagocytosis, chemotaxis, and the production of oxygen radicals.

RA synovial fluid and synovium contain GM-CSF.[183] The major source is synovial macrophages, although IL-1– or TNF-stimulated FLS also express the *CSF2* gene (which encodes GM-CSF). Its ability to induce HLA-DR gene expression on macrophages might be of particular importance in RA. GM-CSF, not IFN-γ, is the major DR-inducing cytokine in RA synovial effusions. Collagen-induced arthritis in mice is less severe in animals that lack a functional *CSF2* gene or are treated with anti–GM-CSF antibody, which supports the hypothesis that *CSF2* is an important pro-inflammatory mediator. Clinical trials demonstrate potential clinical benefit with an anti–GM-CSF antibody in RA, although long-term efficacy and safety still must be evaluated.[184] Some concern exists that pulmonary alveolar proteinosis might be an adverse event because this syndrome develops in *GM-CSF*–deficient mice and in autoimmune diseases marked by spontaneous production of anti–GM-CSF antibodies.

Macrophage CSF (M-CSF) is also expressed by RA synovium and is present in synovial effusions. Its primary pathogenic role in RA probably relates to its osteoclast-differentiating capacity. This factor cooperates with RANKL to facilitate bone erosions. The M-CSF receptor also binds to a novel cytokine that is produced by FLS, namely IL-34. The cytokine is expressed in RA synovium and represents an alternative therapeutic target for M-CSF receptor-driven events in synovitis.[185]

### Interferons

Type I IFNs, especially IFN-β, are expressed by RA synovium. The IFNs possess a range of biologic activities and can be immunostimulatory or inhibitory depending on the context. In murine models of arthritis, exogenous IFN-β has prominent anti-inflammatory activity. However, a similar benefit has not been observed in RA. One possible explanation relates to the mechanism of action in arthritis: In murine models, IFN-β appears to act by increasing IL-1Ra production.[186] Although IL-1 is a key therapeutic target in pre-clinical models, it is less important in RA. Thus IFN-β does actually increase synovial IL-1Ra production in patients with RA, but blocking IL-1 is not sufficient for robust clinical effects. Another IFN, IL-29, has recently been identified in RA synovium. In vitro studies show that IL-29 can increase IL-6, IL-8, and MMP production by cultured FLS.[187]

### Chemokines

Chemokines are a family of related chemoattractant peptides that, with the assistance of adhesion molecules, summon cells into inflammatory sites. They are generally divided into families including CC, CXC, and CX3C based on the position of characteristic cysteine residues.

Many chemokines have been identified in the rheumatoid joint. IL-8, a CXC chemokine that was originally characterized as a potent chemoattractant for neutrophils, along with immune complexes and other chemotactic peptides such as C5a, contribute to the large influx of PMNs into the joint. Immunohistochemical analysis of synovial tissue demonstrates IL-8 protein in sublining perivascular macrophages, as well as in scattered lining cells.[188] Cultured synovial tissue macrophages constitutively produce IL-8, and FLS express the same gene if they are stimulated with IL-1 or TNF. IL-8 accounts for about 40% of the neutrophil chemoattractant activity in synovial fluid. In addition, IL-8 activates neutrophils through G protein–coupled receptors and is a potent angiogenesis factor.

Many other chemoattractant proteins are implicated in RA. Macrophage-inhibitory protein-1α, macrophage-inhibitory protein-1β, MCP-1, and "regulated on activation, normally T cell expressed and secreted" (RANTES)—members of the CC subfamily—are produced by RA synovium, as are many other CC chemokines.

CXCL16 and epithelial neutrophil-activating peptide-78 (ENA-78) are CXC chemokines and are also abundant, along with many others in that family.[189] CXCL16, which binds to CXCR6 on T cells, can contribute to the recruitment of lymphocytes into the synovium. ENA-78 accounts for about 40% of the chemotactic activity for neutrophils in RA synovial fluid. Concentrations of these chemokines are higher in RA synovial effusions compared with non-inflammatory arthritides such as OA. Although the chemokines also can be detected in the blood, the levels are considerably lower than in the joint, thereby providing a gradient that signals cells to migrate into the synovium.

Lymphocyte-specific chemotactic factors contribute to the germinal center architecture of RA. The CXC factor B cell–activating chemokine-1 (BCA-1; CXCL13) binds to specific CXCR5 receptors on B cells. CXCL13 is expressed in the RA synovial tissues, especially by follicular DCs in germinal centers, and likely accounts for B cell migration to these regions.[190] CCL21 and several other factors participate in the anatomic organization of germinal centers, marginal zones, and other regions of lymphoid follicles. Another chemokine, SDF-1, is expressed by synoviocytes and endothelial cells and can play a major role as a chemoattractant for T cells in synovium via its receptor, CXCR4. Unlike other chemokine receptors that can bind multiple members of the family, CXCR4 is highly specific for SDF-1 and is expressed by memory $CD4^+$ lymphocytes.

Chemokines have attracted considerable attention as therapeutic targets to prevent recruitment of immune cells into the synovium. Numerous pre-clinical models support the use of chemokine blockers. For instance, antibodies to fractalkine (CXC3L1) suppress murine collagen-induced arthritis, even though anti–type II collagen antibody production was not affected.[191] Anti-CXCL16 antibody also decreases clinical arthritis in the same model. One problem is that the system is highly redundant and several different chemotactic proteins can bind to the same receptor. No clinical improvement in RA was observed in a study using anti–MCP-1 antibody, perhaps because the antibody also altered the kinetics of MCP-1 metabolism. Anti–IL-8 antibodies have met with limited success in psoriasis. More

promising results were observed with antibody to IP-10 (CXCL10), which demonstrated some efficacy in a phase II study in RA.[192]

Alternatively, chemokines bind to G protein–coupled receptors and can be targeted to block multiple factors. For instance, CCR1 antagonists, which block RANTES and MIP-1α, have been evaluated in clinical trials.[193] Although one compound significantly decreased synovial infiltration by CCR1-expressing cells, no significant improvement was observed. Chemokine receptor blockade has other levels of complexity such as CCR2-deficient mice, which develop more severe arthritis in some models. Several CCR2 antagonists, which block MCP-1, have not demonstrated significant efficacy in RA. These data suggest that the chemokine system, including the receptors, is both redundant, complex, and, in some cases, perhaps protective in arthritis.

Chemokines and other chemotactic factors (such as C5a and $LTB_4$) can signal through a variety of mechanisms, although many pathways converge on PI3K. Of the several PI3K isoforms, PI3Kγ is relatively specific for chemokine signal transduction, which provides an opportunity to block multiple chemokine receptors simultaneously. Proof of concept for this approach was provided by studies in PI3Kγ knockout mice, which have less synovial inflammation in passive and active collagen-induced arthritis than in wild-type mice.[194] Therefore, targeting shared intra-cellular pathways could potentially overcome some of the limitations presented by the complex chemoattractant system.

### Platelet-Derived Growth Factor and Fibroblast Growth Factor

PDGF is a potent growth factor that is both a chemoattractant and a mitogen for fibroblasts and induces collagenase expression. It is the most potent stimulator of long-term growth of synovial cells in culture. PDGF is expressed in vascular endothelial cells, platelets, and other synovial sublining cells in rheumatoid synovium, compared with healthy tissue. Multiple isoforms of this molecule have been identified (PDGF A through D), all of which have been detected in RA synovial membranes. PDGF D has been identified as an especially potent stimulator of MMP-1 expression in cultured synoviocytes. PDGF is a potent activator of the PI3K pathway, which leads to Akt phosphorylation and supports synoviocyte survival through activation of the α and δ isoforms.

FGFs are a family of peptide growth factors with pleiotropic activities. In patients with rheumatoid disease, it is likely that heparin-binding growth factor, the precursor of acidic fibroblast growth factor, is a major mitogen for many cell types and stimulates angiogenesis. Interactions between FGF and proteoglycans is required for biologic activity. It is a potent angiogenic factor, inducing capillary endothelial cells to invade a three-dimensional collagen matrix and form capillaries. Synoviocytes can also be induced to increase expression of RANKL by FGF, thereby enhancing osteoclast activation and bone resorption. FGF is present in RA synovial fluid, and the genes are expressed by synovial cells. Synovial fibroblasts express FGF receptors and proliferate after exposure to the growth factor.

## Immunosuppressive Cytokines and Cytokine Antagonists

The pro-inflammatory cytokine network in RA is offset by a variety of suppressive and anti-inflammatory factors that attempt to re-establish homeostasis. Low production of these suppressive cytokines could contribute to the perpetuation of the synovitis. Many cytokine antagonists or natural immunosuppressives represent potential therapeutic targets for the treatment of inflammatory diseases.

### Interleukin-1 Receptor Antagonist

IL-1Ra is a naturally occurring IL-1 inhibitor that binds directly to types I and II IL-1 receptors and competes with IL-1 for the ligand-binding site. Interaction of IL-1Ra with the IL-1 receptors does not result in signal transduction, and, in contrast to IL-1α or -β, the receptor-ligand complex is not internalized after it binds to the IL-1 receptor. Even though IL-1Ra has high affinity for the IL-1 receptor, it is a relatively weak inhibitor because IL-1 can activate cells even if only a small percentage of IL-1 receptors is occupied. As a result, a substantial excess of the inhibitor is required to saturate the receptor and thereby block IL-1–mediated stimulation (usually 10- to 100-fold excess of IL-1Ra).

IL-1Ra is present in rheumatoid synovial effusions; much of it is produced by neutrophils and macrophages.[195] In RA synovium, IL-1Ra protein is found in perivascular mononuclear cells and in the synovial intimal lining. The IL-1Ra protein and mRNA can be detected in synovial macrophages and, to a lesser extent, in type B synoviocytes (Figure 69-12). The presence of IL-1Ra in synovium is not specific to RA because OA synovial tissue also contains IL-1Ra, albeit in lesser amounts. Normal synovium contains little, if any, IL-1Ra protein. Synovial cell culture experiments show that the amount of IL-1Ra is insufficient to antagonize synovial IL-1.

### Interleukin-10

IL-10 is an immunosuppressive cytokine that was originally characterized as an inhibitor of T cell cytokine production. Its immunosuppressive actions might be important in pregnancy to suppress an immune response directed against paternal MHC antigens, and it might regulate susceptibility to some parasitic infections. As noted previously, IL-10 protein is present in RA synovial fluid, and the gene is expressed by synovial tissue macrophages. Serial synovial biopsies in patients with RA who were treated with recombinant IL-10 did not show any significant histologic improvement, and clinical responses were not impressive in a limited study.

### Transforming Growth Factor-β

TGF-β is a key member of the TGF superfamily, which includes the bone morphogenic proteins that signal through intra-cellular signaling molecules known as *Smads*. TGF-β is widely distributed in different tissues and is produced by many cells, including T cells, monocytes, and platelets. It suppresses the production of collagenase and induces the expression of TIMP. TGF-β accelerates the healing of

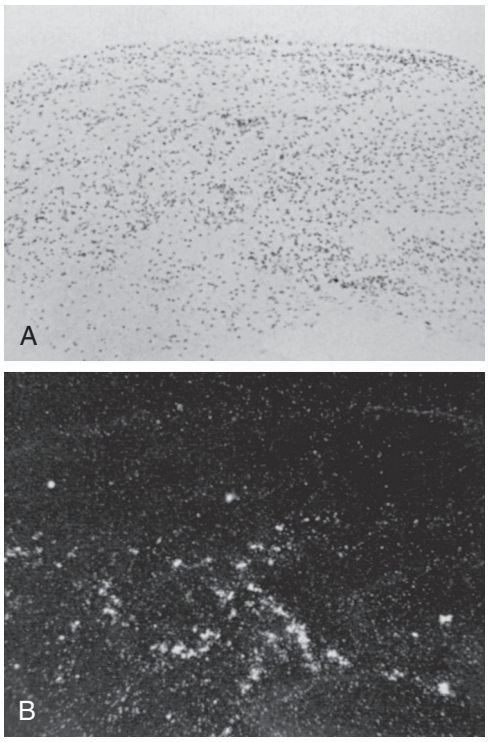



**Figure 69-12** Localization of IL-1 receptor antagonist (IL-1Ra) messenger RNA in rheumatoid arthritis synovial tissue by in situ hybridization. The specific RNA transcript was detected in perivascular cells, especially macrophages. **A,** Bright field view. **B,** The same area using a dark field filter. Silver grains in the dark field view show the location of IL-1Ra$^+$ cells.

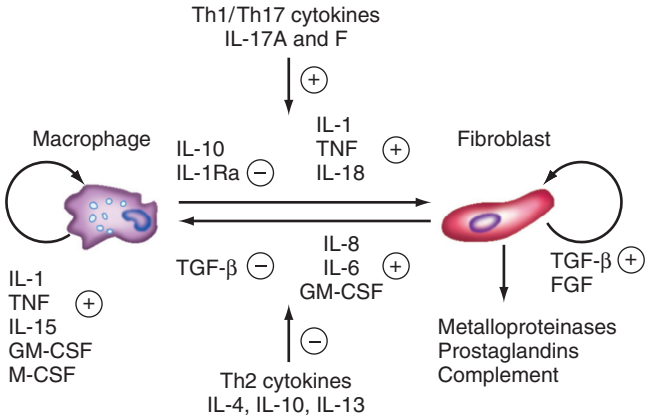



**Figure 69-13** Cytokine networks in rheumatoid arthritis (RA). Paracrine and autocrine pathways can lead to activation of fibroblast-like and macrophage-like synoviocytes in the synovial intimal lining. Both positive (+) and negative (–) feedback loops are present, although in RA the former predominate. Type 1 T helper (Th) or Th17 cytokines can potentially enhance the network, whereas Th2 cytokines are suppressive. FGF, Fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony stimulating factor; TGF, transforming growth factor.

incisional wounds and induces both fibrosis and angiogenesis in experimental models with animals. Substantial amounts of TGF-β are present in synovial fluid, although it is mainly present in an inactive, latent form, and the mRNA can be detected in RA synovial tissue.[196] Although typically considered an immunosuppressive cytokine with wound-healing properties, the role of TGF-β in RA is complex.

In RA, TGF-β is one of the factors responsible for blunted responses of synovial fluid T cells. TGF-β also downregulates IL-1 receptor expression on some cell types, including chondrocytes. When it is injected directly into the knees of animals, fibrosis and synovial lining hyperplasia develop. In streptococcal cell wall arthritis, parenteral administration or systemic gene therapy with the TGF-β gene ameliorates the disease. However, intra-articular administration of anti–TGF-β antibody decreases arthritis in the injected joint but not in the contralateral joint in the same model. Although mainly considered anti-inflammatory, TGF-β also plays a key role in the development of autoimmunity through the differentiation of T cells into the Th17 phenotype.

### Soluble Cytokine Receptors and Binding Proteins

Soluble cytokine receptors and binding proteins can absorb free cytokines and prevent them from engaging functional receptors on cells. Although these inhibitors can block cytokine action, they also could act as carrier proteins that protect cytokines from proteolytic degradation or deliver them directly to cells such as the IL-6 receptor.

TNF receptors are normally expressed as membrane-bound proteins and can be released from the cell surface after proteolytic cleavage. Soluble p55 and p75 TNF receptors have been detected in RA synovial fluid, sometimes in high concentrations. Based on studies with TNF inhibitors and soluble TNF receptor-Fc fusion proteins like etanercept, it is clear that the levels of soluble TNF receptors are not high enough to block TNF biologic activity.

Many other soluble receptors and binding proteins are produced in RA, albeit in concentrations too low to effectively suppress the pro-inflammatory cytokine milieu of the joint. For instance, the IL-1 type II receptor is present in RA synovial fluid, along with lesser amounts of the type I receptor. These soluble receptors can bind to IL-1 or IL-1Ra in synovial effusions. Soluble receptors to IL-15 and IL-17 that neutralize their respective ligands have been characterized, and an IL-18 binding protein also can inhibit cytokine activity.

## Perpetuation of Synovitis by Cytokine Networks

Mapping the cytokine profile in RA led to targeted biologic therapy to inhibit cytokines and supports the concept that cytokine networks play a key role. The network pathway is not autonomous and, except for certain patients with very early disease, discontinuation of anti-cytokine therapy allows the disease to flare. Nevertheless, paracrine and autocrine cytokine networks in the synovial intimal lining contribute largely to inflammatory arthritis in RA (Figure 69-13).

Several cytokines that have been identified in the synovium or synovial fluid can participate in this system and might explain lining cell hyperplasia, HLA-DR and adhesion-molecule induction, and synovial angiogenesis.

The list of potential candidates in this highly redundant system is extensive. Several of these candidates, including IL-1, TNF, and IL-6, now have well-defined roles. The first two, along with numerous other factors (e.g., IL-15, IL-18, and IL-32), are produced by synovial macrophages and stimulate synovial fibroblast proliferation and secretion of IL-6, GM-CSF, and chemokines, as well as effector molecules such as MMPs and prostaglandins. GM-CSF, which is produced by both synovial macrophages and IL-1β– or TNF-stimulated synovial fibroblasts, can, in turn, induce IL-1 secretion to form a positive feedback loop. GM-CSF, especially in combination with TNF, also increases HLA-DR expression on macrophages. Macrophage and fibroblast cytokines also could indirectly contribute to the evidence for local T cell and B cell activation, including RF and ACPA production. Newly implicated members of the macrophage-fibroblast cytokine network such as IL-33 and HMGB1 can activate other cell lineages such as mast cells that have attracted increased attention in recent years.

The cytokines can then recruit other immune and inflammatory cells into the synovium through the production of chemokines that select specific cell lineages for admission and retention in the synovium. Many of these chemokines, including the CXC and CC families, are produced by macrophages and fibroblasts and attract neutrophils, macrophages, and specific subpopulations of T and B cells. The sublining chemokine profile including CXCL13 and CCL20 helps organize these newly infiltrated cells into organized lymphoid structures in some patients. Other factors such as IL-12 and IL-23 differentiate $CD4^+$ T cells into the Th1 phenotype to produce relatively small amounts of IFN-γ and other relevant cytokines. IL-1 and TGF-β produced mainly by the lining can, along with IL-23, support the production of Th17 cells that release the potent IL-17 family members into the local milieu. All of this occurs in the presence of inhibitor factors, soluble receptors, and binding proteins that are overwhelmed by the inflammatory drive. Other cytokines, such as RANKL and M-CSF, activate osteoclasts that remodel bone.

Even though cytokines might not cause RA per se, they clearly orchestrate and perpetuate rheumatoid synovitis. The pivotal cytokine or cytokines that must be blocked could be different for individual patients, and even this could vary with the stage of disease. Ultimately, understanding the genetic predisposition, environmental triggers, and specific patterns of cytokine production could help determine the correct combination of cytokine inhibitors that will be effective.

## SIGNAL TRANSDUCTION AND TRANSCRIPTION FACTORS

**KEY POINTS**

- **Complex intra-cellular signaling mechanisms regulate cytokine production and actions in RA synovium.**
- **NF-κB, MAP kinases, activator protein-1 (AP-1), JAKs, Syk, PI3Ks, and several other pathways are potential therapeutic targets in RA.**
- **Signal transduction inhibitors have demonstrated clinical efficacy in RA.**

Intra-cellular signal transduction systems transmit information from the environment to the interior of a cell, which can then respond appropriately to stress. The remarkable diversity of signaling pathways and transcription factors provides a selective mechanism for orchestrating activation and repression for appropriate genes. Many of the inflammatory responses observed in RA synovium, including the activation of cytokine, proteases, and adhesion-molecule genes, can be traced to specific transcription factors and signal transduction pathways.

### Nuclear Factor-κB

NF-κB is a ubiquitous transcription factor that plays a key role in the expression of many genes central to RA, including IL-1, TNF, IL-6, and IL-8. It is abundant in rheumatoid synovium, and immunohistochemical analysis demonstrates p50 and p65 NF-κB proteins in the nuclei of cells in the synovial intimal lining.[197] Although the proteins also can be detected in OA synovium, NF-κB activation is much greater in RA because of phosphorylation and degradation of IκB in RA intimal lining cells. Nuclear translocation of NF-κB in cultured FLS occurs rapidly after stimulation with many pro-inflammatory cytokines (e.g., IL-1, TNF, and IL-17) or TLR ligands (e.g., peptidoglycan and LPS). NF-κB is also a major survival factor for cells and is responsible for resistance to apoptosis in some cell lineages after activation. This characteristic is especially important in intestinal inflammation, where IKKβ deficiency actually increases mucosal damage because the lack of NF-κB can increase cell death in epithelium.[197a]

The relevance of NF-κB to inflammatory arthritis has been tested in several animal models. Synovial NF-κB is rapidly activated, often long before clinical arthritis is evident. Adjuvant arthritis in rats is ameliorated using intra-articular gene therapy with the dominant negative IKKβ construct that blocks the IKK pathway, and streptococcal cell-wall arthritis is blocked with decoy oligonucleotides or a dominant negative IκB adenovirus.[198] NF-κB inhibition is associated with decreased synovial cellular infiltration, as well as increased apoptosis. Small molecule IKKβ inhibitors also suppress arthritis and joint destruction in murine collagen-induced arthritis.

### Activator Protein-1

Like NF-κB, AP-1 regulates many genes implicated in RA, especially TNF and the MMPs. AP-1 activity can be induced by extra-cellular signals including cytokines, growth factors, tumor promoters, and the Ras oncoprotein. AP-1 includes members of the Jun and Fos families of transcription factors that form Jun homodimers, Jun-Jun heterodimers, or Jun-Fos heterodimers. AP-1–driven gene expression is greatly enhanced when one of its components, including c-Jun, is phosphorylated by the c-Jun N-terminal kinase (JNK).

AP-1 proteins and nuclear binding are elevated in RA synovium, especially in the nuclei of cells in the intimal lining layer.[199] c-Jun and c-Fos proteins are also expressed in the sublining inflammatory infiltrate, albeit to a lesser degree. Localization of AP-1 to the intimal lining correlates with the site where most protease and cytokine genes are overexpressed in RA. AP-1 proteins are usually not detected

in normal synovium, although modest amounts have also been detected in OA.

Cytokines such as IL-1 and TNF and TLR ligands contribute to the activation of AP-1 in RA synovium. These factors are potent inducers of AP-1 nuclear binding in cultured FLS. IL-1 is particularly effective and can dramatically increase AP-1 binding at very low concentrations. The specific Jun family members that constitute AP-1 in synoviocytes have a clear effect on function. For instance, c-Jun increases the production of pro-inflammatory mediators, whereas JunD suppresses cytokine and MMP production.[200] AP-1 decoy oligonucleotides suppress collagen-induced arthritis and inhibit cytokine production by synovial tissue.

## Mitogen-Activated Protein Kinases

MAP kinases are signal transduction enzymes activated in response to cellular stress and are composed of parallel protein-kinase cascades that regulate cytokine and MMP gene expression. There are three main families of MAP kinases known as JNK, p38, and extra-cellular signal-regulated kinase (ERK). MAP kinases phosphorylate selected intra-cellular proteins, including transcription factors, which subsequently regulate the expression of various genes by transcriptional and post-transcriptional mechanisms. MAP kinases are activated by phosphorylation at conserved threonine and tyrosine residues by a cascade of dual-specificity kinases that are, in turn, activated by MAP kinase kinases. The relative hierarchy of the individual MAP kinases depends on the cell type and inflammatory stimulus.

The MAP kinases are widely expressed in synovial tissue and are activated in rheumatoid synovium. Phosphorylated ERK, p38, and JNK can be detected by immunohistochemistry or Western blot analysis. All three kinases and their upstream regulators are constitutively expressed by cultured FLS and can be activated within minutes after exposure to cytokines. They regulate production of pro-inflammatory cytokines and MMPs.

p38 inhibitors are effective anti-inflammatory agents in murine collagen-induced arthritis and rat adjuvant arthritis, possibly by decreasing the production of pro-inflammatory cytokines. In addition, p38 inhibitors block TNF and IL-6 production by cultured macrophages and synoviocytes, as well as cultured synovial tissue cells. Spinal p38 also plays a major role in pain processing, and inhibitors have potential for both analgesic and anti-inflammatory action. Recent studies suggest that p38 in the central nervous system can regulate peripheral inflammation because intrathecal administration of a p38 inhibitor suppresses inflammation and joint destruction in rat adjuvant arthritis.[200a]

Given the putative role of p38 in RA, it is surprising that clinical efficacy of the selective p38 inhibitors is modest, at best.[201] Clinical response rates are rarely greater than 40%, compared with the 60% range for TNF blockers. Perhaps more interesting, an initial decrease in acute-phase reactants is only transient despite adequate drug levels. Recent data in mice that lack p38α in macrophages shed some light on the mechanisms. In the passive K/BxN serum transfer model, paradoxically, disease increases in these mice[202] (Figure 69-14). The p38-deficient macrophages have lower production of the anti-inflammatory cytokine IL-10. In addition, expression of phosphatases that deactivate other MAP kinases is suppressed, resulting in markedly higher levels of phosphorylated JNK and ERK. Thus a compensatory increase in pro-inflammatory pathways abrogates the beneficial effect of p38 inhibition.

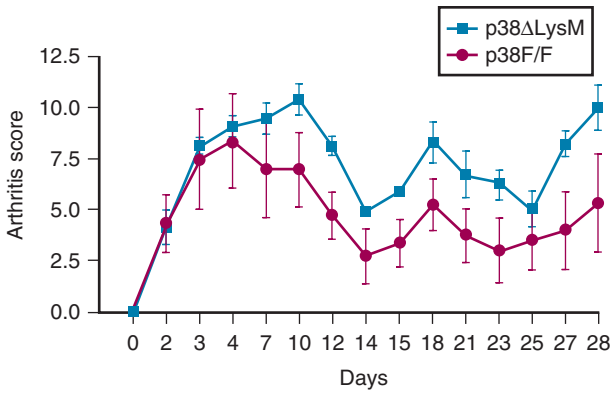



**Figure 69-14** Increased arthritis in mice lacking p38 in macrophages. Mice with p38-deficient macrophages (p38ΔLysM) and control mice (p38F/F) were injected twice with arthritogenic serum from K/BxN mice to create a chronic arthritis model. Note that the mice lacking p38 in macrophages have increased disease severity, most likely because of decreased levels of IL-10 production and increased activation of other mitogen-activated protein kinases. *(From Guma M, Hammaker D, Topolewski K, et al: Anti-inflammatory functions of p38 in mouse models of rheumatoid arthritis: advantages of targeting upstream kinases MKK-3 or MKK-6.* Arthritis Rheum *64:2887-2895, 2012.)*

Instead of blocking p38 directly, an upstream kinase might be targeted. MKK3 and MKK6, both of which regulate p38, are expressed and activated in the rheumatoid synovium. MKK3 knockout mice and MKK6 knockout mice have markedly decreased joint inflammation in the passive K/BxN model and collagen-induced arthritis.[203] Notably, the MKK3 or MKK6 deficiency in macrophages avoids the effect of p38 blockade on ERK, JNK, and IL-10. The upstream kinases represent alternatives to targeting the MAP kinases themselves.

The other two MAP kinases are also potential therapeutic targets. The ERK pathway, which regulates cell growth and some inflammatory mediators, can be blocked by inhibiting its upstream kinases MEK1 and MEK2. One clinical trial with a small molecule inhibitor found minimal benefit, although the placebo response rate was high.

Two of the three JNK isoforms (JNK1 and JNK2) regulate a variety of genes in inflammation, including TNF and metalloproteinases. Using a JNK inhibitor, marked protection of bone destruction was observed in the adjuvant arthritis model, along with decreased synovial AP-1 activation and collagenase gene expression.[204] No benefit was observed in the $JNK1^{-/-}$ mice, and only modest cartilage protection was seen in the $JNK2^{-/-}$ animals.

Like p38, the upstream kinases in the JNK pathway are activated in RA synovium and FLS. MKK4 and MKK7 are the main kinases that modulate JNK function. Only MKK7 is required for cytokine-stimulated JNK activation and MMP expression in cultured synoviocytes.[205] Using antisense oligonucleotides to decrease MKK7 expression, mice have decreased arthritis severity and JNK activation in the passive K/BxN model.[206]

## Janus Kinases and the Signal Transducers and Activators of Transcription

The JAK proteins are key proteins that transduce signals from a wide variety of cytokine and growth factor receptors. Four JAKs have been identified (JAK1, JAK2, JAK3, and TYK1), and they can form heterodimers and homodimers. The specific JAK proteins responsible for individual cytokine responses are not fully elucidated, but some general guidelines are available. JAK3 provides the signaling for many T cell–derived cytokines, and mutations in this gene are responsible for severe immunodeficiency. JAK1/JAK2 is responsible for the IL-6 family and interferons. JAK2/JAK2 signals for growth factors such as erythropoietin and cytokines related to Th17 cell differentiation like IL-23. The distribution and expression levels for JAKs in RA are not well defined. However, all four genes are expressed in cultured FLS and immunohistochemistry studies localize JAK3, especially in sublining DCs.[207]

The JAK proteins have become a focus of interest because of the efficacy demonstrated by several JAK inhibitors in RA, including tofacitinib.[208] The benefit observed is similar to biologic agents, although the importance of toxicity related to host defense, liver enzymes, neutropenia, renal function, and potentially oncogenesis requires further study. The best isoform specificity also needs to be defined to maximize safety and efficacy. Because IL-6 signals through kinases such as JAK1, one could anticipate that this particular signaling molecule would be especially important for RA. On the other hand, a more T cell–directed approach for indications such as transplantation might focus more on JAK3 and could potentially have longer term benefits in complex diseases of innate and adaptive immunity.

The JAK proteins phosphorylate the STATs. The STATs can then translocate to the nuclei, where they can alter gene transcription. STATs have been implicated in the expression of many pro-inflammatory genes. Although there is some variation depending the stimulus, IFNs signal through STAT1, IL-6 signals through STAT3, and IL-12 signals through STAT4.

Multiple STATs are expressed in rheumatoid synovium. STAT1 activation correlates with disease activity in RA (Figure 69-15), and a STAT1 decoy oligonucleotide suppresses antigen-induced arthritis in mice.[209] In addition, studies of rheumatoid tissue have an expression signature suggesting STAT1-regulated gene expression, including IFN-regulated genes. Another study suggested that patients with RA could be divided into a group with a STAT1 signature and the other with a signature reminiscent of tissue repair and remodeling.[210]

A synovial biopsy study in patients receiving the JAK inhibitor tofacitinib showed a close correlation between clinical responses and phospho-STAT1 and phospho-STAT3.[210a] These data suggest that JAK1 inhibition is especially important for clinical efficacy, most likely by blocking IL-6 and type I IFNs.

## Interferon Regulation: IKK-Related Kinases and Interferon Regulatory Factors

IFN signatures have been noted in several autoimmune diseases, including RA. Type I IFNs are expressed in RA synovium, especially by synoviocytes in the intimal lining. Regulation of IFN production and the IFN response genes such as *CCL5*, *CXCL10*, and *CCL2* involves a pathway that runs parallel to the canonical NF-κB pathway and includes two IKK-related kinases, known as IKKε and TANK binding kinase 1 (TBK1). Triggered when TLR3 is ligated by viral dsRNA, IKKε, and TBK1 phosphorylate the transcription factor IFN regulatory factor 3 (IRF3) and induce production of an array of genes that orchestrate this response, including RANTES and IFN-β. IKKε and its substrate IRF3 are expressed and highly activated in RA synovium. Using a combination of $IKK\varepsilon^{-/-}$ mice and genetic constructs that block endogenous IKKε and TBK1 activity, the IKK-related kinases were shown to be a key regulator of IFN-β, RANTES, and MMP expression in cultured FLS.[211] In contrast to DCs, where IRF7 is the primary IRF that regulates IFN responses, IRF3 is the pivotal factor that is responsible for the IFN signature.[212]

Overexpression of some IRF3-driven genes, most notably IFN-β, could have a beneficial impact in inflammatory

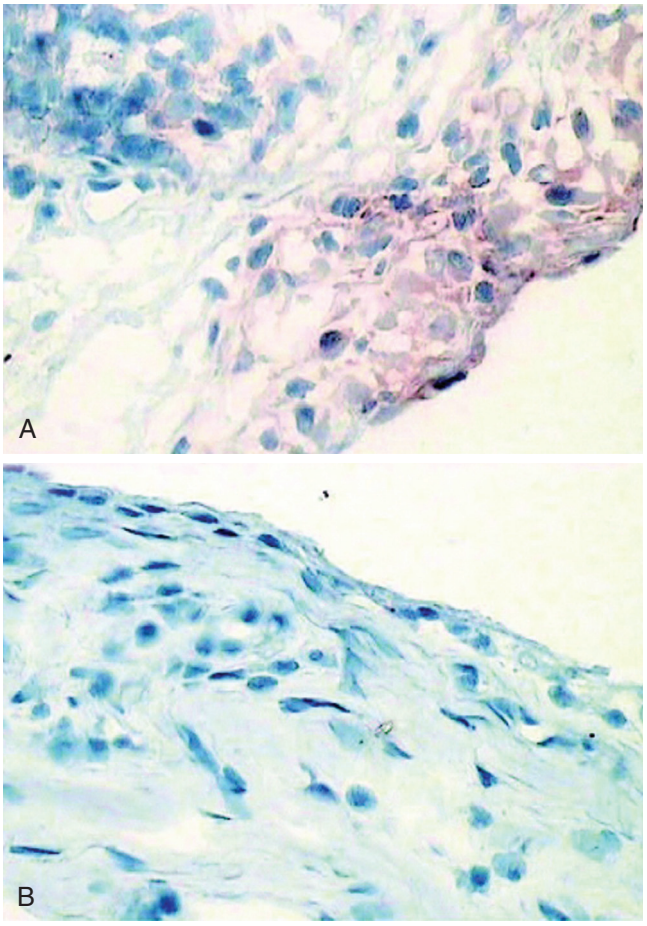



**Figure 69-15** Phospho-STAT1 expression in rheumatoid arthritis synovium. Signal transducer and activator of transcription (STAT)1 is a transcription factor activated by the Janus kinases that regulates interferon responses. **A,** Expression of STAT1 in the synovial intimal lining using immunohistochemistry. **B,** A synovial biopsy from the same patient after treatment with a traditional disease-modifying anti-rheumatic drug. Note that expression markedly decreased after therapy. *(From Walker JG, Ahern MJ, Coleman M, et al: Changes in synovial tissue Jak-STAT expression in rheumatoid arthritis in response to successful DMARD treatment.* Ann Rheum Dis *65:1558, 2006.)*

arthritis. Mice with collagen-induced arthritis that are injected with IFN-β or transduced fibroblasts expressing IFN-β have less severe disease compared with control subjects, including decreased bone and cartilage destruction. As previously noted, a clinical trial of IFN-β in RA showed no benefit, possibly because IFN acts by increasing IL-1Ra. An alternative approach might be to inhibit IKK-related kinases to block the chemokines associated with the IFN response and concomitantly provide treatment with low levels of exogenous IFN-β.[213]

### Spleen Tyrosine Kinase

Several other signaling molecules have been implicated in RA and are potential therapeutic targets. For example, Syk is involved in immunoreceptor signaling in a variety of cell types and plays a critical role in Fc receptor signaling in macrophages and mast cells. It also participates in B cell activation after ligation of the B cell receptor. A small molecule Syk inhibitor has demonstrated efficacy in patients with RA, although the compound fostamatinib also blocks many other kinases.[214] Phase III studies with this compound only showed modest efficacy (ACR20 response rates around 40%) without disease-modifying effect.

### PI3 Kinases and Other Signaling Pathways

The PI3Ks, especially the δ and γ isoforms, are also attractive therapeutic targets because of their role in innate immunity and cell recruitment, respectively. As previously noted, PI3Kγ is the convergence point for chemokine signaling and could permit targeting of several chemoattractants with a single compound. The δ isoform is notable because it not only regulates FLS invasion but also is required for B cell differentiation and activation. Therefore, PI3Kδ is an alternative way to target B cells. Bruton's tyrosine kinase (BTK) inhibitors represent another kinase that can be blocked to suppress B cells as well as innate immune response in myeloid cells. c-Kit blockade has also been evaluated in an open-label study that provided a positive signal. There is no shortage of targets, and many others, including upstream MAP kinase regulators, Ras, IL-1–associated kinases (IRAKs), and sphingosine kinase-1 (SK-1), will be evaluated in the future for a variety of immune-mediated diseases.

## CELL SURVIVAL AND DEATH IN RHEUMATOID SYNOVIUM

**KEY POINTS**

- **Reactive oxygen and nitrogen in RA joints contribute to a toxic environment that can damage cells and increase inflammation.**
- **Inadequate apoptosis, or cell death, can contribute to the accumulation of cells in rheumatoid synovium.**
- **Abnormalities of key regulatory genes such as the p53 tumor suppressor can enhance accumulation of cells in the joint.**
- **Inducing apoptosis can potentially suppress synovial inflammation and joint destruction.**

Studies defining the life cycle of cells have opened a new door to understanding the pathogenesis of neoplastic and inflammatory diseases. In this section, the role of oxidative damage, programmed cell death, and permanent changes in the genome are discussed because they can alter the natural history of RA.

### Reactive Oxygen and Nitrogen

Oxidative stress in the joints of patients with RA results from increased pressure in the synovial cavity, reduced capillary density, vascular changes, an increased metabolic rate of synovial tissue, and locally activated leukocytes. The generation of reactive oxygen species can also be facilitated by repetitive ischemia-reperfusion injury in the joint. Tissue injury releases iron and copper ions and heme proteins that are catalytic for free-radical reactions. Electron transport chains are also disrupted in the mitochondria and endoplasmic reticulum, leading to leakage of electrons to form superoxide.

Increased reactive oxygen species in patients with RA leads to elevated levels of lipid peroxidation products, degradation of hyaluronic acid by free radicals, decreased levels of ascorbic acid in serum and synovial fluid, and increased breath pentane excretion. The levels of thioredoxin, a marker of oxidative stress, are also significantly higher in synovial fluid from patients with RA compared with other forms of arthritis. Peripheral blood lymphocyte DNA from patients with RA contains significantly increased levels of the mutagenic 8-oxohydrodeoxyguanosine, which is a product of oxidative damage to DNA, pointing to the genotoxic effects of oxidative stress.

NO production is also high in rheumatoid synovial tissue. Low levels of NO are constitutively produced by endothelial or neuronal NO synthases, and enzyme levels are substantially increased by inducible NO synthase after stimulation by cytokines or bacterial products. Nitrite levels in synovial fluid are elevated in patients with RA, indicating local NO production. In addition, the urinary nitrate-to-creatinine ratio is increased and inducible NO synthase is present in the synovium.

### Apoptosis and Autophagy

Programmed cell death, or apoptosis, removes cells safely from living tissue and permits remodeling, or cell deletion, without causing an inflammatory response. Apoptosis is a normal process that is tightly regulated and can be initiated by withdrawal of hormones and growth factors. Autophagy represents an alternative adaptive mechanism for cell survival that breaks down some cellular components through lysosomes and maintains critical functions to ensure that the cell does not die.

#### Genes Regulating Apoptosis

The accumulation of cells in RA results from a balance of cell recruitment, cell egress, local proliferation, and local death. Any imbalance can potentially lead to synovial hyperplasia. T cell apoptosis in RA synovial effusions, for instance, is significantly less than lymphocytes from crystal-induced arthropathy. High expression of the anti-apoptotic

molecule Bcl-2 is found in lymphoid aggregates and protects synovial T cells from programmed cell death. Resistance to apoptosis in vitro increases if RA T cells are co-cultured with FLS. The specific adhesion molecules involved are not defined, although the integrin-binding RGD motif (arginine-glycine-asparagine) blocks the protective effects of synoviocytes.

Fas and its TNF superfamily counter-receptor Fas ligand (FasL) are potent regulators of cell death for many cell types, including synovial T cells and synoviocytes. Fas is expressed by rheumatoid synovial fluid T cells, and the number of $Fas^+$ cells in the peripheral blood of patients with RA is greater than in healthy control subjects.[215] Anti-Fas antibody, which cross-links Fas on cell surfaces, rapidly causes apoptosis in synovial fluid B and T lymphocytes in RA, although peripheral-blood T cells are more resistant. Another member of the TNF superfamily, TNF-related apoptosis-inducing ligand (TRAIL), binds to two receptors (DR4 or DR5) to induce caspase-dependent apoptosis. DR5 is expressed in RA FLS but not OA cells, and apoptosis can be induced by either TRAIL or agonistic anti-DR5 antibody.[216]

Studies of apoptosis in RA synovial tissue show only a small number of apoptotic nuclei in the intimal lining and sublining.[217] Electron microscopic studies show rare cells that exhibit the typical findings of programmed cell death. Lymphoid aggregates containing high levels of Bcl-2 have few apoptotic cells. Macrophage apoptosis is also low because they express high levels of the caspase 8 inhibitor FLICE-like inhibitory protein (FLIP), which can inhibit Fas-mediated apoptosis.

It is comparatively difficult to induce apoptosis in synoviocytes through a variety of mechanisms, including Fas ligation. p53, which typically induces cell-cycle arrest and either DNA repair or apoptosis, is also expressed in the synovial lining and sublining. However, one of the main effectors of p53-mediated apoptosis, p53 upregulated modulator of apoptosis (PUMA), is present only in low concentrations in the synovium and cultured synoviocytes. Synoviocytes are resistant to p53-mediated apoptosis even when cells are forced to overexpress the protein using genetic methods.[218] Synoviocyte apoptosis can be initiated by oxidative stress such as hydrogen peroxide or by exposure to NO.

The relative paucity of apoptosis in RA can also be partially explained by patterns of gene expression that favor cell survival. Recently, the protease ADAM15 was implicated in FLS resistance induced by FasL through activation of the focal adhesion kinase pathway.[219] It is of interest that many other genes in same pathway are differentially methylated and could also increase resistance to cell death.[220] Sentrin-1, a ubiquitin-like protein, regulates the cell survival by modifying proteins involved in apoptosis. Sentrin-1 is expressed in RA synovium, especially at sites of cartilage invasion, and protects cells from Fas-mediated death. A second protein, phosphatase and tensin homolog on chromosome 10 (PTEN), was originally defined as a key factor that protects against tumorigenesis through antagonism of PI3K, Akt, and many other proliferative pathways. Underexpression of PTEN in RA has been described in rheumatoid synovial intimal lining, as well as cultured FLS.[221]

### Genes Regulating Autophagy

Genes associated with increased autophagy, such as LC3 and Beclin-1 and the miRNA miR-30a, are increased in the RA synovial intimal lining compared with OA synovium.[222] These genes might program cells for improved survival through autophagy rather than cell death through apoptosis. In RA FLS, induction of autophagy by inhibiting proteasome-mediated protein degradation protected against cell death, whereas endoplasmic reticulum stress increased apoptosis. Therefore, the degree to which autophagy regulates cell survival is context-specific, and conclusions on its role in synovitis remain unclear.

### Possible Therapeutic Interventions that Increase Apoptosis

Fas-induced death has been used successfully in murine collagen-induced arthritis using anti-Fas antibodies and adenovirus encoding for Fas ligand. Anti-Fas antibody also induces synovial cell death in RA synovial tissue explanted in SCID mice. In the SCID mouse model using RA synovial explants, anti-DR5 antibody decreased cartilage erosion. Similarly, adenoviral transfer of TRAIL in a rabbit model of arthritis decreases synovial inflammation.[223] The importance of apoptosis as a regulator of inflammation was confirmed in murine collagen-induced arthritis, where genetic DR5 deficiency exacerbated the disease. Blocking Foxo3a in PMNs is also an effective method of deleting these inflammatory cells and suppressing inflammatory arthritis in mice.[147]

The Bcl-2 homology 3 (BH3) domain-only proteins are potent inducers of apoptosis. The challenge is how to get these proteins expressed in the target cell.[224] One of these proteins, Bim, was engineered into a cell membrane permeable protein (TAT-Bim) and evaluated in the passive K/BxN model. The construct decreased arthritis severity in prophylactic and therapeutic treatment protocols and was associated with apoptosis of cells, mainly in the myeloid lineage. Therefore, targeted cell death of individual lineages can potentially decrease inflammatory arthritis.

Other molecules that regulate apoptosis have also demonstrated potential utility in animal models. For instance, NF-κB blockade in streptococcal cell-wall arthritis induces synovial apoptosis and suppresses arthritis. *TP53* gene therapy in rabbit antigen-induced arthritis induces synovial apoptosis and decreases inflammation.[225] The pleiotropic activities of p53 were demonstrated in collagen-induced arthritis because $Tp53^{-/-}$ mice with the disease developed increased inflammation and greater joint destruction in association with decreased apoptosis. Joint damage was mediated by increased expression of collagenase genes in the knockout mice, most likely because p53 directly suppresses MMP gene transcription.[226]

### Tumor Suppressor Genes

The p53 tumor suppressor is a key regulator of DNA repair and cell replication. p53 protein expression is significantly greater in the rheumatoid synovium compared with OA and normal tissue.[227] It is of interest that p53 protein can also

be detected in RA synovium from patients with very early stage RA. However, its expression is much lower in other inflammatory arthropathies such as reactive arthritis, which might reflect greater DNA damage and oxidative stress in RA.

Somatic mutations in the *TP53* gene, which encodes p53, that can occur in RA synovium could contribute to the unusual phenotype of RA synoviocytes and inadequate apoptosis in synovial tissue.[228] Transition mutations, which are characteristic of damage induced by reactive oxygen or NO, account for more than 80% of the base changes. Some of the mutant *TP53* genes exhibit dominant negative characteristics and suppress the function of the wild-type allele. Microdissection studies identified mutant islands with oligoclonal expansion, and the loss of p53 function in a region of RA synovium was associated with increased IL-6 gene expression in the same location. The data suggest that mutations do not cause RA but, instead, are the result of long-standing oxidative stress. The gene alterations can then potentially increase the aggressive nature of the synovium and alter the natural history of RA. *TP53* also regulates T cell differentiation through a STAT-mediated mechanism. Decreased STAT5 in $CD4^+$ T cells in p53-deficient mice have increased Th17 and decreased Treg function.[229] These data suggest yet another role for this tumor suppressor gene in immune-mediated diseases.

Abnormalities in other genes have also been reported in RA. For instance, synovial T cells in RA have an increased incidence of mutations in the *HPRT1* gene. Although not functionally important, these synovial T cells act as a marker for oxidative damage that occurs in the synovial milieu. Some of these abnormal lymphocytes can also be detected in the peripheral blood, suggesting that articular T cells can migrate out of the joint. Mutations in mitochondrial genes have been described in synoviocytes.

## BLOOD VESSELS AND THE SYNOVIAL VASCULATURE

**KEY POINTS**

- **The synovium in RA is a hypoxic environment.**
- **Angiogenesis is a dynamic process in RA that provides nutrients to expanding synovium.**
- **Angiogenic factors such as IL-8, FGF, and vascular endothelial growth factor can enhance blood vessel proliferation in the synovium.**
- **Microvascular endothelial cells in the synovium express adhesion molecules that guide circulating cells into the joint under the influence of chemoattractants.**

Blood vessels play an active role in such inflammatory processes, not only as a means of selecting which cells should enter the tissue, but also as a determinant of tissue growth and nutrition through the proliferation of new capillaries. Understanding the structure and function of the microvasculature provides insights into how a highly catabolic tissue such as the rheumatoid synovium can flourish.

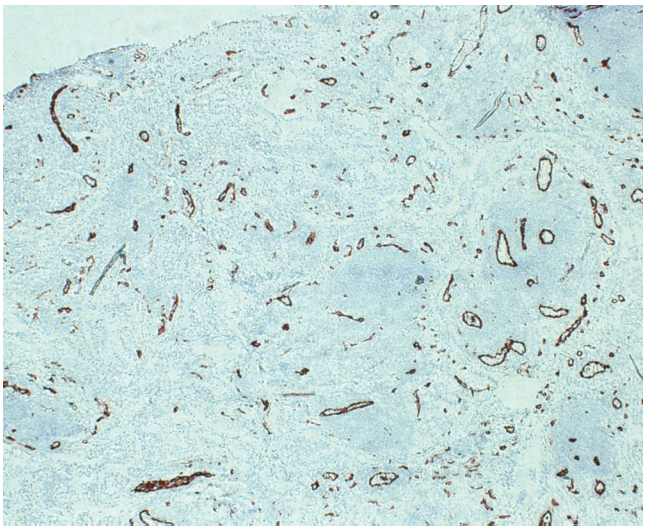


**Figure 69-16** Human rheumatoid synovial membrane stained with antibody to von Willebrand factor to delineate blood vessels. Virtually all of these blood vessels formed in response to angiogenic stimuli after the rheumatoid synovitis had been initiated. *(Courtesy Dr. Paul-Peter Tak.)*

### Angiogenesis: Feeding the Starved Synovium

The importance of luxurious new capillary growth early in the development of synovitis has been recognized for many years. The absolute number of blood vessels is increased in RA synovium (Figure 69-16), with a rich network of sublining capillaries and postcapillary venules in histologic sections stained with endothelium-specific antibodies. These blood vessels are not necessarily normal, however, with a predominance of straight, branching morphologic features compared with tortuous vessels in psoriatic arthritis synovium. The blood vessels in the inflamed synovium are less mature, probably as a result of increased DNA damage and decreased recruitment of pericytes. It is of interest that the immature vasculature is selectively depleted by TNF blockers in RA.[230]

#### Hypoxia

The mass of tissue outstrips angiogenesis in RA as determined by the number of blood vessels per unit area and causes local tissue ischemia.[231] Synovial fluid oxygen tensions are remarkably low, lactate measurements are frequently high, and the pH can be as low as 6.8 in extreme circumstances. The mean rheumatoid synovial fluid pressure of oxygen in the blood ($pO_2$) in samples from rheumatoid knees is approximately 30 mm Hg and occasionally less than 15 mm Hg. Another cause of diminished blood flow is increased positive pressure exerted by synovial effusions within the joint, a process that obliterates capillary flow while producing ischemia-reperfusion injury in the joint. Altered vascular flow may not be the only cause of hypoxia in joints; oxygen consumption of the rheumatoid synovium is 20 times normal.

Hypoxia is a potent stimulus for angiogenesis, and many angiogenic factors are regulated by the hypoxia-sensing protein hypoxia-inducible factor (HIF)-1α. Low oxygen

tension also leads to HIF-1α–induced transcription of VEGF, a specific endothelial cell mitogen that is present in high concentrations in rheumatoid synovial fluid and tissue. Elevated serum concentrations in early disease correlate with subsequent radiographic progress. VEGF also stimulates expression of collagenase, which can degrade the extra-cellular matrix to make room for the advancing vasculature and pannus. VEGF expression is especially high in the synovial intimal lining, and the angiogenesis factor is also produced by cultured FLS that have been exposed to hypoxia and IL-1. HIF-1α has other functions that regulate inflammation; selective deficiency in myeloid lineage cells suppresses inflammation in the passive K/BxN model of arthritis.[232]

HIF-1α regulates a number of other pathways that can modify inflammation and blood vessel formation. For example, it induces the alarmin IL-33 in RA FLS, creating a novel regulatory circuit that connects hypoxia to innate immune responses.[233] HIF-1α also activates STAT3, mimicking some of the sequelae of IL-6 stimulation.[234] Blocking HIF-1α in rheumatoid synovial explants decreases production of IL-6, IL-8, and MMP3. These data suggest that hypoxia not only drives blood vessel proliferation but also cytokine expression and matrix destruction.

### Angiogenic Factors

VEGF can bind to two receptors with tyrosine kinase domains: VEGF-R1/Flt-1 and VEGF-R2. VEGF-R1 regulates inflammatory responses in macrophages such as IL-6 and phagocytosis. Small-molecule VEGF-R inhibitors also suppress acute models of inflammation. Therefore, targeting this receptor with a small molecule might suppress the angiogenic and pro-inflammatory actions of VEGF.

In addition to the hypoxia-driven stimulus for blood vessel growth, the inflammatory cytokine milieu of the joint also encourages angiogenesis. Several pro-inflammatory factors expressed by the rheumatoid joint, including IL-8, FGF, and TNF, are angiogenic. Many of these cytokines further enhance angiogenesis by increasing expression of angiopoietins (Ang-1 and Ang-2) by synoviocytes, which can then bind to their tyrosine kinase receptor, Tie-1, on RA capillary endothelial cells. Co-expression of Tie-1 and Ang-1 in early undifferentiated arthritis correlates with the development of RA.[235] Additional angiogenesis factors derived from activated adhesion molecules on the surface of endothelial cells, such as soluble E-selectin and soluble VCAM, are released in RA synovium and contribute to vascular proliferation.[236] Limited quantities of some anti-angiogenic mediators that inhibit capillary proliferation, such as platelet factor-4 and thrombospondin, are also produced by the joint. Other pathways and factors that contribute to blood vessel formation in RA synovium include TLR5 ligation and expression of the fucosyl transferase Fut1.[237]

Vascular remodeling is an active process that involves the continuous creation and resorption of blood vessels. In RA, new capillaries that form under the influence of pro-angiogenic factors can be identified by the expression of integrins such as $\alpha_v\beta_3$. Endothelial proliferation is especially prominent in synovial tissue regions containing VEGF. Synovial blood vessel involution also can be detected as evidenced by apoptosis of the endothelium in other synovial locations. The ratio of proliferating and involuting blood vessels is significantly higher in RA than in OA or normal synovium.

### Targeting Angiogenesis

The importance of new blood-vessel formation in inflammatory arthritis was elegantly demonstrated in the collagen-induced arthritis model. The disease was markedly attenuated in animals pretreated with an angiostatic compound similar to fumagillin, which is derived from *Aspergillus*.[238] This compound is cytotoxic to proliferating, but not resting, endothelial cells. In addition, established arthritis regressed if treatment was initiated well into the course of the disease. Hence angiogenesis is essential for the establishment and progression of inflammatory arthritis because of the need for blood vessels to either recruit leukocytes or provide nutrients and oxygen to starved tissue.

Targeting HIF-1α with a small molecule inhibitor that blocks nuclear translocation and VEGF induction was effective in adjuvant arthritis.[239] Other small molecule inhibitors of the VEGF-R1 with either antibodies or kinase inhibitors have been successfully tested in pre-clinical models of arthritis as well. One of these, vatalanib, decreased inflammatory knee arthritis in rabbits.

Several other anti-angiogenesis approaches are effective in animal models of arthritis. For instance, gene therapy with NK4, which is a hepatocyte growth factor antagonist, inhibits angiogenesis and inflammatory arthritis in murine models.[240] Thrombospondin 1 overexpression significantly decreased blood vessel density, inflammation, and joint destruction in rat collagen-induced arthritis. Direct intra-articular administration of a cyclic RGD peptide was used in a rabbit model to block $\alpha_v\beta_3$ integrin.[241] The cyclic peptide decreased joint inflammation, increased endothelial cell apoptosis, and suppressed bone and cartilage destruction.

The ability of RGD to bind selectively to proliferating blood vessels was also used to direct a proapoptotic agent to synovial neovasculature in murine collagen-induced arthritis.[242] The cyclic RGD peptide was administered systemically and accumulated in inflamed synovium but not in normal joints or other organs. Apoptosis was induced in synovial blood vessels, and arthritis regressed. The potent angiogenesis inhibitor endostatin has been tested in the SCID mouse model, and it decreased synovial explant inflammatory cell infiltration and capillary density. Despite the compelling rationale for anti-angiogenic therapy, an anti-$\alpha_v$ antibody showed minimal efficacy in a clinical trial, perhaps because other pathways are more important in the synovium.

## Adhesion Molecule Regulation

Endothelial cells activated by cytokines and other mediators express adhesion molecules that bind to counter-receptors on mononuclear cells and neutrophils from the circulation and facilitate their recruitment from the blood (see Chapter 25). Several categories of vascular adhesion molecules exist. The selectins (E-, L-, and P-selectin) are a family of adhesion molecules whose primary ligands are

carbohydrates, especially sialyl LewisX, and related oligosaccharides. The selectins are expressed by RA synovial endothelial cells, which can initiate the influx of blood cells into the joint. A second family is integrins, which are heterodimers that include an α and a β chain. The counter-receptors depend on the specific combination of these chains and are frequently proteins in the immunoglobulin supergene family or extra-cellular matrix proteins.

### Integrins and Their Ligands

As one might expect, adhesion molecule expression is increased in the RA synovium as a result of the rich cytokine milieu. Immunohistochemical techniques localize high levels of ICAM-1 to sublining macrophages, macrophage-like synovial lining cells, and fibroblasts compared with normal tissue.[243] Significant amounts are also present on the majority of vascular endothelial cells. Cultured FLS also constitutively express ICAM-1, which can be markedly increased by TNF, IL-1, and IFN-γ. ICAM-1 and the other ICAM family members can bind to cells expressing the β2 integrins, especially neutrophils.

Adhesion of $\alpha_4\beta_1$(VLA-4)–expressing mononuclear cells such as memory T cells or monocytes to cytokine-activated endothelial cells can be mediated by VCAM-1 or CS-1 fibronectin. A role for VLA-4 in arthritis has been suggested by a number of experimental observations. In adjuvant arthritis in rats, anti-$\alpha_4$ antibody decreases lymphocyte accumulation in the joint but not in lymph nodes, suggesting that VLA-4 is more important in recruitment to inflamed sites than to non-inflamed sites.[244] T lymphocytes isolated from the synovial fluid and synovial membrane of patients with RA exhibit increased VLA-4–mediated adherence to VCAM-1, relative to autologous peripheral blood lymphocytes. These studies also suggest that leukocytes expressing functionally activated VLA-4 are selectively recruited to inflammatory sites in RA. Anti–VLA-4 antibody, which is effective in multiple sclerosis, has potential utility in RA, but enthusiasm is mitigated by effects on host defense observed with natalizumab.

Moderate amounts of VCAM-1 are expressed in RA synovial blood vessels. Surprisingly, the intimal lining is the location of the most intense staining with anti–VCAM-1 antibodies on histologic sections. Even normal synovial tissue expresses VCAM-1 in the lining, albeit less than in RA. Cultured FLS constitutively express small amounts of VCAM-1, and the level is increased by a variety of macrophage and T cell–derived cytokines. VCAM-1 also contributes to T cell adhesion to high endothelial venules in frozen sections of RA synovium.[245]

The integrin $\alpha_4\beta_7$, which also binds to VCAM-1, is a specific adhesion molecule involved in lymphocyte homing to Peyer's patches. Most intraepithelial and lamina propria lymphocytes express $\alpha_4\beta_7$; this molecule is rarely identified in other lymphoid tissues. The expression of $\alpha_4\beta_7$ on peripheral blood lymphocytes from patients with RA is low (similar to normal persons), but up to a quarter of synovial fluid lymphocytes, mostly $CD8^+$ T lymphocytes, express this adhesion molecule and provide an interesting link between arthritis and the gut.[246] $\alpha_2\beta_1$, which binds to native collagen, has also been implicated in Th17 cell differentiation in mice.[247]

### Therapeutic Potential of Blocking Adhesion Molecules

The therapeutic potential for anti-adhesion therapy has been studied in the SCID mouse model. Labeled human peripheral mononuclear cells were injected into engrafted mice, and migration into the tissue was examined.[248] When the mice were treated with TNF, ICAM-1 expression and trafficking into synovium were significantly increased. Anti–ICAM-1 antibody also blocked leukocyte migration into the explant under these conditions. In another study, tonsil mononuclear cells also migrated into the RA synovial grafts in SCID mice. RA clinical trials using anti–ICAM-1 antibody or anti-sense ICAM-1 oligonucleotides produced minimal significant clinical benefit. Surprisingly, mice lacking E- and P-selectin actually had accelerated disease in the collagen-induced arthritis model. This paradoxical result serves as a reminder of the complexity of the inflammatory process.[249]

## CARTILAGE AND BONE DESTRUCTION

**KEY POINTS**

- Cartilage degradation and bone destruction in RA are mediated by distinct mechanisms and cell types.
- Several classes of proteases including metalloproteinases, serine proteases, cathepsins, and aggrecanases are produced by intimal lining cells in RA, especially FLS.
- Synovial lining cells, especially FLS, invade and damage cartilage in RA.
- Bone destruction is mediated by osteoclasts that are activated under the influence of RANKL and other cytokines produced by RA synovium.

### Cartilage Destruction and the Pannus-Cartilage Junction

Macrophage-like and fibroblast-like cells migrate onto the surface of cartilage, and the FLS in particular penetrate into cartilage matrix (Figure 69-17). This invasive "pannus" is more commonly found in metatarsophalangeal joints, compared with hip and knee joints, perhaps explaining why erosions occur more often around small joints.

FLS from the intimal lining are major effectors of cartilage destruction in RA. They produce a prodigious amount of proteases, bind to cartilage, and invade the extra-cellular matrix. The pivotal role of synoviocytes in cartilage destruction was demonstrated in arthritis models using cadherin-11 blockade to disrupt the intimal lining. Cartilage destruction is markedly attenuated, even though bone erosions progress.[250]

Other cells in the joint, including macrophages and synovial fluid neutrophils, could also be responsible for cartilage damage, whereas osteoclasts are responsible for bone erosions. More primitive mesenchymal cells isolated directly from the cartilage-pannus junction express phenotypic and functional features of both synoviocytes and chondrocytes and have also been described in the synovium.

Cartilage is destroyed in RA by both enzymatic and mechanical processes. The enzymes induced by factors such

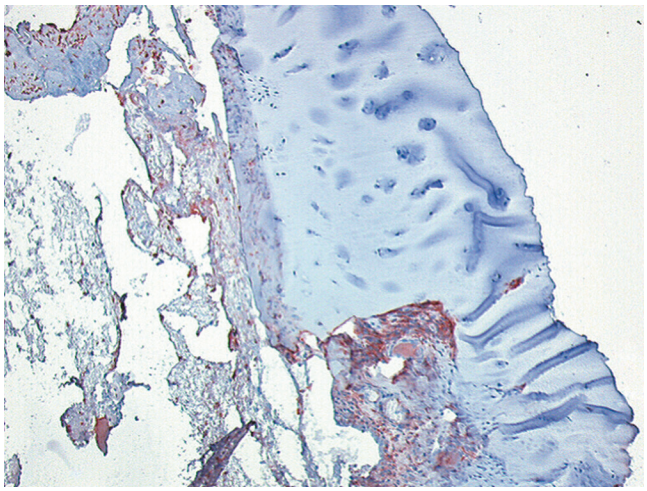


**Figure 69-17** Pannus-cartilage junction. The invasive front of pannus burrows into cartilage matrix in rheumatoid arthritis joints. The pannus is primarily composed of macrophages and mesenchymal cells. Immunostaining with anti-CD68 antibody shows the distribution of macrophages in the invasive tissue. *(Courtesy Dr. Paul-Peter Tak.)*

**TABLE 69-6** Key Proteases and Inhibitors in Rheumatoid Arthritis Synovium

| Protease | Inhibitor |
|---|---|
| Metalloproteinases<br>Collagenase-1<br>Collagenase-3<br>Stromelysin-1<br>92 kDa gelatinase | TIMP family; α2 macroglobulin |
| Serine proteases<br>Trypsin<br>Chymotrypsin<br>Tryptase | SERPINs; α2 macroglobulin |
| Cathepsins<br>Cathepsin B<br>Cathepsin L<br>Cathepsin K | α2 macroglobulin |
| Aggrecanases | Fibronectin |

SERPINs, Serine protease inhibitors; TIMP, tissue inhibitor of metalloproteinases.

as IL-1, IL-17, TNF, phagocytosis of debris by synovial cells, and mechanical trauma degrade the matrix proteins. Early in synovitis, proteoglycans are depleted from the tissue, most likely as a result of the catabolic effect of cytokines such as IL-1 on chondrocytes with the production of MMPs and aggrecanases, and this depletion leads to mechanical weakening of cartilage. As proteoglycans are depleted, cartilage loses elasticity and becomes susceptible to mechanical fragmentation and fibrillation. Eventually the tissue loses functional integrity concurrent with its dissolution by collagenases and stromelysins. Some of the MMPs responsible for this process are also derived from the chondrocytes themselves. Multiple MMPs, especially stromelysin (MMP-3) and collagenases (MMP-1 and MMP-13), are expressed in RA cartilage, and in situ hybridization studies confirm the presence of the mRNA within chondrocytes.[251] Hence the cartilage is under attack from a multitude of sources: It is bathed in protease-rich synovial fluid, under extrinsic attack from the invasive pannus, damaged from within by chondrocytes, and fragmented by mechanical forces.

Enzymes released by PMNs in synovial fluid, including neutrophil collagenase and multiple serine proteases, also contribute to cartilage loss. Immune complexes containing RFs are embedded in the superficial layers of cartilage and can attract and activate neutrophils. Electron microscopic examinations of articular cartilage in RA reveal evidence of breakdown of collagen and proteoglycan as a result of superficial activity of joint fluid enzymes. In a rabbit model of arthritis in which IL-1 was injected directly into the joint, the degree of cartilage damage as measured by proteoglycan levels in synovial fluid correlated best with the stromelysin concentrations in synovial effusions (presumably derived from synoviocytes). Neutrophil depletion of animals did not interfere with subsequent destruction of extra-cellular matrix, suggesting that MMPs derived from the synovium are more important in this model.

Most animal studies suggest that IL-1 is a key regulator of matrix degradation in arthritis. Although TNF blockade has clear anti-inflammatory effects, chondroprotection is less prominent. The joint destruction observed in TNF-dependent models often requires IL-1. IL-17 and TLR ligands can also contribute to joint destruction directly or by synergizing with IL-1 and TNF.

The rate-limiting step in cartilage loss is the cleavage of collagen because proteoglycans are degraded soon after inflammation begins. MMPs, which are released into the extra-cellular space and are active at neutral pH, are probably responsible for most of the effective proteolysis of articular-cartilage proteins, but other classes of enzymes may contribute to joint destruction. Enzymes such as cathepsins B, D, G, K, L, and H may play a role inside and outside cells by degrading noncollagenous matrix proteins. Serine proteinases (e.g., elastase and plasmin) and aggrecanases can also contribute to matrix damage.

## Proteases: Mediators of Joint Destruction

### Matrix Metalloproteinases

The MMPs are a family of enzymes that participate in extra-cellular matrix degradation and remodeling (see Chapter 8). They are usually secreted as inactive proenzymes, and their proteolytic activity requires limited cleavage or denaturation to reveal a zinc cation at the core. Their activation can be mediated by other proteases, including trypsin, plasmin, or tryptase. The substrates for MMPs are varied but quite specific for individual members of the family. Collagenases degrade native collagen, whereas gelatinases are able to degrade denatured or cleaved collagen. Stromelysins have broader specificity and can digest proteoglycans in addition to proteins. They also process procollagenase to the active form, thereby serving as a positive-feedback signal for matrix destruction. Some MMPs such as TNF convertase (TACE) are responsible for the processing and release of cytokines from the cell surface. Many different families of proteinases are found in the joint (Table 69-6), but the MMPs are thought to play a pivotal role in joint destruction.

**Regulation of MMP Production.** The cytokine milieu has the capacity to induce the biosynthesis of MMPs by synovial cells and alter the balance between extra-cellular matrix production and degradation. IL-1 and TNF, in particular, induce MMP gene expression by many cells, especially FLS and chondrocytes. The two cytokines are additive or synergistic when used in combination. Many other cytokines and TLR ligands implicated in rheumatoid synovitis can also induce MMP expression, including IL-17, LIF, LPS, and peptidoglycans.

Culture medium from rheumatoid synovium stimulates cartilage degradation in vitro, mainly because IL-1 stimulates MMP production by synovial intimal lining cells. IL-6 does not induce MMP production by synovial cells but instead increases the production of TIMP-1, a naturally occurring inhibitor of MMPs. TGF-β inhibits collagenase synthesis and enhances the production of TIMP by fibroblasts and chondrocytes. TGF-β also increases collagen production, shifting the balance from destruction to matrix repair. Thus regulation of matrix degradation is a balance between matrix production, protease production and activation, and endogenous inhibitors that inactivate the proteases.

Although multiple upstream regulatory sequences are involved in MMP gene transcription, the dominant element in the promoter is AP-1. Other regulatory sites such as an NF-κB–like region can also contribute to collagenase expression. AP-1 activity is markedly increased in FLS by pro-inflammatory cytokines such as IL-1, TNF, and IL-17, and its transcriptional activity is mediated by increased expression of components such as c-Jun. The MAP kinases are especially important for this activity, and JNK is the most efficient upstream activator. Glucocorticoids markedly inhibit MMP gene expression by blocking AP-1. Engagement of cadherin 11 on the surface of FLS can also increase MMP expression, with associated increases in MAP kinase and NF-κB activation.[252]

Collagenases and stromelysins have the capacity to degrade virtually all the important structural proteins in the extra-cellular tissues within joints. Collagenase-1 (MMP-1) cleaves through the triple-helical collagen molecule at a single glycine-isoleucine bond approximately three-quarters of the distance from the $NH_2$-terminus. This enzyme degrades only the interstitial helical collagens (e.g., types I, II, III, and X). It has little or no activity against types IV, V, and IX and other nonhelical collagens or denatured collagen; the degradation of the latter is primarily accomplished by the gelatinases. MMP-1, however, is a relatively inefficient enzyme, whereas collagenase-3 (MMP-13) has more favorable kinetics. Neutrophil collagenase, or MMP-8, is constitutively stored in neutrophil granules and is released into the milieu after degranulation.

**MMP Expression in Synovium.** The collagenase-1 and collagenase-3 genes are produced by RA synovial tissue, and the latter is highly expressed by chondrocytes in cartilage. In situ hybridization studies show that the primary location of collagenase-1 gene expression in the synovium, like many other MMPs, is the intimal lining, especially in FLS.[253] Subchondral bone is another region in which proteinase expression occurs in RA and could participate in bone resorption. Increased MMP gene expression is an early feature of RA and occurs during the first few weeks of clinically active disease. High expression of collagenase-1, as well as gelatinases such as MMP-2, early in the disease process correlates with rapidly progressive erosions. Similarly, increased blood levels of the proenzymes are also associated with more severe disease.

Stromelysin-1 (MMP-3) and the other members of the stromelysin family effectively degrade type IV collagen, fibronectin, laminin, proteoglycan core protein, and type IX collagen. Stromelysin removes the $NH_2$-terminal propeptides from type I procollagen and can activate procollagenase, creating a positive feedback loop that amplifies damage. Like collagenase, stromelysin gene expression occurs mainly in the synovial intimal lining (Figure 69-18). Despite the putative importance of this enzyme in matrix destruction, stromelysin knockout mice are susceptible to collagen-induced arthritis and develop as much joint destruction as mice with functional stromelysin.[254] This observation substantially decreased interest in stromelysin inhibitors to treat diseases such as RA.

MMP inhibitors are effective in animal models of RA and can suppress bone destruction, as well as the inflammatory synovitis. In models of OA, deletion of MMP genes such as stromelysin does not necessarily improve outcomes. Clinical trials in RA using nonselective inhibitors have had minimal success and significant adverse effects, possibly related to decreased matrix turnover. TACE inhibitors can unexpectedly increase disease severity in RA, perhaps as a result of higher levels of membrane-bound TNF. One of the most consistent adverse effects experienced by patients treated with MMP inhibitors is increased joint stiffness, which is thought to result from deposition of fibrous tissue without sufficient protease activity to permit removal of matrix proteins.

### Cysteine Proteases: The Cathepsins

Cathepsins are an extensive family of cysteine proteases that have broad proteolytic activity, including activity on types II, IX, and XI collagen and proteoglycans. Like MMPs, the cathepsins are regulated by cytokines and by proto-oncogenes such as Ras. IL-1 and TNF induce cathepsin L expression in cultured FLS.[255] In situ hybridization studies demonstrate cathepsin B and L RNA transcripts in RA synovium, especially at sites of erosion. A ribozyme that cleaves cathepsin L decreases FLS invasion and cartilage destruction in the SCID mouse model with implanted cultured synoviocytes, which suggests that this protease has clinical relevance.

Cathepsin K has been implicated in bone resorption by osteoclasts. This protease is unique among the cathepsins because it can degrade native type I collagen. It is expressed in RA synovial tissue by both macrophages and fibroblasts and is present in significantly higher concentrations than in OA.[256] Serum levels of cathepsin K correlate with the extent of radiographic damage. A potential role of cathepsins as mediators of bone destruction in arthritis was confirmed in studies in which cysteine protease inhibitors significantly decrease joint damage in the rodent arthritis models. In the TNF-transgenic mouse model, cathepsin K deficiency decreased, but did not eliminate, bone erosions.[257]

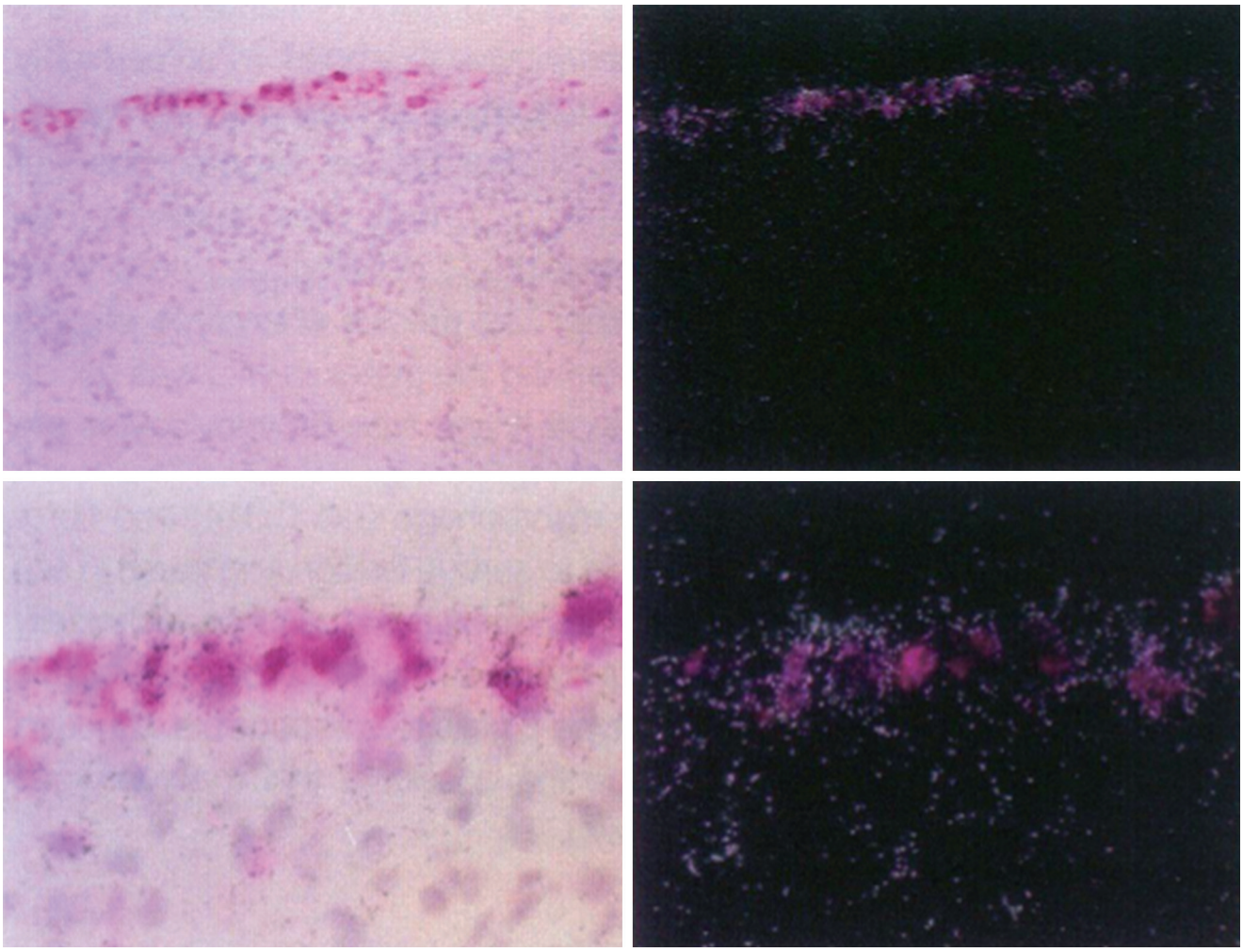


**Figure 69-18** Localization of stromelysin and tissue inhibitor of metalloproteinases-1 (TIMP-1) in rheumatoid arthritis (RA) synovial tissue by in situ hybridization. Stromelysin mRNA *(pink color)* is mainly expressed in the synovial intimal lining, presumably by cytokine-stimulated type B synoviocytes. TIMP mRNA is shown by silver granules in the same region, best viewed in the dark-field view where the silver grains appear as bright granules *(right panels)*. High and low power views of the same field are shown. *(From Paine MM, Firestein GS: Stromelysin and tissue inhibitor of metalloproteinases gene expression in rheumatoid arthritis synovium.* Am J Pathol *140:1309–1314, 1992.)*

### Aggrecanases

Aggrecan is a major proteoglycan component of articular cartilage. Because of its large size and negative charge, it contains a considerable amount of water, which increases compressibility. Two proteolytic sites are available on aggrecan in its globular domain. One site is susceptible to MMP cleavage, whereas the other, located 32 amino acids toward the C-terminus, is the site for cleavage by a family of enzymes known as aggrecanases. The two sites can be identified in tissues using monoclonal antibodies after cleavage when specific neoepitopes are revealed.

Normal cartilage contains a surprising amount of aggrecanase neoepitope, suggesting continuous matrix turnover. The level of aggrecanase cleavage product increases with age. Two aggrecanase genes, aggrecanase-1 and aggrecanase-2, have been cloned and are members of the "a disintegrin and metalloproteinase with thrombospondin motif" (ADAMTS) family of proteins (ADAMTS-4 and ADAMTS-5, respectively). They are expressed in OA and RA cartilage, and their proteolytic activity can be detected in synovial fluids. Especially high levels of the neoepitope are present in arthritic cartilage.[258] IL-1 increases aggrecanase expression in cartilage explants, as well as cultured chondrocytes. Aggrecanase-1 and aggrecanase-2 are constitutively expressed by RA and OA FLS and synovial tissues.[259] Aggrecanase-1, but not aggrecanse-2, is induced in synoviocytes by cytokines, especially TGF-β. Genetic deletion of aggrecanse-1 has no effect on a murine OA model, but loss of aggrecanase-2 prevents degenerative changes.[260]

## Endogenous Protease Inhibitors

$\alpha_2$-Macroglobulin ($\alpha_2$M) accounts for more than 95% of collagenase inhibitory capacity in serum. The mechanism of inhibition by $\alpha_2$M involves hydrolysis by the proteinase of a susceptible region in one of the four polypeptide chains of $\alpha_2$M (sometimes called the "bait"), with subsequent trapping of the proteins within the interstices of the $\alpha_2$M. Ultimately, the protease is covalently linked to a portion of the $\alpha_2$M molecule. The serine protease inhibitors (SERPINs) are also abundant in synovial effusions and

plasma and can serve a dual purpose of directly blocking serine protease function and indirectly decreasing MMP activity by preventing serine proteases from activating MMP proenzymes. One SERPIN, $\alpha_1$–anti-trypsin, has been well characterized in synovial fluid and is frequently inactivated after oxidation by reactive oxygen species. Fibronectin also inhibits aggrecanase-1 (ADAMTS-4).[261] However, citrullinated fibronectin is much less efficient and increases aggrecanase activity, representing another potential pathogenic effect of protein citrullination in RA.

A family of proteins that specifically block MMP activity, called TIMPs, is probably the most important physiologic inhibitor in the inflamed joint. The TIMP proteins block proteinase activity by binding directly to MMPs in a 1:1 molar ratio. TIMP generally binds only to the active enzyme, although exceptions such as TIMP-2 can interact with a progelatinase (MMP-2). The inhibitors bind to MMPs with extremely high avidity. Even though the interaction does not result in new covalent bonds, it is essentially irreversible.

TIMP proteins are present in RA synovial fluid in excess. It is, in fact, difficult to detect free active collagenase and stromelysin because they are quickly bound by the inhibitors after they are activated by limited proteolysis. Immunohistochemical and in situ hybridization studies have localized the TIMPs in hyperplastic synovial lining cells in rheumatoid synovium, but not in the cells of normal synovium. TIMP gene expression is not significantly altered by IL-1 or TNF but is increased by IL-6, oncostatin M, and TGF-β. Not all effects of TIMPs are beneficial in inflammation. For instance, TIMP-3 knockout mice have significantly more synovitis and TNF production in antigen-induced arthritis, perhaps because it is not available to inhibit TACE. Similarly, TIMP-1 or TIMP-3 gene transfer limits rheumatoid FLS invasion into cartilage in an SCID mouse model. The function of these genes can extend beyond protease inhibition and include a number of paracrine functions, as well as induction of apoptosis when expressed intra-cellularly in cultured synoviocytes.

Given the important role of MMPs in tissue destruction, the relative balance between MMPs and TIMPs ultimately determines the fate of the extra-cellular matrix. The ratio in RA, with its more destructive potential, favors degradation, whereas OA has a lower MMP-to-TIMP ratio. The levels of TIMP gene expression are similar in the two diseases and may well be maximal. The higher ratio in RA results from increased MMP production. The balance between protease and inhibitor can potentially be modified in vivo with drug therapy. For instance, methotrexate therapy decreases collagenase but not TIMP-1 mRNA, which could be one mechanism of decreased joint damage in patients treated with this agent.[262]

## Regulation of Bone Destruction

Marginal erosions are the most characteristic location for bone damage in RA, especially in metacarpal phalangeal joints and proximal interphalageal joints, which traditionally has been ascribed to a "bare area" where cartilage does not protect the underlying bone from invasive pannus.[263] However, anatomic pathology and computed tomography studies suggest that erosions actually occur near collateral ligaments in these small joints where microdamage was observed in cadaveric studies. It is possible that local factors related to stress are more important than the anatomic location of overlying cartilage.[263a]

Osteoclasts are the major cells responsible for bone degradation. RANKL, which was originally described for its role in T cell–DC interactions, as well as in lymphocyte and lymph node development, is perhaps the single most important factor that modulates bone resorption. Osteoclast development is complex and involves the differentiation of monocytes under the influence of cytokines such as M-CSF in combination with RANKL. Subsequent osteoclast activation can involve several pathways, most of which also depend on the presence of RANKL. Its receptor, known as RANK, is expressed by the osteoclast precursors. RANKL is produced by many cell types, including activated T cells and FLS.

Abundant evidence implicates this powerful mechanism in bone destruction caused by inflammatory arthritis. For instance, administration of OPG, a RANKL decoy receptor, to rats with adjuvant arthritis inhibits bone destruction but has almost no effect on inflammation or clinical signs of arthritis.[264] $RANKL^{-/-}$ mice are also protected from bone erosions in the passive K/BxN model of arthritis, although cartilage destruction still occurs. Animal models of arthritis point to IL-17 as a mediator of osteoclast generation. Genetic deficiency of IL-17 or anti–IL-17 antibodies have remarkable bone-sparing effects in these experiments.

RANK, RANKL, and OPG (as well as M-CSF and IL-17) have been detected in the synovium and synovial fluid of patients with RA. The ratio of RANKL to OPG is significantly higher in RA synovial effusions than in either OA or gout, which is consistent with the more destructive nature of RA. Osteoclasts expressing tartrate-resistant acid phosphatase (TRAP), which is capable of forming resorption lacunae, can be generated from cultured RA synovial cells (Figure 69-19). This activity is blocked by the addition of exogenous OPG. RA synoviocytes and synovial

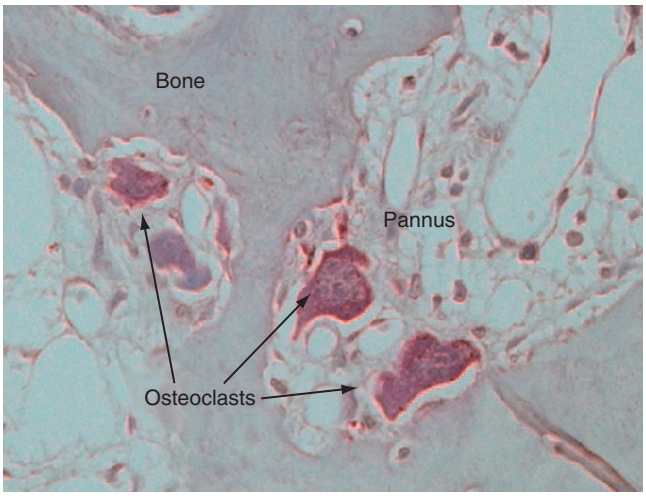



**Figure 69-19** Tartrate resistant acid phosphatase–positive osteoclasts are shown invading bone in rheumatoid arthritis (see *arrows* for examples). This process is regulated by receptor activator of NF-κB ligand (RANKL) in the presence of other cytokines such as macrophage colony-stimulating factor and TNF. *(Courtesy Dr. Steven Goldring, Dr. Ellen Gravallese, and Dr. Allison Pettit.)*

membrane T cells that display RANKL can also induce differentiation of osteoclasts from peripheral blood cells.[265]

The functional relevance of the RANK-RANKL system was confirmed in RA studies in which the anti-RANKL antibody denosumab decreased bone erosions. As predicted from animal model studies, the antibody had no effect on inflammation or clinical signs of synovitis.[266]

A second system that regulates bone remodeling involves the Wnt proteins, which regulate the balance between bone damage caused by osteoclasts and bone formation by osteoblasts. Several members of this family bind to receptors that regulate osteoblast differentiation through effects on β-catenin. Wnt signaling is modulated by many other proteins, most notably the Dickkopf (DKK) family. DKK-1 in particular blocks binding of Wnts to its receptors. In TNF-transgenic mice with arthritis, bone erosions are blocked by inhibiting either TNF or DKK-1. With the latter, proliferative bone lesions such as osteophytes formed instead.[267] Therefore, DKK-1 is a master switch that determines the fate of bone in inflammatory lesions. When present, bone destruction is favored; when absent, bone formation occurs. Other factors can also stimulate osteoclastogenesis, such as IL-21, which increases bone damage in collagen-induced arthritis.[268]

### Tissue Repair

Extra-cellular matrix turnover in RA has been likened to wound healing as a result of the critical role of collagen production, proteases, and protease inhibitors. Remodeling the matrix by removing damaged proteins is a key element in early repair. Subsequently, the balance shifts to protease inhibition, production of cytokine inhibitors, removal of inflammatory cells through apoptosis, and release of anti-inflammatory eicosanoids such as lipoxins to suppress inflammation. Neutralization of oxidants via glutathione reductase or superoxide dismutase further limits tissue damage.

This process then permits either a return to normal architecture or scar formation. TGF-β, in particular, appears to play a key role in that it increases collagen deposition, suppresses MMP expression, and enhances production of the TIMPs. Although TGF-β levels in the rheumatoid joint are substantial, they are mainly in the inactive latent form and are not sufficient to overcome the impressive array of pro-inflammatory cytokines that induce protease expression. Persistent antigen-driven T cell activation, continued antibody-driven complement fixation, or activation of other cell lineages like macrophages or FLS overwhelm the negative feedback loops that attempt to restore homeostasis. Strategies to shift from tissue damage by enhancing endogenous mechanisms could suppress symptoms and enhance appropriate remodeling of the matrix to restore homeostasis.

Because the invasive rheumatoid synovium exhibits very aggressive behavior, the possibility that the tissue contains immature cells or embryonic genes that regulate repair has been explored. The embryonic growth factors from the Wnt and Fz gene families have been demonstrated in RA synovium. Normally, these proteins participate in bone marrow progenitor differentiation and limb bud mesenchyme. Wnt5a and Fz5, in particular, are markedly elevated in RA tissues and cultured synoviocytes. When normal fibroblasts are transfected with the *WNT5A* gene, cytokine expression such as IL-6 increases significantly. Anti-sense *WNT5A* and dominant negative *WNT5A* vectors diminish cytokine expression by synoviocytes.[269]

These data raise the possibility that immature mesenchymal cells populate the synovium in RA, either as a primary event or as a repair mechanism. Similar primitive mesenchymal cells circulate in the peripheral blood of RA and normal persons, and in collagen-induced arthritis they infiltrate the synovium before synovial inflammation becomes clinically apparent.

Tissue repair in RA is a complex process that also involves the ingress or de-differentiation of mesenchymal cells that can remodel the matrix. These cells are modulated by TGF-β and the bone morphogenic proteins (BMPs). The BMPs are members of the TGF-β superfamily and, like TGF-β, they signal through the Smad pathway. Several members, including BMP-2 and BMP-7, are expressed in the joint and facilitate repair, although inappropriate release can also enhance joint damage or lead to ankylosis or enthesophyte formation.[270] BMP function is also regulated by a family of inhibitors such as Noggin, which can limit cartilage damage when it is overexpressed in murine antigen-induced arthritis. Modulating the relative balance and timing of BMP expression ultimately could be used to modify the destructive influence of synovitis or regenerate damaged tissues.

## CONCLUSION

The causes and pathogenesis of RA are complex and involve the interplay between environment and genes. Many years before clinical disease is apparent, evidence of immune dysregulation is observed, including antibody responses to modified peptides and increased production of cytokines and chemokines. Ultimately, a point is reached when antibodies activate complement in the joint and attract other immune cells from the blood. The local synovial milieu is rich with cytokines, inflammatory cells, and proteases that can drive further adaptive immune responses and joint damage. Understanding the diverse pathogenic events involved in RA helps us understand the diversity of responses to targeted agents that block TNF and IL-6, interfere with T cell co-stimulation, deplete B cells, or inhibit cytokine signal transduction. As we gain additional information about the critical events prior to clinical disease, that is, "pre-RA," approaches that prevent rather than treat RA might be feasible.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 70

# Clinical Features of Rheumatoid Arthritis



Alan R. Erickson • Amy C. Cannella • Ted R. Mikuls

**KEY POINTS**

- **Rheumatoid arthritis (RA) is a systemic inflammatory condition that typically appears as a polyarticular small joint arthritis of the hands and feet as well as extra-articular manifestations in some patients.**
- **Rheumatoid factor (RF) and anti-citrullinated protein antibody positivity is associated with pre-clinical RA, erosive and nodular articular disease, and systemic manifestations that include extra-articular involvement and reduced patient survival.**
- **Evidence suggests that pre-clinical and early RA might be triggered in extra-articular mucosal sites (oral and intestinal) in a genetically susceptible patient.**
- **Patients with RA usually are seen with a polyarticular inflammatory arthritis involving the small joints of the hands and feet, morning stiffness lasting longer than 1 hour, and laboratory findings indicative of systemic inflammation along with RF and anti-citrullinated protein antibody positivity.**
- **RA has a typical and several less common presentations, although all can develop typical radiographic findings, including periarticular osteopenia and marginal erosions.**

## EPIDEMIOLOGY AND DISEASE BURDEN

Rheumatoid arthritis (RA) is a systemic autoimmune condition with protean manifestations. The primary expression of disease occurs in the synovial tissues and is characterized by symmetric polyarticular inflammation, which can lead to progressive joint damage. As a result, RA is associated with substantial functional disability, morbidity, and accelerated mortality, which pose an enormous and growing societal burden.

### Disease Frequency

The prevalence of RA is estimated at 1% in most developed countries, with a frequency ranging from less than or equal to 0.1% to 1.9% in surveys from different parts of the world.[1] The highest rates of RA incidence are observed among individuals of Native American ancestry, with a reported disease prevalence approaching 5% to 7% in Pima and Chippewa Indians in the United States, respectively.[2,3] A much lower prevalence has been reported from select African regions and is thought to be the result of a lower population frequency of the human leukocyte antigen *(HLA)-DRB1* shared epitope-containing allele,[4] which is the single strongest known genetic risk factor for RA. A 40-year population-based study from Rochester, Minnesota (1955-1994) demonstrated an overall age- and sex-adjusted annual incidence of 44.6 cases per 100,000 person-years.[5] In this study, incidence rates were approximately twofold higher in women than men, with peaks in disease onset observed at younger ages in women (55 to 64 years) compared with men (75 to 84 years). Notably, these authors reported marked temporal trends, including a 47% decline in overall RA incidence during the study period. A follow-up investigation from the same study population, extending through 2007, suggested a more recent upsurge in disease incidence among women. These results underscore the potential impact of environmental exposures in mediating risk, and the potential relevance of birth cohort effects.[6] Based on this population, the lifetime risk for RA among U.S. adults has been estimated at 3.6% for women and 1.7% for men.[7]

### Disease Outcomes

Because of its systemic and polyarticular nature, RA can negatively impact physical functioning and health-related quality of life. Compared with individuals without arthritis, patients with RA in one study were 36% more likely to report fair or poor general health, twice as likely to report a health-related activity limitation, and almost 30% more likely to require assistance with personal care.[8] A second study of patients with RA with disease onset less than 1 year from enrollment found that 39% were unable to work after just 10 years of follow-up.[9] Rates of disability were highest among older patients, those with lower education, manual laborers, and those reporting more baseline physical impairment. Correspondingly, RA-related expenditures in the United States are staggering. In 2013 alone, health care costs were estimated to have exceeded $10 billion, and societal costs approached $45 billion.[10,11] The impact of RA on patient outcomes and health care spending is further amplified by its robust associations with comorbid conditions, including substantially higher rates of cardiovascular disease, serious infection, lymphoproliferative malignancy, and osteoporosis leading to fracture.[12]

In addition to detrimental effects on physical functioning and quality of life, RA portends an increased risk of mortality. In a pooled analysis of 18 cohort investigations conducted before 2001, Ward reported a mean standardized mortality ratio (SMR) of 1.70 across studies, with numerically lower mortality burdens seen in more contemporary publications and inception cohorts.[13] Factors associated

with reduced survival in RA have been identified and have included older age, poor functional status, comorbid conditions, extra-articular disease, autoantibody status (rheumatoid factor [RF] and anti-citrullinated protein antibody [ACPA]), disease activity, and medication use.[14-16] Although newer, highly effective biologic therapies have been available for more than a decade, the mortality gap in RA does not appear to have receded in recent years and may even be widening.[17] The excess mortality burden in RA appears to be largely driven by cardiovascular disease, a risk that may be partially mitigated by effective disease-modifying therapy, including methotrexate.[18] Additionally, with the recent emphasis on tighter treatment using effective disease measurement tools, it is expected we will realize a decrease in cardiovascular deaths with time.

## PRE-CLINICAL RHEUMATOID ARTHRITIS

Substantial recent gains have been made in our understanding of the risk factors and mechanisms involved in early disease development (see Chapter 69). It is now well established that circulating RF and ACPA (most often measured by using commercially available anticyclic citrullinated protein [anti-CCP] antibody assays) can be detected in approximately one-half of patients, often many years before RA symptom onset. The period of autoimmunity preceding the initial articular signs and symptoms of disease has been termed *pre-clinical* RA. In addition to detectable levels of serum autoantibody, pre-clinical RA is often characterized by the presence of subclinical inflammation that is reflected by increased serum levels of C-reactive protein (CRP) and other pro-inflammatory cytokines and chemokines. Several potentially relevant alterations in these serum biomarkers have been demonstrated that may herald imminent disease onset. Autoantibody changes include increased circulating concentrations and binding avidity, aberrant glycosylation (promoting complement activation and Fc receptor binding), and epitope spreading.

With the use of a multiplex antigen array and pre-clinical samples from the U.S. Department of Defense Serum Repository, investigators have demonstrated a time-dependent expansion of ACPA specificity, with an increasing number of positive ACPA subtypes, showing progression through the pre-clinical period and peaking at the time of RA symptom onset.[19] In this study, early ACPA expansion predicted subsequent increases in serum concentrations of inflammatory cytokines, including TNF, IL-6, IL-12p70, and interferon (IFN)-γ. Among patients in whom seropositive RA develops, the detection of these pre-clinical inflammatory cytokines and chemokines appears to predict a shorter time to disease onset. Furthermore, the concentration of these cytokines predicts disease onset in an age differential manner, and is longer in duration for older individuals (≥40 years) than younger individuals (<40 years).[20]

Mounting evidence suggests that early inflammatory events leading to RA may occur at extra-articular locations, with mucosal sites predominant among these.[21] Evidence supporting mucosal tissues as possible sites of initiation includes (1) the absence of demonstrable synovitis, either histologically or through the use of advanced imaging, in the vast majority of seropositive individuals during the pre-clinical period[22,23]; (2) the predominant role of environmental exposures that are known to cause mucosal injury as independent RA risk factors (e.g., cigarette smoking and, possibly, periodontitis)[24,25]; (3) the robust metrics of immunoglobulin (Ig)A-ACPA in predicting future disease onset[26]; and, perhaps most importantly, (4) evidence of demonstrable mucosal inflammation during the pre-clinical period. In a study of unaffected first-degree relatives (FDRs) of RA probands, individuals with high-risk autoantibody, defined by the presence of ACPA or two or more RF isotypes, were far more likely than seronegative FDRs to have detectable pulmonary airway abnormalities by high-resolution computed tomography (HRCT) (76% vs. 33%, $P = 0.005$),[27] an association that was independent of cigarette smoking. The prevalence and type of airway abnormalities observed in these high-risk FDRs were similar to those observed in patients with early-stage RA.

Other potential mucosal sites of initiation could include the oral or intestinal mucosa,[21] although evidence demonstrating inflammation from these sites in pre-clinical RA is lacking. Periodontitis is increased in frequency, even among patients with early, treatment-naïve RA compared with controls.[28] Moreover, infection with *Porphyromonas gingivalis* (the only prokaryotic organism with the ability to citrullinate protein antigen) has been associated with expression of disease-specific autoantibody in individuals at increased risk for RA.[29,30] In addition, recent work evaluating the intestinal microbiome has shown an abundance of *Prevotella copri* in patients with new-onset untreated RA compared with controls,[31] although mechanisms underpinning this association remain poorly understood.

In many patients, arthralgias herald the end of pre-clinical disease and often precede the onset of synovitis detected by physical examination. A recent study looked for the presence of synovitis by MRI in ACPA$^+$ arthralgia patients without clinical synovitis and compared them with asymptomatic controls. ACPA$^+$ arthralgia patients had more joint inflammation by MRI, differences that were most striking in the wrist joints ($P = 0.02$) with smaller differences observed in the small joints of the hands ($P = 0.06$) and feet ($P = 0.32$).[22] A robust correlation was observed between MRI-detected articular inflammation and measures of systemic inflammation, including CRP and erythrocyte sedimentation rate (ESR). During a 9-month follow-up period, RA developed in more than half (57%) of the ACPA$^+$ arthralgia patients according to the 2010 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) classification criteria. Importantly, the magnitude of subclinical inflammation detected by MRI did not accurately predict those at risk for imminent RA; rather, the best predictors of progression included a greater number of joints involved and seropositivity for ACPA and/or RF.[22]

### Diagnosis

The characteristic patient with RA often reports pain and swelling of the small joints of the hands, wrists, and feet, with prolonged morning stiffness, often lasting more than an hour. This typical articular clinical presentation, along with laboratory and radiographic assessment, tools that are also used in classification criteria, are critical in differentiating RA from other inflammation-driven diseases. This is in

**TABLE 70-1** Factors Useful for Differentiating Early Rheumatoid Arthritis from Osteoarthritis

| | Rheumatoid Arthritis | Osteoarthritis |
|---|---|---|
| Age at onset | Across age spectrum, peak incidence in 50s | Increases with age |
| Predisposing factors | Susceptibility epitopes (HLA-DR4, HLA-DR1)<br>PTPN22, PADI4 polymorphisms, RF and ACPA positivity<br>Smoking | Trauma, overuse<br>Congenital abnormalities (e.g., shallow acetabulum) |
| Early symptoms | Morning stiffness and gelling phenomenon, pain improves with activity | Pain increases through the day and with use |
| Joints involved | Proximal interphalangeal joints, metacarpophalangeal joints, wrists most often; distal interphalangeal joints almost never | Distal interphalangeal joints (Heberden's nodes), Proximal interphalangeal joints (Bouchard's nodes), knees, lumbar spine |
| Physical findings | Soft tissue swelling, warmth | Bony osteophytes, minimal soft tissue swelling early |
| Radiologic findings | Periarticular osteopenia, marginal erosions, symmetric joint space narrowing in large joints | Subchondral sclerosis, osteophytes, asymmetric joint space loss in large joints |
| Laboratory findings | Increased C-reactive protein, positive RF, positive ACPA, anemia, thrombocytosis | Normal |

ACPA, Anti-citrullinated protein antibody; HLA, human leukocyte antigen; RF, rheumatoid factor.

comparison with osteoarthritis (OA), which is a far more prevalent "low-grade" inflammatory articular disease (Table 70-1). Early in the natural course, patients with RA may experience difficulty using their hands with routine activity, and pain in the forefeet with weight bearing or walking. Patients commonly report a "gelling" phenomenon following periods of rest, which improves with activity. Systemic symptoms such as fatigue and low-grade fever may also be present, but polyarthritis accompanied by a high fever (e.g., >38.5° C) suggests an alternative etiology. The joint examination often reveals swelling (or synovitis), tenderness, increased warmth, and limited range of motion. Other findings may include palmar erythema and subcutaneous nodules on extensor surfaces.

Adding to the complexity of early diagnosis, other rheumatic diseases included in the differential diagnosis of RA may share many of these findings. Diseases, which can be characterized by these typical joint findings, may also have a positive RF and include systemic lupus erythematosus (SLE), Sjögren's syndrome, and viral diseases, such as hepatitis B and C, and HIV (Table 70-2). Other diseases in the differential diagnosis include gout, particularly with tophi mimicking the subcutaneous nodules of RA, and calcium pyrophosphate dihydrate deposition (CPPD) arthropathy, with a pseudo-RA presentation. In elderly patients, polymyalgia rheumatica (PMR) and remitting seronegative symmetric synovitis with pitting edema (RS3PE) should be considered, whereas in younger patients, the signs and symptoms from parvovirus B19 infection or a postinfectious reactive arthritis can closely parallel those of RA. Conditions included in the differential diagnosis of RA and distinguishing characteristics are summarized in Table 70-2. The differential diagnosis will guide subsequent laboratory, serologic and radiographic assessments. In RA, the initial laboratory testing often shows the following results:

- Normal peripheral white blood cell (WBC) count and differential
- Thrombocytosis, reflecting increased systemic inflammation
- Mild normocytic anemia (anemia of chronic disease); less often microcytic
- Normal urinalysis
- Normal renal, hepatic, and other metabolic or endocrine testing
- Increased ESR (typically <50 mm/hr), and elevations CRP of similar magnitude. Both may be normal in as many as one-third of patients when first seen by a physician
- Normal serum uric acid
- RF seropositivity in approximately 70% at the time of diagnosis; an additional 15% of patients may "seroconvert" from $RF^-$ to $RF^+$ status early in the disease course
- ACPA seropositivity with a frequency approaching that observed with RF; in contrast to RF, ACPA positivity is highly specific (>95%)
- Negative anti-nuclear antibody (ANA), although as many as 30% of patients may be positive, typically in a low to moderate titer without positive subserologies
- Early in the disease process, radiographs are often normal, other than periarticular soft tissue swelling and evidence of effusions in some large joints

Arthrocentesis, if performed, typically demonstrates inflammatory synovial fluid with less than 50,000 WBC/$mm^3$ with the proportion of polymorphonuclear (PMN) cells typically less than 90%. Higher WBC and PMN counts in synovial fluid are more indicative of an infectious or crystalline etiology. Most often, synovial fluid values range between 5000 to 25,000 WBCs/$mm^3$ in RA, with 60% to 80% polymorphonuclear neutrophils (PMNs). Qualitative and nonspecific, the mucin clot test in RA classically demonstrates either no clot formation or an easily friable clot caused by a reduced concentration of synovial fluid hyaluronic acid in the context of inflammation. Both culture and crystal analyses are nearly universally negative in RA. Although lacking disease specificity, synovial fluid ragocytes (also called "rheumatoid cells" or "inclusion body cells") have been described in RA. Ragocytes are phagocytic cells with distinctive cytoplasmic granules that are thought to be composed of immune complexes. Other synovial fluid testing such as RF, ACPA, lactate, complement, and glucose are of little diagnostic utility in RA and thus are not indicated.

RA classification criteria have been developed for clinical trials and are based on components from the clinical

**TABLE 70-2** Rheumatoid Arthritis Differential Diagnosis and Discriminating Disease Features

| Disease | Distinguishing Features |
|---|---|
| **Other Connective Tissue/Idiopathic Forms of Arthritis** | |
| Adult-onset Still's disease* | Temperature >39° C for longer than 1 week, leukocytosis >10,000/mm$^3$ with >80% PMNs, evanescent rash, arthralgias, sore throat, lymphadenopathy, splenomegaly, liver dysfunction, marked elevations in serum ferritin |
| Systemic lupus erythematosus | Nonerosive arthritis with similar joint distribution and reducible deformities (Jaccoud's arthropathy), positive ANA with positive anti-dsDNA and other serologies, ACPA$^-$, associated internal organ involvement, especially the kidney |
| Spondyloarthropathy | Male predominance, often oligoarticular with predominantly large joint and lower extremity distribution, low back involvement, HLA-B27$^+$, RF/ACPA$^-$, uveitis, other associations, including psoriasis and inflammatory bowel disease |
| Vasculitis | Seronegative polyarthritis in a patient with systemic symptoms, such as fever, evidence of end-organ involvement; marked elevations in ESR or CRP; presence of anti-neutrophil cytoplasmic antibodies |
| Relapsing seronegative symmetric synovitis with pitting edema (RS3PE) | Marked by synovial thickening and joint tenderness with associated pitting edema of the hands, occurs in elderly men, very responsive to glucocorticoids, malignancy association; RF/ACPA$^-$ |
| Polymyalgia rheumatica | Arm and hip girdle involvement; marked elevations in ESR/CRP; RF/ACPA$^-$; association with giant cell arteritis |
| Sarcoidosis | Acute form associated with erythema nodosum and hilar adenopathy (Lofgren's syndrome), large joint involvement with a predilection for the ankle, often RF$^+$ and ACPA$^-$, nonerosive, pathology shows noncaseating granulomas |
| Sjögren's syndrome | Keratoconjunctivitis sicca (dry eyes) or xerostomia (dry mouth); salivary gland enlargement; positive ANA (SS-A/SS-B), often RF$^+$, ACPA$^-$ |
| Fibromyalgia | Myalgia without an inflammatory arthritis, RF and ACPA$^-$; ESR, CRP normal |
| **Infectious-Related** | |
| Bacterial endocarditis | High fever, predominantly large joints, audible murmur, positive blood cultures, evidence of peripheral emboli; can be RF$^+$, ACPA$^-$ |
| Human immunodeficiency virus | Brief acute joint pain associated with initial viremia followed by an oligoarticular process, fever |
| Hepatitis B and C | Nonerosive arthritis similar to RA, RF$^+$, ACPA$^-$, hypocomplementemia, positive HBV/HCV serologies, cryoglobulins |
| Parvovirus B19 | Nonerosive arthritis typically seronegative, self-limited process, parvovirus IgM$^+$, viral presentation |
| Chikungunya | Insect-borne virus, acute febrile illness with rash followed by a polyarthritis |
| Post-streptococcal infection | Nonerosive arthritis in a patient with an antecedent skin or oropharynx group A streptococcal infection, antistreptolysin O$^+$ |
| Rheumatic fever | Polyarthritis that is often additive, symmetric, with large joint lower extremity involvement; subcutaneous nodules, carditis, chorea, rash |
| Lyme disease | May have both an erosive and nonerosive arthritis, RF and ACPA$^-$, history of a tick bite and/or typical rash while in an endemic region |
| **Crystalline Arthropathy** | |
| Calcium pyrophosphate dihydrate deposition disease | Often in elderly women, 5% incidence of pseudo-RA presentation, radiographs may have chondrocalcinosis; associations with hemochromatosis, hyperparathyroidism, hypothyroidism, and hypomagnesemia |
| Polyarticular gout | Often involves distal joints, including first MTP, and may affect the DIP joints of the hands, associated tophaceous nodules, RF/ACPA$^-$, erosions with "overhanging edge"; hyperuricemia |
| **Systemic Manifestations of Other Medical Conditions** | |
| Thyroid disease | Polyarthralgias and myalgias with other manifestations of thyroid disease; carpal tunnel symptoms may be present |
| Malignancy | Often represents a paraneoplastic process and can mimic RA; lymphoma can present like RA; may be RF$^+$ but usually ACPA$^-$ |
| Hypertrophic osteoarthropathy | Predominant involvement in knees, ankles, wrists; associations with chronic lung disease (e.g., cystic fibrosis) and malignancy; bone pain and periostitis |

*See criteria of Yamaguchi M, Ohta A, Tsunematsu T, et al: *J Rheumatol* 19:424–430, 1992.

ACPA, Anti-citrullinated protein antibodies; ANA, anti-nuclear antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DIP, distal interphalangeal; HLA, human leukocyte antigen; MTP, metatarsophalangeal; RF, rheumatoid factor.

**TABLE 70.3** 1987 American College of Rheumatology (Formerly American Rheumatism Association) Criteria for Classification of Rheumatoid Arthritis*

| Criterion | Definition |
|---|---|
| Morning stiffness | Morning stiffness in and around the joints lasting at least 1 hour before maximal improvement |
| Arthritis of ≥3 joint areas | At least 3 joint areas simultaneously having soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician (the 14 possible joint areas are [right or left] PIP, MCP, wrist, elbow, knee, ankle, and MTP joints) |
| Arthritis of hand joints | At least one joint area swollen as described previously in wrist, MCP, or PIP joint |
| Symmetric arthritis | Simultaneous involvement of the same joint areas (as in criterion 2) on both sides of the body (bilateral involvement of PIP, MCP, or MTP joints is acceptable without absolute symmetry) |
| Rheumatoid nodules | Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions, as observed by a physician |
| Serum rheumatoid factor | Demonstration of abnormal amounts of serum rheumatoid factor by any method that has been positive |
| Radiographic changes | Changes typical of RA on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to involved joints (OA changes alone do not qualify) |

*For classification purposes, a patient is said to have RA with presence of four of the seven criteria: criteria 1 through 4 must be present for ≥6 weeks.

MCP, Metacarpophalangeal; MTP, metatarsophalangeal; OA, osteoarthritis; PIP, proximal interphalangeal; RA, rheumatoid arthritis.

history, physical examination, and laboratory and radiographic testing. Satisfaction of the 1987 ACR RA classification criteria requires the presence of objective synovitis for 6 weeks or longer to exclude alternative self-limited forms of arthritis.[32] Other components of these criteria are shown in Table 70-3. Because these criteria include findings more consistent with long-standing disease, such as the presence of radiographic damage and subcutaneous nodules, the 1987 criteria lack sensitivity for the detection of early RA. Recognizing the importance of both early diagnosis and treatment, new RA classification criteria were proposed as part of a joint ACR/EULAR effort in 2010 (Table 70-4).[33] The new criteria place an emphasis on additional serologic tests, including RF as well as ACPA, ESR, and CRP. Articular involvement is qualified by joint swelling and tenderness. Application of these criteria provides a score of 0 to 10, with a score of 6 being indicative of the presence of definite RA. The 2010 criteria render an earlier diagnosis possible, even with a very low (or in some cases absent) number of tender and/or swollen joints. Although the 2010 criteria yield excellent sensitivity (97%) in identifying patients with RA, the specificity is substantially lower than that of the 1987 criteria (55% vs. 76%, respectively).[34] By default, satisfaction of the 1987 criteria satisfies the 2010 criteria, the latter including provisions for the use other imaging modalities (e.g., ultrasound or MRI) to document the presence of synovitis. Although useful for teaching and

**TABLE 70-4** 2010 American College of Rheumatology/European League against Rheumatism Classification Criteria for Rheumatoid Arthritis*

| | |
|---|---|
| Joint Involvement* | (0-5) |
| 1 medium to large† joint | 0 |
| 2-10 medium to large joints | 1 |
| 1-3 small‡ joints (with or without involvement of large joints) | 2 |
| 4-10 small joints (with or without involvement of large joints) | 3 |
| >10 joints§ (at least one small joint) | 5 |
| Serology‖¶ | (0-3) |
| Negative RF **AND** negative ACPA | 0 |
| Low-positive RF **OR** low-positive ACPA | 2 |
| High-positive RF **OR** high-positive ACPA | 3 |
| Acute Phase Reactants‖# | (0-1) |
| Normal CRP **AND** normal ESR | 0 |
| Abnormal CRP **OR** abnormal ESR | 1 |
| Duration of Symptoms** | (0-1) |
| <6 weeks | 0 |
| ≥6 weeks | 1 |

*Joint involvement refers to any swollen or tender joint on examination, or evidence of synovitis on magnetic resonance imaging or ultrasonography. Distal interphalangeal joints, first carpometacarpal joint, and first metatarsophalangeal joint are excluded from assessment. Categories of joint distribution are classified according to the locations and numbers of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

†Medium to large joints refer to shoulders, elbows, hips, knees, and ankles.

‡Small joints refer to the metacarpophalangeal joints, proximal interphalangeal joints, metatarsophalangeal joints 2 through 5, thumb interphalangeal joints, and wrists.

§In this category, at least one of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular).

‖Individuals should be scored by these criteria only if at least one serologic test and at least one acute phase reactant test result are available. If a value for a serologic test of acute phase reactant is not available, that test should be considered as negative/normal.

¶Negative refers to international unit (IU) values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are greater than the ULN but less than or equal to 3 × ULN for the laboratory and assay; high-positive refers to IU values that are greater than 3 × ULN for the laboratory and assay. Where RF is available only as positive or negative, a positive result should be scored as low-positive for RF.

#Normal/abnormal is determined by local laboratory standards.

**Duration of symptoms refers to patient self-report of the duration or signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment.

ACPA, Anti-citrullinated protein antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; ULN, upper limit of normal.

Modified with permission from Aletaha D, Neogi T, Silman AJ, et al: 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative, *Ann Rheum Dis* 69:1580–1588, 2010.

in clinical practice, it is important to recognize that both the 1987 and 2010 criteria were primarily developed with the goal of identifying homogeneous study populations for RA clinical trials and not for the diagnosis of individual patients.

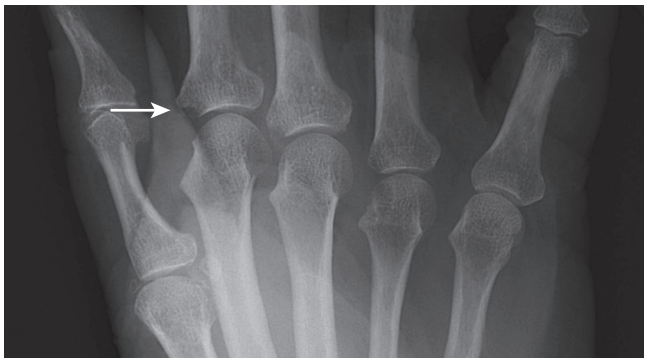


**Figure 70-1** Typical rheumatoid arthritis erosion *(arrow)* along the periarticular area where the synovium wraps-back on itself, the so-called "bare area." *(Courtesy Dr. Gerald Moore.)*

Radiographic assessments are an important element in the RA evaluation, serving as a robust measure of treatment efficacy in therapeutic trials. Although radiographic assessments used in clinical trials focus primarily on quantitative measures of articular damage, including bony erosions and joint space narrowing which is limited to the hands, wrists and feet, it is well recognized that RA affects a number of other joints, with findings that can be highly characteristic and markedly destructive. Often accompanied by periarticular osteopenia, bony erosions in RA are distinctive in location, affecting the so-called "bare area" where synovium wraps back on itself and lays directly on the bone. This bare area is located periarticularly in the proximal and distal metaphyseal regions (Figure 70-1). Other imaging modalities may assist in diagnosis. Both ultrasound and MRI appear to have superior sensitivity over conventional radiography for the detection of bony erosions in RA (see Chapter 58) and for the identification of synovitis when results from physical examination are equivocal. Bony ankyloses, confirmed radiographically, is rare in RA and is associated with increased duration and severity of disease, and is found in joints that have been immobilized by pain, inflammation, treatment, or any of these factors in combination.

## CLINICAL PRESENTATIONS OF EARLY RHEUMATOID ARTHRITIS

The early presentation of RA includes mild systemic and articular signs and symptoms. Extra-articular features are more commonly seen in later disease. The systemic findings include fatigue and, less commonly, weight loss or low-grade fever. Articular symptoms most often begin with stiffness, pain, and swelling involving the small joints of the hands and feet in a symmetric distribution, including the metacarpophalangeal (MCP), proximal interphalangeal (PIP), and metatarsophalangeal (MTP) joints. Understanding that RA-related synovitis can involve almost any synovial joint in the body, the condition characteristically spares the distal interphalangeal (DIP) joints (impacting <10% of cases)[35] and also spares the thoracic and lumbosacral spine. Joint activity in RA rarely remits completely in an involved joint, while developing in another. This additive quality of arthritis distinguishes RA from conditions such as rheumatic fever, serum sickness, or palindromic rheumatism, which can be characterized by a true migratory arthritis pattern. Among patients with an early undifferentiated inflammatory arthritis, 30% to 40% will experience a spontaneous remission, whereas classifiable RA will eventually develop in approximately 1 in 4. In addition to ACPA positivity, other features predictive of RA development in patients with early undifferentiated inflammatory arthritis include polyarthritis, symmetric arthritis, and the presence of radiographic erosions.[36]

### Patterns of Onset

RA onset typically follows one of several different temporal patterns, ranging from an acute or "explosive" polyarticular disease onset to a far more insidious inflammatory arthritis that can be oligoarticular and additive during the initial course.

#### Insidious Onset

Estimated to impact 55% to 65% of patients,[37] an insidious disease onset represents the most common early presentation of RA. The small joints of the hands, wrists, and feet are most commonly affected.[38] The joint pattern is often symmetric, and is accompanied by morning stiffness, a cardinal sign of inflammatory arthritis that may last as long as several hours. Less commonly, onset is characterized by a slowly evolving monoarticular or oligoarticular presentation, with a predilection for larger joints, such as the elbows, knees, or hips. This presentation mimics a seronegative spondyloarthropathy, but with time, most patients experience symptoms in additional joint areas, including the contralateral joint or other small joints, resulting in a pattern of involvement more typical of RA. In older patients (e.g., >65 years), an insidious onset can be dominated by systemic features, including fever, weight loss, fatigue, marked acute phase responses, and occasional proximal girdle involvement, which together parallel the signs and symptoms of PMR or RS3PE. This type of presentation should also prompt consideration of an occult malignancy.[39]

#### Acute or Intermediate Onset

Estimated to impact 8% to 15% of all cases, RA less frequently manifests as an explosive polyarticular arthritis that can be intensely inflammatory and may involve both small and large joints when the patient is first seen by a physician. With acute onset, the pain may be so limiting that the initial evaluation occurs in an urgent ambulatory setting. This dramatic, but uncommon, disease onset appears to have a predilection for the elderly and has been described as one of the characteristic presentations in "elderly-onset" or "later-onset" RA. An intermediate disease onset, in which symptoms develop over days to weeks, impacts approximately 1 in 5 incident cases.

#### Atypical Onset

In a minority of patients with RA, the initial presentation involves a simple bursitis or tendonitis that may evolve into tenosynovitis. Such patients may also be seen with early carpal tunnel syndrome from wrist involvement and subsequent median nerve compression. As with insidious

presentations, the disease in individuals with an atypical presentation often evolves during time to the more typical symmetric small joint findings of RA.

### Other Patterns of Onset

**Palindromic Rheumatism.** Palindromic rheumatism is an uncommon form of disease onset characterized by relatively brief but intense episodes of joint inflammation. Often, by the time a patient is seen by a rheumatologist, they may already be asymptomatic, because of the self-limited nature of palindromic flares, which may last from a few hours to a few days.[40] Joint involvement with palindromic onset is often monoarticular, with maximal inflammation achieved within just hours. Impacting almost any joint, this onset most commonly affects the patient's knees, fingers, or shoulders. Because of the highly inflammatory, and often monoarticular presentation, palindromic RA can closely mimic crystalline arthropathy or septic arthritis. Serologic testing, including RF and/or ACPA, is often helpful in confirming the diagnosis of palindromic rheumatism.[41] If followed over time, one-third to one-half of patients with initial palindromic rheumatism evolve to develop a daily pattern of joint involvement more typical of RA.[40] This disease evolution appears to be more common among those with hand involvement and ACPA positivity.[41] The disease in the remainder of patients tends to continue to palindrome or evolve into a definable crystal-related arthropathy.

**Arthritis Robustus.** As with palindromic onset, arthritis robustus is a relatively uncommon RA subtype that was first described by de Haas et al in 1973.[42] The typical patient profile is an older man, with an occupational history notable for manual labor. Patients seen with arthritis robustus often have a markedly inflammatory arthritis, usually involving the hands and feet. However, affected patients often have few if any symptoms, giving the impression in some cases of a patient who is "blissfully unaware" of the condition.[43] The challenge in the care of these patients is they often do not communicate complaints, which makes the physical examination and laboratory assessments the key components in physician management decisions.[43]

**Extra-articular Disease as Initial Presentation.** Several reports have shown that extra-articular disease manifestations rarely represent the initial disease manifestation in RA. This relatively uncommon phenomenon has perhaps been most widely observed with the appearance of rheumatoid nodules preceding demonstrable synovitis.[44] "Rheumatoid nodulosis" has been classically described by the combination of rheumatoid nodules, subchondral bone cysts, and recurrent joint symptoms, an entity with a favorable prognosis most often seen in the context of RF seropositivity.[45] More rarely, other forms of extra-articular disease have been observed to precede the development of synovitis in patients with RA, most notably lung disease.[46] Fischer and colleagues examined 74 patients who were seen with respiratory symptoms and a positive ACPA but without evidence of RA or alternative connective tissue disease.[46] Investigators noted four distinct radiographic patterns using thoracic HRCT, including isolated airways disease (54%), isolated interstitial lung disease (ILD) (14%), mixed airways disease and ILD (26%), and combined pulmonary fibrosis and emphysema (7%). Among the 33 patients with high-titer ACPA, articular symptoms of RA developed in 3 during a median follow-up of approximately 1.2 years.

## COURSE AND COMPLICATIONS OF ESTABLISHED RHEUMATOID ARTHRITIS

### Articular Manifestations

With its primary manifestation involving synovial tissues, the signs and symptoms of RA are primarily driven by active synovitis that results in joint swelling, stiffness, palpable warmth, and pain. The classic joint distribution in early disease includes the small joints of the hands and feet (MCP, PIP, and MTPs) (Figure 70-2A). With time, intermediate (wrists, elbows, and ankles) and large (hips, shoulders, and cervical spine) joints may become involved. With more advanced and longer duration of disease, RA may also involve atypical joints, including the temporomandibular joint (TMJ), cricoarytenoid, and sternoclavicular joint. In contrast to OA, RA rarely affects the DIP joints and seldom targets the thoracic or lumbosacral spine.

### Hands and Wrists

The hands and wrists are often considered together because they form a single functional unit. For example, wrist involvement is strongly associated with the presence of ulnar deviation of the MCP joints.[47] This may occur via weakening of the extensor carpi ulnaris muscle or tendon, either as a direct or indirect result of inflammation, which leads to radial deviation of the wrist as the carpal bones rotate (the proximal row in an ulnar direction and the distal row in a radial direction). Subsequently, ulnar deviation of the MCP joints, and associated palmar subluxation, may serve as compensatory mechanisms to maintain digital tendons in a normal line with the radius. Together, changes of the wrist and MCP joints result in a highly characteristic "zigzag" deformity (Figure 70-2B and C). Although classically described as irreversible, reducible ulnar deviation of the MCP joints can result from bulky synovitis, tendon laxity occurring after the reduction of synovitis, tendon rupture, or muscle weakness in the hands. However, reversible MCP deviation is more typical of nonerosive processes such as SLE or non-inflammatory neurologic conditions such as Parkinson's disease.

Proliferative synovium contains both the inflammatory and enzymatic machinery sufficient to destroy ligaments, tendons, and the articular regions distal to the ulnar head, resulting in joint subluxation, dislocation, and bony erosions. This inflammatory synovium may also invade and impinge normal tissue spaces, and lead to nerve entrapments syndromes, such as carpal tunnel syndrome (median nerve). The inflammatory and enzymatic degradation of tissue, coupled with naturally occurring forces, act together to impair the integrity of the radiocarpal, radioulnar, and midcarpal joints. The ulnar collateral ligament can become stretched or even destroyed by synovitis of the radioulnar joint. As a result, the ulnar head may "spring up" into dorsal prominence where it "floats" and can be easily depressed (the so-called "piano key styloid") (Figure 70-3). Severe disease progression in the wrist can lead to marked loss of

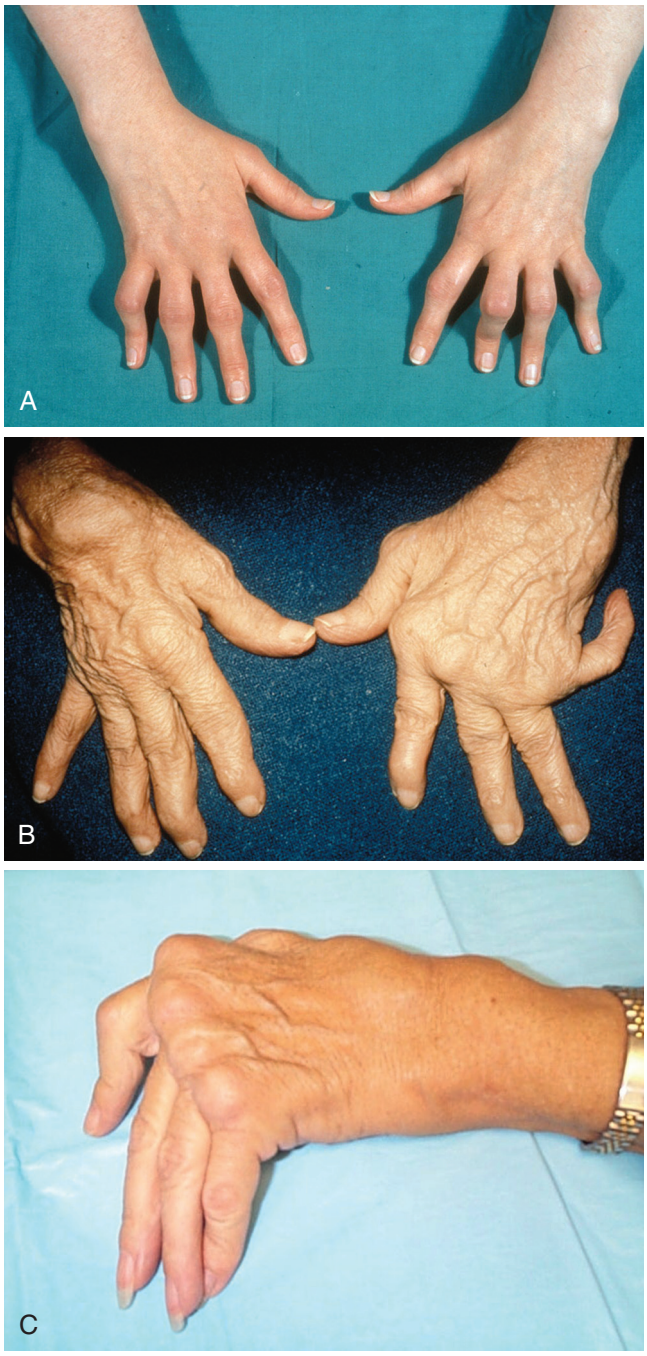



**Figure 70-2** **A,** Polyarticular arthritis with fusiform swelling of the proximal interphalangeal joints along with wrist deformity. **B,** Complete subluxation with ulnar deviation at the metacarpophalangeal joints in a patient with advanced disease. **C,** "Zig-zag" deformity of the hand. RA hand and wrist involvement in a patient with long-standing disease resulting in radial deviation at the wrist and ulnar deviation at the metacarpophalangeal joints. *(**A** and **B**, Courtesy Dr. Iain McInnes; **C**, Courtesy Dr. Gerald Moore.)*

joint space, bone erosions, and ankyloses; the latter is more common with wrist immobilization resulting from unremitting disease.

Perhaps the best recognized of the articular deformities observed in RA are swan neck and boutonnière deformities of the fingers (Figure 70-4Aand B). The swan neck deformity involves DIP flexion with simultaneous PIP hyperextension. This condition is thought to result from direct

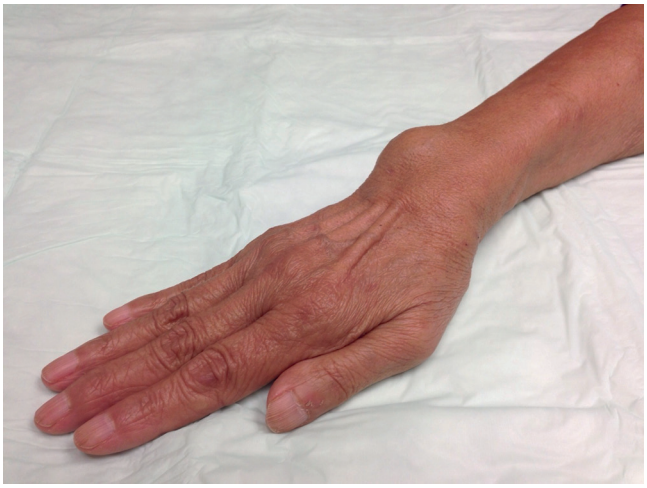


**Figure 70-3** "Piano key styloid." Rheumatoid arthritis wrist involvement that leads to damage of the ulnar collateral ligament with resultant prominence, or floating, of the ulnar head and styloid. *(Courtesy Dr. Amy Cannella.)*

involvement of the PIP and/or MCP joints. Boutonnière deformities result from PIP flexion and DIP hyperextension and are related to relaxation of the central slip, with buttonholing of the PIP joint between its lateral bands. Involvement of the thumb in RA also has unique characteristics. Several corresponding deformities are described, and result from involvement of the first MCP, interphalangeal (IP) or carpal metacarpal (CMC) joints, either alone or in combination. Predominant thumb deformities include a boutonnière-like deformity, which begins with synovitis of the first MCP joint, results in weakening of the joint capsule and surrounding connective tissues, and culminates with the subluxation of the extensor pollices longus (EPL) tendon in both volar and ulnar directions. As a result, the MCP joint is abnormally flexed and the IP joint is secondarily hyperextended, forming a characteristic "Z-deformity" (see Figure 70-4C). The MCP joint may be partially reducible or fixed. Less commonly, RA patients develop a swan neck–like deformity of the thumb, resulting from CMC synovitis and joint subluxation at the base of the thumb. This ultimately leads to exaggerated adduction at the distal end of the MCP joint, markedly narrowing the first web space and effectively reducing the object size that a patient can grasp. In a recent survey of 100 patients with established RA, almost half (47%) had evidence of hand or wrist deformity on examination. Longer disease duration was the only factor found to be associated with these findings.[48] Among patients with deformity, the most common findings included radial deviation of the wrist (91%), ulnar drift of the MCPs (79%), Z-deformity of the thumb (56%), swan neck deformity (49%), boutonnière deformity (43%), palmar subluxation of the MCPs (36%), and wrist subluxation (28%).

One of the most common manifestations of RA in the hands is tenosynovitis in flexor tendon sheaths, which can be a major cause of hand weakness.[49] Along with wrist joint synovitis, flexor tenosynovitis occurs in close proximity to the median nerve, and may result in carpal tunnel syndrome through impingement, similar to synovitis of the carpal joints. Tenosynovitis may occur in as many as one-half of patients with RA, and manifests as diffuse volar swelling of the phalanges or as a palpable grating of the flexor tendon

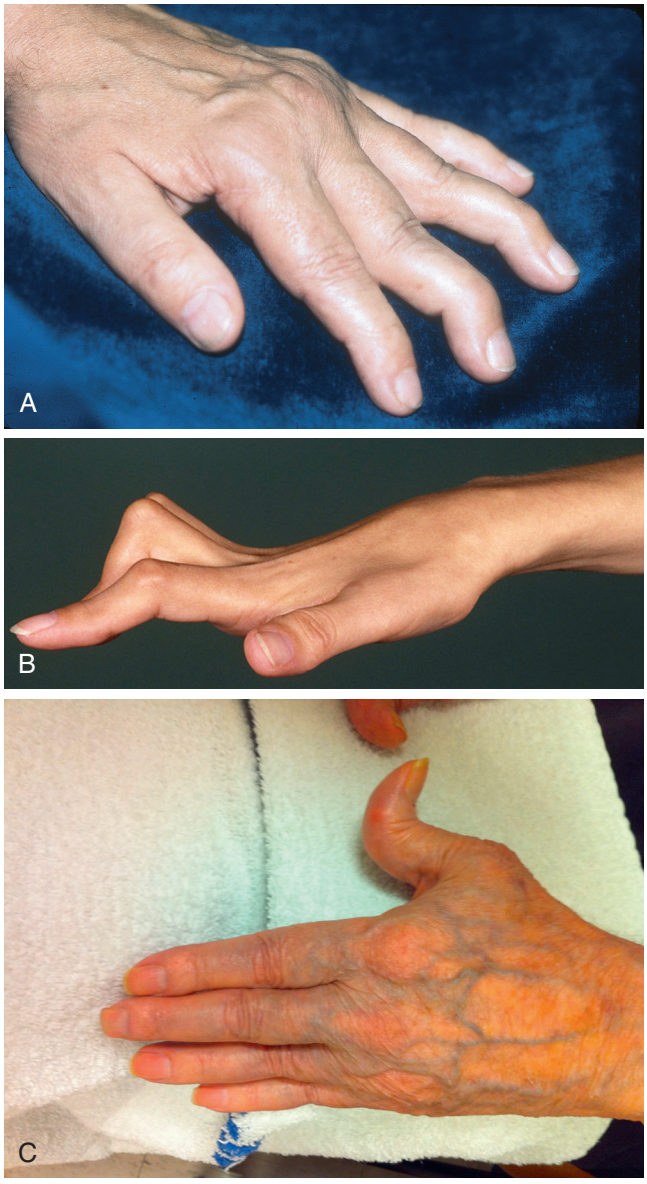



**Figure 70-4** **A,** Swan neck deformity. This deformity is marked by hyperextension of the proximal interphalangeal joint and flexion of the distal interphalangeal joints. **B,** Boutonnière deformity. This deformity is marked by flexion of the proximal interphalangeal joint and extension of the distal interphalangeal joint. **C,** "Z-deformity" of the thumb. *(**A** and **B**, Courtesy Dr. Iain McInnes; **C**, Courtesy Dr. Amy Cannella.)*

sheaths in the palm. Without concomitant and typical RA synovitis, this finding may pose a substantial diagnostic challenge. Nodular thickening may also develop in the tendon sheaths or the pulley system and cause the finger to "lock" in fixed flexion ("trigger finger"), resulting in significant pain. With chronic symptoms or frequent recurrence, trigger finger can be treated with tendon sheath injection or surgical intervention.

RA also represents the most common etiology of tendon ruptures involving the hand. Described by Vaughn-Jackson in 1958, RA can lead to attritional rupture of the digital extensor tendons, occurring at the ulnar head in the wrist.[50] Because surgical intervention can prevent extensor tendon rupture and subsequent loss of function, identifying patients most at risk for this complication is paramount. In extensor



**Figure 70-5** Tendon rupture of the fourth and fifth digits of the hand. Note the significant wrist swelling and prominence of the ulnar styloid region. This condition requires immediate orthopedic evaluation.

tendon rupture, the extensor digiti minimi (EDM) usually ruptures first, a defect that may go unnoticed because the extensor digitorum communis (EDC) extends all four fingers simultaneously. Thus, testing for EDM function, via active independent extension of the little finger, may be valuable in identifying patients that could benefit from prophylactic extensor tendon repair[51] (Figure 70-5). Less frequently, RA may lead to rupture of the extensor pollices longus in addition to attritional ruptures of the flexor pollices longus and the index finger flexor digitorum profundus tendons within the carpal tunnel.[52]

Although more typical of severe psoriatic arthritis, the most serious consequence of RA involving the hand can be seen with marked bone resorption that begins with the articular cartilage and spreads along the diaphysis of involved phalanges. As a result, the digits can become grossly shortened, exposing excess skin folds, and the phalanges can be retracted (or telescoped) into one another and then pulled out into abnormally long extension often without eliciting pain. Typically involving multiple digits, this rare arthropathy has been termed "arthritis mutilans" or the "opera glass hand."

## Feet and Ankles

As with the hand and wrist, the foot and ankle may also be considered as a single functional unit. For example, hindfoot abnormalities that involve the ankle and/or subtalar joint have been shown to directly impact forefoot pressures with loading and weight bearing; results that suggest optimal correction of an existing forefoot deformity is reliant upon simultaneous correction of any residual hindfoot defect. Ankle and foot involvement is common in RA, with forefoot involvement (primarily MTP synovitis) observed in as many as 90% of patients at some point in the disease process, and serving as the initial disease manifestation in approximately 15% of patients.

As a result of forefoot/MTP involvement, pain experienced during push-off in striding results in altered gait mechanics. Plantar subluxation of the metatarsal heads occurs soon after the MTP joints become involved, producing "cock-up" or "claw" toe deformities of the PIP joints. Cystic collections that represent outpouchings of flexor

tendon sheaths often develop under the MTP joints.[53] Pressure necrosis and calluses of the plantar surfaces can develop in patients with subluxation of metatarsal heads, and claw deformities can lead to pressure ulcers on the dorsal surface of PIP joints (Figure 70-6). Metatarsal subluxation leads to increased pressure on the MTP joints, with a sensation described by many patients as "walking on marbles." Concurrently, hallux valgus results in progressive overlap of the second and third toes on top of the great toe. With the development of fixed deformities, including a broadened forefoot, hallux valgus, and claw toes, mechanical foot symptoms often persist in patients, even in the absence of active inflammation.

Involvement of the hindfoot, midfoot, and adjacent joints are also common in RA. Valgus deformity of the hindfoot may occur, likely related to pronation forces applied to weakened and inflamed talonavicular and subtalar joints (Figure 70-7). Similarly, ankle instability may result from stretching and even erosion of ligamentous support tissues. With involvement of the subtalar joint, which provides the major axis for foot inversion and eversion, patients may report difficulty with ambulation on uneven ground. With midfoot involvement, inflammation of the talonavicular and naviculocuneiform joints can result in pes planus (flat foot) deformities. Pes planus can be further exacerbated through insufficiency of the posterior tibialis tendon and the plantar calcaneonavicular (spring) ligament, and result in collapse of the longitudinal arch. Inflammation and subluxation of the tarsometatarsal joints may result in "rocker-bottom" foot deformity, resembling that observed with Charcot's foot disease. Rheumatoid nodules can develop in the Achilles tendon, and spontaneous tendon rupture has been reported to occur in the context of granulomatous inflammation.[54] Parallel to carpal tunnel syndrome in the wrist, tarsal tunnel syndrome can occur in the ankle and results in a compressive tibial neuralgia.



**Figure 70-6** Pressure ulcers are most likely to occur at areas of deformity on or over the plantar region of the metatarsal phalangeal joints. This patient has large rheumatoid nodules on the plantar foot, creating shoe wear issues and increased risk for pressure sores. *(Courtesy Amy Cannella, MD.)*



**Figure 70-7** Ankle valgus and hind foot involvement with metatarsal phalangeal deviation and cross-over deformities. *(Courtesy Dr. Amy Cannella.)*

## Cervical Spine

Cervical spine involvement may be seen in as many as 80% of patients with RA and appears to be most common in those with long-standing and poorly controlled disease. Reviewed by Joaquim and Appenzeller,[55] risk factors for cervical spine involvement in RA include erosive disease, glucocorticoid use, treatment failure, and early age at disease onset. Importantly, the development of cervical spine involvement and its complications may be abrogated by intensive treatment. One study showed that patients treated aggressively with combination disease-modifying therapy were at lower risk for cervical spine disease than patients treated with less aggressive monotherapy.[56]

Of the cervical spine findings observed in RA, the most serious clinical presentations relate to atlantoaxial (C1-C2) subluxation, cranial settling, and subaxial subluxation. Of these, atlantoaxial subluxation is the most common radiologic abnormality of the cervical spine observed[55] and may include:

- The atlas (C1) moving anteriorly on the axis (C2) (most common)
- The atlas moving posteriorly on the axis
- Subluxation of the atlas vertically in relation to the axis (least common)

The earliest and most common symptom of atlantoaxial subluxation is pain radiating into the occiput. Two other serious, but less common, clinical patterns include (1) slowly progressive spastic quadriparesis with painless sensory loss in the hands, and (2) transient episodes of medullary dysfunction associated with vertical penetration of the dens and probable vertebral artery compression. In the latter case, paresthesias may occur in the shoulders or arms during movement of the head. With vertical C1-C2 subluxation, patients may complain of facial pain with decreased sensation in the V1 and V2 distributions of the fifth cranial nerve.

Neurologic symptoms resulting from spinal cord involvement often have little relationship to the degree of C1-C2 subluxation present. When present, symptoms range from simple paresthesias to altered consciousness, loss of sphincter tone, and other well-defined signs of cord compression. Signs of cord compression portend a poor prognosis, often heralding the rapid progression of myelopathy with an approximate 50% 1-year mortality.[57] Risks are further

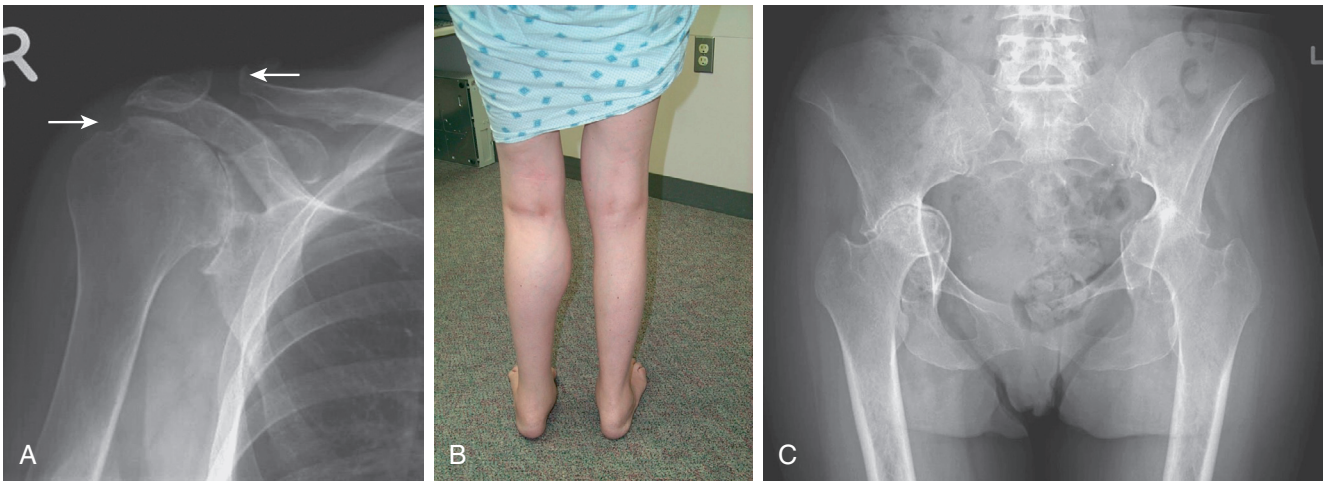



**Figure 70-8** **A**, Shoulder findings in rheumatoid arthritis include glenohumeral joint space narrowing with marginal erosions and cystic changes of the humeral head *(lower arrow)*, a high-riding humeral head indicative of a rotator cuff tear, and tapering of the distal end of the clavicle with widening of the acromioclavicular joint *(upper arrow)*. **B**, Ruptured Baker's cyst of the left calf with an associated pseudothrombophlebitis. **C**, Bilateral protrusio acetabuli. Note the symmetric joint space narrowing with femoral head remodeling and medial migration toward the pelvis. *(**A**, Courtesy Dr. Barbara Weissman; **B** and **C**, courtesy Dr. Gerald Moore.)*

magnified in the context of patient falls, whiplash injury, or the need for general anesthesia with intubation requiring cervical manipulation. The incidence of sustained neurologic deterioration following surgical correction of C1-C2 disease may be as high as 6%, suggesting that a conservative management strategy may be optimal for a substantial proportion of asymptomatic patients.

As with neurologic symptoms, physical examination findings are nonspecific and unreliable in detecting C1-C2 subluxation. Physical findings may include the loss of cervical lordosis, resistance to passive spine motion, or the abnormal protrusion of the axial arch felt with the examining finger on the posterior pharyngeal wall. In symptomatic patients, cervical spine films in flexion should be taken only after radiographs have been taken to rule out odontoid fracture or high-grade atlantoaxial subluxation. Advanced imaging modalities, including CT and especially MRI, have proven to be particularly useful in defining pathology affecting this region in RA (see Chapter 58).

Although not lined by synovium, discovertebral joints of the cervical spine can be involved, leading to osteochondral tissue destruction and disk space narrowing that is visible on lateral radiographs. Patients with cervical spine involvement may report significant neck pain, although passive range of motion may be preserved in the absence of significant muscle spasm. At least two possible mechanisms have been proposed to explain the cervical spine involvement characteristic of RA: (1) extension of inflammation from adjacent synovial-lined neurocentral joints (the joints of Luschka) into the discovertebral area, and (2) chronic cervical instability initiated by apophyseal joint destruction, leading to vertebral misalignment or frank subluxation.

### Intermediate and Large Peripheral Joints

Intermediate and large joint involvement is more indicative of long-standing and often poorly controlled disease. Elbow involvement is reported in approximately one-third to one-half of patients. The earliest clinical manifestation is extension loss, a finding that frequently goes unnoticed by the patient. Bilateral olecranon bursal swelling is observed in RA, and may be encountered in gout or pseudogout, although typically unilaterally. Shoulder involvement impacts not only the glenohumeral joint, but also the subacromial (subdeltoid) bursa, the distal third of the clavicle (related to adjacent inflammatory bursitis), and rotator cuff tendons (Figure 70-8A). In contrast to other joints, shoulder involvement is often quite painful, with symptoms referred to the deltoid region. Patients commonly complain of significantly disturbed sleep with painful awakenings in side sleeping positions. Rotator cuff dysfunction is common in RA, and may relate to the proximity of proliferative synovium to the rotator cuff insertion on the greater tuberosity. Resulting in substantial morbidity, rotator cuff tears have been demonstrated to impact approximately 20% of patients with RA, with another one-fourth having evidence of frayed tendons.[58] Because the rotator cuff acts as a downward stabilizer of the humerus in the glenoid, rotator cuff dysfunction can lead to superior migration of the humeral head from unopposed forces of the deltoid, and resultant secondary degenerative arthritis of the glenohumeral joint.

Hip and knee involvement also results in substantial disability. In contrast to the hip, knee involvement in RA is usually readily identified on examination, with joint effusions that can be large and associated with increases in intra-articular pressures. As with the elbow, loss of full extension may be an early finding of knee involvement. Increased intra-articular pressure, particularly those accompanying flexion, can lead to an outpouching of the posterior components of the joint, resulting in a popliteal or Baker's cyst. In extreme circumstances, a Baker's cyst can rupture, and inflammatory fluid may dissect distally into the calf, resembling acute thrombophlebitis or deep vein thrombosis (see Figure 70-8B). A helpful but uncommon sign of cyst rupture is the presence of a crescentic hematoma inferior to either the lateral or medial malleolus. MRI and ultrasound are often used to identify a Baker's cyst and help to differentiate this complication from other entities.

Unlike the knee, detecting involvement of the hip often poses a challenge. As with other forms of arthritis, including

OA, hip involvement in RA is associated with pain most often localized to the groin or occasionally the buttock. Similar to other forms of inflammatory arthritis involving the hip, RA leads to symmetric joint space narrowing. Symmetric cartilage loss in RA leads to an axial migration of the femoral head. This is in contrast to OA of the hip, which is characterized by asymmetric joint space narrowing that is most evident in the superior aspect of the joint. In advanced cases of RA-related hip involvement, the femoral head can collapse and undergo resorption with remodeling and medial migration of the acetabulum, leading to protrusio acetabuli (see Figure 70-8C). Well recognized in RA, protrusio acetabuli may also be seen in other forms of inflammatory arthritis, including spondyloarthropathies, and has been described in non-inflammatory connective tissue disorders such as Stickler's syndrome.[59]

### Temporomandibular, Cricoarytenoid, Sternoclavicular and Manubriosternal Joints

Though not commonly thought of as synovial joints, both the TMJ and cricoarytenoid joint contain synovial tissue and can be affected in RA. Patients with TMJ involvement often complain of jaw pain, which can be confused with tooth or ear problems or jaw claudication in the elderly, in whom giant cell arteritis with polymyalgia rheumatic may be suspected. As in other joints, radiographic findings of the TMJ in RA can include both joint space narrowing and erosions. Symptoms of cricoarytenoid joint involvement are often far subtler. The cricoarytenoid joints are diarthrodial joints responsible for rotation of the vocal cords as they abduct and adduct. With cricoarytenoid joint involvement in RA, a patient may complain of new-onset hoarseness, sore throat, dysphagia, or pain with speech. In extreme cases, the vocal cords can become immobilized in an adducted midline position, resulting in inspiratory stridor. Laryngoscopy and CT scanning are the most sensitive methods to evaluate for cricoarytenoid arthritis. Finally, as synovial lined joints, both the sternoclavicular and manubriosternal joints can also be involved in RA, although this rarely causes symptoms due to their relative immobility. Overt involvement of the sternoclavicular joint should raise suspicion for possible septic arthritis.

## Extra-articular Manifestations

Recognizing that RA is a systemic inflammatory disease is a key requisite for optimal management. Inflammation in RA involves not only articular structures but may also involve extra-articular tissues, leading to the development of subcutaneous nodules and other mucocutaneous findings, serositis, cardiopulmonary, neurologic, ocular, and hematological complications, to name just a few. The most significant extra-articular features are related to vascular inflammation and vascular damage. Extra-articular manifestations are observed in up to 50% of patients with RA and generally portend a poor prognosis, including increased morbidity and mortality.[7] In a single-center series, mortality risk in patients with RA with extra-articular manifestations was more than twice that of patients without extra-articular disease (relative risk [RR], 2.49; 95% confidence interval [CI], 1.43 to 4.03), and cardiovascular disease accounted for 70% of all deaths in this group.[60] Fortunately, clinically important extra-articular complications are decreasing, likely as a consequence of better treatment practices. However, some of our treatments may further complicate assessments for extra-articular complications in RA, because disease-remitting therapies can lead to adverse effects that closely mimic extra-articular disease manifestations. For example, glucocorticoids exacerbate both osteoporosis and cardiovascular disease risk, conditions already overrepresented in patients with RA. The focus of this section will be on the "true" extra-articular manifestations of RA, and not complications related to therapy, which are covered elsewhere in this textbook.

### Mucocutaneous

Rheumatoid nodules occur primarily in seropositive patients with active disease. In addition to RF positivity, a history of cigarette smoking appears to be a robust risk factor for the development of subcutaneous nodules in RA, particularly among men and heavy smokers.[61] When present and histologically confirmed, rheumatoid nodules are pathognomonic for RA. In rare circumstances, nodules may precede arthritis and may herald the development of other extra-articular manifestations when present early in the disease course. Rheumatoid nodules develop most commonly on extensor surfaces and pressure areas, including the forearm, Achilles and foot regions, and finger joints (Figure 70-9). The appearance of nodules in unusual sites can pose a significant diagnostic dilemma, with reports of rheumatoid nodules involving areas overlying the sacrum, sole and occiput (in bedridden patients), in addition to rare cases involving the heart, lungs (see the following discussion), sclera (rarely leading to perforation of the globe), and central nervous system.[62] In the larynx, rheumatoid nodules on the vocal cords may cause progressive hoarseness and mimic cricoarytenoid arthritis.

On examination, rheumatoid nodules are typically subcutaneous and firm and may be either mobile or adherent to underlying structures. Because the physical findings are nonspecific and subcutaneous nodules can be seen with other forms of inflammatory arthritis, biopsy may be needed to confirm the diagnosis in select circumstances. Other conditions that can result in inflammatory arthritis and nodules include rheumatic fever, tophaceous gout, SLE, and multicentric reticulohistiocytosis (Table 70-5). Histologically, rheumatoid nodules are characterized by a central area of necrosis rimmed by a corona of palisading fibroblasts, which are surrounded by a collagenous capsule with perivascular collections of inflammatory cells. These nodules grow by accumulating cells that expand centrifugally, leaving behind central necrosis of the connective tissue matrix. Inspection of early lesions suggests that the nodule is composed of small inflamed arterioles,[63] suggesting that rheumatoid nodules are a form of small vessel vasculitis. This fact may explain the relationship of this finding to other forms of extra-articular disease, such as cardiovascular disease. Although rheumatoid nodules may regress either spontaneously or with disease-remitting therapy, increased nodulosis is an uncommon but well-recognized complication of methotrexate treatment that often paradoxically occurs in patients with well-controlled disease.[64]




**Figure 70-9** Rheumatoid nodulosis on the extensor surface of the forearm **(A)**, the plantar aspect of the foot **(B)**, and the extensor surface of the fingers **(C)**. *(Courtesy Amy Cannella, MD.)*

Amyloidosis may also complicate long-standing inflammatory disease. The purpura of amyloidosis may be confused with the skin fragility and "senile purpura" seen in patients who have been on long-standing glucocorticoid therapy. Renal disease especially proteinuria or diarrhea with malabsorption in a patient with long-standing RA should prompt investigation for secondary amyloidosis.

Vasculitis in RA may present with multiple skin findings, ranging from relatively benign nail-fold infarcts to a deep, erosive, scarring pyoderma gangrenosum (see Vasculitis). The presence of livedo reticularis, with its characteristic lacey appearance (most prominent on the lower extremities), should raise a concern for a medium-vessel vasculitis or an associated connective tissue disease, such as SLE or anti-phospholipid antibody syndrome (APS).[65]

The prevalence of secondary Sjögren's syndrome (autoimmune exocrinopathy) has been estimated at 17%,[66] resulting in symptoms of keratoconjunctivitis sicca (dry eyes) or xerostomia (dry mouth). These symptoms are accompanied by objective evidence of lacrimal or salivary gland dysfunction. Finally, palmar erythema is often present in patients with active disease.

### Ocular

In addition to keratoconjunctivitis sicca, episcleritis and scleritis complicates the disease course in less than 1% of patients with RA. Episcleritis typically presents as a painless red eye without vision loss or frank discharge, the latter more indicative of conjunctivitis. Inspection reveals engorged blood vessels that move with light pressure and stop at the limbic border. Scleritis also results in scleral injection without discharge, but in contrast to episcleritis, this is typically accompanied by pain reported by the patient and precipitated by palpation of the globe, and may also be associated with areas of dusky discoloration. Manifesting with variable levels of involvement, scleritis can be localized and superficial or more generalized, and extend into deeper tissue layers. In severe cases, deeper involvement can be characterized by granulomatous scleral resorption leading to scleromalacia perforans (Figure 70-10). Whereas episcleritis can usually be managed with topical anti-inflammatory agents, scleritis treatment routinely requires systemic or intraocular immunomodulating therapy. In some cases, the sclera can become thinned, even in the absence of overt inflammation, and lead to scleromalacia.

Other forms of eye involvement in RA include chronic blepharitis or, rarely, perilimbic ischemic ulcers. This latter condition is caused by the deposition of cryoproteins (RF-IgG complexes), which can lead to perforation of the anterior chamber. Iritis and uveitis can occur in RA but are more commonly seen with other rheumatic conditions (e.g., spondyloarthropathies, sarcoidosis, Behçet's disease, and juvenile idiopathic arthritis).

**TABLE 70-5** Differential Diagnosis of the Rheumatoid Nodule*

| Diagnosis | Distinguishing Features |
|---|---|
| Rheumatic fever | Small in diameter (few mm to 1-2 cm), can be transient (rarely lasts >1 month); location more proximal over olecranon rather than proximal forearm; association with carditis |
| Granuloma annulare | Intracutaneous nodule; histologically identical to rheumatoid nodules; RF/ACPA$^-$ without synovitis |
| Xanthomatosis | Yellow tinged; often accompanied by high plasma lipoproteins and cholesterol levels |
| Gouty tophi | Often found in similar locations as RA nodules; aspiration and microscopic analysis which consists of collections of monosodium urate crystals; associated hyperuricemia; RF/ACPA$^-$ |
| Multicentric reticulohistiocytosis | Often located around the cuticles; histologically contain large, lipid-filled macrophages |
| Systemic lupus erythematosus | Accompanied by other signs and symptoms of active SLE (histologically similar to rheumatoid nodules) |

*Other less common disorders to consider include erythema elevatum diutinum, erythema nodosum, Gottron's papules, calcinosis associated with CREST (calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, telangiectasias), acrodermatitis chronica atrophicans, bejel, yaws, pinta, leprosy, and amyloid.

ACPA, Anti-citrullinated protein antibody; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

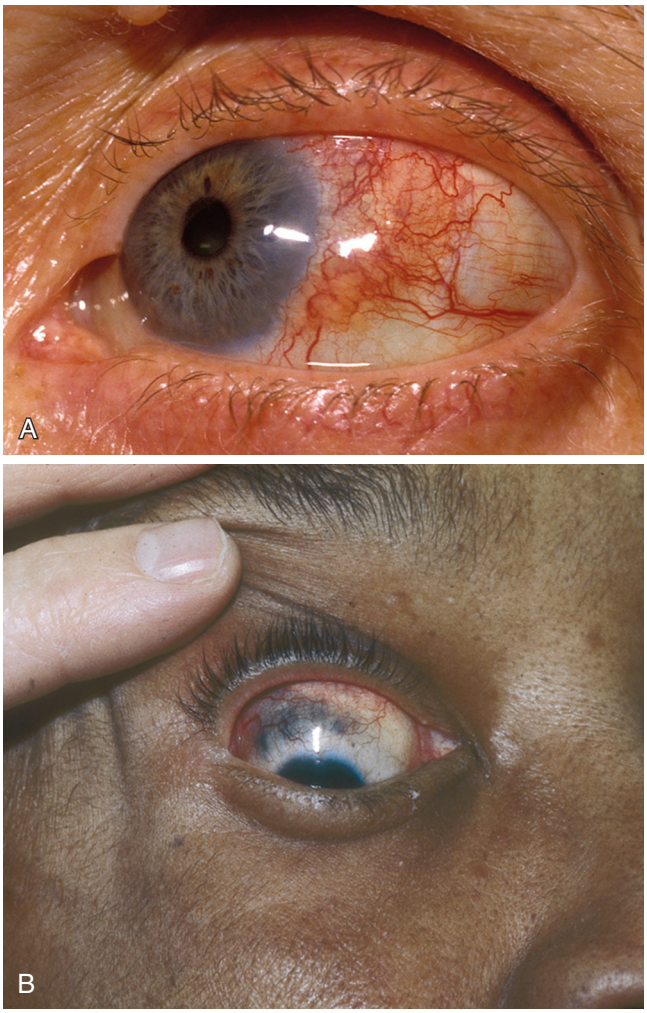



**Figure 70-10** **A**, Episcleritis of the eye. Note that the engorged blood vessels stop at the limbic border. **B**, Scleritis of the eye. Note the dusky bluish area that is indicative of pending scleromalacia perforans, an ocular emergency. *(Courtesy Iain McInnes, MD.)*

## Bone

In addition to periarticular bone loss and local erosions, RA is a well-recognized cause of systemic bone loss, placing patients at a substantially higher risk for the development of osteoporosis and fracture. This risk is further heightened in the context of glucocorticoid therapy. In a prospective series of 395 patients with early RA, more than one-third experienced a fracture over just 5 years of follow-up.[67] In addition to glucocorticoid use, risk factors for bone loss and fracture in RA included older age, disability, longer disease duration, decreased grip strength, low body mass,[68] and increased inflammation.[69] Systemic inflammation in RA results in the increased expression of growth factors, including macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor-κB ligand (RANKL), that promote osteoclast differentiation and ultimately bone resorption.[70] More recently, antigen-specific ACPA have been implicated as direct promoters of the osteoclastogenesis and bone loss that characterizes RA.[71] These findings are bolstered by animal work showing that immune responses to citrullinated self-antigen may serve as a trigger for declines in bone mineral density and bone quality.[72]

## Muscle

Weakness is a common patient complaint in RA. This may be related to associated joint pain and deconditioning or to type II muscle atrophy, complicating glucocorticoid use. There have been reports of inflammatory myopathy presenting with muscle tenderness and increased muscle enzymes. Muscle biopsies demonstrate focal accumulations of lymphocytes and plasma cells in a condition referred to as "nodular myositis." Necrotizing myositis has also been reported as a rare complication of RA.[73]

## Hematologic

Hematologic abnormalities are common in patients with autoimmune disease, and RA is no exception. Patients with RA frequently manifest a mild normocytic normochromic anemia that tends to correlate with increased disease activity and elevated inflammatory markers. Patients with chronic inflammation have an increased synthesis of ferritin and hemosiderin, abnormal retention of iron from senescent red blood cells, and increased lactoferrin, ultimately contributing to the binding and lowering of serum iron. Although mild anemia is common, the

presence of more severe anemia (i.e., hemoglobin <10 g/dL) suggests an alternative etiology. Mixed anemia poses a frequent diagnostic challenge in RA. For instance, a patient might have concomitant microcytic (iron deficiency or less commonly anemia of chronic disease) and macrocytic anemia (vitamin $B_{12}$ or folate deficiency, methotrexate use), a clinical scenario that is characterized by a heterogeneous red blood cell population (reflected in an increased red cell distribution width). In addition to its utility in evaluating causes of anemia, ferritin is an acute phase reactant and is therefore affected by inflammation. In general, however, a serum ferritin less than 50 ng/mL is indicative of iron deficiency, whereas values exceeding 100 ng/mL are indicative of adequate iron stores, regardless of RA disease activity.

Thrombocytosis is frequent with active RA, with platelet counts serving as an indirect measure of systemic inflammation. Platelet counts are positively correlated with both clinical and laboratory parameters of disease activity in RA, including ESR, RF, and several acute phase proteins.[74] Thrombocytopenia, on the other hand, is rare in RA in the absence of treatment-related bone marrow suppression or Felty's syndrome. As with other rheumatic and autoimmune conditions, eosinophilia may accompany active RA.

Associated with the presence of *HLA-DRB1*0401* allele,[75] Felty's syndrome comprises an uncommon but severe disease subset, defined by the triad of RA, splenomegaly, and leukopenia. The syndrome classically occurs in patients with long-standing disease, characterized by RF and ANA seropositivity and the presence of rheumatoid nodules and articular deformities, often in the context of minimal synovitis. In addition to rheumatoid nodules, these patients often have other extra-articular manifestations, and are at increased risk for bacterial infections and chronic nonhealing ulcers, leading to skin infections. The risk of bacterial infection is greatest in patients with more pronounced neutropenia (e.g., absolute neutrophil count [ANC] <100/$mm^3$). Low serum complements may also be seen in Felty's syndrome, with bone marrow biopsy typically yielding either normal or hyperplastic cell lines. In addition to associations with the HLA-DR*0401 allele, Felty's syndrome is characterized by the presence of autoantibodies directed against citrullinated histones that bind to activated neutrophils and neutrophil extra-cellular traps (NETs).[76]

Large granular lymphocyte (LGL) syndrome may also complicate the course of RA and may be related to Felty's syndrome. In addition to marked neutropenia, patients typically demonstrate LGL cells in both the blood and bone marrow. Splenomegaly may be variable.[77] LGL cells represent in vivo–activated cytotoxic T cells (CTC) or natural killer (NK) cells, and they proportionally represent 85% (CTC) and 15% (NK) of LGL syndromes, respectively.[78,79] LGL affects as many as 1% of patients with RA but is also seen in other inflammatory diseases (SLE, Sjögren's syndrome), viral infections, and hematologic malignancies (myelodysplastic syndrome). As opposed to aggressive and fatal subsets of LGL leukemia, the lymphocytosis associated with RA tends to follow an indolent course. In addition to rare malignant transformation, LGL places patients with RA at risk of infection. Although seemingly counterintuitive in the context of marked neutropenia, methotrexate remains the treatment of choice for both Felty's and LGL syndromes complicating RA. Splenectomy is reserved for those patients with Felty's syndrome and documented splenomegaly who are recalcitrant to medical management.

RA is associated with an increased risk of lymphoproliferative malignancy, particularly Hodgkin's disease and non-Hodgkin's lymphoma (NHL).[12] As with assessing the etiology of other extra-articular manifestations, delineating the simultaneous contributions of underlying RA from the effect of immunosuppressive agents used to treat the disease is difficult. Lymphoma risk in RA appears to be closely associated with RA disease activity and severity, even in the absence of RA treatments. In a large case-control study involving more than 11,000 patients with RA (many of whom were treatment naïve), those with higher levels of inflammation had a more pronounced risk of lymphoma, with an odds ratio of greater than 25 among those with the highest cumulative disease activity.[80] Patients with Felty's syndrome had an approximate 12-fold risk for lymphoma compared with patients with RA without this extra-articular manifestation.[81] In the absence of immunosuppressive therapy, RA results in the clonal expansion of $CD5^+$ cells, decreases the number and function of T-suppressor lymphocytes and NK cells, and inhibits T-suppressor function directed against the pro-oncogenic Epstein-Barr virus (EBV), which are all potential contributing factors to an increased lymphoma risk in RA.[12] These immunologic changes may also explain the general state of immunosuppression that characterizes RA even in the absence of immunomodulation therapy.

## Vasculitis

A small- to medium-vessel vasculitis can complicate the natural course of RA, a manifestation that is more common in men and associated with RF positivity (high titer), long-standing disease, and radiographic damage. Clinically, an important clue to the development of vasculitis is low serum complements with associated circulating immune complexes and cryoglobulins, along with the presence of other extra-articular manifestations.[82] Perhaps owing to improvements in treatment, the prevalence of rheumatoid vasculitis appears to have decreased substantially in recent years, with a study from the U.S. Veterans Health Administration showing a 20% to 50% drop in prevalence between 1997 and 2006.[83]

Rheumatoid vasculitis can present in a variety of ways, including:

- Distal arteritis with splinter hemorrhages, nail-fold infarcts, and possible gangrene (Figure 70-11)
- Cutaneous ulceration, including pyoderma gangrenosum
- Peripheral neuropathy with either a mononeuritis multiplex or a sensory-related stocking-glove polyneuropathy[84]
- Palpable purpura
- Arteritis involving viscera, similar to polyarteritis nodosum
- Rheumatoid pachymeningitis (rare), confined to the dura and pia matter

As with other forms of vasculitis, the signs and symptoms of systemic vasculitis in RA stem from vascular occlusion and resulting ischemic injury. Organ involvement is

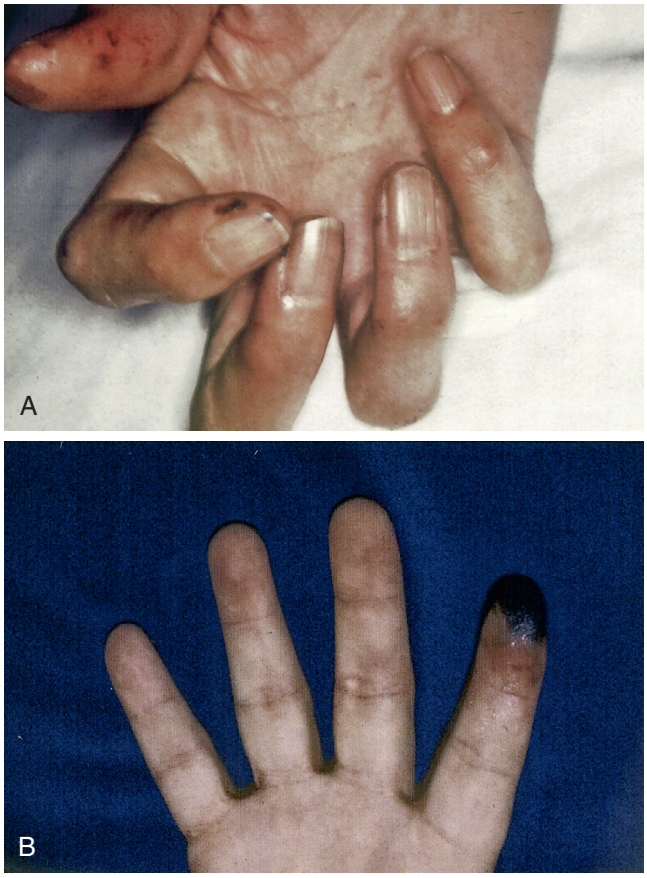



**Figure 70-11** **A**, Digital vasculitis with nail-fold infarcts in a seropositive patient. **B**, Digital gangrene of the index finger related to larger vessel involvement.

variable. Intestinal involvement typically manifests as abdominal pain with bowel infarction representing a surgical emergency. Digital gangrene, intestinal ischemia/infarction with bleeding or perforation, and mononeuritis multiplex are indicative of severe vasculitis and are associated with poor patient outcomes.[85,86] With diagnostic biopsy or tissue resection, histopathology in rheumatoid vasculitis typically demonstrates a "panarteritis" with fibrinoid necrosis. Obliterative endarteritis can be seen with distal gangrene, with the reported demonstration of immune complex deposition in the involved vessels.[87] As with other vasculitides, the treatment of rheumatoid vasculitis depends on the extent and severity of organ involvement. More conservative approaches can be used with splinter hemorrhage or palpable purpura in isolation, and more aggressive high-dose glucocorticoids and cytotoxic therapies are used for organ-threatening involvement.

## Pulmonary

Pulmonary involvement in RA is relatively common, with targets including the pleural or parenchymal tissue. Perhaps because of the increased prevalence of subclinical and clinical pulmonary involvement, patients with RA have a risk of lung cancer that is increased 1.5- to 3.5-fold versus that of the general population.[88] Although reported, clinically relevant small-airway disease and pulmonary hypertension appear to be relatively uncommon in RA,[89,90] and when present, appear to follow a relatively benign course. Several risk factors are associated with pulmonary complications related to RA, but some of the most important include smoking, RF and ACPA positivity, and being male.

**Pleural Disease.** Clinically apparent pleuritis may be evident in as many as 20% of RA cases, whereas postmortem examination suggests this number is higher. Pleural effusions in RA can be large and result in symptomatic dyspnea. Pleural effusions in RA are exudative, with mixed cell counts (predominance of monocytes), high protein and lactate dehydrogenase (LDH) levels, low glucose concentrations (resulting from a defect in the transport of glucose across the pleura), and low pH. Low pH and low fluid glucose, (10 to 50 mg/dL) are highly characteristic of RA-related effusions, although these values can also be observed in infectious etiologies (particularly tuberculosis).[91]

**Interstitial Lung Disease.** Pneumonitis and fibrosis are perhaps the most common and serious forms of pulmonary involvement in RA. On average, patients with RA complicated by ILD experience a 7-year reduction in median survival compared with patients with RA without lung disease.[92] With risk factors including male gender, smoking, older age at disease onset, and autoantibody (ACPA and RF) positivity,[93] ILD prevalence estimates vary widely across study populations, likely reflecting important differences in methods of case ascertainment. The clinical course of ILD in RA is also highly variable, ranging from an asymptomatic and indolent course to rapidly progressive and life threatening in a minority of patients. Radiographs typically demonstrate bibasilar reticular or reticulonodular patterns in both lung fields, with a characteristic honeycombing appearance in more advanced disease. HRCT and lung biopsy remain the gold standard for evaluation. Usual interstitial pneumonia (UIP) is the most common pattern seen on HRCT, with biopsy results showing diffuse fibrosis often in the midst of a mononuclear cell infiltrate (lymphocytes, plasma cells, and histiocytes). Even with limited disease, pulmonary function tests frequently reveal a decreased diffusion capacity with evidence of volume restriction (e.g., diminished vital capacity) becoming evident with more extensive ILD.[93,94]

Caplan's syndrome[95] represents a rare complication of RA, in which extra-articular ILD acts in concert with a pneumoconiosis (related to coal, silica, or asbestosis exposure) to produce a rapidly progressive obliterative granulomatous fibrotic syndrome. Lymphoid interstitial pneumonitis (LIP), a benign lymphoproliferative disorder of the lung, has been observed in patients with RA and Sjögren's syndrome.[96] Characteristic HRCT findings of LIP include ground-glass changes accompanied by thickened bronchovascular bundles, small ill-defined pulmonary nodules, and scattered thin-walled cysts within the lung parenchyma in a perivascular or subpleural location. Consistent with a lymphoproliferative process, approximately 80% of patients with LIP have evidence of a polyclonal or monoclonal IgM gammopathy.[97] Obliterative or constrictive bronchiolitis is an uncommon and severe form of interstitial pneumonitis that progresses to alveolar involvement and bronchiolitis, respiratory failure and, in some cases, death. Pathologically, bronchiolitis is characterized by fibroblast proliferation with proteinaceous exudates and interstitial infiltrates. The course and prognosis is distinct from cryptogenic organizing

pneumonia (COP) (also known as idiopathic bronchiolitis obliterans with organizing pneumonia) and nonspecific interstitial pneumonitis (NSIP), both of which are more responsive to immunosuppression than either UIP or bronchiolitis.

**Obstructive Lung Disease.** Although its links with restrictive lung disease has been long recognized, recent attention has focused on associations of RA with obstructive lung disease (OLD). In a recent population-based study from the Rochester Epidemiology Project, OLD was approximately 50% more likely to develop in individuals with RA than in controls, even after adjusting for age, gender, alcohol use, and smoking history (hazard ratio [HR], 1.54; 95% CI, 1.01 to 2.34).[98] In this cohort, chronic obstructive pulmonary disease (COPD) was the most prevalent form of OLD, with asthma and bronchiectasis far less common. Risk factors independently associated with the development of OLD among patients with RA included smoking, RF positivity, increased ESR, and a history of prior disease-modifying anti-rheumatic drug (DMARD) and steroid use.

**Nodular Lung Disease.** Rheumatoid nodules can develop in the pulmonary parenchyma, presenting either singly or in clusters, and serve as a hallmark of Caplan's syndrome, as already detailed. Characterized by central necrosis, pulmonary nodules from RA can cavitate, leading to the formation of bronchopleural fistulas. Although a core needle biopsy can be used for evaluation, particularly in the context of a solitary pulmonary nodule, a more aggressive diagnostic strategy may be warranted when results are equivocal or the patient is otherwise deemed to be "high-risk," recognizing that pulmonary nodulosis and bronchogenic carcinoma have been reported to co-exist in RA.[99]

### Cardiovascular

Cardiovascular disease (CVD) is common, representing the leading cause of excess mortality in RA. In addition to associations with atherosclerosis and coronary artery disease, extra-articular cardiac manifestations also relate to serositis, valvulitis and fibrosis.

**Atherosclerosis.** Patients with RA are disproportionately impacted by CVD outcomes,[100-102] including angina, myocardial infarction (MI), congestive heart failure (CHF), stroke, and the need for coronary revascularization. In a large prospective study, patients with RA were approximately twice as likely as individuals without RA to develop CHF.[102] Likewise, the rate for MI and stroke in RA are also approximately twofold higher than that of individuals without RA, as assessed in a cohort study of more than 25,000 patients with RA.[103] A recent meta-analysis noted a 50% increase in CVD-related mortality, with a 52% increase in death from cerebrovascular disease as well as a 59% increase in death from ischemic heart disease among patients with RA compared with non-RA controls.[100] The increased risk of CVD in RA appears to be independent of traditional risk factors such as hypertension, hyperlipidemia, and diabetes.[104]

The premature atherosclerosis and resulting CVD risk observed appears to be a consequence of the systemic inflammatory burden posed by RA. Several studies have now demonstrated that greater levels of RA disease activity, including both ESR and CRP, are robust predictors of major acute cardiac events.[105,106] These data underscore the potential importance of disease-remitting therapy, not only as a means of improving articular symptoms of RA, but also of mitigating CVD outcomes. In addition to associations with higher acute phase responses, both RF[107] and ACPA[108] positivity are associated with increased CVD. Although mechanisms linking autoantibody status to CVD have not been well elucidated, recent data from Sokolove and colleagues have shown that citrullinated autoantigens are expressed in atheromatous plaque.[109] Moreover, ACPA targeting citrullinated forms of fibrinogen and vimentin are associated with aortic plaque burden in patients with RA.

**Pericarditis.** Pericarditis is the most common cardiac manifestation of RA and may be seen in as many as 50% of patients at autopsy. Clinically apparent disease is far less common, with cardiac tamponade serving as a rare consequence. With RA involvement, pericardial fluid demonstrates findings similar to that described with RA-related pleuritis; an exudative sample characterized by cellular infiltrate, high protein and LDH concentrations, and a low glucose and low pH. Rarely, constrictive pericarditis can complicate the course RA and may require pericardectomy or pericardial stripping for definitive treatment.[110]

**Myocarditis.** Myocarditis is rare but may be related to granulomatous disease or interstitial myocarditis. The granulomatous process, when present, pathologically resembles rheumatoid nodules and is highly specific for RA.

**Conduction Defects.** Atrioventricular (AV) block is an unusual complication of RA, but if present is probably related to direct granulomatous involvement. Complete heart block has been described and is also more common in patients with seropositive nodular disease.[111] Because RA is a chronic inflammatory process, patients with long-standing, poorly controlled disease are at risk for amyloidosis, which can result in both cardiomyopathy and conduction disturbances.

**Endocardial Inflammation and Granulomatous Aortitis.** Often asymptomatic, echocardiographic studies have demonstrated a high prevalence of mitral valve abnormalities in RA.[112] Although more commonly associated with ankylosing spondylitis, aortic valve disease has also been observed as a consequence of RA.[113] In severe RA, granulomatous disease of the endocardium can spread to the base of the aorta resulting in aortitis and valvular incompetency, which can rarely necessitate emergent valve replacement.[114]

### Prognosis and Individual Patient Follow-up

Several factors have been associated with poor outcomes in RA. These outcomes are defined by reduced survival and increases in disability, related health care costs, the accrual in structural joint damage, and the need for total joint arthroplasty. Some of the factors that have been associated with poor outcomes include:

- Autoantibody (RF and ACPA) positivity[14-16]
- Measures indicative of greater disease activity at baseline[16]
- Extra-articular disease[14]
- Longer disease duration[16]
- Diagnostic and treatment delays[115]
- *HLA-DRB1* shared epitope status[116]

- Cigarette smoking and heavy alcohol use[14,117]
- Occupational factors and socioeconomic status[9]
- Erosive disease[116]
- Comorbid illnesses, including depression, post-traumatic stress disorder, and others[14,16,118]

Recent ACR treatment guidelines "risk-stratify" patients based on characteristics such as RF or ACPA positivity or the presence of radiographic erosions. It is important to recognize, however, that although these factors may be statistically correlated with select outcomes in RA, none of these (either alone or in combination) have been demonstrated to provide robust predictions of any outcome at the level of the individual patient. As emphasized in Chapter 71, improved outcomes have increasingly been achieved through the adoption of a treat-to-target strategy in RA management, an approach that is built on a foundation of routine follow-up accompanied by systematic disease activity assessments and treatment modifications as required to achieve a state of either low disease activity or remission. Through these efforts, we are also realizing fewer extra-articular complications to include fewer meaningful vascular complications.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 71

# Treatment of Rheumatoid Arthritis



James R. O'Dell

**KEY POINTS**

- Rheumatoid arthritis (RA) should be diagnosed early, and disease-modifying anti-rheumatic drug (DMARD) therapy should be initiated at the time of diagnosis.
- Treatment for all patients should be based on a disease activity target—either remission or low disease activity.
- The type of DMARD therapy the patient receives is not as important as ensuring that the treatment meets the disease activity target.
- The initial DMARD and the cornerstone of therapy for most patients is methotrexate.
- Many patients will require combinations of DMARDs with or without biologic agents to achieve the disease activity target.
- Many very effective biologic DMARDs are available; essentially all DMARDs are more effective when administered with methotrexate.
- Nonsteroidal anti-inflammatory drugs may provide useful symptom control but are rarely indicated without concomitant use of DMARDs.
- Glucocorticoids are rapidly effective DMARDs but have adverse effects. Therefore they should be used only with other DMARDs and ideally only as a bridge to effective DMARD therapy.
- The ubiquitous comorbidities of RA—particularly cardiovascular disease—must be addressed aggressively.

The treatment of rheumatoid arthritis (RA) has evolved dramatically during the past 30 years, perhaps more so than for any of the other rheumatic diseases. Every rheumatologist who has had the privilege of caring for patients with rheumatoid disease during that period will immediately and enthusiastically acknowledge how far we have come. **The majority of patients newly diagnosed with RA can expect to experience remission of their disease if it is treated early in the disease process by a rheumatologist.** This outcome is remarkable, and it is appropriate to take a step back and examine the reasons behind this sea change. Many persons quickly point to the fact that biologic agents became available in 1998, and they quickly allocate the lion's share of the credit for the dramatic improvement in outcomes to these remarkable medications. Statements such as, "Since the biologics have come on the market, results have improved dramatically" are commonplace. It is clear that this progress in treatment got a tremendous boost with approval of biologic agents almost 20 years ago. Biologic agents are very effective, and importantly, they have helped invigorate clinicians and patients alike, making both parties believe that higher expectations regarding control of RA are justified. However, it is important to acknowledge all the other changes that occurred in roughly that same time frame and have been universally embraced by rheumatologists (Table 71-1).

It is difficult if not impossible to know which of these changes, all of which have become accepted in the past quarter century, are most important. Clearly, early diagnosis aided by use of anti-citrullinated protein antibodies (ACPAs) and the new classification criteria are critical.[1] The universal acceptance that all patients with RA should be taking a disease-modifying anti-rheumatic drug (DMARD)[2-5] and the effective use of increasing doses of methotrexate (MTX) with folic acid have also played huge roles. Additionally, in the mid-1990s, the use of combination DMARDs became an accepted strategy.[6] Early diagnosis, effective use of MTX and combination DMARDs, and biologic agents are the tools that allow us to do the job. **Treat to target (T2T) defines what that job is**. Therefore T2T arguably has been the most important factor in improved outcomes in RA. The Tight Intensive Control of RA (TICORA) trial (discussed in more detail in a subsequent section of this chapter) was the seminal study that put T2T on the map, because it dramatically demonstrated the benefits of this approach.[7] The TICORA trial demonstrated that if patients are treated aggressively to achieve remission or low disease activity, the outcomes improve significantly both clinically and radiographically, even without the use of biologic agents. Similarly, in the Dutch Behandel-Strategieen (BeSt) study, the aggressive use of therapeutic adjustment (T2T) led to significant clinical improvement regardless of the initial choice of drugs, which included both biologic agents and conventional regimens.[8] Therefore **the most important tenet of successful RA therapy today is not which of the score of DMARDs, conventional or biologic, are used (Table 71-2) but that all patients are treated to meet the disease activity target.**

Despite how far we have come, many challenges remain, including first and foremost the identification of markers that predict in a differential fashion who will respond to or experience adverse effects from which treatment, along with further developing methods to allow us to measure the amount of immunosuppression that our agents are producing so we can titrate doses appropriately.

In this chapter the following topics will be discussed: the broad principles of treatment, the goals of RA therapy, the timing of different therapies, and the strategies of using the plethora of options now available to achieve the best

disease control for each patient. Most of the drugs themselves will not be covered in detail here; rather, this chapter will focus on strategies. Please refer to the many other excellent chapters in this book for specific information on nonsteroidal anti-inflammatory drugs (NSAIDs; Chapter 59), glucocorticoids (Chapter 60), traditional DMARDs (Chapter 61), immunoregulatory drugs (Chapter 62), anticytokine therapies or biologic agents (Chapter 63), and the new Janus kinase (JAK) agents (Chapter 65).

## GOAL OF RHEUMATOID ARTHRITIS TREATMENT

It is truly remarkable that rheumatologists now have more than 20 approved conventional or biologic DMARDs from which to choose (Table 71-2). However, as previously noted, despite all these terrific DMARD options, **the most important paradigm shift for the treatment of RA has been the realization that patients should be treated early and with a target of low disease activity or remission.**[2-5,7] For persons outside the rheumatologic community, this seems like stating the obvious—if patients have hypertension, hyperlipidemia, or diabetes, they are of course treated so that their blood pressure, low-density lipoprotein, or glycosylated hemoglobin (HbA1c) levels, respectively, are reduced to a defined and easily measured goal. The problem with RA has been the lack of valid reproducible measures of disease activity and remission that can be routinely measured and followed up on in the clinic. Unfortunately, no single examination finding or laboratory test satisfactorily measures disease activity in persons with RA.

Many measures have been proposed,[9-15] all of which are composite measures that include information derived from some combination of joint examinations, patient and physician assessment of disease activity, patient function, and laboratory measures of inflammation (erythrocyte sedimentation rate [ESR] or C-reactive protein [CRP] levels). Recently the American College of Rheumatology (ACR) has endorsed a list of disease activity measures that have been shown to correlate with outcomes. Table 71-3 is a partial list of some of the better known of these measures. Each of these measures has strengths and weaknesses[15]; some rely only on data from the patient, some require complete joint counts by clinicians, and some require laboratory tests to measure inflammation. Busy clinicians rarely have time to document disease activity in more than 60 tender and swollen joints or wait for laboratory test results to make treatment decisions during a patient's visit. Therefore measures that simplify this process as much as possible are being embraced, including measures that limit the joints that are examined to 28 (Disease Activity Score in 28 joints [DAS28]), do not require laboratory tests (Clinical Disease Activity Index), or are entirely dependent on patient-reported data (Routine Assessment Patient Index Data). A high correlation exists among these measures, and thus currently in the clinic **it is very important that disease activity be measured and less important which measure is used.**

Because none of our therapies cure RA, it would seem obvious that the next best goal should be remission. However, the concept of remission as a goal for patients with RA is problematic. First, a definition of remission that is both relevant and practical has been elusive. To be relevant, remission should be highly predictive of the absence of disease progression over time. To be practical for

**TABLE 71-1** Improved Outcomes in Patients with Rheumatoid Arthritis: Nonbiologic Changes That Have Moved Us Forward

| |
|---|
| Emphasis of and improvement in early diagnosis |
| Use of DMARDs in all patients |
| Increasing use and dose of methotrexate |
| Use of folic acid with methotrexate |
| Use of combinations of DMARDs |
| **Treating all patients on the basis of a disease activity target** |

DMARDs, Disease-modifying anti-rheumatic drugs.

**TABLE 71-2** Disease-Modifying Anti-rheumatic Drugs as of 2015*

| Conventional | Biologic Agents |
|---|---|
| Methotrexate | Etanercept |
| Hydroxychloroquine | Infliximab |
| Sulfasalazine | Anakinra |
| Leflunomide | Adalimumab |
| Gold, intramuscular and oral | Abatacept |
| Azathioprine | Rituximab |
| Minocycline | Certolizumab |
| Cyclosporine | Golimumab |
| Penicillamine | Tocilizumab |
| Glucocorticoids | |
| Tofacitinib | |

*Drugs that have the ability to slow or halt progression of rheumatoid arthritis, including radiographic progression.

**TABLE 71-3** Instruments Used to Measure Rheumatoid Arthritis Disease Activity

| Instrument | Score Range | Remission | Thresholds of Disease Activity | | |
|---|---|---|---|---|---|
| | | | Low | Moderate | High |
| Disease Activity Score in 28 joints (DAS28) | 0-9.4 | ≤2.6 | ≤3.2 | >3.2 and ≤5.1 | >5.1 |
| Simplified Disease Activity Index (SDAI) | 0.1-86.0 | ≤3.3 | ≤11 | >11 and ≤26 | >26 |
| Clinical Disease Activity Index (CDAI) | 0-76.0 | ≤2.8 | ≤10 | >10 and ≤22 | >22 |
| Rheumatoid Arthritis Disease Activity Index (RADAI) | 0-10 | ≤1.4 | <2.2 | 2.2 and ≤4.9 | >4.9 |
| Patient Activity Scale (PAS or PASII) | 0-10 | ≤1.25 | <1.9 | ≥1.9 and ≤5.3 | >5.3 |
| Routine Assessment Patient Index Data (RAPID) | 0-30 | ≤1 | <6 | ≥6 and ≤12 | >12 |

**TABLE 71-4** American College of Rheumatology/European League Against Rheumatism Definitions of Remission in Rheumatoid Arthritis Clinical Trials

| |
|---|
| **Boolean-Based Definition** |
| At any time point, the patient must satisfy all of the following: |
| Tender joint count ≤1* |
| Swollen joint count ≤1* |
| C-reactive protein ≤1 mg/dL |
| Patient global assessment ≤1 (on a 0-10 scale) |
| **Index-Based Definition** |
| At any time point, the patient must have a Simplified Disease Activity Index score of ≤3.3. |

*Include 28 joints plus feet and ankles.

From Felson DT, Smolen JS, Wells G, et al: American College of Rheumatology/European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials, *Arthritis Rheum* 63:573–586, 2011.

clinicians, a definition of remission should be easy to apply in real time to patients seen in the clinic, as previously discussed with regard to measures of disease activity. A new definition of remission for use in clinical trials has been developed by a joint ACR and European League Against Rheumatism (EULAR) effort (Table 71-4[1]). This definition has been rigorously tested against short-term radiologic outcomes in 1- to 2-year randomized controlled trial (RCT) follow-up. This definition standardizes remission and therefore is a huge step forward for reporting and comparing results across clinical trials.

However, this definition was designed for clinical trials and not for clinical care,[16] where rigorous counts of joints with disease activity are not always performed and the need to have CRP results in real time becomes a major problem for clinical work flow. Versions of disease activity scales that do not require laboratory values have been suggested but have not been fully accepted (e.g., the Clinical Disease Activity Index and Patient Activity Scale). Perhaps more problematic is that many persons who believe that remission is defined by clinical data alone will always underestimate the amount of low-level disease activity that could be found if synovial biopsies or advanced imaging techniques such as ultrasound or magnetic resonance imaging (MRI) were used. Significant data indicate that many and perhaps most patients with RA who meet the definitions of "remission" are found to have active disease when they are assessed by ultrasound or MRI.[17-19] Recently it was reported that all patients believed to be in clinical remission during the Treatment of Early Aggressive Rheumatoid (TEAR) trial (discussed in detail in a subsequent section of this chapter) had evidence of inflammation on MRI scans.[20] Indeed, the newly accepted ACR/EULAR definition allows for a swollen joint, which many persons would argue is not really full clinical remission. Another major problem with the term "remission" is that, according to currently available data, **it is not at all clear that remission, regardless of how it is defined, should be the treatment goal for all patients with RA**.

Many patients do very well despite having low levels of disease activity. This situation may be analogous to the recent studies that show that pushing HbA1c levels below 6.5, which seemed appropriate for diabetic control, was associated with increased cardiovascular mortality, mainly as a result of hypoglycemia in patients with histories of cardiovascular disease.[21] **The following important question remains unanswered: In RA, where is the tipping point between pushing the disease activity ever lower and the toxicity and cost of doing so?** To further complicate matters, it is almost guaranteed that the tipping point will be different for each patient. The following issues need to be considered:

- When do the risks and considerable expense of some of our RA therapies outweigh the benefits of escalating therapy further?
- Which patients who have improved dramatically but still have two tender or swollen joints need to switch to a third biologic agent?
- With regard to the aforementioned diabetic patients with cardiovascular disease, which patients with RA are most at risk for toxicities if we push too hard for remission?
- Finally, with current therapies, the vast majority of remissions in persons with RA require ongoing treatment with DMARDs, so the concept of a true remission—meaning one where no therapy is required—remains beyond our current reach for most patients.

Despite the problems in defining remission or low disease activity, **it is clear that patients do better when clinicians have a goal.** The Scottish TICORA study[7] was the first to convincingly demonstrate this concept in a randomized fashion. In this study, patients who had disease for fewer than 5 years were randomized to receive either routine care or intensive care. Both groups were treated with an algorithm of conventional DMARDs (Figure 71-1A). The routine care group had regular follow-up and monitoring, whereas the intensive group was seen monthly and underwent proscribed escalation of therapy (per a protocol) if they had not achieved the goal of low disease activity (defined in this study as Disease Activity Score [DAS] of ≤2.4). Both groups improved significantly, but the T2T group (i.e., the intensive group) did significantly better, with mean DAS scores (1.6) in the remission range at 18 months (Figure 71-1B). In the intensive group, 71% of subjects achieved an ACR 70% composite improvement (ACR70) compared with 18% in the routine care group ($P$ <0.0001). Further, this clinical improvement translated to significantly less radiographic progression of erosions compared with the routine care group (0.5 vs. 3.0; $P$ = 0.002). Importantly, this improved disease control was not associated with an increase in treatment-associated adverse events. Finally, although it entailed more frequent visits, intensive therapy resulted in a cost savings even in the short term. These results were particularly remarkable considering that they were obtained using conventional DMARDs alone (Figure 71-1A), without use of biologic agents. Findings from other studies have corroborated these findings.[8,22,23] Further, a meta-analysis of tight control strategies[24] has suggested that tight control strategies work best if they are performed according to a protocol, as occurred in the TICORA study.

Although the TICORA investigators selected low disease activity as the target, the target could have been remission, as was previously discussed. Most of the

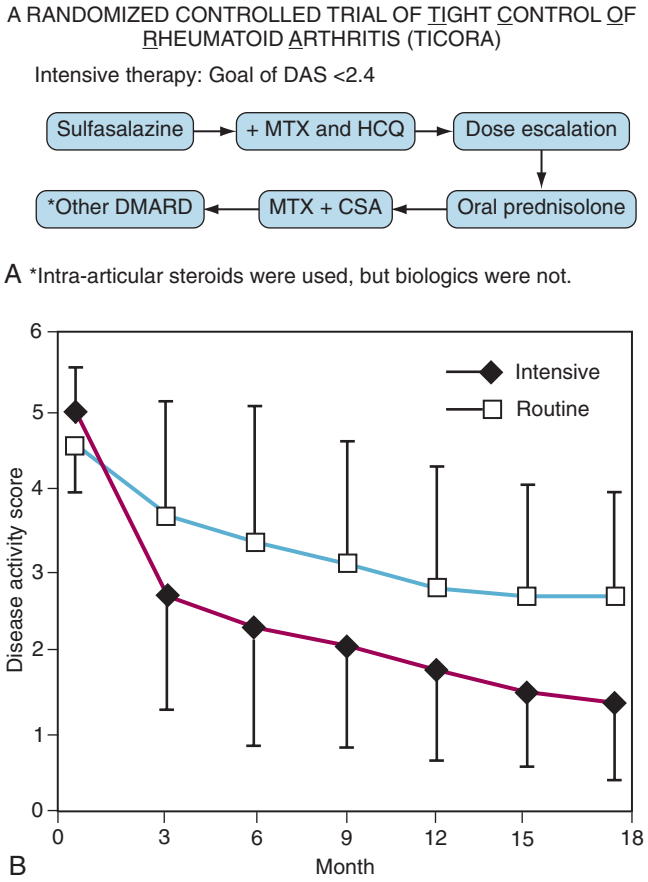



**Figure 71-1** **A,** Treatment escalation used in the Tight Intensive Control of RA (TICORA) trial. **B,** Comparison of intensive and routine treatment groups. CSA, Cyclosporine A; DAS, Disease Activity Score (in 44 joints); DMARD, disease-modifying anti-rheumatic drug; HCQ, hydroxychloroquine; MTX, methotrexate. *(Modified with permission from Grigor C, Capell H, Stirling A, et al: Effect of a treatment strategy of tight control for rheumatoid arthritis [the TICORA study]: a single-blind randomized controlled trial,* Lancet *364:263–269, 2004.)*

aforementioned endorsed measures of disease activity have defined levels that signify "remission" (see Table 71-4). Predictably, the harder we push for remission with increasing numbers and doses of DMARDs, both conventional and biologic, the more that the toxicity and expense (Table 71-5) of our treatments become a concern. Both the ACR and EULAR guidelines, as well as recent reviews,[2-5] state that low disease activity or remission are the goal and leave the decision about which goal is most appropriate for each unique patient's situation to the clinician. **Therefore, until further data elucidate this question, clinicians will need to continue to practice both the art and the science of medicine when selecting the most appropriate target for each patient.**

## CLASSES OF DRUGS

### Disease-Modifying Anti-rheumatic Drugs

#### Methotrexate, Sulfasalazine, Hydroxychloroquine, Leflunomide, and Azathioprine

By definition, a DMARD has the ability to change the course of RA (for the better). The most rigorous application of this definition requires RCTs to show that a DMARD has the ability not only to change the clinical course of the disease but also to decrease or halt the radiographic progression. By this definition, all of the 11 conventional DMARDs and the 9 biologic DMARDs listed in Table 71-2 qualify, with the possible exception of minocycline and hydroxychloroquine (HCQ), for which only weak evidence of radiographic benefits exists. When the DMARDs listed in Table 71-2 are used individually or in combinations of two, three, or four, as is often the case, more than 4000 possible combinations exist for each individual patient, assuming that biologic agents are not used in combination. Obviously, this huge number of choices is both good news and bad news for the clinician; it is great to have all the options but impossible to keep them all straight. Therefore, to use these drugs effectively, the clinician must have goals, strategies, and an up-to-date knowledge of the drugs and their interactions and toxicities.

The most widely used conventional DMARDs—MTX, sulfasalazine (SSZ), HCQ, and leflunomide (LEF)—are discussed in detail in Chapter 61. Together, these four DMARDs, along with glucocorticoids (see Chapter 60), currently account for the vast majority of conventional DMARD use. The new player in this arena is tofacitinib (see Chapter 65); its potential niche in the treatment of RA will be discussed in a subsequent section of this chapter. Although less commonly used, azathioprine, cyclosporine, and the tetracyclines (minocycline and doxycycline) remain important as niche therapies. They are not covered elsewhere in this book and thus will be discussed here. Gold, which has been used in the treatment of RA for more than a century, is an effective therapy, particularly if used intramuscularly,[25-29] but it is cumbersome to administer and has a narrow therapeutic window. At present it is used so rarely that it will not be discussed for the first time in the history of this textbook. Penicillamine is of historical interest[30] but is rarely used and will not be discussed in this chapter.

**TABLE 71-5** Costs of Disease-Modifying Anti-rheumatic Drugs in the United States in 2015

| Medication | Cost |
|---|---|
| **1 Month oral DMARD from Walgreen's** | |
| Methotrexate #32, 2.5-mg tablets | $101 |
| Hydroxychloroquine, 200 mg, #60 | $222 |
| Sulfasalazine, #120, 500-mg tablets | $33 |
| Tofacitinib (Xeljanz), 5 mg, #60 tablets | $3002 |
| Etanercept, 50 mg, syringe #4 | $4082 |
| Adalimumab, 40 mg, syringe #2 | $4224 |
| Adalimumab, 40 mg, pen #2 | $4224 |
| **1 Month IV DMARDs from Good RX*** | |
| Infliximab, 300 mg | $3180 |
| Rituximab, 1500 mg | $2350 |
| Abatacept, 750 mg | $2200 |
| Tocilizumab, 400 mg | $1650 |

*Mean of available data (medication only, not infusion price).
DMARDs, Disease-modifying anti-rheumatic drugs; IV, intravenous.

### Biologic Disease-Modifying Anti-rheumatic Drugs

The biologic DMARDs are discussed in detail in Chapter 63. Within this class of agents we now have the ability to inhibit multiple inflammatory cytokines, including TNF, which is inhibited with three monoclonal antibodies, infliximab, adalimumab, and golimumab; the TNF receptor protein, which is inhibited with etanercept (ETAN); the pegylated Fab fragment, which is inhibited with certolizumab; IL-1, which is inhibited with the IL-1 receptor antagonist anakinra; and IL-6, which is inhibited by the monoclonal antibody to the IL-6 receptor, tocilizumab. We also have the ability or to kill or inhibit cell lines important in inflammation, including B cells (with rituximab) and T cells (with abatacept). **It is an understatement to say that biologic agents have changed the landscape of RA therapy forever** both in terms of therapeutic expectations and the understanding of RA pathogenesis, but as previously discussed, it is also important to acknowledge that they are not the only game changer (see Table 71-1). Because of their often quick onset of action, particularly with the TNF inhibitors and tocilizumab, and their ability to retard radiographic progression of disease, they are increasingly used earlier and more often in persons with RA. The challenge for clinicians is to appropriately integrate conventional and biologic therapies and to use biologic agents when necessary but to make sure the much less expensive conventional therapies have been optimized.

### Glucocorticoids

Glucocorticoids, which are discussed in detail in Chapter 60, have a long and storied history in the treatment of RA. RA was selected as the first disease to be treated with "compound E" at the Mayo Clinic in 1948, and the rest is history.[31] Responses were both rapid and dramatic; an analysis of the first 14 patients treated revealed that in 100%, ESRs improved by more than 50% within 1 to 3 months, and in 80%, ESRs improved by at least 70% (an ESR of 70 is a convenient ACR70). Several landmark studies have proved not only clinical efficacy[32-37] but also the significant radiographic efficacy of glucocorticoids.[35,37] The recent Combinatie therapie Bij Rheumatoide Artritis (COBRA) trial[36,37] and the COBRA arm of the BeSt study (discussed in a subsequent section of this chapter[8,22]) again demonstrated the significant clinical and radiographic benefit that glucocorticoids can provide. However, despite their rapid onset and all the other positive benefits of glucocorticoids in RA, their toxicities are also legend.

Currently glucocorticoids are most often and most appropriately used along with DMARDs as part of initial "induction" therapy to gain rapid control of RA, and then they are aggressively tapered as the slower-acting DMARD starts to kick in. Recent data suggest that patients whose treatment is initiated with the combination of MTX and prednisone initially have better results than do patients who take MTX alone.[38] Historically, the belief has been that once a patient with RA began taking glucocorticoids, he or she would never be able to stop using them. This scenario is clearly not true because of the effective DMARDs that are now available, and successful tapering of glucocorticoids is now the rule.[8,22,39] The inability to taper glucocorticoids successfully or at least to taper them to an "acceptable" low dose is a strong indication that the current DMARD program is not working. Long-term use of doses equivalent to prednisone of greater than 7.5 to 10 mg per day is a clear indication that DMARD therapy needs to be escalated. Importantly**, glucocorticoids should almost never be used to treat RA without concomitant use of DMARDs.**

### Other Conventional Disease-Modifying Anti-rheumatic Drugs

**Azathioprine.** Azathioprine (AZA), 50 to 200 mg/day, has been used to treat RA for more than 50 years. Because it has been a generic drug for many years, little recent research has been performed on AZA. Although clearly it is not a first-line DMARD in contemporary RA treatment, AZA is most commonly used as a substitute for MTX when contraindications or intolerance to MTX exist. Arguably, all the biologic agents work better with concomitant use of MTX, and this phenomenon is particularly true of the TNF inhibitors. One reason for the increased efficacy of TNF inhibitors with concomitant use of MTX is the ability of MTX to prevent the formation of antidrug antibodies. A recent meta-analysis has shown that whereas MTX is most effective at preventing the formation of these antibodies (providing protection at the 77% level), AZA also does so at about the 50% level.[40] Therefore, when patients are being treated with monoclonal antibody therapies and cannot tolerate MTX, the use of AZA should be strongly considered. This situation most commonly arises in patients with the so-called "MTX flu," but other situations, including pregnancy and liver, pulmonary, or renal disease, may be indications for use of AZA in persons with RA. A particularly problematic patient population is patients with RA, frequently male, who have RA interstitial lung disease. Use of MTX and LEF may be problematic, and AZA becomes the best conventional DMARD choice. Currently, AZA is usually used in combination with other conventional or biologic DMARDs. McCarty, who was one of the pioneers of combination therapy in RA, has with several colleagues reported on the combination of MTX, AZA, and HCQ in 69 patients treated in an open-label fashion.[41] With this combination, 45% of patients reached remission according to the previous ACR criteria, and this combination was well tolerated. Further, the combination of low doses of AZA and MTX was more effective than higher doses of either drug alone, although this difference did not reach statistical significance in an underpowered study.[42]

Neutropenia is the most common complication of AZA treatment. Neutropenia can be predicted with a genetic test for polymorphisms of the enzyme thiopurine methyltransferase. Patients who are homozygous for the mutant polymorphism that is nonfunctional (1 in 300 or 0.3% of patients) are very sensitive to bone marrow and other toxicities of AZA. Patients who are heterozygotes (perhaps 10% of the population) may have milder neutropenia.[43] Unfortunately, this test is expensive; in some centers it may cost up to a $1000, and reimbursement is not always assured. Some clinicians elect to start with low doses of 50 mg/day, check complete blood cell counts at 2 weeks, and then increase the dose as needed if the white blood cell count is acceptable. It has been speculated that the subset of patients

with the nonfunctional polymorphisms could be the patients who, when AZA was added to a stable MTX regimen, experienced an acute febrile toxic reaction characterized by fever, leukocytosis, and a cutaneous leukocytoclastic vasculitis.[44]

**Cyclosporine.** In the 1990s, cyclosporine (CSA) gained a foothold in the treatment of RA.[45] CSA, which is mostly used in transplantation procedures to prevent allograft rejection, inhibits the activation of $CD4^+$ helper-inducer T lymphocytes by blocking IL-2 and other T helper type 1 cytokine production[46] and by inhibiting CD40 ligand expression in T lymphocytes.[47] The latter effect prevents T cells from delivering CD40 ligand-dependent signals to B cells. Interest in CSA peaked in the mid-1990s when Tugwell and colleagues[48] showed that the addition of CSA (2.5 to 5 mg/kg/day) to a stable dose of MTX provided substantial additive benefit compared with MTX alone. In the CSA + MTX group, an ACR 20% composite improvement (ACR20) was achieved by 48% of subjects, compared with 16% of subjects for the placebo group (Figure 71-2). Additionally, this therapy seemed to slow radiographic progression of erosions.[49] In this trial, the dose of CSA was decreased if the patient's creatinine level increased to more than 30% of initial values. Unfortunately, follow-up reports revealed that only 22% of patients continued taking this combination at 18 months, with the most common reasons for discontinuation being hypertension or increasing creatinine levels.[49] Treatment of patients with hepatitis C and RA may provide a niche for CSA, because it has anti-viral activity against hepatitis C.[50,51]

## Minocycline and Doxycycline

Tetracycline and its derivatives have a long and somewhat checkered history with regard to the treatment of RA and other arthritidies.[52,53] They are not mainstream therapy for RA but may have a niche in patients for whom laboratory monitoring is not feasible, in patients with frequent infections, or in patients who have concomitant periodontal disease. The mechanism of action of tetracyclines in RA is poorly understood. Tetracyclines are, of course, antibiotics, but they also inhibit metalloproteinases, modulate immune responses, and have anti-inflammatory effects. No evidence exists to support the claim that tetracyclines treat the "infection that causes RA," as was touted by some of the original supporters of this drug.[53] However, it is entirely possible that inhibition of nonspecific infections that upregulate the immune response via IL-1, TNF, and IL-6, such as periodontitis, bronchitis, and gastritis, to name a few, may be helpful in controlling disease in patients with RA.

Increasingly, the microbiome and the role it may be playing in RA pathogenesis, disease activity, and responses

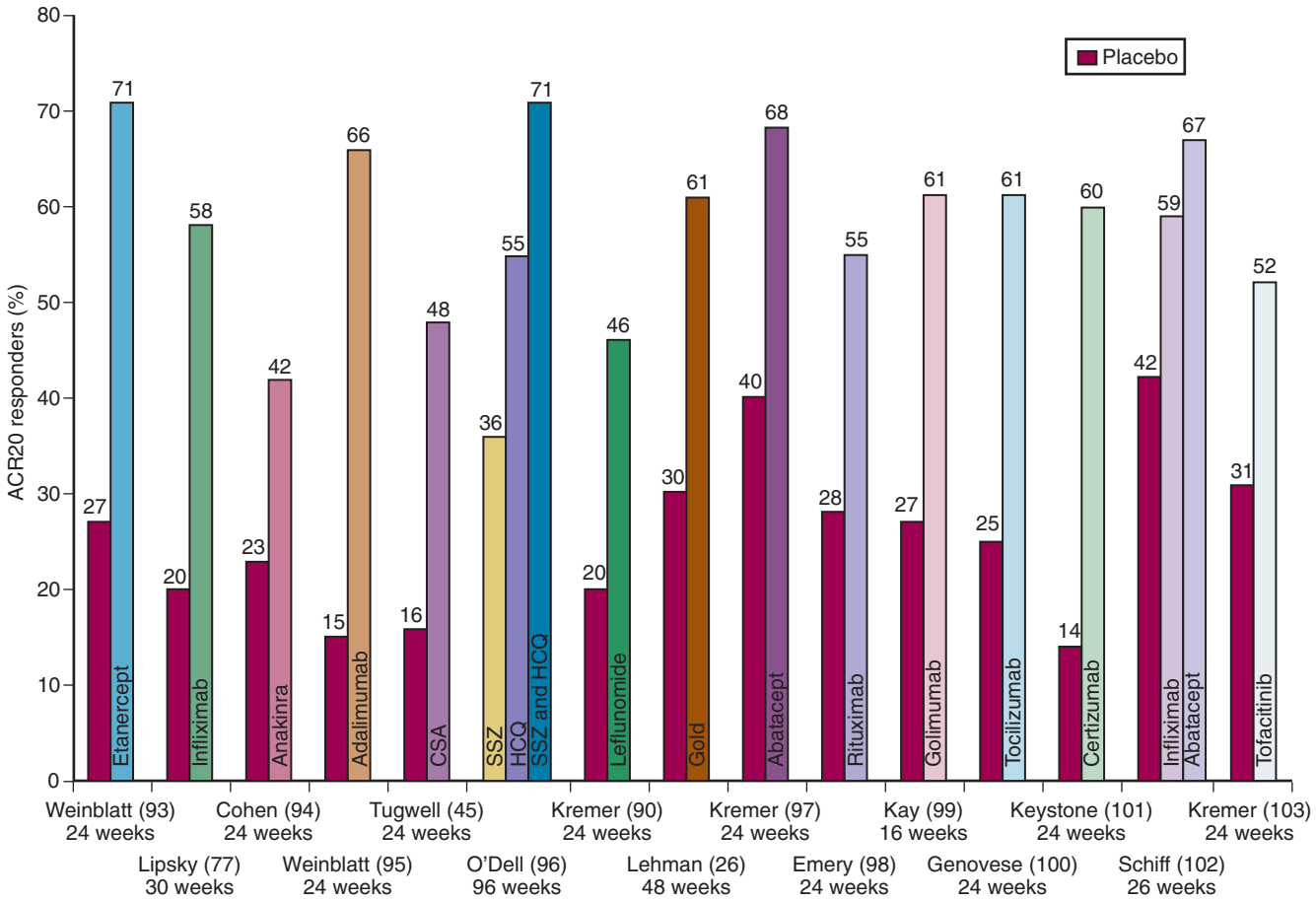



**Figure 71-2** Blinded trials of therapies in patients with active disease despite methotrexate. ACR20, American College of Rheumatology 20% composite improvement; CSA, cyclosporine; HCQ, hydroxychloroquine; SSZ, sulfasalazine.

to therapies is receiving attention.[54,55] Modification of the microbiome by tetracyclines is another possible mechanism of action. Tetracyclines also have the ability to inhibit biosynthesis and activity of matrix metalloproteinases, which have a principal role in degrading articular cartilage in persons with RA. This inhibition has been effective in osteoarthritis treatment. The presumed mechanism is through chelation of calcium and zinc molecules, which subsequently leads to altered molecular conformations of proenzymes sufficiently to inactive them.[56,57] Minocycline has mild but definite inhibitory effects on synovial T cell proliferation and cytokine production and has been shown to upregulate IL-10 production. Further evidence of its ability to modulate the immune system is the fact that it is known to induce anti-DNA antibody positive lupus in some patients, especially when it is used to treat acne.

Given in a dose of 100 mg twice daily, moderate statistically significant improvement in clinical parameters of disease activity was found in patients with established RA who were treated with minocycline compared with placebo.[58,59] Findings in the treatment of early-stage RA have been more impressive. In a study of 46 patients with early-stage rheumatoid factor (RF)-positive RA who had not received previous treatment, researchers reported that 65% of patients experienced 50% improvement in tender and swollen joints, duration of morning stiffness, and ESR (Paulus criteria), whereas only 13% of the placebo recipients improved similarly over a 6-month period.[60] In 2001, the results of a 2-year trial comparing minocycline with HCQ were published.[39] In this small study of patients with early-stage RF-positive RA, the patients treated with minocycline were more likely to achieve an ACR 50% composite improvement (ACR50; the primary endpoint) than were the patients treated with HCQ (60% vs. 33%) and were more likely to experience success in the tapering of glucocorticoids. This study reconfirms the potential utility of minocycline, particularly in early-stage RF-positive patients.

Although doxycycline is less frequently studied, evidence supports the use of this drug in the treatment of RA. In a trial of patients with early RA, doxycycline plus MTX was compared with the use of MTX alone. Investigators studied low-dose (20 mg twice a day) and high-dose (100 mg twice a day) doxycycline in combination with MTX and found that both approaches were superior to MTX alone.[61] It is intriguing to speculate that the doxycycline in this combination alters the gut microbiome to allow MTX to be more effective. Despite the positive results of this study, it needs to be replicated.

Potential adverse effects of tetracyclines include lightheadedness, vertigo, rare liver toxicity, drug-induced lupus, and, with longer-term use, cutaneous hyperpigmentation.[62] Elderly patients appear to be at an increased risk of vertigo. Lupus-like syndromes have developed in patients taking minocycline, complete with autoantibodies, including anti-DNA and occasionally perinuclear anti-neutrophil cytoplasmic antigen.[63] Drug-induced lupus has not been reported to develop with doxycycline or with the use of minocycline in patients with RA. The hyperpigmentation can be impressive and may limit treatment in some persons.[62] Hyperpigmentation resolves with discontinuation of minocycline and occurs much less commonly, if at all, with doxycycline.

### Nonsteroidal Anti-inflammatory Drugs

NSAIDs, including salicylates, which are covered in detail in Chapter 59, have been a ubiquitous part of RA treatment for more than a century. During the past several decades, as toxicities (particularly gastrointestinal,[64,65] cardiovascular,[66,67] and renal[68,69]) have become apparent and our DMARDs have become more effective, the use of NSAIDs, fortunately, has been declining. The somewhat surprising cardiovascular toxicities, which are now known to be strongly associated not only with the COX-2–specific NSAIDs but essentially with all NSAIDs, have been particularly concerning with regard to the RA patient populations in which the main excess mortality is largely due to accelerated cardiovascular disease. As with glucocorticoids, **NSAIDs should rarely be used without concomitant use of DMARDs**. Also as with glucocorticoids, the goal should be to taper use of NSAIDs as soon as possible to avoid gastrointestinal and cardiovascular toxicities.

## TREATMENT APPROACHES AND STRATEGIES

As previously detailed, the goal of treatment for all patients with RA is remission or at least low disease activity; other than toxicity concerns and, obviously, issues relating to expense, **clinicians should not focus on which drug or combination of drugs is used in an individual patient but should focus on getting patients to the disease activity target with whatever drugs achieve that goal.** There is no magic or correct DMARD or combination of DMARDs that is right for all patients. Each patient presents a unique challenge and comes with unique expectations, biases, disease activity level, damage burden, comorbidities, and, in many countries, insurance coverage issues. **One of the most important areas of investigation in RA is to identify parameters that will predict in a differential fashion which patient will respond to which therapy.** Thus far, no clear-cut answers that are applicable to the clinical care of the vast majority of patients have emerged.

Some investigators have suggested that treatment should be different for patients with RA who have good prognosis versus poor prognosis. This concept is problematic, because separating patients into those who have a good versus a poor prognosis is difficult. Although some data suggest that certain features are associated with worse prognosis (Table 71-6), unfortunately, no data exist that suggest that stratifying our therapies based on prognosis at the individual patient level yields better outcomes. An example might be that if we could perfectly score prognosis on a scale of 1 to 10, it would be conceivable that patients with intermediate scores would benefit the most from our most aggressive therapies, whereas patients with low scores do not need them and patients with the highest scores may have an unacceptable benefit-to-toxicity ratio. Most patients who meet the criteria for classification of RA in the clinic and essentially all those included in clinical trials have poor-prognosis RA, with several of the factors listed in Table 71-6. Regardless of the prognostic factors, the goal for each individual patient is to achieve at least a low level of disease activity. Until or unless parameters are identified,

**TABLE 71-6** Factors Associated With Poor Prognosis in Rheumatoid Arthritis

| |
|---|
| Presence of rheumatoid factor and titer |
| Presence of antibodies to CCP and titer |
| Presence of shared epitope and number of alleles |
| Presence of erosive disease at presentation |
| Disease activity at presentation |
| Magnitude of ESR or CRP elevations |
| Presence of nodules or extraarticular features |
| Female gender |
| Smoking currently and in the past |
| Obesity |

CCP, Cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

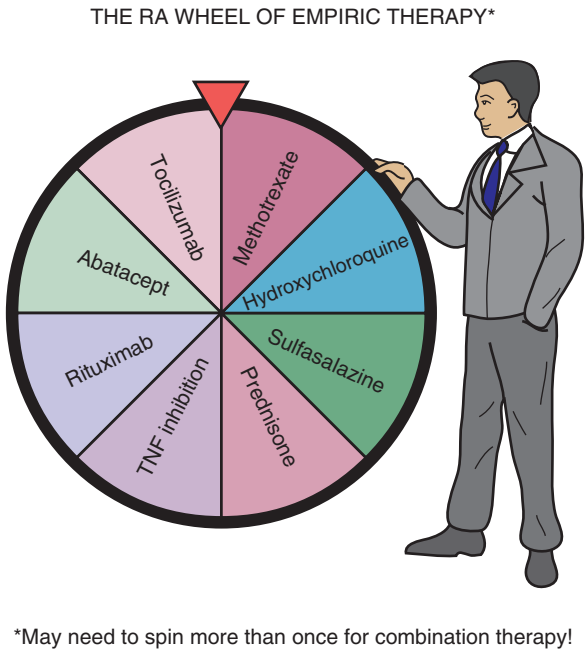



**Figure 71-3** One approach to selecting therapy for patients with rheumatoid arthritis (RA). TNF, Tumor necrosis factor. *(Artwork courtesy Robert Wigton, MD.)*

rheumatologists will of necessity continue to use their clinical judgment at the individual patient level.

Without parameters that predict in a differential manner the response to medications in terms of efficacy or toxicity, one approach to treatment decisions is illustrated in Figure 71-3. Note that several "spins" of the wheel may be required. Figure 71-3 makes the point that we do not have these much-needed parameters. The figure also emphasizes that if we hope to treat RA in a rational, scientific way, it is critical that we find these parameters, and it highlights and reinforces the need to include biobanks with all of our clinical trials. With regard to individual patients, Figure 71-4 (ACR recommendations[3]) is perhaps a more practical approach. Specific patient populations will be discussed in the following sections.

## Treatment of the Disease-Modifying Anti-rheumatic Drug–Naïve Patient

The most critically important principle of treating RA effectively is to **initiate and rapidly advance DMARD therapy as early as possible in the disease process**. Although this principle is universally accepted, a paucity of rigorous data directly addresses this point. Few randomized, double-blind trials have been conducted in which patients are randomized to early-stage treatment versus late-stage treatment,[70-73] and it is very unlikely, not to mention unethical, that any more will be forthcoming. Rather, we accept this foundation principle based on the common and firm belief that treating patients early in the disease process prevents damage and deformity and preserves function. Many trials and case series provide strong, credible evidence to support this central tenet. Evidence to support this commonsense belief comes from cohort studies of early versus delayed therapy,[74] randomized studies of intensive therapy versus usual care,[7] RCTs of combination versus monotherapy,[8,22,36,75-79] and, finally, studies of what was previously defined as pre-clinical RA.[73]

One cohort study of note reported on patients who had recently received a diagnosis of RA. The first cohort received DMARD therapy early (a mean of 123 days after diagnosis), whereas the second cohort received DMARDs very early (a mean of 15 days after diagnosis[74]). The first cohort had significantly more radiographic damage at 2 years and, importantly, continued to have radiographic progression, unlike the second cohort. The findings of the TICORA trial (previously detailed[7]) clearly show the advantages of better control of disease with earlier treatment. Multiple studies in early-stage RA have demonstrated that when groups of patients are compared, those that receive combinations of therapy fare better than do those that receive monotherapy.[8,22,36,75-79] Some examples of these studies are COBRA, with the combination of MTX, SSZ, and prednisolone versus SSZ alone[36]; the Finnish Rheumatoid Arthritis Combination Therapy (FIN-RACo) trial,[75] with the combination of MTX, SSZ, HCQ, and prednisone versus SSZ; the BeSt study, with the multiple combinations compared with step-ups or switches[8,22]; the Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) trial,[77] with the combination of infliximab and MTX versus MTX alone; and the PREMIER study, with the combination of adalimumab and MTX versus each alone.[78] In all these trials and many others, the combinations outperformed monotherapy. However, as discussed in a subsequent section of this chapter, it does not follow that all patients need combination therapy initially.

The new ACR/EULAR RA classification criteria[1] should allow us to classify patients with RA earlier and therefore treat them earlier. The criteria, discussed in detail in Chapter 70, were designed to allow rheumatologists to classify patients in clinical trials with RA as early as possible. Gone is the previous absolute requirement that all patients must have certain features for a minimum of 6 weeks. The 6-week threshold still is acknowledged as important but is no longer required and many patients (perhaps most patients), particularly with poor prognostic features, will fulfill criteria before 6 weeks. Importantly, the presence of

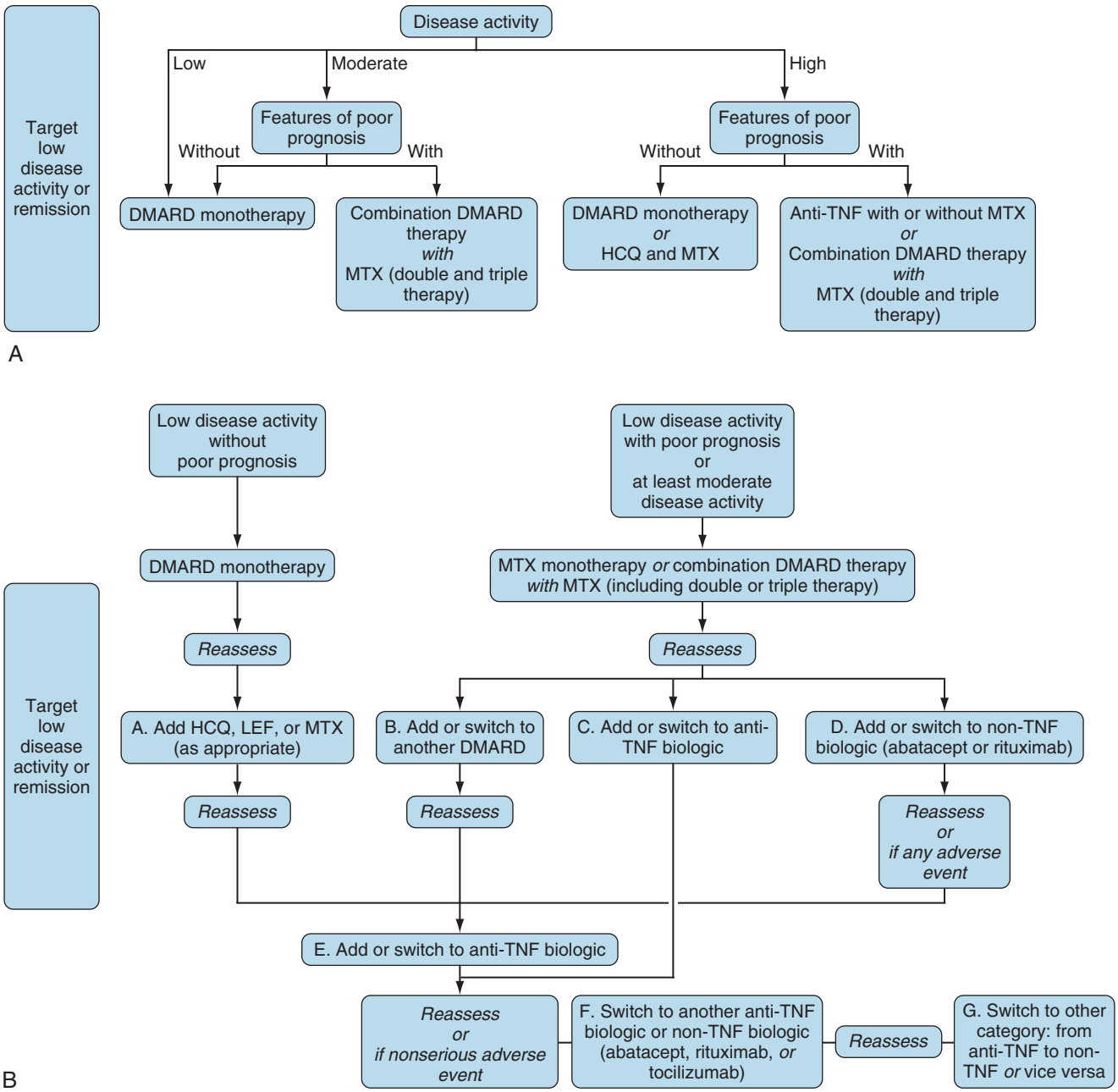



**Figure 71-4** American College of Rheumatology recommendations for treatment of rheumatoid arthritis.[2] **A,** Early disease. **B,** Established disease. DMARD, Disease-modifying anti-rheumatic drug; HCQ, hydroxychloroquine; LEF, leflunomide; MTX, methotrexate; TNF, tumor necrosis factor.

ACPA, particularly in a higher titer, is weighed heavily in the new criteria (3 points).

### Initial Therapy—The First Disease-Modifying Anti-rheumatic Drug

Upon accepting the premise that DMARD therapy should be started as early as possible, we must then ask which DMARD should be initiated and whether we should begin with monotherapy or combination DMARD therapies. Although many of the previously cited studies have shown that combinations outperform monotherapy in RCTs, this does not mean that initial combination therapy should be the standard approach for all patients in the clinic. Most clinicians initially start with DMARD monotherapy; the evidence to support this approach is discussed in a subsequent section of this chapter and is strongly validated by the TEAR trial[79] and the subanalysis of patients treated only with MTX from this trial.[80] The decision of which DMARD to initiate at the individual patient level is complex, and at this time it is clear that no one right answer exists for all patients and all clinical situations (Figures 71-3 and 71-4[2])**—one size, or in this case one drug, clearly does not fit all.** Many factors need to be considered, including but

not limited to, the patient's disease activity, comorbidities, and preference, the relative expense to the patient and to the health care system (weighing the benefits and including direct and indirect costs in our thinking), and importantly, where relevant, the desire of the patient (both female and male) to conceive. Until or unless parameters become available that allow selection based on data, this complicated decision will still require the clinician's best judgment.

Given all these factors, MTX should be the initial DMARD prescribed for the majority of patients. It is inexpensive (see Table 71-5), effective, well tolerated, improves survival rates,[81,82] and, importantly, is the cornerstone of most successful combination therapies (Figure 71-2). In particular, excellent data exist that anti-TNF therapy with all the currently available agents is much more effective with MTX on board both in terms of clinical and radiographic outcomes[78,83,84] (see Chapter 61 on MTX). MTX is usually administered orally at first, although subcutaneous (SC) dosing has more predictable bioavailability (Figure 71-5). In most cases, the dose should be pushed to 25 mg/week if necessary to control disease unless contraindications or tolerance problems exist. Most studies have shown that maximum efficacy may take up to 6 months to achieve but that in most situations, the response at 3 months predicts ultimate success. The bioavailability of oral MTX starts to plateau when doses of 15 to 20 mg are reached (Figure 71-5); therefore, if acceptable efficacy has not been achieved with a single weekly dose of MTX, two options exist. Splitting the weekly dose of MTX into two doses taken 4 to 12 hours apart on the day MTX is taken has been shown to improve efficacy, presumably because of better absorption.[85] If the patient is willing, the best strategy is to switch to SC MTX. MTX bioavailability and clinical response clearly improve if MTX is given in SC form compared with orally.[86,87] Some investigators have suggested that SC MTX, 25 mg/wk, be initiated in all patients, but this promising approach will require further study.[88] No data are available to compare split oral dosing to SC MTX. If MTX is optimally used, approximately 60% of patients will have a good response, and according to consistent data from several trials where MTX was only used at a maximum of 20 mg/wk orally, 30% will achieve low disease activity status[7,8,79,89] and will not require additional therapy.

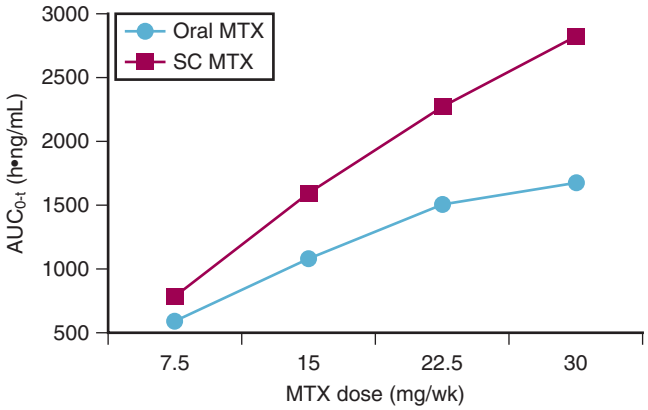



**Figure 71-5** Bioavailability of methotrexate (MTX): oral compared with subcutaneous (SC). AUC, Area under the curve.

### Initiating Treatment with a Single Disease-Modifying Anti-rheumatic Drug versus Combinations of Disease-Modifying Anti-rheumatic Drugs

Although most clinicians still favor starting treatment with monotherapy, combination DMARD therapy has clearly changed the treatment of RA forever. In the early 1990s, the treatment model called for individual DMARDs and then, when necessary, switching to a different DMARD. At that time, combinations of DMARDs were not used. Studies published in the mid-1990s showing the impressive efficacy and tolerability of various combinations of DMARDs dramatically changed the treatment paradigm.[6,48,75] Now the majority of patients with RA are treated with combinations of two, three, or more DMARDs. The first study that put combination DMARD therapy on the map was the so-called "triple therapy study" by the RAIN group of investigators.[6] This study clearly demonstrated that the triple combination of MTX, HCQ, and SSZ was significantly more effective than MTX alone or the combination of HCQ and SSZ. Importantly, this combination of DMARDs did not lead to an increase in toxicity. Multiple publications on other successful combinations of conventional DMARDs soon followed.[26,36,48,75,76,90]

Today the important question of whether treatment of all patients should start with combinations of DMARDs that are then stepped-down or whether monotherapy should be used initially and then stepped-up only if patients are not at target is an ongoing debate. Both approaches have their supporters, and data can be presented to support both sides of this question. On the one hand, there is no doubt that if short-term responses are examined in groups of patients, combinations outperform monotherapy. This outcome is true for combinations of conventional DMARDs,[36,75,76] as well as combinations of conventional DMARDs with use of a biologic agent.[78] On the other hand, the strategy of initial monotherapy with step-ups only in the patients who need it was clearly effective in the TICORA study (as previously discussed[7]) and, importantly, in the BeSt[8,22] and TEAR[79,80] trials (discussed in a subsequent section of this chapter), and although initial responses were better in both of the latter trials in the clinical parameters of patients initially treated with combination therapy, all groups in both the BeSt and TEAR trials had identical DAS or DAS28 scores at the end of 2 years. Clinicians do not treat groups of patients but individual patients, and if the results are similar at 2 years or beyond, **effective treatment approaches that minimize the number of DMARDs and therefore their potential toxicities and certain expenses will be desirable for patients and health care systems alike.**

## The BeSt Study

The BeSt study continues to be an important randomized, multicenter trial where 508 patients with early RA were randomized to receive one of four treatment "strategies" in an open-label study.[8,22,91] The four arms of the study were as follows:

- **Group 1:** Sequential DMARD monotherapy: initial MTX 15 mg/week → MTX 25 to 30 mg/week → SSZ → LEF → MTX + infliximab → etc.

- **Group 2:** Step-up combination therapy: initial MTX 15 mg/week → MTX 25 to 30 mg/week → MTX + SSZ → MTX + SSZ + HCQ → + MTX + SSZ + HCQ + prednisone → MTX + infliximab → etc.
- **Group 3:** Initial combination therapy: initial MTX 7.5 mg/week + SSZ 2000 mg/day + prednisone 60 mg/day (tapered to 7.5 mg/day by 7 weeks) → MTX 25 to 30 mg/week + SSZ + prednisone → MTX + CSA + prednisone → MTX + infliximab → etc.
- **Group 4:** Initial MTX and infliximab; initial MTX 25 to 30 mg/week + infliximab 3 mg/kg → infliximab 6 mg/kg every 8 weeks → infliximab 7.5 → infliximab 10 mg/kg every 8 weeks → etc.

Treatment adjustments were made every 3 months with the target of a DAS less than or equal to 2.4 (low disease activity). The DAS is calculated using the following four variables: Ritchie Articular Index (RAI; a count of 66 tender joints), Swollen Joint Count (SJC; 44 joints); the ESR (mm/hour); and the Global Health Assessment (GH) score (0-100): DAS = $0.53938 \times \sqrt{RAI} + 0.06465 \times (SJC) + 0.33 \times \ln(ESR) + 0.00722 \times (GH)$. The major results at 1 and 2 years are shown in Figure 71-6; both combination

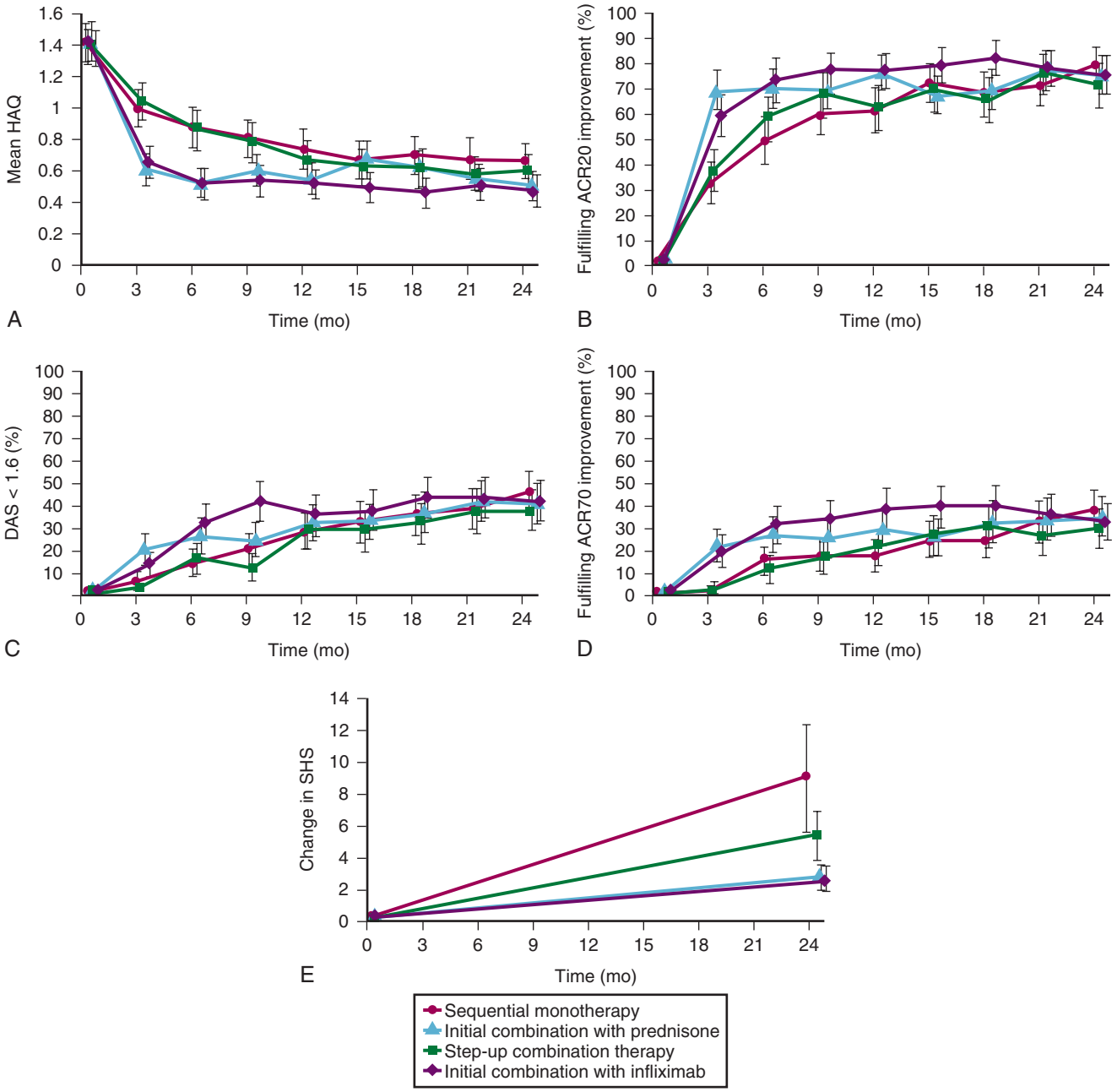



**Figure 71-6** Two-year results of the Dutch Behandel-Strategieen (BeSt) study. The error bars indicate 95% confidence intervals. ACR20, American College of Rheumatology 20% composite improvement; ACR70, American College of Rheumatology 70% composite improvement; DAS, Disease Activity Score; HAQ, Health Assessment Questionnaire; SHS, Sharp–van der Heijde score. *(Modified from Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, et al: Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial,* Ann Intern Med *146:406–415, 2007.)*

groups (groups 3 and 4) improved more quickly than did groups 1 and 2, which was expected, with high-dose prednisone in group 3 and a high initial dose of MTX and also infliximab in group 4. At 1 year, DAS and other clinical outcomes were similar in all four groups and, importantly, at 2 years they were identical. Health Assessment Questionnaire (HAQ) scores improved more in the early combination groups (groups 3 and 4) at 1 year but were not different at 2 years, and radiographic progression at 2 years was greater in group 1 than in group 2 and greater in groups 1 and 2 than in the combination groups (mean Sharp–van der Heijde score progression of 9, 5.2, 2.6, and 2.5, respectively). Progression of the joint space narrowing score (the importance of which is discussed in a subsequent section of htge text), although numerically higher in group 1, was not statistically different among the four groups (4.3, 2.1, 1.5, and 1.2, respectively). Interpretation of these differences is complicated by the fact that despite having disease for only 6 months, group 2 had more radiographic progression at baseline.

### Conclusions from the BeSt Study

One conclusion of the BeSt study is that all patients treated with a disease activity target will do well, even if they are receiving many different therapies. The type of therapy patients receive is not so important; it is only important that they are at low disease activity or in remission. Several important caveats must be noted:

1. The strategy of adding DMARDs, as in group 2, was more effective than the strategy of switching from one DMARD to another, at least for the DMARDs and their order of use in the BeSt trial. Although the clinical outcomes were similar at 2 years for these groups, radiographic progression was greater in group 1 (mean 9 vs. 5.2), more group 1 patients required infliximab (26% vs. 6%), and many group 1 patients ended up taking combinations of drugs anyway.
2. Initial therapy with combinations of conventional DMARDs (group 3) or combinations including biologic agents (group 4) work more quickly than step-up therapy (group 2). However, at 2 years, the clinical results were identical. There was a statistical advantage of the combination groups compared with the step-up group in terms of total radiographic progression (Δ2.5 points over 2 years); a difference was not noted in terms of joint space narrowing. This difference in radiographic progression is of no clinical significance unless it continues to grow at similar rates for years. Further strategies need to be used to further change therapies based on radiographic progress in the small group of patients (perhaps 10%) receiving only conventional therapy where it is relevant.
3. A subset of patients were able to discontinue drug therapy for a period. In the BeSt trial, if a DAS score of less than 1.6 was achieved for 6 months, patients were tapered off all medications; this scenario occurred in 115 patients (23%). Although many patients experienced a relapse, 59 patients (11.6%) were in remission during discontinuation of all treatment (median follow-up: 23 months[91]).

## Treatment of Early Aggressive Rheumatoid (TEAR) Trial

The TEAR trial was a landmark study of initial DMARD therapy in patients with early-stage (mean disease duration = 3.6 months), poor prognosis (all had RF- or ACPA-positive or erosive) RA.[79,80] It is the largest ($n$ = 755) investigator-initiated, randomized, double-blind trial in RA to date. TEAR was a 2-year study and sought to address two critical questions in this early-stage RA population:

1. Should treatment with combination therapy be initiated, or should patients be stepped-up to combinations of drugs only after a trial of MTX monotherapy?
2. Is combination therapy with MTX-ETAN superior to therapy with MTX-SSZ-HCQ (triple) therapy?

The 755 patients were randomized to the four groups, as illustrated in Figure 71-7. Randomization was performed in a 2:1 ratio, with twice as many patients randomized to the ETAN groups. Patients randomized to the MTX-only groups were stepped-up (72% of patients were stepped-up) to their assigned combination at 6 months in a blinded fashion unless their DAS28 scores were less than 3.2.

The major clinical findings are illustrated in Figure 71-8. All four groups had excellent improvement, with a mean ΔDAS28 of 2.8. For the primary endpoint of the study (mean DAS28 between weeks 48 and 102), all four groups were identical. Patients who were started on combination therapy (either triple or MTX-ETAN) had better results than did patients started on MTX alone at the 6-month point. However, this difference disappeared within 12 weeks of the step-up point, and the step-up group's DAS28 scores were identical to those of the other groups for the remainder of the study (Figure 71-8). Data of functional assessments and quality of life measures closely mirrored the DAS28 results. Radiographs were performed at initiation and at 48 and 102 weeks. Radiographic data are presented in Table 71-7, and the cumulative probability plot is shown in Figure 71-9. As shown, the radiographic outcomes of all four

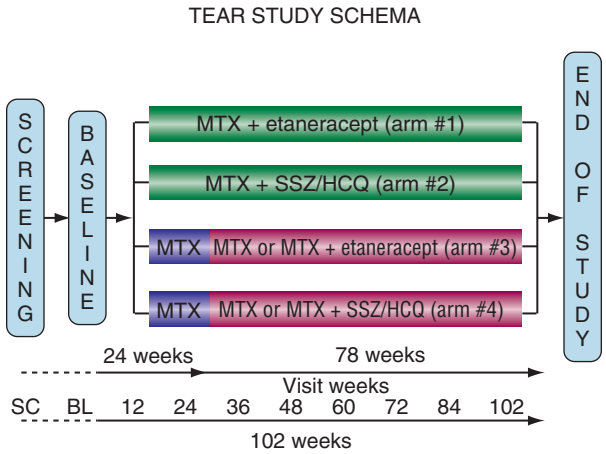



**Figure 71-7** Schematic of the four different treatment groups in the Treatment of Early Aggressive Rheumatoid (TEAR) trial.[71] Patients in arms 3 and 4 were treated with methotrexate (MTX) alone and step-up at 24 weeks if the Disease Activity Score in 28 joints (DAS28) was greater than 3.2. BL, Baseline; HCQ, hydroxychloroquine; SC, screening; SSZ, sulfasalazine.

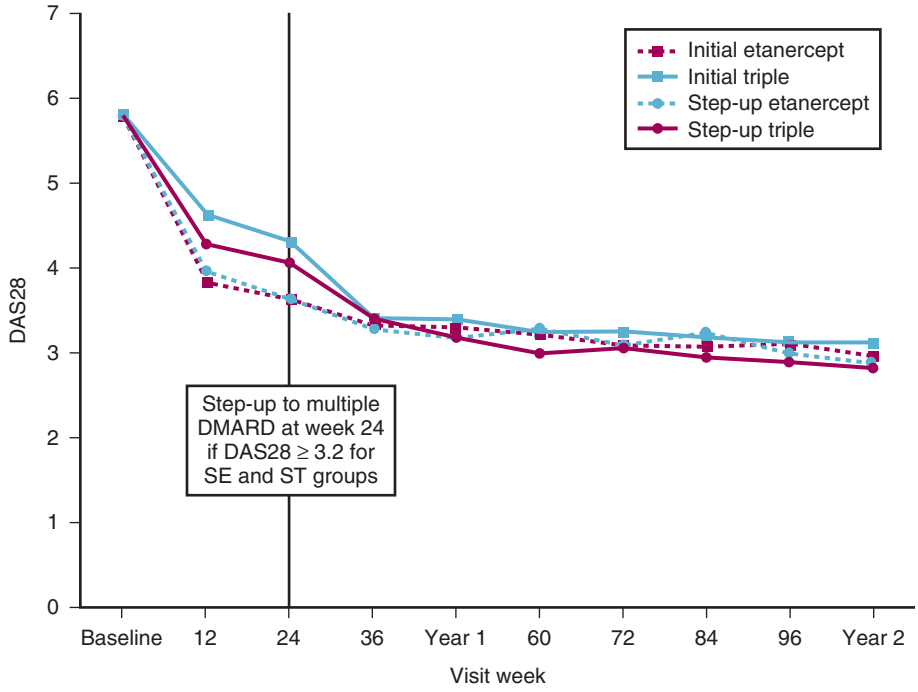



**Figure 71-8** Results of treatment in the four groups of the Treatment of Early Aggressive Rheumatoid (TEAR) trial over 102 weeks.[79] DAS28, Disease Activity Score in 28 joints; DMARD, disease-modifying anti-rheumatic drug; SE, step-up etanercept; ST, step-up Triple. *(Modified from Moreland L, O'Dell J, Paulus H, et al: A randomized comparative effectiveness study of oral triple therapy versus etanercept plus methotrexate in early, aggressive rheumatoid arthritis: the TEAR trial,* Arthritis Rheum *64:2824–2835, 2012.)*

**TABLE 71-7** Treatment of Early Aggressive Rheumatoid (TEAR) Radiographic Results

| Treatment Group | N | ΔTSS (over 2 yr) | SD |
|---|---|---|---|
| Immediate etanercept | 141 | 0.52 | 3.24 |
| Immediate triple therapy | 74 | 1.96* | 9.48 |
| Step-up etanercept | 139 | 0.76 | 2.75 |
| Step-up triple therapy | 63 | 1.36 | 5.00 |

*One outlier in this group had a ΔTSS of +78.5 points.
SD, Standard deviation; TSS, total Sharp score.

groups were superimposable. Table 71-7 shows that there was no difference between the four groups, but when all the ETAN groups are combined and compared with all the triple groups, the ETAN group had less total Sharp score (TSS) progression than did the triple group—ΔTSS of 0.51/year ($P = 0.047$). This small statistical difference was not clinically significant in the 2 years of the trial (see the following discussion about interpreting radiographic changes in clinical trials). Importantly, extensive data on adverse effects revealed no differences between the two groups. Many investigators had speculated that serious adverse advents may be more common in the ETAN-treated group, whereas minor toxicities such as gastrointestinal upset and others would be more common in the triple-treated patients; neither outcome was seen in this large, blinded, clinical trial. Analysis of the MTX-only group from the TEAR trial[80] reveals that the group that responded well to MTX initially continued to do well to the end of the trial (2 years), with the lowest DAS28 scores and radiographic progression of any group.

### Conclusions from the TEAR Trial

1. Strategies that initially treat patients with MTX and progress to step-up to combination therapy only if patients have not achieved their target at 6 months are as effective as initial combination therapy in disease control at 1 and 2 years, with no difference in clinical or radiographic outcomes. At the core of the debate about initial therapy—single DMARD versus combination—is the question of how soon is soon enough when considering control of RA? Is it important to control synovitis and radiographic progression in days or weeks, or is control by 3 to 6 months adequate? No clear long-term data exist to answer this important question. In both the TEAR trial and the BeSt study, as previously discussed, the groups treated with combination therapy early did better more quickly, but in both trials, the step-up groups looked identical to the early combination groups at 2 years in terms of clinical parameters. However, in both studies the early combination groups had very small but statistically significant less radiographic progression at 2 years. In the TEAR trial, this difference was 0.5 TSS/year, and in the BeSt trial, it was 1.3 per year.[22] Therefore central to this issue is the clinical relevance of small degrees of radiographic progression, which will be discussed in detail in a subsequent section of this chapter.
2. The strategy of using triple therapy was as effective as using the combination of MTX-ETAN. This observation is clearly important and should allow clinicians to be comfortable with this conventional therapy combination. Importantly, and somewhat sobering, the mean DAS28 scores of the four groups between 1 and 2 years

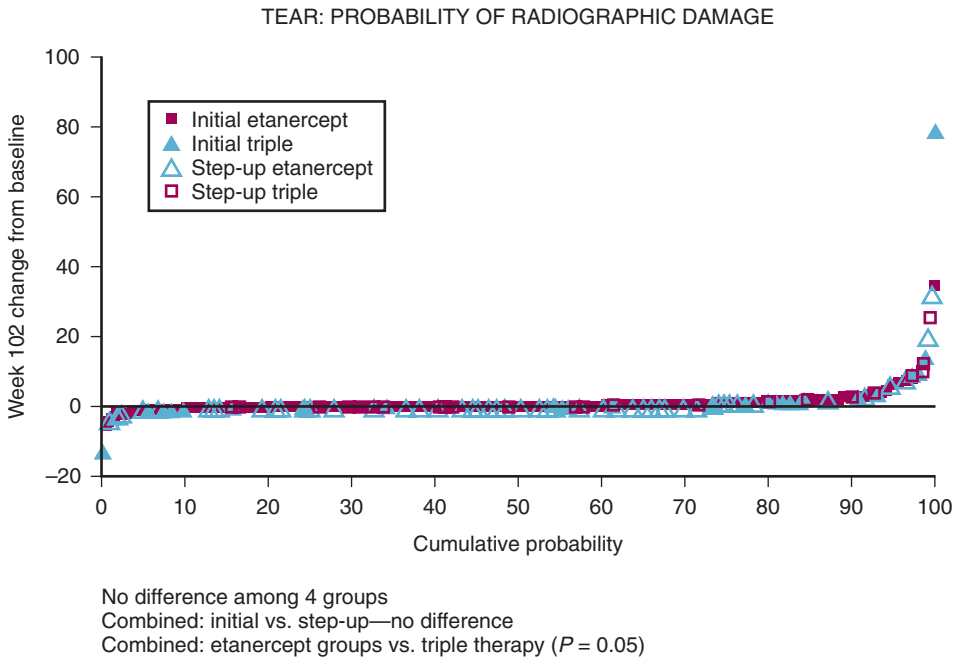



**Figure 71-9** Percentage of patients with radiographic improvement or progression during the 102 weeks of the Treatment of Early Aggressive Rheumatoid (TEAR) trial[79] by treatment group. Measured by total Sharp score.

of the trial was around 3.0, which means that almost half of the patients were not at target (DAS28 <3.2) regardless of treatment assignment. Therefore, in clinical situations, usually after 6 months of therapy, these patients should be switched to other therapies to obtain better control of their disease. For example, patients receiving triple therapy should be switched to or have ETAN added, or patients receiving MTX-ETAN should have HCQ and SSZ added. Trial data on patients who have failed to respond to triple therapy and are then treated with biologic agents or vice versa are discussed in a subsequent section, and both groups have the opportunity to do well.

## Treatment of Patients with Active Disease Despite Treatment with Methotrexate

As previously discussed, excellent data from clinical trials in which patients in general have high disease activity and poor prognostic features show that approximately 30% of patients will achieve a state of low disease activity or better if treated with modest doses of oral MTX. This figure is consistent across three major trials that documented this response.[8,22,79,89] If MTX therapy is optimized by switching to SC delivery and pushing doses to at least 25 mg/week, additional patients will experience disease control. Of the ~50% who have still not achieved this level of control, experience suggests that perhaps a third have improved significantly and that either the patient or the physician is not willing to escalate therapy. This still leaves approximately 40% of patients who will require treatment other than MTX monotherapy. The first question to ask is this: Should patients switch to another DMARD, or should other DMARDs be added to MTX**? Data from multiple sources, including the BeSt trial, as previously discussed, strongly support adding conventional DMARDs or biologic agents to MTX rather than switching to another DMARD.**[8,22,92] A recent trial has addressed the question of stopping MTX versus continuing MTX in patients with active disease who start taking ETAN. This trial randomized 151 patients to either stop taking MTX or continue to take MTX while starting ETAN. The group that continued to take MTX fared much better, with ACR20 scores of 86% versus 64%, and they experienced significantly less radiographic progression as well.[92]

Excellent data now exist that support the efficacy of 16 different clinically available DMARDs or DMARD combinations when added to MTX in this group of patients (i.e., those who have active disease despite treatment with MTX). This is true for all of the commonly used DMARDs, conventional and biologic agents alike. Figure 71-2 highlights the blinded clinical trials that have studied currently approved therapies in this patient population.[26,48,77,93-103] The problem for the clinician is the marked paucity of data with regard to comparing active therapies with each other in this critically important patient population. **Knowing that 16 different treatments are better than a placebo is of limited value because most clinicians do not commonly use placebos to treat patients.**

Two studies included in Figure 71-2 have provided some insight by comparing active therapies to each other in a limited way. In the study by the RAIN group, the combination of SSZ and HCQ appears to be better than either drug alone when added to MTX.[96] In the study by Schiff and colleagues,[102] abatacept was numerically better than infliximab for efficacy, but this difference did not reach statistical significance. This study has been faulted by some investigators because of the low and fixed dose of infliximab that was

used (3 mg/kg every 8 weeks). This criticism is fair with regard to the efficacy comparison, but importantly, even at this "low dose," serious and opportunistic infections were increased in the infliximab group compared with the abatacept group. A recent study in patients not receiving MTX has shown that tocilizumab is superior to adalimumab in terms of short-term efficacy, but this finding is applicable only to this limited population, because most patients should be taking MTX.[103] Fortunately, several recent studies have shed some additional light on patients with active disease despite treatment with MTX, as discussed in a subsequent section of this chapter.

**The really critical decision for clinicians is whether to add additional conventional therapies to MTX or take the leap and add biologic agents or tofacitinib.** Compared with the number of therapies that we know work in patients with active disease despite treatment with MTX, far less data address this question. The open-labeled Swefot study in this population of patients with active disease despite treatment with MTX compared adding SSZ and HCQ (triple) with adding infliximab.[89] At 6 months, both approaches appeared identical, with 25% of patients receiving triple therapy and 26% of patients receiving infliximab and MTX responding (EULAR good response); by 1 year, the results favored the biologic group (26% for triple therapy vs. 39% for infliximab), but this difference was no longer apparent at 2 years.[104] Importantly, in the 2-year data from this trial, there was no advantage to using the biologic agents first with regard to work disability.[105,106]

## Rheumatoid Arthritis: Comparison of Active Therapies Trial

Rheumatoid Arthritis: Comparison of Active Therapies (RACAT)[107] was a double-blind, randomized, controlled, noninferiority trial designed to answer the question, "Should conventional combination therapy be used before biologic agents in patients with active disease despite use of MTX?" RACAT was a multinational trial of 353 patients with RA who had active disease despite taking a mean of 19.6 mg per week of MTX. It was funded by the Veterans Administration, Canadian Institute of Health Research, and the National Institutes of Health. Of critical importance, this study was specifically designed to test the strategy of first adding conventional DMARDs (SSZ and HCQ) compared with first adding a biologic agent (ETAN) to MTX (Figure 71-10). Importantly, while maintaining the blinded condition, both groups were switched to the other treatment if they had not improved a clinically significant amount by 24 weeks. The primary endpoint was the improvement in DAS28 at 48 weeks (ΔDAS28) based on the original group randomization.

The strategy of first adding conventional DMARDs was not inferior to first adding ETAN (Figure 71-10A). Importantly, the switch rate from triple to ETAN was identical (27%) to the switch rate from ETAN to triple therapy (Figure 71-10B). This switch rate would have been nearly identical if the switch point had been ACR20 at 24 weeks (44% compared with 45%) instead of using the switch point of clinically significant improvement (ΔDAS28 >1.2). Further, no differences in radiographic outcomes were found, with the modified Sharp scores of the triple therapy group increasing by 0.54 compared with the ETAN group increase of 0.29 ($P$ = 0.43), and the radiographic cumulative probability curves were completely superimposable. Figure 71-10B also demonstrates the first data from a blinded trail on outcomes when patients are switched from triple therapy to ETAN and vice versa; the answer is that both groups improved clinically to a significant degree ($P$ = 0.001), and their amount of improvement is similar.

During the RACAT trial, more patients in the triple group experienced gastrointestinal adverse effects (30% vs. 22%; $P$ = 0.01), whereas more patients in the ETAN group had infections (37% vs. 25%, $P$ = 0.02). Serious infections occurred in 12 patients in the ETAN group and in 4 patients in the triple therapy group.

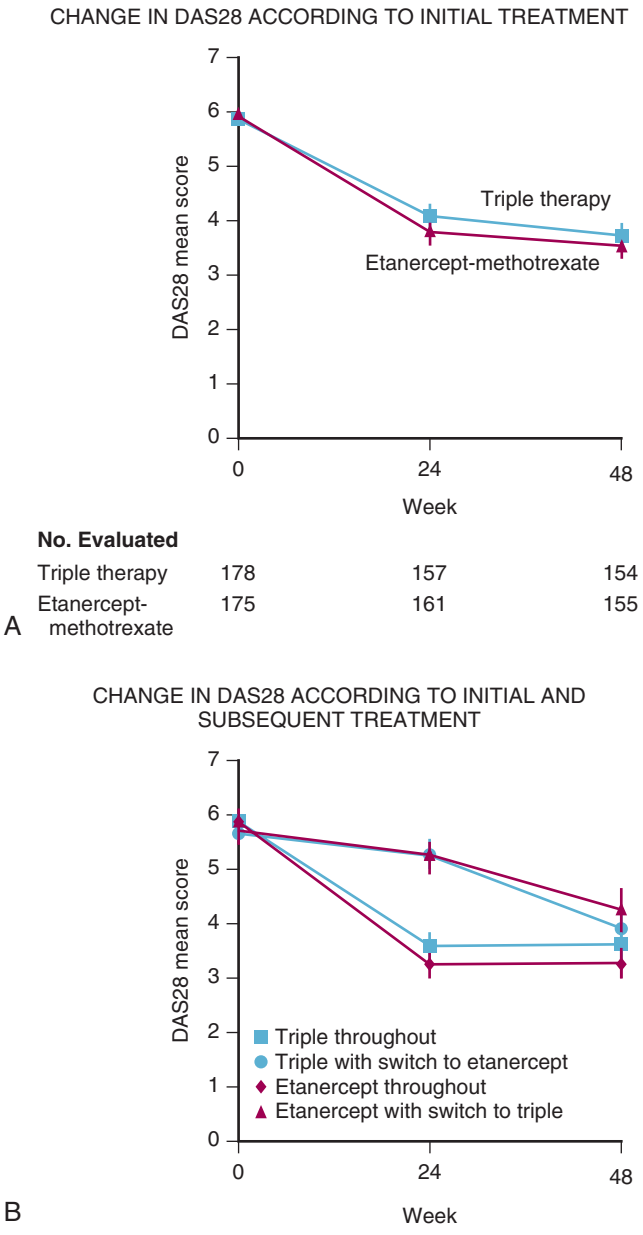



**Figure 71-10** **A,** Primary endpoint of the Rheumatoid Arthritis: Comparison of Active Therapies (RACAT) trial.[107] **B,** RACAT divided by switchers versus nonswitchers. DAS28, Disease Activity Score in 28 joints.

### Conclusions from the RACAT Trial

1. The strategy of first adding SSZA and HCQ to MTX (triple therapy) is not inferior to first adding ETAN clinically or radiographically.
2. Switching to ETAN in patients with active disease who are receiving triple therapy provides additional benefit, as does switching to triple therapy in patients with active disease despite taking MTX and ETAN.
3. Gastrointestinal toxicities were more common in the patients receiving triple therapy, and infections were more common in the patients taking ETAN.

Finally, the TEAR trial, as previously discussed,[79,80] addressed the question of conventional combination first versus biologic agent first indirectly, but importantly, in a double-blind study. Although the TEAR trial was designed to look at initial therapy, half of patients initially were treated with MTX alone and are relevant to this discussion. Of these patients treated with MTX alone, 72% or 271 patients did not achieve a DAS28 score of 3.2 or less and were treated with ETAN or the addition of HCQ/SSZ (triple therapy) in a blinded fashion. As shown in Figure 71-8, both of these groups looked identical 12 weeks after stepping-up therapy and continued to look identical as far as week 102, which was the end of the study. Importantly, with regard to radiographic progression, there was no difference in the radiographic progression of these MTX "failures" in the HCQ/SSZ group versus those in the ETAN group (mean progression of 1.7 vs. 1.1, $P = 0.57$).

Increasingly, demands are being made on rheumatologists to provide value-based care. This demand forces us to consider not only the clinical effectiveness of our therapies but also their economic costs and consciences. The longer term follow-up of the Swefot trial did not show benefit of the biologic arm compared with the triple arm, with fewer work days lost in the triple arm. Economic analysis of Swefot,[108] TEAR,[109] and RACAT[110] have all been presented at major international rheumatology meetings and have shown that the strategy of first using biologic agents rather than triple therapy in patients with active disease despite use of MTX costs approximately $1 to $2 million per quality-adjusted life-year gained.

Until further data are available that allow clinicians to select the best therapy for each patient in this group based on the different mechanisms of action (MOAs) of the many effective therapies (see Table 71-2), the aforementioned data dictate that conventional combinations should be tried first. When conventional combinations fail to help the patient achieve the disease activity target, data now show that TNF inhibition can provide additional benefit.[107] When a biologic agent is needed and until other data exist, largely because of their long track record, most clinicians favor a TNF inhibitor as the first biologic agent to use in this group of patients.

## Treatment of "Refractory" Patients or Those with Active Disease Despite TNF Inhibition

Despite the effectiveness of conventional DMARD combinations and the TNF inhibitors, a subset of patients will continue to have "unacceptable" levels of disease activity. Estimates of the size of this subset range from 10% to 40% of patients with RA. The lack of a universal definition of "refractory" and of "unacceptable" levels of disease activity hampers this discussion. With regard to levels of disease activity, it is clear and probably appropriate that clinicians are willing to tolerate more active disease in this "refractory" group before changing therapy, compared with patients who are naïve to treatment or those treated with active disease despite use of MTX only. Although clear data are needed, this approach currently seems prudent as the risk of toxicity and expense of treatment become significant factors.

Increasingly, as our expectations for our patients increase, we push to control disease sooner and use biologic agents earlier, and patients are being labeled "refractory" much earlier than ever before. When faced with a "refractory" patient, a close inspection of previous treatments that "failed" is in order. It is common for patients to be treated initially with low doses of MTX (≤15 mg by mouth) for 3 months and then be started on a TNF inhibitor with or without MTX, and then when they still have active disease after a few months, the patient inappropriately is labeled as "refractory." It is critical to assess whether MTX therapy has been optimized (see the previous discussion and Chapter 61); unless contraindications are present, most patients should have their dose of MTX pushed to 25 mg per week, and SC administration should be used. As previously discussed, data show that triple therapy is as effective as MTX-ETAN,[79,107] and thus this combination should be considered when resources are limited before progressing too far down the biologic agent road. Triple therapy can provide benefit in patients who have failed to respond to MTX and ETAN.[107]

For patients who truly are refractory to treatment, as with the previous section on patients with active disease despite the use of MTX, we urgently need markers or factors that inform us about the best of many possible approaches. The good news is that we still have multiple options with data to support them even in this difficult patient population, which include switching to another TNF agent,[111-114] starting rituximab,[115] starting abatacept,[116] starting tocilizumab,[117] or adding tofacitinab.[118] Currently, data directing this choice are limited (as discussed in the following text), and thus until markers or parameters are available, we will be making this decision largely empirically (Figure 71-3).

The strategy of TNF inhibition has been in use the longest, and because of its efficacy and the comfort level of clinicians with its use, many clinicians would switch to a second TNF inhibitor before switching to a biologic agent with a different MOA or to tofacitinib. A number of observational studies and one RCT support this approach.[111-114] From the observational studies, a consistent theme has been that if the first TNF inhibitor was discontinued for toxicity or loss of efficacy, patients have a better chance of efficacy with the second TNF than if the first was discontinued because of primary inefficacy (hazard ratio [HR], 2.7; confidence interval [CI], 2.1 to 3.4][111]). Discontinuation rates, particularly as measured in observational trials, are not the same as efficacy and reflect many things, including other treatment options that are available at the time of the study. In the previously cited study published in 2007,

golimumab, certolizumab, tocilizumab, and tofacitinib were not options.

A single randomized trial has addressed this question of switching to a second TNF inhibitor[114]; golimumab was administered after failure of at least one anti-TNF agent, and 43% of subjects responded, compared with 17% in the placebo-treated group. A special case in this patient population may be patients in whom antidrug antibodies have developed. Recently, data have been published with regard to antidrug antibodies in patients treated with adalimumab. After 3 years of treatment, antidrug antibodies developed in 28% of patients (secondary failures); in 67% of cases, these antidrug antibodies developed in the first 6 months.[119] Development of antidrug antibodies was associated with lack of or loss of efficacy (HR, 3.0; CI, 1.6 to 5.5), and patients were less likely to achieve remission (HR, 7.1; 95% CI, 2.1 to 23.4). Importantly, 38% of patients with antidrug antibodies compared with 14% of those without antidrug antibodies discontinued therapy because of lack of efficacy ($P = 0.001$). Perhaps of more concern was a recent finding that the development of antiadalimumab antibodies was associated with thromboembolic events (HR, 7.6; CI, 1.3 to 45.1; $P = 0.25$[120]). These interesting findings raise the possibility that **monitoring patients for development of antidrug antibodies, not only to adalimumab but also to other biologic agents, may be an important strategy to predict not only lack of efficacy but also to prevent toxicities such as thromboembolic events.**

Once the decision has been made to use a biologic agent with a different MOA, three choices are currently available (although the number of choices will likely increase in coming years): rituximab, abatacept, or tocilizumab. Few data are available to help differentiate among them. Based on strong suggestions in the literature that patients who are seronegative for ACPA and RF do not do as well compared with seropositive patients when treated with rituximab,[121,122] most clinicians would opt for either abatacept or tocilizumab in seronegative patients. Randomized trials have shown the efficacy of all three MOAs in this patient population.[115-117] These studies have been similar in design and also in the results of the intervention. Patients taking MTX who have active disease or intolerance to at least one TNF inhibitor have had the TNF inhibitor stopped and are randomized to receive the intervention + MTX or MTX + placebo. Response rates in the three different trials for ACR20 for the active drug versus placebo were as follows: rituximab, 51% versus 18%[115]; abatacept, 50% versus 20%[116]; and tocilizumab, 50% versus 10%.[117] All these responses, however, are less robust than in patients naïve to biologic agents. Based on the strikingly similar response rates of 50% across these three trials, there is little to choose from until further predictors of responses or comparison trial data are available. A limited number of trials have compared biologic agents to each other, but those trials were not in patients who had failed to respond to a TNF inhibitor. In the patients with active disease who were taking MTX and had baseline MTX, abatacept was similar to infliximab, with lower rates of serious infections.[102] In a head-to-head trial, abatacept and adalimumab were similar,[123] and in a monotherapy trial (ADACTA), tocilizumab was superior to adalimumab.[124] The later trial is of interest in patients who cannot take MTX. Some persons have raised concern about treatment with a second biologic agent after treatment with rituximab, which depresses B cell numbers significantly for at least 6 to 12 months. Limited data from observational studies provide some reassurance, because a significant increase in toxicities has not been reported.[125]

## The Niche for Tofacitinib and Potentially Other Janus Kinase Inhibitors

A paucity of data address what the therapeutic niche for tofacitinib should be. (See Chapter 65 for a detailed discussion on the tofacitinib and other JAK agents.) The efficacy of tofacitinib is not in question, but because cost and toxicity have not been major differentiating factors compared with biologic agents, its place in the treatment algorithm remains to be defined. Tofacitinib works well as monotherapy in early-stage disease[126] when added to MTX in patients who have active disease despite use of MTX[103] and in patients who have failed to respond to a TNF inhibitor.[118] One study has suggested that the efficacy of tofacitinib may be similar to that of adalimumab,[127] but other studies comparing tofacitinib and possibly other JAK inhibitors with distinct JAK isoform profiles with biologic agents and with conventional combinations are needed.

## How to Manage Patients in Remission Who Are Taking Disease-Modifying Anti-rheumatic Drugs

It is a powerful testament of how far therapy for patients with RA has come that this section on how to manage the patient in remission is included in this edition. It is now common practice for clinicians to ask how to taper DMARDs in patients who are "in remission." Indeed, a recent ACR expert panel acknowledged the importance of this question and included it as one of the top priorities for future investigation.[128] Putting aside the obvious difficulty in defining "remission," be it on clinical grounds, with radiographic evidence, or by using advanced imaging, clinicians see an increasing number of patients in "remission" or with very low levels of disease activity and are increasingly seeking direction regarding who should undergo tapering of what medications and when.

Unfortunately, again, few definitive data exist. We clearly do not have laboratory tests, inflammatory parameters, or cytokine profiles that can help predict which patients can safely undergo tapering of these agents. Studies that have examined discontinuation of all DMARDs have shown an unacceptably high rate of relapse.[22,129] Reports have suggested that seronegativity[130,131] and the absence of Doppler ultrasound–detected synovitis but not MRI synovitis[132] predict more successful tapering. Because of their well-chronicled toxicities, the highest priority should be discontinuation of glucocorticoids and NSAIDs for all patients. A number of trials[8,22,39] have shown that with effective DMARD therapy, glucocorticoid tapering not only is possible but should be expected. Perhaps the best example of this is from the BeSt trial, in which 92% of group 3 (the group that received high-dose prednisone initially) had stopped taking all prednisone at 2 years.[22] In this trial, prednisone was tapered only if the DAS was at or below 2.4 (a low level of disease activity).

Once the patient is no longer taking glucocorticoids and perhaps is taking only as-needed NSAIDs but is still in remission, it is more difficult to know which drug should be tapered next. A common situation is the patient who is undergoing combination therapy with MTX and a TNF inhibitor. Because of the expense and concerns for long-term toxicities, it would make sense to taper the TNF inhibitor to the lowest dose possible. Many of these patients in practice may have started taking this combination initially or had the TNF inhibitor added after receiving only a very short course of MTX. These patients may be similar to those in the TEAR trial who were started on the combination of MTX-ETAN.[79] For this group of patients, we can infer that ~30% of them did not need the ETAN and could have done extremely well with MTX alone, just as the 28% of patients who started taking MTX alone did not need a step-up in therapy. The MTX-only patients who did not step up not only had the lowest DAS28 score at 2 years (mean 2.7), but they also had the least amount of radiographic progression.[79,80] Unfortunately, unless we are talking about the small minority of seronegative patients,[130,131] we do not know how to select these patients.

If patients have been in remission for 6 months to a year and are being treated with a biologic agent, it seems prudent to decrease the dose or lengthen the interval between injections.[129-131] Although most biologic agents, certainly those administered subcutaneously, are dosed in a fixed, one-size-fits-all approach, we know from some of the earlier trials that doses that are lower than those usually clinically prescribed can be very effective. For example, the ERA trial compared doses of ETAN of 10 mg and 25 mg given twice weekly[133]; although the data were slightly better for the higher dose, the low dose clearly had substantial efficacy nearly equal to "full dose" ETAN, both as measured by clinical (ACR20) parameters and lack of radiographic progression. Therefore a significant percentage of patients may be overdosed, particularly those in remission. Our experience and that of others confirms that a slow and gradual lengthening of the interval between SC injections works well. In our clinic it is not uncommon for patients who are in remission to take 50 mg of ETAN every 2 to 4 weeks or 40 mg of adalimumab every 3 to 6 weeks. Conversely, a recent trial has shown that a fixed tapering schedule does not work.[129] In this trial, patients in remission while taking MTX and ETAN were then randomized to either a decrease in ETAN to 25 mg per week with MTX, taking MTX alone (i.e., use of ETAN was stopped), or taking no therapy. Predictably, the group that continued to take ETAN did best and the no-therapy group did the worst. Unfortunately, an unacceptable number (57%) of even the ETAN group were no longer in remission at 1 year.

Although wondering what to do with patients who are in remission while taking DMARDs is clearly a wonderful problem to have, further direction is certainly needed for this emerging group that fortunately is increasing in size. Trials to address this question with the collection of biomarkers will be increasingly important as patients continue to respond better with current therapies. It is clear that both ultrasound and MRI (discussed in the following text) can detect levels of synovitis that are not apparent on clinical examination. Ultrasound has shown promise in predicting which patients in clinical remission are most likely to experience flares while receiving stable therapy,[134,135] and synovitis on Doppler ultrasound predicts which patients more likely to experience flares when their medication is tapered. Even if, statistically, patients who are shown by ultrasound to have synovitis are more likely to have flares, the difference is of questionable clinical significance.[132] More importantly, ultrasound is not widely available, which significantly limits its practical application. Clearly, parameters that are clinically useful to differentiate patients who can successfully undergo tapering of medications are a high priority but largely remain to be elucidated.

### Use of Combinations of Biologic Agents

Combinations of conventional DMARDs and combinations of conventional DMARDs, especially MTX, with biologic agents have played a critical role in the vast improvement in responses to treatments that we currently see. However, thus far efforts to combine biologic products have not met with success. To date, studies to combine ETAN with anakinra have shown no improvement in disease control, with an ACR50 score of 41% in the ETAN-only arm compared with 31% in the combination arm, and they have shown an increase in serious infections in the combination arm (7.4% vs. 0%[136]). Similarly, in patients with active disease despite use of ETAN, patients were randomized to add abatacept or placebo; efficacy showed a nonstatistically significant trend toward improvement (ACR20 for combination, 48% vs. 31%; $P = 0.07$), but again, an increased risk of serious adverse events in the combination group (17% vs. 3%) and serious infections (4% vs. 0%[137]). Finally, a recent small trial in which patients who had active disease despite use of MTX and either ETAN or adalimumab were treated with the addition of rituximab or placebo showed very modest improvement in efficacy (an ACR20 score of 30% vs. 17%). Again, serious adverse events including infections were numerically greater in the group treated with combination therapy.[138]

Despite these less than encouraging results from early biologic combination trials, it is likely that ultimately patients will be treated with combinations of biologic agents. What is critically needed are good ways to measure the degree of immune suppression we create with our interventions—a thermostat for TNF, IL-1, or IL-6, if you will.[139] Currently, it is likely that suppression is inadequate in some patients, therefore not achieving optimal disease control, and that suppression in other patients is occurring at dangerous levels, leading to increased toxicity. When better immune system monitoring techniques are developed, not only will we be able to use the biologic agents we currently have in a more effective manner, but it is likely that we will be able to safely and effectively combine biologic agents to improve outcomes in terms of both efficacy and safety for patients.

## INTERPRETING RADIOGRAPHIC PROGRESSION AND THE USE OF OTHER IMAGING MODALITIES

What is the clinical relevance of radiographic progression? How much progression is significant, and over what period? Are erosions important, or should we be concerned only

with joint space narrowing? Finally, what role if any should ultrasound or MRI play in the management of RA?

In clinical trials, treatments of RA are evaluated by clinical parameters (e.g., ACR20 responses and DAS) and radiographic progression (TSS or van der Heijde Sharp [VHS]). This form of evaluation is problematic for a number of reasons. Clinical and radiographic progression is not always parallel. Perhaps the most dramatic example of this phenomenon from an RCT is the PREMIER trial, where, in the monotherapy arms, MTX alone was significantly better statistically than adalimumab alone, while at the same time, adalimumab alone was better than MTX alone with regard to radiographic progression (see Figure 71-11[78]). So which treatment was better? Some would say it is moot because the combination of the two outperformed either monotherapy arm in both measures. However, that misses the point about how to balance radiographic progression versus clinic parameters. Because **patients do not come to the clinic complaining of radiographic progression or demanding that it be stopped,** the question of how radiographic progression is related to factors that patients do care about—most importantly, physical function—is highly relevant.

Data exist that relate TSS or similarly total (VHS) progression to changes on HAQs (the gold standard for physical function in RA). These data suggest that a ΔTSS of 1 = a change in HAQ of ~0.01.[140] It is well accepted that a clinically significant ΔHAQ is ~0.22, so it follows that a ΔTSS of 22 is required for a radiographic change to be clinically relevant. This large Δ is never seen in clinical trials in which active therapies are compared. Thus, although many RCTs have shown statistically significant radiographic progression differences, no contemporary active comparator trials have shown clinically significant difference of any therapy versus any other therapy within the 1 to 2 years of the clinical trial. Additionally, recent data suggest that joint space narrowing correlates well with clinical progression but that erosions do not,[141,142] so perhaps we should not be concerned with TSS but only the joint space narrowing component. The next critically important point is as follows: Over what duration should we be concerned about radiographic progression? If a trial shows that therapy A results in two less TSS points progression per year compared with therapy B, then it follows that if those same therapies are used for 11 years, then patients receiving therapy A will have a clinically significant clinical benefit—HAQ 0.22 less than patients receiving therapy B. This is the magnitude of difference (ΔTSS of 1 to 2/year) that has been seen in trials and leads to claims of superiority of one therapy compared with another. Two problems are readily apparent with this type of extrapolation:

1. Within clinical trials, patients are often randomized to a therapy and continue to receive that therapy for the duration of the trial regardless of how they are doing. This approach does not reflect how patients are taken care of in the clinic; if patients are not doing well, therapy is or should be adjusted. Again, the PREMIER trial[78] is an excellent example of this issue; patients were in their assigned groups for 2 years (Figure 71-11B). Many patients were not having an optimal clinical response, and therapy would have been changed if this response had happened in a real-life clinical setting. These patients would not still be in the trial demonstrating their predictable radiographic progression, so in any RCT, radiographic progression that occurs after the time the therapy should have had maximal clinical benefit, and therefore patients would have been switched to a new therapy, is not clinically relevant. In the PREMIER trial, the therapies should have had maximal benefit by 6 months, so if the data after that time point include patients who were not at target (at least at low disease activity), then it is not relevant to patients seen in the clinic.
2. The second point is similar; to extrapolate forward in the example of a ΔTSS of two per year to 11 years of therapy

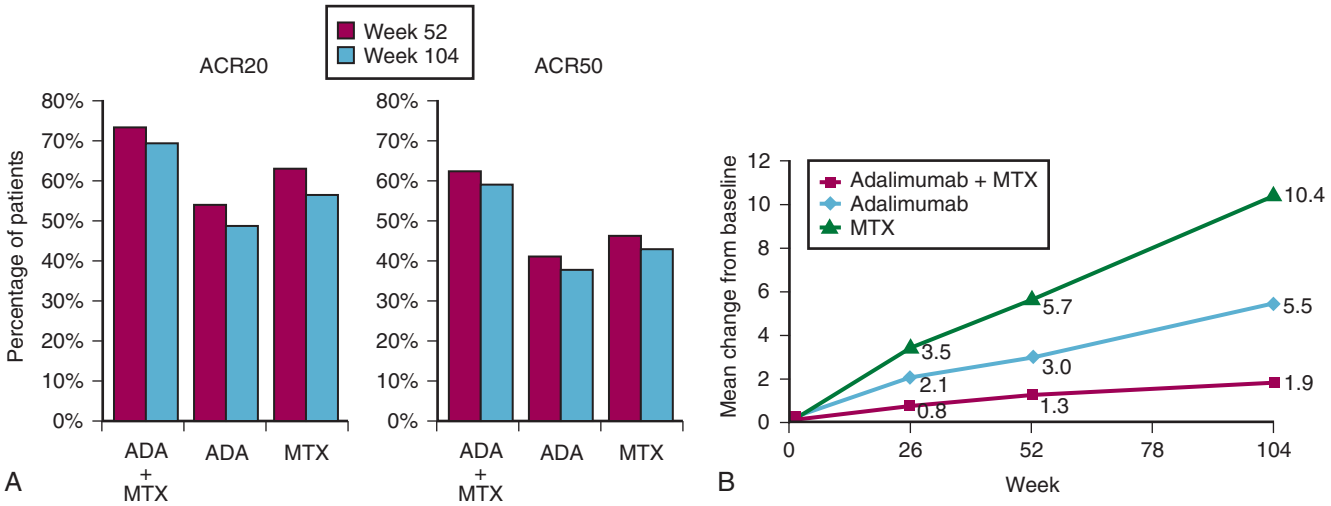



**Figure 71-11** Results from the PREMIER trial.[78] **A,** Clinical improvement measured by American College of Rheumatology (ACR) 20% and 50% composite improvement. **B,** Radiographic progression. ADA, Adalimumab; MTX, methotrexate. *(Modified from Breedveld FC, Weisman MH, Kavanaugh AF, et al: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment,* Arthritis Rheum *54:26–37, 2006.)*

would only be relevant if all patients were doing well clinically. Patients who are not doing well clinically would have their therapies changed before the 2 TSS progressions per year had a chance to become clinically relevant.

Another major reason why radiographic progression is problematic for the clinician is that formal evaluation of radiographic progression is almost never performed outside of clinical trials; few patients have formal Sharp score assessment of radiographs. In this regard, routine yearly radiographs are not indicated in many patients with RA and only add to the expense of care. This is true for patients who are not doing well clinically, for those patients need a change of care regardless of what the radiographs show, and it is also true for patients who are unwilling or unable to change therapies. The small minority of patients who are at the clinical target but who are progressing radiographically are the ones for whom we hope to discover important information with serial radiographs.

Finally, the manner in which radiographic information is presented in clinical trials is problematic. Most commonly, the mean TSS progression of group A is compared with group B, statistical analysis is performed, and we are told that therapy A is superior to therapy B because one or two fewer TSS progressions occur in that group. A much more informative way to examine radiographic outcomes is to look at cumulative probability plots for radiographic changes (Figure 71-12) for the Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO) trial[83,84] and Figure 71-9 for the TEAR trial.[79] When data are looked at this way, it is readily appreciated that a small number of patients, perhaps 15% to 20% in the TEMPO trial and even fewer in many studies, are driving the radiographic outcome. In the TEAR trial, there are no differences across the four groups in the probability plot. **With this epiphany, we must ask the following important questions: Who are these patients, and how do we focus on this small group that would benefit from a different therapy and not subject the other 80% to 95% to additional risks and expense?** Recently, as previously mentioned, Aletaha and colleagues[141] have called into question the relevance of erosions with regard to clinical correlations and have suggested that joint space narrowing correlates much more strongly with clinical progression; therefore, going forward, we need to ensure that we take this factor into account.

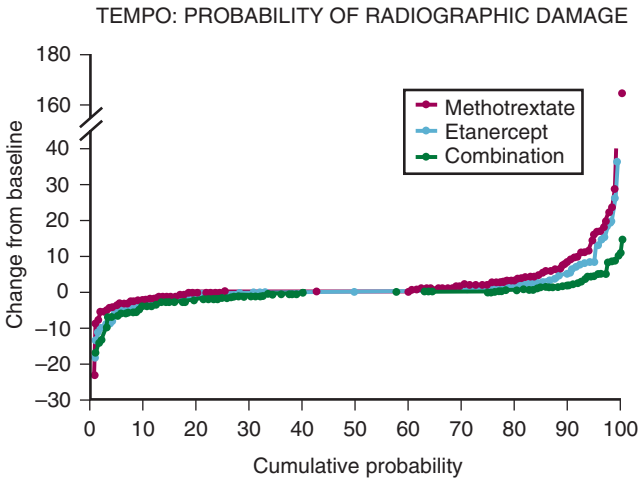



**Figure 71-12** Radiographic probability of progression plot from the Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO).[72,73]

Finally, what is the place, if any, of "advanced" imaging techniques in evaluating patients with RA? Clearly, both ultrasound and MRI are powerful techniques to evaluate early erosions and to detect synovitis. One potential use of these obviously sensitive techniques, as previously suggested, may be to verify the true absence of synovitis in patients who are believed to be in remission on clinical grounds.[134] Until further research is available, these techniques will remain largely research tools with regard to routine care of patients with RA.

## ADJUNCTS TO MEDICATIONS

### Patient Education

RA is a lifelong disease, so it not only makes sense that patients who are educated about their disease do better, but good data exist to show that education leads to better outcomes.[143] Use of the Arthritis Self-Management Program has been shown to lead to less pain, decreased visits to physicians, and economic savings.[144,145] Chronic diseases such as RA affect the entire family, and participation of spouses in educational group sessions leads to additional beneficial effects.[146] Training in stress management is particularly effective in improving measures of helplessness, coping, pain, self-efficacy, and health status.[147] Unfortunately, formal education level, a factor that we cannot modify, is associated with morbidity and mortality rates. Formal educational level is inversely proportional to these outcomes, and this finding cannot be explained by age, duration of disease, joint count, functional measures, or medications.[148]

It is clearly important for patients to take an active role in the management of their chronic disease. The more patients understand their disease and medications, the more control they feel they have over the entire situation. The bond that develops between the patient and physician as they work together to control this lifelong disease is an important factor in outcome and the satisfaction level of both parties. The stronger the bond, the less likely patients will become frustrated and turn to alternative therapies that are not only expensive but may do more harm than good.

### Pain Control

If patients with RA are treated early and effectively with DMARDs and therapy is escalated to achieve excellent control of the active components of disease, the need for specific pain medications, particularly narcotics, can be minimized. If pain is a major problem, the first thing to do is review the DMARD program and modify it to achieve maximum control of any active synovitis. Unfortunately, some patients present later in the course of their disease after substantial joint damage has already occurred, and their pain will need to be addressed. Pain can be the

factor that limits effectiveness of physical and occupational therapy, and as pointed out during a special workshop sponsored by the National Advisory Board for Arthritis and Musculoskeletal and Skin Diseases, it is frequently undertreated in patients with arthritis.[149] In addition to inhibiting function, pain is a major cause of depression in patients with polyarthritis. To maximize therapy in patients with early-stage RA or undifferentiated polyarthritis, pain must be controlled without altering consciousness or generating addiction. Treatment strategies favoring education, rest, exercise, and disease-modifying therapies generally are favored as an approach to pain control in persons with arthritis, and strategies that rely on narcotic derivatives may not address control of active RA. In most medical centers, experts in pain management are available for consultation by rheumatologists and primary care physicians (PCPs). Chapter 67 provides an excellent discussion of analgesics in rheumatic disease.

### Rest and/or Exercise and Activities of Daily Living

Education and supervision of a patient by trained professionals regarding the importance of finding the best balance of rest and exercise for inflamed joints is essential. This component of therapy can be started well before a definitive diagnosis is made. No matter the cause, finding this balance should ensure that a patient develops or retains sufficient strength to support joint function without exacerbating inflammation.

Details of physical and occupational therapy are outlined in Chapter 38. A patient with acutely and severely inflamed joints may not be the ideal exercise candidate and may need application of resting splints to immobilize the joint until anti-inflammatory medications, specifically DMARDs, take effect. Even the most painful joints, when splinted, must be moved passively through a full range of motion each day to prevent flexion contractures, particularly in children. For moderately inflamed joints, isometric exercises with muscles contracted in a fixed position (the resting length of the muscle) provide adequate muscle tone without exacerbating joint inflammation and pain. Maximal contractions, held for 6 seconds and repeated 5 to 10 times, performed several times each day, can prevent further loss of muscle mass around arthritic joints.

Patients with well-controlled arthritis will benefit from variable-resistance programs or high-intensity strength training, which has been shown to provide significant improvements in strength, pain, and fatigue levels. Older patients with RA benefit from progressive resistance exercises, similar to younger patients. In a study of older patients given closely regulated workouts on pneumatic resistance equipment, maximal strength of all major exercised muscle groups was increased 75% without exacerbation of clinical disease activity.[150] Not only does prescribed sustained exercise increase muscle strength, but also it helps the ability of patients to perform daily routines, improves global assessments and moods, and can decrease pain.[151]

Most patients with RA should have one or more sessions with a licensed occupational therapist (OT) to learn how to preserve joint function and alignment while carrying out the necessary and enjoyable activities of daily living and to be introduced to the assistive devices that are available. The basic concept is to avoid excessive force applied across non–weight-bearing joints and to avoid unnecessary impact loading on weight-bearing joints. A prospective and controlled Canadian trial demonstrated that home therapy by OTs produced a statistically significant and clinically important improvement in function in patients with RA.[152]

## TREATMENT OF RHEUMATOID ARTHRITIS COMORBIDITIES AND THE INTERACTIONS OF RHEUMATOLOGISTS WITH PRIMARY CARE PHYSICIANS

The best possible outcomes for patients with RA can be achieved only with a carefully orchestrated collaboration between PCPs and rheumatologists. On the one hand, the ever-increasing complexity of RA management options, combination therapies, and possible toxicities of therapy have made it increasingly essential that all patients with RA be treated by rheumatologists. Good-quality evidence shows that patients with RA are more likely to be taking DMARDs, as well as combination DMARDs, and are happier with their care when they are treated by rheumatologists.[153-155] On the other hand, the realization of the critical nature of the comorbidities associated with RA, especially cardiovascular disease, make ongoing engagement of a PCP essential[156] to produce optimal outcomes.

A significant amount of data demonstrate that patients with RA have better outcomes when rheumatologists are the primary manager of the RA. In one series of 561 patients with definite RA who were followed up for 20 years, the patients treated by a rheumatologist during the first 2 years of their disease fared significantly better than did the patients who were not treated by a rheumatologist during this period.[157] The favorable outcome could be related to an early start of DMARD therapy. Further, data indicate that regular ongoing care by a rheumatologist (a mean of 8.6 visits per year) results in less functional disability than that found in patients who received only intermittent, sporadic care by a rheumatologist.[158] Analysis of these data supported the interpretation that worsening disability was not the reason for intermittent care but rather a consequence of it. Additionally, evidence supporting care by a rheumatologist showed that patients who had access to a rheumatologist had higher performance scores than did patients who were treated only by a PCP. Access to specialist care resulted in significant improvements in arthritis care, comorbid illness, and health care maintenance overall beyond being treated by a PCP alone.[159]

Despite the importance of the rheumatologist, the PCP needs to be closely engaged (Table 71-8). Recently, the comorbidities of RA have been highlighted and include premature atherosclerosis and congestive heart failure and increased risks of osteoporosis, fractures, and infections. A recent review by a panel of cardiologists and rheumatologists highlighted RA as a significant risk factor for cardiovascular morbidity and mortality on par with diabetes.[160] In this regard, the PCP needs to aggressively address traditional cardiovascular risk factors such as hypertension and especially hyperlipidemia in patients with RA. Statins should be used aggressively because the primary reason for

**TABLE 71-8** Role of Primary Care Physicians in Treating Rheumatoid Arthritis

| |
|---|
| Monitor and aggressively treat cardiovascular risk factors |
| Monitor and treat/prevent osteoporosis* |
| Recognize toxicities of RA medications and initiate appropriate and timely workup* |
| Recognize the risks for infections and ensure immunization status is current |

*In partnership with a rheumatologist.
RA, Rheumatoid arthritis.

the excess mortality seen in persons with RA is cardiovascular. As an added bonus, statins, probably as a result of their anti-inflammatory effects, have been shown in animal studies of RA models[161] and in a least one RCT in humans[162] to decrease RA activity. Further, data show that statin use may protect against the development of RA.[163]

Because RA and particularly glucocorticoids are risk factors for osteoporosis, the PCP, in concert with the rheumatologist, need to address this issue. Most patients should be receiving calcium and adequate vitamin $D_3$. Bisphosphonates have been shown to prevent steroid-induced osteoporosis,[164] and their use should be strongly considered in patients taking steroids on a long-term basis unless contraindications exist (e.g., women of childbearing age).

With the increased risk of infections experienced by patients with RA because of their disease and therapies, up-to-date immunizations, including yearly influenza shots, every-5-year pneumococcal vaccinations, and appropriate zoster vaccinations, are critical.[2] The latter is a live-virus vaccination and should be avoided in patients currently receiving biologic agents. Current recommendations are to give the zoster vaccination 2 weeks before starting any of the biologic agents. Influenza and pneumococcal vaccinations can be safely given to patients who are taking biologic agents with reasonable immunologic response[165] except in patients taking rituximab, for whom response is severely impaired.[166,167] The rheumatologist and PCP should push hard for smoking cessation for all the usual reasons plus concern for cardiovascular and pulmonary disease that is already over represented in RA; cessation has the added benefit of potentially making patients more responsive to therapies.[168] Finally, because the PCP often sees the patient first during an illness, the PCP needs to be familiar with the common toxicities of RA medications, including concerns about MTX in the patient with decreasing renal function, MTX pneumonitis, and the need for heightened concern and aggressive workup of all potential infections, including opportunistic infections in immunosuppressed patients, particularly those taking biologic agents. Often the difference between life and death or at least between excellent outcomes and less than optimal results is the rapidity of response to the early warning signs for conditions as simple as cellulitis or a pulmonary infiltrate.

Clearly the best care is achieved when a team of a rheumatologist, a PCP, and an educated patient all work closely together. Timely consultation with physical and occupational therapists and orthopedic surgeons also plays a role in optimal outcomes for patients with RA. Although the need for joint replacement surgery is thankfully decreasing, few interventions have been as successful in improving patient mobility and quality of life as having properly timed hip and knee replacements. With the potential of huge changes in health care delivery on the horizon, at least in the United States, it may be an increasing challenge to ensure that the right team is always engaged to achieve the best outcomes for each patient.

## EVIDENCE THAT PATIENTS WITH RHEUMATOID ARTHRITIS ARE DOING BETTER

The prognosis for patients with RA has improved dramatically. Every rheumatologist who has been fortunate enough to see the changes during the past quarter of a century is only too happy to witness the improvements in care. The clinic days when wheelchairs were commonplace and symptomatic C1 cervical subluxations, chronic leg ulcers, constrictive pericarditis, and corneal melts were not uncommon in patients with RA are, we hope, gone forever. Despite this firmly held perception by clinicians, strong data to support this change have been slow to accumulate.

Recent data from Olmstead County shows that patients with RA diagnosed after 1995 lived significantly longer—almost 9 years longer—than did those diagnosed prior to 1995.[169] In the same cohort of patients in Olmstead County, knee surgery decreased by 46% and hand surgery decreased by 55%.[170] Data from more than 35,000 U.S. veterans with RA showed greater than a 30% reduction in extra-articular manifestations of RA since the year 2000.[171] Ward,[172] using data from California for the years 1983-2001, showed that hospitalizations for RA vasculitis or splenectomy for Felty's syndrome decreased by 33% and 71%, respectively.[172] Further, primary knee arthroplasty for RA decreased by 10%. Early unpublished reports from many centers indicate that joint replacement surgery for RA many be reduced by as much as 50% to 80%. Data from Sweden and Spain[173] both show that disease activity scores and health assessment scores have significantly improved during the past decade.

Investigators postulate that it may take up to 20 years for the results of a change in therapy or approach to fully translate into improvement in long-term outcomes, such as joint replacements and mortality. The aforementioned encouraging data likely reflect our therapies of the early to mid-1990s and, therefore, we expect that these early reports are the leading wave of good news as some of the therapeutic principles in use for the past decade begin to show their full affect.

## RESEARCH AGENDA: UNMET NEEDS

As mentioned numerous times in this chapter, huge gaps remain in our knowledge of how to best utilize the 20 clinically available DMARDs (see Table 71-2) into optimal care for each patient with RA. At the top of the list of important clinical questions is the urgent need for factors or parameters that would allow us to differentiate the probability of response to or toxicity from different DMARDs at the level of individual patients. This information is important for selection of DMARDs for all the categories of patients previously discussed—those who are DMARD naïve, patients with active disease despite taking MTX, and patients who

have not responded to MTX plus a TNF inhibitor. Several studies have suggested that factors such as serologic status,[174] human leukocyte antigen-DRB1 haplotypes,[175] and a variety of findings in synovial tissue[176-178] are statistically associated with responses to therapy, particularly to TNF inhibition. Unfortunately, none of these differences approaches clinical significance. With regard to specific trials needed in each of these patient groups, a recent ACR task force was formed to prioritize the clinical research trials needed to most expeditiously advance the knowledge base needed to move RA treatment forward.[128] After making the strong recommendation that all further trials include biologic samples to aid in our search for factors that differentiate therapeutic responses, they ordered the trial priorities as follows:

- Trials to elucidate the possible role of induction therapy in early disease
- Treatment of active disease despite use of MTX and the first TNF inhibitor
- Tapering therapy for patients in remission
- Treatment of active disease despite use of MTX
- Stratifying patients to determine in advance the most appropriate therapy in order to move beyond the current trial-and-error approach

Except for studies in patients in remission, the task force emphasized the need for trials that compare active therapies to each other. Further trials that demonstrate the superiority of product X over placebo in any of the aforementioned areas where multiple treatments have already been shown to work will not provide useful information to clinicians trying to make important clinical decisions. True comparative effectiveness research is needed to address the multiple options available for patients with RA in all of the previously listed categories. Further, the task force stressed the importance of innovative trial designs that more closely mirror clinical practice.[128] Examples include trials that allow escalation or switching of therapies in a blinded fashion based on clinical response and not allowing patients with ongoing active disease to continue to be treated with fixed therapies after the point of maximal response.

## THE HORIZON

It is always difficult to predict the future, particularly in an area of rapid change such as RA. Certainly, the authors of the early editions of this book could not have predicted the giant strides that have been made in RA treatment. Despite the progress that has been made, no cure exists for RA, and the vast majority of our patients require lifelong DMARD therapy, and thus plenty of opportunity remains for further progress. As Bill Gates remarked, "We always overestimate the change that will occur in the next two years and underestimate the change that will occur in the next ten." By the time the 11th edition of this book is published, we will almost certainly have several new biologic agents and perhaps several new small-molecule DMARDs available for use in treatment regimens. Regardless of new therapies that expand our portfolio of choices, the two areas where we still need to see the most progress are profiling patients based on parameters that predict differential responses to different therapies and monitoring the types and intensity of immunologic modification that we are producing. If substantial progress can be made in these areas, even without new therapies, we should be able to obtain vastly superior and more expeditious disease control.

On a somewhat more pragmatic note, the treatment of RA has become an expensive endeavor, and with all the changes in health care and the continuing increases in its cost, it is imperative that we have appropriate studies that allow us to provide the best value-based care to patients. If we do not have the appropriate research to justify expensive medications, we will have increasing difficulties justifying their use. Therefore it is critical that trials be designed with this factor in mind and that long-term costs, both direct and indirect, of suboptimally controlled RA be part of the equation.

Many observations clearly show that the immune response that becomes RA starts years[179-181] before most patients present with the classic symptom complex. Studies to elucidate this transition are under way,[182] and studies to treat presymptomatic RA are not far behind. Even further, these studies on pre-clinical disease hold the promise of elucidating the triggers of RA and, if so, the ultimate goal of RA prevention cannot be far behind.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 72

# Early Synovitis and Early Undifferentiated Arthritis



Annette H.M. Van Der Helm-Van Mil • Thomas W.J. Huizinga

**KEY POINTS**

- ***Early synovitis*** **refers to synovitis that is detected by physical examination; the symptom duration defined by *early* has changed with time.**
- **Intervention in inflammatory arthritis with symptoms less than 12 weeks can potentially prevent damage later.**
- **Undifferentiated arthritis (UA) is a diagnosis of exclusion. The phenotypic characteristics and outcome depend on the classification criteria used.**
- **Few clinical trials have been performed in UA, and treatment strategies were not adjusted to an individual patients' chance of spontaneous remission versus progression to rheumatoid arthritis.**

## WHAT IS EARLY-STAGE SYNOVITIS?

Recent data suggest that early therapy and achieving low disease activity improve long-term outcomes in inflammatory arthritis.[1,2,3] These observations have increased awareness among rheumatologists of the importance of early diagnosis and treatment. As a result, considerable pathophysiologic research and clinical trials have focused on identifying early-stage synovitis as soon as possible. Stratifying patients into well-characterized diseases, such as rheumatoid arthritis (RA), is essential so that appropriate therapy can be initiated. Equally important, identifying patients with a high chance for spontaneous remission is needed to avoid overtreating individuals with transient undifferentiated synovitis.

Uniform descriptions of patients and accurate diagnostic criteria for early-stage synovitis are essential for proper interpretation of clinical research. At present, no standardized definition for *early-stage synovitis* is widely accepted. In fact, the definition of *early-stage* has evolved with time. Previously, studies used a cutoff of less than 5 years to define early-stage disease for RA. By the 1990s, duration of symptoms for less than 12 to 24 months was considered early stage. This duration was chosen because most RA patients incur significant damage when treated conventionally.

The window for modifying disease outcomes might be narrow, and maximum benefit might require interventions even earlier. Some evidence suggests that treatment within months might be beneficial.[3] For this reason, several early arthritis cohorts in recent years restrict the inclusion criterion of symptom duration and include only patients with a symptom onset of less than 12 weeks; this is referred to as *very early-stage synovitis*. Even this definition probably does not capture the earliest phase of the disease because circulating antibodies might appear years before the onset of symptoms in RA, and biomarkers reflecting bone destruction are elevated before arthritis is present.[4,5] Animal models show that histologic evidence of inflammation and activation of signaling pathways can occur well before clinically apparent disease.

Although the term *synovitis* generally refers to a swollen joint detected by physical examination, alternative definitions are also applied. Physical examination might not be sensitive enough to detect the condition, and clinically undetectable synovitis may be present and relevant to identify. Therefore some studies consider tenderness in the absence of swollen joints as synovitis. For instance, early arthritis clinics might include patients with tenderness but no swollen joints. Others determine synovitis not by physical examination but through the use of imaging modalities such as ultrasound or MRI. Different ultrasound techniques are used; of these, traditional B-mode grayscale ultrasonography provides information on synovial thickness, and Doppler imaging measures the synovial blood flow. Ultrasound is easily accessible, rapid, and inexpensive. An important consideration is the reproducibility between readers. Whereas some studies found good or acceptable inter-reader agreement, other studies observed considerable variation between readers. Ultrasound appears to be valuable, especially in the absence of abnormalities, and the negative predictive value of a normal ultrasound result is high. The prognostic implication of abnormal ultrasound findings is less clear.[6] More long-term studies are needed to establish which ultrasound characteristics have a high positive predictive value for the development of persistent or erosive arthritis.

Until more data become available, the term *early-stage synovitis* generally refers to synovitis that is detected by physical examination. It is important to note that early-stage synovitis refers to a sign and/or symptom but does not reflect a specific diagnosis. The spectrum of final diagnoses in a population of early synovitis patients is presented in Figure 72-1.

## EARLY-STAGE ARTHRITIS CLINICS

Early-stage arthritis clinics are population-based inception cohorts in which patients with synovitis of early onset are included and followed prospectively. These cohorts often

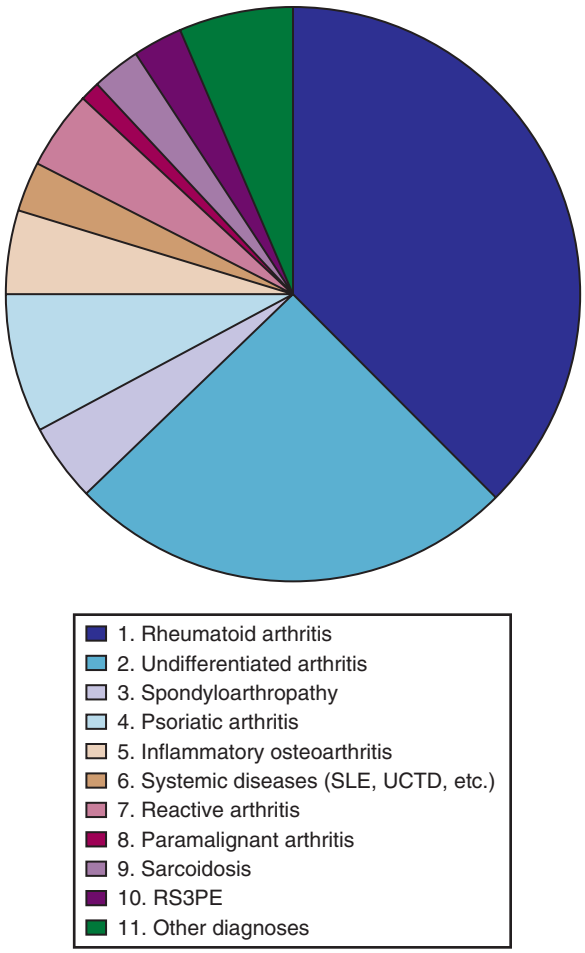



**Figure 72-1** Overview of diagnoses of early arthritis patients. (Presented data are based on 2284 early-stage synovitis patients included in the Leiden Early Arthritis Clinic cohort and their diagnosis after 1 year of follow-up.) RS3PE, Remitting seronegative symmetric synovitis with pitting edema; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease.

contain a wealth of phenotypic information, and the longitudinal design allows the study of factors related to a beneficial or severe course of the disease. Such studies might increase our understanding of the processes involved in arthritis progression. A summary of existing early arthritis cohorts is presented in Table 72-1. Inclusion criteria have changed with time, in parallel with changing interpretations of the term *early-stage*. Whereas the early arthritis clinics started in or before the 1990s allow inclusion of patients with symptoms for as long as 2 years, recently started early-stage arthritis clinics focus on very early-stage arthritis and include patients with symptoms for less than 12 weeks.

## WHAT IS UNDIFFERENTIATED ARTHRITIS?

The term *undifferentiated arthritis* (UA) refers to a subpopulation of early-stage synovitis patients who do not meet criteria for other diseases, including infections, spondyloarthropathies, crystal diseases, and RA. Because it is a diagnosis of exclusion, no classification criteria exist for UA.

European studies of early-stage arthritis cohorts found that 35% to 54% of the patients included do not meet the criteria for other diseases and are considered as UA[7,8]; in the remaining early-stage synovitis patients, a definitive diagnosis could be established at first visits. The frequency of UA depends on symptom duration at the time of the first visit to a rheumatologist. The longer symptoms have existed, the more likely it is that sufficient characteristics are present to support a definitive diagnosis, and the lower the prevalence of UA. In addition, the prevalence of UA will change when classification criteria for rheumatologic diseases change. For example, when the 1987 American College of Rheumatology (ACR) criteria for RA were applied to newly presenting early-stage synovitis patients in the context of an early-stage arthritis clinic in Europe (e.g., Leiden, the Netherlands), approximately 20% of those patients directly fulfilled the 1987 ACR criteria and thus were classified as RA. Initial data from the Leiden clinic suggest that this percentage is increased when the 2010 criteria for RA are applied.[9] The new criteria for RA not only might lead to a lower prevalence of UA, they also may lead to changed patient characteristics in the UA group. Those patients whose symptoms matched those of RA most closely may have been reclassified from UA to RA.[10,11]

### Characteristics of Early-Stage Undifferentiated Arthritis

Characteristics at first presentation in patients with early-stage UA and early-stage RA measured by using the 1987 criteria are somewhat different. Patients with recent-onset UA are younger (mean age, 48 vs. 57 years) and are less likely to be female (58% vs. 66%) than early-stage RA patients. UA patients generally have fewer swollen joints and have a greater likelihood of asymmetric synovitis. UA and RA patients do not differ in the acuteness of the start of their complaints, body mass index (BMI), or frequency of a positive family history for RA.[12] UA patients have fewer bone erosions at baseline; in a recent study, erosions were present in 18% of UA patients and 35% of RA patients.[13] Important differences were also noted with regard to autoantibodies. UA patients are RF positive in only 14% and anti-citrullinated protein antibody (ACPA) positive in 12% of cases, whereas both of these values are 55% in patients with early-stage RA.[6,7] According to both the 1987 and the 2010 classification criteria for RA, the presence of ACPAs in patients with arthritis is not equal to the classification of RA. Particularly in the case of monoarthritis or oligoarthritis, patients may not fulfill criteria for RA and are labeled as UA. However, about 70% of early-stage UA patients with ACPAs meet the 1987 ACR criteria 1 year later.

## REMISSION RATES IN EARLY-STAGE UNDIFFERENTIATED ARTHRITIS AND RHEUMATOID ARTHRITIS

The natural disease course of UA is variable, depending on the inclusion criteria and the duration of symptoms in several inception cohorts. Spontaneous remission occurs in 40% to 55% of UA patients. In contrast, the remission rate in RA is at most 10% to 15%.[14,15] In these studies, remission was defined as the absence of swollen joints for 1 year or

**TABLE 72-1** Overview of Early Arthritis Cohorts*

| Cohort | Inclusion Criteria | Year of Onset | Includes Early-Stage Arthritis (Symptoms <2 yr) | Includes Very Early-Stage Arthritis (Symptoms <3 mo) |
|---|---|---|---|---|
| Wichita Arthritis Center | Undifferentiated polyarthritis syndrome or RA<br>Disease duration <2 yr | 1973 | X | |
| Norfolk Arthritis Register | Early inflammatory polyarthritis<br>Age ≥16 yr<br>≥2 swollen joints<br>Symptom duration ≥4 wk | 1989 | X | |
| Leiden EAC | Synovitis of ≥1 joint<br>Symptom duration <2 yr<br>Age >16 yr | 1993 | X | |
| Austrian Early Arthritis Registry | Inflammatory arthritis<br>Symptom duration <12 wk | 1995 | | X |
| Amsterdam | Synovitis of ≥2 joints<br>Symptom duration <3 yr<br>Age >18 yr | 1995 | X | |
| Leeds Early Arthritis Centre | Undifferentiated arthritis of the hands<br>Symptom duration <12 mo | 1995 | X | |
| Birmingham VEAC | Synovitis of at least one joint<br>Symptom duration ≤3 mo | 2000 | | X |
| ESPOIR Cohort Study | Certain RA and UA that may develop into RA<br>Symptom duration <6 mo<br>Age 18-70 yr<br>≥2 inflammatory joints for the past 6 wk | 2002 | X | |
| Toronto Early Arthritis Cohort | Age >16 yr<br>Symptom duration between 6 wk and 12 mo<br>≥2 swollen joints or 1 swollen MCP or PIP joint with one or more of the following: positive RF, positive ACPA, morning stiffness >45 min, response to NSAIDs, or painful metatarsophalangeal squeeze test | 2003 | X | |
| Berlin EAC | ≥2 swollen joints<br>Symptom duration between 4 wk and 12 mo | 2004 | X | |
| Norwegian Very Early Arthritis (NOR-VEAC) | Synovitis of at least one joint<br>Symptom duration of 16 wk | 2004 | | X |
| New Castle EAC | Synovitis of at least one joint or inflammatory complaints without a synovitis at physical examination | 2006 | X | |
| Rotterdam Early Arthritis CoHort (REACH) | Synovitis ≥1 joint or ≥2 tender joints<br>Symptom duration ≤1 yr | 2007 | X | |

*The presented cohorts include not only early-stage RA patients but also a broader range of early-stage synovitis patients.
ACPA, Anti-citrullinated protein antibody; EAC, Early Arthritis Center; MCP, metacarpophalangeal; NSAIDs, nonsteroidal anti-inflammatory drugs; PIP, proximal interphalangeal; RA, rheumatoid arthritis; UA, undifferentiated arthritis.

longer after discontinuation of eventual disease-modifying anti-rheumatic drug (DMARD) therapy and/or discontinuation of participation in the outpatient clinic because the rheumatologist classified the patient as in remission. Longer-term follow-up is needed to determine whether symptoms recur many years later. In addition to the difference in frequency of spontaneous remission in UA and RA, the disease duration when spontaneous remission is achieved differs as well. A recent study observed that with UA, the median disease duration until spontaneous remission is 17 months, whereas in RA patients, the median period is 40 months before remission occurs.[12] Thus the chance of achieving a natural remission is reduced while the disease process matures. This supports the notion that chronicity might be more easily reversed in the undifferentiated phase of disease.

## JOINT DESTRUCTION IN EARLY-STAGE UNDIFFERENTIATED ARTHRITIS AND RHEUMATOID ARTHRITIS

Validated scoring methods are available to measure joint destruction objectively. Most scoring methods assess a limited number of joints. For example, the Sharp–van der Heijde method evaluates the metacarpophalangeal (MCP), proximal interphalangeal (PIP), wrist, and metatarsophalangeal (MTP) joints but does not evaluate the distal interphalangeal or larger joints. Therefore this method may be less optimal to measure the level of joint damage in patients who are seen with predominant synovitis in large joints. In RA, the extent of joint destruction in small joints adequately reflects joint destruction in larger joints. Such data are missing for UA patients.

Evaluation of the subgroup of UA patients in whom RA developed later in time provides insights that can help physicians to assess prognosis. Radiologic data were compared with those of patients who, when first seen by a physician for the disease, directly fulfilled the 1987 ACR criteria for RA. Radiographic progression rates between these groups were not different. Health Assessment Questionnaire (HAQ) scores and Disease Activity Score (DAS) measures also were not different between these two groups.[16] Thus, although UA patients as a group have a higher rate of spontaneous remission compared with patients with RA, the subgroup of UA patients who progress to RA have an equally severe course compared with patients classified as RA earlier in the disease. Studies that compare the rate of joint destruction in the total group of early-stage UA patients with that of patients with early-stage RA have not been performed.

## BIOLOGIC MECHANISMS IN UNDIFFERENTIATED ARTHRITIS AND DETERMINANTS OF PROGRESSION TO RHEUMATOID ARTHRITIS

Our understanding of the processes responsible for progression from UA to RA is far from complete. The following risk factors were identified as independent predictors for RA development and may provide clues:

- *Age:* The incidence of RA is clearly age dependent, with a rising incidence from 7 in 100,000 individuals for the age group 18 to 34 years to 107 in 100,000 individuals for the age group 75 to 84 years.[17] The reasons for this age-related increase are unknown. Possibilities include age-related alterations in cellular, humoral, and innate immunity.
- *Sex:* Women with UA have a twofold greater chance for RA compared with men who are seen with UA.[17] Sex hormones influence predisposition to autoimmune disease. In general, men are less prone than women. Androgens have anti-inflammatory effects, and estrogens have been reported to suppress arthritis as well.[18,19] Both estrogen and androgen inhibit bone resorption.[20] These results may account for increased progression in postmenopausal females.
- *Number of involved joints and C-reactive protein (CRP):* The number of affected joints and acute phase reactants like CRP reflects the level of inflammation and are frequently reported to be associated with progression of synovitis and worse disease outcomes. Although the number of swollen joints and the level of CRP are correlated on a group level, this is often not the case in individual patients, and both markers have their own independent predictive value. UA patients who are seen with CRP levels higher than 50 mg/L have a five times increased risk of being in an early stage of RA. Likewise, patients classified with UA who have polyarthritis have a 1.5 times increased chance of fulfilling the criteria for RA later on compared with patients with monoarthritis or oligoarthritis. Moreover, the risk that RA will develop in an early-stage UA patient who has more than 10 swollen joints is three times greater than that in patients with monoarthritis or oligoarthritis.
- *Autoantibodies:* The presence of autoantibodies in the serum of patients with RA has been recognized already for more than 70 years. Rheumatoid factors (RF) targeting the Fc part of human IgG were among the first group of autoantibodies discovered. Unfortunately, RF are not specific for RA and can be detected in as many as 15% of healthy individuals. In 1964 and 1979, additional serum factors present in RA patients were described: antiperinuclear factor antibodies and antikeratin antibodies, respectively. Although their specificity for RA was higher, assays to detect them were not routinely performed. Therefore RF was used to aid RA diagnosis in daily practice, and the presence of RF was incorporated into the ACR 1987 criteria for RA. It was not until 1995 when it was realized that antiperinuclear factors and the antikeratin antibodies recognize similar epitopes, and that they both target citrulline residues in proteins (i.e., ACPAs). ACPAs as well as can be present many years before the first clinical symptom of synovitis is noted; they are a risk factor for a persistent and destructive course of synovitis. Spontaneous remission is uncommon in $ACPA^+$ patients. Not only the presence but also the level of ACPAs has prognostic relevance. Despite these strong associations, studies that formally prove that ACPAs themselves are causally related to disease progression are lacking.

  Recently a new class of autoantibodies was identified that discriminates between citrulline- and homocitrulline-containing antigens in the sera of RA patients. IgG antibodies recognizing carbamylated (homocitrulline-containing) antigens were present in the sera of more than 45% of RA patients. Likewise, anti-carbamylated protein (anti-CarP) IgA antibodies were observed in 43% of RA sera. ACPA and anti-CarP antibodies are distinct autoantibodies because, in selected double-positive patients, the anti-CarP antibodies binding to carbamylated antigens could be inhibited by carbamylated antigens, but not by control or citrullinated antigens.[21] Anti-carbamylated protein (anti-CarP) antibodies precede the onset of RA in a subset of patients that are $ACPA^-$. When found in arthralgia patients, they are associated with the subsequent development of RA.[22,23,24]
- *Environmental factors:* Early-stage UA patients who smoke have a higher risk for RA and a destructive disease course. This risk is confined to patients who carry human leukocyte antigen (HLA)-DRB1 alleles that encode for the so-called *shared epitope*. Persons who smoke and also carry an HLA-DRB1 shared epitope allele are particularly prone to ACPAs, which subsequently are associated with disease persistency and erosiveness.
- *Genetic factors:* Apart from the HLA-DRB1 shared epitope alleles, genetic factors associated with progression from UA to RA are not clearly identified. Most genetic risk factors for RA provide risk for ACPA-positive RA when compared with healthy controls. It is not clear whether these factors are associated with progression from UA to RA as well, and if so, whether such an association is independent of the strong association between ACPAs and development of RA. Identification of new genetic factors has fueled the study of their relevance. In a population of UA patients, information on

currently known genetic risk factors for RA does not improve prediction of risk for RA compared with a prediction rule based on common clinical risk factors alone.[25]

- *Additional biomarkers:* The role of biomarkers in the diagnosis of UA is limited. Within RA, it is known that serologic levels of promatrix metalloproteinase 3 (MMP3), receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL), and osteoprotegerin (OPG) correlate with the rate of joint destruction with time.[26] Biomarkers that specifically reflect or predict disease progression in early-stage UA patients thus far remain unknown. A prospective cohort of first-degree relatives of RA probands, which is a population without RA but at increased risk for its future development, was recently studied. Serum IL-6 and IL-9, IL-2, granulocyte macrophage-colony stimulating factor (GM-CSF), and interferon (IFN)-γ levels were associated with positive autoantibodies and could be used as a proxy for RA risk.[27]

## WINDOW OF OPPORTUNITY

The concept of a window of opportunity suggests that there is a period early in the course of the disease when the disease process can be altered or can even be reversed with a complete return to normality. Treatment during this period might have a greater effect than treatment at a later stage in terms of halting disease progression and achieving remission.[27-30] Several different aspects have been studied, including whether very early-stage UA (<12 weeks) may be an immunopathologically distinct phase compared with later disease.

Studies that focused on synovial tissue did not reveal differences.[31] It is difficult to come to a conclusion from negative studies because one never knows whether the relevant processes have been studied. However, studies that focused on the composition of autoantibody responses have demonstrated that profound maturation of the autoantibody response occurs early during disease.[32]

UA patients who are seen at an early-stage arthritis clinic within 12 weeks of symptom onset less often progress toward RA compared with UA patients with symptom duration of longer than 12 weeks. Similarly, RA patients who at their first visit to a rheumatologist reported symptoms for less than 12 weeks had a lower rate of joint destruction with time and achieved sustained DMARD-free remission more often than RA patients who reported a period of symptoms for longer than 12 weeks before seeing a rheumatologist.[33]

Support for the presence of a window of opportunity is also obtained from trials.[34] An open-label study of a single dose of corticosteroids in patients with mild early-stage inflammatory arthritis (median, 20 weeks) found that the strongest predictor of disease remission at 6 months (defined as the absence of symptoms and signs in patients without anti-inflammatory treatment) was disease duration less than 12 weeks at the time of therapy.[35] Clinical and radiologic outcomes were significantly better at 3 years in patients who started DMARD therapy within 3 months ("very early") after disease onset compared with a median duration of 12 months at the start of treatment ("early").[29] Remission was achieved in 50% of the very early-stage group compared with 15% of the early-stage group. These data suggest that treatment in very early-stage arthritis might have a greater effect on disease progression and thus underline the relevance of identification and treatment of arthritis in the very early-stage disease phase.

## TREATMENT OF UNDIFFERENTIATED ARTHRITIS

Although many rheumatologists have experience treating UA patients, formal data providing information on treatment of UA patients are lacking. A systemic literature review revealed eight randomized controlled trials (RCTs), two uncontrolled open-label trials, and three cohort studies. Temporary treatment with methotrexate (MTX), abatacept, and intramuscular corticosteroids were demonstrated in RCTs with 12 months to 5 years follow-up to be more effective than placebo in suppressing disease activity or radiologic progression. One study suggests that DMARD combination therapy is, at least after 4 months, superior to MTX monotherapy in patients with UA at high risk for persistent arthritis. The open-label uncontrolled trials and cohort studies also suggested that early treatment might provide immediate suppression of inflammation. The long-term benefit of early treatment in UA remains unclear, and RA ultimately develops in many patients if therapy is discontinued.[36]

The trials that have been performed in UA patients are summarized in Table 72-2. One trial observed an effect of steroids in early-stage UA patients—an observation that was not reproduced in another study. MTX is reportedly beneficial in UA patients and is associated with delayed progression to RA and a reduction in the rate of joint destruction. This effect appeared to be predominant among $ACPA^+$ UA patients but was negligible in $ACPA^-$ UA patients. However, as noted above, when MTX was discontinued, the rate progression to RA was similar to the placebo arm.

Some biologics have been studied in UA patients. Although surprising lack of efficacy was observed with TNF blockers, abatacept was beneficial. The heterogeneous nature of UA patients and the more favorable disease outcome and spontaneous remission rate make it difficult to observe treatment efficacy in the total UA group. Thus the placebo-treated group can have a good remission rate, and differences from the treatment group might be difficult to detect. None of the published randomized trials in UA patients addressed this problem of heterogeneity and stratified patients having a high chance of developing RA or of having a spontaneous remission.

### Individualized Treatment of Undifferentiated Arthritis

UA has a variable disease course, ranging from spontaneous remission to severe destruction. Because of this, and because DMARD therapy is potentially toxic, treatment of UA patients should be personalized. The chance for individual patients to progress toward RA or to have a persistent erosive disease course can be estimated by using prediction models.[37,38] One of the present prediction rules (Figure 72-2A) is validated by using data from early-stage arthritis clinics from Germany, the United Kingdom, Canada,

**TABLE 72-2** Randomized Controlled Trials in Patients with Early-Stage Undifferentiated Arthritis

| Trial | *n* | Treatment | Follow-up Duration | Outcome | Effect Compared with Placebo |
|---|---|---|---|---|---|
| SAVE trial[46] | 389 | Single IM injection of 120 mg methylprednisolone | 52 wk | Drug-free clinical remission | No effect |
| STIVEA trial[47] | 265 | 3 IM injections of 80 mg methylprednisolone | 6 and 12 mo | Need to start DMARDs | At 6 mo 61% vs. 76% had started DMARDs; At 12 mo 10% vs. 20% had resolved disease |
| PROMPT trial[48,53] | 110 | Methotrexate (MTX) therapy for a period of 12 mo | 60 mo | Fulfilling the 1987 ACR criteria | RA developed in 40% vs. 53%; lower radiologic progression |
| ADJUST trial[49] | 56 | Abatacept treatment for a period of 6 mo | 12 mo | Fulfilling the 1987 ACR criteria | RA developed in 46% vs. 67%; no effect on radiographic progression |
| Saleem et al[50] | 17 | Infliximab at 0, 2, 4, and 16 wk | 26 wk | Clinical remission | No effect observed |
| van Eijk *et al*[51] | 82 | Remission-targeted treatment vs. conventional care | 2 yr | Radiologic progression | Less progression |
| Improved[52] | 122 | MTX + tapered high-dose prednisone | 4 mo | DAS remission (<1.6) | Not applicable |
| TREACH [53] | 281 | MTX + oral GC vs. MTX+ SSZ + HCQ + oral GC vs. MTX+ SSZ + HCQ + i.m GC | 3 mo | DAS, HAQ | Combination better |

ACR, American College of Rheumatology; DAS, disease activity score; DMARDs, disease-modifying anti-rheumatic drugs; GC, glucocorticoids; HAQ, Health Assessment Questionnaire; HCQ, hydroxychloroquine; IM, intramuscular; RA, rheumatoid arthritis; SSZ, sulfasalazine.

FORM TO CALCULATE A PATIENT'S PREDICTION SCORE

| | | | |
|---|---|---|---|
| 1. What is the age? | Multiply with 0.02 | | _____ |
| 2. What is the gender? | In case female: | 1 point | _____ |
| 3. How is the distribution of involved joints? | | | |
| | In case small joints hands and feet: | 0.5 point | _____ |
| | In case symmetric | 0.5 point | _____ |
| | In case upper extremities | 1 point | |
| | *Or:* In case upper and lower extremities | 1.5 points | _____ |
| 4. What is the length of the VAS morning stiffness (range 0-100 mm)? | | | |
| | In case 26-90 mm | 1 point | _____ |
| | In case >90 mm | 2 points | _____ |
| 5. What is the number of tender joints? | | | |
| | In case 4-10 | 0.5 points | _____ |
| | In case 11 or higher | 1 point | _____ |
| 6. What is the number of swollen joints? | | | |
| | In case 4-10 | 0.5 point | _____ |
| | In case 11 or more | 1 point | _____ |
| 7. What is the C-reactive protein level (mg/L)? | | | |
| | In case 5-50 | 0.5 point | _____ |
| | In case 51 or higher | 1.5 points | _____ |
| 8. Is the rheumatoid factor positive? | If yes | 1 point | _____ |
| 9. Are the anti-citrullinated protein antibodies positive? | If yes | 2 points | _____ |
| | | Total score | _____ |

A

**Figure 72-2** Tool to predict an individual early arthritis patient's probability of developing rheumatoid arthritis (RA). With the calculated prediction score **(A),** the probability can be derived **(B).**[29] VAS, Visual analogue scale.

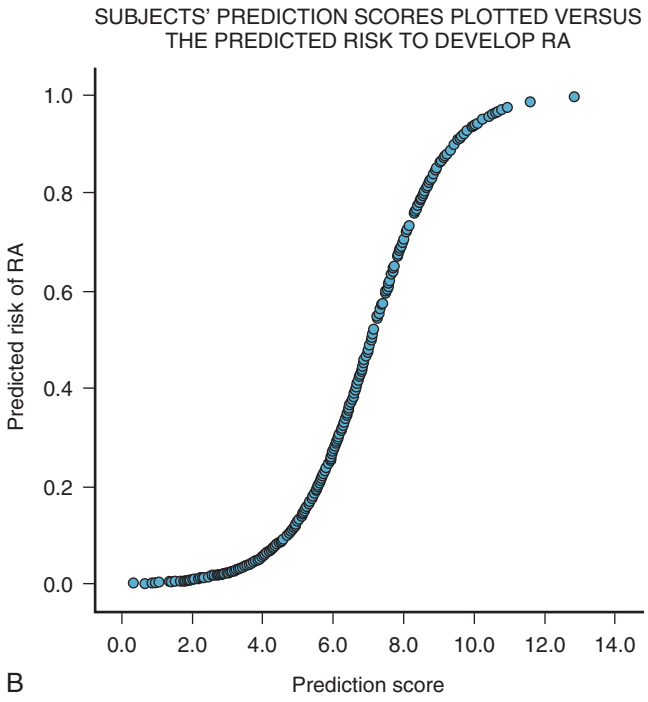



**Figure 72-2, cont'd**

Russia, and Japan; this algorithm is currently used in daily practice in several countries.[37,39-44] The discriminative ability of this model is high. The prediction rule consists of nine variables: age, gender, distribution of involved joints, morning stiffness, numbers of tender and swollen joints, CRP level, and presence of RF and ACPAs. It calculates the risk of development of RA for every UA patient (see Figure 72-2B). Such information can facilitate the decision of whether or not to initiate DMARD therapy and might facilitate patient involvement in decision making. In general, a score of 6 or lower is related to a low chance that RA will develop (91% chance of that RA will not develop) and may be a reason to not initiate DMARD therapy. In contrast, patients with a score of 8 or higher have an 84% chance that RA will develop; this might be a reason to initiate DMARD therapy in some patients.

Can the 2010 ACR/European League against Rheumatism criteria[45] be used to identify UA patients who actually have early-stage RA? The presence of new criteria for RA is relevant in that the absence of up-to-date classification criteria had hampered progress with regard to treatment strategies in early-stage RA. The 1987 ACR criteria for RA were not designed to classify early-stage RA and did not include more recent autoantibody tests or imaging modalities. Most randomized clinical trials included patients who fulfilled the 1987 criteria, hence patients with established RA. It is not clear whether the efficacy observed in these trials is the same for patients with early-stage RA or UA. The new criteria have mainly been derived for classification and research purposes[45] and require validation for use in clinical practice for early-stage synovitis. The 2010 criteria should not be used as diagnostic criteria until it is demonstrated that patients who fulfill them have a high chance for a persistent or destructive disease course, making the 2010 criteria positivity the basis of an argument for starting disease-modifying drugs.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 73

# Sjögren's Syndrome



E. William St. Clair

**KEY POINTS**

- Sjögren's syndrome is divided into primary and secondary forms. The primary form occurs in approximately 0.1% to 0.6% of the general population.
- The clinical hallmarks of Sjögren's syndrome are keratoconjunctivitis sicca (dry eyes), xerostomia (dry mouth), and parotid gland swelling.
- Extraglandular features of primary Sjögren's syndrome include fatigue, Raynaud's phenomenon, polyarthralgia/arthritis, interstitial lung disease, neuropathy, and purpura.
- A chronic mononuclear cell infiltration of the lacrimal and salivary glands is the characteristic histopathologic finding.
- A diagnosis of primary Sjögren's syndrome is made by subjective and objective assessment of dry eyes and dry mouth, testing for serum anti-nuclear antibodies, including anti-Ro/SS-A and anti-La/SS-B antibodies, and labial salivary gland biopsy.
- Treatment of Sjögren's syndrome currently aims to provide symptomatic improvement in the symptoms of dry eyes and dry mouth, as well as control extraglandular manifestations of disease. Disease modifying therapy so far remains experimental.

## HISTORIC PERSPECTIVE

In 1888, Johann von Mikulicz-Radecki described a case of bilateral painless swelling of the lacrimal, parotid, and submandibular glands,[1] an entity that later bore his name. Reports soon followed showing that Mikulicz disease was not a distinct pathologic entity but rather a clinical potpourri of conditions, including leukemia, lymphoma, and tuberculosis. Shortly thereafter, salivary gland disease became linked with dryness of the eyes and oral mucosa when Henri Gougerot, a well-known and successful French dermatologist, wrote in 1925 about three cases of salivary gland atrophy with dryness of the eyes, mouth, and vagina. The modern concept of Sjögren's syndrome was firmly rooted in 1933 when Henrik Sjögren, a Swedish ophthalmologist, reported a series of 19 cases of keratoconjunctivitis sicca, including two cases with swelling of the major salivary glands.[2] During the next 2 decades, Sjögren and others published extensively on various facets of the disease that now carries his name (also called Gougerot-Sjögren disease), with most of these early contributions coming from European ophthalmologists.

In 1953, Morgan and Castleman[3] published their detailed histopathologic findings from 18 patients with enlarged lacrimal and salivary glands. This pathologic treatise consisted of cases with clinical features resembling those of Mikulicz's disease and Sjögren's syndrome. Notably, 15 of the 18 patients were women with lacrimal and salivary gland swelling that developed in the fifth and sixth decades of life. Morgan and Castleman found in the salivary gland tissue a consistent "lymphoid element" accompanied by a striking proliferation of myoepithelial and epithelial cells. The epithelial changes produced a characteristic narrowing or obliteration of the ductal lumen, forming cords of solid cell masses they called *epimyoepithelial islands*. Because of the prominence of these epithelial structures, Morgan and Castleman advanced the theory that Mikulicz disease had its origins in the ductal epithelium. The salivary gland pathology in these cases was similar to that of the patients with keratoconjunctivitis sicca described by Henrik Sjögren. Because Sjögren's patients were mostly middle-aged women, Morgan and Castleman reasoned that Mikulicz disease was a subset of Sjögren's syndrome but with incomplete clinical manifestations. This now classic paper had the effect of unifying Mikulicz disease and Sjögren's syndrome into a single disease entity, a belief that dominated thinking until recently.

When Joseph J. Bunim delivered the Heberden Oration at the Wellcome Foundation in London on December 2, 1960, he described in elegant detail the clinical, pathologic, and laboratory findings of the 40 patients with Sjögren's syndrome evaluated by himself, Kurt Bloch, Martin Wohl, Richard Oglesby, and Irwin Ship at the Clinical Center of the U.S. National Institutes of Health (NIH).[4] All patients in their series had at least two of the three following features: keratoconjunctivitis sicca, xerostomia (with or without enlargement of the salivary glands), and rheumatoid arthritis. Bunim also knew from the work of others that keratoconjunctivitis sicca and xerostomia, or sicca complex, occurred in some patients with systemic lupus erythematosus, scleroderma, polymyositis, and polyarteritis nodosa. In his lecture, he made the point that the exocrinopathy extended beyond the lacrimal and salivary glands to affect the pharynx, larynx, and trachea, as well as the vagina. Many of the patients with Sjögren's syndrome in the NIH cohort also had extraglandular manifestations, such as Raynaud's phenomenon, purpura, pulmonary infiltrates, and peripheral neuropathy. Talal and Bunim took note among the NIH cases of three patients with reticulum cell sarcoma (an older term that includes non-Hodgkin's lymphoma) and a fourth patient with Waldenström's macroglobulinemia, drawing attention for the first time to the increased risk for lymphoma in this disease.[5] In this report, they speculated

that a "chronic state of immunologic hyperactivity and the proliferation of immunologically competent cells producing abnormal tissue antibodies predisposes to the relatively frequent development of malignant lymphoma."[5]

The NIH group also characterized the serologic markers of Sjögren's syndrome. In 1965 they reported that 12 of their 16 patients (75%) with sicca symptoms but no evidence of another connective tissue disease had serum anti-nuclear antibody (ANA) reactivity by indirect immunofluorescence on rat liver tissue.[6] Applying the Ouchterlony plate method, they further showed that sera from 13 of 16 (81%) of these patients contained precipitating antibodies to SjD and SjT, self-antigens subsequently named Ro (SS-A) and La (SS-B). Moreover, Bloch and colleagues[7] set the stage for our modern classification schemes by subdividing their patients into primary and secondary Sjögren's syndrome. Secondary Sjögren's syndrome referred to patients with sicca symptoms in the setting of another connective tissue disease, such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, or dermatomyositis, whereas primary Sjögren's syndrome was the designation when the sicca complex occurred in the absence of another connective tissue disease.

In the late 1960s and early 1970s, studies of labial salivary gland biopsy as a diagnostic test for Sjögren's syndrome led to grading systems for quantifying tissue inflammation. In 1968, Chisholm and Mason proposed a grading system for labial salivary gland biopsies that used a simple grading scale (0 to 4), in which grades 0, 1, and 2 represented absent, slight, and moderate mononuclear cell infiltrates, respectively.[8] The higher grades of 3 and 4 corresponded to focal inflammatory cell scores of 1 or more per 4 $mm^2$ of tissue, in which a focus equated to an aggregate of 50 or more mononuclear cells. In this initial study, 9 of the 10 patients with Sjögren's syndrome were classified as grade 3 or grade 4. By comparison, biopsies with lower grades came from patients with other chronic inflammatory conditions, as well as controls. For example, among 10 patients with rheumatoid arthritis, the highest biopsy grade was only 3. Twenty other patients with a variety of other rheumatic diseases (e.g., osteoarthritis, reactive arthritis, psoriatic arthritis, and scleroderma) had biopsies with grades of 0 or 1. Although it was a small study by today's standards, this work inspired others to validate and refine these findings. Later, post-mortem biopsies from 116 controls with no history of inflammatory disease were scored as having only grade 0, 1, or 2 infiltrates, attesting to the diagnostic specificity of this grading system.[9] However, the Chisholm and Mason grading system had limitations because it was sensitive only at the lower end of the scale and did not distinguish among biopsies with the highest degree of cellular infiltrates or biopsies with two or more foci per 4 $mm^2$ of tissue.

To improve on these earlier efforts, Tarpley and co-workers[10] at the NIH developed a grading system that incorporated scales that not only estimated the extent of inflammation but also quantified the amount of acinar gland destruction. As expected, they found that labial salivary gland biopsies from patients with the sicca complex alone had higher grades of cellular infiltrates and acinar destruction than those with sicca symptoms and rheumatoid arthritis or rheumatoid arthritis alone. However, grading the acinar destruction did not add diagnostic value independent of the severity of the infiltrates. Greenspan and colleagues[11] from the University of California at San Francisco modified the Chisholm and Mason grading scale when they examined labial salivary gland biopsies from 54 patients with definite or probable Sjögren's syndrome together with 21 controls. Their results reinforced the diagnostic relevance of the focus score, including its relationship to focus size, and highlighted the presence of germinal centers (GCs).[11] Grade 4 biopsies (>1 focus per 4 $mm^2$ of tissue) were primarily found in the patients with Sjögren's syndrome, with the highest focus scores in patients with the sicca complex in the absence of another connective tissue disease. The range for the focus score was extended from 1 to 12, in which a score of 12 was arbitrarily assigned to specimens with foci so numerous that they were confluent. The focus score was thereafter adopted as the "gold standard" to quantify chronic inflammation in labial salivary gland biopsies. Other methods were examined to determine salivary gland involvement in Sjögren's syndrome, such as sialometry, chemical analysis of the saliva, sialography, and scintigraphy. However, these measures of salivary secretion and the imaging of duct anatomy proved to be diagnostically nonspecific; sialography and scintigraphy had other shortcomings that limited their clinical use.

With a growing interest in Sjögren's syndrome, investigators began to develop classification criteria for comparison of results across studies. In the 1980s, many groups proposed classification criteria for Sjögren's syndrome. These proposed criteria incorporated the following items: symptoms of the ocular and oral components, objective measures of lacrimal and salivary gland involvement, a focus score of 1 or more obtained from a labial salivary gland biopsy, and the presence of serum autoantibodies. Many investigators from Europe, the United States, and Japan contributed to this effort, leading to the general acceptance in 2002 of the revised version of the European criteria proposed by the American-European Consensus Group (AECG).[12] In 2012, new classification criteria were proposed by the International Sjögren's Syndrome Registry that have been provisionally accepted by the American College of Rheumatology (ACR),[13] which is the latest effort to improve disease classification.

## DEFINITIONS AND CLASSIFICATION CRITERIA

The clinical hallmarks of Sjögren's syndrome are keratoconjunctivitis sicca and xerostomia, or the sicca complex. The term "keratoconjunctivitis sicca" is derived from Latin, and its translation is "dryness of the cornea and conjunctiva." Xerostomia refers to the subjective symptoms of dry mouth. Sjögren's syndrome is subdivided into primary and secondary Sjögren's syndrome. Secondary Sjögren's syndrome describes patients with keratoconjunctivitis sicca, xerostomia, or both, in the setting of another connective tissue disease or chronic inflammatory process, such as rheumatoid arthritis, systemic lupus erythematosus, polymyositis, systemic sclerosis (scleroderma), or granulomatosis with polyangiitis. Patients are diagnosed with primary Sjögren's syndrome if they manifest signs and symptoms of keratoconjunctivitis sicca and xerostomia in the absence of another

connective tissue disease, provided they also meet serologic or histopathologic criteria. Because primary Sjögren's syndrome is associated with extraglandular features that overlap with those of other connective tissue diseases, it may be difficult in some cases to distinguish from secondary Sjögren's syndrome. The challenge in distinguishing between primary and secondary forms of the disease arises most often in patients with primary Sjögren's syndrome and overlapping features of lupus such as rash, arthritis, and leukopenia. This distinction may boil down to semantics, depending on the penchant for "lumping" or "splitting."

Other descriptors for Sjögren's syndrome are *autoimmune exocrinopathy*[14] and *autoimmune epithelitis*.[15] The term *exocrinopathy* emphasizes the generalized glandular involvement in this disease, which affects not only the lacrimal and salivary glands, but also the apocrine sweat glands of the skin and the submucosal glands of the nose, pharynx, larynx, large airways, and vagina. The term *epithelitis* gains footing from the activated epithelial cells seen in the lacrimal and salivary gland tissue and at other sites of glandular involvement.

By the early 1980s, several sets of criteria had been proposed to classify Sjögren's syndrome, including the Copenhagen criteria,[16] the Japanese criteria,[17] the Greek criteria,[18] and the California criteria.[19] Common to each of these criteria sets was the requirement for objective evidence of keratoconjunctivitis sicca and salivary gland involvement. They differed, however, in their item content and weighting, as well as the methods for assessing salivary gland involvement. Movement toward consensus began when the Epidemiology Committee of the European Community conducted a multicenter study involving 26 centers from 12 countries aimed at unifying criteria for the classification of Sjögren's syndrome. This group first agreed on the items that should be included in the classification criteria for Sjögren's syndrome and then tested their operational characteristics in a large sample of patients with a clinical diagnosis of primary Sjögren's syndrome ($n = 246$), secondary Sjögren's syndrome ($n = 201$), other connective tissues diseases without Sjögren's syndrome ($n = 113$), and healthy controls ($n = 133$).[20] Two sets of three questions were selected from a larger body of questions that best correlated with the presence of keratoconjunctivitis sicca and xerostomia, respectively, as judged by clinical experts. The results from objective tests were analyzed by univariate analyses, yielding a tentative list of items based on their sensitivity and specificity for correct classification of the diagnosis. The following six items were chosen for the classification criteria: (I) ocular symptoms; (II) oral symptoms; (III) ocular signs (Schirmer-I test ≤5 mm/5 min or Rose Bengal score ≥4 by the van Bijsterveld scoring system); (IV) histopathologic features (focus score ≥1 on labial salivary gland biopsy; (V) objective evidence of salivary gland involvement by at least one abnormal test (salivary scintigraphy, parotid sialography, or unstimulated salivary flow rate ≤1.5 mL/15 min); and (VI) at least one of the following serum autoantibodies: anti-Ro/SS-A or anti-La/SS-B antibodies, ANAs, or rheumatoid factor. Exclusion criteria were pre-existing lymphoma, acquired immunodeficiency syndrome, sarcoidosis, and graft-versus-host disease. The presence of four of six criteria (accepting only a positive test for anti-Ro/SS-A or anti-La/SS-B antibodies for item VI) had good sensitivity (93.5%) and specificity (94%) for correctly classifying a patient with primary Sjögren's syndrome. It was suggested to be the optimal combination for efficient classification of this condition. For classification of secondary Sjögren's syndrome, the best combination was found to be a positive response for items I or II plus a positive result for any two of items II, IV, and V, resulting in a sensitivity of 85.1% and specificity of 93.9% for this diagnosis.

These preliminary European classification criteria for Sjögren's syndrome were validated in a second study of similar design. Cases of primary Sjögren's syndrome ($n = 81$), secondary Sjögren's syndrome ($n = 76$), other connective tissue diseases without Sjögren's syndrome ($n = 54$), and controls ($n = 67$) were assembled by expert clinicians at 16 centers from 10 countries to validate the initial results.[21] In this study, it was decided not to use items III (a) (Schirmer-I test) and V (c) (unstimulated whole salivary flow) to classify patients older than age 60 years because the tear and salivary flow by these measures were significantly reduced in the elderly controls from the original cohort. The results were similar to those of the earlier study, showing a sensitivity of 97.5% and specificity of 94.2% for correct classification of primary Sjögren's syndrome and a sensitivity of 97.3% and specificity of 91.8% for correct classification of secondary Sjögren's syndrome. A limitation of this type of study design is that the sensitivity and specificity may be artificially inflated by a "circular bias." These preliminary European classification criteria have also been criticized because a patient may be classified with primary Sjögren's syndrome despite a negative biopsy and the absence of serum anti-Ro/SS-A or anti-La/SS-B antibodies.

This conceptual impasse was overcome by the European Study Group on Classification Criteria for Sjögren's Syndrome with a group of American experts. From their previous study, they selected a cohort of 76 patients with primary Sjögren's syndrome, 41 patients with another connective tissue disease but without Sjögren's syndrome, and 63 controls, and tested three different combinations of items from the original classification criteria using a receiving operator curve (ROC) analysis. Based on the ROC analyses, patients may now be classified with primary Sjögren's syndrome if they fulfill four of the six items, including either a positive labial salivary gland biopsy or a positive test for serum anti-Ro/SS-A and/or anti-La/SS-B antibodies. This combination avoids the problem of classifying a patient with primary Sjögren's syndrome in the absence of a positive labial salivary gland biopsy or a positive test for anti-Ro/SS-A and/or anti-La/SS-B antibodies. Alternatively, they may meet the criteria by satisfying any four of the objective items, which was a highly unusual scenario in their data set.

The revised classification criteria proposed by the AECG are shown in Table 73-1. It was further specified that the Schirmer-I test be performed with anesthesia and that other ocular dyes, such as lissamine green (for conjunctival staining) and fluorescein (for corneal staining), should be allowed as replacements for Rose Bengal dye, which was not available in many countries. A positive labial salivary gland biopsy was further defined according to the rules set forth by Daniels and Whitcher.[22] In this case, a positive biopsy must show evidence of focal lymphocytic sialadenitis (FLS), which was defined as dense aggregates of 50 or more

**TABLE 73-1** Revised International Classification Criteria for Sjögren's Syndrome (SS)

I. Ocular symptoms: a positive response to at least one of the following questions:
   1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
   2. Do you have a recurrent sensation of sand or gravel in the eyes?
   3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: a positive response to at least one of the following questions:
   1. Have you had a daily feeling of dry mouth for more than 3 months?
   2. Have you had recurrently or persistently swollen salivary glands as an adult?
   3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:
   1. Schirmer-I test, performed without anaesthesia (≤5 mm in 5 minutes)
   2. Rose Bengal score or other ocular dye score (≥4 according to van Bijsterveld's scoring system)

IV. Histopathology: in minor salivary glands (obtained through normal-appearing mucosa), focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score greater than 1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 $mm^2$ of glandular tissue

V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:
   1. Unstimulated whole salivary flow (≤1.5 mL in 15 minutes)
   2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
   3. Salivary scintigraphy showing delayed uptake, reduced concentration, and/or delayed excretion of tracer

VI. Autoantibodies: presence in the serum of the following autoantibodies:
   1. Antibodies to Ro(SS-A) or La(SS-B) antigens, or both

**Revised Rules for Classification**

**For Primary SS**

In patients without any potentially associated disease, primary SS may be defined as follows:
   a. The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (histopathology) or VI (serology) is positive.
   b. Any three of the four objective criteria items (i.e., items III, IV, V, and VI) are present.
   c. The classification tree procedure represents a valid alternative method for classification, although it should be more properly used in clinical-epidemiologic surveys.

**For Secondary SS**

In patients with a potentially associated disease (e.g., another well-defined connective tissue disease), the presence of item I or item II plus any two from items III, IV, and V may be considered indicative of secondary SS.

**Exclusion Criteria**

Past head and neck radiation treatment
Hepatitis C infection
Acquired immunodeficiency syndrome
Pre-existing lymphoma
Sarcoidosis
Graft-versus-host disease
Use of anticholinergic drugs (since a time shorter than fourfold the half-life of the drug)

From Vitali C, Bombardieri S, Jonsson R; the European Study Group on Classification Criteria for Sjögren's Syndrome: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 61:554–558, 2002.

lymphocytes in perivascular or periductal locations. These aggregates, or foci, must contain only a small proportion of plasma cells and be located adjacent to normal-appearing acini in lobules without duct dilatation or fibrosis. A minimum threshold for positivity was considered to be greater than or equal to 1 focus per 4 $mm^2$ of tissue. These rules are important to follow because many biopsy specimens, especially from elderly individuals, show patterns of inflammation consistent with chronic sialadenitis, namely mixed lymphocytic and plasma cell infiltrates in association with ductal dilation, acinar atrophy, and fibrosis.

An abnormal parotid sialogram has been clarified in the revised criteria as the presence of diffuse sialectasis according to the scoring system of Rubin and Holt.[23] Positivity by salivary scintigraphy was also further defined as delayed uptake, reduced concentration, or delayed secretion of the tracer, by using the method of Schall and colleagues.[24] Parotid sialography and salivary scintigraphy, however, are rarely used clinically in the United States. The revised version also included some modifications to the list of exclusion criteria.

The revised criteria have also not escaped criticism by some experts because of the possibility that patients may be classified as having primary Sjögren's syndrome without subjective or objective evidence of ocular involvement (satisfying items IV, V, and VI).[25] Others have mentioned the "histologic and immunologic bias" of the revised criteria.[26] Some patients with primary Sjögren's syndrome test positive for serum ANAs in the absence of anti-Ro/SS-A and anti-La/SS-B antibodies. If such patients have a falsely negative labial salivary gland biopsy, then they will not be classified with primary Sjögren's syndrome, despite a better than average chance otherwise. Another potential shortcoming is the lack of laboratory standardization for the detection of anti-Ro/SS-A and anti-La/SS-B antibodies. Many different methodologies are available to measure these autoantibodies, including the older Ouchterlony plate assay, various commercial enzyme-linked immunosorbent

assay (ELISA) kits, and the newer multiplex bead technology. ELISA and the multiplex bead assays are used by nearly all of the hospital and commercial laboratories. It is unclear whether the type of methodology influences the sensitivity or specificity of these criteria. Although these classification criteria have been generally accepted by the medical community, they have not been officially endorsed by the ACR or the European League against Rheumatism (EULAR).

Recently, new classification criteria have been published based on data from 1362 participants in the Sjögren's International Collaborative Clinical Alliance Cohort (SICCAC).[13] These criteria have been provisionally approved by the ACR pending their validation. These criteria are derived solely from objective test results and do not require symptoms of dry eyes or dry mouth. To meet the classification of primary Sjögren's syndrome, patients must have at least two of the following: (1) a positive test for serum anti-Ro/SS-A and/or anti-La/SS-B antibodies or a positive test for rheumatoid factor and an ANA titer of greater than or equal to 1:320, (2) labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus greater than or equal to 1 per 4 $mm^2$, and (3) keratoconjunctivitis sicca with ocular staining score greater than or equal to 3. By using an external set of 303 participants, these classification criteria had a sensitivity of 92.5% and a specificity of 95.4%. The 2012 criteria have been criticized as unduly invasive because they require a slit lamp exam and corneal staining and/or lip biopsy, but they are classification criteria designed for the research environment and not for diagnosis.

The operating characteristics of the 2002 AECG and 2012 SICCAC/ACR have been compared in two studies. One study examined 646 participants, 279 of whom met the AECG criteria and 268 of whom met the SICCAC/ACR criteria. There was agreement regarding the classification of 244 of 279 patients (81%). The difference in ocular staining score (ACR ≥3/12 vs. AECG ≥4/9) was a source of discrepancy between the two classification schemes. Among the 35 patients who were classified positive by the AECG criteria but not the ACR criteria, 26 (74.3%) had insufficient ocular staining. Among the 24 patients who met the ACR criteria but not the AECG criteria for Sjögren's syndrome, 17 (70.8%) did so because of differences in the ocular staining score or serologic testing; 7 (29%) meeting the ACR criteria but not the AECG criteria tested positive for ANA and rheumatoid factor but not anti-Ro/SS-A or anti-La/SS-B antibodies.[27] Similar results were found in another study of 105 patients, 42 of whom met the AECG criteria and 36 the ACR criteria; 27 of whom met both. This study also found that a major source of disagreement between the two criteria sets was the different cut-off for ocular staining.[28]

## EPIDEMIOLOGY

Primary Sjögren's syndrome ranks among the most common of the autoimmune diseases, with a prevalence rate ranging from 0.1% to 4.6%.[29,30] However, the epidemiologic data are confounded by variations in the ages of the study populations and differences in the classification criteria used for case identification. Bowman and colleagues[31] estimated the prevalence of primary Sjögren's syndrome at 0.1% to 0.6% in a community from the United Kingdom by using the revised AECG criteria. In a study from Greece that used these same criteria, the age-adjusted mean annual incidence and prevalence rates were 5.3 (confidence interval [CI], 4.5 to 6.1) per $10^5$ people (0.5 for men and 10.1 for women) and 92.8 per 100,000 people (8.4 for men and 177.4 for women), respectively.[32] The incidence and prevalence rates of primary Sjögren's syndrome are significantly higher in women than men (e.g., approximately 20:1), with a peak incidence in the fifth and sixth decades of life.[32] Both gender ratio and age distribution appear to depend on the ethnicity and geographic region of the study population.[33] Primary Sjögren's syndrome occurs infrequently in children, with onset as early as 5 years.[34] The revised AECG criteria may be less sensitive for classifying primary Sjögren's syndrome in children than adults because of differences between these two age groups in the clinical presentation of this disease.[34]

The prevalence of secondary Sjögren's syndrome in patients with rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis has been estimated at 17.1%,[35] 8% to 20%,[36-38] and 14%[39]; respectively. To be classified with secondary Sjögren's syndrome, patients must have symptoms of keratoconjunctivitis sicca or xerostomia and objective evidence of lacrimal or salivary gland involvement.

## ETIOLOGY AND PATHOGENESIS

In primary Sjögren's syndrome, a common theme among models of disease pathogenesis is loss of immunologic tolerance to self-antigens. This breakdown in tolerance is evident in primary Sjögren's syndrome from the production of serum autoantibodies. Because the appearance of serum autoantibodies may precede the onset of clinical disease, the loss of immune tolerance appears to be permissive but not sufficient to induce tissue inflammation and damage. T cells reactive with several candidate self-antigens infiltrate the labial salivary glands of patients with primary Sjögren's syndrome. However, the timing of the evolution of these aberrant T cell responses is unclear with respect to disease onset. Environmental or stochastic factors or both are likely at play in triggering the chronic inflammatory response in the context of a genetically predisposed innate and adaptive immune system.

Because a disproportionately high number of the cases of primary Sjögren's syndrome occur in women, the search for environmental triggers has included studies of abnormal regulation of estrogens and androgens. However, no major differences have been found between patients with primary Sjögren's syndrome and healthy controls in serum levels of sex steroid hormones.[40] In addition, treatment with the hormone dehydroepiandrosterone, which acts on the androgen receptor, has shown no clinical efficacy in women with primary Sjögren's syndrome.[41] Among possible viral triggers, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) have received attention in primary Sjögren's syndrome because of their suppressive effects on T cell immunity and their ability to establish persistent infection. In one study, EBV DNA was found by immunochemical staining in salivary gland biopsies to localize in acinar and ductal epithelial cells[42]; however, the available evidence does not otherwise support a direct role for EBV infection in the pathogenesis of this disease. A 94-base pair (bp) fragment

of coxsackievirus RNA was also differentially expressed in salivary gland biopsies from patients with primary Sjögren's syndrome compared with controls,[43] but these results were not replicated in a later study.[44] If one truly exists, the identity of a possible viral trigger remains elusive.

## Genetics

It has been difficult to estimate the genetic risk for primary Sjögren's syndrome because of the absence of large twin studies. However, the existence of many large families with two or more members with primary Sjögren's syndrome is strong evidence for a genetic component to disease pathogenesis.[45] Primary Sjögren's syndrome is considered a complex genetic disorder, similar to the genetic susceptibility of systemic lupus erythematosus and rheumatoid arthritis. It is now clear from genetic studies of human autoimmune diseases that multiple genes contribute to disease risk and that individually each gene confers only modest effects on disease susceptibility.[46] An exception to this rule is the relatively strong signal associated with the human leukocyte antigen (HLA) locus on human chromosome 6p21.3. In populations of European descent, confirmed HLA associations with primary Sjögren's syndrome include DRB1*0301 (DR3), DRB1*1501 (DR2), DQA1*0103, DQA1*0501, DQB1*0201, and DQB1*0601.[47,48] The disease-associated polymorphisms located in the DRB1*0301 and DRB1*1501 loci account for 90% of the HLA genetic contribution. The HLA locus appears to play a major role in the pathogenesis of autoantibody responses in primary Sjögren's syndrome. In a cohort of patients with this disease, higher titers of anti-Ro/SS-A and anti-La/SS-B antibodies were linked to heterozygosity for the DQA1 and DQB1 alleles.[49]

The genetic susceptibility to primary Sjögren's syndrome includes inheritance of genes identified as risk factors in other autoimmune diseases. Familial clustering of cases of primary Sjögren's syndrome with systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis, as well as other autoimmune diseases, is consistent with these findings.[50-52] Initial efforts to identify disease susceptibility genes in primary Sjögren's syndrome relied on the candidate gene approach. More recently, however, collaborative research networks have been formed to generate large enough sample sizes for genome-wide association studies (GWAS). In one study of 395 individuals with primary Sjögren's syndrome and 1975 population controls of European descent, a strong association was confirmed for the HLA region, with allelic variants at HLA-DQB1*0201, HLA-DQA1*0501, and HLA-DRB1*0301 accounting mainly for this association.[53] Other genes conferring a disease risk in this study, albeit with a lower peak effect, included those encoding interferon (IFN) regulatory factor 5 (IRF5), signal transducers and activator of transcription 4 (STAT4), interleukin (IL)-12A, B lymphoid tyrosine kinase (BLK), C-X-C chemokine receptor type 5 (CXCR5), and TNFAIP3-interacting protein 1.[53] Another GWAS in Han Chinese identified GTF2I as a new susceptibility locus in primary Sjögren's syndrome, and confirmed the previously reported associations with STAT4, TNFAIP3, and the major histocompatibility locus in Europeans.[54] GTF2I encodes TFII-I, which is a phosphoprotein involved in B and T cell signaling pathways.

Genes in the type I IFN pathway are of interest in primary Sjögren's syndrome because they are highly expressed in the peripheral blood and salivary glands of patients with this disease compared with controls.[55] IRF5 in particular is a transcription factor that induces the transcription of IFN-α and the production of several proinflammatory cytokines, including IL-12-p40, IL-6, and TNF. It is among the nine IRFs that signal through Toll-like receptors (TLRs) and is essential for inducing responses through TLR4, 7, and 9.[56] Given the possible inciting roles of viral RNA and DNA, the endosomal TLR7 and TLR9 pathways may activate the type I IFN pathway in primary Sjögren's syndrome.[57] An increased risk of primary Sjögren's syndrome has been associated with a single nucleotide polymorphism in the splicing sequence of exon B of the gene encoding IRF5.[58] A 5-bp insertion/deletion polymorphism (CGGGG insertion/deletion) in the promoter region of the IRF5 transcript has also been linked to primary Sjögren's syndrome.[59] This polymorphism appears to have functional significance because reovirus (double-stranded RNA virus)–infected salivary gland epithelial cells in culture bearing this polymorphism produce higher levels of the IRF5 transcript.[59]

STAT4, a transcription factor involved in IL-12 and IL-23 signaling, is known to promote the development of T helper (Th)1 and Th17 responses. In a case-control study, an increased risk for primary Sjögren's syndrome was associated with a variant haplotype of the transcription factor STAT4.[60] This STAT4 polymorphism increases the risk for systemic lupus erythematosus and rheumatoid arthritis and correlates with the variant identified in the GWAS study of primary Sjögren's syndrome from Europe. Polymorphisms in the *STAT4* and *IRF5* genes appear to be additive in the risk for primary Sjögren's syndrome.[61] IL-12A, another player in the type I IFN pathway, encodes the p35 subunit of the IL-12 heterodimer (p35/p40), which is secreted mainly by monocytes and dendritic cells and is important for Th1 cell differentiation.

BLK is a Src family tyrosine kinase involved in B cell development. Nordmark and colleagues[62] have identified three other B cell–related gene loci associated with an increased risk of primary Sjögren's syndrome—the early B cell factor *(EBF1)* gene, the interval encompassing family with sequence similarity 167 member A and B lymphoid tyrosine kinase *(FAM167A-BLK)*, and the tumor necrosis family member 4 (TNFSF4, or the *OX40L* gene). All three genes are involved in B cell development and in theory might contribute to the hyperactivated state of B cells in this disease. CXCR5, a risk allele identified in a GWAS, is a chemokine receptor expressed on the surface of B cells and follicular helper T cells. In primary Sjögren's syndrome, its expression is upregulated in the salivary gland, where it may promote formation of GC-like structures and T cell–dependent B cell responses.

The human genome contains conserved noncoding elements with functional sequences, including regulatory motifs in promoters and untranslated regions of genes. Some of these conserved noncoding elements encode microRNAs that may regulate gene expression. MicroRNAs influence both innate and adaptive immunity and play a role in late B cell differentiation and development, as well as the establishment of B cell tolerance. Early studies have shown that

microRNA expression patterns in salivary gland tissue can distinguish patients with Sjögren's syndrome from controls, suggesting a pathologic role for microRNAs in the regulation of the chronic inflammatory response.[63]

Epigenetics refers to the study of inherited changes in gene expression caused by mechanisms other than a change in DNA sequence of that gene. Its principal mechanisms include remodeling of chromatic structure by DNA methylation and histone modification. Thus far, little is known about the epigenetics of primary Sjögren's syndrome.

## Adaptive and Innate Immunity

Many insights have been gained about disease mechanisms in primary Sjögren's syndrome in animal models as well as humans with this disease. Experiments in model systems enable hypotheses to be examined in ways not possible in humans, and have contributed to our understanding of the immunoregulatory disturbances underlying the clinical expression of disease (Table 73-2).[64,65] Although animal studies have shed light on possible disease mechanisms, it has been possible to perform highly significant research in humans because of the accessibility of labial salivary gland tissue from patients for immunohistopathologic analysis. Studies of labial salivary gland biopsies from patients with primary Sjögren's syndrome have shown approximately 90% of the infiltrating cells are $CD4^+$ T lymphocytes and B lymphocytes, with the remainder an admixture of plasma cells, $CD8^+$ T cells, $FoxP3^+$ T regulatory cells, $CD56^+$ natural killer (NK) cells, and macrophages, as well as myeloid and plasmacytoid dendritic cells (DCs)[66] (Figure 73-1). Most of the infiltrating T cells bear the memory phenotype (CD45RO) and display a restricted T cell receptor (TCR) repertoire representing several different clonotypes across

**TABLE 73-2** Mouse Models of Sjögren's Syndrome*

| Mouse Model | Phenotype | Comments |
|---|---|---|
| **Spontaneous Disease Models** | | |
| (NZB)NZW F1 mice | Progressive focal sialadenitis | Glandular involvement F > M |
| MRL/*lpr* | Lymphocytic infiltration of lacrimal and salivary glands; anti-Ro/SS-A and M3R antibodies; oligoclonal expansion of T cells and IgA and IgM production in the salivary glands | Normal secretory function; mRNAs for IL-1 and TNF expressed in salivary glands before onset of sialadenitis |
| NOD and its derivatives NOD.H2h4, NOD.Q, and NOD.P, NOD.E2fl$^{-/-}$, NOD.*scid* | Lymphocytic infiltration of lacrimal and salivary glands; NOD.H2h4 strain (but not parental NOD strain) secretes high levels of anti-Ro/SS-A and anti-La/SS-B antibodies; reduced glandular function | Mice also develop diabetes; exchange of H2 haplotype from H2g7 to H2q (NOD.Q) or H2p (NOD.P) does not affect the frequency of sialadenitis; disruption of ICA69 locus prevents lacrimal gland inflammation and reduces salivary gland inflammation; NOD IFN-$\gamma^{-/-}$ mice do not develop glandular disease; blockade of LT-βR signaling pathway reduces salivary gland infiltrates and improves salivary gland function |
| NFS/*sld* | Lymphocytic infiltration of lacrimal and salivary glands; anti–α-fodrin antibodies | Aberrant immune responses to α-fodrin; mice develop autoimmune lesions in other organ systems |
| **Experimentally Induced Models** | | |
| Carbonic anhydrase (PL/J mice) | Lymphocytic infiltration of salivary glands; antibodies to carbonic anhydrase | |
| Ro peptides (Balb/c mice) | Lymphocytic infiltration of salivary glands; glandular hypofunction, anti-Ro/SS-A and anti-La/SS-B antibodies | Disease induction requires multiple injections of peptide emulsified in Freund's adjuvant |
| **Transgenic or Knockout Models** | | |
| Id3$^{-/-}$ | Lymphocytic infiltration of lacrimal and salivary glands; adoptive transfer experiments; anti-Ro/SS-A and anti-La/SS-B antibodies; salivary hyposecretion | *Id3* gene involved in TCR-mediated T cell indicate a role for T cells in the development of disease; treatment with anti-CD20 antibodies ameliorates disease |
| PI3K$^{-/-}$ | Lymphocytic infiltration of the lacrimal glands; anti-Ro/SS-A and anti-La/SS-B antibodies | |
| BAFF transgenic | Lymphocytic infiltration of lacrimal and salivary glands; unique population of marginal zone B cells in salivary glands | No anti-Ro/SS-A or anti-SS-B antibodies; also develop lupus manifestations and anti-DNA antibodies and RF |
| IL-14α transgenic | Lymphocytic infiltration of lacrimal and salivary glands; hypergammaglobulinemia; <25% of animals develop anti-Ro/SS-A and anti-La/SS-B antibodies; glandular hyposecretion; mild immune complex–mediated renal disease and lymphocytic interstitial pneumonitis | IL-14 is a growth factor for B cells; mice develop large B cell lymphomas later in life; role for LT-α in the salivary gland inflammation; infiltrate primarily B cells with relatively few $CD4^+$ and $CD8^+$ T cells |
| IL-12 transgenic | Lymphocytic infiltration of lacrimal and salivary glands; anti-La/SS-B antibodies; glandular hyposecretion; increased acinar cell volume | Mice also develop thyroiditis and lung pathology |

*See references 58 and 59 for further details.

BAFF, B cell–activating factor; F, female; ICA69, islet cell antigen 69; Id3, protein inhibitor of DNA binding 3; IFN-γ, interferon-γ; IL-1, interleukin-1; LT-α, lymphotoxin-α; LT-βR, lymphotoxin-β receptor; M, male; M3R, muscarinic receptor subtype 3; NOD, nonobese diabetic; NZB/NZW F1, New Zealand Black × New Zealand White F1 (mouse hybrid); PIK3, phosphoinositide-3-kinase; RF, rheumatoid factor; TCR, T cell receptor; TNF, tumor necrosis factor.

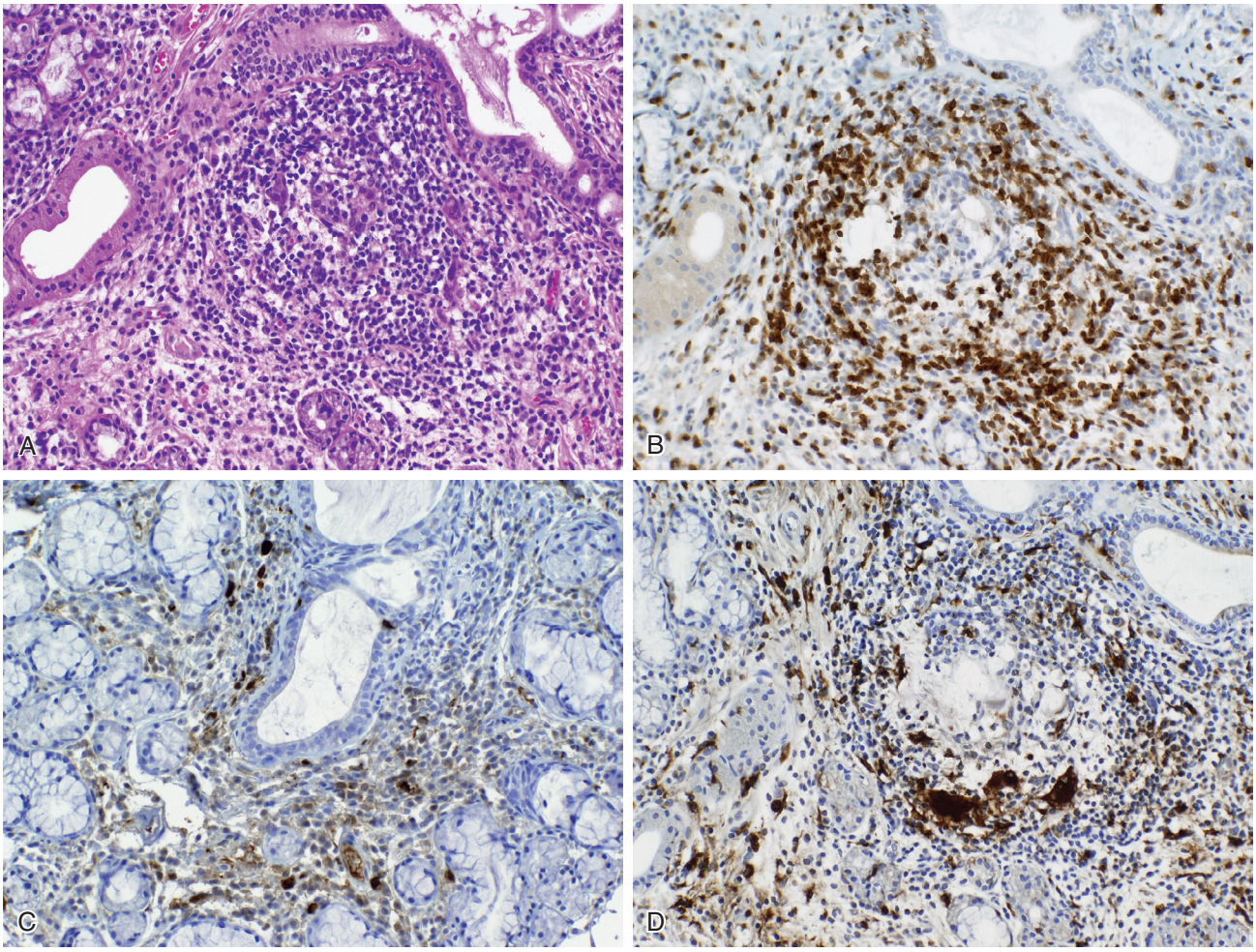



**Figure 73-1** Histopathology of the labial salivary glands in primary Sjögren's syndrome. **A,** Hematoxylin-eosin (20×). **B,** Anti-CD3 staining of T cells (20×). **C,** Anti-CD21 staining of B cells (20×). **D,** Anti-CD68 staining of macrophages (20×). Mononuclear cells aggregate in foci throughout the glands **(A)**. In this biopsy, most of the mononuclear cells are T cells **(B)**, with fewer numbers of B cells **(C)** and macrophages **(D)**.

multiple Vβ families. The proportion of B cells in the infiltrate increases with the severity of the inflammatory lesion.

The infiltrating mononuclear cells tend to coalesce around ducts and blood vessels, and in more severe inflammatory lesions form aggregates organized into GC-like structures. The GC-like structures display well-circumscribed mononuclear cell infiltrates with B and T cell components, Ki-67$^+$ proliferating cells, CD21/CD35$^+$ follicular DC networks, and CD31$^+$ high endothelial venules (HEVs).[67] CXCL13 expression by epithelial cells, HEVs, and within GC-like structures together with the expression of CXCL12 and CCL21 provides a salivary gland microenvironment capable of attracting and retaining B cells.[68-70] The receptor for CXCL13 is CXCR5, which is expressed on the surface of B cells and follicular Th cells. The myeloid DCs and macrophages, the classic antigen-presenting cells, are mostly found in proximity to the ductal epithelium, where they secrete TNF, IL-6, IL-10, IL-12, and IL-18. Minor salivary glands also contain a small number of plasmacytoid DCs, the main producers of the type I IFNs.[71] A robust type 1 IFN signature has been detected both in the salivary glands and peripheral blood of patients with primary Sjögren's syndrome.[71,72] The generation of type 1 IFNs by plasmacytoid DCs is among the first lines of defense against viral infection and is partially dependent on signals through TLR7 and TLR9.

The analysis of T cell cytokines in labial salivary gland biopsies from patients with primary Sjögren's syndrome suggests a predominantly Th1- and Th17-driven response.[73] Studies of the expression of cytokine messenger RNAs (mRNAs) in salivary gland tissue show mainly upregulation of IL-2 and IFN-γ, Th1-specific cytokines, and lesser quantities of IL-4, IL-5, and IL-13, the Th2-specific cytokines. Th17 cells are also found in the minor salivary glands; the salivary gland microenvironment is rich in transforming growth factor (TGF)-β, IL-6, and IL-23, which are cytokines known to promote the development of this subset.[74] In addition, the salivary gland contains FoxP3$^+$ regulatory T cells.[75] These cells typically show suppressive behavior, but as yet they have an uncertain role in regulating the chronic inflammatory lesion.

Several features of primary Sjögren's syndrome implicate B cells in disease mechanisms. B cells are the source of autoantibodies, but they also activate T cells by presenting

antigen peptides, secrete both pro-inflammatory and anti-inflammatory cytokines, and promote the organization of secondary and tertiary lymphoid tissue. The frequent presence of hypergammaglobulinemia, circulating immune complexes, mixed monoclonal immunoglobulin M (IgM) cryoglobulinemia, and serum autoantibodies in patients with primary Sjögren's syndrome provide further evidence that B cells are in a dysregulated state in this disease. Most B cells in the salivary gland tissue exhibit a memory phenotype, express somatically hypermutated immunoglobulin V genes, and show preferential changes in $V_L$ gene usage and the length of CDR3 sequences from $V_H$ gene rearrangements, which are findings characteristic of an antigen-driven response.[76] Salivary gland B cells appear to be responding specifically to antigen, although the site of antigen encounter and activation (salivary gland vs. secondary lymphoid tissue) remains uncertain. GC-like structures found in some of the salivary gland biopsies provide an opportune environment for the selection and differentiation of antigen-driven B cell responses. The distribution of peripheral blood B cell subsets in primary Sjögren's syndrome differs from healthy controls, showing increased proportions of $CD27^-$ (naïve) B cells and decreased proportions of $CD27^+$ (memory) B cells.[77] Among several possibilities, these changes in the peripheral blood may result from abnormal trafficking of $CD27^+$ memory B cells to the target tissues, aberrations in B cell development, or both.

Several intrinsic B cell defects have also been found in patients with primary Sjögren's syndrome, including abnormal retention of preswitch Ig transcripts in circulating postswitch $CD27^+$ memory B cells[78] and enhanced B cell signaling due to altered kinetics of B cell receptor translocation to lipid rafts.[79] Moreover, studies in patients with primary Sjögren's syndrome have shown increased serum and salivary gland tissue levels of B cell–activating factor (BAFF), a B cell prosurvival factor,[80] as well as upregulated salivary gland expression of lymphotoxin (LT)-β mRNA.[81] LT-β is required for the formation of lymph nodes and GCs, whereas the heterodimer LT-α/LT-β can induce the development of ectopic GC-like structures. LT-α in soluble form induces the secretion of IFNs and chemokines, which are elevated in the salivary gland tissue from patients with primary Sjögren's syndrome. Levels of IL-14, another B cell growth factor, also appear to be increased in the serum and saliva of patients with primary Sjögren's syndrome.[82] IL-14 transgenic mice develop a Sjögren's-like phenotype[83] in which the local tissue response is critically dependent on LT-α.[84] Blocking LT-β receptor signaling in nonobese diabetic (NOD) mice ablates the lymphoid organization in the salivary glands and improves their function,[85] further evidence that LTs may play a role in the pathogenesis of Sjögren's syndrome. Finally, abnormal B cell behavior is implied in primary Sjögren's syndrome by the predisposition of this disease toward the development of non-Hodgkin's B cell lymphoma. Interestingly, IL-14 transgenic mice develop large B cell lymphomas later in life.[83]

## Autoantibodies

Anti-Ro/SS-A and anti-La/SS-B antibodies do not appear to have a pathogenic role in disease mechanisms despite their diagnostic and prognostic significance. The stimulating antigen(s) is unknown. An aberrant response to self may be provoked by altered expression of autoantigens. Several models have been proposed to explain the altered expression of autoantigens, including differential expression of protein isoforms, post-translational modification, and abnormal autoantigen presentation via apoptotic blebs, exosomes, or heat shock protein–mediated cross-priming.[86] Ro/SS-A and La/SS-B proteins do in fact show upregulated expression in the vicinity of the immunopathologic lesion[86] where they may induce local immune responses. Saliva from patients with this disease contains anti-Ro/SS-A and anti-La/SS-B antibodies.[87] Although this finding may be the result of local production of autoantibodies, it may also reflect extravasation of proteins from blood vessels into the inflamed tissues.

Among the other autoantibody specificities, those directed against the muscarinic receptor (MR) have attracted the most interest because of their possible role in causing glandular hypofunction. The MR family of receptors is bound by acetylcholine, which mediates the effects of the preganglionic and postganglionic parasympathetic nerve fibers that primarily regulate salivary flow. The muscarinic type 3 receptor (M3R), the target of autoantibodies in primary Sjögren's syndrome, is the subtype that predominantly controls salivary flow. Two lines of evidence support the hypothesis that anti-M3R antibodies reduce exocrine gland function. First, serum immunoglobulins from patients with primary Sjögren's syndrome bind M3R receptors on acinar cell membranes. They also inhibit acetylcholine-evoked $Ca^{2+}$ responses in a salivary gland cell line.[88] This $Ca^{2+}$-sensitive response is tightly regulated through intracellular signaling pathways that open the $Cl^-$ channels on the apical membrane, resulting in an osmotic gradient and movement of water into the duct lumen. Second, passive transfer of the antibody causes glandular hypofunction in NOD mice, an animal model of Sjögren's syndrome.[89] However, the different assays for detecting serum anti-M3R antibodies in patients with primary Sjögren's syndrome have produced inconsistent results. For this reason, it has been difficult to determine the diagnostic sensitivity and specificity of serum anti-M3R antibodies in primary Sjögren's syndrome and the possible relationship of these autoantibodies to glandular hypofunction. Other autoantigens have been identified in patients with primary Sjögren's syndrome and include α-fodrin, poly(ADP)ribose polymerase, carbonic anhydrase, and ICA69 protein. Despite initial enthusiasm for the possible etiologic role of α-fodrin in primary Sjögren's syndrome, recent studies have shown that serum anti–α-fodrin antibodies are not highly specific for this diagnosis.[90] Autoantibodies to carbonic anhydrase, poly(ADP)ribose polymerase, and ICA69 protein occur in only a minority of patients with primary Sjögren's syndrome and therefore do not appear to be important for initiating disease.

## Glandular Epithelium

In primary Sjögren's syndrome, the glandular epithelial cell may also drive the abnormal immune response. In the salivary gland, the mononuclear cells preferentially congregate around ductal epithelium. The epithelial cells show upregulated expression of class I and II HLA

molecules; adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1; CD54), vascular cell adhesion molecule-1 (VCAM-1; CD106) and E-selectin; and costimulatory molecules such as CD80 and CD86[91]; they also produce high levels of cytokine mRNAs for IL-1, IL-6, IL-12, IL-18, and TNF,[92-94] as well as BAFF.[80] Ductal epithelial cells express mRNAs for several different proinflammatory chemokines, including CXCL13, CCL17, CCL21, and CCL22.[95] Cultured salivary gland epithelial cells express functional TLR3 and TLR7 molecules,[96,97] endowing them with a capability to sense pathogens and endogenous molecules produced by injured tissues. Activated epithelial cells are likely drivers of the aberrant innate and adaptive immune responses, not only in the lacrimal and salivary glands, but also in the lungs and kidneys, as well as other extraglandular sites.

At least two models have been proposed to explain the glandular hypofunction in Sjögren's syndrome. In one model, it may be hypothesized that the glandular tissue is destroyed by an immune onslaught perpetuated by persistent exposure to self-antigens or other environmental stimulants (e.g., viral infection), leading to apoptosis of acinar epithelial cells and the irreversible loss of salivary gland function[98] (Figure 73-2A). A weakness of this model is that studies suggest epithelial cells rarely undergo apoptosis in the salivary gland tissue despite the fact they increase expression of mediators of cell death such as Fas, Fas ligand, and Bax (B cell lymphoma 2–associated X protein).[99] Ligand binding to CD40 expressed on epithelial cells in the salivary gland tissue also leads to Fas-mediated cell death by downregulating c-FLIP (cellular FLICE-like inhibitor protein), an inhibitor of Fas-mediated cell death.[100] Because no studies have yet shown that an imbalance truly exists between the rates of glandular proliferation and damage, the relevance of this model to glandular hypofunction is uncertain. It also does not explain why many patients with markedly diminished salivary flow retain substantial amounts of normal-appearing acinar tissue in their salivary glands.[101]

A second model assumes that apoptosis is not a significant factor in the loss of acinar epithelium and, instead, posits that glandular function is inhibited (and not destroyed) by immune-mediated mechanisms (see Figure 73-2B). This model implies a reversibility component to the loss of salivary flow and a pathologic process interrupting M3R activation. Possible mechanisms for such inhibitory effects include a reduction in acetylcholine release, increased breakdown of acetylcholine in the epilemmal space (e.g., acetylcholine must diffuse 100 nm from its release point at the nerve ending to the receptor on the cell), and blockade of M3R by antibodies.[102] Relatively little direct evidence supports any of these mechanisms, except for the experiments showing that anti-M3R antibodies may block receptor function. Nevertheless, it is possible that the cytokine milieu in the inflamed glandular tissue interferes with the mechanisms controlling acinar gland function. In the NOD mouse, for example, impairment of salivary gland secretion does not appear to be readily explained by the destruction of acinar glands.[103] Regardless, the "immune destructive" and "immune inhibited" models are not mutually exclusive and may contribute to glandular hypofunction at various stages of the disease.

## CLINICAL FEATURES

### Keratoconjunctivitis Sicca

Chronic inflammation of the lacrimal glands diminishes secretion of aqueous tears, which if severe, may destroy the conjunctival and bulbar epithelium. Aqueous tear deficiency produces a dry eye, causing symptoms of grittiness or foreign body sensation, burning, photophobia, and eye fatigue. Routine eye examination usually reveals a reduction in the tear flow, as measured by the Schirmer-I test. Additional findings may include the absence of tears in the conjunctival sac and dilated bulbar conjunctival vessels. Thick mucus may also be seen in the inner canthus of the eye. Slit-lamp examination allows for more detailed visualization of the corneal and conjunctival surface. After instillation of lissamine green dye or fluorescein onto the ocular surface, slit-lamp examination may expose devitalized cells or epithelial defects, respectively, signs of corneal and conjunctival damage. Severe dryness may result in corneal abrasion or ulceration.

The anatomy of the tear film informs the differential diagnosis of a dry eye (Table 73-3). The tear film consists of three major layers: the outer lipid layer, the middle aqueous layer, and the inner mucin layer. The middle aqueous layer is diminished in Sjögren's syndrome. Conditions associated with infiltration of the lacrimal glands (e.g., sarcoidosis) and diminished tear flow (e.g., medications, aging, and estrogen deficiency) may also decrease aqueous tear flow. The lipid layer derives from the meibomian glands and traps the aqueous tear film on the eyeball and protects it from rapid evaporation. Meibomian gland dysfunction, or posterior blepharitis, produces dry eyes from rapid evaporation of tears; it may be present with aqueous tear deficiency and be an aggravating factor in patients with keratoconjunctivitis sicca. Meibomian gland dysfunction is often associated with ocular rosacea and seborrheic dermatitis, two conditions encountered often in clinical practice that also lead to symptoms of dry eyes. Lipid degradation resulting from meibomian gland inflammation may produce free fatty acids, which irritate the ocular surface and may cause punctate keratopathy. The mucin layer originates from the goblet cells of the conjunctiva and, if deficient, leads to an uneven distribution of the tear film over the surface of the eye. Vitamin A deficiency and Stevens-Johnson syndrome are examples of conditions associated with an abnormal mucin layer.

### Xerostomia

The changes in the quality and quantity of the saliva are responsible for the signs and symptoms of xerostomia. Although symptoms of dry mouth are relatively common in the general population, they are usually more severe in Sjögren's syndrome and cause incessant difficulties with chewing and swallowing dry food, altered taste (metallic, salty, or bitter), and struggles with prolonged speaking. Patients with xerostomia may have problems wearing dentures. Despite complaints of a dry mouth, many patients will appear to have a normal oral examination because of residual salivary flow. Others with more severe hypofunction will manifest a dry, sticky, or erythematous oral mucosa.

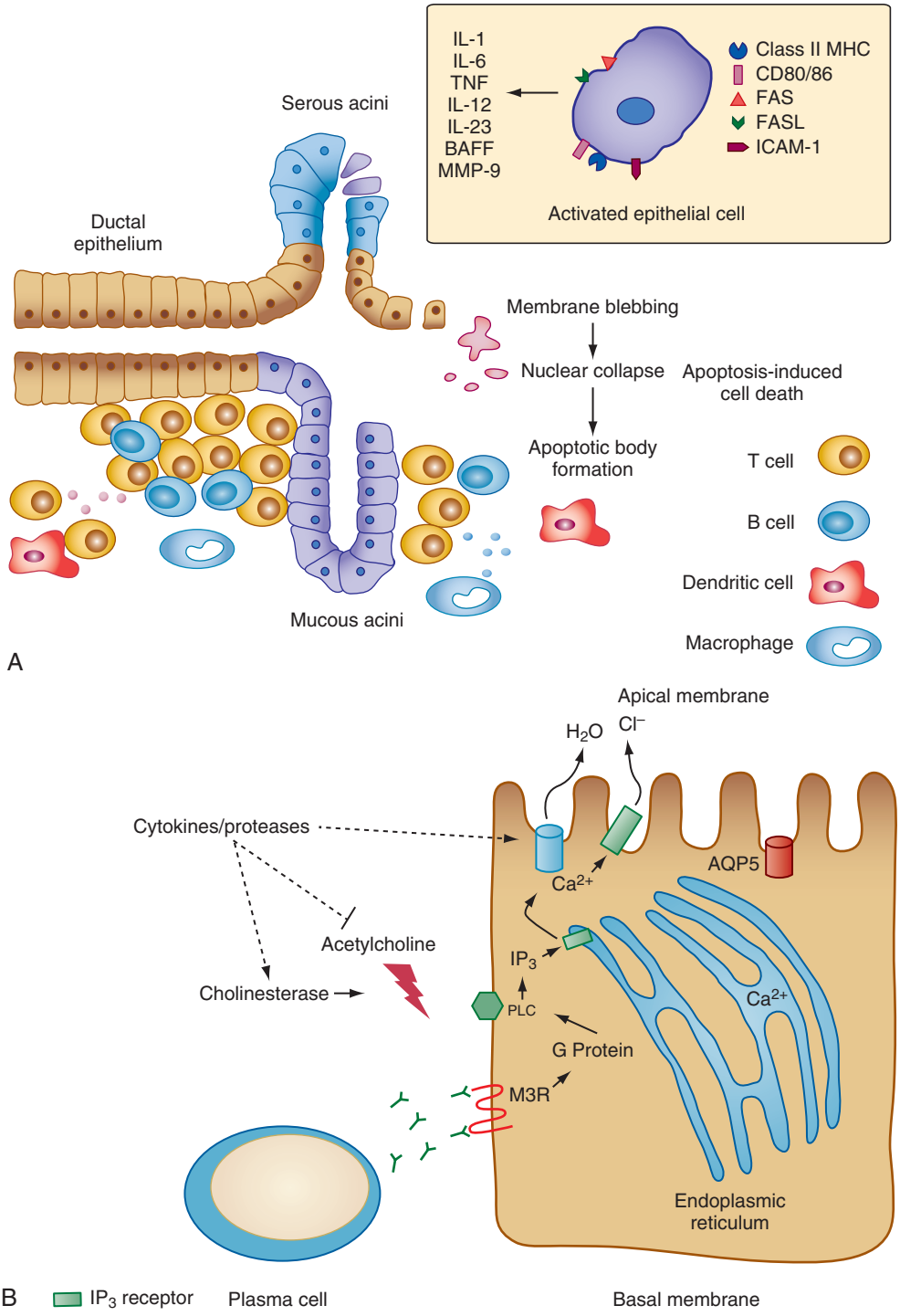



**Figure 73-2** Two models are shown for the pathogenesis of salivary gland inflammation in Sjögren's syndrome. **A,** Glandular hypofunction is explained by tissue loss secondary to immune attack, resulting in cytotoxic cell death and apoptosis. Epithelial cells likely play a central role in this process by several mechanisms: antigen-presentation and T cell activation; production of pro-inflammatory cytokines, such as IL-1, IL-6, TNF, IL-12, and IL-23; and secretion of proteases. Epithelial cells also upregulate expression of Fas (FAS) and Fas ligand (FASL), cell surface molecules involved in the activation of apoptotic pathways. Other immune cells such as T cells, B cells, macrophages, and dendritic cells serve to amplify the chronic inflammatory response. In model **B,** glandular hypofunction results from downregulation of receptor-mediated secretion of salivary fluid into the ductal lumen. Acetylcholine binds to muscarinic type 3 receptor (M3R) on the surface of acinar cells, stimulating production of the second messenger inositol 1,4,5-triphosphate ($IP_3$). $IP_3$ in turn diffuses through the cytoplasm until it binds its receptor, $IP_3R$, on the endoplasmic reticulum. This interaction causes calcium to be released into the cytoplasm, which opens $Ca^{2+}$-sensitive chloride channels on the apical membrane of the cell. Electrochemical neutrality is preserved when $Na^+$ follows chloride across the membrane, while the osmotic gradient propels the water into the ductal lumen. Mechanisms of immune-mediated glandular hypofunction might include inhibition of acetylcholine release by cytokines, increased breakdown of acetylcholine in the epilemmal space by upregulated production of cholinesterases, blockade of M3R by autoantibodies, inhibition of intra-cellular signaling pathways involved in the fluid secretory process, or altered expression of aquaporin 5 (AQP5), which appears to be primarily responsible for water movement through the apical cell membrane. See reference 96 for more details. BAFF, B cell activating factor; ICAM-1, intercellular adhesion molecule-1; MHC, major histocompatibility complex; MMP-9, matrix metalloproteinase-9; PLC, phospholipase C.

**TABLE 73-3** Differential Diagnosis of Primary Sjögren's Syndrome

| Condition | Associated Clinical/Radiologic/Pathophysiologic Features | Immunologic/Histopathologic Profile |
|---|---|---|
| **Dry Eye (Keratoconjunctivitis Sicca)** | | |
| ***Aqueous Tear Deficiency (Failure of Lacrimal Tear Secretion)*** | | |
| Sarcoidosis* | Lacrimal and parotid gland enlargement, cervical and hilar adenopathy, ILD, uveitis, arthralgia/arthritis, erythema nodosum | ANA negative or low titer; noncaseating granulomas in multiple organs (e.g., lymph nodes, lungs, spleen, liver, skin, salivary glands) |
| Chronic hepatitis C infection | Chronic hepatitis | Serum hepatitis C virus antibodies; hepatitis C viremia |
| Chronic GVHD | Occurs after allogeneic hematopoietic stem cell transplantation; other ocular features include sterile conjunctival inflammation and scarring, with progressive loss of goblet cells and dysfunction of meibomian glands | Lacrimal gland fibrosis, with mild chronic inflammation |
| Sensory block | Corneal surgery (LASIK), contact lens wear, diabetes | |
| Motor block | Cranial nerve VII damage | |
| Age-related | None | |
| Systemic drugs† | | |
| ***Evaporative (Excessive Water Loss from the Ocular Surface)*** | | |
| Meibomian gland deficiency | Ocular redness and burning; increased tear break-up time and tear hyperosmolarity; secondary to posterior blepharitis, acne rosacea, seborrheic dermatitis, and drugs (e.g., retinoids) | |
| Cicatricial pemphigoid‡ | Scarring of the meibomian orifices, loss of goblet cells | |
| Poor lid congruity or low blink rate | Proptosis (poor lid congruity), Parkinson's disease (low blink rate) | |
| Vitamin A deficiency | Impaired goblet cell development | |
| **Dry Mouth (Hyposalivation)** | | |
| Amyloidosis | Nephrotic syndrome | ANA negative; monoclonal gammopathy; biopsy often shows amyloid deposits |
| Hemachromatosis | Cirrhosis | Labial salivary gland biopsy with heavy hemosiderin deposits in the ductal epithelium and loss of acinar glands |
| Chronic GVHD | Salivary gland dysfunction less common than lacrimal gland dysfunction; may be associated with oral mucosal involvement of chronic GVHD | Periductal inflammation, fibrosis, and glandular atrophy |
| Chronic hepatitis C infection | See previous text | Labial salivary gland biopsy shows chronic inflammation similar to that of Sjögren's syndrome |
| Diabetes mellitus | Type I or type II diabetes | |
| Radiation therapy | Side effects after radiotherapy for head and neck cancer | Lack of functional acinar cells; damage to ducts, blood vessels, and nerves |
| Anxiety | | |
| Systemic drugs† | | |
| **Parotid Gland Enlargement** | | |
| Sarcoidosis* | See previous text | See previous text |
| IgG4-related diseases | Multiorgan involvement: lacrimal and salivary gland enlargement, chronic sclerosing sialadenitis, autoimmune pancreatitis, sclerosing cholangitis, lung nodules, interstitial pneumonitis, aortitis, interstitial nephritis, prostatitis, orbital pseudotumor, dacroadenitis, meningitis, hypophysitis | High serum IgG4 levels; lymphoplasmacytic infiltrate and abundance of $IgG4^+$ plasma cells in tissues |
| Multicentric Castleman's disease | Lacrimal and salivary gland enlargement (uncommon); fever, lymphadenopathy, hepatomegaly, edema, and ascites (common); increased incidence of non-Hodgkin's lymphoma | Elevated serum IL-6 levels; some patients with increased serum IgG4 levels; lymph node biopsy shows interfollicular plasmacytosis |
| Diabetes mellitus | More common in type II than type I diabetes | Sialadenosis |
| Alcoholism | | Sialadenosis |
| HIV lymphoepithelial lesion | Bilateral involvement | Extensive lymphoid infiltrate with reactive germinal centers ($CD8 > CD4^+$ T cells) |
| Benign tumors | Painless swelling of the parotid gland; usually unilateral but sometimes bilateral | Pleomorphic adenoma (usually unifocal); Warthin tumor: often multifocal and bilateral; oncocytoma; many other types |

**TABLE 73-3** Differential Diagnosis of Primary Sjögren's Syndrome—cont'd

| Condition | Associated Clinical/Radiologic/Pathophysiologic Features | Immunologic/Histopathologic Profile |
|---|---|---|
| Malignant carcinoma | Painless swelling of the parotid gland, facial nerve palsy | Most common: mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic |
| Primary B cell lymphoma | Unilateral or bilateral parotid gland swelling | Marginal zone B cell lymphoma most common subtype (also called *MALT-type*); follicle center and mantle zone lymphomas may also occur in this region; diffuse large B cell lymphoma (rare) |
| Primary T cell lymphoma | Rarely may present as a parotid gland mass; HTLV-1-associated | Anaplastic large cell or diffuse pleomorphic (medium and large cell) |
| Calculous duct obstruction | Painful enlargement of salivary glands | Ruptured ducts and obstructive granulomas |

*May coexist with primary Sjögren's syndrome.
†Common offending drugs include: anticholinergics (e.g., antihistamines, tricyclic anti-depressants, antispasmodics), clonidine, diuretics, isotretinoin, estrogen replacement therapy, amiodarone.
‡May also destroy the lacrimal gland and cause lacrimal duct obstruction late in the clinical course.
ANA, Anti-nuclear antibodies; GVHD, graft-versus-host disease; HIV, human immunodeficiency virus; HTLV-1, human T lymphotropic virus type 1; IL-6, interleukin-6; ILD, interstitial lung disease; MALT, mucosa-associated lymphoid tissue.

Two complications of xerostomia are important to the care of patients, especially those with severe deficits in salivary flow. One complication is rampant dental caries, cracked teeth, and loose fillings. Oral candidiasis, the other frequent complication, typically manifests as the atrophic variant, which is characterized by erythema and atrophy of the oral mucosa and filiform papillae on the dorsum of the tongue, with angular cheilitis. Sometimes, a thin, white exudate may appear on the surface of the tongue. The "thrushlike" variant of oral candidiasis is seen much less frequently in patients with xerostomia except in the face of recent antibiotic therapy.

Approximately one-quarter of patients with primary Sjögren's syndrome suffer enlargement of the parotid or submandibular glands during the course of their disease. Chronic swelling is usually painless and may be unilateral or bilateral; it is often diffuse and firm by palpation. Transiently painful episodes of acute swelling and tenderness may also punctuate the clinical course. Acute swelling of the major salivary glands results mostly from dried mucus transiently obstructing the major ducts; it usually subsides within a few days with conservative therapy. Rarely, bacterial infections may cause acute salivary gland swelling and should be considered as a possible etiology if the patient has a fever or other constitutional complaints. Asymmetric gland enlargement with palpable hard nodules that are increasing in size may indicate a neoplasm such as a lymphoma.

## Involvement of Other Exocrine Glands

Glandular hypofunction may affect the nasal passages (meatal obstruction from dried mucus), larynx (hoarseness), trachea (cough), vagina (dyspareunia), and skin (pruritus), producing symptoms of dryness. Virtually any exocrine gland may be involved in this disease.

## Extraglandular Manifestations

Nearly three-quarters of patients with primary Sjögren's syndrome manifest signs or symptoms of extraglandular disease. Extraglandular involvement is more likely to occur in patients with serum anti-Ro/SS-A and anti-La/SS-B antibodies, as well as hypergammaglobulinemia, cryoglobulinemia, and hypocomplementemia.[104] However, only approximately 25% of patients with primary Sjögren's syndrome develop moderate or severe extraglandular disease.

### Fatigue

Fatigue, a complex and multifaceted phenomenon, occurs in approximately 70% of patients with primary Sjögren's syndrome.[105] It is also a symptom of depression, chronic anxiety, fibromyalgia, and sleep deficit, as well as a side effect of certain medications. The United Kingdom Sjögren's Interest Group has developed an instrument called the Profile of Fatigue and Discomfort–Sicca Symptoms Inventory (PROFAD-SSI) to specifically measure both somatic (needing rest, poor starting, low stamina, weak muscles) and mental fatigue (poor concentration, poor memory) in this disease.[106] In 547 patients with a confirmed diagnosis of primary Sjögren's syndrome, somatic fatigue was the dominant predictor of physical function and general health.[107] The relative contribution of behavioral and cognitive variables to fatigue was studied in 94 patients with primary Sjögren's syndrome.[108] Fatigue was prominent among patients in this study without depression, and it was only partially explained by psychosocial variables. The link between fatigue and "biologic disease activity" is not fully understood in primary Sjögren's syndrome. Increased serum cytokines such as IL-6 and type I IFNs, as well as neuroendocrine and autonomic dysfunction, are postulated to be contributing factors to the physical and mental aspects of fatigue in this setting.[105]

### Raynaud's syndrome

Raynaud's syndrome has been reported in 13% to 33% of patients with primary Sjögren's syndrome, and often precedes the onset of sicca symptoms by several years.[109] Digital ulcers occur only rarely.

### Skin

Among the dermatologic manifestations, the most common are xerosis, or dry skin, eyelid dermatitis, and angular

cheilitis. In addition, many patients develop a variety of other cutaneous manifestations, including annular erythema, purpura, and urticarial vasculitis. Annular erythema has been described in several forms: a donut-ring–like erythema with an elevated border (type I); a subacute cutaneous lupus erythematosus (SCLE)-like lesion with marginally scaled polycyclic erythema (type II); and a papular insect bite–like erythema (type III).[110] Histopathologically, these lesions are characterized by a deep perivascular lymphocytic infiltrate without the epidermal changes associated with lupus.[111] In some cases, immunoglobulin and complement deposition is observed along the basement membrane with liquefaction degeneration in the basal layer of the involved skin. The type I lesion appears to be specific for primary Sjögren's syndrome, occurring predominantly in Asian as opposed to Western populations.

Cutaneous vasculitis may be expressed in several different forms, including palpable purpura, erythematous papules or macules, and ulcers, with lesions predominantly located in the lower limbs.[112] Such lesions are frequently accompanied by other extraglandular manifestations of disease, such as arthritis, peripheral neuropathy, Raynaud's syndrome, anemia, elevated erythrocyte sedimentation rate, hypergammaglobulinemia, serum rheumatoid factor, and serum anti-Ro/SS-A and anti-La/SS-B antibodies. In one study, among the 52 cases of cutaneous vasculitis, 27% and 50% occurred in patients with and without cryoglobulinemia, respectively; 21% of cases were classified as *urticarial vasculitis*.[112] Livedo reticularis has also been described in this setting.

### Joints

Polyarthralgia occurs frequently in patients with primary Sjögren's syndrome. In a retrospective study, articular symptoms in primary Sjögren's syndrome had a prevalence rate of 45%.[113] Although most patients complain only of polyarthralgia, a subset may experience objective signs of synovitis, which is nonerosive, symmetric, and polyarticular of waxing and waning intensity. Joint symptoms may precede the diagnosis of primary Sjögren's syndrome in as many as one-third of cases.[114] In two separate studies, anti-citrullinated protein antibodies (ACPA) were detected in serum from 7.5% and 9.9% of patients with primary Sjögren's syndrome.[115,116] However, in only one of these studies was their presence closely associated with synovitis,[116] and in neither study did it appear that serum ACPA positivity was associated with radiographic erosions or progression to rheumatoid arthritis.

### Lung

Involvement of the airways and lung parenchyma in primary Sjögren's syndrome may take several forms, including xerotrachea and xerobronchitis; nonspecific interstitial pneumonitis (NSIP); lymphocytic interstitial pneumonitis (LIP), now considered a subset of NSIP; usual interstitial pneumonitis (UIP); bronchiolitis; and lymphoma.[117] The estimated prevalence of pulmonary involvement in primary Sjögren's syndrome varies depending on the thoroughness of the evaluation. In one study of 123 patients with primary Sjögren's syndrome, 11.4% showed pulmonary signs or symptoms and/or impaired pulmonary function with abnormal chest computed tomography (CT) findings at the time of evaluation.[118]

Evidence of small airways dysfunction is found often in asymptomatic patients with normal radiologic studies. In symptomatic patients, NSIP appears to be the predominant type of lung involvement. The diagnosis can often be made on the basis of clinical presentation, pulmonary function tests (PFTs), and abnormal chest CT findings. PFTs in patients with NSIP show a restrictive pattern with reduced diffusion capacity of lung carbon monoxide (DLCO). Chest CT scans reveal ground-glass opacities and a reticular nodular pattern. Bronchoalveolar lavage (BAL), which is not usually required for diagnosis, shows evidence of alveolar inflammation, with elevated neutrophil or lymphocyte counts, or both. LIP, a subset of NSIP, is also associated with a restrictive pattern on PFTs. Chest CT findings are ground-glass opacities and thin-walled cysts, with centrilobular nodules, interlobular septal thickening, and bronchovascular bundle thickening (Figure 73-3A). Microscopically, the lung biopsy from patients with LIP shows a diffuse interstitial infiltrate composed of lymphocytes, plasma cells, and histiocytes that expand the interlobular and alveolar spaces (see Figure 73-3). The PFTs in patients with UIP also show a restrictive pattern such as NSIP and LIP, but lower lobe fibrosis, honeycombing, and traction bronchiectasis seen on chest CT distinguish it from these other two types. Follicular bronchiolitis is characterized histopathologically by nodular lymphocytic infiltrates with GC-like structures encircling respiratory bronchioles. In patients with bronchiolitis, PFTs may show a restrictive or obstructive functional defect. The chest CT scan usually displays reticulonodular infiltrates, but it may be healthy in mild cases.

Lung biopsy can reveal evidence of NSIP, bronchiolitis, or other pathologic processes. Ito and colleagues[119] evaluated 33 patients with primary Sjögren's syndrome that had biopsy-proven lung disease and found evidence of NSIP in 20 (61%), non-Hodgkin's lymphoma in four (12%), diffuse bronchiolitis in 4 (12%), and amyloid in 2 (6%). PFTs demonstrated restrictive changes in 19 (58%) patients and obstructive changes in 3 (9%) patients. Analysis of BAL fluid from all of the 28 patients who had this procedure was uniformly abnormal, showing elevated lymphocyte counts in 18 (64%) and elevated neutrophil counts in 19 (68%). In 31 patients, chest CT findings revealed an NSIP pattern in 14 (45%) and a UIP pattern in 4 (13%); 3 patients (10%) showed a pattern of organizing pneumonia. There were no cases of LIP in this series. By comparison, Parambil and colleagues[120] evaluated 18 patients with biopsy-proven lung disease and found a histopathologic pattern of NSIP in 5 (28%) patients, organizing pneumonia in 4 (22%) patients, UIP in 3 (17%) patients, LIP in 3 (17%) patients, lymphoma in 2 (11%) patients, and amyloid in 1 (6%) patient. Pulmonary arterial hypertension is a rare complication of Sjögren's syndrome.[121]

### Kidney

Renal disease of clinical significance in primary Sjögren's syndrome is present only in approximately 5% of patients and includes tubular interstitial nephritis, type I renal

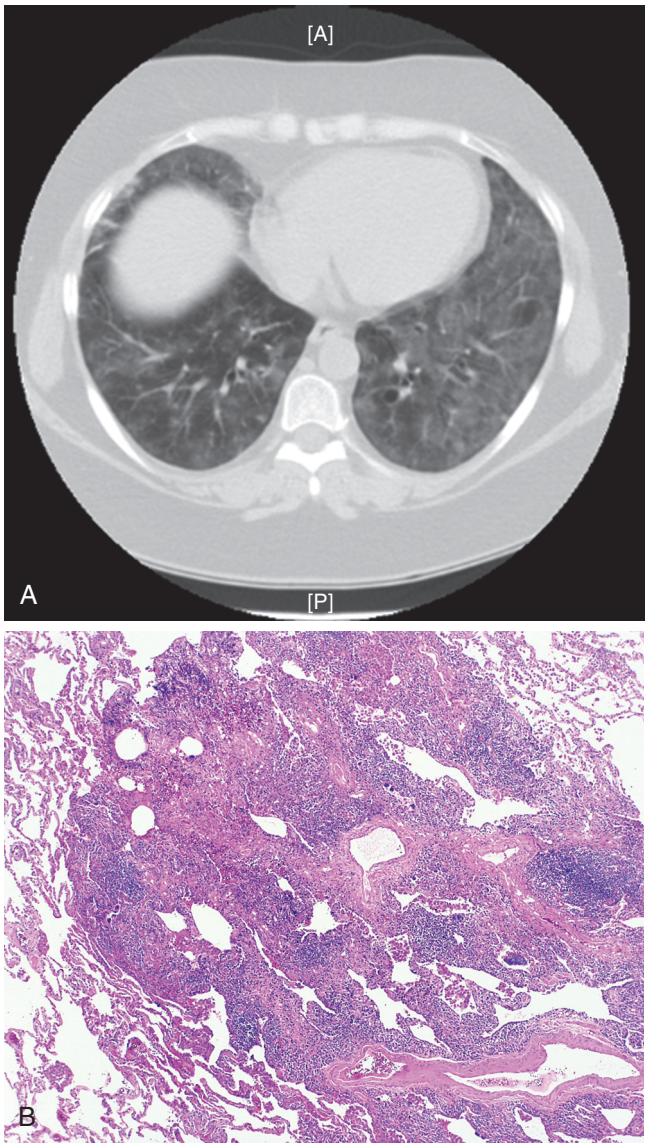



**Figure 73-3** Lymphocytic interstitial pneumonitis (LIP) in a 44-year-old woman with primary Sjögren's syndrome. **A,** A slice through the lower lobes of a chest computed tomography scan shows diffuse ground-glass opacities, a scattering of multiple nodules in the periphery, and many thin-walled parenchymal cysts. **B,** Histopathologic examination from a wedge resection of the lower lobe of this patient shows a patchy, nodular interstitial infiltrate composed of mononuclear cells, with widening of the interlobular and alveolar spaces, consistent with LIP.

tubular acidosis (RTA), glomerulonephritis, and nephrogenic diabetes insipidus. Tubular interstitial nephritis, which is characterized histopathologically by a peritubular lymphocytic infiltrate and fibrosis, rarely progresses to end-stage renal disease (see Figure 73-3A).[122,123] A patient with primary Sjögren's syndrome has been described with tubular interstitial nephritis and acquired Gitelman's syndrome, with absence of a sodium chloride co-transporter in the distal convoluted tubules.[124] Rarely, severe potassium wasting from type I RTA can lead to muscle paralysis.[125] Glomerular disease occurs less commonly in this setting and may take several forms, including membranous, membranoproliferative, mesangial proliferative, and focal crescentic glomerulonephritis. Glomerular disease is most often the result of immune complex deposition in the kidney and is more frequently associated with lymphoma than tubulointerstitial disease.[126]

### Gastrointestinal

Patients with primary Sjögren's syndrome have an increased frequency of gastrointestinal symptoms compared with the general population. Dysphagia and heartburn are particularly common complaints that may result from impaired salivary flow or esophageal motility, or both. Approximately one-third of patients with primary Sjögren's syndrome have varying degrees of esophageal dysfunction, although many studies have been unable to correlate symptoms of dysphagia with a functional abnormality.[127] The results of one study suggest that patients with primary Sjögren's syndrome do not have a primary disturbance of esophageal motility, but rather defective clearance of esophageal acid that exposes the esophageal lining to acid in excessive amounts, which in turn, produces morphologic changes and secondary dysmotility.[128] Other results suggest parasympathetic dysfunction might be at the root of the esophageal abnormalities.[127] A case has also been described of a patient with primary Sjögren's syndrome in whom esophageal achalasia developed in the setting of a sensory ataxic neuropathy, which damaged the myenteric plexus and produced the esophageal abnormality.[129] In addition, chronic atrophic gastritis, which may cause dyspeptic symptoms, has been reported in a small number of cases of primary Sjögren's syndrome.[130]

Liver enzyme abnormalities may develop in patients with primary Sjögren's syndrome for a variety of reasons, most often from an associated disorder such as hepatitis C virus infection, autoimmune hepatitis, primary biliary cirrhosis, or a nonspecific hepatitis.[131] Bowel symptoms such as abdominal pain and constipation occur more commonly in patients with primary Sjögren's syndrome than healthy controls, but their etiology is often obscure.[132]

### Nervous System

Neurologic abnormalities are protean in primary Sjögren's syndrome, with varied patterns of peripheral and central nervous system (CNS) involvement. The prevalence of central nervous system involvement that can be directly attributed to primary Sjögren's syndrome is likely to be in the range of 1% to 2%, but much higher rates have been reported with more liberal case definitions. For example, higher prevalence rates are noted when mood disturbances and minor cognitive and affective disturbances are included in the definition of CNS involvement. Neuropsychiatric symptoms such as depression and minor cognitive disturbances occur in approximately one-third of patients with primary Sjögren's syndrome. However, these neuropsychiatric problems are clinical disorders that occur frequently in the general population and in patients with chronic diseases in general. Rarely, cases have been described in which severe cognitive dysfunction develops in patients with primary Sjögren's syndrome. Brain MRI scans in these instances typically show nonspecific T2-weighted, high-intensity signals in the white matter that have an uncertain relationship to the clinical CNS findings. Focal CNS

deficits have also been rarely described in patients with primary Sjögren's syndrome, and include optic neuropathy, hemiparesis, movement disorders, cerebellar syndromes, recurrent transient ischemic attacks, and motor neuron syndrome.[133] In addition, spinal cord syndromes resembling multiple sclerosis such as transverse myelitis and progressive myelopathy have also been reported in patients with primary Sjögren's syndrome.[134] Patients with primary Sjögren's have been described, too, with neuromyelitis optica in association with serum anti–aquaporin-4 antibodies.[135]

Peripheral nervous system involvement is among the most common of the extraglandular features of primary Sjögren's syndrome. In a cross-sectional study, peripheral neuropathy was diagnosed in 17 (27%) of 62 patients with primary Sjögren's syndrome on the basis of a conventional neurologic examination.[136] However, only 34 (55%) in this group had abnormal nerve conduction velocity studies, including 19 (31%) with a motor neuropathy, 8 (13%) with a sensory neuropathy, and 7 (11%) with a sensorimotor neuropathy. Some of the others with normal nerve conduction velocity studies may have had a small fiber neuropathy. Other peripheral neuropathies that have been reported in patients with primary Sjögren's syndrome are cranial neuropathies, autonomic neuropathies, and multiple mononeuropathies.[133] Most cases of peripheral neuropathy are dominated by sensory symptoms and typically do not progress to cause motor weakness.

### Vasculitis

Beyond lower extremity purpura, systemic vasculitis appears to be a rare manifestation of primary Sjögren's syndrome. A small vessel vasculitis and cryoglobulinemia may develop in patients in the absence of hepatitis C virus infection. A medium-sized vessel vasculitis may rarely occur with features ranging from mononeuritis multiplex to ischemic bowel.

### Risk for Cardiovascular Disease

Patients with primary Sjögren's syndrome have increased risk for cardiovascular disease based on a twofold increase in prevalence of hypertension and hypertriglyceridemia compared with healthy matched controls.[137] The impact of these risk factors on cardiovascular outcomes in patients with primary Sjögren's syndrome is unknown.

## Lymphoma

Non-Hodgkin's lymphoma (NHL) is a complication of primary Sjögren's syndrome with important prognostic significance. In a European study, the prevalence of NHL was determined to be 4.3%, with a median time of approximately 7.5 years from the diagnosis of primary Sjögren's syndrome to the development of NHL.[138] Several histopathologic types of NHL have been described in association with primary Sjögren's syndrome, including marginal zone B cell lymphoma, follicular cell lymphoma, diffuse large B cell lymphoma, and lymphoplasmacytoid lymphoma. Marginal zone B cell lymphoma, which is a family of low-grade B cell lymphomas, is by far the predominant type of NHL associated with this chronic autoimmune disease.

The mucosa-associated lymphoid tissue (MALT) lymphoma, a marginal zone B cell lymphoma, is the most common type to occur in primary Sjögren's syndrome (Figure 73-4A). It develops at extranodal sites in relation

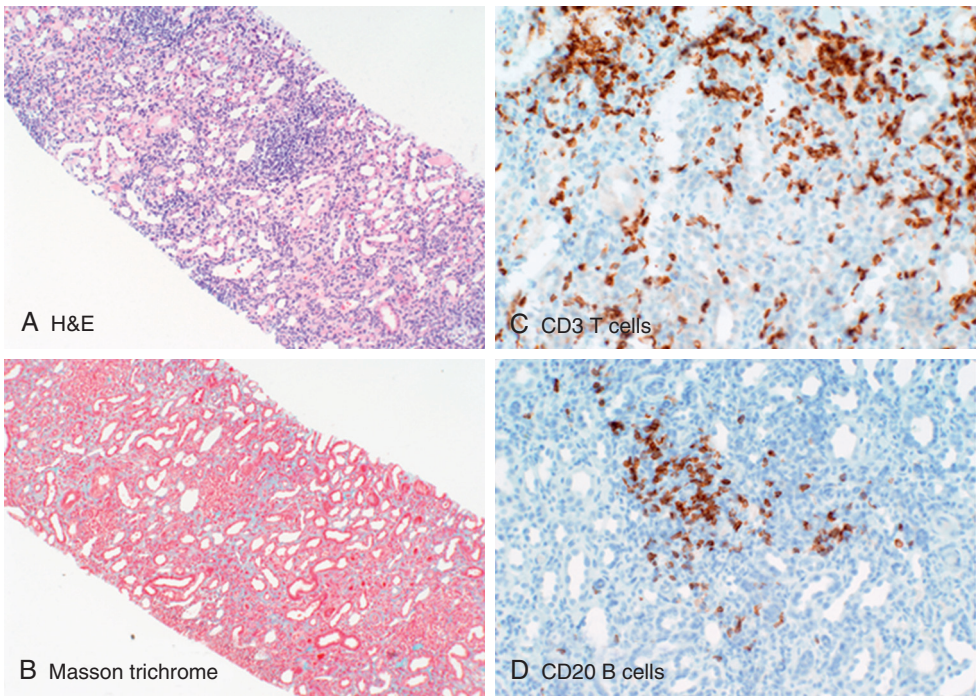



**Figure 73-4** Histopathology of a 57-year-old woman with chronic tubulointerstitial nephritis, consistent with renal involvement in primary Sjögren's syndrome. **A,** Hematoxylin-eosin (H&E) staining. **B,** Masson trichrome staining. **C,** Anti-CD3 staining of T cells. **D,** Anti-CD20 staining of B cells. Dense lymphoplastic infiltrate in the interstitium, with fibrosis and tubular atrophy (**A** and **B**). The majority of the mononuclear cells are T cells **(C),** with fewer numbers of B cells often in small aggregates **(D).**

to mucosal or glandular epithelium, such as the lacrimal and salivary glands, lung, gastrointestinal tract, and skin. The earliest histopathologic feature of MALT lymphoma is the finding of monocytoid B cells surrounding the epithelium. Immunochemical staining of these lesions shows their clonality in revealing monotypic infiltrates of either Igκ- or Igλ-light chains.[138] With progression, these lesions show increasing proliferation of neoplastic cells, replacement of reactive follicles, and ductal dilatation. In primary Sjögren's syndrome, MALT lymphomas most often develop in the salivary glands, but they may also develop at other extranodal sites, especially the lung and gastrointestinal tract. In primary Sjögren's syndrome, a fivefold increased risk for lymphoma is conferred by the presence of parotid gland enlargement, splenomegaly, lymphadenopathy, neutropenia, cryoglobulinemia, or low C4.[139]

### Associated Diseases

Primary Sjögren's syndrome has been associated with a higher risk for other autoimmune diseases, including thyroid disease, autoimmune hepatitis, primary biliary cirrhosis, and celiac disease. A recent large study found an increased frequency of autoimmune thyroid disease among patients with primary Sjögren's syndrome (6.2%) compared with the rate in the general population of 1% to 2%. However, because of varied definitions of thyroid disease this association has been inconsistent across studies.[140,141] Autoimmune hepatitis and primary biliary cirrhosis occur in less than 5% of patients with primary Sjögren's syndrome; more precise estimates of their prevalence are not available because of the absence of well-controlled studies. A Hungarian study showed a 10-fold higher rate of celiac disease in patients with primary Sjögren's syndrome compared with healthy controls.[142]

## DIAGNOSIS AND DIAGNOSTIC TESTS

### Keratoconjunctivitis Sicca and Xerostomia

A diagnostic suspicion of Sjögren's syndrome is initially raised by complaints of dry eyes and dry mouth. However, many patients may not volunteer this information because they do not feel it is important enough to bring to the attention of the physician. Therefore it is essential for the physician to inquire about these symptoms if the patient does not mention them during the initial history taking. In patients with symptoms of dry eyes and dry mouth, the next step is to confirm the diagnosis by objective testing. An easy method to evaluate aqueous tear flow is the Schirmer-I test, which is performed by inserting a sterile piece of filter paper in the middle to lateral third of the patient's lower eyelid, and measuring the distance tears elute during a period of 5 minutes. A distance of 5 mm or less is the usual cutoff for abnormally low tear production. Although a Schirmer-I test has an approximately 20% false-negative rate, normal tear flow in the face of ocular foreign body sensations suggests an alternative diagnosis such as blepharitis (see later). Patients with moderate to severe symptoms of dry eyes are usually referred to an ophthalmologist for slit-lamp examination. With a slit-lamp, the ophthalmologist can carefully examine the ocular surface, evaluating for any signs of damage. Instillation of lissamine green or fluorescein dye onto the surface of the eye displays the integrity of the conjunctival and corneal surface. Lissamine green dye stains epithelial surfaces lacking mucin (Figure 73-5), whereas the fluorescein dye targets areas of cellular disruption on the ocular surface. Rose Bengal dye, which stains dead or degenerated cells, is no longer preferred for evaluating the ocular surface because of its toxic effects on the cornea.

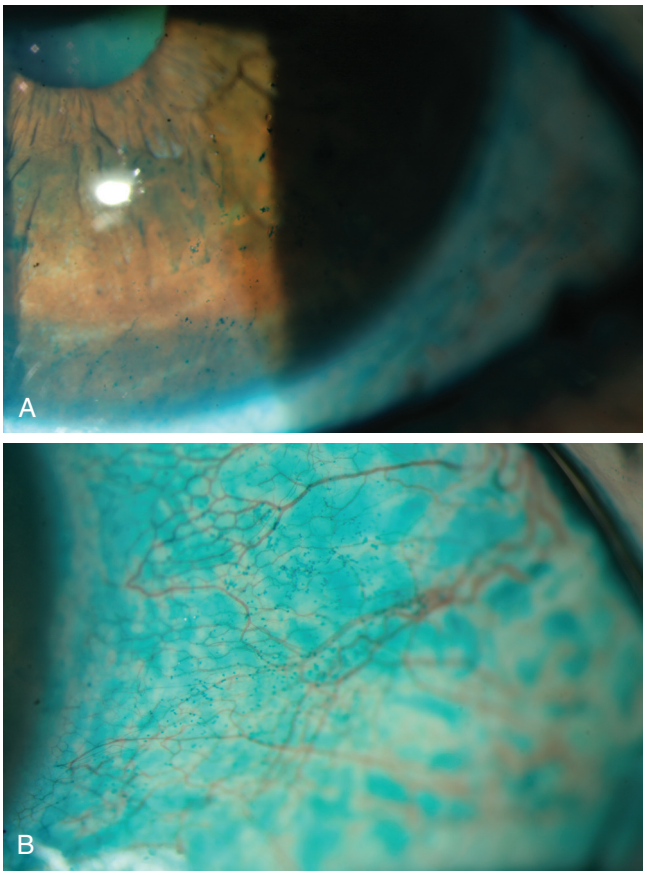



**Figure 73-5** Lissamine staining of a dry eye as shown by slit-lamp examination. **A,** Punctate staining of the cornea. **B,** Punctate staining of the conjunctival epithelium. *(**A** and **B**, Courtesy W. Craig Fowler, MD, Department of Ophthalmology, University of North Carolina at Chapel Hill.)*

Several methods may be used for objective assessment of dry mouth, or xerostomia. Only some patients with xerostomia may show obvious signs of a dry mucosa (e.g., absent sublingual salivary pool, thick and sticky saliva). Sialometry is a method for measuring salivary flow from individual glands (parotid, submandibular, or sublingual) or the mouth as a whole. An unstimulated whole salivary flow rate less than or equal to 1.5 mL/15 min meets the criterion for xerostomia according to the classification criteria developed by the AECG (see Table 73-1). For the collection procedure, patients should keep their heads tilted forward and swallow once to clear the mouth of excess saliva. At this point, the 15-minute collection period is initiated and patients then expectorate as needed, accumulating saliva into a preweighed, 50-$cm^3$ cryovial. Samples are weighed on an analytic balance to determine the volume (1 g = 1 mL) of saliva.

Sialography, another technique for evaluating xerostomia, visualizes radiographic patterns of duct obstruction in

the major salivary glands. It is most useful for differentiating an inflammatory process from a neoplasm. This technique calls for injection of radiographic contrast dye into the salivary duct, followed by serial radiographs to image the pattern of dye flow. A water-based contrast is strongly preferred to an oil-based contrast because the latter may damage the adjacent salivary gland tissue. Sialography is not routinely used in clinical practice because it is an invasive procedure with complications that include duct rupture, pain, and infection. Scintigraphy is a radionuclide technique for measuring salivary gland function. After radiolabeled sodium pertechnetate technetium is injected into the blood, it is absorbed into the salivary gland and secreted into the mouth, allowing for determination of the salivary flow rate. The diagnostic sensitivity and specificity of salivary gland scintigraphy for the diagnosis of primary Sjögren's syndrome has been estimated to be 75% and 78%, respectively.[143] Salivary scintigraphy is not widely available for routine testing.

Ultrasonography and MRI often can detect anatomic abnormalities in the salivary glands of patients with primary Sjögren's syndrome. In comparison with healthy individuals, the detection of parenchymal inhomogeneity by ultrasonography in two or more major salivary gland was found to have a sensitivity of 63% and a specificity of 99% for identifying patients with primary Sjögren's syndrome.[144] Although promising, this technique requires further validation and investigation using appropriate disease controls. MRI with sialography may be more sensitive than ultrasonography for the detection of glandular structural changes,[145] but further studies are necessary to validate this technique as well.

## Labial Salivary Gland Biopsy

Labial salivary gland biopsy has long been considered the gold standard for diagnosing primary Sjögren's syndrome. However, in clinical practice, its use is often reserved for patients in whom the diagnosis remains unclear after a thorough clinical and laboratory evaluation. The biopsy is usually performed by an oral surgeon, otolaryngologist, or other appropriately trained individual. This minor procedure calls for removal of four or more salivary gland lobules through a small incision in the inner lip. A biopsy is considered to be positive if histopathologic analysis shows a focus score of greater than or equal to 1 per 4 $mm^2$ of tissue (a focus is defined as a cluster of 50 or more lymphocytes). With a focus score of greater than or equal to 1 as the cutoff, the sensitivity and specificity of labial salivary gland biopsy for the diagnosis of primary Sjögren's syndrome has been reported at 83.5% and 81.8%, respectively.[20,21] The interpretation of labial salivary gland biopsies is subject to considerable inter-reader variability, depending on the experience of the reader. Therefore it is recommended that the biopsy slides be read by an experienced pathologist or other specialist with an appreciation for the nuances of interpretation.

## Laboratory Evaluation

Most patients with primary Sjögren's syndrome test positive for serum ANAs. In a large study from Spain, ANAs were detected in sera from 85% of patients with primary Sjögren's syndrome.[146] Approximately one-half and one-third of patients with primary Sjögren's syndrome in this study had anti-Ro/SS-A and anti-La/SS-B antibodies, respectively, whereas approximately 50% of patients tested positive for rheumatoid factor. There also appears to be a small subset of patients (<5%) with primary Sjögren's syndrome who test negative for serum anti-Ro/SS-A and/or anti-La/SS-B antibodies, but positive for serum anticentromere antibodies. In one study, patients with serum anticentromere antibodies had a lower prevalence of dry eyes, hypergammaglobulinemia, and anti-Ro/SS-A and anti-La/SS-B antibodies than other patients with primary Sjögren's syndrome, as well as a higher prevalence of Raynaud's syndrome.[147]

Approximately 5% to 10% of patients with primary Sjögren's syndrome have low blood levels of C3 and C4. For example, 9% of the Spanish cohort referred to earlier had low C3 or C4 levels. Approximately the same proportion of patients with primary Sjögren's syndrome have either type II or III cryoglobulinemia or a monoclonal gammopathy.[146] In type II cryoglobulinemia, the monoclonal gammopathy is usually an IgMκ that has rheumatoid factor activity when the patient resides in a Western or European country; patients of Japanese descent express a higher prevalence of IgA and IgG monoclonal gammopathies.[148] Hematologic abnormalities are observed in approximately 5% to 15% of patients with primary Sjögren's syndrome and include leukopenia and thrombocytopenia.

## Approach to Diagnosis

The clinical diagnosis of primary Sjögren's syndrome is highly likely if a patient with symptoms of dry eyes and dry mouth has objective evidence of keratoconjunctivitis sicca and/or xerostomia in conjunction with a positive test for serum anti-Ro/SS-A and/or anti-La/SS-B antibodies. It may not be necessary to obtain histopathologic evidence of salivary gland involvement if the other clinical and laboratory features are consistent with this diagnosis. In some cases, a clinical diagnosis of primary Sjögren's syndrome may be made in the presence of a moderately positive test for ANAs, but in the absence of serum anti-Ro/SS-A and/or anti-La/SS-B antibodies. Because approximately 25% to 50% of patients with primary Sjögren's syndrome test negative for anti-Ro/SS-A and anti-La/SS-B antibodies, a positive test for ANAs may often be the only evidence of an autoimmune process. Serum ANAs of low titer usually have relatively little diagnostic value in this setting unless they are accompanied by anti-Ro/SS-A antibodies.

Biopsy of the labial salivary glands may be warranted in cases in which signs and symptoms of keratoconjunctivitis sicca and xerostomia are not accompanied by convincing serologic evidence of autoimmunity. The labial salivary gland biopsy is used in this scenario to confirm that glandular hypofunction is associated with a chronic inflammatory process and to explore alternative diagnoses, such as sarcoidosis or amyloidosis. In most of these situations, relatively few labial salivary gland biopsies will prove to be positive (focus score ≥1), although a negative result probably reduces the likelihood of primary Sjögren's syndrome to less than 5%.

The diagnosis of primary Sjögren's syndrome in children and adolescents may require special consideration. Compared with adults, children and adolescents are seen more often with recurrent parotitis, which is not included as a criterion in the recent classification schemes derived for adults. It has been shown that adding recurrent parotitis to the AECG classification criteria increases the sensitivity of diagnosing primary Sjögren's syndrome in children and adolescents from 39% to 76%.[34]

## Differential Diagnosis

The differential diagnosis of primary Sjögren's syndrome is broad because of the variety of conditions that also cause dry eyes and mouth, salivary gland enlargement, and a similar profile of extraglandular manifestations (see Table 73-3). It is important to realize that most patients diagnosed with keratoconjunctivitis sicca by an ophthalmologist do not have primary Sjögren's syndrome, despite a deficiency in aqueous tear flow. The etiology of keratoconjunctivitis sicca in these cases is often obscure and presumably related to aging, hormonal changes, or other degenerative processes. Evaporative tear loss secondary to meibomian gland dysfunction is another common cause of dry eyes. In addition, medications with anticholinergic effects contribute to sicca complaints. Because approximately 25% of the general population has symptoms of dry mouth, it is not surprising that dry eyes and dry mouth often may coexist in a patient in the absence of a systemic disease such as Sjögren's syndrome.

Chronic parotitis in association with the sicca complex may result from sarcoidosis. Bilateral diffuse enlargement of the parotid glands from sialadenosis, a non-inflammatory process, occurs in some patients with diabetes mellitus who may also suffer from dry eyes and mouth. In addition, severe hypertriglyceridemia, chronic liver disease, and alcoholism have been associated with sialadenosis and enlargement of the major salivary glands.

Another clinical entity, termed *IgG4-related syndrome*, is emerging as an important consideration in the differential diagnosis of primary Sjögren's syndrome. IgG4-related syndromes are characterized in some cases by high serum levels of IgG4 and tissue biopsies showing a marked infiltration of $IgG4^+$ plasma cells coupled with fibrosis or sclerosis. Compared with primary Sjögren's syndrome, IgG4-related syndromes do not show the same female predominance and are associated with a lower frequency of dry eyes and mouth, arthralgia, and serum ANA positivity, as well as a higher rate of autoimmune pancreatitis.[149]

# TREATMENT

The treatment of primary Sjögren's syndrome aims at reducing the signs and symptoms of dry eyes and dry mouth and controlling the systemic manifestations (Figure 73-6). Sicca complaints may be lessened in general by withdrawal of medications with drying effects. For the treatment of dry eyes, the patient is advised to avoid central heating and air conditioning, windy environments, and medications that reduce tear and saliva production. Tear supplements are available over the counter in various formulations of different viscosities. Low-viscosity tears are administered more often than high-viscosity tear formulations, which are most useful at bedtime for prolonged lubrication during the sleeping hours. Artificial tears are also available with and without preservatives. Preservative-based tears may worsen dry eyes if they are instilled more than four times per day because of their toxic effects on the ocular surface. More frequent instillation of tears warrants use of formulations without preservatives.[150]

An ophthalmic preparation of cyclosporine, 0.05%, (Restasis) has been approved by the U.S. Food and Drug Administration for the treatment of keratoconjunctivitis sicca. In clinical trials, ophthalmic cyclosporine, 0.05%, reduces the signs and symptoms of dry eyes, including a statistically significant improvement in tear flow as measured by the Schirmer-I test. Many patients have trouble tolerating ophthalmic cyclosporine because of its burning effects.

Tears may be conserved by blocking the nasolacrimal drainage channels with temporary or permanent occlusion, a relatively simple procedure performed by an ophthalmologist. This procedure may be performed in patients with persistent symptoms of dry eyes despite use of artificial tears. Plugs are initially placed in the two inferior punctae because 90% of tears drain through these channels. Plugs may be later used to block the two superior punctae. Excessive tearing is the most frequent complication of punctual occlusion, which is the rationale for a trial of temporary blockade with dissolvable collagen plugs before undertaking permanent closure. Long-term punctual closure may be achieved by using silicone plugs, which in some cases are poorly tolerated, or by cauterization or a laser.

Blepharitis often complicates a dry eye state. Patients with blepharitis may benefit from warm compresses and lid scrubs with an eyelid detergent such as Johnson's Baby Shampoo mixed 1:1 with water. Some ophthalmologists avoid the lid scrubs because they can be irritating. More refractory cases of blepharitis may be managed with chronic oral doxycycline therapy. Occasionally, the tear film may contain excessive mucous debris that can be dispersed by instillation of a 10% acetylcysteine ophthalmic solution, a mucolytic agent that can be compounded by a pharmacy.

A dry mouth may be managed by replacing existing saliva or stimulating residual salivary flow. Various over-the-counter preparations of artificial saliva are available containing hypromellose or methylcellulose. However, they provide limited relief in most cases because of their short duration of action. Patients may stimulate salivary flow by sucking on sugarless candy or chewing sugarless gum. Two oral secretagogues are available by prescription that stimulate saliva and tear flow. Pilocarpine (Salagen), which acts by stimulating the M3R, is given at a dose of 5 mg, three to four times daily, and has been shown in controlled clinical trials to improve subjective and objective measures of both dry eyes and dry mouth.[151,152] Cevimeline (Evoxac) also stimulates the M3R and has been similarly shown in trials to improve subjective and objective measures of dry eyes and mouth.[153] Common side effects of these two secretagogues are sweating and flushing, which derive from their mechanism of action. Other possible side effects include visual disturbances, increased urination, nausea, abdominal pain, and diarrhea. Because of their pharmacologic properties, pilocarpine and cevimeline are contraindicated in

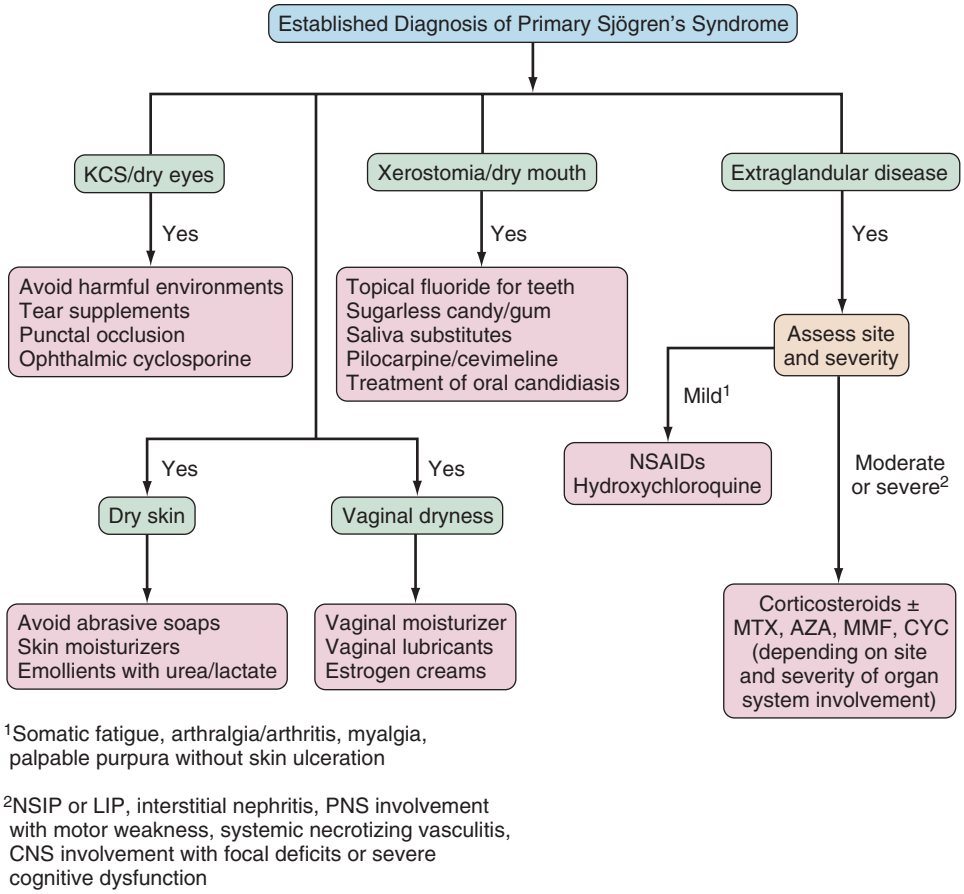



**Figure 73-6** Treatment algorithm for Sjögren's syndrome. The treatment of Sjögren's syndrome usually requires a multidisciplinary approach involving rheumatologists, ophthalmologists, dentists/oral surgeons, otolaryngologists, and other subspecialists, depending on the extent of extraglandular disease. In all cases, it is prudent to minimize the use of medications that can exacerbate the symptoms of dryness, such as antihistamines, antidepressants, muscle relaxers, and other drugs with anticholinergic properties. The treatment of extraglandular disease is individualized according to the site and severity of organ system involvement. The approaches indicated in the algorithm for the treatment of extraglandular disease are not supported by evidence from randomized, controlled trials, but rather from expert opinion based on retrospective case series and clinical experience. AZA, Azathioprine; CNS, central nervous system; CYC, cyclophosphamide; KCS, keratoconjunctivitis sicca; LIP, lymphocytic interstitial pneumonitis; MMF, mycophenolate mofetil; MTX, methotrexate; NSAIDs, nonsteroidal anti-inflammatory drugs; NSIP, nonspecific interstitial pneumonitis; PNS, peripheral nervous system.

patients with iritis, narrow angle glaucoma, and moderate to severe asthma.

Because xerostomia predisposes to dental carries and broken teeth, daily flossing, frequent dental visits, and regular fluoride applications are recommended for prevention purposes. Oral candidiasis, another complication of xerostomia, may be treated for 10 to 14 days with a nystatin elixir, clotrimazole troches, or fluconazole. Clotrimazole cream is beneficial for the treatment of angular cheilitis, which typically accompanies oral candidiasis.

Patients with primary Sjögren's syndrome also suffer from dryness of the lips, skin, and nasal passages. This problem may be addressed by the frequent application of moisturizers, lip balm, and nasal saline spray. The oily content of vitamin E softgel capsules may be used topically on the skin or the lips for its moisturizing effects. Vaginal dryness with dyspareunia is a frequent complaint of women with primary Sjögren's syndrome. This condition may respond to moisturizers and treatment with a topical estrogen cream. Vaginal lubricants may be necessary for treatment of dyspareunia.

Currently, the therapeutic armamentarium of primary Sjögren's syndrome lacks a proven disease-modifying drug. Clinical experience suggests that patient symptoms of fatigue, myalgia, and arthralgia/arthritis may respond favorably to treatment with hydroxychloroquine. A double-blind, parallel group, placebo-controlled trial among 120 patients in France, however, showed that hydroxychloroquine was no better than placebo in reducing dryness, pain, or fatigue.[154] A post-hoc analysis of patients with arthralgia in this study also found that hydroxychloroquine therapy was of no significant benefit in reducing joint pain, but insufficient numbers of patients with synovitis make it difficult to draw any reliable conclusions. In patients with hydroxychloroquine, care is taken to avoid doses exceeding 6.5 mg/kg/day to minimize the risk of retinal toxicity. Annual ophthalmologic examinations are also recommended to monitor for possible retinal complications.

Corticosteroids and other immunosuppressive drugs are often employed for the treatment of organ-threatening extraglandular disease. NSIP and LIP are usually treated with high doses of corticosteroids and other immunosuppressive agents, such as azathioprine, mycophenolate mofetil, or cyclophosphamide. Because virtually no controlled data are available to support the use of these agents

for the treatment of NSIP or LIP, this approach is necessarily empiric and demands judicious monitoring and close follow-up. Bronchiolitis typically improves with high doses of corticosteroids alone, but close follow-up is mandatory for treatment of relapses. Patients who are asymptomatic and have had PFTs showing an isolated mild reduction in DLCO or evidence of small airway disease may be carefully followed up without treatment.

Conservative measures may be the only therapy for a sensory peripheral neuropathy in which medications such as gabapentin and analgesics may help to control the aggravating and painful neuropathic symptoms. High doses of corticosteroids may produce transient improvement in the symptoms of a peripheral neuropathy, but it is unclear if they provide any long-term benefits, and if the benefits outweigh the risks of this treatment. Clinically demonstrable motor loss calls for a more aggressive treatment approach by using corticosteroids and other immunosuppressive agents. Some patients with severe motor involvement may benefit from treatment with intravenous immunoglobulin.

Recurrent lower extremity purpura develops in a minority of patients with primary Sjögren's syndrome. These lesions usually cause burning and stinging and may be associated with lower extremity edema, but they rarely ulcerate. Although moderate to high doses of corticosteroids bring symptomatic relief in most cases, subsequent tapering to low doses and withdrawal usually leads to recurrence of the purpura. Corticosteroid therapy may be avoided in the absence of severe and uncontrollable symptoms or progression to skin ulceration. Support stockings provide symptomatic relief in many cases and can be used in combination with analgesics, antihistamines, or NSAIDs to manage the painful symptoms. The patients are usually left with postinflammatory changes in the skin, which may be an acceptable outcome given the long-term side effects of corticosteroids.

Biologic therapies have been investigated recently for their clinical efficacy and safety in primary Sjögren's syndrome. Infliximab and etanercept, which are TNF inhibitors, have failed in controlled clinical trials to demonstrate therapeutic benefit.[155,156] Rituximab has shown promise in several small trials, including evidence of improvement in fatigue, sicca symptoms and disease activity, although study results were frequently conflicting.[157-160] However, the results of a randomized, double-blinded, placebo-controlled, parallel-group trial involving 120 patients with primary Sjögren's syndrome were unable to show that rituximab provided any significant clinical benefit versus placebo.[160] Other biologics, including belimumab and abatacept, have been tested in small, open-label samples of patients for the treatment of primary Sjögren's syndrome, and others are expected to be evaluated in the near future.[161,162]

EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) are measures developed and validated to assess disease activity and patient-reported outcomes in primary Sjögren's syndrome.[163] These indices have not been used consistently across studies and may be relatively insensitive for detecting improvement in patients with relatively low levels of extraglandular disease activity.

## OUTCOME

Overall mortality is not increased in patients with primary Sjögren's syndrome compared with the general population, although the subgroup of patients with extraglandular disease has an increased risk of morbidity and death. Ioannidis and colleagues[164] showed that, compared with the general population, mortality was not significantly higher in a cohort of 723 patients with primary Sjögren's syndrome. In this study, the patients were subdivided into two groups, types I and II, according to their risk for complications and death. Approximately 20% of the patients fell into the high-risk, type I group, whereas the remainder in the type II group had no increase in the risk for complications or death. In the type I group, patients presenting with palpable purpura and low C4 levels had a higher risk for long-term complications and death.[164] Similar results were found in another study showing no increase in all-cause mortality among individuals with primary Sjögren's syndrome.[165] Hypocomplementemia has been confirmed as a risk factor for adverse outcomes in another cohort of 336 patients with primary Sjögren's syndrome from Spain.[166]

The outcome of primary Sjögren's syndrome is likely to be influenced by disease mechanisms. The labial salivary gland tissue, a target tissue, is readily accessible for study, and new technologies are enabling the discovery of the complex pathways contributing to the aberrant innate and adaptive immune responses in this disease. Genetics and genomics are beginning to provide important clues about disease pathogenesis. An unanswered question is the cause of the glandular hypofunction. It appears that in the earlier stages of disease, glandular damage does not adequately explain the impaired salivary flow, which suggests that autoantibodies, the cytokine milieu, or other mediators are contributing to these functional abnormalities. This knowledge is critical to understanding the potential reversibility of glandular dysfunction and the best targets and timing of therapeutic interventions.

Although primary Sjögren's syndrome cannot yet claim a disease-modifying therapy, much can be done for patients with this disease by effectively managing the sicca component and monitoring for disease complications. Although biologics such as rituximab may yet prove to be useful for treating Sjögren's syndrome, the discovery of new therapeutic targets will shape the future development of new disease-modifying agents. Proof of therapeutic efficacy will require the development of new instruments for quantifying disease activity and damage. Discovery of the first drug with properties of disease modification will be a great progression for the field and will open the door to testing of new therapies in the years to come.

## CONCLUSION

Primary Sjögren's syndrome occurs nearly as often as rheumatoid arthritis and more than 10 times more frequently than systemic lupus erythematosus. Therefore it ranks second in prevalence only to rheumatoid arthritis among the autoimmune disorders in the domain of rheumatology. Many patients with mild forms of primary Sjögren's syndrome probably never come to the attention of the rheumatologist, but rather are managed exclusively by

primary care physicians, ophthalmologists, otolaryngologists, and dentists. Others may never be referred for a diagnosis because of the misperception that little can be done for patients with Sjögren's syndrome. However, primary Sjögren's syndrome is a systemic autoimmune disease that may cause serious organ system involvement. Patients with this disease are also at increased risk low-grade B cell lymphoma. The rheumatologist is aptly trained to accurately diagnose primary Sjögren's syndrome and to lead a multidisciplinary team of specialists who can effectively manage the multisystem complications of this disease.

Full references for this chapter can be found on ExpertConsult.com.

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# SPONDYLOARTHROPATHIES

PART 10

CHAPTER 74

# Etiology and Pathogenesis of Spondyloarthritis



Keith A. Sikora • Gerlinde Layh-Schmitt • Robert A. Colbert

**KEY POINTS**

- Genetics play a major role in the etiology of spondyloarthritis.
- The largest genetic contribution in the population is the B27 allele of human leukocyte antigen-B.
- TNF is a major mediator of inflammation and is currently the principle therapeutic target.
- The IL-23/IL-17 axis plays an important role in pathogenesis of spondyloarthritis.
- New bone formation as well as bone destruction occurs during the course of ankylosing spondylitis.
- There may be a complex temporal relationship between inflammation and new bone formation.

*Spondyloarthritis* refers to a group of diseases with overlapping clinical features and pathogenic mechanisms, yet there are important clinical and outcome differences. Considered to be largely genetic in etiology, environmental triggers in the form of gastrointestinal and genitourinary infections are well recognized for reactive arthritis, but have not been established for ankylosing spondylitis. There is growing interest in whether commensal organisms that are recognized to play important roles in shaping our immune responses also contribute to spondyloarthritis. In the last several years, multiple areas of investigation have converged to provide strong evidence that implicates the IL-23/IL-17 axis in spondyloarthritis pathogenesis. In this chapter we discuss pathogenic mechanisms, drawing largely from studies in ankylosing spondylitis, but whenever possible examine other forms of spondyloarthritis. To fully explain pathogenesis we need to understand what initiates and drives spontaneous chronic inflammation (Figure 74-1), why the enthesis and the axial skeleton are targeted, the role of gastrointestinal inflammation, and the mechanisms driving aberrant bone formation and bone loss, often occurring simultaneously and in close proximity.

## ETIOLOGY OF SPONDYLOARTHRITIS

Spondyloarthritis is a consequence of a complex interaction between genetic polymorphisms and environmental factors. The relative contributions of genes and environment may vary between different forms of spondyloarthritis. For ankylosing spondylitis, heritability is estimated to be more than 90%, suggesting that environmental components are ubiquitous.[1] Gastrointestinal and genitourinary pathogens that are recognized triggers for reactive arthritis (Chapter 76) have no proven role in ankylosing spondylitis. Interestingly, commensal gut microbiota, first shown to be directly involved in the development of human leukocyte antigen (HLA)-B27-associated spondyloarthritis in an animal model more than 20 years ago,[2,3] are now important considerations in the search for the environmental component of human spondyloarthritis.

### Genetics

The class I major histocompatibility complex (MHC) allele, HLA-B27, is the dominant genetic risk factor for spondyloarthritis[4,5] and is present in as many as 90% of individuals with ankylosing spondylitis[6] compared with less than 10% of the population, although frequency varies geographically. Although it is almost necessary to have HLA-B27 for ankylosing spondylitis to develop, it is not sufficient. Less than 5% of HLA-B27 carriers are affected,[7] and despite this striking relationship, it should be emphasized that ankylosing spondylitis occurs in HLA-B27-negative individuals.

Although HLA-B27 has a dominant genetic presence, it accounts for less than 25% of the overall heritability of ankylosing spondylitis.[8] In recent years, genome-wide association studies (GWAS) incorporating thousands of patients and controls have identified at least 30 additional genes or genetic regions that contribute to susceptibility.[9,10] The excitement of these discoveries is tempered by the reality that they account for only an additional 3% of overall heritability.[8] "Missing heritability" is a common problem in complex genetic diseases and may be a consequence of multiple factors. It has been suggested that overall heritability has been overestimated, and thus the proportion discovered may actually be higher,[11] or that the models used to estimate heritability ignore substantial gene-gene interactions (epistasis).[12] Rare variants that are unknown and thus not represented on current single nucleotide polymorphism (SNP) maps may also contribute. Although our understanding of the genetic etiology of ankylosing spondylitis and other spondyloarthritides may be incomplete, the discoveries to date have implicated important immune response

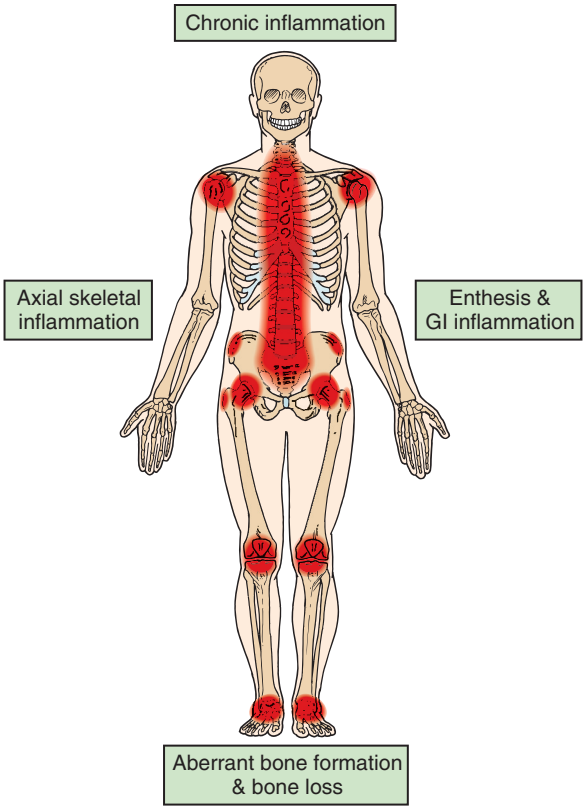



**Figure 74-1** Key components of spondyloarthritis pathogenesis. Chronic inflammation may be revealed systemically, or be primarily local. Entheses are a primary site of inflammation and pathology, with both axial *(red)* and peripheral *(yellow)* sites involved. Root joints (hips and shoulders) are frequently affected. Gastrointestinal (GI) inflammation is common and may be linked to articular manifestations.

pathways and revealed considerable overlap with other immune-mediated inflammatory diseases, most notably inflammatory bowel disease (IBD) (Crohn's disease and ulcerative colitis), psoriasis and psoriatic arthritis, and Behçet's disease[9,10] (Figure 74-2). Relevant pathways and potential mechanisms will be further considered in the context of pathogenesis.

## Environment

Reactive arthritis, defined here as arthritis that follows infection and exhibits features of undifferentiated spondyloarthritis, provides a clear example in which known environmental exposures trigger disease in the susceptible host. The majority of organisms causing reactive arthritis are gastrointestinal pathogens (e.g., *Salmonella spp*., *Campylobacter jejuni* and *Campylobacter coli*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, *Shigella flexneri*, and *Clostridium difficile*), with genitourinary and respiratory infection with *Chlamydia trachomatis* and *Chlamydia pneumonia*, respectively, also implicated. Clinical features, course, and classification of reactive arthritis are covered elsewhere (Chapter 76). For the purpose of this discussion, it is important to emphasize that although cultures are frequently positive for the triggering pathogen at the site of infection, synovial fluid is sterile. For *Chlamydia*-induced reactive arthritis, the situation may be different.[13] Because the organism is difficult to culture even when present, negative results are less informative,[14] and in fact *Chlamydia* can exist and persist inside monocytes or macrophages in the inflamed joint.[15,16] Their presence is best documented by nucleic acid detection by using polymerase chain reaction (PCR), or specific lipopolysaccharides by enzyme-linked immunosorbent assay (ELISA).[15] In the large majority of patients with reactive arthritis, recovery is complete without joint damage or chronic disease. However, some patients will progress to chronic spondyloarthritis including axial involvement with radiographic changes.[17] Thus conceptually, reactive arthritis-associated infectious organisms may serve as a trigger for chronic spondyloarthritis, but this accounts for only a small percentage of those affected. Genetic variants, including HLA-B27, are likely to play a significant role in determining the long-term outcome of reactive arthritis.

The human intestinal tract is colonized by as many as 100 trillion ($10^{14}$) bacteria representing more than 1000 different species.[18] The intestinal microbiome plays a critical role in development of the immune system and maintaining immune cell homeostasis, and is increasingly recognized for its importance in chronic inflammatory diseases.[18] Changes in the gut microbiome, or dysbiosis, are implicated in the pathogenesis of IBD with direct links to predisposing genes involved in innate immunity such as NOD2,[19] an intra-cellular pathogen recognition receptor, and ATG16L1 and IRGM, which regulate intra-cellular bacterial killing via autophagy and GTPase activity, respectively.[20] Dysbiosis in IBD is associated with a loss in diversity and relative reductions in *Firmicutes* and *Bacteroidetes*, and increases in *Enterobacteriaceae*.[21,22] There is evidence that innate immune inflammation may drive gut dysbiosis,[23] although the converse may occur as well, making it difficult to sort out cause and effect. In spondyloarthritis, gut inflammation without overt IBD is common, with as many as 60% of ankylosing spondylitis patients showing subclinical inflammation, and IBD developing in 6.5% during the course of their disease,[24] consistent with overlapping in genetic susceptibility.

From another perspective, as many as 30% of IBD patients may fulfill the criteria for spondyloarthritis,[25] with 11% to 52% exhibiting asymptomatic sacroiliitis, and as many as 10% meeting criteria for ankylosing spondylitis.[24] In both IBD[26] and ankylosing spondylitis,[27] antibodies against gut microbes can be detected, suggesting loss of tolerance.[28] In HLA-B27 transgenic rats that develop spondyloarthritis, animals remain healthy in an environment completely free of known microbes.[2,3] Reintroduction of normal commensal gut bacteria is sufficient to trigger colitis and arthritis.[3] More recently, HLA-B27 transgenic rats were shown to have differences in their gut microbiome, with an increase in *Prevotella spp*. and *Bacteroides vulgatus*, and a decrease in *Rikenellaceae* in the cecum.[29] This is consistent with the idea that one effect of HLA-B27 might be to alter the gut microbiome in a manner that promotes inflammatory disease.[30] Consistent with this hypothesis, patients with HLA-B27$^+$ ankylosing spondylitis exhibit several differences in their terminal ileal microbial communities compared with healthy controls, including a higher abundance of *Prevotellaceae* and *Bacteroidaceae*.[31] However, in both the rat and human studies, it remains unclear whether the microbial content differences are the

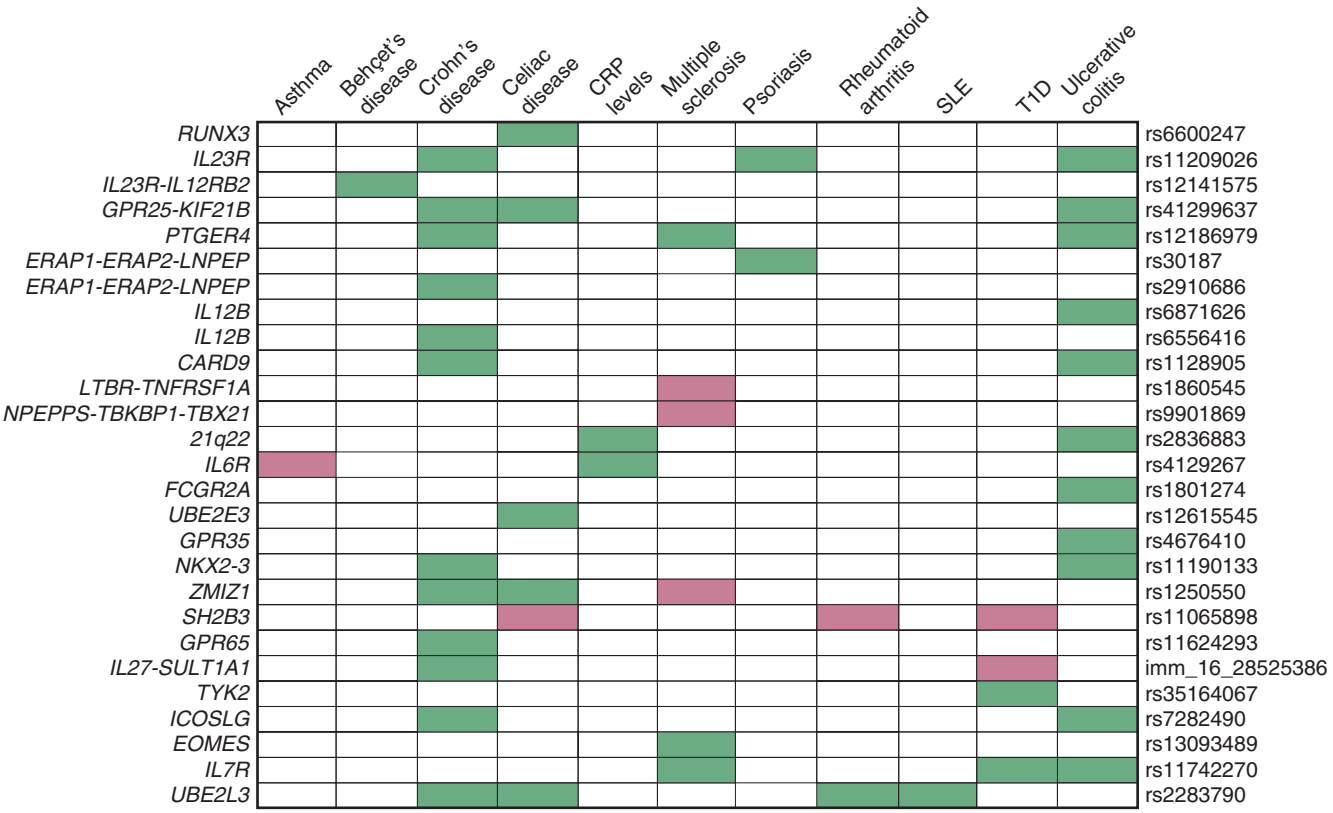



**Figure 74-2** Ankylosing spondylitis susceptibility genes or genetic loci overlap with those of other immune-mediated inflammatory and autoimmune diseases. Columns list diseases, and ankylosing spondylitis susceptibility loci are represented in rows. *Green* indicates shared susceptibility loci where the effect size is concordant, and *purple* represents discordant effect sizes. CRP, C-reactive protein; SLE, systemic lupus erythematosus; T1D, type I diabetes. *(From International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, et al: Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci.* Nat Genet *45:730 -738, 2013; Macmillan Publishers Ltd., with permission.)*

cause or the consequence of inflammation. More work needs to be done to better understand the impact of the gut microbiome on spondyloarthritis.

# PATHOGENESIS OF SPONDYLOARTHRITIS

## Functional Genomics

### HLA-B27

Understanding the functional consequences of genetic variants that create susceptibility to immune-mediated inflammatory disease is a key step in unraveling pathogenic mechanisms. For spondyloarthritis, efforts to understand the role of HLA-B27 have been a focus of research since the relationship was initially discovered in the 1970s,[4,5] yet fundamental questions remain unanswered. For example, what aspect(s) of the biology of HLA-B27 that distinguish it from closely related alleles are critical for promoting an immune-mediated inflammatory disease? Is the role of HLA-B27 in undifferentiated spondyloarthritis or reactive arthritis the same as in ankylosing spondylitis? What are the key cell types in which HLA-B27 exerts its effect(s), and does it trigger an abnormal immune response, inflammation, or other biologic effects? Historic perspectives on the biology of HLA-B27 and disease provide more detailed consideration of these questions.[32,33] The three main concepts currently gaining the most attention can be divided into two categories (Figure 74-3). The first encompasses the canonical function of HLA-B27, and suggests that this allele triggers disease through interactions with $CD8^+$ T cells by presenting self-peptides that become the target of an autoimmune response. Based on this hypothesis (arthritogenic peptides),[34] cytotoxic T cell responses to HLA-B27 expressed at various anatomic locations (e.g., entheses, sacroiliac joints, uveal tract) would generate tissue-specific inflammation to explain the spondyloarthritis phenotype. The second category encompasses abnormal features of HLA-B27 such as the tendency for the heavy chain to misfold during assembly in the endoplasmic reticulum (ER) (HLA-B27 misfolding),[35] and for unfolded/misfolded dimers or monomers of HLA-B27 heavy chains free of $\beta_2$m to be displayed on the cell surface (free heavy chain dimers/monomers).[36] These potential mechanisms are not mutually exclusive, and each is fundamentally related to the interaction between the HLA-B27 heavy chain and peptide. The quality (sequence/length) and quantity (supply) of peptides available to, and eventually bound by, HLA-B27 (i.e., peptide sequence and supply) can have substantial effects on its biology. A few aspects of these concepts will be further discussed.

### Arthritogenic Peptides

HLA-B27 and human $\beta_2$m transgenic rats (referred to as *HLA-B27 transgenic rats*) develop highly penetrant enterocolitis and less frequent peripheral and axial arthritis, nail dystrophy, and orchitis/epididymitis.[37] The disease is dependent on the bone marrow compartment,[38] as well as T cells. Surprisingly, $CD4^+$ cells T cells are necessary, whereas $CD8^+$

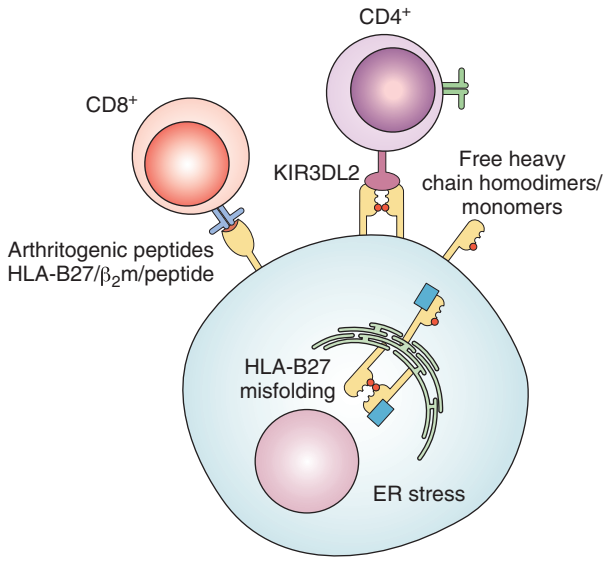



**Figure 74-3** Hypotheses accounting for canonical and noncanonical features of human leukocyte antigen (HLA)-B27 in spondyloarthritis pathogenesis. Arthritogenic peptides presented by properly folded complexes of HLA-B27 with $\beta_2$m and peptide. Free heavy chain homodimers/monomers lacking $\beta_2$m and peptide that engage killer immunoglobulin-like receptor 3DL2 (KIR3DL2) and trigger IL-17 production from $CD4^+$ T cells. Misfolding of HLA-B27 during assembly in the endoplasmic reticulum (ER) generates cellular stress, one consequence of which can be activation of the unfolded protein response. *(Modified from Colbert RA, et al:* Molecular immunology *57:44-51, 2013.)*

T cells that directly interact with HLA-B27 do not cause disease.[39-41] There are striking expansions of $CD4^+$ Th17 cells that express IL-17A, and cells that make IL-17A plus IFN-γ, and to a lesser extent IFN-γ–expressing Th1 cells.[42,43] Thus, arthritogenic peptides do not play a key role in spondyloarthritis in transgenic rats, whereas the IL-23/IL-17 axis is activated.[44] This scenario is consistent with the lack of direct evidence for arthritogenic peptides in human spondylarthritis, and strong evidence for involvement of the IL-23/IL-17 axis.[45] However, it should be emphasized that mechanisms of disease in transgenic rats could differ from those in humans. Although it seems unlikely that entirely distinct mechanisms would result in the striking phenotypic overlap between rat and human spondyloarthritis, and the common elements of pathogenesis revealed to date, arguments supporting a possible role for arthritogenic peptides persist and should be considered.[46,47]

## HLA-B27 Misfolding

The unique peptide-binding specificity of HLA-B27 also promotes misfolding of newly synthesized heavy chains.[35,48] Misfolding of membrane bound or secreted proteins has consequences that can range from loss-of-function to gain-of-function. Some of the latter effects are caused by the generation of stress in the ER, enhanced cell death, and activation of autophagy.[49] The majority of work on HLA-B27 misfolding has focused on the generation of ER stress and activation of the unfolded protein response (UPR) although other consequences are possible.[50,51] This is readily detectable in HLA-B27 transgenic rats that have multiple copies of the transgene, but only in some cell types and under certain conditions,[52] and not in rats that overexpress HLA-B7.[50] ER stress and UPR activation promote Toll-like receptor-mediated IFN-β and IL-23 induction[32,42] via the UPR target gene *Ddit3* (CHOP),[53] providing a potential link between HLA-B27 and $CD4^+$ Th17 cell-driven disease. Evidence of UPR activation in cells or tissues from patients with HLA-B27 and spondyloarthritis has been more variable. Macrophages from synovial fluid express more of the UPR target gene *Hspa5/BiP*,[54] however, this has not been evident in macrophages derived from peripheral blood monocytes.[55,56] UPR activation was reported in bulk peripheral blood mononuclear cells (PBMCs) isolated from ankylosing spondylitis patients but not in HLA-B27-negative healthy controls,[57] whereas others have not found evidence of UPR target gene overexpression.[58] Differences in methodology and experimental conditions between these studies need to be explored.

In ileal mucosal tissue from ankylosing spondylitis patients with subclinical gut inflammation, autophagy was activated without the UPR being apparent.[59] Colocalization of free HLA class I heavy chains with HRD1 (encoded by SYVN1), a ubiquitin ligase involved in ER-associated degradation (ERAD) of misfolded HLA-B27,[60] suggested that in gut tissue misfolding might be associated with ERAD and activation of autophagy precluding the need to activate the UPR. Inhibition of autophagy increased the frequency of cells expressing IL-23p19, suggesting that inhibition of autophagy might increase IL-23 production, as has been reported.[61] Autophagy does not appear to be activated in synovial tissue from ankylosing spondylitis patients.[62] Taken together, the evidence is consistent with the idea that the consequences of HLA-B27 misfolding are cell type-dependent, and may impact ER function only under certain conditions such as during upregulation when misfolded heavy chains can accumulate.[52] Further work with human cells containing a single copy of HLA-B27 is needed to determine whether misfolding can promote IL-23 production through the UPR and/or autophagy, or whether other biologic effects emerge.

There is also evidence that dendritic cells from HLA-B27 transgenic rats are dysfunctional, exhibiting altered class II MHC expression and viability, resulting in preferential loss of a tolerogenic $CD103^+$ population.[63-66] The dendritic cells have the property of reduced immune synapse formation and tend to promote Th17 development,[43] and thus may play a role in disease promotion. The molecular property of HLA-B27 that is responsible for these effects remains unclear.

## Free Heavy Chain Dimers and Monomers

Another unusual feature of HLA-B27 is its tendency to exist on the cell surface as $\beta_2$m-free heavy chains, either in the form of disulfide-linked homodimers or monomers,[36,67] in addition to the trimolecular complexes containing peptide and $\beta_2$m that predominate. Homodimers are "aberrant" forms of HLA-B27 that are covalently linked via the unpaired cysteine at position 67. They form during endosomal recycling of cell surface complexes and do not appear to emerge from the pool of misfolded dimers in the ER.[68,69] Free heavy chain monomers may arise from loss of peptide and $\beta_2$m on the cell surface. Suboptimal (low affinity)

peptides that are loaded in antigen-processing pathway-deficient cells dissociate more readily from HLA-B27, and promote the formation of free heavy chain monomers as well as homodimers.[48]

HLA-B27 homodimers can trigger leukocyte receptors in the killer immunoglobulin receptor (KIR) and leukocyte immunoglobulin-like receptor (LILR) families.[69] KIRs are highly polymorphic cell surface receptors expressed on natural killer (NK) cells, and subsets of $CD4^+$, $CD8^+$, and NK T cells.[70] Different forms of LILRs are expressed on NK, B, T and dendritic cells (LILRB1), or monocytes and dendritic cells (LILRB2). Both KIRs and LILRs bind class I MHC molecules in an allele-specific fashion, and certain variants bind HLA-B27 dimers. While many interactions have been demonstrated, of particular importance is the binding of KIR3DL2 because it does not interact with properly folded forms of HLA-B27. $KIR3DL2^+$ $CD4^+$ T cells and NK cells are expanded in the blood and synovial fluid of spondyloarthritis patients,[71,72] and can be triggered to produce IL-17 after encountering cells that express large amounts of HLA-B27 dimers on the cell surface.[72] IL-17 production is inhibited in the presence of antibodies that bind to HLA-B27 dimers or KIR3DL2, indicating the need for direct interaction. Free heavy chain monomers can interact with KIRs and LILRs, although the relative contributions of free heavy chain monomers and dimers to $CD4^+$ T cell stimulation is unclear. Many of these studies have been performed by using engineered tetramers of homodimers, or cells transfected with HLA-B27 that overexpress cell surface homodimers because of a deficiency in the class I assembly pathway, and thus the extent to which cells derived from spondyloarthritis patients trigger KIR3DL2-expressing $CD4^+$ T cells in vivo remains an important consideration.

## ERAP1

Variants of endoplasmic reticulum aminopeptidase-1 (ERAP1), which encodes an important component of the class I MHC antigen processing and assembly pathway, are associated with ankylosing spondylitis[73,74] as well as other diseases including those with class I MHC associations.[75,76] ERAP1 trims N-terminal amino acids from peptides in the ER that are destined to be displayed by class I MHC molecules.[77-79] It is also involved in cytokine receptor shedding, regulation of angiogenesis and blood pressure, and macrophage activation.[80-83] The genetic effect of ERAP1 in ankylosing spondylitis is limited to $HLA\text{-}B27^+$ individuals, in which variants reported to be loss-of-function were associated with protection.[74] However, recent studies have demonstrated functionally distinct ERAP1 haplotypes (referred to as *allotypes*).[84,85] When allotypes associated with ankylosing spondylitis were assessed, they were poor at generating optimal peptides for HLA-B27 and other class I MHC molecules, indicating that loss-of-function confers risk rather than protection.[85] These and other data strongly suggest that the role of ERAP1 in trimming peptides in the ER is critical for its association with ankylosing spondylitis. However, the functionally different ERAP1 allotypes that influence peptide supply are likely to alter various properties of HLA-B27 from folding and assembly to cell surface dimerization,[48] as well as the presentation of individual epitopes to T cells.[86,87]

## Genetic Susceptibility Distinct from the Major Histocompatibility Class I Pathway

IL-23 receptor gene *(IL23R)* variants associated with inflammatory bowel disease,[88] psoriasis,[89] and ankylosing spondylitis[73] have implicated the IL-23 signaling pathway in distinct but related diseases.[45] IL-23 is a critical cytokine for promoting proliferation and differentiation of $CD4^+$ Th17 T cells.[90,91] It also stimulates IL-17 production from Th17 as well as innate immune cells, including γδ T cells, certain NK cells, invariant NK T cells, Paneth cells, mast cells, and lymphoid tissue inducer cells, and a population of $CD4^-$/$CD8^-$/$CD3^+$ T cells.[92,93] The *IL23R* SNP (rs11209026) that is most strongly associated with protection from ankylosing spondylitis and IBD produces a nonsynonymous Arg381Gln substitution that reduces IL-23 responsiveness.[94] Subsequent GWAS in ankylosing spondylitis have identified additional genes that may influence IL-23 production *(CARD9, IL12B, PTGER4)*, the number or frequency of IL-23 responsive cells *(IL6R, IL27)* and IL-23 signaling (*TYK2* and *STAT3*, in addition to *IL23R*), have strengthened the evidence that the IL-23/IL-17 axis is involved in susceptibility[10,74,95,96] (Figure 74-4). The functional impact on immune cell development and function of most individual genetic variants or risk haplotypes remains to be worked out. However, combinatorial effects of risk/susceptibility alleles in a diverse population of spondyloarthritis patients revealed that the protective *IL23R* variant was associated with decreased expression of Th17 and Th1 genes, whereas patients with greater numbers of IL-23/IL-17 pathway risk SNPs exhibited greater expression.[97]

## IL-23 and the Spondyloarthritis Phenotype

Systemic expression of IL-23 in mice causes peripheral and axial enthesitis and sacroiliitis, and entheseal new bone formation in the absence of any initial synovial inflammation. This led to the discovery of a unique population of $CD3^+$/$CD4^-$/$CD8^-$ T cells situated in the enthesis that also express the "Th17" transcription factor RAR-related orphan receptor-γt (ROR-γt), and the IL-23 receptor.[93] In response to IL-23, the entheses produce IL-6, IL-17, IL-22, and CXCL1, consistent with the Th17-like phenotype of the T cells. IL-22 was implicated in promoting osteoblast mediated bone remodeling (Figure 74-5), although the cellular target of IL-22 remains to be determined. A similar phenotype that also exhibits gut inflammation can be induced in ZAP-70 mice when animals are treated with microbial β-glucans.[98] Beta-glucans such as curdlan and zymosan are microbe-associated molecules that are potent inducers of systemic IL-23 through the dectin-1/CARD9 pathway. SKG mice accumulate autoreactive $CD4^+$ Th17 T cells because of a mutation in the ZAP-70 T cell receptor subunit that alters T cell selection, and they overproduce Th17 cytokines in response to IL-23.[98] Together, these models demonstrate that IL-23 can produce a remarkable phenocopy of human spondyloarthritis and the potential importance of innate immune signaling stimulated by microbial products. They also suggest ways in which crosstalk between the innate immune system and adaptive $CD4^+$ Th17 T cells can be important. The existence of entheseal $CD3^+$/$CD4^-$/$CD8^-$/ROR-$\gamma t^+$/$IL\text{-}23R^+$ T cells provides a novel explanation for

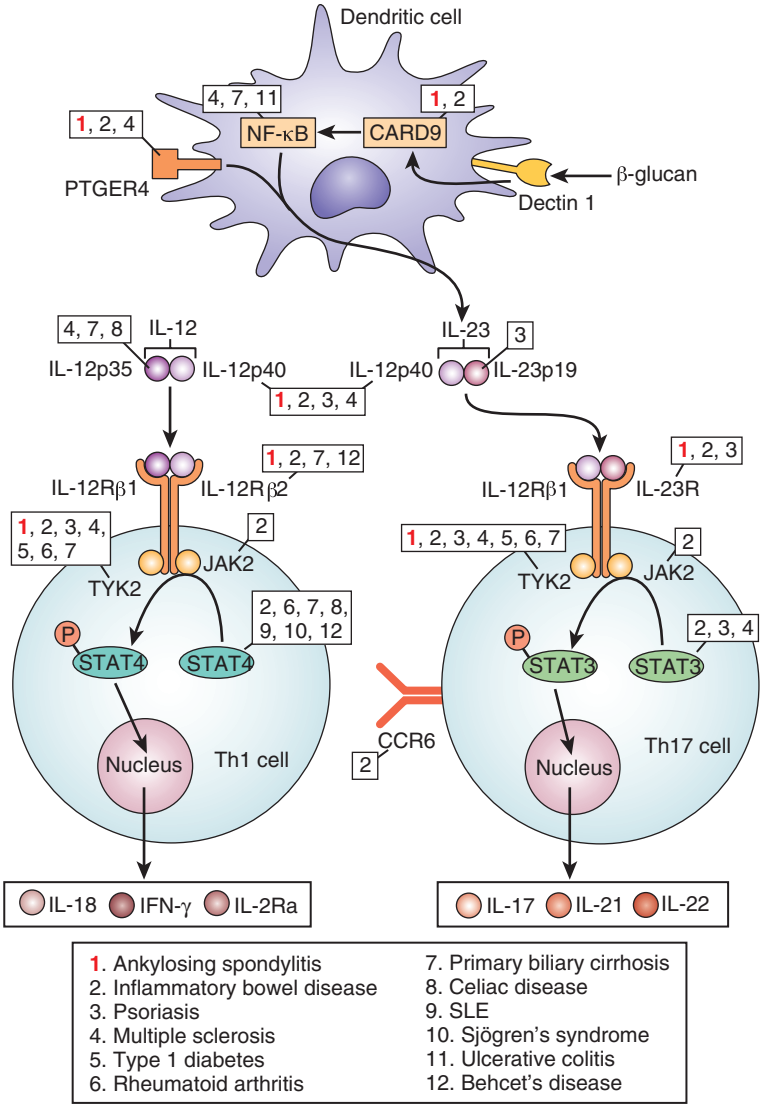



**Figure 74-4** Components of the IL-23/IL-17 axis and Th1 pathway showing genes implicated in pathogenesis of several immune-mediated inflammatory diseases. Multiple components of the IL-23 production and IL-23 receptor (IL-23R) response pathway show significant genetic associations. In addition to conventional T helper (Th1) and Th17 lymphocytes, it is now recognized that other T cells and innate immune cells are highly responsive to IL-23 and likely influenced by genetic factors. These various cell types are candidates to have key roles in various diseases. *Red circles* highlight ankylosing spondylitis associations. CARD9, Caspase recruitment domain-containing protein 9; CCR6, CC chemokine receptor 6; IFN-γ, interferon-γ; JAK2, Janus kinase 2; NF-κB, nuclear factor-κB; PTGER4, prostaglandin E2 receptor EP4 subtype; STAT3, signal transducer and activator of transcription 3; TYK2, tyrosine kinase 2. *(From Parkes M, Cortes A, van Heel DA, et al: Genetic insights into common pathways and complex relationships among immune-mediated diseases.* Nat Rev Genet *14:661-673, 2013; Macmillan Publishers Ltd.)*

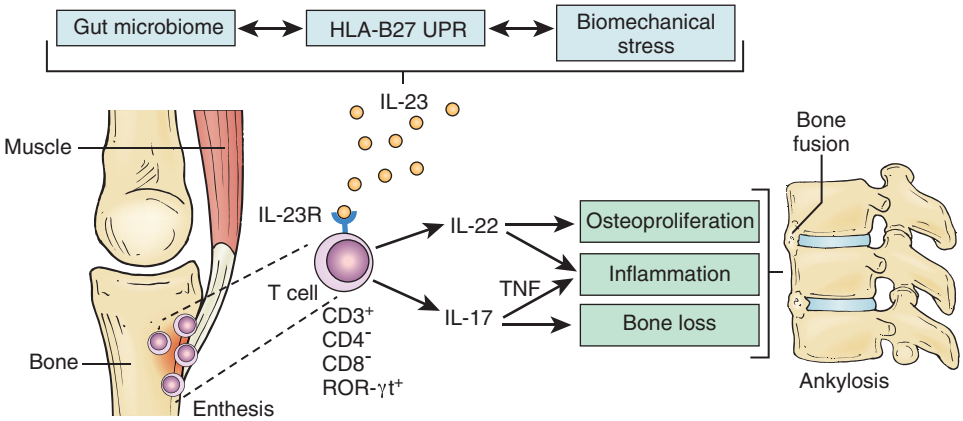



**Figure 74-5** IL-23 drives entheseal-resident T cells in the pathogenesis of spondyloarthritis in mice. Various pathways may lead to increased IL-23 expression. IL-23 can activate resident T cells within the enthesis, which can promote local inflammation and bone remodeling through a variety of cytokines, including IL-17 and IL-22. These actions may culminate in new bone formation and ankylosis. TNF, Tumor necrosis factor; UPR, unfolded protein response. *(From Sherlock JP, Joyce-Shaikh B, Turner SP, et al: IL-23 induces spondyloarthropathy by acting on ROR-gammat+CD3+CD4−CD8− entheseal resident T cells.* Nat Med *18:1069-1076, 2012; Macmillan Publishers Ltd.)*

how the unique spondyloarthritis phenotype might be generated in early disease, without invoking $CD8^+$ T cell-mediated autoreactivity or other IL-17-producing cells.

### Cytokines and Cytokine-Producing Cells in Spondyloarthritis

Considerable evidence from human studies indicates that the IL-23/IL-17 axis is activated in spondyloarthritis.[45] Serum and synovial fluid levels of IL-17 and/or IL-23 can be elevated,[99-102] although serum IL-23 correlates poorly with disease activity in spondyloarthritis compared with rheumatoid arthritis.[103] $CD4^+$ Th17 cells, including those that express KIR3DL2, may be increased in number or frequency in the peripheral blood and synovial fluid of spondyloarthritis patients,[72,104-108] consistent with cytokine data. It should be noted that other studies have not found increases in $CD4^+$ Th17 cells,[109-111] including those that express KIR3DL2,[108] and in one study more IL-23$R^+$ γδ T cells were observed.[111] Variability in spondyloarthritis subtypes, disease duration, and disease activity may be important factors that contribute to these differences. Perhaps more important in spondyloarthritis is the consideration that peripheral blood and synovial fluid do not reflect pathologic processes active in the sacroiliac joints, vertebral bodies, facet joints, and bone marrow adjacent to these axial inflammatory lesions. This is exemplified by TNF, in which serum levels are not uniformly increased in spondyloarthritis[112,113] and can even be lower in ankylosing spondylitis than healthy controls,[114] yet this cytokine is overexpressed in actively inflamed sacroiliac and hip joints,[115,116] and it is a major therapeutic target. Several studies have documented expression of IL-17 and IL-23 at sites of inflammation in spondyloarthritis. Mast cells in the synovium of patients with peripheral spondyloarthritis contain IL-17,[117] whereas in ankylosing spondylitis neutrophils (myeloperoxidase $[MPO]^+/CD15^+$) and mononuclear cells express IL-17 in facet joints.[109] IL-23 overexpression in inflamed ileum and bone marrow has also been documented.[118,119] In ileal biopsies from patients with subclinical inflammation, IL-23 localized to infiltrating monocytes and Paneth cells,[118] whereas in the marrow of spinal facet joints IL-23 was predominantly localized to myeloid precursors ($MPO^+/CD15^-$), and to a lesser extent in $CD68^+$ or $CD163^+$ macrophages.[119] Evidence from GWAS, animal models, and translational studies in humans, together with emerging results from clinical trials blocking IL-17A[120] and IL-12/23,[121] provides strong support the importance of the IL-23/IL-17 axis in spondyloarthritis pathogenesis.

## STRUCTURAL DAMAGE IN ANKYLOSING SPONDYLITIS

Until this point the focus has been on mechanisms that promote chronic inflammation in joints, entheses, and the gut in spondyloarthritis. Structural damage is an important contributor to morbidity in ankylosing spondylitis, and deserves special consideration. Although erosions occur, and in the sacroiliac joint may be seen as irregular margins or joint space widening on plain radiographs, they are often limited in severity. Bone inflammation, or osteitis, is frequently visualized as bone marrow edema and can lead to significant vertebral trabecular bone loss even in early disease,[122] and contributes to increased fractures.[123] Bone loss in ankylosing spondylitis is problematic, but the principal causes of structural damage are aberrant bone formation that results in fusion of the sacroiliac and zygapophyseal joints, and syndesmophytes that eventually bridge vertebral bodies and result in ankylosis.[124] The principal paradox in ankylosing spondylitis is that bone loss and bone formation, often regulated by opposing signals, occur simultaneously and frequently in juxtaposition. This is most notable in and around vertebral bodies. Aberrant bone formation is not explained by gross differences in the inflammatory infiltrate in spondyloarthritis, which is similar to more erosive arthritic conditions.[125]

### Bone Homeostasis and Aberrant Bone Formation

Bone homeostasis is a tightly regulated balance between osteoclasts responsible for bone resorption and osteoblast lineage cells that form bone. Several factors may disrupt this balance in spondyloarthritis, and thus are worth considering in greater detail. Osteoclasts are derived from bone marrow hematopoietic stem cells that differentiate into monocytes and then macrophages under the influence of macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL).[126] Pro-inflammatory cytokines such as TNF, IL-17, IL-6 ,and IL-1 promote osteoclastogenesis and bone degradation either directly or through effects on RANKL expression in other cells,[127] whereas IFN-β is an inhibitor.[128] Osteoprotegerin (OPG) also inhibits osteoclastogenesis by direct inhibition of RANKL. Analysis of tissue from patients with ankylosing spondylitis reveals evidence of persistent osteoclast activity and bone destruction, even in long-standing disease.[129] Increased osteoclast precursors in peripheral blood of patients with ankylosing spondylitis has been noted[130] and could be caused in part by a pro-osteoclastogenic milieu with a reduced OPG/RANKL ratio in the serum.[131] Other pro-osteoclastogenic cytokines may contribute, and HLA-B27 misfolding in transgenic rats promotes osteoclast formation through increased IL-1α production.[132]

In contrast to osteoclasts, osteoblasts are derived from mesenchymal stem cells. Several osteoblast lineage cells are involved in bone and cartilage formation (e.g., mesenchymal progenitors, preosteoblasts, mature osteoblasts, and osteocytes). They are poorly defined histologically but express different transcription factors critical to their developmental programs.[126] Bone formation and growth occur through two different processes. Endochondral bone formation is characterized by bone marrow-derived mesenchymal stem cells differentiating into hypertrophic chondrocytes that form cartilage tissue matrix. Mesenchymal cells that differentiate into osteoblasts are attracted to the matrix, which is eventually replaced by bone matrix from osteoblasts. During membranous bone formation, mesenchymal cells differentiate directly into osteoblasts that then produce calcifying bone matrix. Both endochondral and membranous or direct bone formation are involved in the pathogenesis of ankylosing spondylitis.[133-137] New bone formation, often referred to as *osteoproliferation*, has been characterized as a tissue remodeling process as opposed to merely bone

proliferation,[138] but also differs from classic remodeling in which osteoclast and osteoblast activity are tightly balanced. New bone is thought to originate from the enthesis and periosteum, and primarily involves the sacroiliac, zygapophyseal and costovertebral joints, and vertebral bodies. Syndesmophytes grow along the vertical axis between edges of vertebral bodies by apposition of new bone resulting in fusion of the vertebrae. There can be ongoing loss of trabecular matrix in vertebral bodies, whereas cortical bone is simultaneously added to growing syndesmophytes at the vertebral margins, implying that local factors must be important.

Bone development is regulated by several soluble factors, including bone morphogenetic proteins (BMPs), wingless-related integration site proteins (Wnts), hedgehog, and fibroblast growth factors (FGFs). BMP and Wnt signaling pathways have been implicated in aberrant bone formation in spondyloarthritis,[137] and will be discussed in more detail. BMPs stimulate endochondral bone formation through effects on chondrocyte differentiation and hypertrophy.[139] They are also important in early developmental processes and homeostasis of other organ systems.[140,141] BMPs are antagonized by noggin, which is secreted by chondrocytes, and osteocyte-derived sclerostin. Wnt family proteins are also important regulators of early bone development and homeostasis, and similar to BMPs, are critical for the development of other organs and tissues.[142,143] Wnts are expressed by osteocytes and can be regulated by mechanical stresses. They stimulate bone formation by direct effects on osteoblasts, and also influence cartilage homeostasis.[144] Wnts signal through a receptor known as frizzled, which is regulated by binding to low-density lipoprotein receptor-5 (LRP5) and LRP6. Wnt signaling is inhibited by sclerostin, which binds to LRP5, and thus sclerostin inhibits bone formation.[145] Other Wnt antagonists include Dickkopf-1 (Dkk1), which, similar to sclerostin, binds to LRP5, and secreted frizzled-related protein (SFRP), which binds directly to Wnt proteins.

BMP signaling has been linked to excessive bone formation in DBA/1 mice that develop spontaneous peripheral ankylosing enthesitis.[146] Administration of the BMP antagonist noggin was able to reduce and prevent bone formation and peripheral joint ankylosis in BDA/1 mice. Enhanced BMP signaling in a biopsy from a patient with Achilles enthesitis and new bone formation suggests that increased BMP signaling might be linked to excessive bone formation in humans with spondyloarthritis. The Wnt pathway has also been implicated in aberrant bone formation in spondyloarthritis through Dkk1 and sclerostin. Dkk1 and sclerostin are upregulated by TNF and in a peripheral inflammatory arthritis model, inhibiting Dkk1 reduced bone destruction and promoted osteophyte formation, revealing its normal role in limiting destructive damage and even excessive repair.[147] In another animal model of spondyloarthritis in which engineered overexpression of TNF causes sacroiliitis, Dkk1 inhibition promoted sacroiliac joint fusion.[148] Abnormally low levels of Dkk1 were reported in patients with ankylosing spondylitis,[147] and although Dkk1 levels seem to go down after TNF inhibition in patients with rheumatoid arthritis, this was not the case in ankylosing spondylitis.[148] Higher levels of Dkk1 were associated with protection from syndesmophyte formation in ankylosing spondylitis.[149] There is also seemingly contradictory evidence that Dkk1 is elevated in ankylosing spondylitis, but in this study it was dysfunctional.[150] Differences in methods used to detect Dkk1 may be responsible for the discrepancy and need to be resolved.[151] Overall, these data suggest Dkk1 (or lack of it) could play an important role in structural damage in ankylosing spondylitis.

Sclerostin, another Wnt pathway inhibitor, has also been reported to be lower in patients with ankylosing spondylitis, which correlates with radiographic progression.[152] Reduced sclerostin levels were confirmed in a recent study, whereas Dkk1 was not low except in individuals with the greatest structural damage.[153] This study revealed elevations in Wnt-3a that correlated positively with structural damage, providing the first demonstration that Wnt agonists might also be useful biomarkers. Interestingly, autoantibodies recognizing noggin and sclerostin that could promote osteoproliferation and structural damage in ankylosing spondylitis were recently noted.[154]

## Inflammation and New Bone Formation in Spondyloarthritis

The relationship between inflammation and new bone formation in spondyloarthritis continues to be the subject of debate.[155] The argument that these are distinct, uncoupled processes is predicated in large part on studies that show no benefit of TNF inhibitors in patients with ankylosing spondylitis,[156,157] and from the ankylosing enthesitis DBA/1 mouse model in which clear evidence exists that TNF inhibition has no impact on entheseal new bone formation.[158] The opposing view that inflammation is in some way coupled with new bone formation is now supported by two studies that compare cohorts of patients treated with TNF inhibitors with cohorts who were not exposed.[159,160] The first study provides evidence for reduced radiographic progression with use of TNF inhibitors and suggests that early initiation and longer follow-up are important variables.[159] Consistent with these data, a long-term follow-up study of TNF inhibitor treated and untreated cohorts revealed fewer new syndesmophytes and lower damage scores (Modified Stoke Ankylosing Spondylitis Spine Score) after 8 years in patients on TNF inhibitors, whereas no differences were seen after the first 4 years.[161] Both of these studies from patients treated with TNF inhibitors are consistent with the small reduction in radiographic progression seen with continuous NSAID use and suggest a link between inflammation and aberrant bone formation.[162] The DBA/1 ankylosing enthesitis model, in which TNF inhibitors had no effect on the incidence or severity of joint ankylosis, may simply be an example of a mouse model that reflects poorly on what happens in humans.

A 2-year longitudinal MRI study suggests that resolution of inflammation (bone marrow edema) and erosions around the sacroiliac joints are independently associated with development of "backfill" and "fat metaplasia" lesions on MRI. Fat metaplasia was used to describe high T1 signal intensity (fat signal) in regions of noneroded bone marrow, whereas backfill referred to a fat signal in areas where erosions had occurred. Reduction in erosions and increased fat metaplasia were associated with ankylosis. These findings were used to support a model in which ankylosis develops after repair of erosions, with fat metaplasia and backfill seen

as important intermediate steps.[163] In another study of vertebral lesions, inflammation and fatty degeneration at baseline and fatty degeneration at 2 years even in the absence of inflammation at baseline, were both associated with syndesmophyte formation at 5 years in patients on TNF inhibitor therapy.[164] Although this evidence contradicts the idea that inflammation is a prerequisite for new bone formation, other studies have shown that MRI is insufficiently sensitive to detect all inflammation, at least in the sacroiliac joint.[165] Thus important limitations exist in the interpretation of lack of inflammation in imaging studies. Efforts in this area are significantly hampered by the location of lesions in axial spondyloarthritis and inherent difficulties in accessing the tissue. Despite the encouraging results suggesting that TNF inhibitors may impede progression of structural damage, it is important to note that results to date suggest that syndesmophytes still form in TNF inhibitor treated patients. This evidence implies that suppression of inflammation with these agents does not completely prevent new bone formation. A clear need exists for a better understanding of pathogenesis and to identify ways of stopping and preventing structural damage.

Several genes identified by GWAS (*ANTXR2*, *PTGER4*, *HAPLN1*, *EDIL3*, and *ANO6*)[74,166] have the potential to impact bone formation in ankylosing spondylitis.[124] For example, *PTGER4* encodes a receptor for prostaglandin E2, an inflammatory mediator that promotes mineralized bone nodule formation. *ANTXR2* encodes the anthrax receptor, also known as capillary morphogenesis protein 2 (CMG2). It interacts with the low-density lipoprotein receptor-6 (LRP6) and LRP5, both of which are involved in regulating Wnt signaling. *HAPLN1* encodes hyaluronan and proteoglycan link protein 1 (also known as Crtl1), which is implicated in endochondral bone formation. An SNP in *HAPLN1* has been associated with osteophyte formation and disc degeneration.[167] In addition, *ANO6* is expressed in osteoblasts as well as other cell types, where it is required for normal mineralization.[168] Once disease-associated SNPs or haplotypes are identified, functional studies can be initiated that may shed light on the role of these genes in ankylosing spondylitis.

## CURRENT AND FUTURE THERAPEUTIC TARGETS IN SPONDYLOARTHRITIS

### Targeting the IL-23/IL-17 Axis

TNF is well established as an important therapeutic target in ankylosing spondylitis. Surprisingly little is known about its predominant cellular source, the mechanisms that drive its production in spondyloarthritis, or whether genetic factors influence the cellular response to this cytokine. TNF may be a relatively early upstream trigger in early disease, and/or a mediator of downstream mechanisms with positive and negative feedback loops in more chronic inflammation that influence its overall effect.[169] The accumulated evidence implicating dysregulation of the IL-23/IL-17 axis in spondyloarthritis is strong, and early results from clinical trials of both IL-17A and IL-23/IL-12 blockade are encouraging.[120,121] Targeting this axis may offer additional options and therapeutic benefit, with the potential to positively impact long-term outcomes for patients with spondyloarthritis.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 75



# Ankylosing Spondylitis

Sjef van der Linden • Matthew Brown • Tony Kenna • Walter Maksymowych • Philip Robinson

**KEY POINTS**

- **The spectrum of axial spondyloarthritis comprises both ankylosing spondylitis (AS) according to the modified New York criteria and nonradiographic axial disease.**
- **Magnetic resonance imaging of the sacroiliac joints may show inflammation before structural changes appear on conventional radiographs.**
- **Nonradiographic axial spondyloarthritic disease occurs approximately 2 to 3 times as often as AS according to modified New York criteria, and may show comparable disease activity.**
- **Nonradiographic axial spondyloarthritis is more heterogenous than AS according to modified New York criteria, human leukocyte antigen (HLA)-B27 prevalence varies widely, there is no male preponderance, and patients may or may not progress to radiographic sacroiliitis. The natural history of nonradiographic axial spondyloarthritis is not well known.**
- **Applying classification criteria for axial spondyloarthritis as a diagnostic tool should be avoided to reduce the risk of a false-positive diagnosis of axial spondyloarthritis.**
- **In many populations, AS occurs in 1% to 3% of *HLA-B27*$^+$ individuals. The disease is more common, occurring in approximately 10% of first-degree relatives of *HLA-B27*$^+$ AS patients.**

Ankylosing spondylitis (AS) belongs to the group of diseases known as the *spondyloarthropathies* or, better, *spondyloarthritides*. This group of disorders constitutes a family of related but heterogeneous conditions, rather than a single disease with different clinical manifestations[1] (Tables 75-1 and 75-2).

Radiographic sacroiliitis is considered a hallmark of AS. The inflammation of the sacroiliac (SI) joints and the spine eventually may lead to bony ankylosis. Spinal ankylosis tends to appear in late stages of the disease and does not occur in many patients with mild disease.

Many patients with AS have onset of back pain during the third decade of life. It takes, on average, 6 to 8 years from the onset of back pain until a clinician establishes a definite diagnosis of AS. This diagnostic delay in the majority of patients results mainly from the relatively late appearance of definite radiographic sacroiliitis on conventional plain radiographs.[2,3] Active sacroiliitis on MRI has been shown to predict the later appearance of sacroiliitis on radiographs.[4] Thus many patients at an early stage of AS typically are seen with characteristic clinical symptoms of AS, but definite sacroiliitis may not show up on radiographs. Therefore they may not be classified with AS according to the modified New York criteria.[5] In such patients with suspected early-stage AS, sacroiliitis may best be detected on MRI.[3] Radiographic sacroiliitis (i.e., structural damage of SI joints) may develop in a substantial proportion of such patients with time (approximately 20% to 25% over a period of 15 years) and will progress to definite AS, fulfilling the modified New York criteria.[5,5a] This finding also implies that a significant proportion of patients will remain at this (nonradiographic) stage of disease, with inflammation of SI joints at some point, but without radiographically detectable damage during the subsequent years.[4] Therefore, to describe these patients correctly, the term *early AS* has been dropped in favor of the more fitting term *nonradiographic axial spondyloarthritis* (nr-axSpA)[6] (Figure 75-1). The term *axial spondyloarthritis* (axSpA) comprises both nonradiographic AS and classic AS (according to the modified New York criteria). The total prevalence of axSpA has been estimated at two to three times that of AS according to the modified New York criteria.[7-9] Contrary to the modified New York criteria, there is no male preponderance for AS among patients with nr-axSpA.[8] In population-based studies, human leukocyte antigen *(HLA)-B27* prevalence varies widely (from 20% to 97%) depending on how many patients were included.[8,9] Such patients may have active disease that needs appropriate treatment. The efficacy of conventional disease-modifying anti-rheumatic drugs (DMARDs) in these patients is largely unknown. At the group level, these patients tend to respond less favorably to treatment with TNF inhibitors than AS patients by modified New York criteria.[10] The course and natural history of axSpA without radiographic sacroiliitis is not yet well known. This chapter focuses on radiographic AS. Although this disease may be more homogenous than the full spectrum of axSpA, many aspects also apply to patients with nonradiologic AS or axSpA.[11,12]

## CLASSIFICATION

### Criteria for Ankylosing Spondylitis and Axial Spondyloarthritis

Classification criteria are intended for clinical and epidemiological studies and also for research on the etiopathogenesis of diseases. Such criteria should create homogenous groups of patients who have already been diagnosed clinically by various, but varying, professionals. Inappropriate use of classification criteria as diagnostic criteria increases

**TABLE 75-1** Spondyloarthritis

| |
|---|
| Ankylosing spondylitis and nonradiograhic axial spondyloarthritis |
| Reactive arthritis |
| Arthropathy of inflammatory bowel disease (Crohn's disease, ulcerative colitis) |
| Psoriatic arthritis |
| Juvenile chronic arthritis and juvenile-onset ankylosing spondylitis |

**TABLE 75-2** Clinical Characteristics of Spondyloarthritis

| |
|---|
| Typical pattern of peripheral arthritis—predominantly of lower limb, asymmetric |
| Absence of rheumatoid factor |
| Absence of subcutaneous nodules and other extra-articular features of rheumatoid arthritis |
| Overlapping extra-articular features characteristic of the group (e.g., anterior uveitis) |
| Significant familial aggregation |
| Association with *HLA-B27* |

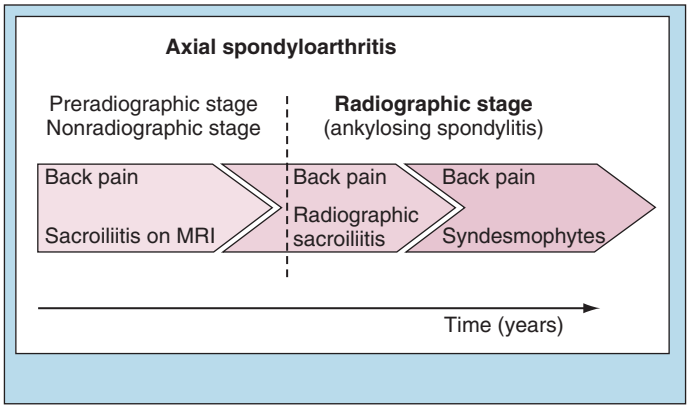



**Figure 75-1** Concept of axial spondyloarthritis as an umbrella for patients without radiographic damage and patients with such damage. MRI, Magnetic resonance imaging.

**TABLE 75-3** Criteria for Ankylosing Spondylitis

| |
|---|
| **Rome, 1961** |
| Clinical Criteria |
| 1. Low back pain and stiffness for more than 3 months not relieved by rest |
| 2. Pain and stiffness in thoracic region |
| 3. Limited motion in lumbar spine |
| 4. Limited chest expansion |
| 5. History or evidence of iritis or its sequelae |
| Radiographic Criterion |
| Radiograph showing bilateral sacroiliac changes characteristic of ankylosing spondylitis (this excludes bilateral osteoarthritis of sacroiliac joints) |
| Definite Ankylosing Spondylitis |
| Grade 3 or 4 bilateral sacroiliitis with at least one clinical criterion |
| or |
| At least four clinical criteria |
| **Modified New York, 1984** |
| Criteria |
| 1. Low back pain of at least 3 months' duration improved by exercise and not relieved by rest |
| 2. Limitation of lumbar spine in sagittal and frontal planes |
| 3. Chest expansion decreased relative to normal values for age and sex |
| 4. Bilateral sacroiliitis grade 2 to 4 |
| 5. Unilateral sacroiliitis grade 3 or 4 |
| Definite Ankylosing Spondylitis |
| Unilateral grade 3 or 4, or bilateral grade 2 to 4 sacroiliitis and any clinical criterion |
| Grading of Radiographs |
| Normal, 0; suspicious, 1; minimal sacroiliitis, 2; moderate sacroiliitis, 3; ankylosis, 4 |

Data from van der Linden SM, Valkenburg HA, Cats A: Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. *Arthritis Rheum* 27:361–368, 1984.

the likelihood of false-positive diagnoses, especially in settings in which the prior probability of the disease is low.

The diagnosis of AS is based on clinical features. The disease is "primary" or "idiopathic" if no associated disorder is present, and it is "secondary" if the disease is associated with psoriasis or chronic inflammatory bowel disease. In daily practice, a presumptive clinical diagnosis of AS is usually supported by evidence of sacroiliitis on conventional pelvic radiographs. Although radiographic sacroiliitis is frequent in AS, it is by no means an early or obligate manifestation of the disease.[13] Contrary to the New York criteria, radiographic evidence of sacroiliitis is not obligatory to fulfill the Rome criteria (Table 75-3). Both the Rome and New York criteria were primarily intended for use in epidemiologic studies. Lack of either sensitivity or specificity led to a modification of the New York criteria for AS[5] (see Table 75-3). Two criteria: limitation of lumbar spine motion and limitation of chest expansion, appear to reflect disease duration. They are usually not present in early disease.[14] Therefore the modified New York criteria are not useful for inclusion of early cases of AS because of a lack of sensitivity. MRI is useful to detect axial manifestations before the presence of radiographic sacroiliitis.[15] To encompass both nonradiographic AS and classic AS (according to the modified New York criteria), classification criteria have been proposed for the full spectrum of AS (Table 75-4).[11] The term *axial spondyloarthritis* provides an umbrella for the entire spectrum of patients with predominant axial involvement, regardless of the presence of structural damage on radiographs. Thus axial spondyloarthritis includes nr-axSpA and classic AS (fulfilling the modified New York criteria). According to the Assessment of SpondyloArthritis (ASAS) classification criteria,[11] a patient with chronic back pain and age at onset younger than 45 years can be classified (but not diagnosed) with axSpA if sacroiliitis on imaging (radiographs or MRI) is present, plus at least one other spondyloarthritic (SpA) feature, or, in the absence of sacroiliitis on imaging, if *HLA-B27* plus at least two further SpA features are present (see Table 75-4). The sensitivity of the entire set of ASAS criteria for axSpA was 83%, and the specificity was rather low (84%).[11] In the ASAS study on these new criteria, 30% of all patients considered to have axSpA had definite radiographic sacroiliitis and fulfilled the modified New York criteria for AS. Therefore two-thirds were classified with nr-axSpA.[11] It should be noted that patients classified with axSpA according to the ASAS criteria, tend to be more heterogenous than AS patients classified by modified New York criteria.[11,12,15a]

**TABLE 75-4** ASAS Classification Criteria for Axial Spondyloarthritis in Patients with Back Pain 3 Months or More and Age at Onset Younger Than 45 Years

| Sacroiliitis on Imaging plus ≥1 SpA Feature | OR | *HLA-B27* plus ≥2 Other SpA Features |
|---|---|---|

| SpA Features | Sacroiliitis on Imaging |
|---|---|
| Inflammatory back pain<br>Arthritis<br>Enthesitis (heel)<br>Uveitis<br>Dactylitis<br>Psoriasis<br>Crohn's disease/ulcerative colitis<br>Good response to NSAIDs<br>Family history for SpA<br>*HLA-B27*<br>Elevated CRP* | Active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA<br>OR<br>Definite radiographic sacroiliitis according to modified New York criteria |

*Elevated CRP is considered a SpA feature in the context of chronic back pain.

ASAS, Assessment of Spondyloarthritis International Society; CRP, C-reactive protein; HLA-B27, human leukocyte antigen-B27; IBP, inflammatory back pain; MRI, magnetic resonance imaging; NSAIDs, nonsteroidal anti-inflammatory drugs; SpA, axial spondyloarthritis.

From Rudwaleit M: New classification criteria for spondyloarthritis. *Int J Adv Rheumatol* 8:1–7, 2010.

The full spectrum of AS includes patients defined by the modified New York criteria as well as patients with nr-axSpA by ASAS classification criteria. However, fulfilling the ASAS criteria does not guarantee AS. Inappropriate use of the ASAS criteria as diagnostic criteria for individual patients may cause incorrect labeling as axial spondylitis due to insufficient specificity of the criteria in diagnostic settings.[15a]

## EPIDEMIOLOGY

### Prevalence

The prevalence of AS closely parallels the frequency of *HLA-B27*. This holds true for those B27 subtypes that are associated with the disease, but it is not true for populations in which the *HLA-B27*06* subtype, which lacks a strong association with AS, occurs frequently.[16-18]

Among whites, the estimated prevalence rate of AS, as defined by the modified New York criteria, ranges from 68 per 100,000 in a population older than 20 years in The Netherlands, to 197 per 100,000 in the United States.[19,20] In the general population, AS is likely to develop in approximately 1% to 2% of *HLA-B27*$^+$ adults who have a disease-associated B27 subtype, although there may be regional or geographic differences.[19,21] For example, in northern Norway, AS may develop in 6.7% of *HLA-B27*$^+$ people.[21]

The disease is much more common among *HLA-B27*$^+$ first-degree relatives of *HLA-B27*$^+$ AS patients; roughly 10% to 30% of them have signs or symptoms of AS.[19] In fact, a positive family history of AS is a strong risk factor for the disease.

### Incidence

There is no adequate evidence that the incidence of AS has changed in the past few decades.[22] Clinical features, age of onset, and survival time have remained stable.[23] This study revealed an overall age and gender-adjusted incidence of 7.3 per 100,000 person-years.

### Racial Distribution

AS occurs in all parts of the world, but there are race-related differences in prevalence. This might reflect differences in the distribution of *HLA-B27* among races. Approximately 90% of white patients with AS possess *HLA-B27*, whereas AS and *HLA-B27* are nearly absent (prevalence of B27 <1%) in African blacks and Japanese. In African-Americans, because of racial admixture with whites, 2% possess B27, but only approximately 50% of black patients with AS possess B27. Correspondingly, African-Americans are affected far less frequently than American whites.

### Burden of Disease

AS is associated with a considerable burden to the patient and society. Apart from axial and articular manifestations, extra-articular manifestations, such as enthesitis and acute anterior uveitis, and comorbidities, such as inflammatory bowel disease and psoriasis, contribute to the burden of disease. In addition, a large proportion of patients has spinal osteoporosis, leading to vertebral fractures and thoracic kyphosis. All of these features result in a decreased quality of life. Disease status scores for physical functioning and disease activity correlate clearly with psychological scores for anxiety and depression.[24] The impact of AS can also be seen in various aspects of employment, ranging from required assistance at work to increased sick leave and withdrawal from the workforce.[25,26] AS patients have an important impact on health care and non–health care resource utilization, resulting in mean total costs (direct and productivity) of approximately $6,700 to $9,500 per year per patient.[27-29]

The burden of illness increases with duration of disease. Because the burden caused by AS reduces quality of life, and because all types of costs associated with AS result from loss of function and disease activity, early diagnosis and treatment are necessary to prevent or reduce functional decline and improve patient outcome.[30]

## GENETICS

Twin and family studies have shown that the risk for AS runs strongly in families and is largely genetically determined, and that genetic factors also play major roles in determining disease activity and severity.[31,32] Major progress has been made in recent years in defining the alleles (genetic variants) that underlie this genetic predisposition, with 41 known loci (genetic regions) associated with the disease, including different loci within the major histocompatibility complex (MHC), and in non-MHC regions.

### Major Histocompatibility Complex Associations with Ankylosing Spondylitis

The association of *HLA-B27* with AS was first reported in the early 1970s, and since then increasing numbers of HLA-B27 subtypes have been identified, with more than

100 subtypes now known. The majority of these subtypes are too rare for us to know whether they are disease associated. The ancestral HLA-B27 subtype is *HLA-B*2705*, from which the other subtypes have arisen largely by point mutation. AS has been reported to occur with the following HLA-B27 subtypes: *B*2702*, *B*2703*, *B*2704*, *B*2705*, *B*2706*, *B*2707*, *B*2708*, *B*2710*, *B*2714*, *B*2715*, and *B*2719*. Two subtypes appear to show reduced or possibly absent association with AS; *HLA-B*2706*, which is found in Southeast Asia, and *HLA-B*2709*, which is found in Sardinia. The impact of subtype variation on AS risk has been a cornerstone of research into the mechanism by which HLA-B27 induces AS. HLA-B27 is found in 85% to 95% of cases of primary AS compared with a prevalence in most populations of European descent of approximately 8%.[33] A lower prevalence of HLA-B27 (~60%) is found in AS associated with psoriasis or inflammatory bowel disease.

*HLA-B27* homozygotes have roughly double the risk of AS, compared with B27 heterozygotes. The odds ratio for AS for *HLA-B27* (as defined by the tag single nucleotide polymorphism [SNP] rs116488202) for *HLA-B27* heterozygotes is 62 and for homozygotes 105.[34] *HLA-B27* is widely used in clinical practice as a diagnostic aid in chronic back pain patients; indeed, the clinical arm of the ASAS axSpA classification criteria requires the presence of *HLA-B27* to classify the condition. *HLA-B27* typing is also of use in determining the risk of recurrence of AS in families, with the risk of recurrence extremely low in the absence of *HLA-B27* (<1% chance). Because the prevalence of AS is quite low in the community, and because only approximately 5% of *HLA-B27* cases develop AS, population screening that uses HLA-B27 is not recommended. Disease develops in *HLA-B27*$^-$ individuals on average a decade later than *HLA*-B27$^+$ individuals, but their disease activity and severity of ankylosis is not different. There is some evidence to suggest that *HLA-B27*$^-$ individuals have lower responses to TNF inhibitors; nonetheless, these agents are clearly effective overall in treating *HLA-B27*$^-$ AS. Whether other genetic effects influence responses to TNF inhibitors is unknown in AS, but given that large studies in rheumatoid arthritis have failed to show any convincing evidence of a genetic association with response to TNF inhibitor treatment in that disease, it is unlikely that such an effect will be seen in AS. It will be particularly interesting, however, to see whether genetic variants influence the risk of development of antibodies against TNF inhibitors, which are associated with loss of effectiveness to these agents.

The prevalence of AS worldwide roughly matches the prevalence of HLA-B27. Regions with high *HLA-B27* prevalence include Scandinavia and some North America indigenous populations, and low prevalence is found through most of Africa and in Australian Aboriginals. This variation may be the result of population bottlenecks in human history or natural selection for HLA-B27 in some populations. It should be noted that *HLA-B27* is protective for HIV infection. It has recently been suggested that *HLA-B27* may influence survival, potentially through an effect on cardiovascular risk. This finding suggests another potential selective pressure on *HLA-B27* prevalence.[35]

*HLA-B27* is not the only HLA-B allele associated with AS. Multiple studies confirm a role for *HLA-B60* (also known as *HLA-B40*), which further increases AS risk by approximately 1.5-fold, and it is likely that other *HLA-B* alleles also contribute to the risk.[36] Controlling for association at HLA-B, association is also evident with *HLA-A*0201*.[34] There is suggestive evidence for the association of class III HLA genes, including *MICA* alleles with AS, which, if confirmed, would be consistent for a role of natural killer (NK) cells with the disease.[37] It is likely that other MHC genes are also associated with AS. Distinguishing true positive associations from linkage disequilibrium with the known associations, in particular *HLA-B27*, is extremely challenging because of the complex and extensive linkage disequilibrium that occurs in this region.

## Non–Major Histocompatibility Complex Associations of Ankylosing Spondylitis

The majority of the genetic risk for AS is thought to be determined by loci outside the MHC. To date 40 non-MHC loci have been convincingly demonstrated to be associated with the disease, and these have cast new light on the disease pathogenesis (summarized in Table 75-5).

## Aminopeptidase Genes and Ankylosing Spondylitis

Three genes in the aminopeptidase pathway are robustly associated with AS: *ERAP1* and *ERAP2* encoded at chromosome 5q15 and *NPEPPS* (puromycin-sensitive aminopeptidase) at chromosome 17q21. At least two associated haplotypes are present at *ERAP1*, with one driven by the

**TABLE 75-5** Loci with Established Association with Ankylosing Spondylitis*

| Locus | Gene | SNP | Odds Ratio | Heritability Explained (%) | Putative Mechanism of Action |
|---|---|---|---|---|---|
| 1p31 | *IL23R* | rs11209026 | 1.650 | 0.352 | Activation/differentiation of IL-23R–expressing cells |
| 1p31 | *IL23R* | rs12141575 | 1.147 | 0.100 | Activation/differentiation of IL-23R expressing cells |
| 1p36 | *RUNX3* | rs6600247 | 1.164 | 0.138 | T lymphocyte differentiation |
| 1q21 | *IL6R* | rs4129267 | 1.176 | 0.151 | Th17 lymphocyte differentiation, multiple other immunologic effects |
| 1q23 | *FCGR2A* | rs1801274 | 1.123 | 0.080 | Unknown |
| 1q23 | *FCGR2A* | rs2039415 | 1.088 | 0.037 | Unknown |

*Continued*

**TABLE 75-5** Loci with Established Association with Ankylosing Spondylitis*—cont'd

| Locus | Gene | SNP | Odds Ratio | Heritability Explained (%) | Putative Mechanism of Action |
|---|---|---|---|---|---|
| 1q32 | *GPR25-KIF21B* | rs41299637 | 1.201 | 0.162 | Unknown; ?Bacterial sensing |
| 1q32 | *HHAT* | rs12758027 | 1.094 | 0.048 | Unknown |
| 2p15 | Intergenic | rs6759298 | 1.308 | 0.407 | Unknown |
| 2q11 | *IL1R2-IL1R1* | rs4851529 | 1.103 | 0.055 | Influence on IL-1 response |
| 2q12 | *IL1R2-IL1R1* | rs2192752 | 1.112 | 0.047 | Influence on IL-1 response |
| 2q31 | *UBE2E3* | rs12615545 | 1.109 | 0.062 | Ubiquination |
| 2q37 | *GPR35* | rs4676410 | 1.131 | 0.060 | ?Bacterial sensing |
| 3p24 | *EOMES* | rs13093489 | 1.119 | 0.067 | T lymphocyte differentiation |
| 4q21 | *ANTXR2* | rs12504282 | 1.136 | 0.096 | Unknown |
| 5p13 | *PTGER4* | rs12186979 | 1.093 | 0.047 | Induction of IL-23 expression, in turn driving activation/differentiation of IL-23R–expressing cells; bone anabolism |
| 5p13 | *IL7R* | rs11742270 | 1.113 | 0.054 | T lymphocyte differentiation |
| 5q15 | *ERAP1* | rs30187 | 1.318 | 0.411 | Peptide trimming before class I HLA presentation |
| 5q15 | *ERAP1* | rs1065407 | 1.171 | 0.136 | Peptide trimming before class I HLA presentation |
| 5q15 | *ERAP2* | rs2910686 | 1.171 | 0.147 | Peptide trimming before class I HLA presentation |
| 5q33 | *IL12B* | rs6871626 | 1.117 | 0.066 | Activation/differentiation of IL-23R–expressing cells |
| 5q33 | *IL12B* | rs6556416 | 1.107 | 0.054 | |
| 6p21 | *HLA-B*27* | rs116488202 | 60 | 20.089 | Presentation of peptides to T cells, or misfolding leading to endoplasmic reticulum stress reaction |
| 6p21 | *HLA-A*02* | rs2394250 | 1.214 | 0.220 | Presentation of peptides to T cells |
| 6q15 | *BACH2* | rs17765610 | 1.172 | 0.064 | B cell or CD4 T lymphocyte differentiation |
| 6q15 | *BACH2* | rs639575 | 1.081 | 0.034 | B cell or CD4 T lymphocyte differentiation |
| 7q31 | *GPR37* | rs2402752 | 1.108 | 0.052 | ?Bacterial sensing |
| 9q34 | *CARD9* | rs1128905 | 1.124 | 0.082 | Th17 activation after β-glucan exposure |
| 10q22 | *ZMIZ1* | rs1250550 | 1.110 | 0.059 | T lymphocyte differentiation |
| 10q24 | *NKX2-3* | rs11190133 | 1.181 | 0.136 | T lymphocyte differentiation |
| 12p13 | *LTBR-TNFRSF1A* | rs1860545 | 1.131 | 0.086 | TNF signaling |
| 12p13 | *LTBR-TNFRSF1A* | rs7954567 | 1.113 | 0.062 | TNF signaling |
| 12q24 | *SH2B3* | rs11065898 | 1.129 | 0.060 | TCR signaling |
| 14q31 | *GPR65* | rs11624293 | 1.234 | 0.086 | ?Bacterial sensing |
| 16p11 | *IL27-SULT1A1* | imm_16_28525386 | 1.112 | 0.064 | Th17/Th1 lymphocyte differentiation balance |
| 16p11 | *IL27-SULT1A1* | rs35448675 | 1.236 | 0.007 | Th17/Th1 lymphocyte differentiation balance |
| 17q11 | *NOS2* | rs2531875 | 1.122 | 0.074 | Nitric oxide synthesis |
| 17q11 | *NOS2* | rs2297518 | 1.129 | 0.055 | Nitric oxide synthesis |
| 17q21 | *NPEPPS-TBKBP1-TBX21* | rs9901869 | 1.146 | 0.111 | Innate lymphoid cell differentiation/peptide trimming before class I HLA presentation, respectively |
| 19p13 | *TYK2* | rs35164067 | 1.155 | 0.080 | Signaling from cytokine receptors, including IL-23R |
| 19p13 | *TYK2* | rs6511701 | 1.098 | 0.036 | Signaling from cytokine receptors, including IL-23R |
| 21q22 | Intergenic | rs2836883 | 1.190 | 0.140 | Unknown |
| 21q22 | *ICOSLG* | rs7282490 | 1.100 | 0.052 | T lymphocyte differentiation |
| 22q11 | *UBE2L3* | rs2283790 | 1.124 | 0.052 | Ubiquination |

*Heritability explained assumes an additive model, disease prevalence 0.5%. Allele frequency and odds ratio are as reported for samples of European ancestry from Cortes et al, and for *ANTXR2* from Karaderi et al.

IL, Interleukin; HLA, human leukocyte antigen; SNP, single nucleotide polymorphism; TCR, T cell receptor; TNF, tumor necrosis factor.

From Cortes A, Hadler J, Pointon JP, et al: Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Gen* 45:730–738, 2013; and Karaderi T, Keidel SM, Pointon JJ, et al: Ankylosing spondylitis is associated with the anthrax toxin receptor 2 gene *(ANTXR2). Ann Rheum Dis* 73:2054–2058, 2014.

noncoding SNP rs30187, and the second haplotype less well defined, and tagged by the SNP rs10050860.[34] At *ERAP2* the genetic association is very broad, but on the key disease-associated haplotype, one protective variant (rs2248374[G]) leads to a transcribed RNA product that undergoes nonsense-mediated decay and thus no translated protein product.[38] This variant is thus the major functional variant at *ERAP2*.

Gene-gene interaction between *ERAP1* and *HLA-B27* is the first example of replicated, statistically significant interaction in any human disease and indicates that the mechanism by which HLA-B27 induces AS must involve aberrant peptide processing.[39] It is now known that the same variants in *ERAP1* are associated with psoriasis, and interaction is observed with *HLA-Cw6*, the major psoriasis class I HLA allele.[40] Similarly, in Behçet's disease, there is *ERAP1* association restricted to *HLA-B51* cases, the major Behçet's disease class I HLA allele.[41] This suggests that *HLA-B27*, *HLA-Cw6*, and *HLA-B51* operate in these diseases through similar mechanisms.

### Interleukin-23 Pathway Genes and Ankylosing Spondylitis

The genetic discovery of the association of *IL23R* with AS was the first demonstration of the involvement of this pathway in AS and has driven the repositioning of inhibitors of the IL-23 pathway for use in AS. There are now multiple polymorphisms identified with independent associations at *IL23R* that are associated with AS, inflammatory bowel disease (IBD), psoriasis, and other associated diseases. Several other IL-23 pathway genes have also now been associated with AS, including *IL12B*, *TYK2*, and *JAK2*, as well as other genes that interact with the IL-23 pathway such as *IL1RL1*, *IL1RL2*, *IL6R*, *PTGER4*, and *CARD9*. The molecular mechanisms underlying these associations largely await dissection, but these findings clearly demonstrate that this is a major pathway in AS pathogenesis. Consequently, it is a major target for development of therapies for AS. The association of *IL23R* is found in both *HLA-B27*$^+$ and *HLA-B27*$^-$ disease, and thus the pathway is relevant to both AS subsets.[42] Whether these genes play any role in controlling disease activity, severity of ankylosis, or clinical subsets of AS such as those that develop anterior uveitis, is the subject of ongoing research.

### Lymphocyte Development and Activation Genes

The association of AS with multiple cytokines, cytokine receptors, and transcription factors involved in controlling lymphocyte development and activation points to the key involvement of the affected cell types in AS pathogenesis. Associated genes include *EOMES*, *RUNX3*, *TBX21*, *ZMIZ1*, *IL7*, and *IL7R*.[34,42,43] The functional significance of these findings is discussed in more detail in subsequent text.

## PATHOGENESIS

Better understanding of the genetics of AS has advanced understanding of AS pathogenesis. Of the 41 loci associated with susceptibility to developing AS to date several fall into pathogenic "pathways" that may be important immunopathogenic drivers of AS: classic and nonclassic roles of HLA-B27; ERAP1 and other aminopeptidases involved in peptide trimming and antigen presentation; IL-23 and IL-17 cytokines.[34]

### HLA-B27

Despite more than 40 years of research, the precise role of HLA-B27 in AS remains unclear. Current models for how HLA-B27 operates in AS can be divided into classic models of how class I alleles operate in antigen presentation versus nonclassic models associated with irregular properties of HLA-B27, including slow folding rate and cell-surface homodimer formation.

The dominant role of class I HLA molecules, such as HLA-B27, is in presentation of antigens to CD8 T lymphocytes. In the case of infected or transformed cells, presentation of foreign or tumor antigens provide danger signals to CD8 lymphocytes. The "arthritogenic peptide" model of AS pathogenesis suggests that in genetically predisposed individuals, aberrant presentation of self-peptides by HLA molecules results in recognition of innocuous self-antigens as harmful, inducing autoreactivity from CD8 T cell responses. Self-reactive T cells have been isolated from synovium of AS patients,[2] but these studies have not been convincingly replicated.[44-46] This model is not well supported by studies in B27-transgenic rats, in which disease pathogenesis is independent of CD8 T cells. One caveat to this model is that B27-transgenic rats express in the region of 40 to 50 copies of HLA-B27 per cell compared with, at most, two copies in humans. The effects of such overexpression of class I alleles in this animal model require cautious interpretation of data from such experiments because it is possible that an AS-like condition may arise from different pathogenic mechanisms.

During antigen processing and presentation, the highly polymorphic heavy chain of HLA-B27 noncovalently associates with β2-microglobulin, and that complex is loaded with a peptide before transport of this complex to the cell surface. The highly ordered process of HLA:peptide assembly, which occurs in the endoplasmic reticulum (ER), happens more slowly for HLA-B27 than any other HLA molecule.[47] The unfolded protein response (UPR) theory suggests that slow assembly of HLA-B27 complexes initiates endoplasmic reticulum stress pathways that lead to the secretion from macrophages of IL-23. Support for the UPR theory is, again, strongest in B27-transgenic rats but has also been reported in the synovium of AS patients.[48,49] However, recent data from AS patients have found little evidence for ER stress in circulating cells in AS patients.[50]

Following transport to the cell surface, β2-microglobulin can dissociate from the heavy chain, leaving a free heavy chain. Free heavy chains can then dimerize to form cell surface heavy-chain homodimers. Recent reports have shown that subsets of T cells and NK cells can recognize the B27 homodimers through specific killer immunoglobulin receptors (KIRs). Recognition of cell surface B27 heavy-chain homodimers by KIR3DL2$^+$ CD4 T cells leads to IL-17 secretion by those T cells.[51]

## ERAP1 and Other Aminopeptidases

Outside the *HLA* locus, *ERAP1* is the strongest association with AS susceptibility. ERAP1 is an aminopeptidase involved in trimming of peptides that have been processed through the proteasome from approximately 13 to 15 amino acids in length down to 8 to 9 amino acid peptides. ERAP1 is therefore considered to be a "molecular ruler," shaping peptides before loading onto class I MHC molecules. The association with AS has been described and validated in several studies.[39,52] At least two AS-associated *ERAP1* haplotypes have been described, tagged by the nonsynonymous SNPs rs30187 and rs10050860.[34] These SNPs only show association with HLA-B27$^+$ patients, thus demonstrating gene-gene interaction between *ERAP1* and *HLA-B27*.[39] Despite this new knowledge, our understanding of the precise role of ERAP1 in AS pathogenesis remains limited.

ERAP1 exists in either a closed (enzymatically active) or open (enzymatically inactive) state. X-ray crystallography revealed that the main AS-associated SNP rs30187 (K528R) in *ERAP1* is located at the hinge region of the enzyme that controls the switch between open and closed conformations, and studies using recombinant ERAP1 and in cell lines have shown that this variant is associated with reduced ERAP1 function.[39,53]

*ERAP1* polymorphisms could contribute to all three models for how HLA-B27 operates in AS. Altered rates of peptide trimming mediated by ERAP1 could lead to cell surface presentation of aberrant peptides in the context of HLA-B27. Altered enzyme activity could affect the rate at which HLA-B27:peptide complexes fold within the ER, thereby contributing to the UPR and ER stress. Recently, ERAP1 variants have been reported to alter levels of HLA-B27 cell surface free heavy chains.[54]

Controlling for the association of *EPAP1*, variants in two additional aminopeptidases falling within the chromosome 5q15 locus, *ERAP2* and leucyl/cystinyl aminopeptidase *(LNPEP)*, have also been identified.[34] Association with the lead SNP in *ERAP2* remains significant in HLA-B27$^-$ cases; whether *LNPEP* is independently associated with AS is unclear. The cytosolic aminopeptidase *NPEPPS* at chromosome 17q21 is the fourth aminopeptidase demonstrating an association with disease, involved in peptide processing prior to transport of substrates into the ER.[34] As with *ERAP1*, loss-of-function variants in *ERAP2* are protective against AS. The protective allele at rs2248374 produces truncated *ERAP2* transcripts that undergo nonsense-mediated decay, knocking down expression of the protein completely in homozygote individuals and by approximately 50% in heterozygotes.[38] Further understanding of how each of these aminopeptidases functions in AS is warranted, but inhibition of ERAP1 and ERAP2, at least, appears as an attractive therapeutic strategy for AS.

## IL-23 Signaling Pathway

IL-23 signals through a cell surface receptor composed of an IL-23–specific subunit (IL-23R) and a subunit shared with IL-12R (IL-12RB1). IL-23 signaling is necessary for the development of IL-17– and IL-22–secreting cells. As described previously, polymorphisms in *IL23R* and, more recently, downstream components of the IL-23 signaling pathway, *JAK2* and *Tyk2*, and the IL-23 subunit *IL12B*, have been associated with AS.[34,52]

Under normal physiologic conditions, IL-23, IL-17, and IL-22 regulate gut homeostasis. IL-23, IL-23R, and IL-17 are increased in AS. Inhibition of either IL-12/IL-23 or IL-17 with monoclonal antibodies ustekinumab or secukinumab, respectively, are promising new therapeutics in AS, displaying similar efficacy to TNF antagonists.

Improved understanding of the cells involved in IL-23 signaling in AS has shone light on new immune cell subsets involved in AS pathogenesis. Until recently, CD4 T cells were thought to be the dominant cell type involved in IL-23–mediated immune responses. However, studies in IL-23R–green fluorescent protein (GFP) reporter mice revealed that CD4 T cells are only a small component of the IL-23 signaling family. Several other cell types in these mice express high levels of IL-23R, including γδ T cells, macrophages, dendritic cells, and lymphoid tissue inducer-like (LTi) cells. In AS patients, several "noncanonical" IL-23–responsive cells have been described, including γδ T cells, KIR3DL2$^+$ CD4 T cells, mast cells and neutrophils.[51,55-57] The precise hierarchy of these cell types remains unclear, but it is conceivable that each cell type may play tissue-specific roles in regulating IL-23 responses in AS. The prominent source of IL-23 in AS also remains unclear.

# STRUCTURAL REMODELING AND ANKYLOSIS

A major distinguishing feature of seronegative from seropositive arthropathies is that although, at least early in both groups of diseases, inflammation typically causes erosion; in later stages, seronegative disease osteoproliferation occurs. There is little direct evidence in humans about the processes involved in osteoproliferative disease because of the difficulty in obtaining suitable specimens for histopathologic examination. Therefore most of the information that is available comes from imaging studies or from studies of murine models of disease.

Inflammation is clearly a critical early step in the osteoproliferative process. Baseline C-reactive protein (CRP) is a predictor of future radiographic change, and there is increasing evidence that although TNF inhibition does not altogether prevent bone formation, it does significantly slow ankylosis.[58] It had been postulated that TNF inhibition released Wnt-pathway–driven bone formation due to reduction in expression of the Wnt inhibitor DKK1.[59] However, this would be expected to lead to accelerated ankylosis in TNF inhibitor–treated patients, when in fact the converse appears to be the case.

Similarly, chronic nonsteroidal anti-inflammatory drug (NSAID) use, particularly in patients with elevated acute phase reactants, also slows ankylosis, albeit marginally.[60] This may relate to the known association of AS with genetic variants in *PTGER4*,[39] which encodes a receptor for prostaglandin $E_2$; $PGE_2$ is produced at entheses in response to physical stress, driving IL-23 production and bone formation.

MRI studies suggest that bone marrow fat metaplasia is an intermediate step prior to new bone formation, and that once fat metaplasia has occurred, new bone formation may

develop. The cell and molecular processes that are involved are not well understood, but animal model studies suggest involvement of the Wnt and BMP pathways, which are the major bone anabolic pathways in bony animals.[61] These may prove useful targets for reducing the rate of ankylosis in AS, but medications that specifically target inflammation-induced bone formation will be required to avoid generalized osteoporosis as a therapeutic side effect.

Recently noggin (NOG) and sclerostin (SOST) have been associated with the disease, and they might contribute to neoossification in AS patients.[62]

Currently, it remains unclear whether early suppression of inflammation leads to a decrease of the risk of new bone formation in AS.[63]

## CLINICAL MANIFESTATIONS

### Skeletal Manifestations

#### Low Back Pain and Stiffness

Back pain is an extremely common symptom, occurring in as much as 80% of the general population. Therefore it is important to note that back pain in AS and axSpA has special features that differentiate it from mechanical back pain[64-66] (Table 75-6). In clinical practice, inflammatory back pain is often not well recognized.[67] Back pain is the most prevailing diagnostic feature of AS (Table 75-7).

The pain is initially felt primarily deep in the gluteal region, is dull in character, is difficult to localize, and is insidious in onset. The pain can be severe at this early phase of the disease; it localizes in the SI joints but is occasionally referred toward the iliac crest or greater trochanteric region or down the dorsal thigh. Radiation of buttock pain may suggest root compression of the sciatic nerve. The buttock pain typically alternates from side to side. Coughing, sneezing, or other maneuvers that cause a sudden twist of the back may accentuate pain. Although the pain is often unilateral or intermittent at first, within a few months, it usually becomes persistent and bilateral, and the lower lumbar area becomes stiff and painful. The pain is associated with a feeling of low back stiffness that is worse in the morning and may awaken the patient from sleep, particularly during the second half of the night. Many patients do not differentiate between low back pain and stiffness. The morning stiffness may last as long as 3 hours. Both the stiffness and the pain tend to be eased by a hot shower, an exercise program, or physical activity; they do not improve with rest. Fatigue as a result of chronic back pain and stiffness may be an important problem and can be accentuated by sleep disturbances due to these symptoms.

**TABLE 75-6** Aspects of Inflammatory Back Pain in Ankylosing Spondylitis and Axial Spondyloarthritis

| |
|---|
| Onset of complaints before age 45 years |
| Duration of symptoms more than 3 mo (chronic pain) |
| Located at the lower back |
| Alternating buttock pain |
| Awaking due to back pain during the second half of the night |
| Morning stiffness for at least 30 min |
| Insidious onset of complaints |
| Improvement with exercises |
| No improvement of back pain with rest |
| Improvement with use of nonsteroidal agents |

**TABLE 75-7** Diagnostic Features of Ankylosing Spondylitis

| |
|---|
| Chronic inflammatory spinal pain |
| Chest pain |
| Alternate buttock pain |
| Acute anterior uveitis |
| Synovitis (predominantly of lower limbs, asymmetric) |
| Enthesitis (heel, plantar) |
| Radiographic sacroiliitis |
| Positive family history of ankylosing spondylitis |
| Chronic inflammatory bowel disease |
| Psoriasis |

#### Chest Pain

With subsequent involvement of the thoracic spine (including costovertebral and costotransverse joints) and the occurrence of enthesitis at the costosternal and manubriosternal joints, patients may experience chest pain accentuated by coughing or sneezing, which is sometimes characterized as "pleuritic." The chest pain is often associated with tenderness over the costosternal junctions. Mild to moderate reduction of chest expansion may be detectable in an early stage of AS. Chest pain occurs relatively often in *HLA-B27*$^+$ relatives, even in the absence of radiographic evidence of sacroiliitis.[68]

#### Tenderness

Extra-articular tenderness at certain loci is a prominent complaint in some patients. These lesions are caused by enthesitis. Common tender sites are the costosternal junctions, spinous processes, iliac crests, greater trochanters, ischial tuberosities, tibial tubercles, and heels (Achilles tendinitis or plantar fasciitis). Radiographically, bone spurs may develop at these sites.

#### Joints

The girdle or "root" joints (hips and shoulders) are the most frequently involved extra-axial joints in AS, and pain in these areas is the presenting symptom in as many as 15% of patients. Shoulder involvement, but especially hip involvement, may cause considerable physical disability. Coexisting disease in the lumbar spine often contributes significantly to disability of the lower extremities. Hips and shoulders are involved at some stage of disease in as many as 35% of patients. Hip disease is relatively more common as a presenting manifestation if the disease starts in childhood (juvenile AS). In boys 8 to 10 years of age, hip disease as a manifestation of juvenile AS is the most frequent type of chronic arthritis. These children with hip disease are mostly *HLA-B27*$^+$, and negative for anti-nuclear antibodies.

The knee joint may also be affected in AS, often as an intermittent effusion. The temporomandibular joint is involved in approximately 10% of patients.

## Extraskeletal Manifestations

Constitutional symptoms such as fatigue, weight loss, and low-grade fever occur frequently. Other extraskeletal manifestations are more localized.

### Eye Disease

Acute anterior uveitis or iridocyclitis is the most common extra-articular manifestation of AS, occurring in 25% to 30% of patients at some time during the course of the disease. There is no clear relationship between activity of the articular disease and this extra-articular manifestation. The onset of eye inflammation is usually acute and typically unilateral, but the attacks may alternate. The eye is red and painful, with visual impairment. Photophobia and increased lacrimation may be present. If the eye remains untreated or if treatment is delayed, posterior synechiae and glaucoma may develop. Most attacks subside in 4 to 8 weeks without sequelae if early treatment is provided. Acute anterior uveitis is more common in $B27^+$ than $B27^-$ patients with AS.[69] Relatives who have acute anterior uveitis seem to be at higher risk for AS. The calculated incidence of acute anterior uveitis in a Swiss family study was 89 attacks per 1000 patient-years for AS patients, but only 8 attacks per 1000 person-years among healthy $B27^+$ relatives.[70]

### Cardiovascular Disease

Cardiac involvement may be clinically silent or may cause considerable problems. Manifestations of cardiac involvement include ascending aortitis, aortic valve incompetence, conduction abnormalities, cardiomegaly, and pericarditis. In rare situations, aortitis may precede other features of AS. Aortic incompetence was noted in 3.5% of patients who had the disease for 15 years and in 10% after 30 years.[71] Inflammation and dilation of the aorta are the main causes of aortic valve incompetence. Cardiac conduction disturbances are seen with increasing frequency with the passage of time, occurring in 2.7% of those with disease of 15 years' duration and in 8.5% after 30 years.[71] Both aortic incompetence and cardiac conduction defects occur twice as often in patients with peripheral joint involvement. In AS the prevalence of myocardial infarction is increased (4.4%) in AS patients compared with 1.2% in the general population.[72]

### Pulmonary Disease

Lung involvement is a rare and late manifestation of AS. It is characterized by slowly progressive fibrosis of the upper lobes of the lungs, appearing, on average, 2 decades after the onset of AS. Patients may complain of cough, dyspnea, and sometimes hemoptysis.[73]

High-resolution computed tomography (CT) may be helpful in detecting interstitial lung disease in patients with respiratory symptoms whose chest radiographs are normal.[74,75]

Pulmonary ventilation is usually well maintained; an increased diaphragmatic contribution helps compensate for chest wall rigidity, which is due to involvement of the thoracic joints in the inflammatory process. Vital capacity and total lung capacity may be moderately reduced as a consequence of the restricted chest wall movement, whereas residual volume and functional residual capacity are usually increased.

### Neurologic Involvement

Neurologic complications of AS can be caused by vertebral fracture, instability, compression, or inflammation. Traffic accidents or minor trauma can cause fractures of a vulnerable osteoporotic spine.[76] The C5-C6 or C6-C7 level is the most commonly involved site.

As in RA, atlantoaxial joint subluxation, atlantooccipital subluxation, and upward subluxation of the axis may occur in AS as a consequence of instability resulting from the inflammatory process. Spontaneous anterior atlantoaxial subluxation is a well-recognized complication in approximately 2% of patients and manifests with or without signs of spinal cord compression.[77]

Nontraumatic causes of neurologic complications caused by compression include ossification of the posterior longitudinal ligament (which may lead to compressive myelopathy), destructive intervertebral disk lesions, and spinal stenosis.

The cauda equina syndrome is a rare but serious complication of long-term AS. The syndrome affects lumbosacral nerve roots. This gives rise to pain and sensory loss, but frequently there are also urinary and bowel symptoms. Gradual onset of urinary and fecal incontinence, impotence, saddle anesthesia, and occasionally loss of ankle jerks occurs. Motor symptoms, if present, are usually mild. CT and MRI allow the accurate noninvasive diagnosis of this complication of AS.[78] Arachnoiditis and arachnoid adhesions may be important in the pathogenesis.

### Renal Involvement

Renal complications may be elevated among patients with AS.[79] Immunoglobulin (Ig)A nephropathy has been reported in many patients with AS. These patients often have an elevated IgA level (93%) and renal impairment (27%) at presentation.[80] Microscopic hematuria and proteinuria may occur in as many as 35% of patients. The significance of these findings in terms of subsequent deterioration of renal function is unclear.[81] Amyloidosis (secondary type) is a rare complication.[82]

### Osteoporosis

Osteopenia is seen in the early stages of AS.[83] In patients with this disease, osteoporotic deformities of the thoracic spine contribute significantly to abnormal posture, particularly fixed hyperkyphosis.[84] Radiographic damage to the cervical and lumbar spine, thoracic wedging, and disease activity are determinants of hyperkyphosis in AS.[85] An increased occiput-to-wall distance is associated with vertebral fractures. The prevalence of symptomatic osteoporotic spinal fractures is increased in AS.[86] Neurologic

complications occur rather frequently, even after minor trauma.[87] Proper assessment of bone density in the spine is difficult in the presence of syndesmophytes because they may give rise to falsely high values.

## PHYSICAL EXAMINATION

### Spinal Mobility

To arrive at an early diagnosis, the physician must perform a thorough physical examination. On examination of the spine, there may be some limitation of motion of the lumbar spine as elicited by forward flexion, hyperextension, or lateral flexion. Early loss of the normal lumbar lordosis is often the first sign and is easily assessed on inspection.

The Schober test (or its modifications) is useful to detect any limitation of forward flexion of the lumbar spine, although it is typically normal in early disease. While the patient stands erect, one mark is placed with a pen on the skin overlying the fifth lumbar spinous process (usually at the level of the posterosuperior iliac spine or the "dimple of Venus"), and another mark is placed 10 cm above in the midline. The patient is then asked to bend forward maximally, without bending the knees. If the distance between both marks is less than 15 cm, this indicates reduced lumbar spine mobility. Lateral flexion may also be diminished, and spinal rotation may cause pain.

### Chest Expansion

Mild to moderate reduction of chest expansion is often detectable in early stages of AS. Normal values are age and sex dependent, and there is considerable overlap between normal values and those obtained from AS patients. Reduction below 5 cm in young persons with an insidious onset of chronic, inflammatory low back pain strongly suggests AS. Chest expansion should be measured on maximal inspiration after forced maximal expiration at the level of the fourth intercostal space in males and just below the breasts at the xiphisternal level in females.

### Enthesitis

Examination of the ischial tuberosities, greater trochanters, spinous processes, costochondral and manubriosternal junctions, supraspinatus insertion, and iliac crests can determine the presence of enthesitis. Heel pain, especially when getting out of bed, is a characteristic manifestation of Achilles tendon enthesitis and plantar fasciitis.

### Sacroiliitis

Direct pressure over the SI joints may elicit pain, as may special testing maneuvers, although the latter lack specificity and sensitivity. These signs may also be negative in early disease or may become negative in late stages as inflammation is replaced by fibrosis or bony ankylosis.

### Posture

Over the course of the disease, the patient may lose normal posture. Involvement of the cervical spine is manifested by pain and limitation of neck movement. The physician can detect a forward slope of the neck by having the patient stand against a wall and try to position his or her occiput against it.

After many years of progression in patients with severe disease, the entire spine may become increasingly stiff, with loss of normal posture from gradual loss of lumbar lordosis and the development of thoracic kyphosis.[84,85] The abdomen becomes protuberant; breathing is primarily by diaphragmatic action. These typical deformities usually evolve after disease duration of 10 years or more.

## LABORATORY TESTS

Generally, routine blood tests are not helpful. A normal erythrocyte sedimentation rate (ESR) or normal CRP level does not exclude active disease. An elevated ESR or CRP is reported in as many as 75% of patients, but it may not correlate with clinical disease activity.[88] Neither ESR nor CRP is superior in assessing disease activity.[89] A mild normochromic anemia may be present in 15% of patients. Elevation of serum alkaline phosphatase (derived primarily from bone) is seen in some patients but is unrelated to disease activity or duration. Some elevation of serum IgA is frequent in AS. Its level correlates with acute phase reactants. Active disease is associated with decreased lipid levels, particularly high-density lipoprotein cholesterol, resulting in a more atherogenic lipid profile.[90]

## IMAGING STUDIES

### Conventional Radiography

The typical radiographic changes of AS are seen primarily in the axial skeleton, especially in the SI, discovertebral, apophyseal, costovertebral, and costotransverse joints. They evolve during many years, with the earliest, most consistent, and most characteristic findings seen in the SI joints. The radiographic findings of sacroiliitis consist of blurring of the subchondral bone plate, followed by erosions and sclerosis of the adjacent bone. The changes in the synovial portion of the joint (i.e., the lower one-third of the joint) primarily result from inflammatory osteitis of the adjacent subchondral bone.[91] Progression of the subchondral bone erosions can lead to pseudowidening of the SI joint space. With time, gradual fibrosis, calcification, interosseous bridging, and bony ankylosis occur. It is practical to grade radiographic sacroiliitis according to the New York criteria (Table 75-8).

Bony erosions and osteitis ("whiskering") at sites of osseous attachment of tendons and ligaments are frequently seen, particularly at the calcaneus, ischial tuberosities, iliac

**TABLE 75-8** Grading of Sacroiliitis: New York Criteria

| |
|---|
| Grade 0, normal |
| Grade 1, suspicious |
| Grade 2, minimal sacroiliitis |
| Grade 3, moderate sacroiliitis |
| Grade 4, ankylosis |

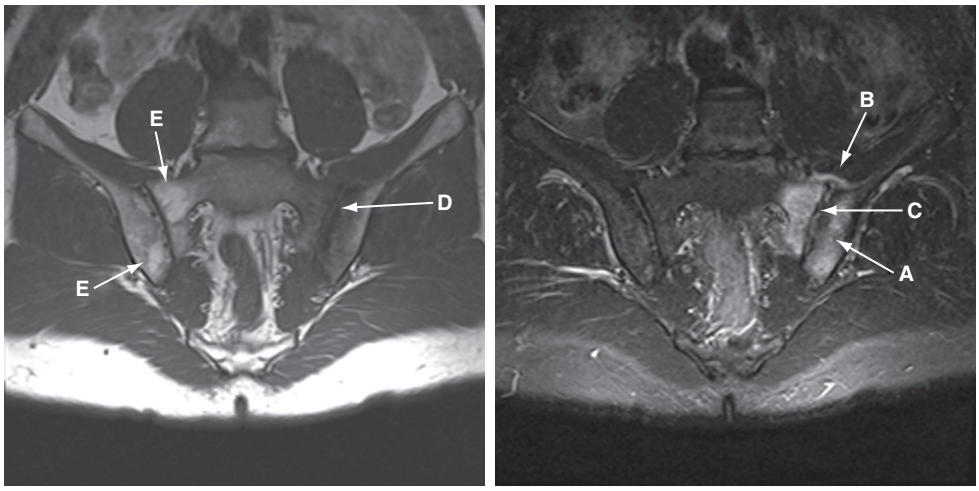



**Figure 75-2** T1-weighted *(left)* and short-tau inversion recovery sequence magnetic resonance *(right)* images of 23-year-old male with inflammatory back pain and equivocal pelvic radiograph demonstrating the following features: *A,* Bone marrow edema in left iliac and sacral bones; *B,* capsular inflammation; *C,* joint space inflammation; *D,* diffuse erosion of left iliac bone with widening of joint space; *E,* fat infiltration in right sacrum and ilium.

crest, femoral trochanters, supraspinatus insertion, and spinous processes of the vertebrae. In the early stages of the evolution of syndesmophytes, there is inflammation of the superficial layers of the annulus fibrosus, which leads to erosion of the adjacent corners of the vertebral bodies visible on radiography. Tissue repair leads to "squaring" of the vertebral bodies and reactive bone sclerosis, which constitute the Romanus lesion seen on radiography. Gradual ossification of the annulus fibrosus may lead to complete bony "bridging" between vertebrae.[92] Concomitant ankylosis often occurs in the apophyseal joints, with ossification of the adjacent ligaments. In a number of patients, this may result in complete fusion of the vertebral column ("bamboo spine").

Hip involvement may lead to concentric joint space narrowing, irregularity of the subchondral bone with subchondral sclerosis, osteophyte formation at the outer margin of the articular surface, and, ultimately, bony ankylosis.

Several validated scoring methods are available to quantify structural damage in AS: the Bath AS Radiology Index (BASRI), the Stoke AS Spondylitis Score (SASSS), and the modified SASSS.[93-95] The BASRI includes scores for the cervical and lumbar spine, as well as the SI and hip joints. The SASSS evaluates the lumbar spine only; the modified SASSS assesses the anterior cervical and lumbar spine. These scoring methods are most suited for use in clinical trials and observational studies.

## Computed Tomography and Magnetic Resonance Imaging

CT may detect bone abnormalities such as sclerosis and erosion sooner than plain radiography, but its use is limited by radiation exposure, and it does not detect changes in soft tissues or bone marrow where early features of sacroiliitis develop. MRI sequences that are used in routine practice to image the SI joint can detect sacroiliitis in at least 50% of patients with nonradiographic SpA.[96] These sequences include fat-saturating techniques, such as short-tau inversion recovery (STIR), that are very sensitive in the detection of bone marrow edema, which is a frequent finding in SpA (Figure 75-2). The T1-weighted (T1W) sequence allows detection of erosions and fat metaplasia, which may occur early in the disease and are associated with resolution of inflammation. Diagnostic MRI should include both sequences, whereas the role of contrast enhancement with gadolinium is still unclear because of its substantial cost and the prolonged time for imaging, which some patients find difficult to endure.

Spinal inflammation can only be visualized by MRI, where it is typically seen as bone marrow edema in the vertebrae at both anterior and posterior vertebral corners, as well as around the intervertebral disk. Lateral and posterior elements, such as the costovertebral and costotransverse joints, facet joints, pedicles, and spinal ligaments, can also show inflammatory lesions.[97] Resolution of inflammation is associated with development of fat metaplasia in these same locations. MRI and ultrasonography can be useful to assess enthesitic problems such as Achilles tendinitis and heel pain. Definitions for the assessment of enthesitis by ultrasound have been established.[98] Quantification of spinal inflammation by using MRI is highly sensitive to change and is now recognized as an essential component of clinical trials. MRI scores for inflammation correlate well with CRP, but only weakly with symptoms in patients with AS. Evidence supports an association between MRI inflammation and the future development of structural changes on radiography.[6,99] Resolution of inflammation, especially with TNF blockers, is associated with the development of fat metaplasia in the sacroiliac joints and the spine.[100,101] The histopathology of this lesion remains unclear, but it may be an important intermediary tissue associated with the development of ankylosis.[102,103] Evidence suggests that inflammatory lesions with no signs of concomitant fat metaplasia may resolve completely without sequelae, provided effective anti-inflammatory therapy is administered.[63,104] On the other hand, inflammatory lesions that already demonstrate fat metaplasia may be at higher risk of developing into new bone formation, even if the inflammation resolves with treatment. Consequently, the concept of a window of opportunity for effective treatment

with anti-inflammatory agents has been proposed and will require further testing in prospective studies.

## DIAGNOSIS

Clinical manifestations of AS usually appear in patients in late adolescence or early adulthood; only rarely do they appear in adults older than 40 years.[19] The diagnosis of AS at an early stage of disease depends primarily on a careful history and physical examination. Two features of the history are critical: (1) the presence of inflammatory low back pain and stiffness, and (2) a positive family history for AS.

Low back pain in AS has typical "inflammatory" features (see Table 75-6). A positive family history increases the pretest probability of AS from 0.1% for a person belonging to the general population to approximately 10% for any first-degree relative of an AS proband.[19] The probability that a first-degree relative with a positive family history of AS will have AS increases from 10% to nearly 50% if this relative has inflammatory low back pain. In contrast, the likelihood that a person who has inflammatory back pain (without any other inflammatory indications listed in Table 75-9) but has a negative family history for AS will have AS increases from 0.1% to only 1%. A definite diagnosis of AS is usually established by radiographic evidence of bilateral sacroiliitis. The plain anteroposterior (AP) view of the pelvis is usually adequate for diagnostic purposes. A major challenge to early diagnosis by using radiography is the complex anatomy of the joint. The cartilaginous portion, which is the region primarily affected in early disease, is convex anteroinferiorly in the anterior one-third of the joint. In addition, the joint cavity is oblique, with the posterior aspect located medially and the anterior located laterally. This creates difficulty in the interpretation of early subchondral changes by using the conventional AP view. Overlying bowel gas and soft tissues further confound detection of abnormalities. Erosion may therefore be quite severe in the SI joint before detection, and this has led to the use of various imaging approaches in clinical practice to optimize radiographic assessment of the SI joint. Reliable detection of radiographic sacroiliitis is difficult, even for experienced readers, and after extensive training and calibration.[105,106]

There is little evidence to support the use of specific SI joint views, such as oblique views or the Ferguson view, versus the conventional AP of the pelvis. Moreover, there is minimal additional diagnostic utility to the use of specific SI joint views in which the AP of the pelvis is equivocal for sacroiliitis, which comes at the expense of additional exposure to radiation. In the setting of suspicion of early AS, in which an AP pelvic radiograph is reported as equivocal, MRI of the pelvis would be a more useful option than additional radiography.

**TABLE 75-9** Proposed Criteria for Inflammatory Back Pain in Young to Middle–Aged Adults* with Chronic Back Pain

| |
|---|
| Morning stiffness of at least 30-min duration |
| Improvement of back pain with exercise but not with rest |
| Awakening because of back pain during second half of night only |
| Alternating buttock pain |

*Younger than 50 years.

From Rudwaleit M, Metter A, Listing J, et al: Inflammatory back pain in ankylosing spondylitis: a reassessment of the clinical history for application as classification and diagnostic criteria. *Arthritis Rheum* 65:569–578, 2006.

In most adult patients, AS can be diagnosed clinically without the *HLA-B27* test. This assessment often has no additional value in established disease or as a pure screening tool.[107] However, in young patients with inflammatory chronic back pain, a positive *HLA-B27* test increases the likelihood of having AS, particularly if imaging of the SI joints does not provide conclusive results. In this setting, MRI may help to classify patients with SpA or non-SpA.[96,108]

An approach based on pretest probabilities and likelihood ratios has been proposed to diagnose early disease with predominantly axial manifestations before convincing evidence of radiographic sacroiliitis.[65] The majority of patients diagnosed clinically with nr-axSpA have MRI evidence of SpA,[96] and the severity of bone edema on MRI has been shown to predict the development of radiographic sacroiliitis on follow-up.[6] Consequently, MRI is now an acceptable imaging criterion for the diagnosis of SpA and is especially useful when the history points to inflammatory back pain, the pelvic radiograph is normal or equivocal, and the patient is positive for HLA-B27 or has a positive family history for AS. MRI studies in such settings should include T1W and STIR of the sacroiliac joint. Imaging is conducted in the oblique coronal plane with consecutive slices parallel to the dorsal surface of the sacrum so that the cartilaginous portion of the joint can be adequately visualized. Imaging in the axial plane may provide better visualization of the ligamentous portion, but it has not been shown that this enhances diagnostic utility. Contrast enhancement with gadolinium may be useful in demonstrating lesions such as synovitis and capsulitis, but these often occur concomitantly with subchondral bone marrow edema so that diagnostic utility is not enhanced.[109] Bone marrow edema in subchondral bone is detected by using fat-suppressed sequences such as STIR and is the most frequent lesion in early disease. A definition of a positive MRI has been proposed according to consensus by Assessment for Ankylosing Spondylitis/Outcome Measures in Rheumatology (ASAS/OMERACT), which states that a single coronal slice that clearly demonstrates bone marrow edema in several locations is sufficient to designate a scan as positive for SpA.[110] A single lesion must be evident in two consecutive slices for a scan to be considered positive for SpA. Assignment of a positive MRI by using this definition can be done more reliably than for radiographic sacroiliitis.[111]

Studies with age- and sex-matched controls have shown that false positives may occur in 15% to 20% with use of this definition, and that assessment of structural lesions, especially erosions, may enhance detection of SpA.[96,112] Fat metaplasia may be diagnostically useful when it is observed adjacent to subchondral bone, has a distinct border, and a homogeneous increase in T1W signal. However, it is unusual to see fat metaplasia alone, and it is often observed with other structural lesions as well as bone marrow edema.[113] A key principle for evaluation of MRI scans is the simultaneous evaluation of both T1W and STIR sequences, because each often provides information that enhances confidence

in overall diagnostic ascertainment. For instance, subtle bone marrow edema may by itself not be definitive for SpA, but if the T1W scan also demonstrates an erosion or a region of fat metaplasia, the sum of the MRI evaluation may be definitive for SpA.

Spinal MRI is conducted in the sagittal plane, and the spine is imaged in cervicothoracic and thoracolumbar halves. It may demonstrate vertebral corner bone marrow edema and/or fat metaplasia in nr-axSpA. However, spinal MRI is not recommended for routine evaluation of SpA unless the SI joint scan is equivocal and the clinical index of suspicion for SpA is high. Vertebral corner lesions demonstrating bone marrow edema and/or fat metaplasia may be observed at two to three spinal levels in healthy individuals, especially in patients with mechanical disorders.[114] Conventional spinal imaging does not extend sufficiently laterally to cover all lateral spinal structures that could be inflamed. Specific MRI protocols for spinal imaging in axSpA have been developed to address this potential shortcoming.[97]

At the other end of the age scale, juvenile-onset AS is not uncommon among young patients. Such patients tend to have enthesitis and peripheral arthritis, which may be severe and disabling.

## ANKYLOSING SPONDYLITIS IN MALES AND FEMALES

Clinically, AS is more common in males, with a reported male to female ratio of approximately 2:1 to 3:1. In contrast, studies of patients with nr-axSpA usually show a slight female preponderance.[115] One possible explanation could be that structural changes are less likely to develop in women. Another possibility might be lack of specificity of the ASAS classification criteria.[11] This would cause false-positive inclusion of female patients who fulfill the criteria but do not really have axSpA. Of course, both possibilities are not mutually exclusive.

Disease expression of AS by modified New York criteria is thought to be different in males and females. A case-control study comparing 35 female patients with 70 male patients as controls showed no differences in spinal symptoms, chest expansion, peripheral arthritis, extra-articular manifestations, or functional outcome. The males with AS more often had radiographic spinal changes and hip joint involvement than their female counterparts. There is still some controversy, but overall, there are no significant clinical or radiographic differences between women and men with AS. However, on average, the disease seems to be more severe in men.[116,117]

Female patients with AS have normal fertility.[118] Most patients (50% to 60%) do not experience major changes in disease activity during pregnancy, but an increase in morning stiffness and low back pain, particularly at night, may occur at approximately the 20th week of gestation and last for a few days to weeks.[119] In approximately 50% of patients, an exacerbation of symptoms is seen within the first half year after delivery. Sacroiliitis including complete ankylosis of the SI joints does not constitute a contraindication for vaginal delivery. Epidural anesthesia is usually possible because most patients have a rather short duration of disease and do not have extensive spinal syndesmophytes. The fetal outcome is not impaired in patients with AS. Every pregnancy in patients with this disease should be considered potentially high risk, however, and such pregnancies require close collaboration between rheumatologists and obstetricians.

An uncontrolled study among 612 AS patients (mean age, 50.8 years; 71.6% males) reported a substantial impact on sexual relationships (response rate, 38%). Poor function, depression, disease activity, unemployment, and poor self-efficacy were independently associated with greater impact on patients' sexual relationships.[120]

## OUTCOME

The course of AS is highly variable, characterized by spontaneous remissions and exacerbations. Its prognosis has generally been considered rather favorable. The disease may run a relatively mild or self-limited course. However, the disease may also remain active during many years. Life expectancy is somewhat reduced, particularly after 10 years of disease.[121] A study from Finland indicates that the risk of dying for patients with AS is increased by 50% compared with controls matched for age and gender. Causes of death include complications of the disease such as amyloidosis and spinal fractures, as well as cardiovascular, gastrointestinal, and renal disease.[122] No differences exist between familial and sporadic AS in terms of age at onset, age at diagnosis, or prevalence of peripheral arthritis and acute anterior uveitis.[123]

Functional limitations increase with disease duration. Although structural damage seen on radiographs is clearly associated with physical function and spinal mobility at the group level, individual patients with normal radiographs might exhibit a major reduction in spinal mobility, whereas those with severe radiographic abnormalities might function quite well in everyday tasks.[124]

The functional prognosis of AS is less favorable than was previously thought. Withdrawal from work in those with paid jobs varies from 10% after 20 years of disease duration to 30% after 10 years, depending on the characteristics of the patients included and the social security system considered.[125-128] The age- and sex-adjusted withdrawal rate from labor-force participation was 3.1 times higher among Dutch patients compared with the general population.[128] Older age at disease onset, manual work, lower educational level, and coping strategies characterized by the limitation and pacing of activities were associated with a higher risk for work disability.[126-128] Vocational counseling, job training, easy access to the workplace, and support of colleagues and management may reduce the probability of withdrawal from work.[125,129] Sick leave in those with paid jobs was linked to disease activity and presence of extraspinal disease manifestations.[125,128,130] Patients with peripheral joint involvement are more likely to take sick leave than are AS patients with axial manifestations only.

Overall, the first 10 years of disease are particularly important with respect to subsequent outcome. Most of the loss of function among patients with AS occurs within this period and is associated with the presence of peripheral arthritis, spinal radiographic changes, and development of a so-called *bamboo spine*.[131,132] Although radiographic progression is highly variable among patients, radiographic

evidence of spinal involvement, especially the presence of syndesmophytes, appears to be the primary factor that independently predicts further radiographic progression.[133]

Increasing evidence supports the importance of inflammation in predisposition to radiographic progression. Clinical parameters of inflammation, notably the Ankylosing Spondylitis Disease Activity Score (ASDAS) and CRP, are associated with prognostic risk, especially early in the disease course, which is consistent with MRI data that evaluate prognostic risk of inflammatory lesions in the spine.[58,134,135] This is also consistent with preliminary data from an observational cohort that introduction of anti-TNF therapy early in the disease course may be associated with less risk for radiographic progression. Smoking and NSAID utilization have also been shown to influence prognostic risk, although it is unclear whether this is related to inflammation.[136-138] Biomarkers reflecting connective tissue remodeling (e.g., metalloproteinase 3 and citrullinated vimentin) and formation of bone (e.g., sclerostin and dickkopf-1) have been associated with radiographic progression.[139-142]

The long-term results of total hip replacement in AS are satisfactory. The outcome of 138 total hip replacements and 12 revisions was good or very good in 86% of patients, and 63% of patients had no pain. Mobility was good or very good in 44% of patients. The mean follow-up was 7.5 years (range, 1 to 34 years). Altogether, 69% of the male hip recipients younger than 60 years were at work at the time of the survey.[143]

## ASSESSMENT AND MONITORING

Signs and symptoms such as spinal pain and limitation of motion might be the result of current disease activity or to damage. A plethora of tools is available to assess these dimensions. For example, there are many ways to measure limitation of motion of the lumbar spine. New instruments have been developed to assess various aspects of the disease, including the Bath and Edmonton AS Metrology Indices, the Bath AS Global Index, the BASRI, the Bath AS Disease Activity Index, and the Dougados Functional Index.[144-149] However, standardization and validation of many of these instruments are lacking or incomplete. An international ASAS working group was formed with the aim of selecting, proposing, and testing core sets of measures for different settings.[150] It was thought that a certain set of variables should be targeted to a specific task. For example, when assessing the efficacy of physical therapy, it would not be realistic to include measures of radiographic changes of the spine. Clearly, the set of measures for a drug's disease-modifying capabilities will differ from a set that measures analgesic effectiveness only. Four settings have been defined: disease-controlling anti-rheumatic therapy; symptom-modifying anti-rheumatic drugs, such as NSAIDs; physical therapy; and clinical record keeping in daily practice (Table 75-10).[150] Criteria to assess the response of individual patients have also been developed and validated. These ASAS-20 improvement criteria are frequently used in clinical trials.[151] In addition, more stringent improvement criteria—ASAS-40 and ASAS-5/6—have been proposed,[152] as well as criteria to define partial remission. The three sets of improvement criteria and the partial remission criteria are presented in Table 75-11.[151,152] The recently developed ASDAS enables physicians to define disease activity states and to demonstrate improvement by applying cutoff values.[135]

A needs-based quality-of-life instrument specific for AS has been developed. It is well accepted by patients and easy to perform, and has shown good scaling, psychometric properties, and sensitivity to change.[153,154]

**TABLE 75-10** World Health Organization–International League of Associations for Rheumatology Core Sets for Ankylosing Spondylitis

| Domain | Instrument |
|---|---|
| Function | BASFI or Dougados Functional Index |
| Pain | VAS: last week, spine pain at night due to AS<br>VAS: last week, spine pain due to AS |
| Spinal mobility | Chest expansion and modified Schober's test and occiput-to-wall distance (lateral spinal flexion or BASMI) |
| Patient global assessment | VAS: last week |
| Stiffness | Duration of morning spine stiffness, last week |
| Peripheral joints and entheses | Number of swollen joints (44-joint count); validated enthesis index |
| Acute phase reactants | Erythrocyte sedimentation rate |
| Spine radiographs | Lateral view of lumbar spine and lateral view of cervical spine |
| Hip radiographs | Pelvic radiograph, including sacroiliac joints and hips |
| Fatigue | VAS on fatigue from BASDAI |

*Disease-controlling anti-rheumatic therapy domains: 1-10; symptom-modifying anti-rheumatic drug domains: 1-5, 10; physical therapy domains: 1-5, 10; clinical record-keeping domains: 1-7.

AS, Ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; VAS, visual analogue scale.

From van der Heijde D, Calin A, Dougados M, et al: Selection of instruments in the core set for DC-ART, SMARD, physical therapy, and clinical record keeping in ankylosing spondylitis: progress report of ASAS Working Group—assessments in ankylosing spondylitis. *J Rheumatol* 26:951–954, 1999.

## MANAGEMENT

### KEY POINTS

**NSAIDs, physiotherapy, and patient education are first-line therapy.**

**TNF inhibitors are effective to treat the signs and symptoms in moderate to severe disease. Biologics that target other cytokines, such as IL-17A, also demonstrate clinical efficacy.**

**Some rather weak evidence supports the view that continuous use of NSAIDs may reduce radiographic progression. However, results of studies are conflicting; weighing advantages and disadvantages, it is currently unclear whether or when AS patients should be offered continuous NSAID treatment.**

**TABLE 75-11** Assessment in Ankylosing Spondylitis International Working Group Improvement Criteria and Partial Remission Criteria

| |
|---|
| **ASAS-20 Improvement Criteria** |
| At least 20% improvement and 10 units improvement in 3 of the 4 following domains, without 20% or more worsening and 10 units worsening in the remaining domain: |
| BASFI |
| Morning stiffness |
| Patient global assessment |
| Pain |
| **ASAS-40 Improvement Criteria** |
| At least 40% improvement and 20 units improvement in 3 of the 4 following domains, without any worsening in the remaining domain: |
| BASFI |
| Morning stiffness |
| Patient global assessment |
| Pain |
| **ASAS-5/6 Improvement Criteria** |
| At least 20% improvement in 5 of the 6 following domains: |
| BASFI |
| Morning stiffness |
| Patient global assessment |
| Pain |
| Acute phase reactants |
| Spinal mobility |
| **ASAS Partial Remission Criteria** |
| A value below 20 units in all 4 domains of the ASAS-20 improvement criteria |

ASAS, Assessment in Ankylosing Spondylitis; BASFI, Bath Ankylosing Spondylitis Functional Index.

From Anderson J.J, Baron G, van der Heijde D, et al: Ankylosing spondylitis assessment group preliminary definition of short-term improvement in ankylosing spondylitis. *Arthritis Rheum* 44:1876–1886, 2001; and Brandt J, Listing J, Sieper J, et al: Development and preselection of criteria for short term improvement after anti-TNFα treatment in ankylosing spondylitis. *Ann Rheum Dis* 63:1438–1444, 2004.

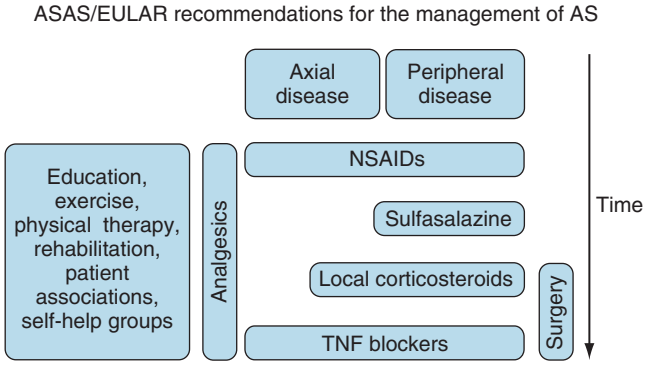



**Figure 75-3** Recommended management of ankylosing spondylitis (AS), based on clinical expertise and research evidence. The disease progression with time moves vertically from top to bottom. These recommendations were developed before the availability of secukinumab. Secukinumab is approved but was not available at the time of these recommendations. ASAS/EULAR, Assessment in Ankylosing Spondylitis/European League against Rheumatism; NSAIDs, nonsteroidal anti-inflammatory drugs; TNF, tumor necrosis factor.

Updated recommendations that emphasize the key evidence-based components of disease management have been compiled[154a,155,156] (Tables 75-12 and 75-13; Figure 75-3). Most patients do not experience significant extraskeletal manifestations except for acute anterior uveitis, which occurs in approximately 30% to 40% of patients. The number of patients who have experienced uveitis increases with disease duration. Usually, this eye disease can be well managed with eye drops that contain corticosteroids to reduce inflammation and with pupil-dilating, mydriatic agents to prevent or diminish synechiae (when the iris adheres to the cornea or lens). At the outset, patients should be warned that acute anterior uveitis may occur at any time during the course of the disease.

The treatment objectives in AS are to relieve pain, stiffness, and fatigue and to maintain good posture and good physical and psychosocial functioning.[157] Evidence is lacking that any of the conventional DMARDs, including sulfasalazine and methotrexate (MTX) alter or inhibit the inflammation seen in the spine and entheses in AS.

A full explanation of the disease, its course, possible complications (e.g., acute anterior uveitis), and its prognosis is very helpful to achieve treatment adherence by the patient. Self-help groups provide important information and social support. In addition, patient organizations often provide access to hydrotherapy and group physiotherapy. Exercises are a mainstay of treatment. Preferably, they should be started after a hot shower or a hot bath. Swimming and extension-promoting exercises or sporting activities such as volleyball or cross-country skiing are appropriate. These activities counteract the kyphotic effects of pain and fatigue on posture and reduce stiffness. Patients should avoid vigorous or contact sports if the spine has become fused or osteoporotic because such a spine is susceptible to fracture.

Appliances such as driving mirrors may improve comfort and safety, especially if there is considerable involvement of the cervical spine. In that case, appropriate neck support is also required to reduce the risk of fractures in the vulnerable osteoporotic cervical spine as a consequence of traffic accidents.

## Physiotherapy

Evidence indicates that physiotherapy in the form of exercises is effective, at least in the short term (as long as 1 year). In a randomized, controlled trial, a program of supervised physiotherapy in groups was superior to individualized programs in improving thoracolumbar mobility and fitness. The program, which consisted of hydrotherapy, exercises, and sporting activities twice weekly for 3 hours per session, resulted in improved overall health and less stiffness, as reported by the patient.[158] An intensive 3-week SpA exercise therapy program resulted in marked improvement in both subjective and objective assessments that lasted for as long as 9 months. Health resource use, in particular NSAID use and sick leave, was significantly reduced during this 9-month follow-up period. The clinical benefits of such treatments can be achieved at acceptable costs.[159,160] A Cochrane Review concluded that a home exercise program is better than no intervention, supervised group physiotherapy is better than home exercises, and that combined inpatient spa-exercise therapy followed by supervised outpatient weekly group physiotherapy is better than weekly group physiotherapy alone (level A evidence) (Table 75-14)[161] The tendency toward positive effects of physiotherapy in the management of AS calls for further research in this field. New trials should also address other physiotherapy interventions commonly used in clinical practice.

**TABLE 75-12** First Update of the ASAS/EULAR Recommendations for the Management of Ankylosing Spondylitis

| |
|---|
| The over-reaching principles of the management of patients with ankylosing spondylitis (AS) are: |
| AS is a potentially severe disease with diverse manifestations, usually requiring multidisciplinary treatment coordinated by the rheumatologist |
| The primary goal of treating the patient with AS is to maximize long-term health-related quality of life through control of symptoms and inflammation, prevention of progressive structural damage, preservation/normalization of function, and social participation |
| Treatment of AS should aim at the best care and must be based on a shared decision between the patient and the rheumatologist |
| The optimal management of patients with AS requires a combination of nonpharmacologic and pharmacologic treatment modalities |
| **General Treatment** |
| The treatment of patients with AS should be tailored according to: |
| The current manifestations of the disease (axial, peripheral, entheseal, extra-articular symptoms and signs) |
| The level of current symptoms, clinical findings, and prognostic indicators |
| The general clinical status (age, gender, comorbidity, concomitant medications, psychosocial factors) |
| **Disease Monitoring** |
| The disease monitoring of patients with AS should include: |
| Patient history (e.g., questionnaires) |
| Clinical parameters |
| Laboratory tests |
| Imaging |
| All according to the clinical presentation as well as the ASAS core set |
| The frequency of monitoring should be decided on an individual basis depending on: |
| Course of symptoms |
| Severity |
| Treatment |
| **Nonpharmacologic Treatment** |
| The cornerstone of nonpharmacologic treatment of patients with AS is patient education and regular exercise |
| Home exercises are effective. Physical therapy with supervised exercises, land or water based, individually or in a group, should be preferred as these are more effective than home exercises |
| Patient associations and self-help groups may be useful |
| **Extra-articular Manifestations and Comorbidities** |
| The frequently observed extra-articular manifestations (e.g., psoriasis, uveitis, and inflammatory bowel disease) should be managed in collaboration with the respective specialists |
| Rheumatologists should be aware of the increased risk of cardiovascular disease and osteoporosis |
| **Nonsteroidal Anti-inflammatory Drugs (NSAIDs)** |
| NSAIDs, including coxibs, are recommended as first-line drug treatment for AS patients with pain and stiffness |
| Continuous treatment with NSAIDs is preferred for patients with persistently active, symptomatic disease |
| Cardiovascular, gastrointestinal, and renal risks should be taken into account when prescribing NSAIDs |
| **Analgesics** |
| Analgesics, such as paracetamol and opioid (like) drugs, might be considered for residual pain after previously recommended treatments have failed, are contraindicated, and/or are poorly tolerated |
| **Glucocorticoids** |
| Corticosteroid injections directed to the local site of musculoskeletal inflammation may be considered |
| The use of systemic glucocorticoids for axial disease is not supported by evidence |
| **Disease-Modifying Anti-rheumatic Drugs (DMARDs)** |
| There is no evidence for the efficacy of DMARDs, including sulfasalazine and methotrexate, for the treatment of axial disease |
| Sulfasalazine may be considered in patients with peripheral arthritis |
| **Anti–Tumor Necrosis Factor (TNF) Therapy** |
| Anti-TNF therapy should be given to patients with persistently high disease activity despite conventional treatments, according to the ASAS recommendations |
| There is no evidence to support the obligatory use of DMARDs before or concomitant with anti-TNF therapy in patients with axial disease |
| There is no evidence to support a difference in efficacy of the various TNF inhibitors on the axial and articular/entheseal disease manifestations, but in the presence of inflammatory bowel disease, a difference in gastrointestinal efficacy needs to be taken into account |
| Switching to a second TNF blocker might be beneficial, especially in patients with loss of response |
| There is no evidence to support the use of biologic agents other than TNF inhibitors in AS |
| **Surgery** |
| Total hip arthroplasty should be considered in patients with refractory pain or disability and radiographic evidence of structural damage, independent of age |
| Spinal corrective osteotomy may be considered in patients with severe disabling deformity |
| In patients with AS and an acute vertebral fracture, a spinal surgeon should be consulted |
| **Changes in the Disease Course** |
| If a significant change in the course of the disease occurs, causes other than inflammation, such as a spinal fracture, should be considered, and appropriate evaluation, including imaging, should be performed |

These recommendations were developed before the availability of secukinumab. Secukinumab is approved but was not available at the time of these recommendations.

ASAS, Assessment in Ankylosing Spondylitis; EULAR, European League against Rheumatism.

From van der Heijde D, Sieper J, Maksymowych WP, et al: 2010 update of the international ASAS recommendations for the use of anti-TNF agents in patients with axial spondyloarthritis. *Ann Rheum Dis* 70:905–908, 2010.

**TABLE 75-13** SPARTAN Key Recommendations for the Treatment of Patients with Ankylosing Spondylitis or Nonradiographic Axial Spondyloarthritis

| |
|---|
| In adults with active AS, we strongly recommend treatment with NSAIDs over no treatment with NSAIDs. |
| In adults with active AS despite treatment with NSAIDs, we strongly recommend treatment with TNF-i over no treatment with TNF-i.* |
| In adults with active AS, we do not recomment any particular TNF-i as the preferred choice, except for patients with concomitant IBD or recurrent iritis. |
| In adults with active AS and IBD, we strongly recommend treatment with TNF-i monoclonal antibodies over treatment with etanercept. |
| In adults with active AS, we strongly recommend against treatment with systemic glucocorticoids. |
| In adults with active AS, we strongly recomment treatment with physical therapy over no treatment with physical therapy. |
| In adults with active AS and advanced hip arthritis, we strongly recomment treatment with total hip arthroplasty over no surgery. |
| In adults with active nonradiographic axial SpA despite treatment with NSAIDs, we conditionally recommend treatment with TNF-i over no treatment with TNF-i. |

*These recommendations were developed before the availability of anti-IL-17 antibodies. Anti-IL-17 antibodies are approved but were not available at the time of these recommendations.

AS, Ankylosing spondylitis; IBD, inflammatory bowel disease; NSAIDs, nonsteroidal anti-inflammatory drugs; TNF-i, TNF inhibitor; SpA, spondyloarthritis; SPARTAN, Spondylitis Association of America/Spondyloarthritis Research and Treatment Network.

From Ward MM, Deodhar A, Akl EA, et al: American College of Rheumatology/Spondylitis Association of America/Spondyloarthritis Research and Treatment Network 2015 recommendations for the treatment of ankylosing spondylitis and nonradiographic axial spondyloarthritis. *Arthritis Care Res (Hoboken)* 2015 Sep 24. [Epub ahead of print].

**TABLE 75-14** Cochrane Review of Physiotherapeutic Interventions and Spondyloarthritis Therapy for Patients with Ankylosing Spondylitis: Conclusions

| |
|---|
| Home exercise programs are better than no intervention |
| Supervised group physiotherapy is better than home exercise |
| Combined inpatient spondyloarthritis and exercise therapy followed by supervised outpatient weekly group physiotherapy is better than weekly group physiotherapy alone |

From Dagfinrud H, Kvien TK, Hagen KB: Physiotherapy interventions for ankylosing spondylitis. *Cochrane Database Syst Rev* (4):CD002822, 2004.

Moreover, the impact on physical functioning of physiotherapy in patients who respond well to biologics needs to be assessed.

Lying prone for 15 to 30 minutes once or several times a day is useful to reverse the tendency toward kyphosis, which is aggravated by pain and fatigue, as well as flexion contractures of the hip joints. Patients should sleep fully supine on a firm mattress with only a small neck-support pillow.

## Pharmacotherapy

### Nonsteroidal Anti-inflammatory Drugs

The efficacy and effectiveness of NSAID therapy for the alleviation of symptoms have been well established (level A evidence). When patients take them for prolonged periods of as long as a year, there may be improvement in spinal mobility and acute phase reactants.[162] Many NSAIDs are effective in patients with AS, and no NSAID has documented superiority in terms of efficacy. Selective cyclooxygenase-2 (COX-2) inhibitors have similar efficacy to conventional NSAIDs (level A evidence).[163] A nonselective NSAID, such as naproxen, is appropriate initial therapy for AS. A COX-2–selective agent (coxib) may be used in the presence of risk factors for peptic ulceration, although both categories of NSAIDs may exacerbate inflammatory bowel disease. Once-daily drug regimens may improve patient compliance. As long as 2 weeks may be required to demonstrate maximal symptomatic benefit from an NSAID. If symptomatic relief is inadequate, a switch to another NSAID is often worthwhile; failure of two NSAIDs should prompt an exploration of other management strategies. In early disease, 1000 mg of naproxen daily was sufficient to put 35% of patients with imaging-positive axial SpA into partial remission, and 57% of patients had an ASAS-40 response.[164] This trial compared naproxen with infliximab, so the placebo rates are unknown.

Given the gastrointestinal and cardiovascular risks of taking NSAIDs or coxibs, one must consider whether this treatment should be on a daily or an "on-demand" basis. A 2-year randomized, prospective, controlled trial in patients with AS compared the efficacy of continuous NSAID therapy with that of intermittent on-demand use. The results suggest that continuous therapy retards radiographic disease progression.[60] A reanalysis of this trial showed that patients with an elevated CRP benefited most in terms of reducing radiographic progression.[165] This study's results agree with an older retrospective study that also suggested a possible disease-controlling effect of continuous therapy.[166] Another observational study from a longitudinal cohort has also found that NSAIDs retard radiographic progression of disease.[167] These findings require confirmation in further randomized trials that eliminate the biases that can be present in observational studies. One randomized multicenter trial showed that continuous treatment with diclofenac over 2 years did not reduce radiographic progression compared with on demand treatment in AS.[154b]

Whether patients should take NSAIDs primarily for retardation of radiographic progression, whether or not they are on a TNF inhibitor, is not definitively known at present. However, based on these data, patients at high risk of radiographic progression (e.g., those with pre-existing syndesmophytes and/or raised CRP) could take continuous NSAIDs to try to reduce their progression rate. This is an individual decision and one that should take into account the risk of radiographic progression, the strength of evidence we have for the effect of NSAIDs on modifying this risk, and the risk of gastrointestinal and cardiovascular complications that NSAID contribute.

### Second-Line Drugs

Borrowing well-established concepts in the treatment of RA, disease-controlling therapy for AS has been defined as an agent that decreases inflammatory manifestations of disease, sustains or improves function, and prevents or decreases the rate of progression of structural damage. Although most of the second-line agents developed

primarily for RA have been studied in patients with AS, none can be considered disease controlling in AS. Most data are available for sulfasalazine, which was first proposed as a therapy for AS in 1984, based on the common association between inflammatory bowel disease and SpA, the description of inflammatory lesions in the ileum of patients with SpA, and its success in the treatment of intestinal inflammation.[168] A total of 11 double-blind, placebo-controlled trials have been published, as well as two meta-analyses. The results of the two largest trials were consistent in demonstrating no significant benefit for sulfasalazine in AS, although subgroup analysis showed that patients with (peripheral) polyarthritis—mostly those with psoriatic arthritis, but also AS patients with peripheral joint involvement—had a significant but modest response.[169,170] An important limitation in most of these studies was the long disease duration (>10 years) of recruited patients, and it has been suggested that early disease may be more responsive to therapy. However, a 24-week placebo-controlled trial that recruited 230 patients who met the European Spondyloarthropathy Study Group (ESSG) criteria for SpA and who had symptom duration of less than 5 years confirmed that sulfasalazine was ineffective.[171] The most recent meta-analysis, based on 11 trials, concluded that this agent has a significant impact only on the ESR and the severity of spinal stiffness (level A evidence).[172] The primary indication for the use of sulfasalazine in routine practice is a patient who has concomitant peripheral arthritis and has had an inadequate response to NSAIDs and physical modalities.

The evaluation of MTX in AS has been limited to case reports and open analyses. These studies have included limited numbers of patients for periods of 6 months to 3 years, at doses from 7.5 to 15 mg weekly. The results have been mixed, with some benefit noted in patients with concomitant peripheral arthritis. Two small placebo-controlled trials assessed MTX in doses of 10 and 7.5 mg weekly for 24 weeks, with contradictory findings.[173,174] A meta-analysis concluded that there was no evidence of efficacy and that higher-quality trials, larger sample sizes, longer durations of treatment, and higher dosages of MTX were necessary before any definitive conclusions could be drawn (level B evidence).[175] Corticosteroids may be effective for local intra-articular treatment in AS, including the SI joints, if given under fluoroscopic guidance (level B evidence). High-dose intravenous pulse corticosteroids have been studied in trials and found to have effect in patients with AS who have flared.[176,177] This therapy was used primarily in treatment-resistant patients before the availability of TNF inhibitors. Systemic oral steroids for AS treatment have been assessed in a 2-week placebo-controlled trial of daily 50 mg prednisolone, 20 mg prednisolone, or placebo. The study found no different in primary endpoint (50% improvement in BASDAI) in either treatment group, but a difference was noted in secondary endpoints (mean BASDAI, BASMI, ASDAS). The mean decrease in BASDAI in the 50-mg prednisolone group was higher compared with placebo (2.39 vs. 0.66, respectively).[178] Leflunomide has been studied in AS, and although an open-label study suggested a benefit in patients with peripheral arthritis, a small placebo-controlled study reported no benefit (level B evidence).[179,180] A controlled dose-response (60 mg vs. 10 mg) evaluation of a bisphosphonate vs. pamidronate, given intravenously on a monthly basis for 6 months, showed evidence of symptomatic efficacy, primarily in patients with only axial disease (level B evidence).[181] The bisphosphonate neridronate was compared with infliximab in a 6-month, open-label uncontrolled study, and no difference was found between these two treatments in their primary efficacy measure: BASDAI reduction. Both agents showed clinically significant improvements compared with baseline.[182]

Thalidomide has been used in two open-label studies in AS because of its known anti-TNF effect, through enhanced degradation of the TNF messenger ribonucleic acid. In a Chinese study, improvement was reported in 80% of patients, with deterioration 3 months after treatment discontinuation. Frequent side effects are drowsiness, constipation, and dizziness (level B evidence). Particular caution is required because of other major thalidomide-associated side effects, such as teratogenicity and peripheral neuropathy.[183]

Considering just nr-axSpA, there is a paucity of evidence regarding the effect of traditional oral DMARDs, but their effect is likely to be similar or better to established AS. As with other agents in rheumatology, early institution of therapy may increase response rates, but this fact is not currently known. Better reporting of separate patient groups (AS and nr-axSpA) in trials will yield more conclusive results.

## Biologic Therapies

TNF inhibitors have revolutionized the treatment of AS. Their introduction was based on the finding of TNF expression in SI joint biopsies of AS patients, the observation that overexpression of TNF leads to sacroiliitis in animal models, and earlier clinical trial data demonstrating the efficacy of one TNF inhibitor, infliximab, in Crohn's disease. Five TNF inhibitors are of proven benefit in AS according to pivotal phase III trials (level A evidence): infliximab, etanercept, adalimumab, certolizumab, and golimumab. Infliximab is an $IgG_1$ chimeric monoclonal antibody, with the Fab portion derived from the mouse. It is given in a dose of 5 mg/kg every 6 to 8 weeks after loading at 0, 2, and 6 weeks. Etanercept is a recombinant TNF receptor $IgG_1$ fusion protein that is self-administered by subcutaneous injection either once (50 mg) or twice (25 mg) weekly. Adalimumab and golimumab are human monoclonal antibodies that are self-administered by subcutaneous injection on alternate weeks (40 mg) or monthly (50 mg), respectively. Certolizumab is a pegylated Fab fragment of a humanized TNF inhibitor monoclonal antibody that is given as a subcutaneous injection either as a 200-mg fortnightly or a 400-mg monthly dose. None of these agents require concomitant therapy with MTX.

In phase III trials in patients with AS all of these agents demonstrated ASAS-20 response rates of 55% to 60% and ASAS-40 response rates of 45% to 50%.[184-188] Even higher response rates have been observed in patients with nr-axSpA and short disease duration who received adalimumab or infliximab in placebo-controlled or active comparator trials.[164,189,190] Partial remission rates of 62% at week 28 were seen in a trial of imaging positive axSpA comparing infliximab with 1000 mg of naproxen in those with disease duration of less than 2 years. This is superior to 22% partial remission rate at week 24 in the trial of infliximab in

established AS.[164,184] Response rates in nr-axSpA are also improved in those with an elevated CRP or an MRI scan showing inflammatory lesions.[191]

Improvement in symptoms is evident by 2 to 4 weeks and is sustained as long as the patient remains on treatment. Virtually all patients with AS relapse by 6 months after discontinuation of treatment.[192] However, withdrawal of therapy in nr-axSpA patients treated early results in a substantial minority (30% to 40%) maintaining remission or partial remission at or beyond 6 months.[193,194] A trial of imaging-positive nr-axSpA patients who had symptoms for less than 2 years and were treated with infliximab for 6 months, 87% to 94% had low disease activity, and 40% to 48% maintained partial remission 6 months after withdrawal of infliximab therapy.[193]

Significant improvement is also observed in function, spinal mobility, peripheral synovitis, enthesitis score, and quality of life with TNF inhibitors. Sick leave and work disability are reduced. Only two patients with AS need to be treated for one to experience at least 50% improvement in disease activity (95% CI, 1 to 6). Objective parameters of disease activity that show improvement include acute phase reactants, synovial histopathology, and MRI features of inflammation in the spine and SI joints.[195] Response to treatment in patients with established AS appears to be increased in those with high disease activity and worse in those with long disease duration, impaired function, and no discernible evidence of inflammation on MRI.[196] Adverse events in patients with AS are no different from those reported in patients with RA, and infusion reactions in patients receiving infliximab have been no more frequent than in patients with RA on concomitant MTX. All TNF inhibitors are effective for psoriasis, and the monoclonal anti-TNF antibodies, infliximab and adalimumab, also have demonstrated efficacy in both uveitis and colitis. Patients who do not respond to one TNF inhibitor may respond to an alternative TNF inhibitor.

Some observational data suggests TNF inhibitors may reduce radiographic progression during a long time period (8 to 10 years).[58,197] The evidence supporting this effect is still preliminary, and based on observational data that is prone to bias. Therefore this requires additional validation.

Recommendations for the use of anti-TNF therapies in axSpA have been developed (Table 75-15).[154a,173,198] These new therapeutic modalities identify important clinical questions to be answered by further research. All of the anti-TNF agents examined to date have symptom-modifying properties, but their long-term disease-controlling effects in terms of preventing structural damage have yet to be definitively demonstrated.

No other biologic therapies are approved for AS or axSpA. Trials of rituximab in AS have shown some efficacy in patients who are TNF inhibitor naïve, but had poor results in TNF inhibitor failure patients. Trials of abatacept (CTLA4-Ig), tocilizumab (anti–IL-6R), sarilumab (anti–IL-6R) and anakinra (anti–IL-1) have failed to show efficacy in AS or axSpA.

The genetic association between AS and the IL-23R gene and the proven functional role that IL-23R genetic variants have on immune system function suggests that agents targeting IL-23 and the downstream cytokine IL-17 hold great promise.

Secukinumab has now been approved for use in AS by the FDA; abstract data reports ASAS20 response rates at week 16 of 61.1% with secukinumab 150 mg versus 28.4% with placebo treatment. The approved dosage includes both a regimen with a loading dose and one without, with 150 mg every four weeks being the maintenance dose. ASAS40 response in the same trial was 36.1% for secukinumab versus 10.8% for placebo. Because of the recent nature of the approval, the place of secukinumab in treatment algorithms is yet to be defined. A phase II trial of ustekinumab (anti–IL-12/23p40) has shown efficacy, and definitive phase III trial data is awaited.

## Anterior Uveitis

TNF inhibitors have been shown to reduce the occurrence of flares of acute anterior uveitis in AS patients.[199,200] The rate of flares was reduced by 51% in all patients and 68% in patients with recent uveitis in a prospective trial of adalimumab.[200] Combined data from 4 trials of infliximab and etanercept showed a reduction from 15.6 flares per 100 patient-years in the control groups to 6.8 fares per 100 patient-years in the TNF inhibitor–treated groups.[199] Other trials have found broadly similar rates of reduction in flares with TNF inhibitors.[201,202]

Retrospective studies suggested that the fusion protein etanercept did not have the same positive effect on uveitis reduction as the monoclonal antibodies, and perhaps even caused an increase in uveitis flares.[203-205] An analysis of eight etanercept trials showed that the rate of uveitis on etanercept was less than placebo (8.6 and 19.3 per 100 patient-years, respectively). In active comparator trials against sulphasalazine, the rate of uveitis on etanercept was numerically lower (10.7 per 100 patient-years) but not statistically significantly different to the flare rate on sulphasalazine (14.7 per 100 patient-years).[202] Based on retrospective analyses, etanercept probably has a reduced level of efficacy on uveitis compared with the monoclonal antibodies, but head-to-head studies have not been conducted to determine this definitively and if so the magnitude of difference.[203,206] Flares of uveitis happen on all of the TNF inhibitors, but if they are recurrent or persistent, then a change of TNF inhibitor may be warranted.

There is some evidence that oral anti-rheumatic agents are effective at decreasing the rate of uveitis in SpA patients. In a small open trial of MTX that assessed 9 patients, the mean recurrence rate of uveitis in the year before MTX use was 3.4, and this dropped to 0.9 during the following year.[207] In this study, most patients were on 7.5 mg of MTX per week. Two studies have shown that sulphasalazine also reduces the flare rate of uveitis. In a 3-year randomized controlled study, the reduction in mean attacks per patient per year was 0.2 to 0.8.[208] An observational study of flare rate presulphasalazine and postsulphasalazine treatment showed a reduction from 3.4 flares per year to 0.9 flares per year.[209] There is also some suggestive data that NSAIDs reduce flares of uveitis.[210]

## Surgery

Involvement of the hip joint may cause serious disability. After 30 years of disease duration, 12% to 25% of patients

**TABLE 75-15** 2010 Update of Recommendations for the Use of Anti–Tumor Necrosis Factor Agents in Patients with Axial Spondyloarthritis

| RECOMMENDATION | |
|---|---|
| **Patient Selection** | |
| Diagnosis | Patients fulfilling modified New York Criteria for definitive AS* or the ASAS criteria for axial SpA |
| Active disease | Active disease for ≥4 wk<br>BASDAI ≥ 4 (0-10)† and a positive expert opinion‡ |
| Treatment failure | All patients should have had adequate therapeutic trials of at least two NSAIDs. An adequate therapeutic trial is defined as at least two NSAIDs over a 4-wk period in total at maximum recommended or tolerated anti-inflammatory dose unless contraindicated<br>Patients with predominantly axial manifestations do not have to take DMARDs before anti-TNF treatment can be started<br>Patients with symptomatic peripheral arthritis should have an insufficient response to at least one local steroid injection if appropriate and should normally have had an adequate therapeutic trial of a DMARD, preferably sulfasalazine<br>Patients with symptomatic enthesitis must not have responded to appropriate local treatment |
| **Assessment of Disease** | |
| *ASAS Core Set of Daily Practice* | |
| Physical function (BASFI or Dougados Functional Index)<br>Pain (VAS for spine at night from AS in the past week and VAS for spine from AS in the past week)<br>Spinal mobility (chest expansion, modified Schober's test and occiput-to-wall distance, and lateral lumbar flexion)<br>Patient's global assessment (VAS for the past week)<br>Stiffness (duration of morning spine stiffness in the past week)<br>Peripheral joints and entheses (number of swollen joints [44 total], enthesitis score such as developed in Maastricht, Berlin, or San Francisco)<br>Acute phase reactants (erythrocyte sedimentation rate or C-reactive protein)<br>Fatigue (VAS) | |
| *BASDAI* | |
| VAS for overall level of fatigue or tiredness in the past week<br>VAS for overall level of AS neck, back, or hip pain in the past week<br>VAS for overall level of pain or swelling in joints other than neck, back, or hips in the past week<br>VAS for overall discomfort from any areas tender to touch or pressure in the past week<br>VAS for overall level of morning stiffness from time of awakening in the past week<br>Duration and intensity (VAS) of morning stiffness from time of awakening (up to 120 min) | |
| **Assessment of Response** | |
| Responder criteria | BASDAI: 50% relative change or absolute change of 2 (on 0-10 scale) *and* expert opinion in favor of continuation |
| Time of evaluation | After at least 12 wk |

These recommendations were developed before the availability of secukinumab. Secukinumab is approved but was not available at the time of these recommendations.

*Modified New York criteria (van der Linden et al, 1984): radiologic criterion (sacroiliitis, grade ≥2 bilaterally or grade 3 to 4 unilaterally) and at least two out of three clinical criteria (low back pain and stiffness for more than 3 mo that improves with exercise but is not relieved by rest; limitation of motion of the lumbar spine in both the sagittal and frontal planes; limitation of chest expansion relative to normal values correlated for age and sex).

†BASDAI assessed on a 0-10 VAS or NRS.

‡The expert is a doctor, usually a rheumatologist, with expertise in inflammatory back pain and the use of biologic agents. Experts should be locally defined. An expert opinion should consider clinical features (history and examination) as well as either serum acute phase reactant levels or imaging results, such as radiographs demonstrating rapid progression or MRI scans indicting inflammation.

ASAS core set for daily practice: physical function (BASFI); pain (VAS/NRS, last week; spine at night, caused by AS and VAS/NRS, last week; spine caused by AS); spinal mobility (chest expansion, cervical rotation, occiput-to-wall distance, modified Schober's test, and lateral lumbar flexion or BASMI); patient's global assessment (VAS/NRS, last week); stiffness (duration of morning stiffness, spine, VAS/NRS, last week); peripheral joints and entheses (number of swollen joints [44-joints count], enthesitis score such as developed in Maastricht, Berlin, or San Francisco); acute phase reactants (preferably C-reactive protein); fatigue (VAS/NRS).

AS, Ankylosing spondylitis; ASAS, Assessment of SpondyloArthritis (International Society); BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; DMARD, disease-modifying anti-rheumatic drug; NRS, numeric rate scale; NSAIDs, nonsteroidal anti-inflammatory drugs; SpA, axial spondyloarthritis; TNF, tumor necrosis factor; VAS, visual analogue scale.

From van der Heijde D, Sieper J, Maksymowych WP, et al: 2010 update of the international ASAS recommendations for the use of anti-TNF agents in patients with axial spondyloarthritis. *Ann Rheum Dis* 70:905–908, 2010.

have at least one hip replaced.[211] Ectopic bone formation may occur, with the rate of heterotopic ossification reported to be 30% in one study.[212,213] One large study of cemented implants with more than 10 years of follow-up showed 96% had low pain scores, and implant survival was excellent.[214,215] Cemented implants generally have a longer life than non-cemented implants.

Vertebral osteotomy may be required in selected cases to correct marked flexion deformity when forward vision is severely impaired. Diaphragmatic herniation may result from the procedure.

In patients with AS, careful consideration of neck involvement is required pre-operatively, including imaging. General anaesthesia needs to be planned well because it requires intubation that involves neck manipulation. These movements may increase the risk of fracture through syndesmophytes or osteoporotic vertebrae. If there is extensive cervical spine disease, special positioning may be required because of kyphosis. Respiratory assessment is also important in established disease because lung function may be adversely affected.

## Osteoporosis

Screening for osteoporosis should occur in those with longer disease duration (>10 years), especially if they have active

disease. Dual energy x-ray (DEXA) scans are appropriate screening tool, but care must be exercised in assessing the lumbar spine measurements because syndesmophytes can artifactually increase bone mass density (BMD) readings. Lateral and volumetric DEXA images may improve the usefulness of lumbar DEXA for assessment.[216] Once diagnosed with osteoporosis, secondary causes or exacerbating factors should be screened for, including hyperthyroidism, hypogonadism, and vitamin D deficiency.

Currently, it is unclear whether any specific antiosteoporotic to prevent spinal fractures therapy, such as bisphosphonates or denosumab, is effective.[76] Reducing disease activity of AS might be more promising in controlling osteoporosis of the vertebral spine. In a 3-year study of 111 patients with AS, treatment with TNF inhibitors has been shown to increase BMD of the spine and hip.[217]

## CONCLUSION

Better understanding of AS genetics has very much improved our knowledge about the pathogenesis of the disease. The spectrum of AS is wider than AS by modified New York criteria. Nonradiologic AS is, however, more heterogeneous than radiologic AS. Inappropriate use of axSpA classification criteria for diagnostic purposes increases the risk of false-positive labeling of individual patients as nonradiologic AS or axSpA. NSAIDs, physiotherapy, and patient education remain first-line therapy. It is currently unclear whether early suppression of inflammation with biologic treatment decreases the risk for new bone formation of the axial skeleton.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 76



# Undifferentiated Spondyloarthritis

John D. Carter • Alan P. Hudson

**KEY POINTS**

- **Undifferentiated spondyloarthritis can have both peripheral and axial features.**
- **Undifferentiated spondyloarthritis is a provisional diagnosis—many cases will evolve into other forms of spondyloarthritis.**
- **Treatment of undifferentiated spondyloarthritis depends on whether axial or peripheral disease is predominant.**

In 1978, a group of inflammatory arthritides that were rheumatoid factor–negative and shared clinical and radiographic features were recognized as a unified entity.[1] These originally were termed *seronegative polyarthritis* but are now known as the *spondyloarthritides* (SpA). Although the SpAs share overlapping features, enough phenotypic heterogeneity exists to permit subclassification. Currently recognized subtypes include ankylosing spondylitis (AS), psoriatic arthritis (PsA), inflammatory bowel disease–related arthritis, reactive arthritis (ReA), and undifferentiated SpA. The term *undifferentiated spondyloarthritis* (uSpA) is used to designate patients with clinical and radiographic features consistent with the SpAs but who do not fulfill the criteria for any of the established disease categories. More recently it has been suggested that these subtypes represent different presentations of one disorder, rather than related but distinct entities.[2] Whether or not these are distinct subtypes, or whether they truly represent one disease process, may have important implications for clinical practice.

Several classification criteria now exist for the SpAs. In 1991, the European Spondyloarthropathy Study Group (ESSG) criteria were developed to establish criteria for the SpAs[3] (Figure 76-1). These criteria have been demonstrated to be 87% sensitive and specific for the diagnosis of SpA. They are, however, somewhat less well suited for diagnosis of early disease but extremely useful for established disease. In 1995, the Amor criteria for SpA were developed[4] (Table 76-1). These are similar to the ESSG criteria but include the human leukocyte antigen (HLA)-B27 antigen. Other classification criteria that have been proposed include the Calin and Berlin criteria[5,6] (Tables 76-1, 76-2 and 76-3). More recently, the Association of SpondyloArthritis International Society (ASAS) developed classification criteria[7] (Figure 76-2). These divide the SpAs into axial or peripheral disease, and more emphasis was placed on the clinical presenting features along with advanced imaging, specifically MRI, in an attempt to classify patients earlier. These classification criteria sets are primarily intended to facilitate research, allowing for rather homogeneous study populations, but they can be used as a guide in clinical practice.

## EPIDEMIOLOGY

Because of the nature of the diagnosis, data regarding the incidence and prevalence of uSpA are limited and variable. The general prevalence of SpA in Caucasian populations is estimated to be 0.5% to 2.0%.[8] However, recent data from the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) program suggests a higher prevalence. In this survey, the frequency of chronic back pain has risen to 19.4%. with nearly one-third of this number having inflammatory back pain; of these, 1% to 1.4% of the adult U.S. population may have axial spondyloarthritis.[9] These same survey data suggest that the prevalence of inflammatory back pain does not differ significantly among age groups or between genders; non-Hispanic white individuals are significantly more likely to have it compared with non-Hispanic blacks.[10] Interestingly, these same data demonstrate a decreasing frequency of HLA-B27 antigen with increasing age, suggesting a negative impact on long-term survival. The true prevalence of uSpA is difficult to assess quantitatively in these or other groups because of a lack of specific diagnostic criteria, and the fact that it is often unrecognized. This might be particularly true of uSpA patients who are seen primarily with peripheral symptoms.

In spite of these limitations, a few studies have attempted to define the prevalence of uSpA. A large study in Germany demonstrated the overall prevalence of SpA at 1.9%, with AS the most common type (0.86%), followed by uSpA (0.67%).[11] A prospective cohort study that followed 175 patients in a dedicated outpatient SpA clinic classified 23% of patients with uSpA.[12] Another report suggested that uSpA is more than twice as common as AS: 40% of study patients included had uSpA.[13] uSpA was demonstrated in 16.1% of patients with SpA in a study in Spain,[14] 17.8% in Sweden,[15] and 28.2% of early-stage SpA multiracial patients.[16] uSpA also has been associated with HIV infection, with a prevalence of 2.2% to 11.1%.[17] As described previously, uSpA may be the most underdiagnosed subtype of SpA. Another study in Spain evaluated 514 patients with anterior uveitis and found that 53% of these patients were not diagnosed with SpA until after their first episode of uveitis. Of patients who were undiagnosed, 91% had uSpA.[18]

The disease in many patients with uSpA will progress to one of the better defined subtypes if followed up with time.

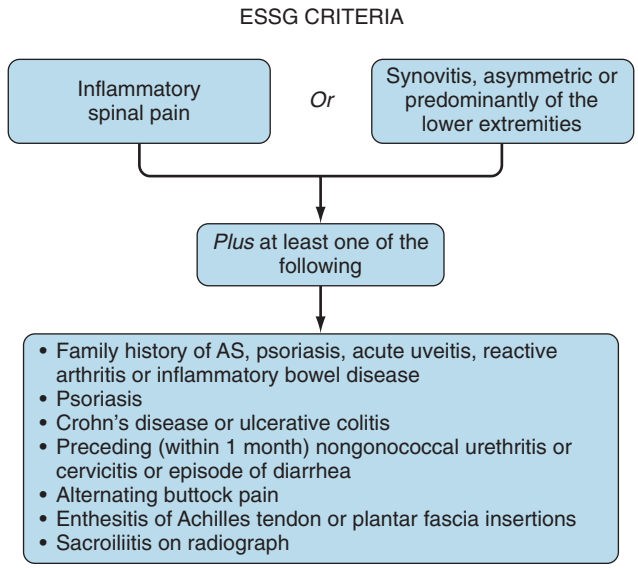



**Figure 76-1** According to the European Spondyloarthropathy Study Group (ESSG) criteria, for a patient to be classified as having spondyloarthritis, he or she has to satisfy one of two entry criteria: inflammatory spinal pain or synovitis (typically asymmetric synovitis, predominantly of the lower limbs). If one or both are present, the patient must have at least one of the features in the bottom box to fulfill the classification criteria. Inflammatory back pain: Back pain is considered inflammatory if four of the following five criteria are found: (1) onset of back discomfort before the age of 40 years, (2) insidious onset, (3) persistence for at least 3 months, (4) association with morning stiffness, and (5) improvement with exercise. AS, Ankylosing spondylitis; ReA, reactive arthritis. *(From Dougados M, van der Linden S, Juhlin R, et al: The European spondyloarthropathy study group preliminary criteria for the classification of spondyloarthropathy.* Arthritis Rheum *34:1218–1227, 1991; and Gomariz EM, del M, Guijo VP, et al: The potential of ESSG spondyloarthropathy classification criteria as a diagnostic aid in rheumatological practice.* J Rheumatol *29:326–330, 2002.)*

In a Brazilian cohort of patients with uSpA and a predominance of female patients, peripheral symptoms were the most prevalent at disease onset, followed by a high frequency of axial manifestations during an 8-year follow-up period.[19] In this same cohort after 2.5 years of follow-up, 16.7% were classified with AS and 2.7% with PsA. A similar study with a predominance of males demonstrated similar progression rates of 24.3% and 2.7% to AS and PsA, respectively.[20] HLA-B27 positivity and buttock pain are significant predictors of progression. For patients with uSpA that transitions to AS, radiographic sacroiliitis can take years to develop[21]; however, the disease burden is similar with and without radiographic sacroiliitis.[22,23]

Although the disease in patients with uSpA can progress, some also experience spontaneous resolution of symptoms. In the same study referenced previously demonstrating that uSpA can progress to AS or PsA, 22.5% of uSpA patients also experienced remission.[20] This has been demonstrated in other studies as well. The fact that uSpA can spontaneously remit draws parallels with ReA, suggesting that some patients with uSpA might in fact have ReA with an asymptomatic or unrecognized triggering infection. Another analysis of SpA patients suggests that disease onset in individuals 40 years of age or younger are seen with predominance of axial symptoms, whereas those with later disease onset favor peripheral manifestations.[24]

**TABLE 76-1** Amor Criteria

| Clinical Symptoms or History | Points |
|---|---|
| Lumbar or dorsal pain during the night, or morning stiffness of lumbar or dorsal spine | 1 |
| Asymmetric oligoarthritis | 2 |
| Buttock pain<br>If affecting alternately the right or the left buttock | 1<br>2 |
| Sausage-like toe or digit (dactylitis)* | 2 |
| Heel pain or any other well-defined enthesopathy (enthesitis)* | 2 |
| Iritis | 2 |
| Nongonococcal urethritis or cervicitis accompanying, or within 1 month before, the onset of arthritis | 1 |
| Acute diarrhea accompanying, or within 1 month before, the onset of arthritis | 1 |
| Presence or history of psoriasis, balanitis, or inflammatory bowel disease (ulcerative colitis or Crohn's disease) | 2 |
| **Radiologic Finding**<br>Sacroiliitis (grade >2 if bilateral; grade >3 if unilateral) | 3 |
| **Genetic Background**<br>Presence of HLA-B27, or familial history of ankylosing spondylitis, Reiter syndrome, uveitis, psoriasis, or chronic enterocolopathies | 2 |
| **Response to Treatment**<br>Good response to NSAIDs in less than 48 hr, or relapse of the pain in less than 48 hr if NSAIDs discontinued | 2 |

*A patient is considered to have spondyloarthritis if the sum of the point counts is 6 or more. A total point count of 5 or more classifies for probable spondyloarthritis.

**TABLE 76-2** Calin Criteria for Inflammatory Back Pain*

| |
|---|
| Age at onset <40 years |
| Duration of pain >3 months |
| Insidious onset |
| Morning stiffness |
| Improvement with exercise |

*Requires the presence of 4/5 criteria

**TABLE 76-3** Berlin Criteria for Inflammatory Back Pain*

| |
|---|
| Morning stiffness of >30 minutes duration |
| Improvement in back pain with exercise but not rest |
| Nocturnal awakening (second half of the night only) |
| Alternating buttock pain |

*Sensitivity of 70% and specificity of 81% if two of the four criteria are fulfilled.

## GENETICS, PATHOGENESIS, AND ETIOLOGY

Because uSpA is poorly described, data regarding its etiology and pathogenesis are extremely limited. Although HLA-B27 is clearly associated with the risk of SpA, it is not the only contributory factor. In addition to HLA-B27, other genetic factors, including HLA-Cw*0602, HLA-B38,

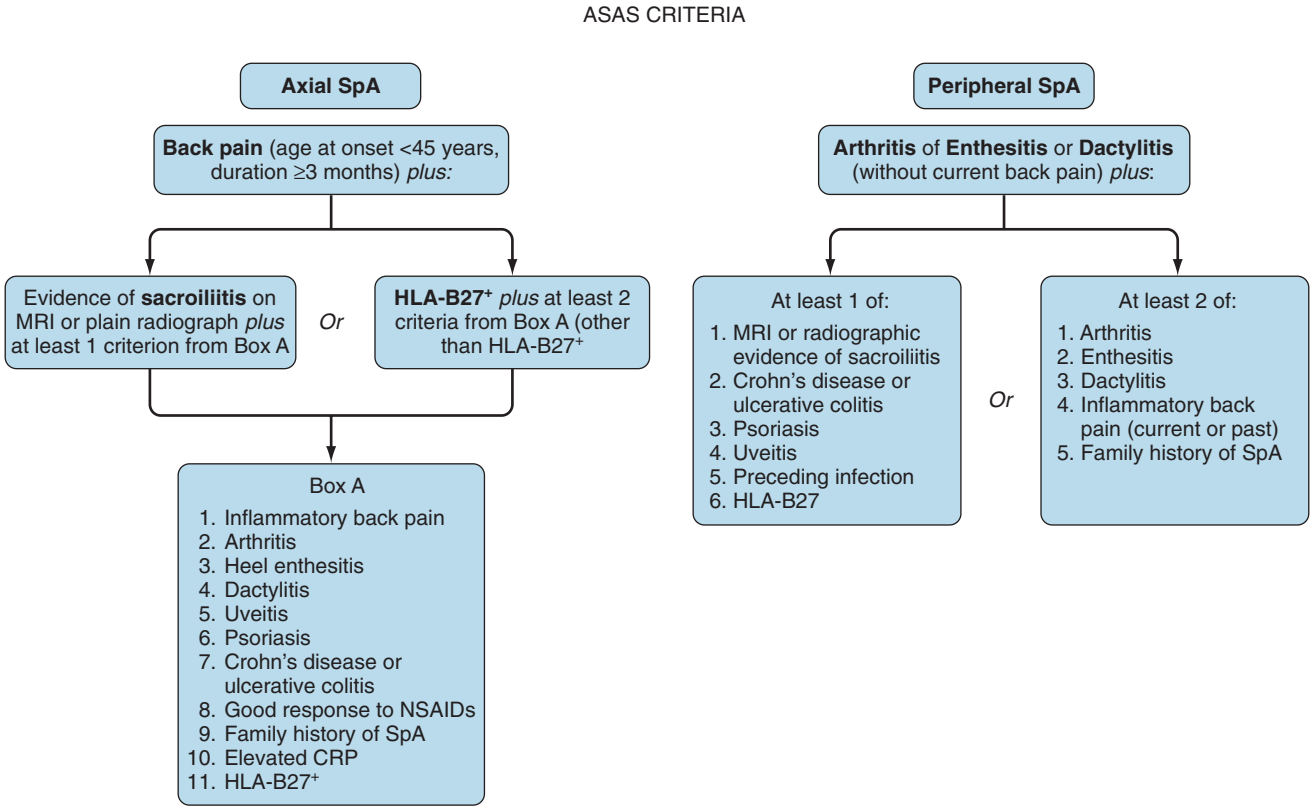



**Figure 76-2** The Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axial and peripheral spondyloarthritis (SpA). To fulfill the criterion of sacroiliitis, the patient must have active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA or definite radiographic sacroiliitis according to the modified New York criteria. CRP, C-reactive protein; HLA, human leukocyte antigen; MRI, magnetic resonance imaging; NSAIDs, nonsteroidal anti-inflammatory drugs. *(From Zeidler H, Amor B: The Assessment in Spondyloarthritis International Society [ASAS] classification criteria for peripheral arthritis and for spondyloarthritis in general: the spondyloarthritis concept in progress.* Ann Rheum Dis *70:1–3, 2011; Rudwaleit M, Landewé R, van der Heijde D, et al: The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis [part I]: classification of paper patients by expert opinion including uncertainty appraisal.* Ann Rheum Dis *68:770–776, 2009; and Rudwaleit M, van der Heijde D, Landewé R, et al: The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis [part II]: validation and final selection.* Ann Rheum Dis *68:777–783, 2009.)*

HLA-B39, HLA-B60, HLA-DRB1, as well as those outside the major histocompatibility complex (e.g., ERAP-1 and IL-23R) have been associated with the SpAs in general. Because phenotypic congruity exists between SpA and uSpA, these genes likely play a role in uSpA as well. Approximately 50% to 70% of patients with uSpA are HLA-B27+.[25] The HLA-B*5703 allele was shown to be significantly increased in HIV+ individuals with uSpA in a Zambian population.[26] HLA-B60, HLA-C3, HLA-DR12, CXCR4, IL-1β, IL-8, and integrin-1 all also have been directly linked with uSpA.[27,28] Gene expression profiles in peripheral blood mononuclear cells from patients with AS and uSpA identified RGS1 as a candidate biomarker, especially for uSpA.[29]

Studies have been performed to investigate a potential bacterial trigger for uSpA. These studies have concentrated on the known triggers of ReA, and the resulting data have suggested potential causative links with chlamydiae and salmonellae.[30,31] However, DNA from a wide spectrum of bacterial species, including those known to be involved in ReA and others not previously associated with ReA, also have been detected in patients with uSpA. The pathogenic significance of these findings remains unclear.

## CLINICAL FEATURES

The clinical picture of uSpA is congruent with those of other forms of SpA. Most types of SpA begin around the ages of 15 to 45 years, including uSpA. However, like other types of SpA, uSpA can begin in childhood or later in life as well. Most individuals with uSpA lead long and productive lives, but certain complications can lead to disability. By the very definition of the condition, uSpA encompasses a host of symptoms that are shared in the SpA family without any unique or identifying characteristics. Although the various classification criteria are meant to facilitate clinical research and are not intended for clinical diagnosis, they are useful as a group to identify the various potential phenotypic features of uSpA. Channeling bias might lead to a diagnosis of uSpA in those who are seen with axial radiographic features versus those with only peripheral manifestations.

The clinical presentation of uSpA is dominated by inflammation that can affect the axial skeleton (spine and sacroiliac [SI] joints), peripheral joints (especially the large joints of the lower extremities), enthesitis, dactylitis, and extra-articular sites. Extra-articular manifestations are

heterogeneous and shared among all members of the SpA group; exceptions include mucocutaneous lesions in PsA and ReA, genitourinary manifestations in ReA, and the gastrointestinal manifestations in inflammatory bowel disease (IBD) and, possibly, AS. However, ocular and cardiac manifestations can be present in uSpA. Of uSpA patients, 20% to 30% experience mild intermittent abdominal symptoms or short episodes of diarrhea. Gut inflammation is mainly subclinical and can only be demonstrated by ileocolonoscopy.[32] In addition to genetic background and the possible role of environmental triggers, mechanical stress also appears to play a role in the articular features of uSpA. The male to female ratio of uSpA has been estimated to be 3:2.[25]

Recent data suggest that patients with uSpA are younger and tend to have shorter disease duration than those with AS or PsA.[33] These patients typically are seen with a mixed axial and peripheral arthritis, with the former dominating, and nearly half the patients have radiographic sacroiliitis.[33] Their overall disease activity tends to be similar to the other subtypes of SpA. Bone loss in the hip has been reported in patients with early uSpA and is related to disease activity.[34]

## LABORATORY, RADIOGRAPHIC, AND ADVANCED IMAGING FINDINGS

No specific laboratory findings have been described in patients with uSpA. Patients can be seen with elevated or normal acute phase reactants such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR). Those that do have elevated acute phase reactants, however, tend to have poorer outcomes. The plain radiographic features of uSpA are similar to those of the other subtypes of SpA without unique features. Although radiographic sacroiliitis of AS- and IBD-related SpA tend to be bilateral and symmetric, the radiographic features in uSpA tend to be asymmetric and/or unilateral, as might be seen in PsA or ReA. Because many patients with uSpA have a short disease duration and can progress to a classic subtype of SpA (such as AS), recent efforts have been made to identify patients with "nonradiographic" axial spondyloarthritis, including uSpA. MRI of the SI joints can demonstrate synovitis and bone marrow edema (osteitis). These lesions can be detected months to years before the plain radiographic appearance of sacroiliitis. However, it should be noted that these MRI findings are highly suggestive but not unique to uSpA or the SpAs in general.

## DIAGNOSIS

No specific diagnostic test for uSpA currently exists. Patients are typically described as seronegative (i.e., absence of rheumatoid factor). However, uSpA and SpA in general are often quite easily distinguished from rheumatoid arthritis by the clinical presentation. Other helpful but nonspecific findings can include anemia of chronic disease and inflammatory synovial fluid (>2000 white blood cells/mL, with a predominance of neutrophils). As described previously, HLA-B27 testing is of limited value.

The newer ASAS classification criteria might be the most useful. The ASAS classification criteria for axial SpA are defined as follows: the presence of sacroiliitis by radiography or MRI, plus at least one SpA feature, or the presence of HLA-B27 plus at least two clinical features. The specificity of these criteria was higher than that of the ESSG modified for MRI and modified Amor criteria.[35] There are ASAS classification criteria for patients with peripheral manifestations only as well (see Figure 76-2).

## TREATMENT

Until recently, treatment options for patients with SpA have been limited. NSAIDs have been used as first-line drugs because they have been shown to help the symptoms of patients with SpA, especially when they are suffering from axial symptoms. TNF inhibitors have been studied extensively in patients with AS and PsA in recent years and have demonstrated excellent efficacy at treating the clinical symptoms of both conditions. These same medications have demonstrated radiographic protective effect on the peripheral joints of patients with PsA, but they appear to have little to no effect on the radiographic axial features of patients with SpA. Interestingly, large retrospective studies suggest an axial radiographic protective effect with NSAIDs in AS.[36] There appears to be a clear role for regular physiotherapy in AS to prevent loss of spinal mobility.

Far fewer data exist regarding treatments specifically effective for uSpA. As described previously, uSpA should not be considered a milder form of AS or other type of SpA. Data demonstrate comparable disease activity between uSpA and AS in terms of measures of disease activity, enthesitis, and uveitis.[37] Most treatment recommendations are extrapolated from AS and PsA studies. NSAIDs, cyclooxygenase (COX)-2 inhibitors, and disease-modifying antirheumatic drugs (DMARDs) are the main therapeutic agents used in uSpA, particularly those with peripheral manifestations. Of the DMARDs specifically, sulfasalazine or methotrexate are most often used. Sulfasalazine is the only traditional DMARD that has demonstrated potential efficacy in a prospective clinical trial in uSpA.[38] Local injections of corticosteroids can be effective in cases of monoarthritis or oligoarthritis, enthesitis, bursitis, or tenosynovitis.[39] In patients with axial involvement or severe manifestations despite conventional treatment, TNF inhibitors should be considered. TNF inhibitors effectively treat the clinical symptoms, decreasing acute phase reactants, and improving the MRI findings in patients with uSpA.[25]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 77

# Psoriatic Arthritis



Oliver FitzGerald • Musaab Elmamoun

## KEY POINTS

- Psoriatic arthritis should be suspected in a patient with an asymmetric joint distribution pattern who may have additional clinical features, such as dactylitis, enthesitis, or inflammatory-type back pain, and who is negative for rheumatoid factor.
- New classification criteria, the Classification of Psoriatic Arthritis (CASPAR) criteria, have been validated.
- Psoriatic arthritis is a progressive disease. Erosions develop in 47% of patients within 2 years of diagnosis. Polyarticular disease and an elevated erythrocyte sedimentation rate are markers of poor outcome.
- An essential core set of domains and instruments is necessary for inclusion in clinical trials.
- Studies of synovial tissue demonstrate increased vascularity and the presence of neutrophils, which might help distinguish spondyloarthropathy from rheumatoid arthritis. Change in synovial $CD3^+$ T cell synovial infiltration might be associated with clinical response to treatment.
- Prominent entheseal involvement with bone marrow edema at entheseal insertions on magnetic resonance imaging has prompted the hypothesis that psoriatic arthritis may originate at the enthesis.
- $CD8^+$ T cells and the innate immune response could participate in disease pathogenesis.
- Although there is a paucity of evidence for efficacy of disease-modifying anti-rheumatic drugs in psoriatic arthritis, TNF inhibitors have proved effective for skin and joint disease.

Psoriatic arthritis is a member of the spondyloarthropathy family and may be defined as an inflammatory arthropathy associated with psoriasis and usually negative for rheumatoid factor. Until the 1950s, an inflammatory arthritis that occurred in the presence of psoriasis was thought to represent rheumatoid arthritis (RA) occurring coincidentally with psoriasis. Based primarily on clinical and radiologic grounds and with use of the rheumatoid factor, the distinction between RA and psoriatic arthritis was gradually accepted. Wright described the classic clinical features in 1959, and with his colleague Moll, he published his classification criteria in 1973.[1,2] Until recently, these criteria have been the simplest and the most frequently used in clinical studies. The American Association of Rheumatism included psoriatic arthritis as a distinct clinical entity in the classification of rheumatic diseases for the first time in 1964.[3]

## EPIDEMIOLOGY

Epidemiologic studies have supported the concept that psoriatic arthritis is a unique disease entity separate from RA. The prevalence of inflammatory arthritis is increased among patients with psoriasis, ranging from 7% to 25% compared with a general population estimate of 2% to 3%. The prevalence of psoriasis among patients with arthritis also is increased, at a rate of 2.6% to 7% compared with a general population estimate of 0.1% to 2.8%.[4]

Psoriasis affects approximately 2% of the population. The prevalence varies: 5% to 10% of Russians and Norwegians are affected, but only 0% to 0.3% of West Africans or Native Americans are affected.[5] Onset of psoriasis may occur at any age, but it most frequently peaks in the twenties. Although no gender predilection has been reported, a genetic predisposition has been noted.

Among patients with psoriasis, arthritis develops in 7% to 42%. This figure varies widely in part because of a lack of widely accepted diagnostic criteria; it also varies according to which population is studied. The exact prevalence and incidence of psoriatic arthritis are unknown. The reported prevalence of psoriatic arthritis ranged from 0.056% to 0.28% in a large population-based study in the United States.[6] Cases were defined as patients who reported a "physician diagnosis" of psoriasis and psoriatic arthritis. Prevalence was calculated at 0.25% (95% confidence interval [CI], 0.18% to 0.31%). Kay and colleagues[7] did a prevalence study in northeast England to evaluate records from six general practices; 81 of 772 patients with psoriasis had inflammatory arthritis, with a prevalence of 0.28%. A study by Mease and colleagues[8] that looked at prevalence of rheumatologist-diagnosed psoriatic arthritis in patients with psoriasis in Europe/North American dermatology clinics found that almost a third of patients with psoriasis had psoriatic arthritis (of 949 patients evaluated, 285 [30%] had psoriatic arthritis).[8] The reported incidence of psoriatic arthritis has varied from 3 to 23 cases per 100,000. Data from Rochester, New York, have shown an incidence rate of 6.59 per 100,000, whereas in Finland, 16 new cases of psoriatic arthritis were identified in a population of 87,000, yielding a mean incidence rate of 23 per 100,000.[9,10] By using the Icelandic genealogy database, risk ratios (RRs) for the development of psoriatic arthritis in a sample that spanned five generations were estimated.[11] Results confirmed a strong and complex genetic component with a significant RR up to the fourth-degree relatives of psoriatic arthritis patients, as well as an important environmental contribution.

## CLINICAL FEATURES

Plaque psoriasis, or *psoriasis vulgaris*, is the most common skin phenotype in patients with psoriatic arthritis. Other patterns of skin involvement may be seen (Figure 77-1). Although arthritis usually develops in a setting of an established diagnosis of psoriasis, some patients may be unaware that they have psoriasis, or psoriasis may develop after the onset of arthritis in approximately 15% of cases.[12] In a clinical study, the incidence of development of arthritis in patients with psoriasis remained constant (74 per 1000 person-years), whereas the prevalence increased with time since diagnosis of psoriasis, reaching 20.5% after 30 years.[13] If a patient is first seen with the classic articular




**Figure 77-1** Clinical phenotypes in psoriasis: plaque psoriasis (psoriasis vulgaris). **A** and **B,** At extensor surface of elbow and on scalp. **C,** Genital psoriasis. **D,** Inframammary and umbilical flexural psoriasis. **E,** Guttate psoriasis in a father and child. **F,** Erythrodermic psoriasis on the trunk and upper limbs.







**Figure 77-1, cont'd** **G,** Pustular psoriasis on the foot. **H,** The Koebner phenomenon on a surgical abdominal wound.










**Figure 77-2** Nail dystrophic changes. **A,** Nail pitting. **B,** Onycholysis. **C,** Severe destructive change with nail loss and pustule formation.

manifestations of psoriatic arthritis but does not report psoriasis or the presence of a rash, the physician must examine the patient's skin carefully, including the scalp and nails, because psoriasis frequently lurks in such areas. Examples of nail dystrophic changes are shown in Figure 77-2.

Although a more recent U.S. study suggests that the prevalence of psoriatic arthritis among psoriasis patients increases with psoriasis severity,[6] in clinical practice, little relationship has been observed between severity of skin involvement and severity of arthritis. In one prospective study, only 35% of patients reported that their skin and joint components flared at the same time.[12] Other systemic features of joint inflammation are common in patients with psoriatic arthritis, including stiffness after rest and fatigue. In one study of fatigue, a number of factors, including disease activity, physical disability, pain, and psychological distress, contributed to fatigue, with comorbid fibromyalgia and hypertension further adding to the challenge.[14] Compared with other core outcome measures, fatigue has been found to be an independent outcome measure that is sensitive to change.[15]

Patients with psoriatic arthritis present with symptoms and signs of joint, entheseal, or spinal inflammation. The joints involved at presentation in 129 early psoriatic

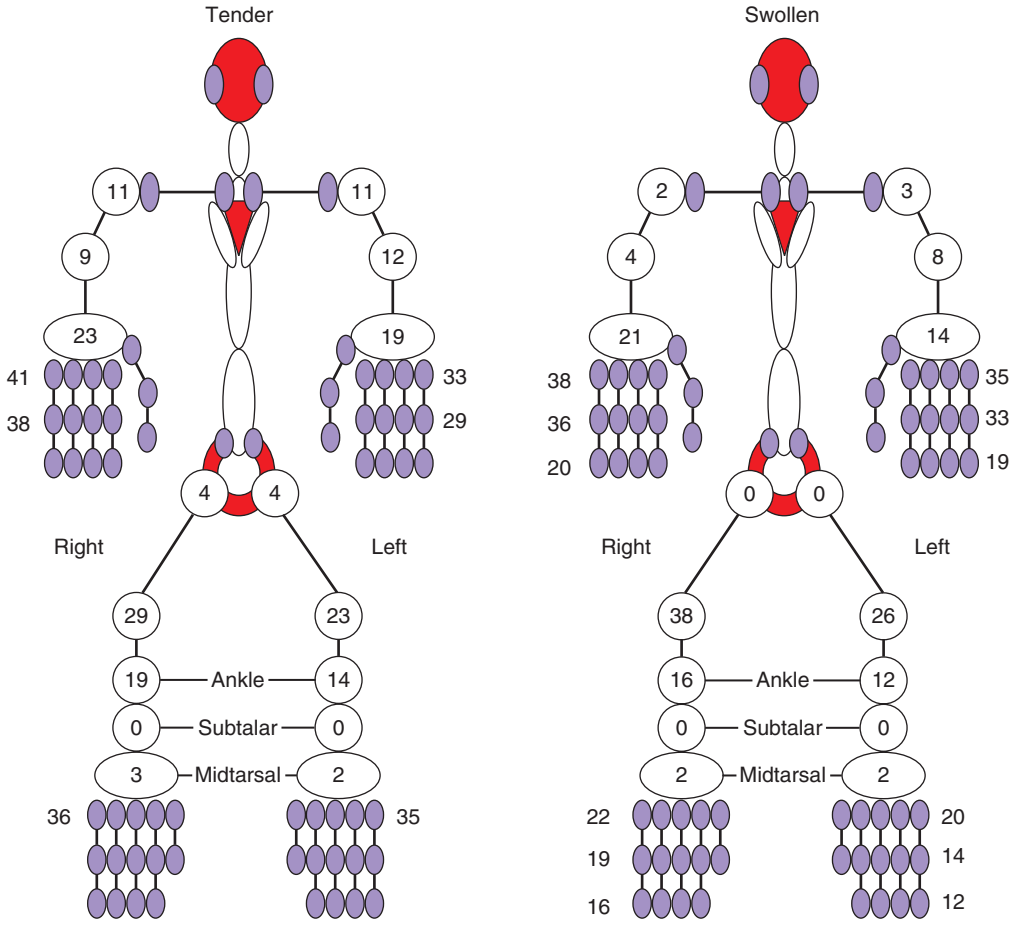



**Figure 77-3** Frequency (%) of peripheral limb joint involvement in 129 patients with early psoriatic arthritis as assessed by joint tenderness and swelling (distal interphalangeal joints of hand and proximal and distal interphalangeal joints of feet not assessed for tenderness as part of Ritchie Articular Index).

arthritis patients are shown in Figure 77-3. In one of their seminal papers on psoriatic arthritis, Wright and Moll[16] described five clinical patterns of psoriatic arthritis (Figure 77-4):

1. Asymmetric oligoarthritis
2. Symmetric polyarthritis
3. Predominant distal interphalangeal (DIP) joint involvement
4. Predominant spondyloarthritis
5. Destructive (mutilans) arthritis

These classification criteria are the most commonly quoted, although many alternative criteria have been proposed. Variability in the definition of terms has led to differences in the reported frequency of psoriatic arthritis in subsets among the different studies. The pattern of joint involvement is not fixed. The patient's disease may fluctuate and may be influenced by treatment. In a study of 129 patients with early psoriatic arthritis, 53 of 77 initially classified as polyarticular were reassessed at 2 years; 26 of 53 (49%) patients were subsequently classified as oligoarticular, 19 of 53 (36%) remained classified as polyarticular, and 12 of 53 (23%) were in remission.[17]

The Classification of Psoriatic Arthritis (CASPAR) study has included in the analysis a breakdown of disease pattern subtypes.[18] This multicenter study included data on 588 psoriatic arthritis cases and 536 controls. In contrast to the original Moll and Wright paper, but similar to many subsequent publications, approximately 63% of patients had polyarticular joint involvement, compared with 13% with oligoarticular disease. The other patterns of joint involvement described by Moll and Wright occurred much less commonly. Predominant DIP disease was found in less than 5%, but DIP involvement can occur in any of the subtypes. Predominant spondyloarthritis is uncommon, although spinal involvement may be found in 40% to 70% of psoriatic arthritis cases, depending on whether or not radiographs are taken.[19] Risk factors for spinal involvement include severe peripheral arthritis and human leukocyte antigen (HLA)-B27.[20] Finally, arthritis mutilans, a destructive form of arthritis associated with flail joints, is rare, although this form of joint involvement may develop in more patients with time if their disease is not properly controlled. The Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) has developed a broad consensus on the features of arthritis mutilans that include both clinical and radiologic features. The consensus was that the definition of arthritis mutilans should involve peripheral joints, especially of the hands and feet, but not axial joints. Involvement of one joint was considered sufficient. Rapidity of joint destruction was not considered




**Figure 77-4** Patterns of peripheral joint disease: asymmetric polyarticular disease. **A,** Distal interphalangeal joint involvement and forearm lymphedema. **B,** Toe dactylitis with skin and nail change. **C,** Predominant distal interphalangeal joint involvement. **D,** Arthritis mutilans.

important. Both clinical and radiographic features were important, but radiographic features were believed to be more sensitive. Clinical criteria include shortening of digits, digital telescoping, and/or flail joint not caused by subluxation. Radiographic criteria include erosion involving entire articular surfaces on both sides of the joint and/or pencil-in-cup change, osteolysis was considered to be the defining feature. Although ankylosis was a feature of severe joint destruction, it was placed into a different category distinct from arthritis mutilans.[21]

Features that are typical of psoriatic arthritis, including dactylitis and enthesitis, are helpful in diagnosis. Dactylitis, which is characterized by a sausage-shaped swelling of the fingers or toes (see Figure 77-4B), may be found in 29% to 33.5% of patients with psoriatic arthritis at first presentation, and 48% may have an episode of dactylitis during follow-up.[17,22] Ultrasound and magnetic resonance imaging (MRI) studies have shown that joint and tenosynovial inflammation are prominent in involved digits.[23,24] Enthesitis, inflammation at tendon and ligament insertion into bone, is a feature of all of the spondyloarthropathies and may be a presenting feature in psoriatic arthritis. Overall, enthesitis is found in 38% of patients at presentation.[17] The most common entheseal sites involved are the Achilles and plantar fascia insertions. Other sites include the insertions of the quadriceps and patellar tendons, the iliac crest, the rotator cuff, and the epicondyles at the elbow. Patients have reported pain at these sites, with tenderness and sometimes swelling noted on examination. Entheseal involvement may be asymptomatic, and ultrasound is more sensitive than clinical palpation. Often spurs are detected on radiograph, although spurs are not always associated with symptoms.

With the obvious exception of psoriasis and nail dystrophic change, extra-articular disease is less common in psoriatic arthritis than in RA. Iritis or uveitis occurs in 7% to 18%, more bilateral than in ankylosing spondylitis, but is usually found in patients with spinal involvement.[12,25] Numerous studies have suggested that patients with psoriatic arthritis have a higher prevalence of inflammatory bowel disease, sometimes asymptomatic and detected only on biopsy specimen.[26,27] Whether this inflammatory bowel disease is coincidental or is possibly related to medication effects remains to be clarified. Distal limb edema or lymphedema may occur more commonly in psoriatic arthritis; one case-control study found it in 21% of psoriatic arthritis patients compared with 4.9% of controls (see Figure 77-4A).[28] Finally, amyloid is rare but is described in psoriatic arthritis.

**TABLE 77-1** Clinical Features that Distinguish Psoriatic Arthritis from Rheumatoid Arthritis

| | Psoriatic Arthritis | Rheumatoid Arthritis |
|---|---|---|
| Psoriasis | + | – |
| Symmetric | + | ++ |
| Asymmetric | ++ | + |
| Enthesopathy | + | – |
| Dactylitis | + | – |
| Nail dystrophy | + | – |
| Human immunodeficiency virus association | + | – |

## DIFFERENTIAL DIAGNOSIS

Certain articular features, if present, are useful in distinguishing psoriatic arthritis from RA, including dactylitis, DIP involvement, and inflammation at entheseal sites (Table 77-1; see Figure 77-4). In addition, inflammatory-type back pain or sacroiliitis on plain radiograph or MRI should raise the suspicion of psoriatic arthritis because spinal involvement is uncommon in RA. The absence of rheumatoid nodules or other systemic features common to RA can be another useful differentiating feature.

Distinguishing psoriatic arthritis from other spondyloarthropathies is also important. Dactylitis may be a feature in reactive arthritis, in which a palmoplantar pustular rash (keratoderma blennorrhagicum) may be clinically and histologically indistinguishable from pustular psoriasis (see Figure 77-1G). In relation to spinal involvement, sacroiliitis may be unilateral more frequently, and the spinal changes on plain radiography may be more asymmetric in psoriatic arthritis than with classic ankylosing spondylitis. Finally, crystal-associated arthropathies occasionally can confuse, especially with monoarticular disease, and are best distinguished by synovial fluid crystal analysis. Serum urate levels may be increased in patients with psoriatic arthritis, adding to the confusion. To aid dermatologists and general physicians in identifying those with psoriatic arthritis, a number of screening tools for arthritis in patients with psoriasis have been proposed and are the subject of a current head-to-head comparative study.

## LABORATORY FEATURES

No diagnostic laboratory test for psoriatic arthritis is known. Although the absence of rheumatoid factor is considered an important distinguishing feature from RA, low levels of rheumatoid factor may be found in patients (5% to 16%) with typical psoriatic arthritis features. Until a more definitive diagnostic test becomes available, it is difficult to be categorical about diagnosis in these patients. Citrullinated protein antibodies (CPA) initially were thought to be specific to RA, but it is now recognized that CPAs are found in approximately 5% of patients with psoriatic arthritis as well.[29] Acute phase markers, such as erythrocyte sedimentation rate, C-reactive protein (CRP), and serum amyloid A, may be elevated in patients with psoriatic arthritis, but less commonly and to a lesser degree than in patients with RA. These markers are elevated in particular in patients with polyarticular disease, and act as a marker of poor prognosis.[30] Finally, as mentioned previously, hyperuricemia may be found in association with metabolic abnormalities in patients with psoriatic arthritis and may not reflect the extent of skin involvement.

## RADIOGRAPHIC FEATURES

Although substantial advances have been made in the application, in particular, of musculoskeletal ultrasound (MSUS) and of MRI in patients with arthritis, including psoriatic arthritis, plain radiographic imaging remains the "gold standard" for assessing bony changes in peripheral joints in psoriatic arthritis.

### Plain Radiography

Of patients with established psoriatic arthritis, 67% have radiographic abnormalities, 12% and 47% of patients with recent-onset psoriatic arthritis will have developed erosions within 2 years of disease onset.[17] Distinctive radiographic features reflect in some cases the clinical phenotype (Figure 77-5). These features include asymmetric joint involvement; involvement of the interphalangeal joints of the fingers and toes, with features of bony erosion and resorption sometimes seen together and resulting in the classic "pencil-in-cup" deformity; joint space narrowing or involvement of entheseal sites, often with bony spurs or development of periostitis; and spinal involvement, frequently less severe and asymmetric than in classic ankylosing spondylitis.

Radiographic progression in psoriatic arthritis is slow in early stages of the disorder, with the mean modified Sharp (to include DIP joints in the hands) erosion score at presentation increasing from 1.2 to 3 at 2 years.[17] Larson and Sharp scoring systems have been used in psoriatic arthritis, but neither the Larson nor the Sharp score has been developed specifically for psoriatic arthritis nor has it been extensively validated. Other scoring systems that have been proposed for use in psoriatic arthritis includes the modified sharp score (MSS), the Sharp/van der Heijde modified method (SHS), the modified Steinbrocker method, and the psoriatic arthritis Ratingen score. With the exception of the Ratingen method, these scoring methods were designed and validated for use in RA and subsequently modified for use in psoriatic arthritis. None of the existing radiographic measures are both sufficiently feasible and sensitive to change to be easily applied in large longitudinal observational studies.[31]

## MUSCULOSKELETAL ULTRASOUND

Many MSUS applications are useful in psoriatic arthritis; these applications are likely to develop further while the technology (in particular, power Doppler) that can be used to allow identification of blood flow is further advanced (Figure 77-6). It has already been shown that MSUS is more sensitive than clinical examination in detecting subclinical synovitis in early psoriatic arthritis—in this study, 96% of patients had at least one joint with subclinical

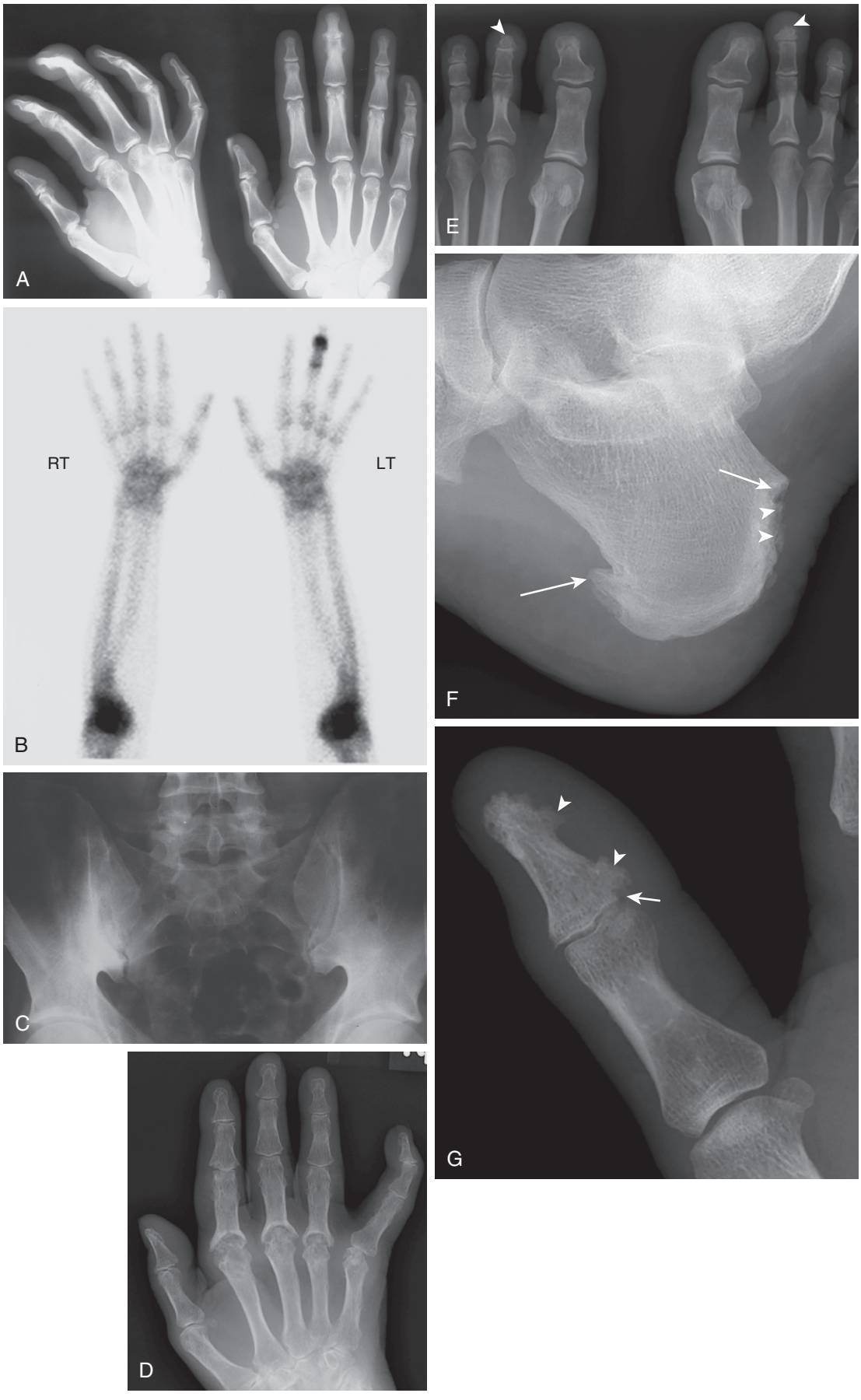



**Figure 77-5** Radiologic features in psoriatic arthritis. **A,** Third left distal interphalangeal joint monoarthritis with prominent new bone formation. **B,** Bone scan from same patient as in **A. C,** Asymmetric right-sided sacroiliitis. **D,** Arthritis mutilans. Severe destructive changes with multiple erosions and "pencil-in-cup" deformities. **E,** Acro-osteolysis. Destruction of the tufts of the distal phalanges of both second toes *(arrowheads)*. **F,** Enthesitis. Lateral view of the calcaneus shows a large erosion at the dorsal aspect of the calcaneus at the Achilles tendon insertion *(large arrow)*. There is some associated periosteal new bone formation *(arrowheads)*. A large plantar calcaneal spur is also demonstrated *(small arrow),* indicating enthesopathy at the plantar fascia origin. **G,** Periostitis. Radiograph of the thumb shows a marginal erosion of the base of the distal phalanx *(arrow),* with extensive cloaking of the surface of this phalanx with irregular periosteal new bone *(arrowheads)*. RT, Right; LT, left. *(Courtesy Dr. Robin Gibney and Dr. Eric Heffernan.)*

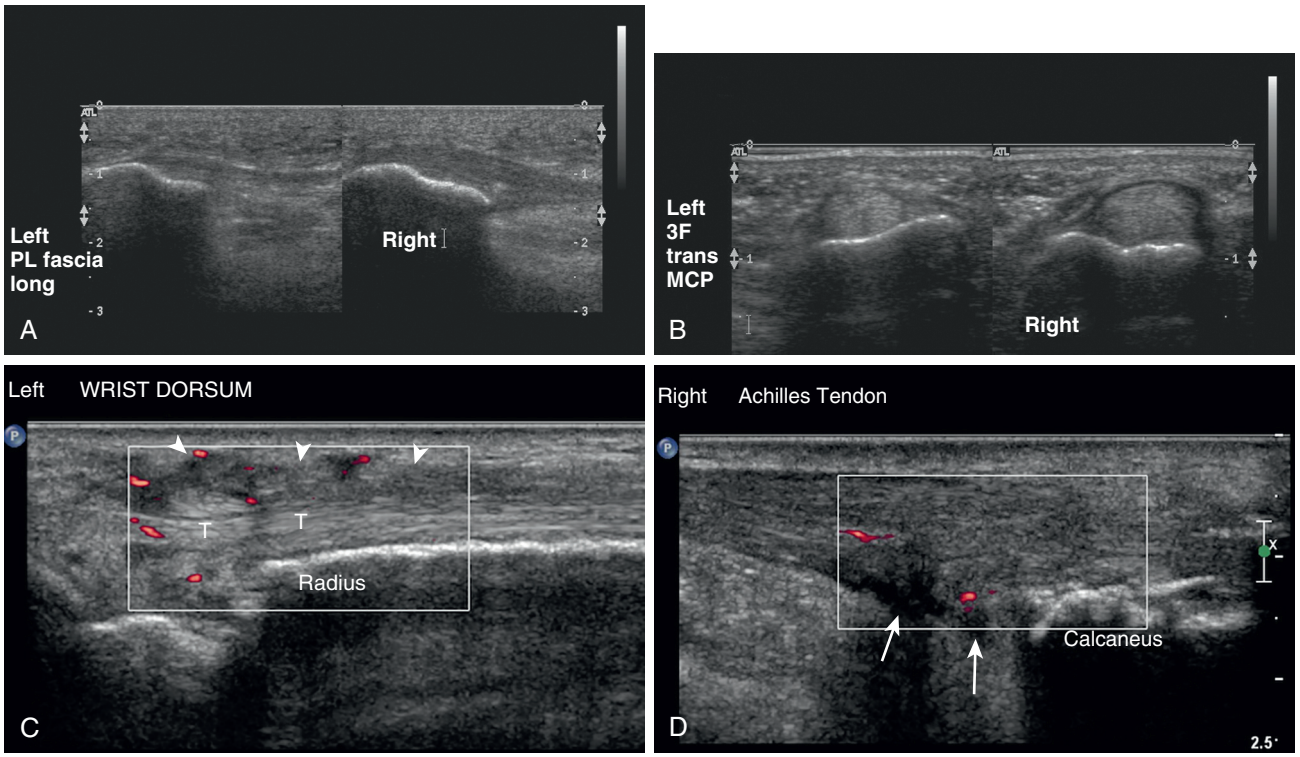



**Figure 77-6** Musculoskeletal ultrasound features in psoriatic arthritis. **A,** Right plantar fascia thickening compared with the left. **B,** Transverse section through left third finger at the metacarpophalangeal joint showing right tenosynovitis. **C,** Extensor tenosynovitis. Longitudinal ultrasound image of the dorsum of the wrist shows lobulated, hypoechoic thickening of the extensor tendon sheath *(arrowheads)* surrounding an extensor digitorum tendon *(T)*. Power Doppler has been used and shows hyperaemia *(red and orange pixels)* in the thickened tendon sheath. **D,** Retrocalcaneal bursitis and enthesitis. Longitudinal ultrasound image with Power Doppler shows hypoechoic thickening of the retrocalcaneal bursa *(arrows)*, deep to a severely thickened distal Achilles tendon. Abnormal color flow is demonstrated in the tendon, indicating neovascularization. There is also irregularity of the cortex of the calcaneus at the tendon insertion caused by erosions. *(Courtesy Dr. Robin Gibney and Dr. Eric Heffernan.)*

synovitis—and led to the majority of patients clinically diagnosed with oligoarthritis being reclassified with polyarthritis.[32] This reclassification may result in significant changes in prognosis and therapy. Finally, MSUS has been used in objective monitoring of the response of synovitis to therapy.[33]

MSUS features at the enthesis include entheseal thickening, hypoechoic change, increased vascularity as shown on power Doppler, tenosynovitis, and bony erosions or enthesophyte formation.[34,35] MSUS is a valid noninvasive tool, and it has been shown to be more sensitive than clinical examination in detecting lower limb enthesopathy. MSUS has also been shown to detect a high percentage of occult subclinical entheses abnormalities in patients with early psoriatic arthritis, independent of clinical examinations and symptoms.[35,36] MSUS has been used in studies of dactylitic digits. With MRI, MSUS has shown dactylitis to be caused by a combination of synovial and tenosynovial inflammation.[23,24] Finally, MSUS guidance for small joint or entheseal aspiration or injection may have particular application in patients with psoriatic arthritis.

## Magnetic Resonance Imaging

MRI studies have been particularly useful in offering new insights into disease pathogenesis in psoriatic arthritis. Based on the prominent entheseal-related bone marrow edema seen on MRI, McGonagle and colleagues[37] have proposed that psoriatic arthritis, in contrast to RA, is an entheseal-based disease. MRI can be used to study all aspects of joint involvement, including the enthesis, but use of MRI as a routine clinical tool in psoriatic arthritis has not been clarified. Application of MRI to the spine or sacroiliac joints in psoriatic arthritis may prove especially helpful, as has been shown in ankylosing spondylitis, especially in HLA-B27 related active axial psoriatic arthritis.[38] Preliminary studies have suggested that MRI can be useful as an outcome measure in the detection of synovitis or of vascularity in patients with psoriatic arthritis undergoing biologic therapies (Figure 77-7). More detailed studies are required. A new MRI scoring system has been proposed to measure articular inflammation and damage in patients with psoriatic arthritis (PsAMRIS).[39]

## Other Imaging Modalities

The use of other imaging modalities, such as computed tomography (CT) or scintigraphy, has largely been superseded by MRI. CT is now reserved mainly for patients for whom MRI is contraindicated, or for whom MRI is unavailable. Positron emission tomography (PET) has been found to be comparable with MSUS and MRI in the knees of patients with RA. An exploratory study used high-resolution [$^{18}$F] PET to explore the pattern of DIP joint bone metabolism in psoriatic arthritis compared with osteoarthritis and healthy joints. The authors conclude that this technique

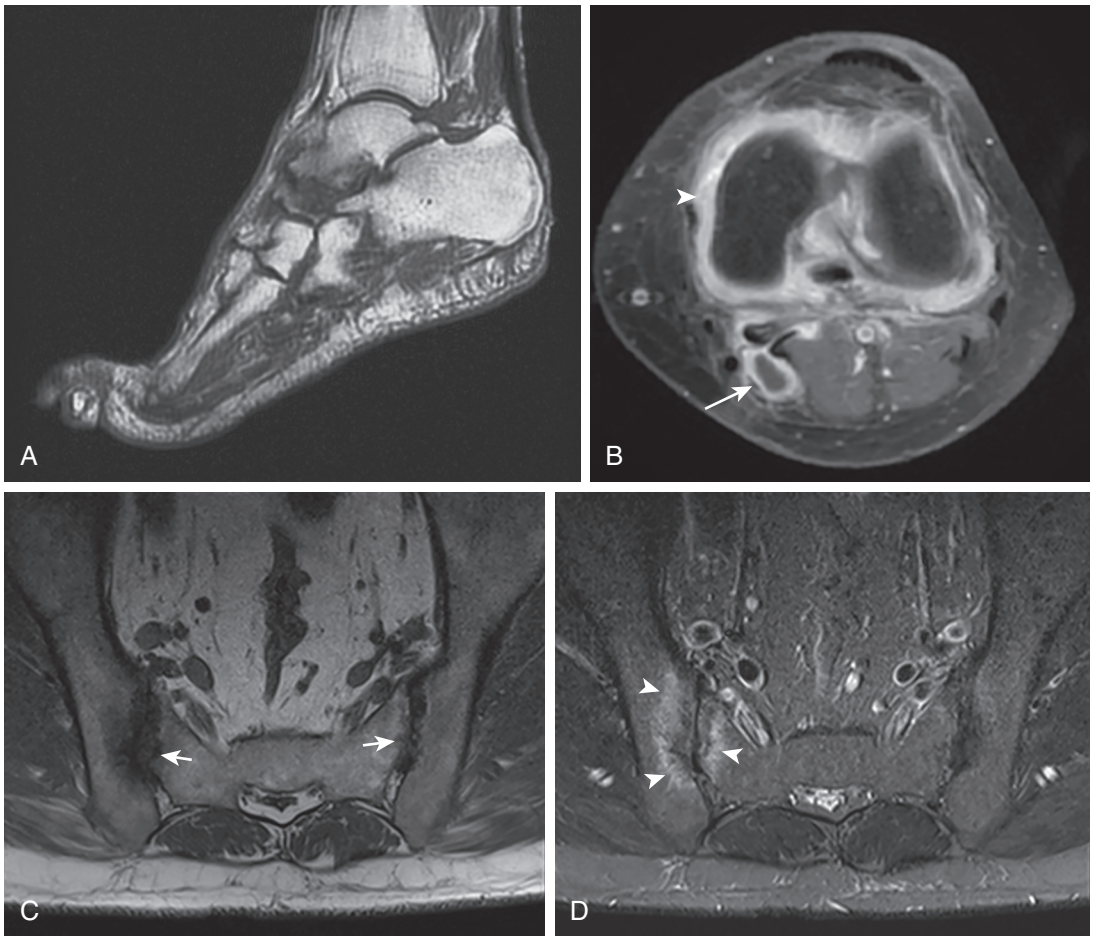



**Figure 77-7** **A,** T1-weighted magnetic resonance imaging (MRI) of left foot confirming severe talonavicular disease with bone edema. **B,** Knee synovitis. Transverse T1-weighted fat-saturated contrast enhanced MRI shows diffuse severe thickening of the synovium of the knee *(arrowhead)*. The synovitis extends to involve a small Baker's cyst in the popliteal fossa *(arrow)*. **C,** Asymmetric sacroiliitis. Transverse T1-weighted image shows low signal sclerosis around the right sacroiliac joint, in addition to bilateral erosions *(arrows)*. **D,** Transverse short tau inversion recovery image from same patient as in **C**, shows florid edema on both sides of the right sacroiliac joint *(arrowheads)*, with minimal edema on the *left*. *(Courtesy Dr. Robin Gibney and Dr. Eric Heffernan.)*

potentially offers a novel explanation for the known phenotypic expression of psoriatic arthritis and helps improve understanding of the bone-enthesis-nail complex.[40] In a study that used high-resolution CT imaging, structural bone changes were compared between patients with RA and patients with psoriatic arthritis. Smaller Ω-shaped and tubule-shaped erosions, as well as large, sometimes corona-shaped osteophytes, are typical of psoriatic arthritis. Another longitudinal study that described distribution, pattern, and development of erosions and proliferations in the hands of patients with hands by using high-resolution CT demonstrated that radiographs have low sensitivity, but high specificity for erosions and proliferations, when CT is considered the gold standard reference.[41]

## DIAGNOSIS

A diagnostic test for psoriatic arthritis is currently unavailable. Nevertheless, in its simplest form, psoriatic arthritis can be considered as an arthritis occurring in the presence of psoriasis, but in the absence of rheumatoid factor. Most patients with psoriatic arthritis meet this simple definition. The arthritis can be predominantly spinal, it may involve only entheseal sites, psoriasis may present after arthritis in 15% of cases, and low-titer positive rheumatoid factor may be found. Recognizing these difficulties, the CASPAR group published new classification criteria based on an analysis of 588 patients with psoriatic arthritis and 536 controls (Table 77-2). These criteria have now been well validated in a number of studies, both prospective and retrospective, with high sensitivity and specificity, including early psoriatic arthritis patients.[42] In the setting of clinical research, the CASPAR criteria have a specificity of 0.987 and a sensitivity of 0.914. For the individual patient, an algorithm for diagnosis is suggested in Figure 77-8.

## CLINICAL COURSE AND OUTCOME

Five early psoriatic arthritis cohorts have been studied.[17,43-46] The mean disease duration in these cohorts was 6 to 12 months, the median patient age at onset of psoriasis was 27 to 31 years, and the median patient age at onset of arthritis was 38 to 52 years. Overall, little relationship was noted between skin disease severity and onset of psoriatic arthritis.

**TABLE 77-2** CASPAR Classification Criteria for Psoriatic Arthritis

| |
|---|
| Inflammatory articular disease (joint, spine, or entheseal) with ≥3 points from the following: |
| 1. Evidence of psoriasis (one of a, b, or c)<br>a. Current psoriasis*: psoriatic skin or scalp disease present today as judged by a rheumatologist or dermatologist<br>b. Personal history of psoriasis: history of psoriasis that may be obtained from patient, family physician, dermatologist, rheumatologist, or other qualified health care provider<br>c. Family history of psoriasis: history of psoriasis in a first-degree or second-degree relative according to patient report |
| 2. Psoriatic nail dystrophy: typical psoriatic nail dystrophy, including onycholysis, pitting, and hyperkeratosis observed on current physical examination |
| 3. Negative test for rheumatoid factor: by any method except latex, but preferably by enzyme-linked immunosorbent assay or nephelometry, according to the local laboratory reference range |
| 4. Dactylitis (one of a or b)<br>a. Current swelling of an entire digit<br>b. History: history of dactylitis recorded by a rheumatologist |
| 5. Radiologic evidence of juxta-articular new bone formation: ill-defined ossification near joint margins (but excluding osteophyte formation) on plain radiographs of hand or foot |

Specificity 0.987, sensitivity 0.914 for CASPAR criteria.
*Current psoriasis scores 2, whereas all other items score 1.
CASPAR, Classification of Psoriatic Arthritis.

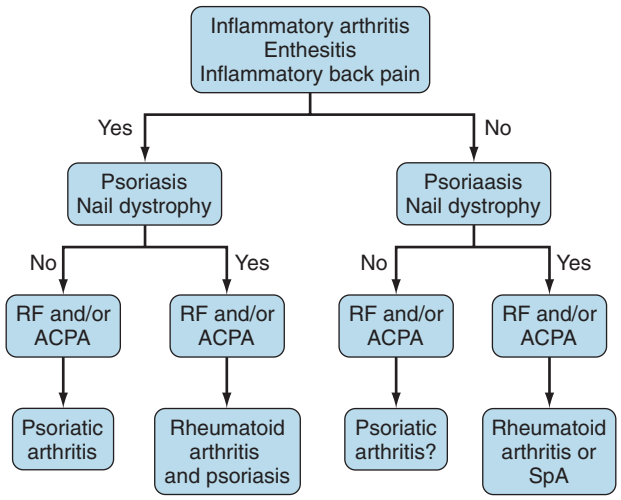



**Figure 77-8** Algorithm to be used in the diagnosis of individual patients presenting with possible psoriatic arthritis. Some patients may present with typical articular manifestations of psoriatic arthritis, but in the absence of skin or nail disease. They can be diagnosed as having definite psoriatic arthritis only when psoriasis subsequently develops. ACPA, Anti-citrullinated protein antibody; RF, rheumatoid factor; SpA, spondyloarthropathy.

The small joints of hands and feet were most commonly involved; the DIP joints were involved in one-third of patients, usually in association with nail disease, which was present in two thirds; dactylitis and enthesitis were present in one third; and spinal involvement only was found in 2% to 4%, but was present in 20% overall. At follow-up, disease had continued to progress in most patients. Erosive disease developed in 47% of patients within 2 years.[17] Markers for progression included polyarticular disease and an elevated erythrocyte sedimentation rate. Long-term follow-up studies have shown significant morbidity and increased mortality in psoriatic arthritis: 17% of patients have five or more deformed joints, 40% to 57% have a deforming arthritis, 20% to 40% have spinal involvement, 11% to 19% are disabled, and mortality is increased compared with the general population.[12,47,48]

## COMORBIDITIES IN PSORIATIC ARTHRITIS

Cardiovascular morbidity and mortality are increased in patients with psoriatic arthritis The risk of myocardial infarction, ischemic heart disease, hypertension, diabetes, and dyslipidemia was increased in cross-sectional and longitudinal prospective cohorts.[49] A number of studies have highlighted cardiovascular risk factors and the metabolic syndrome in psoriatic arthritis. In one study of 109 psoriatic arthritis patients compared with 699 RA and 122 ankylosing spondylitis controls, the adjusted odds ratio (OR) for the metabolic syndrome was 2.44 (1.48 to 4.01; $P < 0.001$) with adjusted ORs significantly increased for central obesity, impaired fasting glucose, and low high-density lipoprotein (HDL) cholesterol.[50] In another study of patients with psoriatic arthritis, 44% had metabolic syndrome ($n = 283$) and 16% had insulin resistance ($n = 263$). A significant association was also noted between metabolic syndrome and more severe psoriatic arthritis (OR 4.47, $P < 0.001$).[51] Psoriasis is associated with diabetes, independent of obesity and other traditional risk factors. Diabetes risk may also be elevated in psoriatic arthritis caused by systemic inflammation from both skin and articular disease. In a study that used a large U.K. general practice database, The Health Improvement Network (THIN), the authors evaluated the risk of incident diabetes among patients with psoriatic arthritis, psoriasis, and RA. They suggested that the overall risk of diabetes is increased in patients with psoriatic arthritis. Age- and sex-matched HRs for diabetes were 1.72 (95% CI, 1.46, 2.02) in psoriatic arthritis, 1.39 (95% CI, 1.32, 1.45) in psoriasis, and 1.12 (95% CI, 1.01, 1.25) in RA. After adjustment for BMI, smoking, alcohol, baseline glucocorticoid use, and co-morbidities, the HRs were 1.33 (1.09, 1.61) in psoriatic arthritis, 1.21 (1.15, 1.27) in psoriasis, and 0.94 (0.84, 1.06) in RA.[52] A large, population-based study by Ogdie and colleagues[53] used the same database to measure the risk of all-cause mortality among patients with psoriatic arthritis compared with the general population and with patients with psoriasis only and RA. It demonstrated an increased mortality in patients with severe psoriasis or RA but no statistically significant increase in mortality among patients with psoriatic arthritis. HR (age- and sex-adjusted) 1.02 (95% CI, 0.92 to 1.12), 1.56 (95% CI, 1.51 to 1.62), and 1.10 (95%, CI, 1.06 to 1.13) for psoriatic arthritis, RA, and psoriasis, respectively.

Surrogate markers of atherosclerosis were all unfavorable in psoriatic arthritis in terms of the increase in cardiovascular risk. Subclinical carotid artery atherosclerosis has been found to play a part in cardiovascular risk in patients with inflammatory arthritis. Several studies have assessed subclinical carotid atherosclerosis among patients with psoriatic arthritis by using carotid intima-media thickness (cIMT). cIMT appeared consistently greater in psoriasis and psoriatic arthritis compared with the general population.

Eder et al[49] compared the extent of subclinical atherosclerosis between patients with psoriatic arthritis and patients with psoriasis only. In the study, 125 patients with psoriatic arthritis and 114 patients with psoriasis were compared. Two surrogate measures of subclinical atherosclerosis were measured, cIMT and carotid total plaque area (TPA). The authors suggest that patients with psoriatic arthritis have more severe subclinical atherosclerosis, as reflected by higher carotid TPA, compared with psoriasis patients (TPA [square root of area in $mm^2$] 3.33 ± 3.34 vs. 2.43 ± 2.72, $P = 0.03$). The difference in cIMT between the groups did not achieve statistical significance.[49] Finally, a systematic review looking at cardiovascular comorbidities in psoriatic arthritis suggests an increased prevalence of cardiovascular disease in patients with psoriatic arthritis compared with the general population, as well as increased prevalence of cardiovascular risk factors.[54] It is important to note that when assessing cardiovascular disease and comorbidities, we must carefully discriminate between comorbidities associated with the psoriatic disease process itself versus those that are associated with and/or modified by co-existing cardiovascular risks and arthritis treatment. The European League Against Rheumatism (EULAR) has published evidence-based guidelines for cardiovascular risk management in patients with inflammatory arthritis.[55]

## OUTCOME DOMAINS AND INSTRUMENTS

Measuring response to treatment of psoriatic arthritis in clinical trials has been the subject of much interest for members of the GRAPPA and Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT). Many of the data that have been used to date in clinical trials have been adapted from RA and have not been validated. Controversial issues have included the number of joints to count, the usefulness of the acute-phase response in psoriatic arthritis, and how important it is to include a measure of function or quality of life in the trial. An essential core set of domains that must be included in clinical trials has now been agreed on, with other domains necessary but not mandatory, and still others that require considerably more research (Figure 77-9). Instruments for many of these domains have yet to be developed and validated, and some instruments, such as the Psoriasis Assessment Severity Index (PASI), have acknowledged limitations. Table 77-3 lists the currently available instruments for the core domains. Instruments for dactylitis and enthesitis have been proposed and validated.[56,57]

In the setting of clinical trials, numerous composite scores (e.g., American College of Rheumatology [ACR]20, ACR50, ACR70; EULAR Disease Activity Score [DAS] response criteria) have been used in psoriatic arthritis, most again adapted from RA and not extensively validated in psoriatic arthritis. One scoring system, the PsARC, was developed for psoriatic arthritis. Although it has been used in numerous studies, it has not been extensively validated and is considered perhaps insufficiently responsive and discriminant.[58] Much work is required to develop a validated and responsive composite instrument for psoriatic arthritis. A composite measure is one way of assessing all relevant clinical outcomes in a single instrument. Ideally, a composite index should combine practicability and feasibility with validity and clinical relevance. It should also provide a measure of disease activity, as well as response to therapy. Recently, minimal disease activity criteria (MDA) were developed and validated.[59] MDA provides an easy method to measure acceptable patient disease activity state. The definition of MDA was agreed at OMERACT as a state of disease activity deemed a useful target of treatment by both

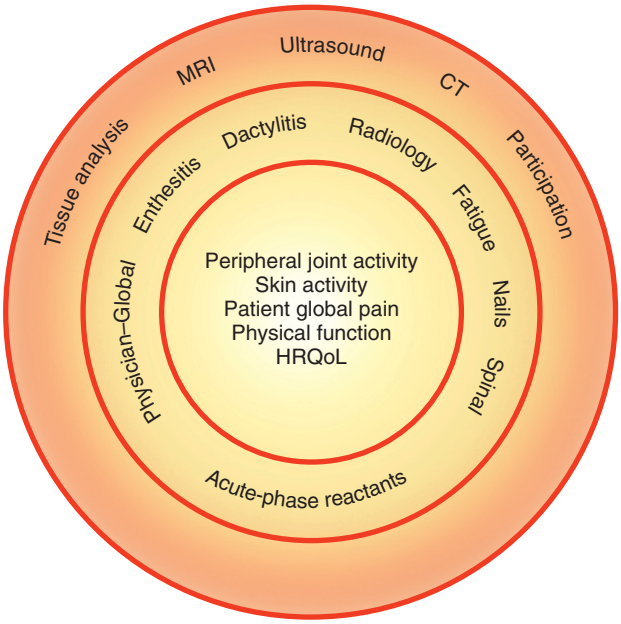



**Figure 77-9** Outcome domains in psoriatic arthritis. The central core domains are considered essential for inclusion in clinical trials. The middle circle contains domains that are considered important but not essential. The outer circle contains domains that all require further research and validation. CT, Computed tomography; HRQoL, health-related quality of life; MRI, magnetic resonance imaging.

**TABLE 77-3** Core Set Instruments Proposed for Use in Clinical Trials

| Domain | Instrument |
|---|---|
| Peripheral joint inflammation | Tender/swollen joint count 68/66 |
| Patient global assessment | Instrument under study* |
| Skin assessment | Psoriasis Assessment Severity Index (if body surface area ≥3%)<br>Lesion score (erythema, induration, scale)<br>Body surface area |
| Pain | Visual analogue scale or numeric rating scale |
| Physical function | HAQ/SF-36 physical function composite |
| Health-related quality of life | Generic disease specific (e.g., DLQI, PsAQoL) |

*Cauli A, Gladman DD, Mathiew A, et al: Patient global assessment in psoriatic arthritis: a multicenter GRAPPA and OMERACT study. *J Rheumatol* 38:898–903, 2011.

DLQI, Dermatology Life Quality Index; HAQ, health assessment questionnaire; PsAQoL Psoriatic Arthritis Quality of Life; SF-36, Short Form health survey.

the patient and physician, given current treatment possibilities and limitations. MDA encompasses both remission and low disease activity as acceptable targets for therapy. It can be used as a responder index in addition to a target for treatment interventions. Patients with psoriatic arthritis are considered to have MDA when they meet 5 out 7 of the following criteria:

- Tender joint count ≤1
- Swollen joint count ≤1
- PASI ≤ 1, or body surface area (BSA) ≤3
- Patient pain, visual analogue scale (VAS) ≤15
- Patient global activity VAS ≤20
- Health Assessment Questionnaire (HAQ) ≤5
- Tender entheseal points ≤1

Two joint-related disease composite indices have been proposed. First, the Disease Activity Index for Psoriatic Arthritis (DAPSA), which is an adaptation of the Disease Activity index for Reactive Arthritis (DAREA), has been developed from a clinical cohort and validated using clinical trial data. DAPSA calculates the following components: tender joint count (0-68), swollen joint count (0-66), CRP level (mg/dl), patient VAS for pain (0-10), and patient VAS for global disease activity (0-10).[60] Second, a weighted articular responder index, the Psoriatic Arthritis Joint Activity Index (PsAJAI), a measure of response reflecting joint involvement in psoriatic arthritis, has been developed from randomized placebo-controlled trials of biologic agents in psoriatic arthritis.[61] The PsAJAI is a weighted sum of 30% improvement in core measures with weights of two given to joint count measure, CRP level, and the physician's global assessment of disease activity measure. Weights of one given to the remaining 30% improvement measures include pain, patient global assessment of disease activity, and the HAQ.

Three domain-based approaches have been proposed. First, the Composite Psoriatic Disease Activity Index (CPDAI) assesses as many as five domains: peripheral joints, skin, entheses, dactylitis, and spinal manifestations.[62] For each domain, instruments are used to assess both the extent of disease activity and the effect of involvement in that domain on patient function and health-related quality of life. Domains are scored 0 to 3, with empiric cutoffs for disease severity/activity proposed in each, largely based on the literature. Individual domain scores are summed to give an overall, composite score (range 0-15). This instrument has been validated in a large clinical trial data set, the Psoriasis Randomized Etanercept Study in Subjects with Psoriatic Arthritis (PRESTA). CPDAI better reflects all of the domains potentially involved in psoriatic arthritis. Unlike the DAPSA, this instrument could distinguish global treatment responses between two doses of etanercept in the PRESTA trial dataset. This suggests that the CPDAI is a more sensitive instrument to detect change in domains beyond joints and patient global, particularly in domains such as enthesitis, dactylitis, and skin that are important multidimensional components of psoriatic arthritis.[63] Second, the Grappa Composite Exercise (GRACE) project[64] derived two composite disease activity measures for psoriatic arthritis from a longitudinal study: the Psoriatic Arthritis Disease Activity Score (PASDAS) and Arithmetic Mean of Desirability Functions (AMDF). The PASDAS was developed by using linear regression and physician-defined cutoffs for disease activity. It is a weighted index incorporating patient and physician assessments of global disease activity, tender and swollen joint counts, dactylitis and enthesitis, the physical component of the Medical Outcome Study Short Form 36, and CRP. The AMDF (recently renamed the GRACE measure) is a composite index based on score transformations, incorporating measures of skin and joint involvement together with physical function; domains include tender joint count, swollen joint count, patient skin VAS, patient joint VAS, patient global VAS, HAQ, the Psoriatic Arthritis Quality of Life (PsAQoL) instrument, and PASI. Both instruments performed well and were able to distinguish active from inactive disease, but were less able to discriminate in subgroups of patients with oligoarthritis and patients with severe skin disease. A comparison of PASDAS, AMDF, CPDAI, and DAPSA was performed by using data from the GO-REVEAL study. This analysis allowed a comparison of their performance in a trial of a highly effective intervention. All indices were able to discriminate treatment from placebo. PASDAS, AMDF, and modified CPDAI better reflected domains such as skin, enthesitis, and dactylitis in addition to the articular component of the disease. The composite indices have demonstrated both face, content, and convergent validity and response to change; however, further testing of these indices in reliability studies is needed.[65] Finally, the questionnaire has been developed and validated in an international cross-sectional and longitudinal study in 13 countries. Two preliminary patient-derived weighted questionnaires to assess the impact of psoriatic arthritis on the patient's life were proposed. One questionnaire was for clinical trials and included nine domains, and the other was for clinical practice and included 12 domains. The domains included pain, fatigue, and skin problems, ability to work and participate in leisure activities, functional capacity, discomfort, sleep disturbance, coping, and anxiety. The domains of embarrassment and/or shame, social participation, and depression were added for the clinical practice questionnaire.[66]

## PATHOGENESIS

Many studies have explored key components of disease pathogenesis, including the contribution of genetic factors, the role of infection or trauma, studies of animal models or involved sites of disease, and the importance of components of the immune system, such as cytokines.

### Genetic Factors

Familial clustering of psoriasis and psoriatic arthritis is well described. Twin studies in psoriasis have shown a high rate of concordance in monozygotic twins.[67] The genetic basis for this clustering has been the subject of extensive investigations in psoriasis, but has been much less well studied in psoriatic arthritis. Studies of psoriatic arthritis have often included patients as a subset of larger psoriasis cohorts, and the diversity of clinical phenotypes has not often been recognized.

A strong association between psoriasis and the HLA-C region of the major histocompatibility complex (MHC) has long been recognized. Whether this was HLA-C*06 itself, found in approximately 60% of psoriasis cohorts, or a region

telomeric to this has been the subject of much controversy. Elder[68] definitively showed that the HLA susceptibility region for psoriasis is HLA-C*06, which is often in linkage disequilibrium with other HLA-B alleles such as HLA-B57, HLA-B37, and HLA-B13. The presence of HLA-C*06 is associated with an earlier patient age of onset of psoriasis (type 1 disease, <40 years old) and with more extensive and severe disease. Winchester et al,[69] confirmed by Eder et al,[70] have shown that in patients with psoriatic arthritis, the frequency of HLA-C*06 was lower than that in patients with psoriasis (28.7% vs. 57.5%; $P = 9.9 \times 10^{-12}$). Additional associations in psoriatic arthritis have been found with HLA-B27, HLA-B08, HLA-B38, and HLA-B39. It was also observed that arthritis develops much closer to the appearance of psoriasis in the HLA-B*27:05:02 or B*39:01:01 subset than in the HLA-C*06:02 subset of patients with psoriatic arthritis. Patients with B*27:05:02 had similar penetrance for musculoskeletal and skin disease, and they were roughly contemporaneous in onset; nearly a third of B*27:05:02 patients had the onset of skin disease after the onset of musculoskeletal disease, psoriatic arthritis sine psoriasis. Conversely for HLA-C*06:02 patients, the interval between skin disease and musculoskeletal disease was more than a decade, and the HLA-C*06:02 gene appeared to specify an effect that included highly penetrant severe cutaneous disease and delayed milder musculoskeletal disease with greatly reduced penetrance.

In a further exploratory study, HLA-B27 was also strongly associated with symmetrical sacroiliitis, enthesitis, and dactylitis. In contrast, HLA-B08 was associated with predominant asymmetrical sacroiliitis, joint fusion, joint deformities, and dactylitis. HLA-C06 was negatively associated with asymmetrical sacroiliitis.[71]

These findings have been interpreted to suggest that the MHC association with psoriasis lies close to the HLA-C region, whereas the MHC association with the articular manifestations more likely lies in or close to the HLA-B region. A study of a large cohort of patients with psoriatic arthritis in the United Kingdom found HLA-C*06 to be in linkage disequilibrium with HLA-DRBI*07, and that possession of both alleles was associated with fewer involved or damaged joints (Pauline Ho, personal communication, 2007).[72]

Other genes within the MHC region have been explored in psoriasis and psoriatic arthritis. Tumor necrosis factor (TNF) promoter polymorphisms or a gene in linkage disequilibrium with TNF may predispose the patient to or increase susceptibility to psoriasis and to psoriatic arthritis. One study has found a further association between the TNF-308 A allele and disease progression in early psoriatic arthritis.[73] Whole-genome scans in psoriasis have identified additional non-MHC susceptibility regions, known as the *PSORS regions* on chromosomes 4, 6, and 17. To date, no candidate genes have been identified.

Increasing evidence suggests that an additional or distinct genetic contribution is responsible for the development of psoriatic arthritis. Investigators have pointed to an class I MHC chain-related A (MICA)-A9 polymorphism, which confers additional relative risk, in particular for polyarticular disease, in patients with psoriasis who carry C*0602.[74] MICA-A9 polymorphism was found in linkage disequilibrium with HLA-B alleles (B*5701, B*3801). These results suggest that the *MICA* gene or other nearby genes may be involved in the development of psoriatic arthritis. Additionally, a genome scan identified a paternally influenced locus on chromosome 16—a region not known to be implicated in psoriasis susceptibility.[75]

Finally, two genome-wide association studies in psoriatic arthritis have been reported.[76,77] These studies have confirmed associations with HLA-C and interleukin (IL)-12B, and they have identified a new susceptibility region TRAF3IP2 on chromosome 6p, which encodes a protein involved in IL-17 signaling, and which interacts with members of the Rel/nuclear factor-κB (NF-κB) transcription factor family. Further studies[78] have provided genome-wide significant evidence for the involvement of the REL locus in psoriatic arthritis susceptibility, and confirmed the previously reported association with psoriasis vulgaris. A genome-wide significant association was found between psoriatic arthritis and a single-nucleotide polymorphism (SNP; rs13017599), detected at 2p16 near the REL locus encoding c-Rel (rs13017599, $P = 1.18 \times 10^{-8}$, OR, 1.27, 95% CI, 1.18-1.35).

## Environmental Factors

The role of environmental factors in triggering skin or joint disease in patients with psoriasis or psoriatic arthritis has been supported largely by clinical observations, although the mechanism is poorly understood. A strong association between guttate psoriasis and preceding streptococcal infections in children has long been recognized.[79] That this association might be related to a streptococcal superantigen has been proposed. Some authors have found bacterial antigens in synovial tissue samples from psoriatic arthritis patients, but this may be no different from those in non-inflammatory control participants.[80]

The Koebner phenomenon (see Figure 77-1H) has been reported to occur in 52% of patients with psoriasis. The Koebner phenomenon is the development of psoriasis along the site of skin trauma. It has been proposed that trauma may play a role in triggering episodes of joint inflammation, and the term *deep Koebner phenomenon* has been coined. Although the role of trauma has not been proved, in one study 24.6% of patients reported a traumatic event before the onset of arthritis.[81]

Finally, a link between stress and exacerbation of psoriasis has been proposed, supported largely by clinical observational studies. A similar association may exist in psoriatic arthritis, but this has not been systematically examined.

## Animal Models

Although spondyloarthropathy has been detected in a variety of primates, rodent models have proved helpful in deciphering pathogenic pathways. In rodents transgenic for class I HLA-B27 molecules, skin, nail, and joint features have been described that mimic some of the features of the human phenotype.[82] When HLA-B27 transgenic rats were raised in a germ-free environment, they seemed to be protected from joint disease. Mice genetically lacking class II MHC have developed skin and joint disease, but the disease is confined to the distal phalanges, with skin and nail disease also on the affected digits.[83] Involvement of the distal

phalanges and nails was reported in aging male DBA/1 mice from different litters that were caged together from 12 weeks.[84] In these animals, dactylitis, periostitis, and ankylosing enthesitis were observed.

Finally, JunB protein was shown to be expressed in healthy and in clinically uninvolved psoriatic skin, but expression was considerably reduced in involved psoriatic lesions.[85] Epidermal deletion of JunB and c-Jun in a mouse model resulted in skin and joint disease with 100% penetrance and a clinical and histologic phenotype highly consistent with human psoriasis and psoriatic arthritis. In further experiments, the same authors showed that the joint disease, but not the skin disease, required T and B cells and intact TNF receptor 1 signaling.

## Immunopathology

The key pathologic events in psoriatic arthritis occur in the skin, synovium, entheseal sites, and cartilage and bone. Pathobiologic features in the skin and synovium have been well described, but only a few studies have focused on the enthesis. In relation to cartilage and bone, studies have shown the presence of osteoclasts at the cartilage-pannus junction and high numbers of circulating osteoclast precursors in the circulation of psoriatic arthritis patients. Detailed studies similar to those done on RA on the synovial-cartilage-bone interface possibly could yield valuable information regarding joint destruction in psoriatic arthritis.

### Psoriasis Skin

Involved psoriasis skin is characterized by epidermal hyperplasia, mononuclear leukocytes in the papillary dermis, neutrophils in the stratum corneum, and an increase in various subsets of dendritic cells.[86] $CD8^+$ T cells are the predominant T cell subset chiefly found in the epidermis, whereas dermal T cells contain a mixture of $CD4^+$ and $CD8^+$. Most T cells in skin lesions express addressin, a cutaneous lymphocyte antigen, in contrast to circulating T cells and T cells found in the inflamed synovium in psoriatic arthritis.[87] Finally, vascular changes are prominent in psoriasis with impressive growth and dilation of superficial blood vessels.

### Psoriatic Synovium

Many early studies of synovial pathology in psoriatic arthritis highlighted the presence of prominent and striking vascular changes. In the first study that compared psoriatic arthritis and RA synovial tissue, quantitative immunopathologic analysis confirmed these prominent vascular changes and found that vessel number was significantly increased in psoriatic arthritis.[88] Lining layer hyperplasia was less marked in psoriatic arthritis, and fewer macrophages were seen trafficking into the synovium and out to the lining layer. The numbers of T lymphocytes and their subsets and the number of B cells were similar to the frequency found in RA. Although neutrophil infiltration was not assessed, this study examined adhesion molecule expression further in the two patient subgroups and found E-selectin expression to be considerably reduced in psoriatic arthritis. Many of these observations have been confirmed by other authors.

In a study by Kruithof and co-workers,[89] the synovial immunopathologic features in patients with spondyloarthropathy, including psoriatic arthritis, were compared with the features seen in RA.[89] Using a semiquantitative scoring system, the authors identified many features characteristic of the spondyloarthropathy group as a whole and in the psoriatic arthritis subgroup alone. Increased vascularity, higher neutrophil numbers (also seen in involved psoriasis skin), and a higher number of infiltrating $CD163^+$ macrophages, a marker of mature tissue macrophages, reliably distinguished spondyloarthropathy from RA. No significant differences were seen between oligoarticular versus polyarticular psoriatic arthritis.

The important role of the vasculature in psoriatic arthritis pathogenesis is perhaps most elegantly shown by the large numbers of tortuous and dilated blood vessels observed through an arthroscopic view of psoriatic joints.[90] An interaction of key growth factors is thought to regulate closely the new vessel formation or angiogenic process. Growth factors, including TNF, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), angiopoietins (ANG-1, ANG-2), and vascular endothelial growth factor (VEGF), have been described in skin and synovial tissue.[91,92] Because this expression is found at an early stage of inflammation, it may represent a primary event in psoriatic arthritis versus a reaction or response to hypoxia. One possibility is that a genetic predisposition exists to endothelial activation, which results in new vessel formation and increased cellular trafficking.

Of interest, two studies have identified a change in synovial $CD3^+$ T cell infiltration as correlating with clinical response in patients commencing biologic therapies[93] (Pontifex, personal communication). Changes in CD3 infiltration also correlated with changes in MRI assessment of synovitis (Pontifex). Changes in CD3 infiltration may well prove to be a useful tissue biomarker of treatment response.

### Entheseal Sites

Laloux and associates[94] described the immunopathologic features of the enthesis in patients undergoing joint replacement surgery with spondyloarthropathy, including psoriatic arthritis, and compared them with RA. The number of patients was small in this study, but a consistent increase in $CD8^+$ T cell expression was observed at the enthesis in patients with psoriatic arthritis compared with RA patients. Ultrasound-guided biopsy of five sites of acute enthesitis in early spondyloarthropathy confirmed an inflammatory response with increased vascularity and cellular, predominantly macrophage, infiltration.[95] These findings are consistent with the well-described association of psoriatic arthritis with class I HLA antigens. They also are consistent with the previously described dominance of activated and mature $CD8^+$ T cells in psoriatic arthritis synovial fluid samples compared with RA.[96] It is attractive to suggest that enteseally derived antigens might trigger an immune response in adjacent synovial tissue. To date, evidence for this hypothesis has not been found, although this is clearly an area for future study. A search for candidate antigens common to the enthesis and the skin might be informative.

## Cytokines

Synovial explant tissues obtained from psoriatic arthritis joints have been shown to produce higher levels of the T helper (Th) type 1 cytokines IL-2 and interferon-γ protein than explants similarly cultured from osteoarthritis and RA patients.[97] This Th1 lymphocyte profile also has been observed in psoriasis plaques.[98] The cytokines IL-1β and TNF were released by psoriasis synovial explants in high concentrations. In contrast, IL-4 and IL-5 were not identified, but IL-10 was highly expressed in psoriatic synovium, although not in skin. A similar pattern of cytokine production in psoriatic arthritis synovium was shown by using immunohistochemical and gene expression techniques.[99,100] Other innate cytokines, such as IL-18 and IL-15, are present in psoriatic arthritis synovial tissue and are downregulated by methotrexate therapy.

The role of Th17 cells has been explored, and an important immunomodulatory role has been better established in psoriasis than in psoriatic arthritis. Comparison of lesional skin gene expression in responders versus nonresponders to etanercept revealed rapid downmodulation of innate IL-1β and IL-8 sepsis cascade cytokines in both groups, but only responders downregulated IL-17 pathway genes to baseline levels.[101] An increase in circulating Th17 cells has been found in psoriatic arthritis,[102] and an increase in IL-17 has been found in skin, synovial tissue, and synovial fluid of psoriatic arthritis patients.[103] Menon and co-workers demonstrated that IL-17$^+$CD4 T cells, consisting predominantly of CD8$^+$ T cells, are present at increased levels in psoriatic arthritis synovial fluid as compared with paired samples of psoriatic arthritis peripheral blood and synovial samples from patient with RA. In addition, the frequency of these synovial fluid IL-17$^+$CD4$^-$ T cells is correlated with serologic, clinical, and imaging measures of psoriatic arthritis disease activity and erosive disease status.[104] Further supporting a key role for the Th17 pathway, genetic polymorphisms in key cytokines involved in differentiation of T cells to Th1 and Th17 subtypes, IL-12 and IL-23, have been found to be associated with susceptibility to both psoriasis and psoriatic arthritis,[105] and inhibition of the common p40 subunit of IL-12/IL-23 has led to clinical improvement in both skin and joint manifestations.[106] Further observations by Benham et al[107] revealed a higher proportion of IL-17-producing cells within the peripheral blood mononuclear cells (PBMCs) of psoriatic arthritis and psoriasis patients compared with that of healthy controls (1.0% and 1.1% vs. 0.57%, respectively; $P$ <0.05, $P$ <0.01, respectively). The percentage of IL-22$^+$ CD4$^+$ T cells were also increased in PBMCs from patients with psoriatic arthritis compared with those of healthy controls (0.95% vs. 0.51%; $P$ <0.05), but the percentage increase in psoriasis patients relative to controls (0.81% vs. 0.51%; $P$ >0.05) was not statistically significant. There was no difference in the frequency of IL-17$^+$ and IL-22$^+$CD4$^+$ T cells between patients with psoriasis alone and those with psoriatic arthritis. In patients with psoriatic arthritis, CCR6$^+$ and IL-23R$^+$ T cell numbers were elevated in synovial fluid compared with peripheral blood. In addition to genetic associations described above, there are now substantial data implicating Th17-Th22-IL-23 axis in mouse models of both psoriasis and spondyloarthropathy; Sherlock and colleagues[108] have shown that the anatomic sites that are inflamed in spondyloarthropathy are primed to rapidly react to IL-23 by the presence of a previously unidentified population of IL-23R$^+$ resident cells and also in vivo exposure to IL-23 is sufficient to induce highly specific entheseal inflammation in the absence of Th17 cells and with rapid kinetics. The previous observations strengthen the case for the central involvement of the Th17-Th22-IL-23 axis in both psoriasis and psoriatic arthritis.

TNF levels are elevated in psoriatic skin, synovium, and joint fluid of patients with psoriatic arthritis.[97,109] Several lines of evidence support the concept that TNF is an important cytokine in the psoriatic joint. TNF transgenic mice exhibit extensive bone destruction similar to that observed in some psoriatic arthritis patients. In a study of 129 patients with early psoriatic arthritis, patients with erosions were significantly more likely to have the TNF-308 A allele, an allele associated with high TNF production.[73] As mentioned earlier, immunohistochemical and gene expression studies have shown marked upregulation of TNF in the psoriatic synovial membrane. Histopathologic analysis of synovial specimens from eight spondyloarthropathy patients, four of whom had psoriatic arthritis, treated with the anti-TNF monoclonal antibody infliximab revealed decreased vascularity, synovial lining thickness, and mononuclear cell infiltration after therapy.[110,111] In another study, a significant reduction in the quantity of infiltrating macrophages, the CD31$^+$ vascular area, αvβ3$^+$ neovessels/Ulex europaeus agglutinin–positive vessels, VEGF and its receptor KDR/flk-1 (VEGFR-2), and SDF-1–positive vessels in psoriatic arthritis synovium was noted after 8 weeks (three infusions) of infliximab treatment.[112]

## Matrix Metalloproteinases and Cartilage Destruction

Radiographs of psoriatic joints often reveal cartilage loss manifested as joint space narrowing. Similar to RA, matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) were identified in psoriatic arthritis synovial lining and sublining layers.[113,114] In particular, immunohistochemical studies revealed that MMP-9 localized to blood vessel walls, whereas MMP-1, MMP-2, MMP-3, TIMP-1, and TIMP-2 showed a cellular and interstitial staining pattern in the synovial lining. Serum levels of MMP-3 exhibited a marked and rapid decrease after successful anti-TNF therapy, raising the possibility that this molecule may serve as a biomarker. In another study, similar levels of MMP-1 and MMP-3 mRNA were detected in RA and psoriatic arthritis synovial tissue despite the fact that RA patients exhibited more erosions on plain radiographs.[115] The elevated ratio of MMPs to TIMP-1 in synovial tissue favored cartilage degradation, although expression of MMPs was not significantly elevated at the cartilage-pannus junction compared with other sites. These reports indicate that MMPs are upregulated in psoriatic arthritis synovium, but their precise functions remain to be defined.

## Bone Remodeling

Radiographs of psoriatic arthritis joints can reveal markedly altered bone remodeling in the form of bone resorption (tuft resorption or osteolysis, large eccentric erosions, and

pencil-in-cup deformities) and new bone formation (periostitis, spur or enthesophyte formation, bony ankylosis). Important in bone resorption, psoriatic joint biopsy specimens show large multinucleated osteoclasts in deep resorption pits at the bone-pannus junction.[116] Osteoclastogenesis (differentiation of monocytes into osteoclasts) is a contact-dependent process directed by osteoblasts and stromal cells in the bone marrow. These cells release signals necessary for differentiation of an osteoclast precursor derived from the $CD14^+$ monocyte population into an osteoclast.

One of these signals is the receptor activator of NF-κB ligand (RANKL), a member of the TNF superfamily that binds to receptor activator of NF-κB (RANK) on the surface of osteoclast precursors and osteoclasts. This ligand-receptor interaction stimulates proliferation and differentiation of osteoclast precursors and activation of osteoclasts. It has been proposed that the relative expression of RANKL and of its natural antagonist osteoprotegerin (OPG) ultimately controls osteoclastogenesis. In psoriatic arthritis synovial tissues, marked upregulation of RANKL protein and low expression of osteoprotegerin were detected in the adjacent synovial lining.[116] Osteoclasts also were noted in cutting cones traversing the subchondral bone, supporting a bidirectional attack on the bone in psoriatic joints. In addition, osteoclast precursors, derived from circulating $CD14^+$ monocytes, were markedly elevated in the peripheral blood of patients with psoriatic arthritis compared with healthy controls. Treatment of patients with psoriatic arthritis with anti-TNF agents significantly decreased the level of circulating osteoclast precursor, supporting a central role for TNF in the generation of this precursor population. Although abundantly expressed, there appeared to be little relationship between expression of RANKL, OPG, and RANK and both systemic and local inflammation,[117] and also little relationship between the decline in osteoclast precursor numbers and bone marrow edema following etanercept treatment.[118]

The mechanisms responsible for new bone formation in the psoriatic joint are poorly understood. Higher circulating levels of bone formation markers, in particular bone alkaline phosphatase, in patients with psoriatic arthritis compared with patients with RA both before and after TNF inhibitor therapy has been demonstrated (Szentepetery, personal communication). TGF-β and VEGF may be pivotal in this process of new bone formation, given that TGF-β is strongly expressed in synovial tissues isolated from patients with ankylosing spondylitis and synergizes with VEGF to induce bone formation in animal models.[119] De Klerck and colleagues[120] showed that the bone morphogenetic proteins (BMPs) BMP-2 and BMP-7 are upregulated in regions of pathologic new bone formation. These investigators showed that expression of phosphorylated Smad-1 and Smad-5, important signaling molecules downstream of BMP, was markedly increased in regions of new bone formation taken from the calcaneus in a patient with Achilles tendinitis and periostitis. These studies provide evidence that potential mediators of ankylosis and periostitis in the psoriatic enthesis and joint include BMP molecules and possibly VEGF and TGF-β. Finally, the Wnt pathway and the balance between Wnt and the Wnt antagonist dickkopf-1 (DKK-1), which binds to the Wnt receptor complex on the surfaces of osteoblast lineage cells, may well be of considerable importance in the disordered bone remodeling found in psoriatic arthritis.

## CONCLUSION

In considering a model for disease pathogenesis in psoriatic arthritis, we have to take into account genetic susceptibility, the role of the environment, cellular immunologic mechanisms, secreted cytokines, chemokines, and other proteins. For some time, the primary hypothesis has been that psoriatic arthritis is an class I HLA-restricted, antigen-driven immune process (Figure 77-10). Considerable evidence has been presented to support this hypothesis; however, despite careful analysis of T cell-receptor phenotype, no antigen-driven process other than that driven by Epstein-Barr virus has been identified.[121] The potential role of components of the innate immune response, such as Toll-like receptors or cells bearing natural killer receptors, is currently under active investigation. It is possible that the interaction of environmental factors, such as those derived from pathogens or expressed after trauma, with Toll-like receptors in a genetically susceptible individual, may set in motion intra-cellular signaling events that lead to cytokine release, immune activation, and release of destructive enzymes such as MMPs (Figure 77-11).

## TREATMENT

In considering treatment strategies for psoriatic arthritis, the diverse nature of the clinical phenotype (peripheral arthritis, skin and nail disease, axial disease, dactylitis, and enthesitis) may complicate therapeutic decisions because not all treatments are effective for all features, and patients often display a mixture of all of the features simultaneously. EULAR has published recommendations for the treatment of psoriatic arthritis based on a systematic literature review. The reader is referred to this publication for a detailed review of the evidence (Table 77-4; Figure 77-12).[122] Treatment choices may be driven by the disease feature considered most severe at the time of evaluation. Finally, in reviewing the evidence for therapeutic effect presented in the following section, recommendations from the Agency for Health Care Policy and Research were used, wherein interventions are scored by categories of evidence (level 1 through 4) and strength of recommendation (grade A through D).[123]

### Traditional Agents

Although little published evidence exists of a favorable therapeutic effect in psoriatic arthritis, nonsteroidal anti-inflammatory drugs are most often the agents first used in psoriatic arthritis, whatever the clinical phenotype (level 1b, grade A).[124] Expert opinion supports the use of nonsteroidal anti-inflammatory drugs, although occasional exacerbations of psoriasis have been reported. The use of systemic corticosteroids is not evidence based (level 4, grade D), although 24% of patients in one study were taking prednisolone.[125] Concerns have arisen suggesting that exacerbations of psoriasis may follow corticosteroid withdrawal. No randomized controlled trials have examined intra-articular steroids in psoriatic arthritis or the use of local entheseal or

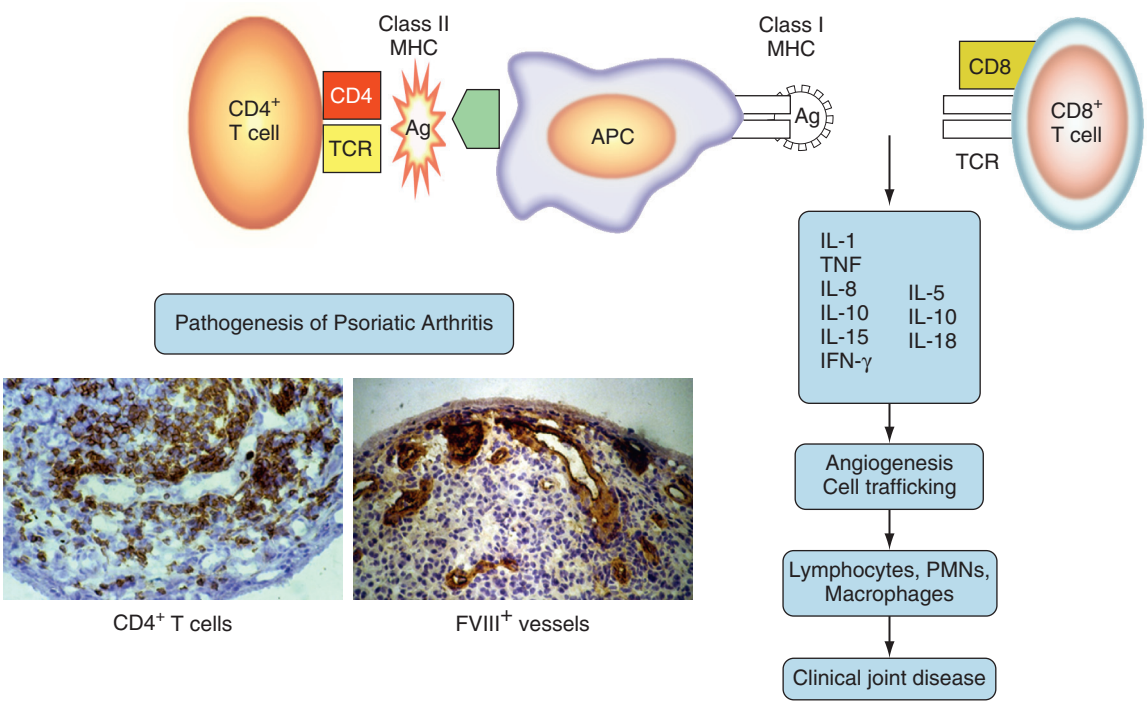



**Figure 77-10** Traditional disease pathogenesis model in psoriatic arthritis. APC, Antigen-presenting cell; FVIII, factor VIII; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; PMNs, polymorphonuclear neutrophils; TCR, T cell receptor; TNF, tumor necrosis factor.

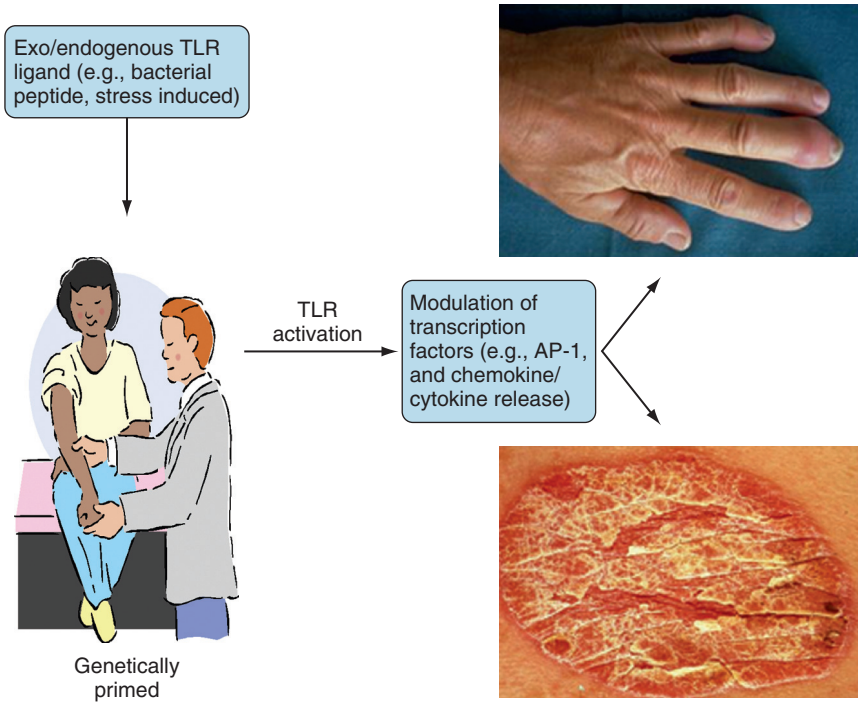



**Figure 77-11** Alternative model incorporating new disease pathogenesis concepts. TLR, Toll-like receptor.

dactylitis injections. Expert opinion indicates that intra-articular steroids can be quite effective, especially in oligoarticular disease, or where localized entheseal involvement is present, as in plantar fasciitis (level 4, grade D). Mild skin disease (PASI <10) is usually controlled with topical steroids or vitamin D derivatives; the latter are best used for maintenance therapy.[126]

Systemic therapy is considered in patients with three or more inflamed joints despite conventional therapy, as described previously; persistent or treatment-resistant axial, entheseal, or dactylitic disease, especially in which multiple sites are involved; or moderate or severe psoriasis (PASI >10). Randomized controlled trials of disease-modifying antirheumatic drugs (DMARDs) are few and limited by size.

**TABLE 77-4** Updated EULAR Recommendations for the Management of PsA with Levels of Evidence, Grade of Recommendations, and Level of Agreement

| | Overarching principles | Level of agreement (mean ± SD) |
|---|---|---|
| A. | PsA is a heterogeneous and potentially severe disease, which may require multidisciplinary treatment. | 9.6 ± 1.1 |
| B. | Treatment of patients with PsA should aim at the best care and must be based on a shared decision between the patient and the rheumatologist, considering efficacy, safety, and costs. | 9.2 ± 1.7 |
| C. | Rheumatologists are the specialists who should primarily care for the musculoskeletal manifestations of patients with PsA; in the presence of clinically significant skin involvement, a rheumatologist and a dermatologist should collaborate in diagnosis and management. | 9.5 ± 0.8 |
| D. | The primary goal of treating patients with PsA is to maximize health-related quality of life through control of symptoms, prevention of structural damage, normalization of function and social participation; abrogation of inflammation is an important component to achieve these goals. | 9.6 ± 1.0 |
| E. | When managing patients with PsA, extra-articular manifestations, metabolic syndrome, cardiovascular disease, and other comorbidities should be taken into account. | 9.5 ± 1.0 |

| | Recommendations | Level of evidence | Grade of recommendation | Level of agreement (mean ± SD) |
|---|---|---|---|---|
| 1. | Treatment should be aimed at reaching the target of remission or, alternatively, minimal/low disease activity, by regular monitoring and appropriate adjustment of therapy. | 1b | A | 9.6 ± 0.9 |
| 2. | In patients with PsA, NSAIDs may be used to relieve musculoskeletal signs and symptoms. | 1b | A | 9.6 ± 0.8 |
| 3. | In patients with peripheral arthritis, particularly in those with many swollen joints, structural damage in the presence of inflammation, high ESR/CRP, and/or clinically relevant extra-articular manifestations,[a] csDMARDs should be considered[b] at an early stage,[a] with methotrexate preferred in those with relevant skin involvement.[b] | [a]: 3<br>[b]: 1b | B | 9.4 ± 0.8 |
| 4. | Local injections of glucocorticoids should be considered as adjunctive therapy in PsA[a]; systemic glucocorticoids may be used with caution at the lowest effective dose.[b] | [a]: 3b<br>[b]: 4 | C | 9.1 ± 1.2 |
| 5. | In patients with peripheral arthritis and an inadequate response to at least one csDMARD, therapy with a bDMARD, usually a TNF inhibitor, should be commenced. | 1b | B | 9.5 ± 0.7 |
| 6. | In patients with peripheral arthritis and an inadequate response to at least one csDMARD in whom TNF inhibitors are not appropriate, bDMARDs targeting IL-12/23 or IL-17 pathways may be considered. | 1b | B | 9.1 ± 1.1 |
| 7. | In patients with peripheral arthritis and an inadequate response to at least one csDMARD in whom bDMARDs are not appropriate, a targeted synthetic DMARD, such as a PDE4 inhibitor, may be considered. | 1b | B | 8.5 ± 1.4 |
| 8. | In patients with active enthesitis and/or dactylitis and insufficient response to NSAIDs or local glucocorticoid injections, therapy with a bDMARD should be considered, which, according to current practice, is a TNF inhibitor. | 1b | B | 9.1 ± 1.2 |
| 9. | In patients with predominantly axial disease that is active and has insufficient response to NSAIDs, therapy with a bDMARD should be considered, which, according to current practice, is a TNF inhibitor. | 1b | B | 9.6 ± 0.6 |
| 10. | In patients who fail to respond adequately to a bDMARD, switching to another bDMARD should be considered, including switching between TNF inhibitors. | 1b | B | 9.6 ± 0.7 |

The level of evidence was determined for different parts of the recommendation (referred to as a and b) where necessary.

The level of agreement was computed as a 0–10 scale.

bDMARD, Biological DMARD; CRP, C-reactive protein; csDMARDs, conventional synthetic DMARDs, such as methotrexate, sulfasalazine or leflunomide; DMARD, disease-modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; NSAIDs, nonsteroidal anti-inflammatory drugs; PDE, phosphodiesterase; PsA, psoriatic arthritis; TNF, tumour necrosis factor.

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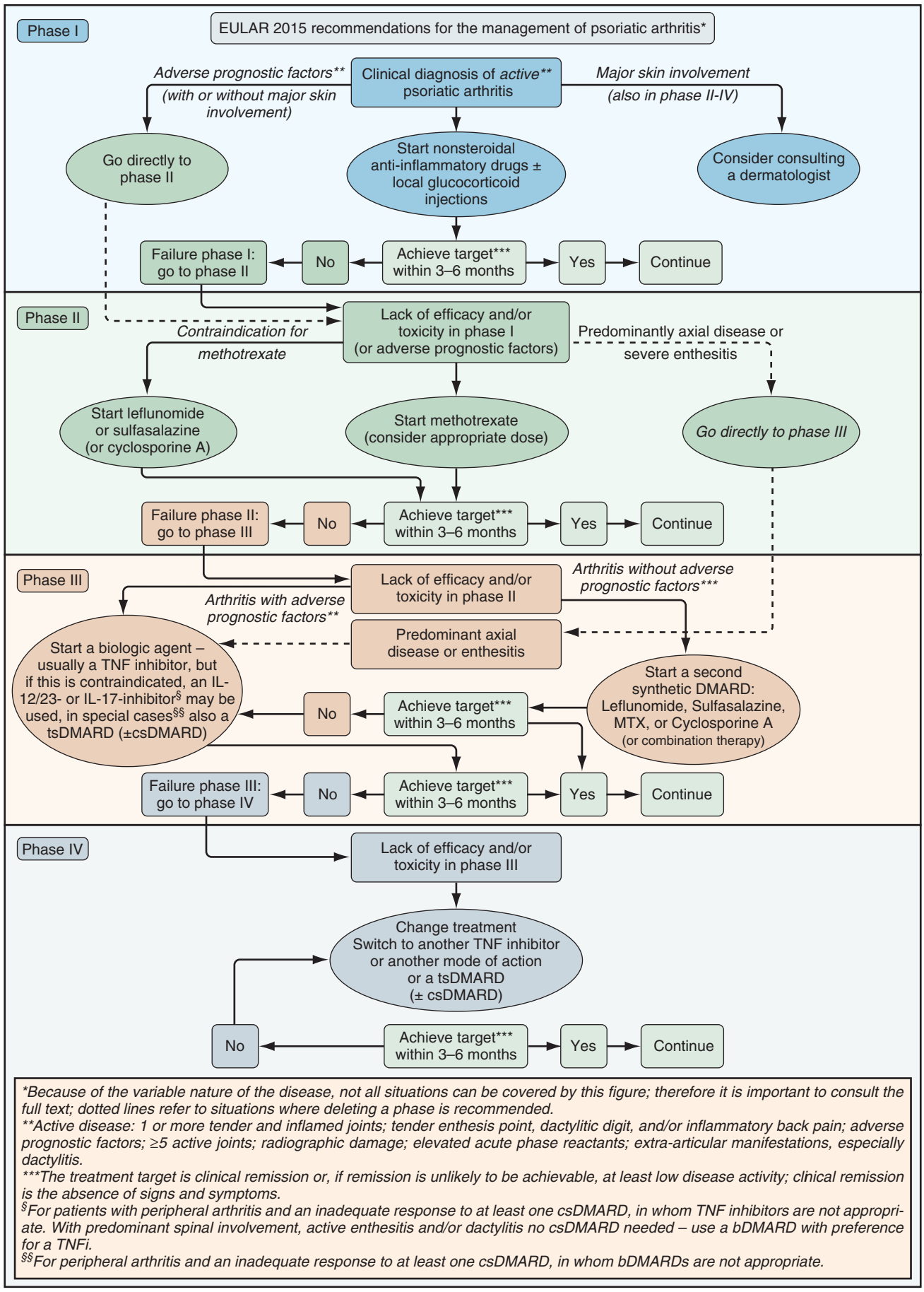



**Figure 77-12** The EULAR 2015 algorithm for treatment of PsA with pharmacological nontopical treatments. bDMARD, Biological DMARD; csDMARDs, conventional synthetic DMARD; DMARD, disease-modifying anti-rheumatic drug; EULAR, European League Against Rheumatism; IL, interleukin; MTX, methotrexate; PsA, psoriatic arthritis; tsDMARD, targeted synthetic DMARD. *(Reproduced from Gossec L, Smolen JS, Ramiro S, et al: European League Against Rheumatism [EULAR] recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update.* Ann Rheum Dis *2015 Dec 7. [Epub ahead of print], with permission from BMJ Publishing Group Ltd.)*

Based on evidence and expert opinion, nearly all DMARDs may have small to moderate beneficial effects on peripheral joints, enthesitis, and dactylitis.[124,127,128] Axial features and nail disease do not seem to respond.[129] Good or moderate improvements in skin disease have been reported with some of the older systemic agents, such as methotrexate, cyclosporine, sulfasalazine, leflunomide, and acitretin (all level 1b, grade A).[126]

The best evidence for disease-modifying anti-rheumatic drug (DMARD) use comes from studies of peripheral joint disease and of psoriasis. Six randomized controlled trials have studied the use of sulfasalazine in psoriatic arthritis (level 1a, grade A). The largest study included 221 patients. Of the patients using the PsARC scoring system, 59% achieved a therapeutic response, but consistent with other studies, a high therapeutic response (42.7%) was also noted in placebo-treated patients.[58]

Methotrexate remains for many rheumatologists the DMARD of first choice for patients with psoriatic arthritis, but evidence for its use is limited (level 3, grade B). A small, prospective randomized controlled trial concluded that methotrexate was as effective as cyclosporine. Another study of 72 patients with active psoriatic arthritis and an incomplete response to methotrexate, in which cyclosporine was added, showed significant differences only in synovitis as detected by MSUS and PASI scores in favor of the combination therapy.[33,130] Although evidence for methotrexate is lacking, an open study reported significant reductions in synovial cellular infiltration and in cytokine gene expression after 3 months of therapy.[100] Another randomized placebo-controlled trial of methotrexate in psoriatic arthritis that included 221 patients found no evidence that methotrexate improved synovitis in patients with active psoriatic arthritis,[131] It is worth noting that this trial had a number of limitations. First, it included patients with mild disease (≥1 acute peripheral joint). Second, it allowed for a maximum dose of only 15 mg/week and, finally, it used PsARC as the primary outcome measure, when it has been shown to be associated with high placebo response.

Although evidence suggests that cyclosporine may be as effective as methotrexate, its use is limited because its toxicity profile is considered to be high (level 1b, grade B).[132]

Perhaps the best randomized controlled trial in psoriatic arthritis of a DMARD examined the use of leflunomide (level 1b, grade A).[133] This trial included 190 patients who received leflunomide or placebo for 24 weeks. Of patients treated with leflunomide, 59% of patients met the primary response criteria (PsARC),compared with 30% of patients given placebo, with significant, although small, improvements in other individual parameters, including joint scores, health assessment questionnaire results, PASI, and Dermatology Life Quality Index scores. Regarding some older DMARDs, such as gold salts and anti-malarials, no evidence of treatment benefit has been found; exacerbation of psoriasis has been reported, and these agents cannot be recommended. One small, randomized controlled trial with azathioprine suggested benefit with a reduction in Ritchie score (level 2b, grade B). Finally, apart from the cyclosporine/methotrexate study referred to earlier, little or no evidence suggests that DMARD combination therapy is beneficial or safe in psoriatic arthritis.

With the exception of psoriasis, there is a paucity of evidence that DMARDs are beneficial for the other features of psoriatic arthritis, including dactylitis, axial disease, or enthesitis. Absence of evidence does not mean absence of an effect, however. Further randomized controlled trials that specifically examine these features in psoriatic arthritis are required. In psoriasis, methotrexate and cyclosporine have been shown to be highly and probably equally effective (level 1b, grade A).[126] Adverse effects, in particular with cyclosporine, may limit usage in some patients.

## BIOLOGICS

The approach to treatment in psoriatic arthritis has changed considerably with the introduction of biologic therapies. As a result of numerous large, well-conducted, randomized controlled trials, accumulating evidence indicates that anti-TNF treatments are effective in controlling peripheral arthritis symptoms and signs, improving quality of life, and preventing radiologic progression (overall level 1b, grade A).[124] Indeed, higher rates of DAS28 remission are found in patients with psoriatic arthritis compared with patients with RA after 1 year of anti-TNF therapy.[134] Patients receiving 25 mg subcutaneously twice weekly of etanercept showed significant improvement in ACR20 responses (59% vs. 15%) at 12 weeks compared with placebo.[135] At 12 months, radiographic disease progression (modified total Sharp score) was inhibited in the etanercept group (–0.03 U) compared with worsening of +1.00 U in the placebo group.

Although the anti-TNF therapies have not been compared in any study, their effect on peripheral arthritis seems to be similar. Evidence indicates that anti-TNF therapies are also effective for other disease features, such as nail disease, enthesitis, and dactylitis.[127,128,136] These studies are limited by the absence of a validated instrument to measure these features. With psoriasis, the effects of anti-TNF therapies can be quite dramatic.[137] In particular with antibody therapy, highly significant improvements in PASI scores were achieved (e.g., PASI = 75 in 59% of adalimumab-treated patients vs. 1% in placebo-treated patients after 24 weeks; PASI = 90 in 50%).[138] Better skin but not articular responses have been demonstrated in etanercept patients treated with 50 mg twice weekly as compared with once weekly.[139]

Other TNF inhibitors that have been licensed for the treatment of psoriatic arthritis include golimumab and certolizumab pegol. In a study of 409 patients, ACR20 response was significantly greater in certolizumab pegol, 200 mg every 2 weeks, and 400 mg every 4 weeks-treated patients than placebo (58.0% and 51.9% vs. 24.3% [$P$ <0.001]) at week 12.[140] Golimumab was evaluated in 405 patients. Forty-eight percent of patients receiving golimumab achieved an ACR20 response, compared with 9% of patients in placebo group.[141] Five year data on golimumab confirms clinical efficacy, safety, and inhibition of radiographic progression (mean change in total SHS ≤0.3).

Other novel therapies that target different pathways in psoriatic arthritis have been developed in recent years. Ustekinumab is a fully human IgG1κ monoclonal antibody that binds to the common p40 subunit shared by IL-12 and 23. It has now been approved for psoriasis and psoriatic

arthritis. It has shown significant and sustained improvements in psoriatic arthritis signs/symptoms in a diverse population of patients with active psoriatic arthritis (ACR20, 43.8% ustekinumab-treated group vs. 20.2% placebo). At 12 months, ustekinumab was shown to inhibit radiographic progression of joint damage (Change in PsA-modified van der Heijde-Sharp (vdH-S) score from baseline to week 52; 1.2 ± 5.4, 0.6 ± 2.6, 0.7 ± 3.7 for placebo, ustekinumab, 45 mg and 90 mg, respectively).[142] There is accumulating evidence that IL-17 is central to the pathogenesis of psoriatic arthritis. The blockade of IL-17A has been proposed as a therapeutic target in the treatment of psoriatic arthritis. Secukinumab is a fully human, high-affinity, anti-IL-17A monoclonal antibody that binds to and neutralizes IL-17A. Secukinumab has been recently approved for both psoriatic arthritis and ankylosing spondylitis. In a phase 3, double-blind, placebo-controlled trial, 397 patients with psoriatic arthritis were randomly assigned to receive subcutaneous secukinumab 300 mg ($n$ = 100), 150 mg ($n$ = 100), 75 mg ($n$ = 99), or placebo ($n$ = 98) once a week from baseline and then every 4 weeks from week 4. A significantly higher proportion of patients achieved an ACR20 at week 24 with secukinumab 300 mg (54%, $P$ <0.0001), 150 mg (51%, $P$ <0.0001), and 75 mg (29%, $P$ = 0.039) versus placebo (15%). Moreover, 52-week data from the same study suggests that efficacy of secukinumab is sustained over long periods.[143] Inhibition of IL-17 signaling by brodalumab—a human monoclonal antibody against IL-17 receptor A (IL-17RA)—also induced significant clinical responses in patients with psoriasis. In a recent study in patients with PsA, ACR20 response at week 12 was achieved in 37% and 39% in patients receiving 2 doses of brodalumab versus 18% receiving placebo. These findings show that IL-17RA is a potential target for the treatment of psoriatic arthritis, with the inhibition of downstream pathways associated with improvements in arthritis, psoriasis and physical function.[144] There is early promise of JAK inhibitors in both psoriasis and psoriatic arthritis. A possible mechanism might be down regulation of the IL-23 receptor, which in turn might affect IL-17 expression.[144a] Finally, apremilast—a non-biologic small molecule that inhibits phosphodiesterase 4—has now been approved in the United States by the U.S. Food and Drug Administration for the treatment of psoriatic arthritis. At week 16, more apremilast, 20 mg BID (31%), and 30 mg BID (40%) patients achieved ACR20 versus placebo (19%) ($P$ <0.001). Apremilast demonstrated an acceptable safety profile and was generally well tolerated.[145]

Recently, the role of methotrexate as a co-medication in anti-TNF inhibitor treatment for psoriatic arthritis has been evaluated by using the Norwegian DMARDs registry. The study analyzed 440 patients, 170 on anti-TNF only and 270 on combination therapy methotrexate-anti-TNF. Results showed that anti-TNF drug survival of the group on co-medication with methotrexate was superior to those not on methotrexate. The effect was mainly seen in those on infliximab, which leaves open the possibility of a role for methotrexate in inhibiting development of drug antibodies when patients are treated by monoclonal antibodies.[146] The first study that used a treat-to-target approach in psoriatic arthritis has very recently been published. In the study, 206 patients were randomly assigned to either tight control (step-up regimen aiming for minimal disease activity by using methotrexate, combination DMARDs, and anti-TNF) or to standard care. In the tight control group, 61.7% achieved ACR20 response compared with 44.6% in the standard care group. There were more adverse events in the tight control but no unexpected serious adverse event.[147] This study highlights the possibility of adopting a treat-to-target approach in psoriatic arthritis and accepting minimal disease activity as a potential target in the future.

Alefacept, a fusion protein of soluble lymphocyte function antigen 3 with Fc fragments of IgG1, that was approved for the treatment of psoriatic arthritis, has since been withdrawn because of a business decision by its manufacturer. Efalizumab, a monoclonal antibody that was approved for the treatment of psoriasis, has also been withdrawn because of safety issues.

In summary, the therapeutic options for psoriatic arthritis continue to expand, and modern biologic therapies mean that psoriatic arthritis patients are reaching levels of disease control previously not thought possible.

However, these drugs are expensive and place a huge burden on health systems. It is noteworthy that some patients do not respond to biologics, and some treatments may lose efficacy with time, or patients may begin to experience intolerable side effects.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 78

# Enteropathic Arthritis



Frank A. Wollheim

**KEY POINTS**

- The gut wall is a leaky barrier exposed to commensal and pathogenic microorganisms.
- Microbiota are essential for maturation and regulation of the immune system.
- Gastrointestinal lymphoid tissue–microbiota interaction balances between inflammatory defense and tolerance.
- Genetic polymorphisms in Crohn's disease can result in relative immune deficiency.
- Some types of joint disease in inflammatory bowel disease are genetically determined.
- Human leukocyte antigen-B27 locus interacts with non-major histocompatibility complex genes as a determinant of disease risk.
- Celiac disease is surprisingly common in adults, 25% of whom have joint manifestations.
- Patients with Whipple's disease often present with joint symptoms.
- Microscopic colitis is accompanied by extraenteric autoimmune manifestations.

Interest in gut microbiota and their role in the pathogenesis of human disease has increased in recent years, resulting in more than 2000 publications on the topic in 2013 alone. A number of diseases, including obesity, irritable bowel syndrome, and rheumatoid arthritis (RA), appear to be linked to changes in the gut microbiota. This chapter will begin with an update on gut physiology, intestinal microbiota, and the gut-associated lymphoid system (GALT). It will then address musculoskeletal problems associated with inflammatory bowel disease (IBD), celiac disease (CeD), and other less common conditions with prominent gut involvement. Reactive arthritis, although often triggered by pathogenic microorganisms in the gastrointestinal (GI) tract, is addressed in Chapter 76. Knowledge regarding the signals from the gut which generate joint disease still remains incomplete. The fine tuning of permeability, the interactions between the microbiota and cells in the gut mucosa, and the biology of GALT and its role in the development of arthritis are the main topics addressed in this chapter.

## GUT MUCOSA BIOLOGY

**KEY POINTS**

- The gut is covered by mucin, which forms an unstirred layer.
- The gut epithelium is impermeable to most hydrophilic solutes.
- The epithelial tight junctions regulate gut wall permeability.
- Myosin light-chain kinase is a regulator of tight junction and is influenced by TNF.
- Disturbed gut wall permeability is present in several diseases, including non-alcoholic fatty liver disease.

The GI tract has three major functions. It serves as a barrier against hostile environmental factors, it performs fluid and food absorption and excretion of waste products, and it produces major trophic host functions. The surface area of the gut mucosa was previously considered as large as a tennis court. However, new research indicates a surface measurement of "only" 32 $m^2$.[1] The mucosa is constantly exposed to potentially harmful environmental agents against which defense is essential, but tolerance toward normal microflora, referred to as *microbiota*, must also be maintained. To fulfill its diverse functions, the gut can be considered an immunologically privileged organ.[2]

The gut epithelium functions as a so-called *leaky* barrier. It consists of intestinal epithelial cells, including Paneth cells in deep crypts, mucus-secreting goblet cells, M cells, and cells of the innate and adaptive immune system. It is covered by a thin layer of fluid separated from luminal flow and peristalsis of the intestine by mucin, the "unstirred layer," which slows down the diffusion of solutes and reduces loss of digestive enzymes. An increased thickness of the unstirred layer may contribute to malabsorption in CeD. Underneath the basal membrane is the lamina propria, which contains immune cells, including lymphocytes, dendritic cells (DCs), and macrophages.[2] The plasma membrane of the epithelial cells is impermeable to most hydrophilic solutes in the absence of specific transporters. The structure of the interepithelial paracellular space consists of the tight junction, the adherens junction, and the desmosome (Figure 78-1B). The tight junction is considered

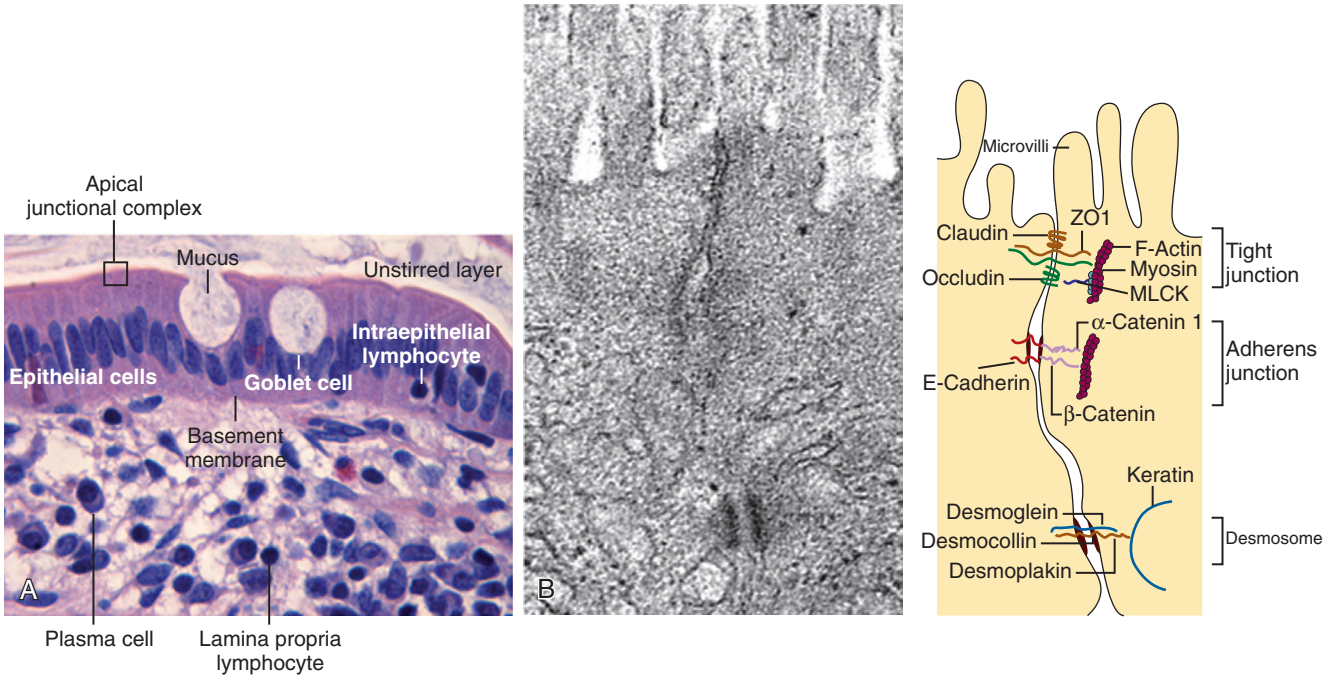



**Figure 78-1** Anatomy of the mucosal barrier. **A,** The human intestinal mucosa is composed of a simple layer of columnar epithelial cells, as well as the underlying lamina propria and muscular mucosa. Goblet cells, which synthesize and release mucin, as well as other differentiated epithelial cell types, are present. The unstirred layer, which cannot be seen histologically, is located immediately above the epithelial cells. The tight junction, a component of the apical junctional complex, seals the paracellular space between epithelial cells. Intraepithelial lymphocytes are located above the basement membrane but are subjacent to the tight junction. The lamina propria is located beneath the basement membrane and contains immune cells, including macrophages, dendritic cells, plasma cells, lamina propria lymphocytes, and, in some cases, neutrophils. **B,** An electron micrograph and corresponding line drawing of the junctional complex of an intestinal epithelial cell. Just below the base of the microvilli, the plasma membranes of adjacent cells seem to fuse at the tight junction, where claudins, zonula occludens 1 (ZO1), occludin, and F-actin interact. E-cadherin, α-catenin 1, β-catenin, catenin δ1 (also known as *p120 catenin;* not shown), and F-actin interact to form the adherens junction. Myosin light-chain kinase (MLCK) is associated with the perijunctional actomyosin ring. Desmosomes, which are located beneath the apical junctional complex, are formed by interactions among desmoglein, desmocollin, desmoplakin, and keratin filaments. *(From Turner JR: Intestinal mucosal barrier function in health and disease.* Nat Rev Immunol *9:799–809, 2009.)*

rate limiting for permeability. A perijunctional actomyosin ring condensation is regulated by myosin light-chain kinase (MLCK), which has a central role in regulating tight junction transport and in TNF-induced permeability increase.[3] TNF inhibition will influence permeability via inhibition of MLCK transcription. Overexpression of MLCK, on the other hand, results in increased permeability and activation of immune cells in transgenic mice.[4] Although initially healthy, these mice were prone to accelerated colitis if challenged.

Solutes smaller than 5000 Da can pass through the epithelium freely, whereas bacterial products and dietary antigens are dependent on active transport mechanisms. Altered gut permeability can be observed in several diseases, including nonalcoholic fatty liver disease.[5] In this condition, alterations in the expression of zona occludens 1 (ZO1) result in perturbation of permeability. Genetic and exogenous factors, such as drugs, nicotine, microorganisms,[6] and cytokines, are potential triggers of pathology by influencing paracellular functions in the gut epithelium.

Mucosal permeability and transport can be assessed by oral feeding of lactalbumin, lactoglobulin, polyethylene glycol particles, $^{51}$Cr-labeled ethylenediaminetetraacetic acid (EDTA), and sugars such as lactulose and mannitol, followed by urinalysis. In addition, intestinal permeability and function can be studied by regional perfusion with the help of endoscopic techniques that close off segments of the gut with inflatable balloons.[7] A study that applied enzyme-linked immunosorbent assays to fluid collected by this technique showed marked local immunity to a number of food-related antigens in patients with RA.[8] Ethnic differences in gut permeability have also been described.[9]

## GUT MICROBIOTA

**KEY POINTS**

**The gut microbiota contain 10 times more bacteria than the cells in the human body. Segmented filamentous bacteria cause dysbiosis by stimulating IL-17 formation.**

**Bacteria stimulate Toll-like receptor 5 signals in the mucosa, which enhances systemic immune response.**

**Environmental influences on risk for arthritis include antibiotics, smoking, and stress.**

The human gut lumen harbors 90% of all cells of our biome and contains trillions of microorganisms and more than 1000 different species.[2] This gut microbiota consists of a mixture of native bacteria acquired at birth or shortly thereafter that retains a relatively constant composition. It also has a smaller population of transient bacteria of varying

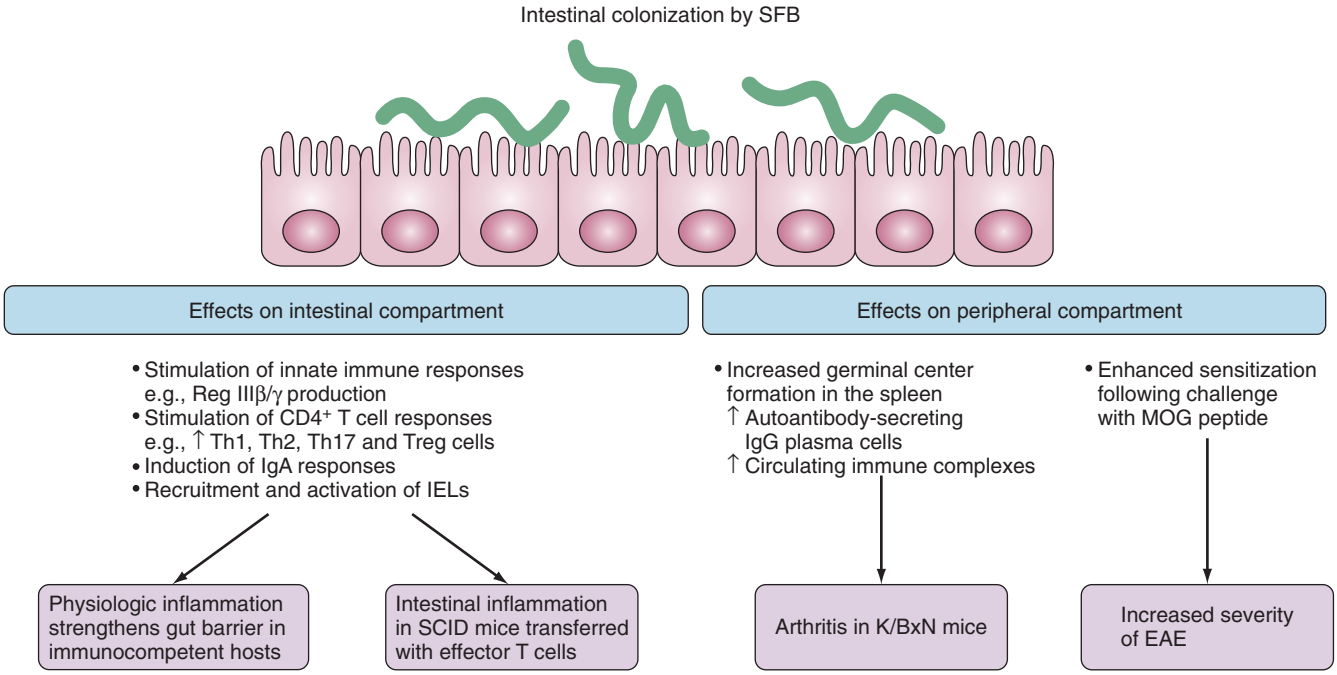



**Figure 78-2** Effects of colonization of the gut immune system. Segmented filamentous bacteria (SFB) are spore-forming bacteria that are related to the genus *Clostridium*.[51] Inherited from the mother microbiota, SFB develop strong interactions with the ileal mucosa, and in immunocompetent mice, the bacteria can largely recapitulate the inducing effects of the whole microbiota on the postnatal maturation of the gut immune system. SFB induce the production of Reg IIIβ/γ microbicidal peptides,[23,45] which protect against colonizing pathogens.[46] Additionally, SFB simultaneously activate strong secretory immunoglobulin (Ig)A responses,[44] induce the recruitment and activation of cytotoxic intraepithelial lymphocytes (IELs), and drive various T cell responses, including a robust T helper (Th) 17 cell response.[28,45] In immunocompetent mice, SFB-induced pro-inflammatory and regulatory responses balance each other, which results in physiologic inflammation that strengthens the gut barrier. By contrast, colonization by SFB promotes the development of colitis in severe combined immunodeficient (SCID) mice that have been reconstituted with effector T cells.[52] Intestinal colonization by SFB can also promote the development of inflammatory diseases outside of the gut. SFB promote arthritis in autoimmune nonobese diabetic (NOD) mice that express a transgenic T cell receptor (TCR) that is specific for a self-peptide (known as K/BxN mice), an effect ascribed to the induction of Th17 cells.[54] SFB also enhance the severity of myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE). These aggravating effects may reflect the strong adjuvant properties of SFB. Treg, T regulatory. *(From Cerf-Bensussan N, Gaboriau-Routhiau V: The immune system and the gut microbiota: friends or foes?* Nat Rev Immunol *10:735–744, 2010.)*

composition. The former are essential for health and live in symbiosis; the latter contain potential pathogens. Prolonged administration of antibiotics to neonates impedes colonization and increases the risk of late-onset sepsis, demonstrating the protective role of the microbiota.[26] Whereas the stomach and duodenum normally contain less than $10^3$ mucosa-adhering bacteria, the number of bacteria increases to $10^4$ in the jejunum and $10^7$ in the ileum. Most of the latter group comprises gram-negative aerobic species. In the colon, the bacterial density is $10^{12}$ or more, consisting mostly of anaerobic bacteria. Transit time is fast in the upper gut and slow in the distal gut, but the immunologic impact of the microbiota is higher in the proximal parts of the gut.[10] One can also distinguish between luminal and mucosal microbiota.

In addition to culturing, the microbiota can be studied by molecular methods, the most sensitive of which is analysis of the 16S bacterial ribosomal RNA gene sequence. This analysis has revealed the presence of a bewildering number of phylotypes. Consequently, the number of genes in the microbiota is estimated to be 150 times greater than that of the human genome.[11] Interactions with host cells has a profound influence on adaptive immunity. The molecular and cellular mechanisms have mainly been studied in animal models. The phenotype of the K/BxN mouse model of autoimmune arthritis was found to be strongly attenuated under germ-free conditions and in mice fed a pathogen-free mix of bacteria. The introduction of a single species of segmental filamentous bacteria to these animals, however, triggers arthritis by activating autoreactive Th17 effector cells in lamina propria (Figure 78-2).[45] Researchers have found that the effect is mediated by interfering with intrinsic T cell desensitization in the mucosa.[12] Vaccination of germ-free animals results in subnormal responses. Introduction of certain bacteria can normalize the response in mice; this process is mediated by enhanced release of Toll-like receptor 5 (TLR5) from the gut.[13] A similar mechanism could be involved in the mediation of enteropathic arthritis.

Bacteria digest food carbohydrates into short-chain fatty acids, which facilitate the absorption of $Ca^{2+}$, $Mg^{2+}$, and $Fe^{2+}$ ions. They also synthesize amino acids and vitamins, and secrete antibacterial protective substances. Genetic and environmental factors, including stress, may alter the gut microbiota and shift a state of homeostasis into one of imbalance or *dysbiosis*, as will be discussed later. Understanding host-microbiota interactions still is hampered by methodological challenges. Extrapolation of mouse data should be made with some caution.[14] However, exploration of the microbiota is expected to give better insight into the pathophysiology of several conditions, including enteropathic arthritis, and help to generate therapeutics.

## Gastrointestinal-Associated Lymphoid Tissue

**KEY POINTS**

- Microbiota influences regulate gastrointestinal-associated lymphoid tissue.
- Innate and adaptive immune cells constitute 80% of the intestine.
- Retinoic acid promotes immunoglobulin (Ig)A and lymphocyte homing.
- Flagellin stimulates DCs to induce T helper 17 cells.
- Vascular adhesion protein 1 is expressed in the gut and synovium and is a putative therapeutic target.
- Gut homing lymphocytes express integrins like α4β7 and αEβ7 as well as the chemokine receptor CCR9.

GALT is the largest lymphoid organ of the body, constituting 25% of the mucosal mass. Cellular components of GALT are localized in Peyer's plaques, gut lymphoid follicles, lamina propria, and intraepithelial T cells. GALT is part of a complex defense and tolerance-regulating system that involves specialized DCs and intestinal epithelial cells IECs (Figure 78-3). Commensal bacteria interact with IECs, which send signals to DCs mediated by anti-inflammatory substances (e.g., thymic stromal lymphopoietin [TSLP]) and to lamina propria DCs. Bacteria also interact directly with DCs by means of their polysaccharide A. A subset of lamina propria DCs expresses $CD11b^{hi}$, $CD11c^{hi}$, and TLR5. When exposed to bacterial flagellin, these DCs promote the differentiation of T helper (Th)17 cells. These DCs also produce retinoic acid. IEPs also produce retinoic acid from dietary vitamin A, APRIL (a proliferation-inducing ligand), and B cell–activating factor (BAFF). Retinoic acid stimulates the expression of the integrin α4β7 and CCR9, thereby influencing gut-homing lymphocytes.[15] Wnt–β-catenin signaling is required for the stimulation of expression of anti-inflammatory mediators by intestinal DCs.[16] Bacteria can also directly penetrate Peyer's patches (PPs) and create a symbiotic environment with host cells.[17] Cytokines such as IL-10 and transforming growth factor (TGF)-β serve anti-inflammatory functions. Retinoic acid, APRIL, and BAFF contribute to the dominating IgA production in local lymphoid cells. Gut enterochromaffin cells produce 5-hydroxytryptamine, which has anti-inflammatory effects and chromogranins with both pro-inflammatory and anti-inflammatory effects.[18]

Some of these not fully understood interactions are depicted in Figure 78-4. Plasma cells in the lamina propria become programmed to produce IgA, but the production is also skewed to formation of dimeric IgA, polymeric immunoglobulin A (pIgA), in which the monomers are connected by joint, or J, chains.[19] Intestinal epithelial

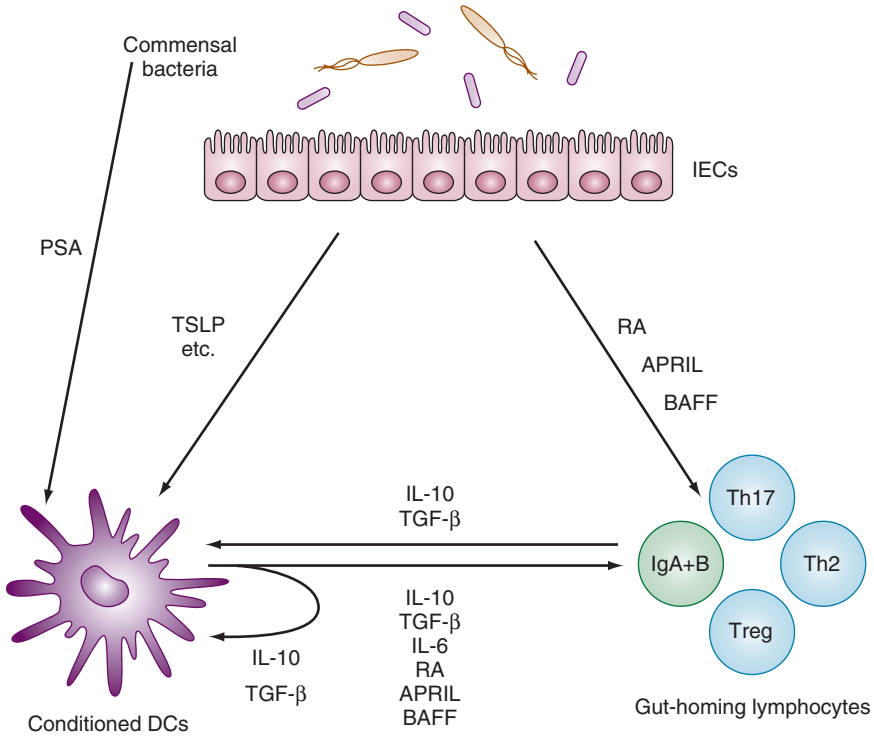



**Figure 78-3** Tolerance induction machinery in the gut. Under the influence of commensal bacteria and their products, conditioned intestinal epithelial cells (IECs) constitutively produce anti-inflammatory molecules, such as thymic stromal lymphopoietin (TSLP), to limit inflammation-induced signals, leading to the development of tolerogenic dendritic cells (DCs). Tolerogenic DCs might also be induced by direct stimulation with *Bacteroides fragilis*–derived polysaccharide A (PSA). Once the tolerogenic DCs are developed, they preferentially produce critical factors for the induction of Th2 cells, T regulatory (Treg), Th17 cells, and immunoglobulin $(Ig)A^+$ B cells. IECs also produce retinoic acid (RA), a proliferation-inducing ligand (APRIL), and B cell–activating factor (BAFF) belonging to the tumor necrosis factor family, which probably affect the development of these lymphocyte subsets. Communication among the commensal flora, IECs, and gut immune system is bidirectional, but only a part of the bidirectional communication is shown in this figure. IL, Interleukin; TGF-β, transforming growth factor-β. *(From Tezuka H, Ohteki T: Regulation of intestinal homeostasis by dendritic cells.* Immunol Rev *234:247–258, 2010.)*

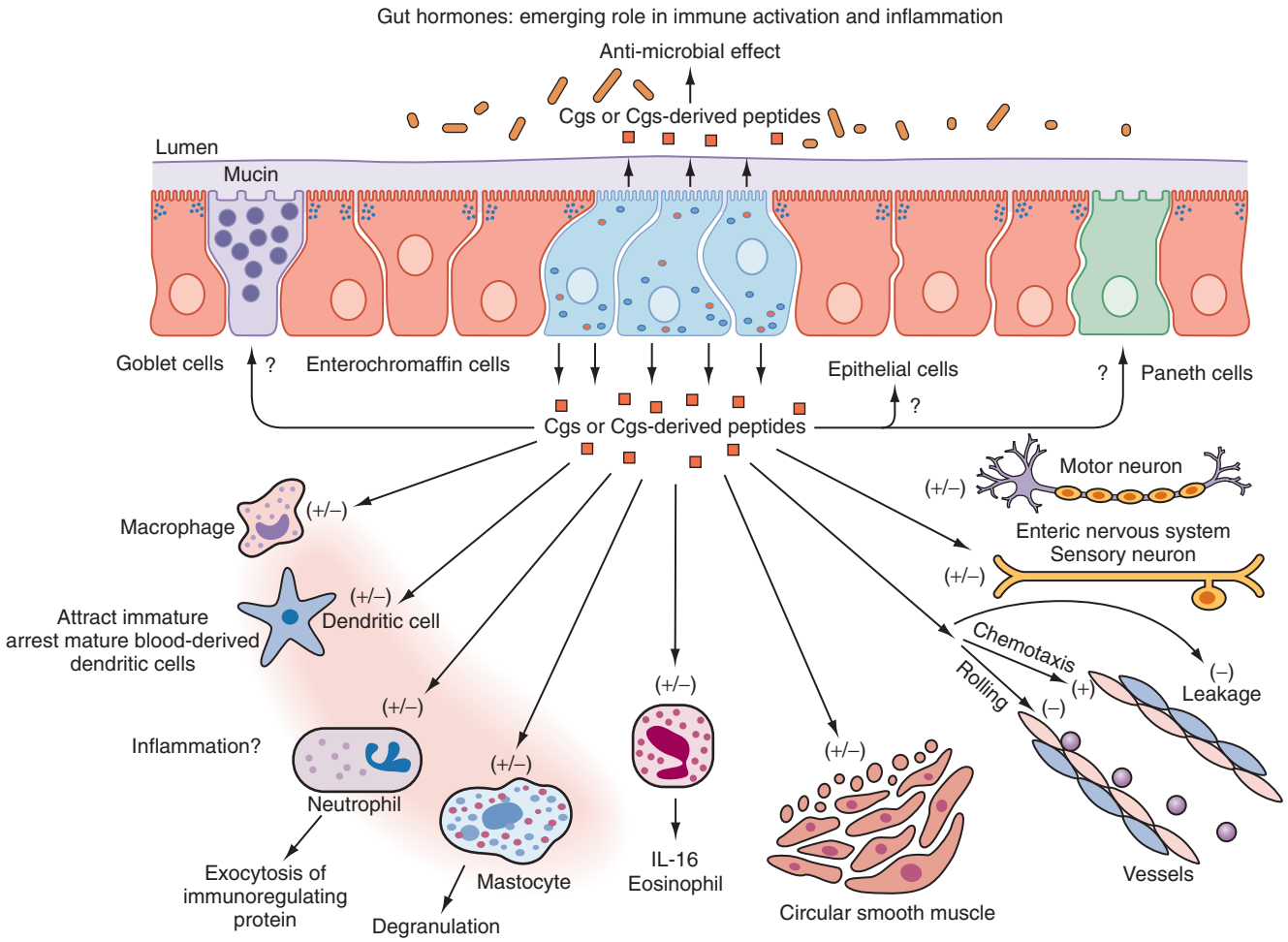



**Figure 78-4** Putative role of chromogranins (Cgs) in immune activation and inflammation. Luminal or internal inflammatory stimuli causes alteration in Cgs or Cgs-derived peptides release. They may act locally on Paneth, goblet, and epithelial cells, as well as on immune cells such as macrophages, dendritic cells, neutrophils, mastocytes, and eosinophils. Endothelial permeability, chemotaxis, rolling, smooth muscle contractility, and the enteric nervous system can also be modulated. IL-16, Interleukin-16; –, inhibition; +, activation. *(From Khan WI, Ghia JE: Gut hormones: emerging role in immune activation and inflammation.* Clin Exp Immunol *161:19–27, 2010.)*

glycoprotein pIgR, a polymeric immunoglobulin receptor that is also called *secretory component* (SC), is produced in IECs. It is a 100 kDa transmembrane receptor for pIgA and IgM. SC/pIgR is abundantly present in PPs in the distal ileum. The pIgR in IECs combines with pIgA and to a lesser extent with IgM to form secretory IgA, secretory IgA (sIgA), and sIgM, respectively, which are exported into the lumen and constitute a non-inflammatory, non–complement-binding first line of defense. It is estimated that a healthy adult secretes 3 to 5 g of sIgA into the gut daily (Figures 78-5 and 78-6).[19] Breastfeeding provides the newborn with abundant sIgA and sIgM, which confers passive protection and also regulates much of the child's immune system[20] (Figure 78-7).

From the PP, primed B lymphocytes disseminate throughout the body's mucous membranes, notably to other parts of the alimentary tract (Figure 78-5). Primed T lymphocytes also disseminate into the circulation and lymph nodes and home into target organs such as salivary glands (in Sjögren's disease), lungs, and synovium.[20] Vascular adhesion protein 1 (VAP-1) expressed on synovial epithelial cells is involved in lymphocyte homing, and P-selectin is a part of macrophage recruitment. VAP-1 is a bifunctional glycoprotein with both adhesive and amino-oxidative properties. Both monoclonal antibodies and small molecular inhibitors are in development for potential use in inflammatory and malignant disease.[21] Monoclonal antibodies are protective against collagen-induced arthritis.[21] Most T lymphocytes in the mucosal lamina propria are $CD4^+$, whereas intraepithelial T cells are mostly $CD8^+$. Gut-associated lymphocytes preferentially express the integrins $\alpha4\beta7$ and $\alpha E\beta7$ and the integrin receptor CCR9 on stimulation by intestinal DCs.

Functional characterization of innate (ILC) and adaptive lymphoid cells distinguish new subsets of ILCs, natural killer (NK) cells, ITi, ILC-22, ILC-17, and type 2 ILC. They activate different sets of effector T cells and are instrumental in the fine tuning of local immune responses. Type 2 ILCs, originally referred to as *nuocytes*, are involved in stimulating IL-13 production and mediating experimental colitis.[22]

Intestinal DCs play a key role in regulation of the intestinal immune response to foreign antigens. DCs are present

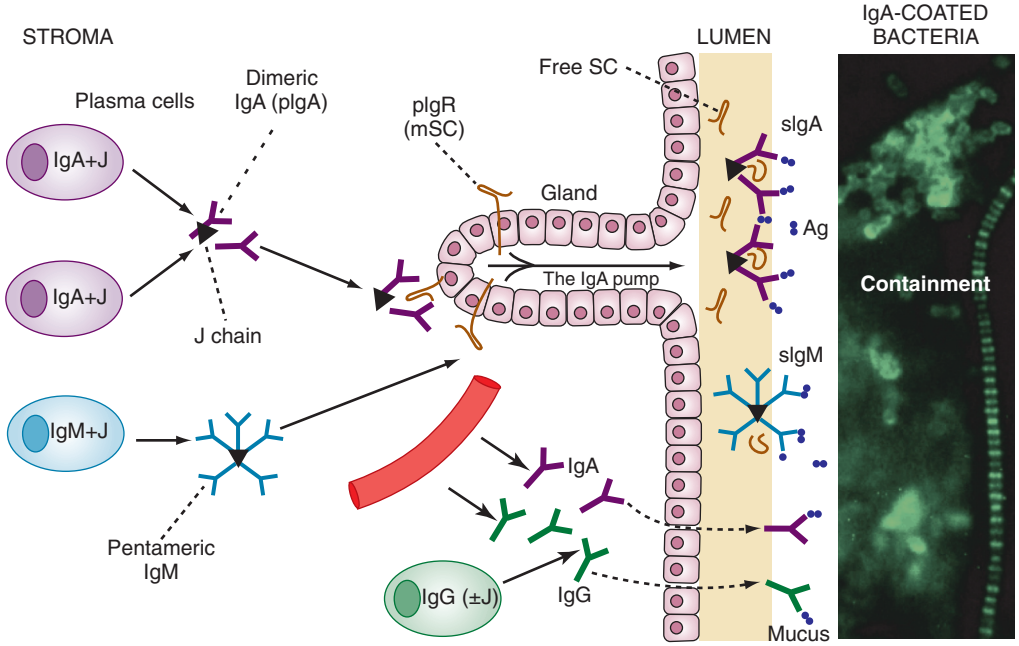



**Figure 78-5** Receptor-mediated export of dimeric immunoglobulin (Ig)A and pentameric IgM to provide secretory antibodies (sIgA and sIgM) that function in immune exclusion of antigen (Ag) at the mucosal surface. Polymeric Ig receptor (pIgR) is expressed basolaterally as membrane secretory component (mSC) on secretory epithelial cells and mediates transcytosis of dimeric IgA and pentameric IgM, which are produced with incorporated J chain (IgA + J and IgM + J) by mucosal plasma cells. Although J chain is often produced by mucosal IgG plasma cells (70% to 90%), it does not combine with this isotype and is therefore degraded intra-cellularly as denoted (± J). Locally produced (and serum-derived) IgG is therefore not subject to pIgR-mediated transport, but can be transmitted paracellularly to the lumen together with monomeric IgA as indicated. Free SC (depicted in mucus) is generated when pIgR in its unoccupied state *(top basolateral symbol)* is cleaved at the apical face of the epithelium-like bound SC in sIgA and sIgM. Commensal bacteria in the right-hand panel are coated in vivo with sIgA, which aids their containment and thereby promotes host-microbial mutualism. *(From Brandtzaeg P: Mucosal immunity: induction, dissemination, and effector functions.* Scand J Immunol *70:505–515, 2009.)*

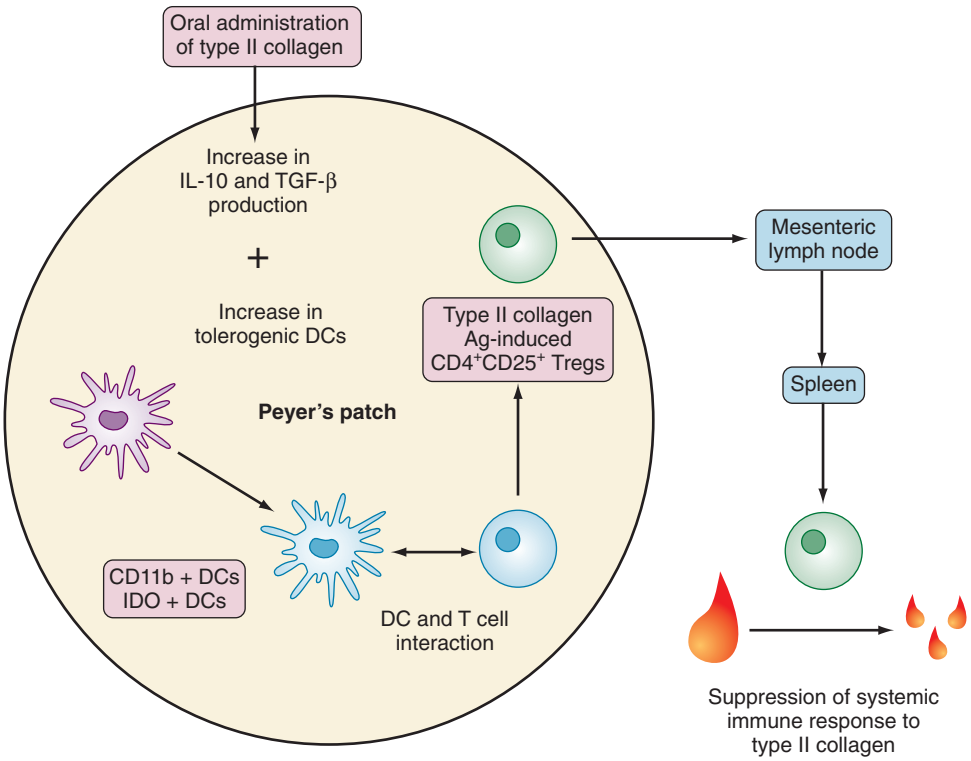



**Figure 78-6** Role of Peyer's patch (PP) dendritic cells (DCs) in type II collagen oral tolerance. As a result of repeated oral administration of type II collagen, the production of anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor-β (TGF-β) by PP cells is enhanced, and the populations of $CD11c^+CD11b^+$ DCs and $IDO^+$ DCs are increased in PP. Then, through DC–T cell interaction, type II collagen inducible $CD4^+CD25^+Foxp3^+$ and regulatory T cells (Tregs) are generated in the PP. Tregs generated in the PP move to the mesenteric lymph node and then enter the systemic circulation, where they suppress the systemic immune response to CII. *(From Park KS, Park MJ, Cho ML, et al: Type II collagen oral tolerance; mechanism and role in collagen-induced arthritis and rheumatoid arthritis.* Mod Rheumatol *19:581–589, 2009.)*

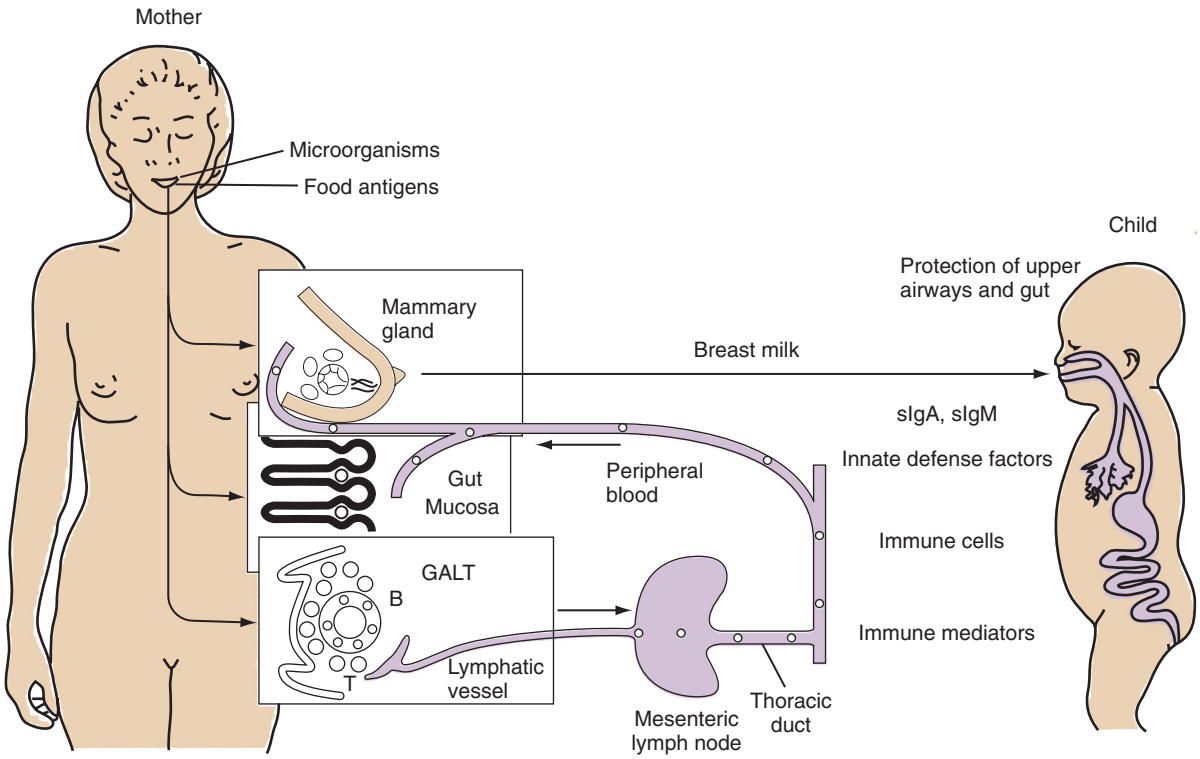



**Figure 78-7** Integration of mucosal immunity between mother and newborn. Primed B (and probably T) cells from Peyer's patch migrate via lymph and peripheral blood to the lactating mammary gland, resulting in the presence in breast milk of secretory immunoglobulin (Ig) antibodies (sIgA and sIgM) specific for enteric antigens. GALT, Gastrointestinal-associated lymphoid tissue. *(From Brandtzaeg P: Mucosal immunity: integration between mother and the breast-fed infant.* Vaccine *21:3382–3388, 2003.)*

throughout the GALT compartment, and migratory DCs can also be identified in mesenteric lymph nodes.[23] Intestinal DCs can be distinguished from macrophages and monocytes by distinct surface markers and their uptake and presentation of the antigenic material monitored. The dynamic control of DC functions by transcription factors and their trafficking to mesenteric lymph nodes indicates that the systemic effects of intestinal DCs may play a key role in the pathogenesis of several gut associated diseases. They may become future targets for therapy.[22] From the circulation thus activated leukocytes can penetrate joint tissue.[24,25]

Induction of oral tolerance to type II collagen has demonstrated how GALT activation may ameliorate inflammatory joint disease (see Figure 78-6). A chain of events in the pathogenesis of enteropathic arthritis can begin with GI infection with the appropriate microorganism in a genetically predisposed patient. This causes local inflammation in the gut mucosa, formation of sIgA, increased permeability, absorption of foreign material, and triggering of T lymphocytes. Circulating immune complexes and memory T cells localize to joints and cause synovitis.

In conclusion, the gut wall is a highly diversified immunologic, endocrine, and digestive organ. Interaction of the gut wall with microbiota results in adjuvant effects, and potentially mediates both protective and harmful signals, resulting in either homeostasis or dysbiosis[26] (see Figure 78-2).

## INFLAMMATORY BOWEL DISEASE

### Epidemiology

**KEY POINTS**

- **The occurrence of IBD has remained constant in Western countries but is increasing elsewhere in the world.**
- **Twin concordance is higher in Crohn's disease than in UC.**
- **Smoking increases the risk in Crohn's disease and ulcerative colitis.**
- **Smokeless nicotine (snuff) may be inert or protective.**
- **Ethnic background influences risk and severity.**

The prevalence and incidence of IBD has historically been markedly higher in North America, Western Europe, Australia, and New Zealand than in other parts of the world. In recent decades, IBD is becoming common in developing countries, South America, Asia, and Eastern Europe, coinciding with the change to more "Western" conditions, such as lifestyle and standard of living. Typically, ulcerative colitis (UC) increases earlier than Crohn's disease (CD). Environmental influences, changes in lifestyle and, consequently, of the microbiota are contributing factors, the study of which could provide clues to the pathogenesis of IBD.[27] The prevalence of CD and UC is approximately equal in many countries and varies between approximately 250 and 150 per 100,000 population for UC, and 300 and 40 for CD

in high-prevalence populations. Prevalence figures below 10 have been reported from most other countries. However, in Japan a notable increase from 5.5 to 63.3 for UC and from 5.9 to 21 for CD, all per 100,000, was noted between 1965 and 2005. Corresponding incidence figures vary between 5 and 20 for UC and 3 and 30 for CD in areas rich in IBD and less than 4 in other places.[28] Ethnic influences also occur. For example, the Jewish population has a higher susceptibility in general, but prevalence approaches that of the background population. In Hungary, Romanies have a remarkably lower risk for IBD compared with the Hungarian population at large.[29]

The incidence of UC and CD in a large Swedish population-based study was 18 and 11 per 100,000 person-years, respectively.[30] Importantly, ever smokers had an elevated relative risk for UC and CD of 1.3 and 1.5, respectively. The overall concordance in monozygotic twins is 36%, but only 16% in UC. This finding indicates a stronger environmental influence in UC.[31] In addition to arthritis, asthma was more prevalent in relatives of Canadian patients with IBD.[32]

## Genetics

**KEY POINTS**

**Genome-wide association studies identified at least 163 single-nucleotide polymorphisms associated with inflammatory bowel disease, 110 of which are common for both Crohn's disease and ulcerative colitis.**

***NOD2* (previously known as *CARD15*), *IRGM,* and *ATG16L1* are associated with DCs.**

***IL23R* and *MDR1* are associated with Crohn's disease and ulcerative colitis.**

***IL23R* deletion protects against colitis in mice.**

The common forms of UC and CD are genetically complex diseases. As of 2012, genome-wide association studies (GWAS) have identified 163 associated single-nucleotide polymorphisms (SNPs), of which 110 are associated with both diseases. Of these SNPs, 23 were associated only with UC, and 30 were associated only with CD, confirming the close relationship but also the distinct nature of the two conditions.[33] The strongest associations, apart from human leukocyte antigen (HLA), are with the *NOD2* (formerly known as *CARD15*) gene on chromosome 16 and the *IL23R* gene on chromosome 1. *NOD2* and *NOD1* are cytosolic sensors for the bacterial peptidoglycan muramyl dipeptide, and trigger the synthesis of antibacterial α-defensins.[34,35] Reduced mucosal expression of these defensins is found in patients with CD.[36] IL-23 signaling can result in activation of Th17 cells in the gut. The importance of IL-23 is supported by the finding that *IL23R*$^{-/-}$ mice are resistant to induction of colitis.[37] Also, IL-23 is upregulated in patients with CD.[38] Another association is found with the transcription factor signal transducer and activator of transcription (STAT)3, which is involved in Th17 activation. STAT3 is present on innate and reactive immune cells and is present in increased amounts in the gut mucosa.[39] Another confirmed genetic association is that with *ERAP1*, an endoplasmic reticulum aminopeptidase previously known as *ARTS1*.[40] *ERAP1* functions by trimming the length of peptides before loading into the grove of HLA, and therefore it could play a role in the triggering of immune reactions in various locations such as gut and joint. A Spanish study of patients with CD looked at the Fc receptor–like 3 *(FcR3)* gene, and found an association with peripheral arthritis.[41] Polymorphisms in this gene are associated with susceptibility to RA and other autoimmune diseases in some populations and, interestingly, *FcR3* may interfere with the Treg function.

*NOD2* mutations were present in 43% of patients in the initial French study,[42] but later population studies found a much lower prevalence in northern European populations, and no correlation in Asians. *NOD2* mutations are not related to susceptibility in the United States. High expression of *NOD2* mRNA has been found in the small intestine, and it is believed to be a regulator of nuclear factor-κB (NF-κB) signaling after the engagement of TLRs. *IBD3* on chromosome 6 has shown the most constant association with IBD, and *HLA-DRB1*0103* has been linked to severe UC in several studies.[24] Further, a TNF microsatellite gene factor was associated with CD but not with UC. HLA-DR2 and DR3 associations have been linked with UC but not with CD.

Mutations of the detoxifying ATP-binding cassette, subfamily B, member 1 gene *(ABCB1)*, also known as *multidrug resistance 1 gene (MDR1)*, are strongly downregulated in unaffected colonic tissue of both CD and UC. *TLR4* and *TLR5* associations have been identified in several populations; this may be of special interest because they act in synergy with *NOD2* and *CARD4* in the induction of proinflammatory cytokines. TLR inhibitors are under investigation for therapeutic efficacy. Recently, researchers found a role for a virus as the contributing trigger in gene-manipulated mice, when mice with a disrupted autophagy gene, *ATG16L1*, were infected with a specific strain of murine norovirus and developed CD-like disease.[38]

A concluding comment regarding genetic association in IBD is that polymorphisms involved in a large number of signaling pathways with relevance for pathogenesis are affected, and that different associations are found in different populations.

## Pathogenesis

IBD is the result of an imbalance between aggressive influences from the lumen and defensive mechanisms in the gut wall. Precise abnormalities of the dysbiosis have been identified in animal models, several but not all of which can be confirmed in patients with IBD. Dysbiosis has been found to be characterized by less diversity of commensals, belonging to *Bacteroides* and Firmicutes phyla, and an increased presence of Proteobacteria.[43,44] Preliminary twin studies indicate different patterns in UC and CD.[45] Dietary factors and treatment with antibiotics could contribute to pathogenic dysbiosis in IBD.

The IECs that form the first line of defense show a diminished formation of defensins in IBD. IECs also have a lower expression of a class I major histocompatibility (MHC)–like transmembrane molecule, CD1d. CD1d cross-linking results in formation of IL-10 and, as a consequence, less IL-10 is formed, which aggravates inflammation.[46]

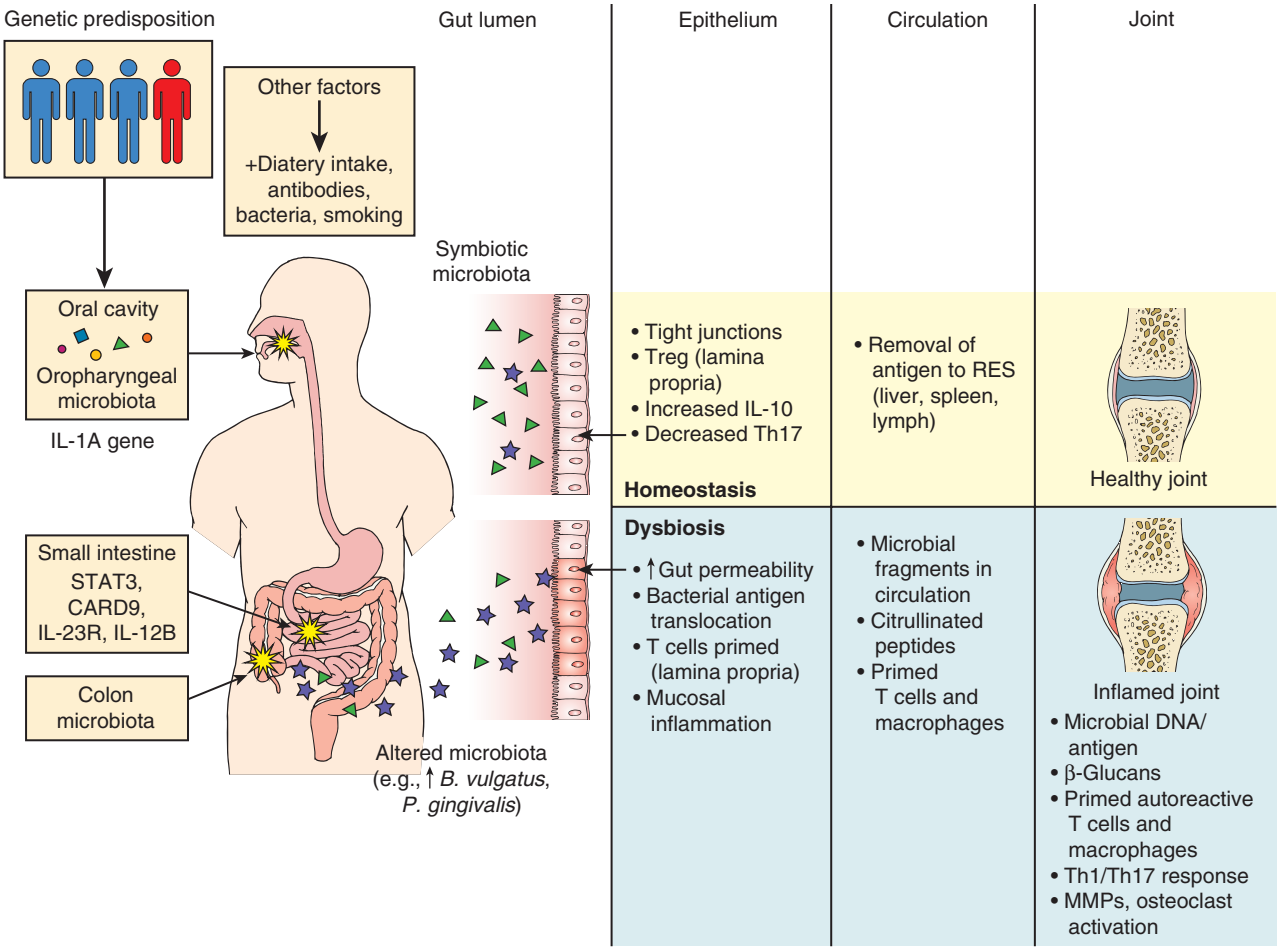



**Figure 78-8** Microbiota-host interaction. Homeostasis versus dysbiosis and putative generation of enteropathic arthritis. **Homeostasis:** Host genetic factors help to preserve the integrity of the epithelial barrier in the oral cavity and intestinal tract. Continual sampling of gut luminal contents enables immune regulation of the inflammatory response (oral tolerance). T regulatory (Treg) cells in the lamina propria assist with an anti-inflammatory environment where such cytokines as TGF-β and IL-10 predominate. Bacteria and antigenic material from the gut which enter the circulation are removed via the reticuloendothelial system (RES) and do not enter the joints. **Dysbiosis:** Host genetic factors contribute to increased intestinal permeability. In the mouth, IL-1A is associated with chronic periodontitis; in the gut, IL-23R, signal transducer and activator of transcription (STAT)3, and CARD9 are associated with intestinal inflammation. Proportions of potentially harmful pro-inflammatory bacteria (e.g., *Bacteroides vulgatus*, *Klebsiella* spp, and sequential batch reactors) are believed to change. Primed T cells and $CD163^{+}$ macrophages from the lamina propria migrate to joints, initiating a pro-inflammatory response mediated by Th1 and Th17 cytokines. Microbial fragments, including β-glucans are lodged in the joints, perpetuating inflammation. In rheumatoid arthritis, citrullinated peptides formed by the action of *Porphyromonas gingivalis* are presented via the shared epitope human leukocyte antigen (HLA)-DRB1, leading to a pro-inflammatory cascade. Within the joint, cytokines upregulate matrix metalloproteinases (MMPs) and osteoclasts, resulting in cartilage destruction and bone erosion. SRBs, Sulfate-reducing bacteria. *(From Yeoh N, Burton JP, Suppiah P, et al: The role of the microbiome in rheumatic diseases.* Curr Rheumatol Rep *15:314, 2013.)*

Several other cytokine disturbances are present in IBD: increased activation of the IL-1 system and decreased ratio of IL-1Ra/IL-1; IL-17A is increased, and IL-17F is present in both UC and CD; IL-33 is increased in UC only. IL-22, on the other hand, promotes IEC healing by STAT signaling, and TNF, IL-6, and IFN-γ are generated by cells both from the innate and adaptive immune system. TNF has a central role in the pathogenesis. Figure 78-8 shows some of these interactions.[47] These cytokine disturbances may contribute to the occurrence of extra-articular manifestations.[48]

Increased gut permeability has already been identified as an important factor in pathogenesis.[7,49] Bacteria recovered from the gut lumen in IBD are covered by immunoglobulin, part of which is circulatory IgG.[50] Increased leakage of tissue fluid from the inflamed mucosa allows the egress of complement-binding IgG, which may contribute to inflammation and further augment permeability. The altered immune response to bacteria differs between CD and UC.[51] Increased gut permeability in IBD is under genetic influence. Basal permeability was healthy in a study of relatives of patients with CD, but it was abnormally increased after the ingestion of acetylsalicylic acid.[52] Environmental influences on permeability may be partly mediated by bacterial endotoxin. An in vitro perfusion study on rat gut showed that serosal rather than mucosal application of endotoxin impairs the barrier.[53] Absorbed bacterial material could therefore add to an already damaged barrier.

The pathogenesis of extraintestinal manifestations (EIM) and complications of IBD involve immune cell migration from the gut. Migratory DCs that carry gut commensals normally are cleared in mesenteric lymph nodes (MLNs), allowing only degraded microbial material to reach the liver via the blood. In a mouse model, MLNs are

overwhelmed and intact microorganisms are cleared first in the liver. The liver may be an important mediator between the gut and EIM in IBD.[49] Not surprisingly, genetics influence the different patterns of EIM. Polymorphisms linked to shared pathogenic pathways can be identified and may, in part, explain the diversity of manifestations.[48]

## Clinical Features

**KEY POINTS**

- **Peripheral arthritis in Crohn's disease and ulcerative colitis occurs as axial disease or peripheral joint disease.**
- **Enthesitis pain is more pronounced than in ankylosing spondylitis.**
- **Uveitis in inflammatory bowel disease is prone to become chronic.**
- **Sclerosing cholangitis develops in 5% of patients with inflammatory bowel disease.**

EIM involve several organ systems in addition to the musculoskeletal and are major contributors to the morbidity and impact of IBD and underline their systemic nature (Table 78-1). Differences exist between UC and CD (Table 78-2). The overall prevalence of EIM may exceed 35%. Spinal involvement occurs in 10% to 20% of cases and may be the only articular manifestation or accompany oligoarthritis.[54] Spinal involvement is often silent, thus its prevalence is underestimated; it may precede the onset of IBD or appear later.[7] In contrast to classic ankylosing spondylitis (AS), there is an equal sex distribution.[55] Radiographic changes are more discrete, squaring of the vertebrae more prevalent, and Romanus lesions unusual. Spinal symptoms do not correlate with intestinal symptoms. Silent CD, on the other hand, is found on biopsy in as many as 30% of patients with early-stage AS.[56] In full-blown IBD-related AS, the prevalence of B27 is only between 50% and 70%.[7] Interestingly, MRI shows more extensive bone marrow edema in IBD-related spondyloarthritis than in AS without IBD.

Peripheral arthritis develops in between 5% and 15% of patients in most studies, slightly more often in patients with CD than in patients with UC (Table 78-3). Peripheral arthritis is more likely to develop in CD patients with large bowel disease (~70%) and intestinal complications, such as fistulas or abscesses, than in patients with disease limited to the ileum.

IBD arthritis is often nondestructive and reversible, but erosive changes may also occur. Limited histopathologic evidence indicates the presence of granulomas in CD and nonspecific synovitis in UC.[7] In CD patients, rapidly destructive septic arthritis has been reported in the hip. Joint symptoms tend to coincide with gut activity in UC but not in CD. Total colectomy is associated with remission of arthritis in half of the patients with UC, but paradoxically, arthritis may also begin after surgery.[57] This may represent a form of bypass arthritis, and is related to altered gut microbiology in a blind loop.

**TABLE 78-1** Extraintestinal Manifestations of Inflammatory Bowel Disease

| | |
|---|---|
| Musculoskeletal | Peripheral arthritis in 5%-20% of patients<br>Type I self-limiting oligoarthritis, activity related<br>Type II progressive polyarthritis, unrelated to activity<br>Proximal arthritis/ankylosing spondylitis in 15%-20%, unrelated to activity |
| Mucocutaneous | Erythema nodosum 10%-15%, related to activity<br>Pyoderma gangrenosum, 1%-5%<br>Sweet syndrome, activity related<br>Aphthous stomatitis, 10% activity related |
| Ocular | Scleritis or episcleritis, 2%-3%, activity related<br>Uveitis, 2%-6% |
| Hepatobiliary | Primary sclerosing cholangitis, as many as 7%<br>Acute pancreatitis |
| Thromboembolic | Both venous and arterial, 1%-6%, activity related |
| Pulmonary | Asthma, chronic obstructive pulmonary disorder<br>Subclinical and clinical inflammation, activity related |
| Neurologic | Demyelinating disease, multiple sclerosis, hearing loss |
| Other | Amyloidosis rare in Crohn's disease, not reported in ulcerative colitis |

Modified From Ott C, Schölmerich J. Extraintestinal manifestations and complications in IBD. *Nat Rev Gastroenterol Hepatol* 10:585-595, 2013.

**TABLE 78-2** Distinct Features of Inflammatory Bowel Disease

| Feature | Crohn's Disease | Ulcerative Colitis |
|---|---|---|
| Replicated non–human leukocyte antigen (HLA) genetic associations | *CARD15/NOD2, IRGM, ATG16L1, IL23R* | *IL23R* |
| Concordance in monozygotic twins | 36% | 19% |
| Non-HLA genes | Several | Several |
| Gut permeability sensitive to acetylsalicylic acid on genetic base | Yes | ? |
| T lymphocyte response in gut | Th1, Th17 (IFN-γ↑) | No Th1-Th2 imbalance, Th17 |
| Fas ligand expression | No | Yes |
| Effect of smoking | Negative | Negative |
| Correlation of gut activity to arthritis symptoms | No | Yes |
| Intercellular adhesion molecule-1 antisense therapy | Beneficial | No response (?) |
| Response to TNF inhibitor therapy | Effective | Probably effective |

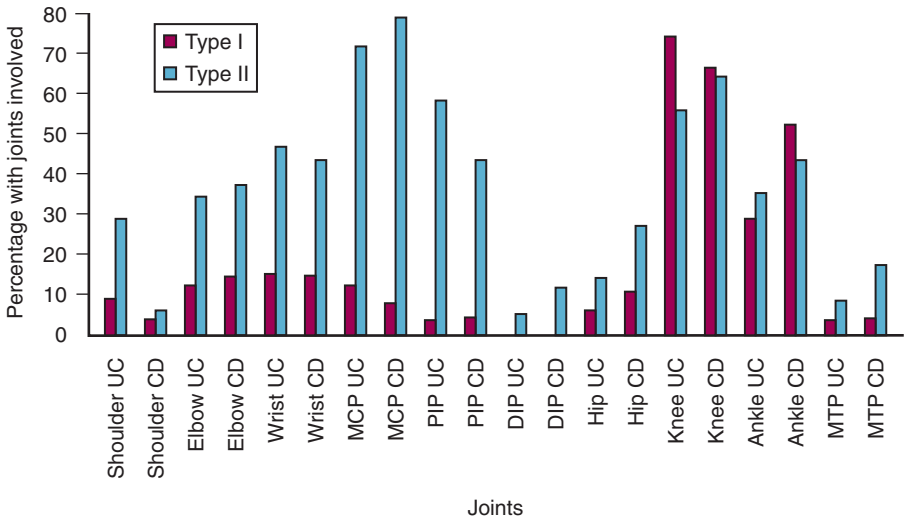



**Figure 78-9** Articular distribution of peripheral arthropathies in inflammatory bowel disease. CD, Crohn's disease; DIP, distal interphalangeal joint; MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; PIP, proximal interphalangeal joint; UC, ulcerative colitis. *(From Orchard TR, Wordsworth BP, Jewell DP: Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history.* Gut *42:387, 1998.*

**TABLE 78-3** Peripheral Joint Disease in Inflammatory Bowel Disease

| Feature | Type II (>5 Joints) | Type I (<5 Joints) |
|---|---|---|
| Ulcerative colitis | 3% of all UC; 3% of all CD | 2% of all UC; 5% of all CD |
| Crohn's disease | 6% of all UC; 0% of all CD | 4% of all UC; 6% of all CD |
| Clinical course | Persistent, often destructive, arthritis<br>A spondyloarthritis subset | Self-limited, not erosive arthritis<br>Reactive arthritis–like |
| Course of IBD | Relapsing in <85% | Relapsing in 30%-40% |
| MHC association | *HLA-B27, B35, DRB1*0103* | *HLA-B44* |

CD, Crohn's disease; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; MHC, major histocompatibility complex; UC, ulcerative colitis.

From Orchard TR, Wordsworth BP, Jewell DP: Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. *Gut* 42:387, 1998.



**Figure 78-10** Pyoderma gangrenosum in a case of Crohn's disease. *(From Rothfuss KS, Stange EF, Herrlinger KR: Extraintestinal manifestations and complications in inflammatory bowel diseases.* World J Gastroenterol *12:4819-4831, 2006.)*

On the basis of examination of approximately 1500 patients with IBD, an important distinction was made between two forms of peripheral arthritis[58] (Figure 78-9). Oligoarthritis, or type I, affects fewer than five joints, whereas polyarthritis, or type II, involves more than five joints. The highest prevalence was found in metacarpophalangeal, proximal interphalangeal, knee, and ankle joints. Shoulder involvement was more common in UC, but joint involvement was otherwise strikingly similar. The majority of type I arthritis cases were acute and resolved within 6 weeks, whereas the type II cases persisted.[59] Type I arthritis was 12 times more prevalent in carriers of the rare *HLA-DRB1**0103 allele, type II arthritis was associated with HLA-B44, and sacroiliitis in CD was associated with *NOD2* polymorphisms.[60] This is an example of genetic influence on disease phenotype and may be a clue to pathogenesis.

Clubbing of fingers, uveitis, and skin manifestations are other extraintestinal manifestations of IBD, with a higher frequency in patients with CD. Erythema nodosum, which is usually self-limited, is most frequent in young female patients with UC. Pyoderma gangrenosum is a more severe, painful, ulcerating skin reaction that is frequently associated with systemic disease[61] (Figure 78-10). In a series of 86 patients with pyoderma gangrenosum seen at the Mayo Clinic between 1970 and 1983, 31 patients had IBD.[7] Erythema nodosum, uveitis, and peripheral arthritis commonly occur together in IBD and have been linked to *HLA-DRB1**0103 and *TNF* gene polymorphism.[62] Uveitis is also a feature of other forms of spondyloarthritis, such as AS and reactive arthritis. In IBD, however, uveitis is more often bilateral and more prone to chronicity.[63,64] Primary sclerosing cholangitis (PSC), a progressive liver disease that can lead to end-stage liver fibrosis, can develop in as many as 5% of the patients with UC and CD. The risk for colorectal cancer is increased in IBD and is related to the extent of colonic involvement and disease duration. Colorectal cancer mortality has decreased significantly in the past few decades, mainly because of better surveillance and earlier diagnosis.[67,68]

## Diagnosis

| KEY POINTS |
|---|
| MRI in cases of suspected inflammatory back pain is recommended. |
| Fecal calprotectin is a useful screening test for inflammatory bowel disease. |
| Large joint oligoarthritis may mimic reactive arthritis. |
| Monogenic inflammatory bowel disease-like disease has been reported in children. |

In young children, very rare instances of monogenic IBD-like disease, for instance, with IL-10 mutations, have been identified.[69] These conditions are often combined with peripheral arthritis, typically familial, and their study can be informative to understand the pathogenesis of the classic genetically complex forms of IBD. No specific tests exist to confirm a suspicion of IBD-related arthritis. Careful history and clinical examination remain the principal diagnostic tools. Arthropathy can precede intestinal symptoms in a subgroup of patients, and thus colonoscopy with histologic exploration can be informative regarding the origin of occurring joint symptoms. When inflammatory back pain is suspected, MRI of the sacroiliac joints is mandatory.[70] Fecal calprotectin has emerged as a sensitive screening test for IBD, and it helps to select cases for endoscopic exploration.[65] As mentioned earlier, genetic mapping has shown interesting clinical correlates, but genotyping is not part of the routine clinical workup, except perhaps for HLA-B27. Stool cultures should be performed when infection with special pathogens is suspected. In patients with apparent IBD and monoarthritis, joint aspiration is important to exclude septic arthritis, especially before immunosuppressive therapy is begun.

## Treatment

| KEY POINTS |
|---|
| Arthralgia is common in inflammatory bowel disease. |
| NSAIDs should be avoided. |
| "Nonradiographic" spondyloarthritis can precede intestinal symptoms. |
| Early treatment with TNF inhibitors can potentially prevent spinal destruction. |
| Patients with inflammatory bowel disease are at higher risk for complications after total hip replacement. |
| The efficacy of probiotics remains unproven. |

Management of musculoskeletal IBD requires awareness of other EIMs. Ocular, cutaneous, and hepatic manifestations are most prevalent, but pulmonary and neurologic manifestations also need attention. Arthralgias are by far the most common peripheral arthritis complaint, but because of increased risk of exacerbation of intestinal disease NSAIDs should be avoided. Type I peripheral arthritis, that is, a large joint oligoarthritis, has features of reactive arthritis coinciding with intestinal activity. It is usually self-limiting, may remit, and requires symptomatic treatment, for instance, with intra-articular glucocorticoids.

Spondyloarthritis, with or without polyarticular type II polyarthritis, is a more severe manifestation. When inflammatory back pain is suspected, MRI of the sacroiliac joints is mandatory. If positive, treatment with a biologic agent may be indicated.

Several randomized trials have shown efficacy in treating IBD with monoclonal anti-TNF antibodies.[71-76] Only one small trial of etanercept was negative. The reason remains unknown. Inhibition of TNF is also effective against spondyloarthropathies with IBD. Treatment should be started early to prevent or delay structural damage to the spine.[77]

Vedolizumab is an all human monoclonal antibody against the $\alpha_4\beta_7$ integrin receptor involved in gut homing of T cells. Based on a few positive phase III trials, it has been approved for the therapy of UC and CD. Efficacy in IBD-related spondyloarthritis is not known. Inhibition of the IL-12/IL-23 pathway using IL-17 inhibitors secukinumab and brodalumab failed in treatment of patients with CD, whereas ustekinumab had an effect in patients with CD in whom treatment with TNF inhibitors failed. Development of an IFN-$\gamma$ inhibitor, fontolizumab, as well as abatacept, seems ineffective.[78]

Probiotics, although theoretically attractive, have not been found to be effective in treating CD, and are of unconfirmed limited efficacy in UC.[79] Their safety remains to be documented.[80] Azathioprine and glucocorticoids used in intestinal IBD could be beneficial against arthritis, but no controlled trials have been done. Azathioprine has been widely used to maintain remission in IBD. It has proven long-term efficacy in both UC-related colitis and CD, according to a large European study.[71] It should not be combined with 5-aminosalicylic acid, because of a pharmacokinetic interaction.[72] Destructive hip disease may require total joint replacement. Patients with IBD are at higher risk of infection and need of revision.

## Outcome

The outcome of type I peripheral arthritis is linked to bowel activity, but it is generally benign. Type II peripheral arthritis is erosive and progresses independently of disease activity in the gut. The outcome of IBD-associated spondyloarthritis is considered to be similar to that of spondyloarthritis in general. Information on the effects on musculoskeletal involvement of the biologics is incomplete. A Swedish study shows higher survival among male patients with peripheral arthritis who are on drug therapy.[81]

# *BRUCELLA* ARTHRITIS

| KEY POINTS |
|---|
| Brucellosis is a leading zoonosis in the Middle East. |
| Brucellosis is rampant in developing countries as a consequence of urbanization. |
| Stray dogs and unpasteurized cattle food consumption spread the infection. |

### Epidemiology

Some years ago, there was hope that brucellosis would soon be extinguished. However, an estimated half million new cases occur annually worldwide and make it a leading zoonosis and a common cause of musculoskeletal problems in endemic areas, where the incidence may reach 200 cases per 100,000 of the population.[82] It is the dominating zoonosis in some African and Middle East countries, but high prevalence is also seen in India and other parts of Asia. Traditionally, brucellosis was a rural disease, affecting people working with infected cattle or consuming unpasteurized dairy products.[83-85] However increasing prevalence has been observed in big cities in China, usually caused by consumption of dairy products or raw meet.[86] In the Western world travelers can import brucellosis. Urbanization and widespread infection in sheep, cattle, and perhaps also in small ruminants constitutes an increasing threat in Nigeria and many sub-Saharan countries as well as in China.[86]

### Etiology and Pathogenesis

Sheep- and goat-derived *Brucella melitensis* is the dominating infecting species. Ten members of the genus *Brucella* are known. *Brucella abortus* from cattle and *Brucella suis* from swine are unusual causes of human disease, whereas *Brucella melitensis* dominates because of the less effective eradication of animal reservoirs among sheep and goat. *Brucella canis* from stray or domesticated dogs is rare in humans.[82] The small Gram-negative organisms enter GI mucosa and survive in macrophages, migrating to lymph nodes where they multiply and spread to the circulation.

### Clinical Features and Diagnosis

Brucellosis causes arthritis in approximately one-third of cases. The main locations are the spine in adults and the peripheral joints in children and adolescents. Knees, hips, and ankles are the dominant peripheral locations. Sacroiliitis can be extremely acute and painful. Rising titers of serum antibodies and a confirmatory culture solidify the diagnosis. Case reports describe septic prepatellar bursitis[84] and olecranon bursitis[85] and indicate that fluid from these lesions can be diagnostic. Several serologic and polymerase chain reaction (PCR) tests are available. Blood cultures prove a septic state, whereas synovial cultures rarely are positive.[82]

### Treatment and Outcome

The World Health Organization guidelines for treatment include using doxycycline (100 mg p.o. twice a day) for six weeks, in combination with either streptomycin (1 g i.m. once a day) for two to three weeks or rifampin (600-900 mg p.o. once a day) for six weeks. The former regimen is thought to be more effective by some investigators. As with all regimens, the clinical circumstances of the patient could require a change in dose or duration of therapy.[86a] The arthritis can become destructive unless treated early. Spinal stenosis may be a complication.

## BOWEL-ASSOCIATED DERMATOSIS-ARTHRITIS SYNDROME

**KEY POINTS**

- BADAS develops as a consequence of bacterial overgrowth.
- BADAS responds to anti-microbial therapy.

### Epidemiology

Bowel-associated dermatitis-arthritis syndrome (BADAS) was discovered in the 1970s as "bypass arthritis" and later renamed because the syndrome was also seen without bypass surgery. The prevalence is not known, but originally it was reported in as many as 20% of patients after abdominal surgery. Newer surgical techniques have decreased the risk some, but awareness remains important in patients with impaired bowel function, including IBD and less common conditions (e.g., short bowel syndrome and systemic sclerosis).[87,88]

### Etiology and Pathogenesis

BADAS by definition is caused by disturbances in the GI canal. It can occur after all kinds of GI surgery. The supposed mechanism is dysbiosis often secondary to disturbed peristalsis or blind loop created by surgery or intestinal disease. Absorbed microbial products result in neutrophile dermatoses, which can be oral aphthous ulcers, pustular skin lesions, erythema nodosum, or pyoderma gangrenosum.[89] Recurrent arthritis, predominantly in the legs, arthralgia, and episodes of fever, indicate the systemic nature of BADAS. The molecular mechanisms have not been characterized. Bacterial overgrowth and neutrophil accumulation is present, and a role for immune complexes caused by absorbed microbial products has been postulated.[89]

### Clinical Features and Diagnosis

The main features seen in patients in the 1970s were an intensely painful oligoarthritis of large and small joints and spine, without structural changes, and a recurrent papulopustular rash (Figure 78-11). Tendinitis and enthesitis is another feature. Recurrent episodes of exacerbated skin and



**Figure 78-11** Relapsing pustulosis in a patient with bypass arthritis-dermatitis.

musculoskeletal manifestations may become chronic or disappear spontaneously.

### Treatment and Outcome

Antibiotic therapy with metronidazole, neomycin, or minocycline can be effective. Symptomatic therapy with local glucocorticoids or, sometimes, oral glucocorticoids can be used. Overtreatment should be avoided because the outcome is usually benign. Physical health and mobility is often improved following successful bariatric surgery.

## CELIAC DISEASE

| KEY POINTS |
|---|
| Adult CeD affects about 1% of the population and diagnosis is often delayed. |
| CeD is more common in women. |
| HLA-DQ2 or DQ8 confers susceptibility, with an odds ratio (OR) of 10. |
| Gluten triggers and maintains CeD. |
| Sensitivity to gliadin, endomysium A, and tissue transglutaminase are the causal factors. |
| Nonspecific symptoms cause delayed diagnosis. |
| Arthralgia and arthritis can be the initial symptoms. |
| A gluten-free diet is the only effective therapy. |

### Epidemiology

CeD is emerging as a common but underdiagnosed condition. It used to be considered a disease of Caucasians in Western countries, but now CeD occurs globally and in all ethnic groups.[90] The reported prevalence of 1% in adults may be an underestimate.[91] CeD is more common in women.[92] CeD has been found in 6% of patients with idiopathic juvenile arthritis.[92]

### Genetics

The association of CeD with HLA-DQ2 was discovered 30 years ago. To be susceptible, an individual must carry HLA-DQ2 or DQ8. This explains 40% of genetic susceptibility and confers an OR of more than 10. GWAS analysis has revealed 40 non–HLA-associated SNP polymorphisms with ORs less than 2.[91] As expected, several of the associations are shared with other immune-regulated diseases. Taken together, the replicated associations only explain 5% to 10% of CeDs heritability.[93,94]

### Etiology and Pathogenesis

Gluten intolerance and immunization against the identified antigens gliadin, endomysium A, and tissue transglutaminase explain much of the pathogenesis of CeD. It was shown in 2002 that dietary gluten is partly digested by gastric enzymes to generate a stable 33–amino-acid peptide that is deamidated by tissue transglutaminase.[95] The peptide is then presented in the context of HLA-DQ2 or HLA-DQ8 to $CD4^+$ T cells, resulting in IFN-γ release and inflammation. Early presence of impaired endothelial integrity[95] confirms the importance of a defect of the mucosal barrier. Villus atrophy is the end result. Malabsorption and systemic features from the skin, the endocrine glands, and the musculoskeletal system follow.

### Clinical Features and Diagnosis

Diarrhea and sprue symptoms are the classic symptoms, but patients with CeD more often present with atypical abdominal pain, nonspecific fatigue, headache, and arthralgia.[92] Correct diagnosis is often delayed for 10 or more years. CeD is a systemic disease involving type 1 diabetes, anemia, osteoporosis, neuropathies, and joint symptoms in as many as 25% of patients.[97] Asymmetric oligoarthritis or polyarthritis and axial involvement are common. Arthritis can be the presenting symptom of the disease.[98]

A small bowel biopsy showing atrophic villi is diagnostic, and serum for IgA anti–tissue transglutaminase antibodies and IgA antiendomysial antibodies are present in 95% of patients and confirms the diagnosis.[95]

### Treatment and Outcome

Elimination of gluten from the diet is the rational therapy, and is often the only one required. In addition, various experimental approaches have been discussed but are not yet supported by data. These include the administration of IL-10 to boost regulatory T cells, the induction of tolerance by nasal application of gluten peptides, and gene therapy.[99] No specific therapy has been established for the joint problems. Osteomalacia may require administration of vitamin D.

Children with verified CeD still have abnormal mucosa in adulthood and must continue the dietary restriction.[100]

## WHIPPLE'S DISEASE

| KEY POINTS |
|---|
| Anti–*Tropheryma whipplei* serology is found in 50% to 70% of adults. |
| Acute *T. whipplei* gastroenteritis outbreaks occur. |
| *T. whipplei* is transmitted by oral-oral and fecal-oral contact. |
| Asymptomatic carriage is the rule. |
| Restricted immune deficiency may be the cause of classic Whipple's disease. |
| Immune histology of gut biopsy establishes the diagnosis, and real-time PCR of saliva supports it. |
| Doxycycline and hydroxychloroquine is preferred versus trimethoprim-sulfamethoxazole. |

### Epidemiology

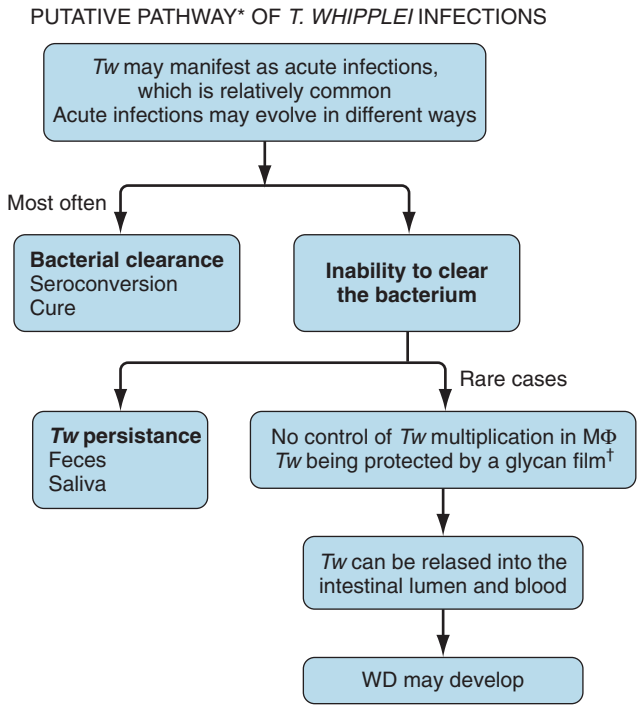
The discovery of *Tropheryma whipplei* in 1992, followed by the development of sensitive and reliable assays, have revealed that in contrast to the very rare classic Whipple's disease, infection with *T. whipplei* is extremely common in humans. The pathogen has been identified in sewers but no

animal reservoir is identified. The infection is spread by oral-oral and fecal-oral contact and is therefore more prevalent in populations with poor hygiene. Carriage is endemic among sewer workers. Positive serology is present in 50% to 70% of adult populations.[102] Acute outbreaks of gastroenteritis have been linked to the organism. The incidence and prevalence of classic Whipple's disease are unknown, but some clustering (e.g., in eastern parts of France) has been observed.

## Genetics and Pathogenesis

George Hoyt Whipple (1878-1976), an American pathologist, published the case of a 36-year-old white medical missionary with migratory polyarthritis, cough, diarrhea, weight loss, malabsorption, and mesenteric lymphadenopathy in 1907. Dr. Whipple observed "great numbers of a rod-shaped organism" in a lymph node.[102] In 1992, the nature of the organism as a Gram-positive actinomycete was finally identified with the bacterial 16S microRNA technique.[103] Five years later, Swiss investigators succeeded in culturing *T. whipplei* in deactivated human macrophages.[104] Classic Whipple's disease, originally named *intestinal lipodystrophy*, is rare, but 2% to 13% of Europeans carry live *T. whipplei* in their stool. The initial infection may be silent or manifest as an acute infection, which usually resolves but causes seroconversion. In a minority of the infected, the bacteria will not be cleared, resulting in an asymptomatic carrier state.[105,106] Then, for some unknown reason, the organisms begin to replicate inside macrophages, and cause the rare classic disease (Figure 78-12). In a cohort of 122 European patients, an association between the HLA alleles *DRB1*13* and *DQB1*06* and Whipple's disease was found, supporting a genetic contribution to susceptibility.[107]

Six different genotypes have been confirmed in cultures from diseased tissue.[108] Reinfection with different strains indicates that immunodeficiency restricted to *T. whipplei* with the inability to produce an adequate T cell response exists in some individuals.[102] The organism lives in macrophages, and these elicit a skewed lymphocyte response, with suppressed Th1 dominating Th2 cells. Expression of the cytokine IL-16 stimulates growth of the pathogen.[105] In vitro monocytes from patients with Whipple's disease express CD163, possibly explaining a reduced oxidative impact against *T. whipplei*.[106]




*This pathway is incomplete but this is the way the authors see the progression of the disease.

†Bonhomme CJ, Renesto P, Desnues B, et al: *Tropheryma whipplei* glycosylation in the pathophysiologic profile of Whipple's disease. *J Infect Dis.* 199:1043-1052, 2009.

**Figure 78-12** *Triphyroma whipplei* (TW) infection and its development into Whipple's disease. *(From Fenollar F, Lagier JC, Raoult D:* Tropheryma whipplei *and Whipple's disease.* J Infect Dis *69:103-112, 2014.)*

## Clinical Features

The disease can have many faces and may remain undiagnosed for decades. A correlation between different clinical manifestations and variable genotypes of the bacterium has not been identified, and host factors seem to be crucial for the kind of manifestation.[107] Altered gut permeability may be a factor in joint involvement. Dominating features can consist of recurrent fever, malaise, hematologic, pulmonary, cardiac disturbances, neurologic, or ophthalmic symptoms. Articular symptoms, however, are present early in two-thirds of cases, whereas intestinal symptoms are initially seen in only 15%. With time, diarrhea, abdominal pain, and malnutrition develop in 83% of patients. Arthralgia and arthritis are most frequent in knee joints but can localize in any peripheral joint, as well as in spinal joints and disks. Sacroiliitis has been described.

## Diagnosis

Immune histology, with the occurrence of periodic acid–Schiff–positive material, an abundance of $CD68^+$ macrophages, and staining with antisera specific for *T. whipplei* establishes the diagnosis. Real-time PCR serology of saliva and stool support the diagnosis.[109] PCR of cerebrospinal fluid can detect central nervous system involvement. The organism can grow out in culture, which takes an average of 30 days. In one study, only 2 of 10 small bowel specimens were culture positive; the yield is higher with sterile cardiac or nerve tissue.[104] Culture, however, remains a research tool.

## Treatment

The efficacy of oral trimethoprim-sulfamethoxazole is controversial. The recommended treatment consists instead of 12 months administration of doxycycline, 200 mg, combined with hydroxychloroquine, followed by life long prophylactic doxycycline.[102]

## Outcome

Without treatment, Whipple's disease is chronic or relapsing, usually progressive, and ultimately fatal. With adequate antibiotic therapy, clinical remission is usually complete or near complete.

## MICROSCOPIC COLITIS

Microscopic colitis (MC) is a name given to two conditions that present with profuse diarrhea. The first, collagenous colitis (CC), was described in 1976 and the second, lymphocytic colitis (LC), in 1989. A rare third condition, collagenous gastritis (CG), was described in 2009. Its frequent association with arthritis, arthralgia, diabetes, and thyroid disease motivates the inclusion in this chapter.[110]

### Epidemiology

Although initially considered rare, it is now evident that MC is a common cause of watery diarrhea in several populations, with an annual incidence of 5 to 6/10,000 for CC and for LC. It is 5 to 10 times more common in individuals older than 65 years and distinctly more common in women.[100] The female-to-male ratio is 7 : 1 in CC and 2 : 1 in CC. The peak incidence is seen in patients 60 to 80 years old. The total prevalence of 100 to 200/100,000 makes MC a common condition.[110]

### Etiology

Environmental factors in association with genetic susceptibility are involved. Smoking is an important contributing factor, and MC develops in smokers an average of 10 years earlier than in nonsmokers. MC is associated with *HLA-DQ2* or *DQ1,3*, the *HLA-DR3-DQ2* haplotype, and the *TNF2* allele, regardless of the presence of concomitant CeD.[110] Drug exposure is a likely trigger and may in part explain some disease associations. Drugs that are prime suspects include acarbose, aspirin and NSAIDs, proton-pump inhibitors (in particular lansoprazole), ranitidine, sertraline, and ticlopidine.[111] CC, LC, and CG all seem to be associated with CeD, autoimmune thyroid disease, and arthritis, presumably secondary to dysbiosis and abnormal GALT signaling.

### Pathogenesis

No infectious agent has been identified. Luminal factors are strongly suggested by the almost complete histologic normalization in patients after ileostomy is performed, and the recurrence of pathology and symptoms after its closure.[116] Connective tissue growth factor was markedly increased in the subepithelial zone of biopsies from CC patients analyzed by reverse transcription PCR, indicating a pathogenic role for connective tissue growth factor (CTGF).[117] Imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMP) and activation of myofibroblasts may contribute to the pathogenesis of CC. Abnormal cytokine signaling has been observed in LC,[112] and increased IL-17 signaling has been identified.[113]

### Clinical Features

Chronic and intermittent (or sometimes chronic and persistent) painful and watery stools, weight loss, and fatigue are the cardinal intestinal symptoms. There is no difference between collagenous and lymphocytic colitis in this regard. The course is acute or chronic but usually benign.

Autoimmune conditions are seen in 30% to 50% of MC cases.[110] These include rare cases of systemic lupus erythematosus (SLE) and scleroderma, Sjögren's syndrome, nondestructive oligoarthritis, migratory arthralgias, sacroiliitis, and rheumatoid arthritis.

### Diagnosis

The diagnosis can be made only by histology obtained with colonoscopy. Endoscopic examination is essentially normal. The Malmö pathologist Claes Lindström found a characteristic thickening of the collagen layer under the gut epithelium. This layer is normally 3 μm, but in CC, it is more than 10 μm and may reach 50 to 100 μm (Figure 78-13). In addition, one can see inflammation and an increased number of lymphocytes. The histology of LC shows an abundance of epithelial lymphocytes (Figure 78-14). In both conditions, the gut epithelium remains intact, although colonic mucosal tears are occasionally present.[116]

### Treatment and Outcome

Any suspected harmful drugs should be eliminated. The presence of CeD should be considered. Initial antidiarrheal therapy consists of 2 to 16 mg of loperamide and cholestyramine, 4 g/day. Bismuth subsalicylate is recommended.

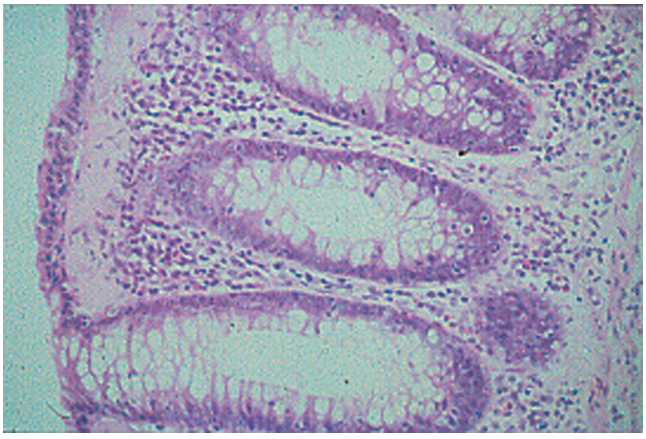


**Figure 78-13** Collagenous colitis. Note the intact epithelium and massive subepithelial collagen layer. *(Courtesy Dr. Claes Lindström.)*

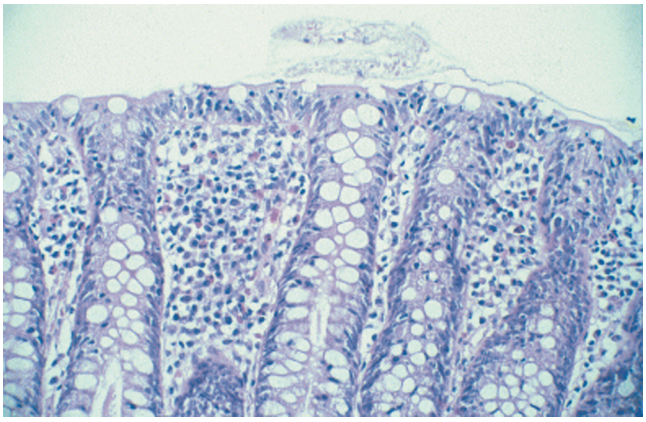


**Figure 78-14** Lymphocytic colitis. Note the epithelial lesions with intraepithelial lymphocytosis and inflammation of the lamina propria. *(From Wollheim FA: Collagenous colitis and rheumatology.* Curr Rheumatol Rep *2:183–184, 2000.)*

Budesonide, 9 mg/day, induces remission in at least 80% of patients, but relapse of diarrhea is common after the treatment is ended. When severe symptoms persist, TNF inhibitors, azathioprine, and even temporary ileostomy may be effective. The long-term prognosis is good, although arthralgia and other symptoms may persist for years.[114]

## PONCET'S DISEASE AND BACILLE CALMETTE-GUÉRIN–INDUCED ARTHRITIS

In 1897 the famous French professor of surgery in Lyon, Antonin Poncet published his paper "*La polyarthrite tuberculeuse simulant des lésions rhumatismales chroniques déformantes*," in which he described a patient with tuberculosis and arthritis without signs of local presence of tubercle bacillus. Similar cases have been identified during the years in children and adults with a diagnosis of tuberculosis and, more recently, in patients treated with bacille Calmette-Guérin (BCG) vaccine to stimulate immunity to malignant diseases. The existence of Poncet's disease (PD) remains controversial because it is rare and often a diagnosis of exclusion. The global surge of tuberculosis, with 10 million new cases per year, motivates a higher awareness of possible musculoskeletal manifestations.[118,119]

### Epidemiology

An extensive search of the literature identified 199 published case reports of PD. The following numbers of cases of PD have been reported globally: India, 70 cases; Brazil, 36 cases; Mexico, 20 cases; the United Kingdom, 12 cases; Nigeria, 6 cases; in Russia, Spain, the United States, and Pakistan, 5 cases; Iran, 4 cases; and in Japan, Korea, and France, 3 cases in each country.[120] One or two cases were published from several other countries. The incidence of BCG-induced cases is unknown, but is probably very low.

### Etiology and Pathogenesis

The vast majority of musculoskeletal manifestations of tuberculosis are of a septic nature. Active tuberculosis, or the administration of BCG, also can elicit nonseptic arthralgia and benign arthritis, which by definition is reactive. The enteropathic nature is not firmly established. HLA-B27 may be a susceptibility factor in post-BCG arthritis.[7] *Mycobacterium* heat shock protein 65 has been implicated in both of these sterile forms of arthritis, as well as in others.[121] Mycobacterial and human heat shock proteins are 50% homologous, and one hypothesis is that both the therapeutic efficacy and the arthritis are caused by cross-reactive T lymphocytes. Heat shock protein also has homologies with proteoglycan and HLA-DR. The pathogenesis of arthritis after intravesical instillation of BCG might be different and related to antigen persistence.

### Clinical Features and Diagnosis

PD is most often seen in younger adults of both sexes. Onset is insidious with fever, arthralgia, and malaise. Knee joints and ankles, followed by elbows and wrists, are the most common arthritis localizations. In contrast to septic tuberculosis arthritis, the condition is nondestructive. Several authors report rapid improvement upon therapy against tuberculosis.

The diagnosis is based on the clinical findings, presence, or a history of active tuberculosis, and exclusion of other causes of arthritis.

### Treatment and Outcome

Post-BCG cases usually heal without treatment within 3 months. Although culture results are negative, it is common practice to treat for 4 to 6 months with conventional antituberculosis therapy.[122] The positive outcome associated with this practice hints at sensitivity to bacterial elements.

## ENTEROVIRAL AND HEPATITIS VIRUS–ASSOCIATED ARTHRITIS

### Epidemiology

*Enterovirus* infections, similar to other common virus infections, often give rise to arthralgias.[123] The precise prevalence is not known. Hepatitis B and hepatitis C virus (HCV) infection causes joint manifestations in 2% to 38% of cases.[124] Considering that HCV infection alone causes 3 to 4 million new cases each year, this is not a trivial problem.[125]

### Genetics and Pathogenesis

No genetic factors have been identified. *Enterovirus* species can invade joints and may rarely be isolated from joint fluid.[126] Immune complex–mediated activation may result in viral hepatitis. A preferential deposition of such immune complexes in joints results in arthralgias and arthritis. In hepatitis C disease, precipitation of cryoglobulins is often observed. Molecular mimicry has been proposed but not proved. Viral persistence in host cells with continuous production of viral protein expressed on cell surfaces has been observed.[127] Immunosuppressive therapy may reactivate latent virus growth, and may be misinterpreted as increased disease activity in patients with other rheumatologic conditions.

### Clinical Features

Hepatitis B may be associated with a variety of general symptoms, including fever, abdominal pain, nausea, and vomiting after a prodromal period of as long as 6 months. Articular symptoms also develop in 10% to 25% of these patients, symptoms that may present during the prodromal phase. The preferential locations are hands and knees. In addition to morning stiffness, migratory arthritis is common. Interestingly, a coincident appearance of urticaria-like rash, predominantly of the legs, is common. The arthritis characteristically disappears with the onset of jaundice.[128]

A chronic disease with persistent or remitting articular manifestations develops in less than 5% of hepatitis B–infected individuals. The disease is self-limited, and requires only symptomatic treatment.[126]

HCV is associated with joint symptoms in 20% of cases. Other frequent symptoms are myalgia, glomerulonephritis, and vasculitis or essential mixed cryoglobulinemia, which may become severe. Oligoarthritis occurs in one-third of the cases, whereas polyarticular disease develops in two-thirds of cases.[129] The early symptoms can mimic RA. RF may be positive in as many as 80% of patients, whereas anti-citrullinated protein antibody (ACPA) only occurs in 4% to 7% of cases. Another mimic is SLE. HVC is among the suspected triggers of SLE.[125]

Enteroviruses, coxsackie viruses, and echoviruses are only associated with arthropathy in less than 1% of cases, but because of the high prevalence of the primary disease, the number of arthritis cases is not trivial.[129] After an incubation period of 3 to 5 days, the patients experience fever, fatigue, headache, pharyngitis, myalgia, rash, pleuritic pain, and conjunctivitis or, alternatively, nausea, abdominal pain, enteric cramps, vomiting, and diarrhea. Joint symptoms are self-limited and disappear within days, although recurrence can occur. Any peripheral joint may be affected.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 79

# Etiology and Pathogenesis of Systemic Lupus Erythematosus



Mary K. Crow

## KEY POINTS

- Systemic lupus erythematosus (SLE) results from chronic and recurrent activation of the immune system, with production of antibodies and other protein products contributing to inflammation and tissue damage.
- SLE is most common in females and typically begins in the childbearing years. This suggests a role for both hormones and as yet uncharacterized sex-related factors in disease pathogenesis.
- Progress in genetic analysis has resulted in identification of genetic variants associated with SLE, with most related to innate and adaptive immune system function. Complement deficiencies confer the highest risk of disease. Mutations or common variants in genes that encode components of the endosomal Toll-like receptor pathway or regulators of endogenous nucleic acids contribute to innate immune system activation.
- Environmental factors contribute to initiation of lupus and lupus flares.
- A contribution of nucleic acid–containing immune complexes as stimuli for endosomal Toll-like receptors has added an important new role for immune complexes in the pathogenesis of SLE.
- Production of type I interferon (IFN), broad expression of type I IFN–inducible genes, and the effects of IFN on immune system activation and function have emerged as central mechanisms of lupus pathogenesis.
- T cell subsets, including T follicular helper cells, cytokines, and soluble survival factors such as B lymphocyte stimulator (BLyS) promote B cell differentiation and autoantibody production.
- Platelets and neutrophils, along with neutrophil extra-cellular material, have gained new attention as important pathogenic effectors. Complement remains an important mediator of inflammation.

Systemic lupus erythematosus (SLE) is one of the most widely studied diseases in medicine. Predominantly a disease of young women in their childbearing years, and with the potential to cause significant physical disfigurement, morbidity, and, occasionally, mortality, lupus is the focus of strong advocacy to support research that will generate insights into disease pathogenesis. In fact, characterization of the immunologic contributors to lupus, the prototype systemic autoimmune disease, has been the focus of particularly intense study since the flowering of the discipline of immunology in the 1950s and 1960s. Recent efforts to define the genetic variations that underlie susceptibility to lupus have supported the central role of the immune system in disease pathogenesis, but have extended the view of lupus pathology beyond the important role of autoantibodies and T cells to include a significant contribution of the innate immune system to disease. An underlying role for the vasculature as a target of the immune system and its products is gaining renewed interest as an important component of lupus pathogenesis. Together, these recent advances provide important insights into how the intersection of genetic variations with environmental triggers amplifies immune system activation and target organ vulnerability to generate the classic manifestations of lupus and its clinically significant comorbidities. Figure 79-1 provides a schematic overview of many of the contributors to SLE and how their interactions drive autoimmunity and tissue damage.

## HISTORIC VIEW OF LUPUS PATHOGENESIS

Insights into the immunopathogenic mechanisms that account for development of autoimmunity in patients with SLE and the tissue damage that results in disease have occurred periodically during many decades. Progress has been informed by the scientific tools available at the time, along with the acute observations of clinicians and research scientists. Descriptions of the clinical manifestations of disease have suggested a multisystem disease that typically involves skin and joints, with renal, cardiac, and neurologic pathology suspected and then documented with histologic studies. Alterations of blood vessels in many organs were recognized as an important component of the disease process in early studies, with the pathognomonic "onion skinning" of splenic arterioles, cellular infiltration and damage to renal glomeruli, and vasculopathy in skin and brain demonstrated clinically by manifestations such as nephritis, livedo reticularis, and stroke. The lupus erythematosus (LE) cell, described by Hargraves in 1948, suggested that engulfment of cellular debris, a mechanism that remains an important concept in considerations of lupus pathogenesis, was active at sites of inflammation.

The recognition that antibodies directed at cellular components, particularly cell nuclei, were present in the

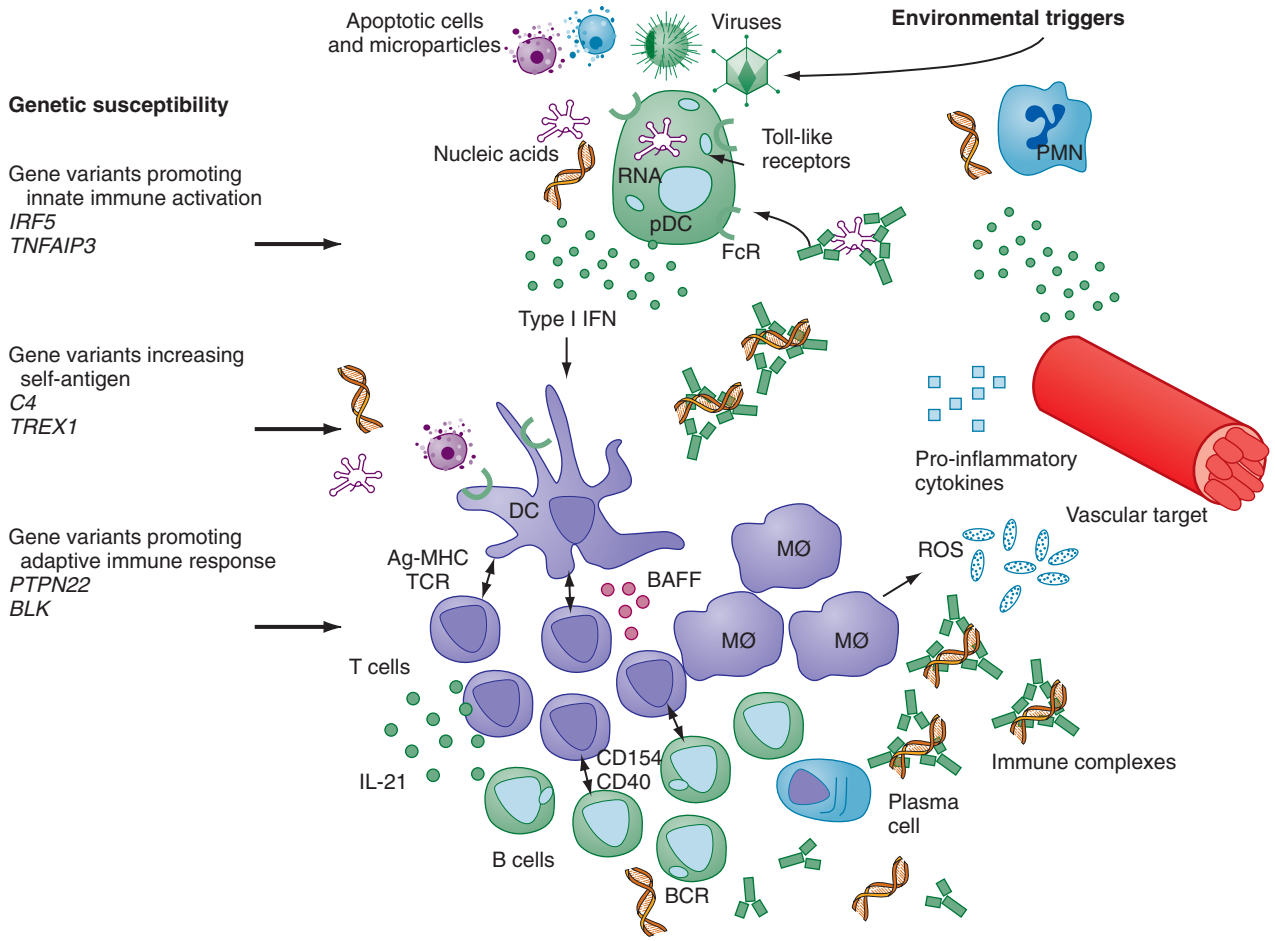



**Figure 79-1** Contributors to systemic lupus erythematosus (SLE) pathogenesis. Mechanisms that promote development of SLE are related to the underlying genetic profile of the individual. Many of the disease-associated genetic variants (examples are illustrated) contribute to excessive production or impaired clearance of stimulatory nucleic acids; increased generation of products of the innate immune response, particularly type I interferon (IFN); or an altered threshold for activation or efficiency of signaling of cells of the adaptive immune response. In most cases, multiple genetic risk variants are required to establish a state of immune activation that is receptive to environmental triggers that promote development of autoimmunity. In rare cases, a mutation of a critical regulator of immune activation can be sufficient to initiate the altered immune state that can lead to disease. IFN is a product of plasmacytoid dendritic cells (pDCs). Activation of the cells by intra-cellular nucleic acids or exogenous triggers, such as a virus or debris derived from damaged or dying cells, might represent mechanisms of initiation of disease. Once IFN-α is produced, it mediates numerous effects on immune system cells that mimic the response to a viral infection. The antigen-presenting capacity of myeloid dendritic cells can be augmented, promoting activation of self-reactive T cells and differentiation of B cells toward production of pathogenic antibodies. Activated T cells express CD154 (CD40 ligand) and produce interleukin-21 (IL-21), providing effective help for B cells to generate antibody-producing plasma cells. IFN-α also supports the production of B cell–activating factor (BAFF, also known as B lymphocyte stimulator), a survival and differentiation factor for B cells. Once autoantibodies are produced, immune complexes amplify immune activation by accessing endosomal Toll-like receptors in pDCs and B cells and depositing them directly in the vicinity of blood vessels, inducing complement activation, inflammation, and tissue damage. Reactive oxygen species (ROS) and pro-inflammatory cytokines produced by monocytes and macrophages contribute to tissue damage, as does IFN-α, which stimulates endothelial cells and is associated with poor vascular repair and sclerosis. Ag-MHC, Antigen–major histocompatibility complex; BCR, B cell receptor; DC, dendritic cell; FcR, Fc receptor; MØ, macrophage; PMN, polymorphonuclear neutrophil; TCR, T cell receptor.

sera of patients with SLE directed the attention of researchers toward the immune system. The conclusion was reached that lupus reflected an autoimmune process. Pathologic studies of kidneys from patients with lupus nephritis showing glomerular deposition of immunoglobulin and complement components, combined with the elution of anti-DNA autoantibodies from lupus kidneys, contributed to the concept that autoantibodies, particularly anti-DNA antibodies, were pathogenic. Isolation from lupus sera of complexes of antibody with DNA or DNA-binding proteins, such as histones, was an important factor in the classification of SLE as predominantly an immune complex-mediated autoimmune disease. Although lupus pathogenesis does depend on those immune complexes and the inflammatory responses that they induce, subsequent investigation identified virtually all cell components and many soluble immune system products as contributors to the immune system dysfunction that ultimately accounts for disease in SLE.

The development of the field of cellular immunology in the 1970s and the later identification of the T cell antigen receptor, along with families of co-stimulatory molecules that mediate interactions between T cells and antigen-presenting cells or B cells, led to the implication of self-reactive T cells as important regulators of immune responses, as well as helpers for B cell differentiation to autoantibody-producing cells in the case of patients with SLE. Numerous deficiencies in T cell function were described, including

altered production of typical T cell cytokines, such as interleukin (IL)-2, and studies of murine lupus models strongly supported the essential role that T cells play in lupus pathogenesis.

The discovery of Toll-like receptors (TLR) in the 1990s, the recognition that those receptors recognize common determinants expressed by microbes, and the demonstration that TLRs mediate immune system activation was a major advance and perhaps the most significant milestone in many decades. These findings have led to gains in understanding of lupus pathogenesis. Characterization of the typical exogenous ligands of each of the TLRs has been followed by identification of endogenous ligands for their receptors. Nucleic acids are the most relevant for amplification of immune system activation and autoimmunity in SLE. It is not yet clear whether the TLRs act to augment immunologic activity that is initiated by other molecular pathways or if they are also essential as sensors for the primary initiators of autoimmune disease.

In spite of these considerable gains in elucidating the pathogenesis of SLE, the environmental triggers and genetic susceptibility factors that lead to the initiation of autoimmunity in some individuals but not others remain largely undefined. Regarding triggers of disease, clinical observations have pointed to exposure to sunlight, microbial infection, and certain drugs as factors that can lead to initiation or exacerbation of lupus. Whether there is a common thread among these environmental triggers is not clear, but ultraviolet (UV) light–mediated DNA damage and modification of DNA methylation are among the processes that might render self-nucleic acid stimulatory to the immune system. Oxidative damage to cell proteins could alter cell function and impair immune system regulation.

Significant technologic advances in recent years, together with development of consortia of investigators who have cooperated to share biologic samples from patient cohorts and control participants, have supported important progress in defining the genetic variants that show a statistical association with a diagnosis of SLE. These recent genome-wide association studies (GWAS) have used the long-standing observations of the high risk of disease in patients with complement deficiencies to help identify lupus-associated genes that encode important components of molecular pathways that promote production of type I interferon (IFN), alter the threshold for activation of lymphocytes, or generate immune stimuli. In addition, new concepts suggested by single-gene mutations that alter regulation of genome integrity and nucleic acid degradation, resulting in lupus-like clinical syndromes, point to the significance of endogenous nucleic acids as candidate disease triggers.[1,2]

Together, these scientific advances have led to the recent, long-awaited activity in drug development programs that target mechanisms likely to affect lupus disease activity or clinical progression. This chapter reviews recent studies that have illuminated the immunopathogenesis of SLE.

## GENETIC CONTRIBUTIONS TO LUPUS PATHOGENESIS

Current concepts in genetic studies of complex human diseases, including SLE, identify common variants with a small impact on risk of disease, as well as low-frequency mutations that can have a great impact on the risk for lupus or a lupus-like syndrome.

Observations of the occurrence of several individuals with SLE within a family, along with a high frequency of concordance of SLE in identical twins, have pointed to a strong genetic contribution to SLE. Data suggest that concordance of clinical lupus disease in twins is 10 times more frequent in monozygotic than in dizygotic twins, although the highest reported concordance rate is still only 57%. Moreover, the suggestion that multiple autoimmune diseases are associated with common genetic susceptibility factors is supported by the aggregation of several distinct autoimmune diseases within a family. The pace of discovery of common genetic variants, typically identified by statistical analysis of single nucleotide polymorphisms (SNPs) in relation to a diagnosis of SLE, has markedly accelerated in recent years because the cost of genotyping thousands of patient and control samples has made studies feasible. Publication of eight collaborative GWAS, along with meta-analyses, have identified more than 50 genes or genomic loci associated with SLE.[3] A common theme among many of the lupus-associated genes is that they encode proteins implicated in immune system function. Although this result is not surprising, it confirms the essential contribution of the immune system to the autoimmunity, inflammation, and tissue damage that characterize lupus disease and points to the most significant molecular pathways that mediate altered immune function. Additional disease-associated sequence variants and studies defining DNA hypersensitive sites, potentially reflecting chromatin that is accessible to DNA-binding proteins, have been identified through the Encyclopedia of DNA Elements (ENCODE) consortium and suggest a role for regulatory elements that orchestrate complex gene expression programs relevant to lupus pathogenesis.[4]

The lupus-associated genes that contribute to lupus pathogenesis can be considered in relation to their roles in immune function (Tables 79-1 and 79-2). In parallel to the requirements for immune system activation stimulated by foreign antigens, SLE-associated genes are involved in generation of self-antigen, activation of the innate immune response, and activation of the adaptive immune response.

**TABLE 79-1** Genetic Variants Associated with Systemic Lupus Erythematosus—Major Histocompatibility Complex Genes

| **Major Histocompatibility Complex Genes Associated with SLE** |
|---|
| Homozygous deficiencies of early complement components (C2, C4A, C4B) (increase risk 5- to 10-fold) |
| *HLA-DR2* (increases relative risk twofold to threefold) |
| *HLA-DR3* (increases relative risk twofold to threefold) |
| *DR2/DRX* associated with anti-Sm antibodies |
| *DR3/DRX* associated with anti-Ro and anti-La antibodies |
| *DR2/DR3* associated with anti-Ro, anti-La, and/or anti-Sm and also associated with anti-dsDNA antibodies |
| *DR3/DR3* associated with anti-Sm antibodies |

**TABLE 79-2** Genetic Variants Associated with SLE—Non-MHC Genes

| Non-MHC Genes Associated with SLE |
|---|
| Homozygous deficiency of C1q (increases risk 5- to 10-fold) |
| **Associations Based on Linkage Studies** |
| Fc gamma receptor IIa *(FCGR2A)* |
| Fc gamma receptor IIIa *(FCGR3A)* |
| Fc gamma receptor IIb *(FCGR2B)* |
| Programmed cell death 1 *(PDCD1)* |
| **Associations Based on Candidate Gene Studies** |
| C-reactive protein *(CRP)* |
| Interleukin-10 *(IL-10)* |
| Interferon regulatory factor 5 *(IRF5)* (supported by GWAS) |
| Protein tyrosine phosphatase 22 *(PTPN22)* (supported by GWAS) |
| **Associations Based on or Confirmed by Genome-Wide Association Studies** |
| AF4/FMR2 family, member 1 *(AFF1)* |
| AT-rich interactive domain 5B *(ARID5B)* |
| Autophagy-related 5 *(ATG5)* |
| B cell scaffold protein with ankyrin repeats *(BANK1)* |
| B lymphocyte-specific tyrosine kinase *(BLK)* |
| *CD80* |
| Cyclin-dependent kinase inhibitor 1B *(CDKN1B)* |
| C-type lectin domain family 16, member A *(CLEC16A)* |
| C-Src tyrosine kinase *(CSK)* |
| DNA-damage regulated autophagy modulator *(DRAM1)* |
| E74-like factor 1 *(ELF1)* |
| V-ETS avian erythroblastosis virus E26 oncogene homolog 1 *(ETS1)* |
| Intercellular adhesion molecule 1/4/5 *(ICAM1/4/5)* |
| Interferon induced with helicase C domain 1 *(IFIH1/MDA5)* |
| IKAROS family zinc finger 1 *(IKZF1)* |
| IKAROS family zinc finger 3 *(IKZF3)* |
| Interleukin-21 *(IL-21)* |
| IL-1 receptor-associated kinase/methyl-CpG-binding protein 2 *(IRAK1/MECP2)* |
| Interferon regulatory factor 7 *(IRF7)* |
| Interferon regulatory factor 8 *(IRF8)* |
| Integrin alpha M *(ITGAM)* |
| JAZF zinc finger 1 *(JAZF1)* |
| V-yes-1 Yamaguchi sarcoma viral–related oncogene homolog *(LYN)* |
| *MIR146A* |
| Neutrophil cytosolic factor 2 *(NCF2)* |
| Nicotinamide nucleotide adenylyltransferase 2 *(NMNAT2)* |
| Pyruvate dehydrogenase complex, component X/CD44 *(PDHX/CD44)* |
| PHD and ring finger domains 1 *(PHRF1)* |
| PR domain-containing 1, with ZNF Domain (BLIMP1) *(PRDM1)* |
| Protein kinase C, beta *(PRKCB)* |
| Pituitary tumor-transforming 1 *(PTTG1)* |
| PX domain-containing serine/threonine kinase *(PXK)* |
| RAS guanyl-releasing protein 3 *(RASGRP3)* |
| Solute carrier family 15, member 4 *(SLC15A4)* |
| Signal transducer and activator of transcription 4 *(STAT4)* |
| Tet methylcytokine deoxygenase 3 *(TET3)* |
| Toll-like receptor 7 *(TLR7)* |
| Transmembrane protein 39A *(TMEM39A)* |
| Tumor necrosis factor-α–induced protein 3 *(TNFAIP3)* |
| TNFAIP3-interacting protein 1 *(TNIP1)* |
| Tumor necrosis factor superfamily, member 4 *(TNFSF4/OX40L)* |
| Three-prime repair exonuclease 1 *(TREX1)* |
| Tyrosine kinase 2 *(TYK2)* |
| Ubiquitin-conjugating enzyme E2L 3 *(UBE2L3)* |
| UHRF1-binding protein 1 *(UHRF1BP1)* |
| WDFY family member 4 *(WDFY4)* |
| XK, Kell blood group complex subunit–related family, member 6 *(XKR6)* |
| **Rare Genetic Mutations Associated with Lupus** |
| Three-prime repair exonuclease 1 *(TREX1)* |
| Ribonuclease H2, subunit A-C *(RNASEH2A-C)* |
| SAM domain and HD domain 1 *(SAMHD1)* |
| Adenosine deaminase, RNA-specific *(ADAR)* |

AT, Adenine, thymine; ETS, E26 transformation-specific; GWAS, genome-wide association study; MHC, Major Histocompatibility Complex; PHD, plant homeodomain; PR, positive regulatory; SAM, *S*-adenosylmethionine; SLE, systemic lupus erythematosus.

Although in most cases the specific functional alterations that are conferred by the lupus-associated variant compared with the more common variant have yet to be defined in detail, sufficient information exists regarding its function for hypotheses to be developed and tested in functional genetic studies.

The rare but high-risk deficiencies in complement pathway gene products, including C2, C4, and C1q, are thought to contribute to lupus pathogenesis by impairing clearance of cellular debris, a function that is typically supported by those complement components. Increased availability of nuclear debris can provide sufficient self-antigen for induction of self-reactive T cells, or serve as an endogenous adjuvant for activation of the innate immune response. The ancestral major histocompatibility complex (MHC) 8.1 haplotype block, human leukocyte antigen (HLA)-B8/DR3/DQw2/C4AQO, that is associated with lupus susceptibility, encodes the alleles B8 and DR3 and bears a short *C4B* gene and no *C4A* gene, whereas other nonrisk haplotypes carry either a longer *C4B* segment and/or one or more copies of *C4A*. In fact, the relative risk related to the *C4A* null allele is twice that of either HLA-B8 or DR3, pointing to the significance of the C4 genes in disease risk.[5] It should be noted that this risk haplotype is also associated with accelerated disease in patients infected with human immunodeficiency virus (HIV), insulin-dependent diabetes mellitus, and several other autoimmune diseases. Deficiency of C1q, the recognition protein for the classical complement pathway, might contribute to disease on the basis of its important role in promoting clearance of apoptotic cell debris by mononuclear phagocytes.[6] C1q plays an additional role in inhibition of IFN-α production by directing stimulatory immune complexes to monocytes rather than IFN-producing plasmacytoid dendritic cells (pDCs). Through this mechanism C1q deficiency can augment IFN-α and promote broad immune dysregulation.[7] C-reactive protein (CRP), a member of the pentraxin family, also contributes to clearance of apoptotic debris. Polymorphisms in *CRP* have been associated with SLE and with decreased levels of CRP, but it remains unclear how much of the genetic contribution to basal CRP levels is caused by variations within the gene itself versus variations in other genes.

Recent studies of families with a lupus-like disease, the Aicardi-Goutières syndrome (AGS), suggest that mutations in genes that regulate the integrity of endogenous DNA or RNA might result in excess stimulatory nucleic acid and innate immune system activation.[8-11] AGS is characterized by skin lesions, autoantibodies, central nervous system disease, and high levels of type I IFN, but unlike SLE it is typically diagnosed in young children. Mutations in the *TREX1* gene, encoding a DNase that is required to degrade potentially stimulatory reverse-transcribed DNA, were identified in patients with AGS, and a functional connection between *TREX1* and control of type I IFN production was demonstrated in a murine model in which *TREX1* deficiency resulted in increased levels of IFN-β.[12] A recent analysis of more than 8000 lupus patients found *TREX1* mutations in approximately 0.5% of patients. In addition, a common variant in the *TREX1* gene conferred an odds ratio for diagnosis of lupus of 1.73 compared with healthy controls.[13] Mutations in *SAMHD1*, encoding an enzyme that

removes phosphates from deoxyribonucleotide triphosphate (dNTPs), reducing available nucleotides for reverse transcription, have also been identified in AGS patients and are associated with triggering of a DNA damage response and type I IFN production.[10] Mutations in members of the *RNASEH2* family that remove ribonucleotides from RNA-DNA hybrids also activate an innate immune response and are associated with a diagnosis of AGS.

A recent study of parents of AGS patients with *RNASEH2* mutations implicated that gene in protection from autoimmunity. Parents who were heterozygous for the variant *RNASEH2* demonstrated an intermediate autoimmune phenotype characterized by anti-nuclear antibodies, and showed accumulation of ribonucleotides in their genomes, generation of DNA damage characterized by elevated cyclobutane pyrimidine dimer formation, and activation of the p53 pathway.[14] *ADAR*, with mutations also reported in association with AGS, encodes an RNA-specific adenosine deaminase that converts adenosine to inosine in dsRNAs, particularly those enriched in Alu sequences, and typically functions as a suppressor of the cell intrinsic type I IFN response.[11] Each of these gene products plays a role in control of the quality of endogenous nucleic acids, reduces the risk to the genome of production of endogenous virus-like transposons, and appears to be important for avoiding aberrant activation of an innate immune response characterized by IFN pathway activation. Collectively, these syndromes have been termed *interferonopathies* and provide important clues to pathogenic mechanisms relevant to SLE.[11]

A syndrome with some features consistent with the vasculopathy that characterizes many patients with SLE or systemic sclerosis develops in young children with mutations in *TMEM173*, encoding STING (stimulator of interferon genes), a cytosolic DNA sensor and an essential component of the TLR-independent pathway of immune system activation.[15] Skin rash, a livedo-like vascular pattern, distal extremity ulcerations, interstitial lung disease, markers of systemic inflammation, and low-titer autoantibodies are among the clinical characteristics of patients with *STING* gain-of-function genetic alterations. These patients show increased transcription of IFN-β and IFN-stimulated genes and constitutive phosphorylation of signal transducer and activator of transcription (STAT)1, a signaling component of the Janus kinase (JAK)-STAT pathway that is activated downstream of IFNAR, the type I IFN receptor. In vitro studies that use a JAK inhibitor support targeting the effector arm of the IFN pathway in this syndrome.[15] Taken together, these recent demonstrations of the association of rare genetic variants of enzymes that regulate degradation of nucleic acids with SLE or lupus-like syndromes point to the central role of those nucleic acids as triggers for immune system activation and disease.

In addition to the information provided by the rare mutations described previously, a large number of lupus-associated SNPs found in genes that encode proteins involved in induction of type I IFN or response to that family of cytokines draw additional attention to the important role of type I IFN and the innate immune response in SLE pathogenesis.[16-18] IFN regulatory factor 5 (IRF5) and IRF7 are cytoplasmic proteins that translocate to the nucleus after effective activation of the endosomal TLRs by DNA or RNA. IRF5 and IRF7 then act as transcription factors to initiate transcription of IFN-α and other pro-inflammatory mediators. *TNFAIP3* encodes A20, a protein that regulates several pro-inflammatory cellular mechanisms, including signaling through endosomal TLRs and activation of nuclear factor-κB (NF-κB).[19] Genetic variants in *IRF5*, *IRF7*, *TNFAIP3*, *TNIP1*, and numerous other lupus-associated genetic variants can be mapped to molecular pathways responsible for induction of innate immune system activation or responsiveness to its products, particularly IFN-α.[3]

A third set of lupus-associated gene variants contributes to altered thresholds for lymphocyte activation or efficiency of cell signaling. The ancestral MHC 8.1 haplotype that is strongly associated with a diagnosis of SLE, along with other autoimmune diseases, appears to influence the early stages of immune system activation. Although more efficient presentation of self-antigens by disease-associated class II MHC alleles would seem to be the most likely mechanism by which the MHC risk haplotype confers predisposition to autoimmunity, the available data point to a number of immune alterations, many focused on the T cell, in healthy individuals who carry the ancestral haplotype.[5] The impact of those alleles is best defined clinically as determining immune responses that generate particular autoantibody specificities, and the alleles seem to be important in determining whether anti-DNA autoantibodies, antibodies specific for RNA-associated proteins, or both. can be induced and produced through T cell–dependent B cell differentiation.[20] Additional lupus-associated variants that alter adaptive immune system activation are involved in cytokine signaling, such as *STAT4*, or efficiency of signaling downstream of the T and B cell surface antigen receptors, such as *PTPN22* in the case of both T and B cells, and *LYN*, *BANK*, *BLK*, *TNFAIP3*, and others in the case of B cells.[3] MicroRNAs are RNA transcripts that regulate longevity of target mRNAs and represent another source of genetic variability that can impact adaptive immune system regulation.[21]

A fourth category of lupus-associated genetic variants defines determinants of target organ damage. The understanding of genetic determinants of target organ vulnerability to immune-mediated or oxidative damage is less well developed than the role of genetic variants in altered immune system function in SLE. In addition to SNPs in *TNIP1*, encoding a protein associated with TLR signaling and risk of lupus nephritis, recent studies have found an association between *MTMR3*, which encodes myotubularin-related protein 3, with lupus nephritis, and a link between alleles of *APOL1*, involved in cholesterol transport, and end-stage renal disease. The link in the latter disease occurs particularly in African Americans.[22-25] Table 79-1 and 79-2 list many of the genetic variants documented to have a statistical association with a diagnosis of SLE.

Although impressive progress based on GWAS has identified statistical association of sequence variations in genes or genetic loci with a diagnosis of SLE, the functional consequences of those variations have not been extensively characterized. The best developed insights regarding the impact of lupus-associated variants on immune function have derived from studies of gene products involved in production of type I IFN. The risk alleles for *IRF5* and *IRF7* are associated with increased serum type I IFN activity in

those patients who demonstrate autoantibodies targeting DNA or RNA-associated proteins.[16,26] Those studies support the hypothesis that nucleic acid–containing immune complexes are important stimuli that act through endosomal TLRs such as TLR7 and TLR9, those TLRs that mediate cell signals through the IRF5 and IRF7 transcription factors.[1,2]

The impressive collaborative efforts that have successfully collected sufficient patient and control samples and performed the required genotyping and statistical analyses have defined or at least confirmed those molecular pathways that are central to the immunopathogenesis of SLE. The strong support for activation and altered regulation of the TLR pathway, along with variations in lymphocyte signaling, point to potential therapeutic targets for future study. In addition, the recent identification of rare mutations in enzymes required for degradation or regulation of intracellular nucleic acids suggests that further study of the TLR-independent pathways of innate immune system activation, mediated by nucleic acid sensors and their associated kinases and adaptor molecules as well as DNA damage pathways might be fruitful areas for further investigation.

Subphenotype analysis will continue to amplify the information that can be gleaned from patient and control genotyping. A study of anti-dsDNA$^+$ and anti-dsDNA$^-$ SLE patients, along with healthy controls, identified HLA-DR3, *STAT4*, and *ITGAM* as significantly associated with the presence of anti-dsDNA antibodies.[27] A case-only analysis additionally associated *PTPN22*, *IRF5*, and *PTTG1* with anti-dsDNA antibodies. In contrast, a distinct group of lupus-associated genes showed comparable association between anti-dsDNA positive and negative patients, including *FCGR2A*, *OX40L*, *IL10*, *PXK*, *UHRF1BP1*, *PRDM1*, *BLK*, and *IRAK1*. Although it is possible that some of these variants are associated with distinct autoantibody specificities, it is more likely that they represent risks for general immune system activation, inflammation or tissue damage.

Whether the insights regarding specific genetic susceptibility factors can be used to predict development of lupus or particular manifestations of disease is as yet unclear. Recent studies have attempted to determine the predictive value of accumulated genetic risk variants. Although increased numbers of lupus-associated alleles are associated with some increased risk of disease and the number of lupus-associated genetic alleles is significantly higher in patients with anti-dsDNA antibodies than in those without those autoantibodies, at this time, it would not appear that genotyping for lupus risk is sufficiently informative to warrant practical application in patient management.[28]

## FEMALE PREDOMINANCE OF SYSTEMIC LUPUS ERYTHEMATOSUS

A discussion of genetic contributions to SLE pathogenesis cannot ignore the dramatic 9:1 female predominance of the disease. Of all of the characteristic clinical features of lupus, it is the extreme sex skewing that remains least understood. Hormonal contributions to immune system activation are likely to represent a component of the female predominance of the disease; estrogen can modulate lymphocyte and pDC activation, and prolactin is expressed at increased levels in lupus serum.[29]

Even given the contribution of hormones to increased immune activation, additional factors should be examined to understand why 9 or 10 females are diagnosed with SLE for every male lupus patient. It is intriguing that Klinefelter's syndrome, characterized by a 47,XXY genotype, is increased 14-fold among men with SLE compared with men without SLE.[30] These data are proposed to suggest an X chromosome gene dose effect as an important contributor to SLE pathogenesis. Identifying the X chromosome as a possible risk factor for SLE provides a basis for new hypotheses regarding disease susceptibility, but the nature of that risk remains undefined. A possible role for altered regulation of epigenetic processes, such as DNA methylation, has been raised, and murine studies have supported the impact of duplications of portions of the X chromosome that encode the *TLR7* gene in activation of the innate immune system, production of type I IFN, and generation of autoimmunity.[31] Of note, recent data that used a humanized mouse model support roles for both estrogen and X chromosome dosage in augmented pDC production of type I IFN in response to TLR7 ligation.[32] Investigation of altered gene dosage in human patients has not confirmed a duplication of the *TLR7* gene, and studies of DNA methylation are most suggestive of altered DNA methylation reflecting generalized immune activation, with a particular focus on type I IFN–stimulated genes, rather than a primary etiologic event.[33] However, the *TLR7* gene is located on a region of the X chromosome in which escape from X chromosome inactivation has been documented, so further studies of epigenetic regulation of X chromosome structure and gene expression are warranted.[34]

A potential pathogenic role for the distinct events that occur in the ovary compared with those in the testes also deserves further study. The onset of SLE most typically occurs in the childbearing years, after menarche and before menopause. A positive association between early menarche and SLE has been observed, and breastfeeding confers a protective effect.[35] Both observations might be consistent with molecular and cellular events related to ovulation as factors contributing to lupus pathogenesis. It is intriguing to consider that the biology of germ cell maturation, with female germ cells undergoing a second meiotic division before ovulation each month, might involve mechanisms and mediators that affect immune recognition. Although not yet fully understood, the carefully orchestrated demethylation and remethylation of DNA, along with the production of regulatory RNAs and RNA-associated proteins such as so-called PIWI proteins, in the germ cells and associated somatic cells might provide a source of stimulatory nucleic acid–containing complexes that could access TLR-dependent or TLR-independent pathways and result in immune activation.[36] The challenge in pursuing such concepts is the obvious limitation on access to ovarian tissue for study, although murine models might be helpful in that regard.

## ENVIRONMENTAL TRIGGERS OF LUPUS

Concordance rates for development of SLE in identical twins range from 24% to 57%, indicating that that both environmental factors and stochastic events (i.e., chance) contribute to development of SLE in an individual.

**TABLE 79-3** Environmental Factors That Might Play a Role in the Pathogenesis of Systemic Lupus Erythematosus

| |
|---|
| **Definite** |
| Ultraviolet B light |
| **Probable** |
| Estrogen and prolactin: In humans, the female-to-male ratio is 9:1 between menarche and menopause. The ratio is 3:1 in pre-menarche children |
| Epstein-Barr virus (EBV) |
| Lupus-inducing medications* |
| Hydralazine |
| Procainamide |
| Isoniazid |
| Hydantoins |
| Chlorpromazine |
| Methyldopa |
| Penicillamine |
| Minocycline |
| TNF inhibitors |
| IFN-α |
| **Possible** |
| Dietary factors |
| Alfalfa sprouts and related sprouting foods containing canavanine |
| Pristane and other hydrocarbons |
| Infectious agents other than EBV |
| Bacterial DNA |
| Smoking |
| Human retroviruses or endogenous retroelements |
| Endotoxins, bacterial lipopolysaccharides |
| Vitamin D deficiency |

*Although each drug listed and many others can induce lupus-like symptoms in predisposed individuals, there is little evidence that they can induce true SLE or even activate disease in individuals with spontaneous, established SLE. If the clinical care of a patient with SLE would benefit from the use of one of these drugs, the drug should not be withheld.

SLE, Systemic lupus erythematosus.

Although some environmental triggers of disease have been identified on the basis of clinical observation and epidemiologic studies (Table 79-3), in general there is incomplete understanding of the range of factors that can induce disease and the mechanisms by which they do so.[37] Socioeconomic factors have been demonstrated to contribute to poor outcomes in lupus patients, likely related to poor access to care.[38] The distinct contributions of low socioeconomic status versus genetic factors that impact disease severity in certain ethnic groups are difficult to dissect.

Clinical manifestations that are often present at the time of diagnosis, including fatigue and arthralgias, have suggested that a virus might initiate the disease. In fact, epidemiologic studies demonstrating higher prevalence of antibodies specific for Epstein-Barr virus (EBV) antigen in children with SLE compared with the general population, as well as higher frequency of anti-EBV antibodies in a military cohort before diagnosis of lupus, support a possible role for that virus in disease pathogenesis.[39] In addition, SLE patients demonstrate increased EBV DNA in blood.

Several potential mechanisms might account for a pathogenic role for EBV in SLE, including production of EBV-encoded RNAs that induce type I IFN, utilization of B cell signaling pathways to promote B cell activation and differentiation, and generation of autoantibodies reactive with DNA- or RNA-binding proteins through a molecular mimicry mechanism.[40] EBV-encoded small RNAs, or EBERs, are expressed in cells latently infected with EBV. EBERs induce expression of type I IFN after binding to the dsRNA-dependent protein kinase (PKR) and activating signaling through a TLR-independent pathway. Latent membrane protein 1, encoded by EBV, can act as a mimic of CD40 and promote B cell dysfunction and autoimmunity in lupus-susceptible mice.[41] A recent study demonstrates that antibodies specific for the virus-encoded EBNA-1 protein can also react with dsDNA.[42] The molecular basis of this apparent cross-reactivity is not known, although DNA can associate with EBNA-1 and might account for the described antibody reactivity. T cell responses specific for EBV have been studied in patients with lupus and found to be deficient, perhaps contributing to the increased numbers of EBV-infected mononuclear cells and increased copy number of EBV DNA found in SLE patients.[43]

Although EBV may or may not be an important etiologic agent in SLE, recent studies of murine models of persistent infection with lymphocytic choriomeningitis virus provide a useful system for study of pathogenic mechanisms that appear highly relevant to the immunopathogenesis of SLE.[44-46] Sustained production of type I IFN and a shift from expansion of T effector cells to expansion of T follicular helper cells accompanies the organ inflammation and damage that characterize those models of persistent virus infection. In the mice with prolonged production of IFN, blockade of the type I IFN receptor abrogates tissue damage. Data from comparisons of immune system function in patients with HIV-1 infection to macaques with infection with the related but less lethal simian version of the virus further support a view of chronic production of type I IFN as an important mechanism that promotes widespread inflammation similar to that seen in patients with lupus.[47] These models of chronic viral infection provide a guide for further study of key mechanisms that sustain disease in patients with lupus, and the growing interest in the impact of the microbiome on immune function will likely generate additional new hypotheses.

Environmental toxins are of interest, yet their potential role in lupus pathogenesis has not been comprehensively explored. Data support current smoking as a risk factor for SLE, with a dose response relating pack years of smoking to risk. As is currently proposed with regard to pathogenesis of rheumatoid arthritis, smoking might provide an inflammatory stimulus to epithelial or mononuclear cells in the lungs, promoting protein modification or nonspecific inflammation. Silica has also been proposed as a potential pathogenic factor in SLE on the basis of its known capacity to serve as an adjuvant.[48]

Two well-described triggers of lupus, ultraviolet light and certain drugs, are likely to promote lupus pathogenesis through their effects on DNA. UV light has many effects on skin cells, including induction of DNA breaks that might alter gene expression or lead to apoptotic or necrotic cell death. Even in the absence of cell death, DNA breaks or prolonged maintenance of DNA-protein cross-links might provide either an adjuvant or antigenic stimulus to the immune system. In that regard, recent data show UV-induced generation of cyclobutane pyrimidine dimers and induction of IFN-stimulated genes in cells from individuals heterozygous for mutations in *RNASEH2* genes, encoding enzymes that remove ribonucleotides from genomic DNA.[14] Altered DNA methylation has been proposed as a likely mechanism

of drug-induced lupus.[49] In the case of hydralazine, the drug inhibits extra-cellular signal-regulated kinase (ERK) pathway signaling, resulting in decreased expression of DNA methyltransferase (DNMT) 1 and DNMT3a, two enzymes that mediate DNA methylation.[50] Modification of ERK signaling can be mediated by the effects of oxidizing agents on protein kinase C delta, an upstream regulator of ERK.[51] Altered DNA methylation modifies gene expression and might also expose potential ligands for nucleic acid sensors, promoting immune system activation.

## INNATE IMMUNE SYSTEM ACTIVATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

Arguably the most significant recent advance that has improved understanding of the pathogenesis of SLE and other autoimmune and inflammatory diseases is the discovery of the TLRs and, more generally, the elucidation of the central role of innate immune system activation in regulation of the adaptive immune response, inflammation, and tissue repair.[52] The TLRs are composed of an ectodomain with leucine-rich repeats, a transmembrane domain, and a cytoplasmic domain that associates with adaptor molecules that initiate signaling, resulting in activation of members of the IFN-regulatory factor family, NF-κB, and members of the mitogen-activated protein (MAP) kinase family. TLR-independent innate immune activation can be initiated by intracytoplasmic RNA sensors, such as RIG-I and MDA5, that signal through a mitochondrial adaptor protein, MAVS, to activate IKK-ε and TBK1.[53,54] DNA sensors include IFI16, DAI, and the nucleotidyltransferase cGAS, which generates a 2′-5′ linked cyclic dinucleotide, cGAMP, that binds the endoplasmic reticulum protein STING, also resulting in activation of TBK1.[55-57] Recognition of both RNA and DNA leads to activation of IRF3 as well as NF-κB, which serve as transcription factors that generate IFN-β and other pro-inflammatory mediators.[58] Although these nucleic acid sensing pathways likely evolved to detect infectious viruses, they can also be triggered by endogenous nucleic acids. During the past decade, studies of nucleic acid–responsive TLRs and TLR-independent nucleic acid sensors that result in production of type I IFNs and other pro-inflammatory mediators have identified a direct pathogenic role for nucleic acids and nucleic acid–containing immune complexes as mediators of immune dysfunction in lupus. These studies complement the demonstration of broad expression of type I IFN–inducible genes in peripheral blood cells of lupus patients, referred to as the "IFN signature."[59-61] Together, the data point to the innate immune response and its products as significant factors in lupus pathogenesis. Table 79-4 describes many of the key components of the TLR-dependent and TLR-independent immune system pathways that are important in activation and control of lupus immune responses.

The endosomal TLRs, particularly TLR7, reactive with single-stranded RNA, and TLR9, reactive with unmethylated CpG-rich DNA, have been associated with lupus pathogenesis on the basis of in vitro studies that demonstrate activation of those TLRs by immune complexes that gain access to their intracytoplasmic compartment with the help of Fc receptors that bind the Fc fragment of immunoglobulin in the complex.[62-64] Clinical data demonstrating association of autoantibodies with specificity for RNA-binding proteins (such as Ro, La, Sm, and RNP) with high expression of IFN-induced genes in patient peripheral blood cells point to a significant role for RNA-containing immune complexes in innate immune activation and IFN production.[65] Associated neutrophil-derived proteins, including

**TABLE 79-4** Key Components of TLR-dependent and TLR-independent Innate Immune Response Pathways Relevant to the Pathogenesis of SLE

| TLR-Dependent Pathway |
|---|
| **Receptors** |
| Endosomal TLRs (TLR7 and TLR9; TLR8?) |
| TLR4 |
| TLR3? |
| **Adaptors** |
| Myeloid differentiation primary response gene 88 *(MyD88)* |
| Toll/IL-1 receptor (TIR)-domain–containing adaptor protein (TIRAP) |
| TIR-domain–containing adaptor protein inducing interferon-β (TRIF) |
| TRIF-related adaptor molecule (TRAM) |
| TNF receptor–associated factor 6 (TRAF6) |
| **Kinases** |
| Interleukin-1 receptor-associated kinase-1 (IRAK1) |
| IRAK4 |
| Mitogen-activated protein kinase kinase kinase 7 (MAP3K7/TAK1) |
| I-Kappa B kinase alpha and beta (IKKα/β) |
| I-kappa b kinase gamma (NEMO) |
| **Transcription Factors** |
| Interferon regulatory factor 3 (IRF3) |
| IRF5 |
| IRF7 |
| Nuclear factor-κB (NF-κB) |
| **Regulators** |
| MyD88 short (MyD88s) |
| Toll-interacting protein (TOLLIP) |
| A20 (encoded by *TNFAIP3*) |
| **TLR-Independent Pathway** |
| **Receptors** |
| Retinoic acid–inducible gene 1 *(RIG-I)* |
| Melanoma differentiation-associated gene 5 *(MDA5)* |
| DEXH box polypeptide 58 (DHX58/LGP2) |
| MAB21 domain–containing protein 1 (MB21D1/cGAS) |
| Z-DNA binding protein 1 (ZBP1/DAI) |
| Interferon-γ–inducible protein 16 (IFI16) |
| Eukaryotic translation initiation factor 1–alpha kinase 2 (EIF2AK2/PKR) |
| **Adaptors** |
| Mitochondrial anti-viral-signaling protein (MAVS/IPS-1/VISA/CARDIF) |
| Fas-associated via death domain (FADD) |
| TRAF3 |
| Transmembrane protein 173 (TMEM173/STING) |
| **Kinases** |
| Receptor-interacting serine threonine kinase 1 (RIP1) |
| IKKε |
| TANK-binding kinase 1 (TBK1) |
| **Transcription Factors** |
| IRF3 |
| NF-κB |
| **Regulators** |
| Tripartite motif–containing protein 25 (TRIM25) |
| Ring finger protein 135 (RNF135) |
| RNF125 |
| Cyclindromatosis (CYLD) |
| Heat shock protein 90 (HSP90) |

SLE, Systemic lupus erythematosus; TLR, Toll-like receptor.

high mobility group box 1 (HMGB1) and the cathelicidin protein LL37, can facilitate access of those immune complexes to the TLR-containing endosome.[66] Lupus mice made deficient in one or another TLR have supported the role of TLR7 in generating type I IFN, autoantibodies specific for RNA-binding proteins, and disease.[67] TLR9 activation can also generate IFN-α, but the findings from *TLR9*-deficient MRL/*lpr* (Fas-deficient) mice have been somewhat confusing because those mice show more severe disease, suggesting that the activation of TLR9 might actually be protective. It appears that TLR9 activation can regulate the *TLR7* pathway, resulting in reduced production of RNA-related autoantibodies.[68] In addition to demonstrating the important role of the endosomal TLRs in lupus pathogenesis, the data link TLR pathway activation with production of particular autoantibody specificities. These significant advances in characterization of the role of TLRs and the innate immune response in lupus disease have suggested new therapeutic targets that are currently under consideration (Figure 79-2).

The elucidation of the mechanisms of induction of type I IFN, with pDCs the major producers, have promoted studies that identified roles for additional cell types in amplification of this pathway. Platelets, largely ignored in studies of SLE pathogenesis, were shown to promote IFN production by pDCs through signals mediated by CD40 ligand (CD154) on activated platelets and CD40 on the pDCs.[69] One of the initial microarray data sets demonstrating an IFN gene expression signature in peripheral blood cells of pediatric lupus patients also detected expression of genes that are typically expressed in granulocytes.[59] Recent studies from several groups have extended this observation

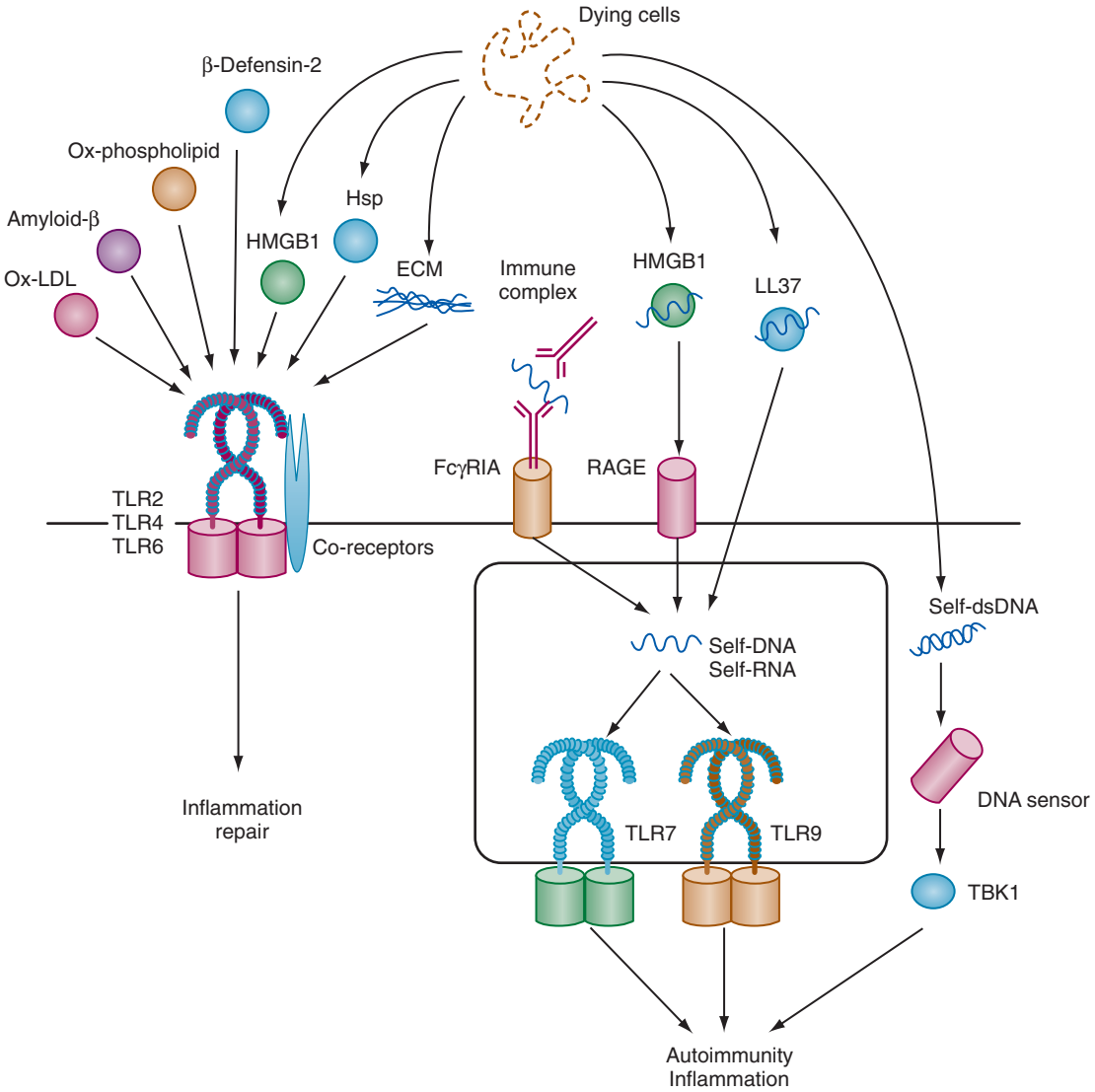



**Figure 79-2** Endogenous stimuli for innate immune system activation. Endogenous molecules released by dying cells, such as high-mobility group box 1 (HMGB1) and β-defensins, are recognized by cell surface Toll-like receptors (TLRs) and induce production of inflammatory mediators. Self-DNA or self-RNA either in association with HMGB1 or LL37 or in the form of immune complexes that bind Fc receptors, is internalized into endosomal TLRs and transducer signals that result in transcription of type I interferon or pro-inflammatory cytokines. Self-nucleic acids can also be recognized by cytoplasmic sensors and trigger gene expression that contributes to immune activation, autoimmunity, and inflammation. ECM, Extra-cellular matrix; Hsp, heat shock protein; LDL, low-density lipoprotein; Ox, oxidized; RAGE, receptor for advanced glycation end products; TBK1, TANK-binding kinase 1. *(From Kawai T, Akira S: The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors.* Nat Immunol *11:373–384, 2010.)*

and have documented low-density granulocytes in the circulation of lupus patients.[70]

Neutrophil extra-cellular traps (NETs) have gained attention as potential mediators of innate immune system activation. The NETs derive from a discrete cellular process, termed *NETosis*, in which aggregates of nuclear or mitochondria-derived DNA and its associated histones, HMGB1, LL37, elastase, and myeloperoxidase are systematically extruded into the extra-cellular environment. Generation of the NETs can be induced by interaction of neutrophils with vascular endothelial cells, activated platelets, or various cytokines. Recent data also implicate nucleosomes and RNA-containing immune complexes in the induction of NETosis.[71-73] Although NETs have only recently been linked to SLE, they present an intriguing and possibly significant mechanism that might account for observed alterations in immune function. They have the capacity to induce production of type I IFN by pDCs, serve as a source of relevant self-antigens for presentation to T lymphocytes, and mediate vascular damage and thrombosis.[74]

As described earlier, the elucidation of a significant role for the nucleic acid–sensing TLRs in recent years has drawn attention to the important role of autoantigen in driving disease in lupus. The contribution of apoptotic debris, either through increased apoptosis and/or impaired clearance of apoptotic cells, has been assumed for a number of years to be at least one likely mechanism that drives immune system activation and focuses its specificity on nucleic acids and their associated proteins. Apoptotic blebs are enriched in the typical targets of autoantibodies, such as Ro and Sm. Recent attention on so-called *microparticles*, small membrane-enclosed particles released by activated and dying cells, has generated data demonstrating binding of those particles by some autoantibodies in lupus plasma.[75] A combination of increased generation of nuclear material, perhaps modified by oxidative stress, and impaired clearance may be important mechanisms of lupus immunopathogenesis.[76] The role of C1q and complement components in the clearance of apoptotic debris has been suggested as an explanation for the strong association of C1q, C2, and C4 deficiencies with SLE.[77] A second role for complement components that likely reduces the pathogenicity of immune complexes is the capacity of complement to solubilize immune complexes, reducing the likelihood that those complexes would deposit in tissue and cause damage.[78]

## ADAPTIVE IMMUNE SYSTEM ALTERATIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS

T cell function has been carefully studied in SLE patients for several decades, and deficiencies or alterations in signaling pathways, production of cytokines, cell proliferation, and regulatory functions have been documented.[79] $CD4^+$ T cells are viewed as a requirement for development of lupus on the basis of their essential role in providing helper signals that drive differentiation of B cells to autoantibody-producing cells. Although some in vitro experiments support the capacity of cytokines such as IL-21 and B cell–activating factor (BAFF)/B lymphocyte stimulator (BLyS) and TLR ligands to mediate antibody production by B cells, T cells are recognized as the most efficient drivers of B cell differentiation.[80]

The activation status and signaling mechanisms of lupus T cells are distinct from those of T cells from healthy individuals. Among the alterations in function is decreased proliferation in response to self–non–T cells or allogeneic–non–T cells or in response to presentation of soluble antigens. In contrast, T cell proliferation induced by direct activation of the T cell antigen receptor is at least as strong as observed in control cells. Lupus T cells readily express CD40 ligand (CD154) after activation and maintain expression of that important co-stimulatory molecule longer than control T cells, leading to augmented help for activation and differentiation of B cells exposed to those T cells. Another long-standing observation is that lupus T cells produce less IL-2 than control T cells, perhaps one factor that contributes to impaired generation of IL-2–dependent T regulatory cells. The molecular basis of the altered T cell activation in lupus patients is complex, but at least one factor might be the observed substitution of the T cell receptor zeta chain with the common gamma chain that is a component of the Fc receptor signaling machinery. Correction of this defect can normalize T cell signaling and IL-2 production.[81] Augmented calcium responses following T cell receptor ligation have been observed, and hyperpolarization of mitochondria in T cells has been associated with altered T cell activation and function.[82]

An interesting observation from studies of lupus T cells might reflect more global epigenetic alterations to the lupus genome that impact autoimmunity. Treatment of mouse and human T cells with 5-azacytidine resulted in increased expression of the lymphocyte function antigen-1 (LFA1; CD11a) adhesion molecule and increased proliferative responses to self–non–T cells.[83] Lupus T cells studied ex vivo show hypomethylation of CG-rich areas of the genome, and a recent report of genome methylation in identical twins discordant for lupus demonstrated relatively less methylation of the twin with active lupus.[84] Multiple mechanisms have been postulated to contribute to DNA demethylation in lupus lymphocytes, including increased expression of growth arrest and DNA damage–induced 45alpha (GADD45alpha), a protein that removes methyl groups from DNA, decreased expression of DNMT1, and altered ERK pathway signaling.[50]

Generalized lymphopenia is typical of SLE, but expansions of specific T cell populations have been described. The T follicular helper population, characterized by expression of ICOS, CXCR5, and Bcl-6 and by production of IL-21, mediates important signals that promote differentiation of autoantigen-specific B cells.[85,86] A population of $CD8^+$ cells with a memory phenotype is associated with poor prognosis, possibly based on tissue damage mediated by those cells.[87]

The regulation and function of T regulatory cells (Tregs), with capacity to suppress immune responses, and Th17 cells, which promote inflammation by production of IL-17, have been intensively studied in recent years. Some studies of lupus patients do show a relative depletion of Tregs and increased Th17 cells and IL-17.[88] The functional impact of those alterations in human lupus is still not clear.

Cytokine production is altered in SLE, with decreased production of IL-2 a characteristic feature of patient T cells, as it is of individuals carrying the HLA 8.1 haplotype.[5] Although this deficiency in IL-2 was initially linked to the

often poor proliferative responses of lupus T cells stimulated with autologous or allogeneic T cells or soluble antigen, the recognition that IL-2 is important for maintenance of Tregs suggests another mechanism through which impaired production of IL-2 might contribute to immune system activation and autoimmunity.[89]

B cell regulation is also impaired in SLE, contributing to differentiation to production of autoantibodies and cytokines. The activated phenotype of SLE B cells has the potential to promote efficient presentation of specific self-antigens to T cells. It has not been entirely clear whether altered B cell function is strictly secondary to increased availability of T cell help, B cell survival, proliferation and differentiation factors, and signaling through TLRs or whether primary B cell dysfunction also contributes to autoimmunity. Recent studies characterizing the B cell repertoire by using single-cell polymerase chain reaction and cloning of individual immunoglobulin transcripts will permit improved understanding of the role of altered B cell tolerance mechanisms in the bone marrow versus secondary effects of T cell and cytokine help in generating self-reactive antibody specificities. BAFF/BLyS, IL-10, and IL-21 are among the candidate therapeutic targets that could modify the B cell differentiation program, a suggestion supported by successful clinical studies of BAFF/BLyS blockade. An additional approach toward improved regulation of B cell function is suggested by recent data demonstrating an association between vitamin D deficiency and presence of antinuclear antibodies in healthy individuals and increased B cell activation and serum type I IFN activity in vitamin D–deficient SLE patients.[90]

The baseline activation status of lupus B cells, as well as their response to antigen stimulation, is altered when compared with B cells from healthy individuals, and current studies are exploring the functional implications of lupus-associated genetic variants in several kinases, phosphatases, and adaptor molecules such as BLK, BANK, and PTPN22 for B cell function. Studies of the risk variant of *PTPN22* in B cells from healthy participants point to the influence of that lupus-associated variant on impaired counterselection of self-reactive B cells.[91] *LYN* deficiency in mice is associated with a lupus-like phenotype, and similar deficiencies in the LYN kinase have been described in lupus B cells.[92] Polymorphisms in the gene encoding Lyn studied in European-descent individuals could potentially impact B cell signaling and autoimmunity.[93] The gene encoding the inhibitory Fc receptor, *FCGR2B*, is polymorphic, and several variants have been associated with SLE.[94,95] SLE B cells show decreased expression of this FcR and altered cytokine production when engaged by immune complexes. Interestingly, the inhibitory capacity of FcγRIIb can be exploited by using an engineered bifunctional antibody that coligates CD19 and FcγRIIb. This antibody was found to suppress B cell functions in a mouse engrafted with human B cells.[96]

Current investigations of antibody-producing cells are defining several categories of B lineage cells that contribute to autoimmunity and disease. Long-lived plasma cells that are maintained by chemokines and stromal cell products in protective niches in the bone marrow are proposed as sources of those lupus autoantibodies such as anti-Smith (Sm) and anti-Ro that are chronically maintained at fairly constant levels. Those antibodies have been refractory to modulation by immunosuppressive or B cell depletion therapy.[97] In contrast, circulating preplasma cells or plasmablasts are sources of anti-dsDNA antibodies that fluctuate in some patients in association with variations in disease activity and might be more amenable to anti–B cell therapy.[98]

The T and B cell alterations that have been described in studies of lupus immune function are undoubtedly a reflection of multiple genetic variations that play out in altering threshold for cell activation and efficiency of cell signaling. They are also the result of interactions among those lymphocytes, along with antigen-presenting cells and their products, that with time amplify the overall level of immune activation. Increased production of type I IFN and presence of anti-nuclear antibodies in healthy family members of lupus patients likely reflect the consequences of genetic variations that are among the factors that predispose to SLE.[99] When the circuits of immune activation are amplified by environmental triggers, conditions are in place to generate the characteristic T and B cell autoimmunity that is required for tissue damage and disease.

## AUTOIMMUNITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

Autoantibodies are traditionally viewed as essential mediators of pathology in SLE, particularly when they are in the form of immune complexes. Virtually all lupus patients demonstrate a positive anti-nuclear antibody test, and the majority of patients have one or more of the characteristic autoantibody specificities that have been associated with lupus (see also Chapter 55). Among those, anti-dsDNA and anti-Sm are most specific for SLE. Interestingly, those specificities have been linked functionally in murine studies that demonstrate that dsDNA can bind to a peptide comprising amino acids 83 to 119 of the SmD1 protein, and T cells specific for that peptide provide help for production of anti-dsDNA antibody.[100,101] Anti-Ro, anti-La, and anti-RNP antibodies are characteristic of SLE but are also seen in other systemic autoimmune diseases. These characteristic autoantibodies in SLE can be categorized in relation to their targets, including DNA and DNA-binding proteins, typically aggregated in nucleosomes that contain histones; RNA and RNA-associated proteins, typically aggregated in cytoplasmic or nuclear ribonucleoprotein (RNP) particles; phospholipids exposed in plasma membranes and associated proteins such as $\beta_2$-glycoprotein I; and cell membrane proteins, typically those expressed on blood cells. Some of these self-antigens typically targeted by lupus autoantibodies are most likely accessed by antigen-presenting cells in the form of cell membrane–enclosed blebs derived from apoptotic cells or microparticles, small aggregates of cellular material derived from cells and generated after flipping of phosphatidylserine to the outer aspect of the cell membrane in the setting of cell activation or apoptosis. Although some patients who present with a clinical picture characteristic of SLE do not have significant titers of those autoantibodies, it is likely that those patients have undefined autoimmunity targeted at unspecified antigens.

The pathogenic antibodies in SLE tend to be those produced by cells that are far along in the B cell differentiation

process, either preplasma cells or plasma cells, and have undergone immunoglobulin class switching, a process that is driven by $CD4^+$ T helper cells and in some cases by TLR ligands together with B cell differentiation factors such as IL-21 and BLyS/BAFF.[80] It is not possible to define the earliest step in the generation of pathogenic autoantibodies, and in fact it is probable that the generation of autoimmunity can begin in any number of ways. Presentation of self-antigen by an excessively activated antigen-presenting cell, stochastic activation of low-avidity self-reactive T cells that are present in healthy individuals but have the potential to expand and drive differentiation of B cells with broad specificity for self-antigens, or direct activation of self-reactive B cells in the presence of activating TLR ligands, such as those provided by demethylated CpG-rich DNA from bacteria or viruses or self-derived nuclear debris might all be starting points for development of pathogenic autoimmunity. The process might begin with production of type I IFN by a virus-infected cell or a cell with impaired degradation of intra-cellular nucleic acids, altering the threshold for activation of the immune system by subsequent exposures to self-antigens. Oxidative or UV-mediated DNA damage by environmental stressors might synergize with altered maintenance of genome integrity to jump-start immune activation. Less important than the precise starting point is the observation that autoimmunity develops and builds with time. The presence of autoantibodies can be observed more than 5 years before clinical manifestations of disease appear, and the scope of autoantibody specificities expands with time.[102] Data from analysis of the prevalence of several lupus autoantibodies among sera collected from members of the military in whom SLE developed later demonstrate an intriguing sequence. Anti-Ro antibodies typically present earliest, anti-dsDNA antibodies appear several years later, and anti-Sm, the antibody specificity most specific for a diagnosis of SLE, appears approximately at the time of clinical diagnosis. This pattern, along with studies from murine models that demonstrated determinant spreading of autoantibody specificities not only to distinct proteins within a nucleic acid–protein particle but also to other self-antigen targets, provides clues to lupus pathogenesis that are not yet understood but hold promise for future breakthroughs in elucidating important pathogenic mechanisms.[103] Additional autoantibody specificities that are associated with lupus activity and with proliferative lupus nephritis and are thought to be pathogenic include those reactive with C1q. Anti-C1q antibodies appear to recognize neoepitopes of C1q when it is bound to early apoptotic cells.[104]

A shift from a predominant polyclonal immunoglobulin (Ig)M picture toward polyclonal IgG, driven by T cell help and cytokines, occurs with time in most patients with lupus and with development of tissue pathology and damage. In fact, some IgM antibodies that have self-reactivity are viewed as protective, with the switch from IgM to IgG or IgA representing an important point of altered immune regulation that contributes to the immunopathogenesis of SLE. Some of these IgM natural antibodies react with apoptotic cells and actually inhibit their activation through TLRs.[105]

In addition to immunoglobulin class, with class-switched antibodies better able to access extravascular spaces compared with IgM antibodies, amino acid sequence and charge of the antigen-binding site can influence pathogenicity. Arginines in the CDR3 region of anti-dsDNA antibodies are characteristic and influence binding to their DNA target. Molecular mimicry is a concept that has been applied to reactivity of antibodies specific for a microbial protein that also react with self-antigens, but the concept can also be applied to those antibodies that unexpectedly bind to two distinct self-antigens. For example, some anti-dsDNA antibodies were found to bind to a peptide that is a feature of glutamate receptors on central nervous system neurons.[106] Beyond features of Ig class and antigen-binding site that influence access to tissue and affinity of binding, glycosylation and complement-fixing capacity are important determinants of the antibody's capacity to bind to Fc receptors and promote complement activation, resulting in target cell death or inflammation.

The role of SLE autoantibodies in the pathogenesis of the disease has traditionally focused on the deposition of immune complexes in skin, renal glomerulus, and other sites of tissue injury, along with a potential contribution of direct targeting of antibodies to local or "planted" antigens. But in recent years, with the recognition that nucleic acid–containing immune complexes can directly induce cell signaling and new gene transcription after accessing endosomal TLRs, an additional important pathogenic role for autoantibodies as immune modulators has been defined.

Particular lupus autoantibody specificities have been associated with specific clinical manifestations of disease (Table 79-5). Perhaps the best developed characterization of the mechanisms by which an autoantibody mediates disease is the role of maternal-derived anti-Ro antibody in the neonatal lupus syndrome. After transplacental transfer of anti-Ro antibody, RNA-containing immune complexes are thought to form, activating the production of cytokines that contribute to fibrosis of the cardiac conduction system.[107] This scenario, similar to that which mediates production of type I IFN by pDCs, identifies nucleic acid–autoantibody immune complexes as regulators of cytokine production that results in tissue damage. Anti-Ro and anti-La antibodies, although common to several autoimmune diseases, are also characteristic in association with sicca symptoms, subacute cutaneous lupus, and, as noted, in neonatal lupus. Anti-RNP antibodies are seen in both SLE and mixed connective tissue disease.

Anti-phospholipid antibodies, with lupus anticoagulant the most informative antibody profile, contribute to placental damage and vascular thrombosis, as well as thrombocytopenia in the anti-phospholipid syndrome and in some patients with lupus. Studies in a murine model indicate that tissue damage and thrombosis depend on activation of the complement system by the anti-phospholipid antibodies.[108] Similar mechanisms might be relevant in forms of lupus renal disease characterized by microangiopathy.

## MECHANISMS OF TARGET ORGAN DAMAGE

Clinical disease is ultimately a reflection of tissue damage mediated by the inflammatory sequelae of the described autoimmunity and immune system activation, along with an exaggerated or aberrant repair response. The classic view

**TABLE 79-5** Correlations Between Clinical Manifestations of Systemic Lupus Erythematosus and Autoantibodies, Immune Complexes, and T Cells

| Manifestation | Autoantibodies | Immune Complexes | T Cells |
|---|---|---|---|
| Nephritis | Anti-dsDNA<br>Anti-Ro<br>Anti-C1q<br>Ids 16/6, 3I, and GN2 | + | + |
| Arthritis | ? | + | + |
| Dermatitis | Anti-Ro<br>Anti-dsDNA<br>Id 16/6 | | + |
| Vasculitis | Anti-Ro | + | + |
| Central nervous system | Antiribosomal P<br>Antineuronal<br>Anti-NR2 | + | |
| Hematologic | | | |
| Lymphopenia | Antilymphocyte | | |
| Hemolysis | Antierythrocyte | | |
| Thrombocytopenia | Anti-platelet | + | |
| Clotting | Anti-phospholipid | | |
| Fetal loss | Anti-phospholipid | | |
| Neonatal lupus | Anti-Ro | | |
| Sicca syndrome | Anti-Ro | | + |
| Mild disease | Anti-RNP without other autoantibody except anti-nuclear antibody | | |

RNP, Ribonucleoprotein.

of the mechanisms that result in tissue damage involves activation of the complement system by immune complexes deposited in tissue, as well as release of products of phagocytes, including enzymes released from neutrophil granules and reactive oxygen intermediates from macrophages. Recent studies in mouse and human systems involving extensive analysis of renal infiltrating cells at various points in the disease process have identified a monocyte population that has undergone differentiation to generate a functional phenotype that mediates what is apparently uncontrolled tissue repair, contributing to sclerosis and organ dysfunction.[109] Infiltrating T and B cells can also contribute to renal damage, with in situ production of autoantibodies specific for vimentin recently demonstrated.[110,111] Studies in a murine model have shown that IFN-α can also contribute to development of crescents in lupus kidneys.[112]

The role of the vasculature as a significant target organ contributing to disease in lupus has once again come to the fore, after several decades in which researchers have focused predominantly on the immune system and the contributions of autoantibodies to pathology.[113] Altered structure and function of both venous and arterial vessels have been noted for many years, including the periarteriolar concentric onion skinning seen in spleen, microangiopathy and associated microthrombi seen in some organs, and the endothelial dysfunction that has been associated with premature atherosclerosis in lupus patients. Recent studies have focused on the potential role of type I IFN on endothelial cells and endothelial cell progenitor cells, and have postulated that increased production of IFN is at least one contributor to impaired vascular repair.[114] A role for granulocytes and pro-inflammatory lipids has also been proposed.[74,115] Characterization of the micropathology of the vasculature in Degos disease, a syndrome with some common pathologic features with SLE, has drawn attention to the association of activation of the IFN pathway with vascular sclerosis and endothelial damage.[116] A possible direct contribution of type I IFN to vascular injury supplements the previously described role of complement activation products C3a and C5a and expression of endothelial cell surface adhesion molecules E-selectin, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1, in lupus flares.

## CONCLUSION

Considerable gains in understanding the mechanisms of lupus pathogenesis have established the knowledge base that will underlie future advances in development of targeted therapies to achieve improved patient outcomes.[117,118] The recognition of a central role for innate immune system activation, including the role of type I IFN in lupus pathogenesis, is a major advance of recent years. Rapid progress in characterizing the genetic variants that are associated with a diagnosis of lupus has provided strong evidence that alterations in molecular pathways that regulate genome integrity, nucleic acid degradation, TLR-dependent and -independent innate immune signaling, and lymphocyte activation thresholds and signaling efficiency are important mechanisms that determine disease susceptibility. The products of the immune system, including autoantibodies and their immune complexes, cytokines, and complement components, along with pro-inflammatory mediators and reactive oxygen products released from neutrophils and macrophages, remain key mediators of tissue damage. However, an additional pathogenic role for nucleic acid–containing immune complexes as immune modulators through their important capacity to access and activate endosomal TLRs, as well as insights into regulation of endogenous cytoplasmic nucleic acids, are important new concepts that can guide future therapeutic approaches. Targeting the components of innate immune system activation that affect the TLR pathway along with modulation of pathogenic T cell help for B cell differentiation to autoantibody-producing cells, particularly that mediated by T follicular helper cells, would appear to be a rational therapeutic strategy in light of our current understanding of pathogenic mechanisms.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 80

# Clinical Features of Systemic Lupus Erythematosus



Maria Dall'Era • David Wofsy

**KEY POINTS**

- Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that is characterized by a relapsing-remitting course and a highly variable prognosis.
- SLE is characterized by the production of a broad array of autoantibodies. Anti-nuclear antibodies have the greatest sensitivity for the diagnosis, and anti–double-stranded DNA and anti-Smith antibodies have the greatest specificity.
- Women of childbearing age and African-American, Asian, and Hispanic populations have the highest incidence and prevalence of disease.
- Constitutional symptoms, rash, mucosal ulcers, inflammatory polyarthritis, photosensitivity, and serositis are the most common clinical features of the disease.
- Lupus nephritis is the most common of the potentially life-threatening manifestations.
- Atherosclerosis, a complication of long-standing SLE, requires aggressive risk factor modification.

Systemic lupus erythematosus (SLE) is the prototypic systemic autoimmune disease, characterized by diverse multisystem involvement and the production of an array of autoantibodies. Clinical features in individual patients can be quite variable and range from mild joint and skin involvement to severe, life-threatening internal organ disease. Criteria for the classification of SLE were initially developed by the American College of Rheumatology (ACR) in 1971, revised in 1982, and revised for a second time in 1997[1,2] (Table 80-1). A person must fulfill 4 of 11 criteria to be classified as SLE, all other reasonable diagnoses having been excluded. A patient does not have to manifest all 4 criteria simultaneously; the required 4 of 11 criteria can be fulfilled during a period of weeks or years. The ACR criteria were developed as a way to classify patients with SLE for the purpose of inclusion in clinical and epidemiologic studies. In clinical practice, these criteria are often cited to support a diagnosis of SLE. However, it should be emphasized that fulfillment of these classification criteria is not an absolute requirement for diagnosis. Rather, diagnosis typically rests on the judgment of an experienced clinician who recognizes a characteristic constellation of symptoms and signs in the setting of supportive serologic studies, after exclusion of alternative differential diagnostic possibilities. Despite widespread acceptance of the ACR criteria, several limitations affect their utility. The ACR criteria include only a subset of the various manifestations that might be present in an SLE patient. For example, despite the variety of neurologic syndromes that have been described in SLE, only seizures and psychosis are included in the ACR criteria. Proteinuria and urinary cellular casts are the only two renal criteria. Notably, a positive renal biopsy is not included in the criteria.

Against this background, the Systemic Lupus International Collaborating Clinics (SLICC) undertook an effort to revise the ACR criteria.[3] Through review of more than 700 SLE and control patient scenarios and validation in a separate sample, 17 elements were identified for inclusion into the revised criteria (11 clinical and 6 immunologic). A patient is classified with SLE if he or she (1) has biopsy-proven lupus nephritis with a positive anti-nuclear antibody (ANA) or anti–double-stranded (ds) DNA antibody or (2) fulfills four of the criteria, including at least one clinical criterion and one immunologic criterion. In contrast to the ACR criteria, the SLICC criteria would not allow a patient to be classified with SLE on the basis of clinical features alone. The revised criteria include more dermatologic and neurologic manifestations, as well as low complement levels and positive direct Coombs test in the absence of hemolytic anemia. Although the SLICC revision provides alternative criteria for use in research studies, it remains to be seen whether these criteria will ultimately replace the ACR criteria.

## EPIDEMIOLOGY

Prevalence and incidence rates of SLE vary widely in the literature. Reported prevalence frequencies range from 20 to 240 per 100,000 persons, and reported incidence rates range from 1 to 10 per 100,000 person-years.[4] This variation is partly the result of methodological differences between studies (e.g., different case definitions of SLE and methods of case ascertainment) and differing racial/ethnic compositions of the study populations. To determine more accurate and contemporary epidemiologic estimates for SLE in the United States, the Centers for Disease Control and Prevention (CDC) funded five large-scale, population-based lupus registries to conduct extensive lupus surveillance. The completed Georgia and Michigan lupus registries, composed primarily of Caucasian and African-American patients, reported incidence rates of 5.6 per 100,000 person-years and prevalences of 72.1 to 74.4 per 100,000 persons. African-American women had the highest rates. Peak disease incidence occurred during reproductive years; African-Americans had a lower mean age of onset compared with Caucasians.[5,6] Registries from California, New York, and the Indian Health Service are nearing completion and will

**TABLE 80-1** 1997 Update of the 1982 Revised American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus*

| Criterion | Definition |
|---|---|
| Malar rash | Fixed erythema, flat or raised, over the malar eminences, sparing the nasolabial folds |
| Discoid rash | Erythematous raised patches with adherent keratotic scale and follicular plugging; atrophic scarring may occur in older lesions |
| Photosensitivity | Skin rash as a result of unusual reaction to sunlight, determined by patient history or physician observation |
| Oral ulcers | Oral or nasopharyngeal ulceration, usually painless, observed by a physician |
| Arthritis | Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion |
| Serositis | Pleuritis-convincing history of pleuritic chest pain or rub heard by a physician or evidence of pleural effusions *or* pericarditis-documented by electrocardiogram or rub or evidence of pericardial effusion |
| Renal disorder | Persistent proteinuria >0.5 g/day, >3+ if quantification not performed *or*<br>cellular casts: may be red blood cell, hemoglobin, granular tubular, or mixed |
| Neurologic disorder | Seizures: in the absence of offending drugs or known metabolic derangements (e.g., uremia, acidosis, electrolyte imbalance) *or*<br>Psychosis: in the absence of offending drugs or known metabolic derangements (e.g., uremia, acidosis, electrolyte imbalance) |
| Hematologic disorder | Hemolytic anemia with reticulocytosis *or*<br>Leukopenia <4000/mm$^3$ *or*<br>Lymphopenia <1500/mm$^3$ *or*<br>Thrombocytopenia <100,000/mm$^3$ in the absence of offending drugs |
| Immunologic disorder | Anti-DNA: antibody to native DNA in abnormal titer *or*<br>anti-Smith: presence of antibody to Sm nuclear antigen *or*<br>Positive finding of anti-phospholipid antibodies based on (1) abnormal serum concentration of immunoglobulin (Ig)G or IgM anti-cardiolipin antibodies, (2) positive test result for lupus anticoagulant by using a standard method, or (3) false-positive serologic test for syphilis known to be positive for at least 6 mo and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test |
| Positive anti-nuclear antibody | An abnormal titer of anti-nuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with drug-induced lupus syndromes |

*The presence of four or more criteria is required for systemic lupus erythematosus classification. Exclude all other reasonable diagnoses.

provide robust incidence and prevalence estimates in the Asian, Hispanic, and Native-American populations. Previous studies suggest a higher prevalence of SLE in Asian and Hispanic populations.

Most patients with SLE are seen with their disease when they are between 15 and 64 years old.[7] Patients with pediatric-onset SLE (<16 years old) are more likely to be African-American than patients with later-onset SLE.[7] SLE tends to be more severe in men and in pediatric patients. Late-onset SLE (>50 years old) is characterized by a more insidious onset, with a higher occurrence of serositis and pulmonary involvement, and a lower incidence of malar rash, photosensitivity, alopecia, Raynaud's phenomenon, neuropsychiatric disease, and nephritis.[7]

## CLINICAL FEATURES

SLE has protean clinical manifestations that may differ dramatically from patient to patient. Just as the signs and symptoms of SLE vary widely among patients, so does the severity of disease. Some patients will have relatively mild disease that is no threat to a life-sustaining organ; other patients' disease will progress rapidly to life-threatening severity.

The great variability in the expression and severity of SLE constitutes a major challenge to accurate diagnosis. Although it is difficult to pinpoint their precise frequencies, it is clear that the most common presenting manifestations are constitutional symptoms (fever, fatigue, and/or weight loss), cutaneous manifestations (e.g., malar rash), and articular manifestations (arthritis and/or arthralgia). Each of these manifestations appears to be present in at least 50% of patients with lupus at the time of diagnosis. The other clinical features of SLE are much less likely to be presenting manifestations, although virtually any of them might be the first clue to the correct diagnosis. More commonly, these manifestations appear while the disease evolves. Taken together, various descriptive studies in the literature[1,8–12] support a cumulative frequency of symptoms and signs that is summarized in Table 80-2.

### Mucocutaneous Involvement

Mucocutaneous involvement is very common in SLE. Gilliam and colleagues have categorized cutaneous lupus erythematosus (LE) lesions as "lupus specific" or "lupus nonspecific" on the basis of the histopathologic finding of interface dermatitis[13,14] (Table 80-3). In a patient with lupus, the presence of lupus-specific lesions confirms the diagnosis of cutaneous LE; lupus nonspecific lesions can occur in diseases other than lupus. Lupus-specific lesions are further subdivided into acute cutaneous lupus erythematosus (ACLE), subacute cutaneous lupus erythematosus (SCLE), and chronic cutaneous lupus erythematosus (CCLE) lesions, on the basis of additional clinical and histopathologic information. Discoid lupus is the most common subtype of

**TABLE 80-2** Frequencies of Various Manifestations of Systemic Lupus Erythematosus*

| Manifestation | Frequency (%) |
|---|---|
| Constitutional symptoms (fatigue, fever, weight loss) | 90-95 |
| Mucocutaneous involvement (malar rash, alopecia, mucosal ulcers, discoid lesions, etc.) | 80-90 |
| Musculoskeletal involvement (arthritis/arthralgia, avascular necrosis, myositis, etc.) | 80-90 |
| Serositis (pleuritis, pericarditis, peritonitis) | 50-70 |
| Glomerulonephritis | 40-60 |
| Neuropsychiatric involvement (cognitive impairment, depression, psychosis, seizures, stroke, demyelinating syndromes, peripheral neuropathy, etc.) | 40-60 |
| Autoimmune cytopenia (anemia, thrombocytopenia) | 20-30 |

*Systemic lupus erythematosus is a heterogeneous disease that can affect virtually any organ system in variable ways.



**Figure 80-1** Localized acute cutaneous lupus erythematosus (malar rash, butterfly rash). This lesion is characterized by macular or papular erythema in a malar distribution, sparing the nasolabial folds.

CCLE. SCLE and CCLE can occur as distinct isolated entities or as one of several manifestations of SLE. The risk of SLE varies with each cutaneous subset. One study of 161 patients with lupus-specific lesions showed that the classification criteria for SLE were present in 72% of patients with ACLE, 58% with SCLE, 28% with any form of discoid lupus, and 6% with localized discoid lupus confined to the head and neck. Many patients displayed more than one type of cutaneous lesion.[15]

### Acute Cutaneous Lupus Erythematosus

ACLE lesions can be localized or generalized. The hallmark feature of ACLE is localized to the malar region ("butterfly rash") and is characterized by confluent, macular, or papular erythema that last days to weeks, and appearing symmetrically on the cheeks and bridge of the nose. They do not appear on the nasolabial folds, which are sun-protected areas (Figure 80-1). Induration and scaling may occur.

**TABLE 80-3** Gilliam Classification of Skin Lesions Associated with Lupus

A. Lupus erythematosus (LE)-specific skin lesions
   1. Acute cutaneous LE (ACLE)
      Localized ACLE
      Generalized ACLE
   2. Subacute cutaneous LE (SCLE)
      Annular SCLE
      Papulosquamous SCLE
   3. Chronic cutaneous LE (CCLE)
      Classic discoid LE (DLE): (a) localized; (b) generalized
         Hypertrophic DLE/verrucous DLE
         Lupus panniculitis/profundus
         Mucosal DLE
         LE tumidus
         Chilblain LE
      Lichenoid DLE (DLE-lichen planus overlap)

B. LE-nonspecific skin lesions
   1. Cutaneous vascular disease
      Vasculitis
         Leukocytoclastic vasculitis
         Polyarteritis nodosa–like
         Vasculopathy
            Degos disease–like
            Atrophe blanche–like
            Periungal telangiectasia
            Livedo reticularis
            Thrombophlebitis
            Raynaud's phenomenon
            Erythromelalgia
   2. Nonscarring alopecia
      "Lupus hair"
      Telogen effluvium
      Alopecia areata
   3. Sclerodactyly
   4. Rheumatoid nodules
   5. Calcinosis cutis
   6. LE-nonspecific bullous lesions
   7. Urticaria
   8. Papulonodular mucinosis
   9. Cutis laxa/anetoderma
   10. Acanthosis nigricans (type B insulin resistance)
   11. Erythema multiforme (Rowell's syndrome)
   12. Leg ulcers
   13. Lichen planus

Erythema also commonly occurs on the forehead, eyelids, chin, and neck. The malar rash of SLE can be mimicked by a variety of other facial rashes, including acne, rosacea, seborrheic dermatitis, perioral dermatitis, atopic dermatitis, and erysipelas. If the diagnosis remains uncertain after an extensive clinical and serologic evaluation, biopsy of the rash can help to distinguish cutaneous lupus from other dermatologic conditions. It is important to remember that other forms of lupus-specific skin lesions, such as discoid lupus, can also occur in the malar distribution.

The generalized form of ACLE refers to widespread macular or maculopapular erythema occurring in a photosensitive distribution on any area of the body. The palmar surfaces, dorsa of the hands, and extensor surfaces of the fingers are commonly involved. In contrast to Gottron's



**Figure 80-2** Subacute cutaneous lupus (papulosquamous variant). Lesions typically appear on the back, neck, shoulders, and extensor surfaces of the arms, and usually spare the central area of the face. Lesions heal without scarring.

papules of dermatomyositis, the erythema of ACLE spares the metacarpophalangeal joints, and typically is located between the interphalangeal joints. In severe forms of ACLE, a widespread bullous eruption similar to toxic epidermal necrolysis (TEN) can occur. ACLE lesions heal without scarring, although temporary postinflammatory hyperpigmentation may be observed.

### Subacute Cutaneous Lupus Erythematosus

Subacute cutaneous lupus erythematosus (SCLE) is characterized by the presence of nonscarring, photosensitive lesions that can take one of two distinct forms: (1) papulosquamous lesions that resemble psoriasis, or (2) annular-polycyclic lesions with peripheral scale and central clearing (Figure 80-2). These two forms can occur concurrently in the same patient. SCLE has a predilection for the back, neck, shoulders, and extensor surfaces of the arms and usually spares the central face. The lesions typically last for weeks to months and heal without scarring. Rarely, a severe TEN-like eruption can evolve from SCLE lesions after sun exposure.[16] SCLE, particularly the annular subtype, is strongly associated with the presence of anti-SS-A/Ro antibody.[17] Several drugs are known to induce SCLE: angiotensin-converting enzyme inhibitors, terbinafine, hydrochlorothiazide, and calcium channel blockers are common culprits. Finally, SCLE has been implicated as a paraneoplastic syndrome.[18]

### Chronic Cutaneous Lupus Erythematosus

CCLE refers to a variety of subtypes of photosensitive lesions that can lead to skin atrophy and scar and that may persist for several months or years. Discoid lupus (DLE) is the most common subtype of CCLE. DLE is subdivided into localized discoid lupus (limited to head and neck) and generalized discoid (occurring above and below the neck) (Figures 80-3 and 80-4). The term "discoid" refers to the sharply demarcated disk-shaped appearance of the lesions, which are raised, erythematosus plaques with adherent



**Figure 80-3** Discoid lupus erythematosus on the face and scalp. Discoid lesions are a form of chronic cutaneous lupus and are commonly found on the scalp, face, and external ears. If untreated, these lesions can lead to permanent alopecia and disfigurement.



**Figure 80-4** Discoid lupus erythematosus on the dorsa of the hands. The lesions spare the proximal interphalangeal joints, a characteristic feature of lupus-specific rashes.

scales that commonly occur on the patient's scalp, face, and neck. The cheeks, nose, ears, and upper lip are classic locations for lesions. Typically, a raised, erythematous border denotes the actively expanding component. Follicular plugging is a characteristic finding. Left untreated, DLE can result in permanent alopecia and disfigurement. Squamous cell carcinoma has been described as a sequela of long-standing DLE; thus active surveillance of known lesions and

evaluation of changing lesions are critical.[19] Other subtypes of DLE include mucosal DLE (described in a subsequent section) and hypertrophic LE. Hypertrophic LE consists of chronic, indurated lesions that are covered by hyperkeratotic, multilayered scales. These lesions can be a source of diagnostic confusion because they may visually and histologically resemble squamous cell carcinoma.[20]

Other forms of CCLE include lupus panniculitis/profundus, chilblain lupus, and lupus tumidus. Lupus panniculitis is a lobular panniculitis that has a predilection for the scalp, face, arms, buttocks, and thighs. The lesions can result in depressed, sunken areas. When a cutaneous discoid lesion overlies the panniculitis, the entity is referred to as *lupus profundus*.[21] Biopsy is often necessary to secure the diagnosis because reports have described cases of T cell lymphoma that mimic panniculitis. However, biopsy should be performed carefully because the lesions have a tendency to break down. Lupus panniculitis is one of the few panniculitides that can occur above the waist. Lupus panniculitis is associated with a low risk of concomitant SLE. Chilblain lupus manifests as tender, erythematous, or violaceous papules or plaques occurring on acral areas, especially the fingers, toes, heels, nose, and ears. The lesions are brought on by cold, damp air. Chilblains lupus is more likely than idiopathic chilblains to be present even in warm weather.

Lupus tumidus lesions are characterized by edematous, erythematous plaques with a smooth surface. These lesions are highly photosensitive and have a predilection for the zygomatic region of the face. The epidermis is not involved; thus there is a lack of overlying scale or follicular plugging.

### Other Systemic Lupus Erythematosus Skin Lesions

Lupus-nonspecific skin lesions such as cutaneous leukocytoclastic vasculitis, bullous lesions, periungual erythema, and livedo reticularis can develop in patients with SLE. Cutaneous leukocytoclastic vasculitis most commonly presents as palpable purpura on the lower extremities. Bullous lupus erythematosus is a rare cutaneous manifestation characterized by subepidermal vesiculobullous skin changes, manifested by a nonscarring bullous eruption[22] (Figure 80-5). SLE may be associated with other bullous disorders such as bullous pemphigoid and dermatitis herpetiformis. The physical examination finding of periungal erythema represents dilation of the capillaries at the base of the nail. The physician can visualize these capillaries at the patient's bedside with a dermatoscope or ophthalmoscope. Other disorders associated with periungual erythema include scleroderma and mixed connective tissue disease (MCTD). Unlike scleroderma and MCTD, SLE is not associated with capillary dropout. Livedo reticularis is characterized by an erythematous to violaceous reticular or netlike pattern of the skin. It is highly associated with anti-phospholipid antibody syndrome.

### Photosensitivity

Photosensitivity occurs frequently in SLE. In one study, photo provocation testing caused an abnormal skin reaction to ultraviolet A, ultraviolet B, or visible light in more than 90% of patients with lupus.[23] Most abnormal skin reactions occurred 1 to 2 weeks after exposure to light, and persisted for weeks to months. Photosensitive patients may report worsening of their systemic disease symptoms, such as fatigue and joint pain, following sun exposure. During evaluation of a photosensitive patient, polymorphous light eruption (PMLE) and phototoxic medications are important diagnostic considerations.[24] Accurate differentiation between PMLE and lupus is essential because PMLE is treated by ultraviolet radiation phototherapy, but this treatment makes lupus worse. PMLE can occur concomitantly in patients with known SLE.

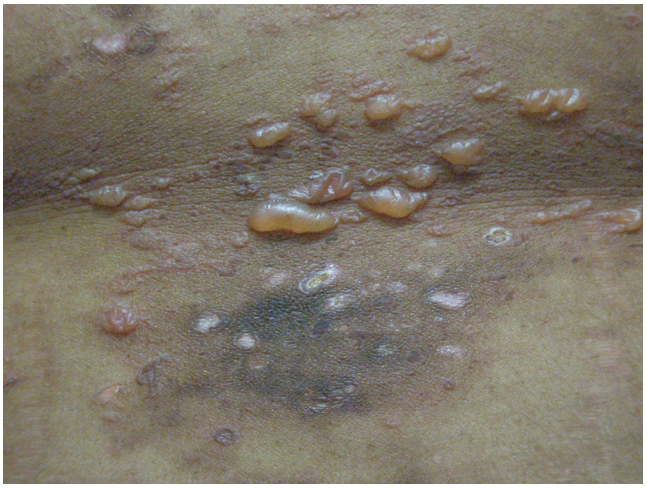


**Figure 80-5** Bullous lupus erythematosus. These lesions are a rare manifestation of lupus and are characterized by nonscarring bullous lesions.

### Alopecia

Scarring alopecia is a common complication of discoid lupus. Scalp discoid lesions most frequently develop on the vertex and parietal areas.[25] Nonscarring alopecia in patients with SLE can take several forms. "Lupus hair" is characterized by short, irregularly sized hair at the frontal hairline and is associated with active systemic disease.[26] Telogen effluvium manifests as diffuse hair thinning. Last, the incidence of alopecia areata (discrete areas of hair loss) is believed to be increased in cases of SLE.[27]

### Mucosal Ulcers

Nasal or oral lesions that represent the mucosal counterparts of cutaneous lupus commonly develop in patients with SLE.[28] Acute oral lupus lesions present as red macules, palatal erythema or petechiae, erosions, or ulcerations. These lesions are usually painless. Subacute oral lesions are rare, and are characterized by well-demarcated, round, red patches. Oral discoid lesions present as painful, well-demarcated, round, red lesions with white radiating hyperkeratotic striae. The buccal mucosa is the most commonly involved site. When the lesions evolve, they may take on a honeycomb appearance. Oral discoid lupus frequently involves the patient's lip and spreads from the vermilion border to the skin of the lip. Mucosal discoid lesions can

also occur on the conjunctiva and genital areas. In general, lupus oral ulcers have a gradual onset and can occur anywhere on the oral mucosa, with the most common locations the hard palate, buccal mucosa, and vermilion border.[29] These lesions are most commonly unilateral or asymmetric. The relationship between the presence of oral lesions and systemic disease activity remains unclear. Note that oral candidiasis and oral lichen planus can take on a similar appearance to SLE oral ulcers. The histopathology and immunopathology of mucosal lesions are similar to the alterations seen in the skin. Vasculitis is absent.

### Dermatopathology and Immunopathology

A skin biopsy is useful in the diagnosis of cutaneous lupus in the setting of an atypical clinical presentation. Immunofluorescence should always be performed, along with conventional histology. "Lupus-specific" skin lesions are characterized by an interface dermatitis consisting of a mononuclear cell infiltrate at the dermal-epidermal junction. Other pathologic findings present in lupus skin lesions include basal layer vasculopathic degeneration of keratinocytes, perivascular and periadnexal inflammation, follicular plugging, mucin deposition, and hyperkeratosis. These findings occur to different degrees in various lupus-specific skin lesions; discoid lesions show the most profound changes.[30] In contrast, early ACLE lesions may have minimal histopathologic findings and only a sparse lymphocytic infiltrate.

Immunofluorescence demonstrates granular deposition of immunoglobulin and complement components along the dermal-epidermal junction. Immunoglobulin (Ig)G and IgM are the most common immunoglobulin subtypes deposited. Various complement components, including C3, C1q, and the membrane attack complex, have also been identified. Direct immunofluorescence (DIF) of nonlesional, sun-exposed skin is referred to as the "lupus band test." Although a positive lupus band test is often seen in patients with SLE, this may also be seen in patients with other rheumatic diseases, as well as in healthy people. In one study, 20% of healthy young adults had a positive DIF in sun-exposed skin.[31] It is important to note that serologic testing with ANA, anti-dsDNA, and anti-Smith (anti-Sm) has largely supplanted use of the lupus band test in confirming the diagnosis of SLE. DIF of nonlesional, sun-protected skin is believed to be more specific for SLE.

## Musculoskeletal Involvement

### Arthritis

Arthritis and arthralgias are very common manifestations of SLE, present in as many as 90% of patients at some point during the course of their disease.[32] Severity of involvement can range from mild joint pain to deforming arthritis. Although any joint can be involved, lupus arthritis is characterized by a symmetric, inflammatory arthritis that predominantly affects the knees, wrists, and small joints of the hands.[33] Synovial effusions are typically small and not as inflammatory as those present in rheumatoid arthritis (RA). Hand deformities can occur as a result of ligamental and/or joint capsule laxity and joint subluxation. This manifestation is referred to as *Jaccoud's-like arthropathy* because it resembles the arthropathy that develops in patients with rheumatic fever (Figure 80-6). These deformities are typically reducible, although sometimes they can become fixed and are associated with disability. Jaccoud's-like arthropathy sometimes occurs in the foot, also.[34]



**Figure 80-6** Jaccoud's-like arthropathy. These hand deformities resemble those that develop in patients with a history of rheumatic fever and are caused by ligamentous and/or joint capsule laxity. Deformities in the hands, such as ulnar drift at the metacarpophalangeal joints, swan neck and boutonnière deformities, and hyperextension at the interphalangeal joint of the thumb, closely resemble the deformities seen in rheumatoid arthritis. The absence of erosions on radiographs and their reducibility distinguish this condition from the deforming arthritis of rheumatoid arthritis. *(Courtesy Dr. D. Vassilopoulos.)*

Although lupus arthritis is not classically associated with erosions on plain radiography, erosive disease has been described in a small subset of patients.[35] In addition, MRI studies have shown occasional erosions in some patients with lupus arthritis.[36] A subset of patients with SLE who also meet classification criteria for RA have historically been referred to as "rhupus." One study demonstrated the presence of marginal joint erosions on plain radiography in 60% of such patients [37] Erosive arthritis is commonly a feature of MCTD. Some studies have demonstrated an association between lupus erosive arthritis and the presence of anti-citrullinated protein antibodies (ACPA).[35] In addition to arthritis, tendinitis or tenosynovitis is frequently observed in patients with SLE.[38] Tendon rupture is a very uncommon occurrence.

Synovial biopsies from patients with lupus arthritis have shown a variety of abnormalities, including deposition of fibrin-like material, focal or diffuse synovial lining cell proliferation, vascular congestion, perivascular mononuclear cell infiltration, vasculitis, and obliteration of vessel lumina.[39] Radiographic studies of patients with SLE have revealed changes such as cystic bone lesions, periarticular soft tissue swelling, demineralization, acral sclerosis, joint subluxations, and erosions.[40,41]

### Avascular Necrosis

Avascular necrosis (AVN), also referred to as *aseptic necrosis* and *ischemic necrosis*, is a painful and disabling condition that occurs in some patients with SLE.[42] AVN is the end result of interruption of the blood supply to bone, leading to reactive hyperemia of adjacent bone,

demineralization, and then collapse. The most commonly affected sites include the femoral heads, tibial plateaus, and femoral condyles, but smaller joints can be involved as well. AVN is often bilateral, and joint effusions may occur. AVN of the femoral head should be suspected in a patient with SLE who has groin pain that is worsened with weight bearing and movement of the hip. The pain may radiate down the side of the thigh, and a limp might be evident. Although both plain radiographs and MRI can be helpful in the diagnosis of AVN, MRI is the more sensitive test. One prospective MRI study of 45 patients with SLE on glucocorticoid therapy demonstrated that silent osteonecrosis of the femoral head developed in 34% of patients.[43] However, MRI studies may be too sensitive an indicator, in that some lupus patients with suggestive MRI findings never progress to clinical symptoms of AVN. Therefore MRI findings should always be interpreted in the context of the clinical setting. The use of high doses of glucocorticoids is a well-known risk factor for AVN, but AVN has also been described in patients with SLE who have never used glucocorticoids. An MRI study of 72 newly diagnosed patients with SLE demonstrated that AVN typically developed within the first 3 months of initiation of high-dose glucocorticoids.[44] Epidemiologic studies have shown that high disease activity, as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), and the use of cytotoxic medications are also associated with AVN.[45,46]

### Myositis

Although myalgias occur commonly in SLE, true myositis is relatively rare. One study of patients with SLE at the National Institutes of Health (NIH) found a prevalence of myositis of 8%.[47] In most of those patients, myositis was one of the presenting features of SLE. Myositis usually involves the proximal upper and lower extremities. Histologic findings in SLE myositis are often less pronounced than those observed in polymyositis.

A biopsy study of 55 unselected patients with SLE demonstrated that several pathologic changes, including type II fiber atrophy, lymphocytic vasculitis, and myositis, were increased in patients with SLE compared with control patients.[48]

It is important to distinguish myositis secondary to SLE from drug-induced myopathies caused by glucocorticoids, antimalarial agents, colchicine, or statins because the treatment is very different. Muscle enzymes such as creatine phosphokinase (CPK) and aldolase are typically normal in patients with both glucocorticoid- and hydroxychloroquine-induced myopathy. Biopsy specimens usually reveal characteristic findings, including vacuolar changes in hydroxychloroquine myopathy and type II fiber atrophy in glucocorticoid myopathy in the absence of inflammation. Colchicine can result in a myopathy or neuromyopathy with elevation of serum CPK levels. Muscle histology reveals a vacuolar myopathy. Finally, it is important to think broadly about other potential causes of myopathy and myositis in patients with SLE, including thyroid disease, electrolyte abnormalities, and infectious myositis. One must also consider the possibility of MCTD because myositis can be a prominent feature of that disorder.

## Renal Involvement

### General Considerations

Renal involvement is common in SLE and is a significant cause of morbidity and mortality.[49,50] It is estimated that as many as 90% of patients with SLE will have pathologic evidence of renal involvement on biopsy, but clinically significant nephritis will develop in only 50%. The clinical presentation of lupus nephritis is highly variable, ranging from asymptomatic hematuria and/or proteinuria, to frank nephrotic syndrome, to rapidly progressive glomerulonephritis with loss of renal function. Lupus nephritis typically develops within the first 36 months of the disease, although there are exceptions. Thus periodic screening for the presence of nephritis is a critical component of the ongoing evaluation and management of patients with SLE. Routine screening procedures include inquiry about new-onset polyuria, nocturia, or foamy urine, and examination of the patient for the presence of hypertension or lower extremity edema. It is important to screen at regular intervals for the presence of proteinuria and/or hematuria and a change in serum creatinine. In patients with active SLE, screening at 3-month intervals is prudent.

### Types of Renal Involvement in Systemic Lupus Erythematosus

Several forms of renal involvement have been described in SLE, including immune complex–mediated glomerulonephritis (the most common form), tubulointerstitial disease, and vascular disease. Glomerulonephritis is characterized by immune complex deposition and inflammatory cell infiltration into the glomerulus. The pattern of glomerular injury is primarily related to the site of immune complex deposition. Tubulointerstitial and vascular disease can occur with or without immune complex–mediated glomerulonephritis. Tubulointerstitial disease has been observed in as many as 66% of SLE renal biopsy specimens[51] and is characterized by inflammatory cell infiltrates, tubular damage, and interstitial fibrosis. The presence of tubulointerstitial disease is a strong predictor of poor long-term renal outcome.[52]

Renal vascular lesions in SLE include "lupus vasculopathy," thrombotic microangiopathy (TMA), vasculitis, and nonspecific vascular sclerosis.[53,54] Lupus vasculopathy is defined as the presence of immunoglobulin and complement-containing thrombi within the glomerular capillary or arteriolar lumina. Inflammatory changes to the vascular wall are absent. TMA is characterized by the presence of fibrin thrombi within the glomerular capillary or arteriolar lumina and may be associated with the presence of antiphospholipid antibodies. The finding of TMA should prompt consideration of anti-phospholipid antibody syndrome nephropathy (APSN). The frequency of TMA was 24% in a study of 148 patients with biopsy-proven lupus nephritis.[55] The majority of patients with documented TMA were thought to have renal-limited TMA in that there was no identifiable alternative disease process to explain the TMA, such as thrombotic thrombocytopenic purpura hemolytic uremic syndrome (TTP-HUS), malignant hypertension, or the presence of anti-phospholipid antibodies. The patients with concomitant biopsy-proven lupus nephritis and TMA

had higher levels of proteinuria, higher serum creatinine concentrations, and a worse renal prognosis. Although exceedingly rare, true vasculitis characterized by leukocyte infiltration and fibrinoid necrosis of arterial walls can occur. Nonspecific sclerotic vascular lesions characterized by fibrous intimal thickening are commonly observed. The presence of such vascular lesions is associated with decreased renal survival.[56] In one study of 341 patients with lupus nephritis, renal vascular lesions were identified in 82% of patients. Vascular immune deposits were the most common lesion observed (74% of patients).[57] In addition to the lupus-related renal lesions described previously, unrelated renal abnormalities may develop in patients with SLE. Such pathologic lesions include focal segmental glomerulosclerosis (FSGS), hypertensive nephrosclerosis, diabetic nephropathy, and thin basement membrane disease.[58] In an SLE patient in whom renal disease is suspected, renal biopsy is critical in distinguishing between these potential causes and in guiding appropriate management decisions.

## Laboratory Evaluation

### Urinalysis

A urinalysis with microscopy is essential in the screening and monitoring of lupus nephritis.[59] Hematuria, pyuria, dysmorphic red blood cells, red blood cell casts, and white blood cell casts may all be present. Red blood cell casts are very specific, but not sensitive, for the diagnosis of glomerulonephritis. Early morning urine specimens, which tend to be concentrated and acidic, are ideal for the detection of red blood cell casts. White blood cells, red blood cells, and white blood cell casts may indicate the presence of tubulointerstitial involvement. Hematuria in the absence of proteinuria might be to the result of urolithiasis, menstrual contamination, or bladder pathology, particularly transitional cell carcinoma in a patient with previous cyclophosphamide exposure.

Accurate measurement of proteinuria is critical because proteinuria is a very sensitive indicator of glomerular damage. In addition, studies of chronic kidney disease have shown that the magnitude of proteinuria is a strong predictor of glomerular filtration rate decline.[60] Normal daily protein excretion is less than 150 mg. Although the gold-standard tool is an accurately collected 24-hour urine protein, this test can be cumbersome for patients and is prone to errors in undercollection and overcollection. Thus many clinicians are currently using the random spot urine protein-to-creatinine ratio for convenience. Use of the spot ratio is controversial because data suggest that the spot ratio often is not representative of the findings in a timed collection, especially in the range of 0.5 to 3.0 (the range of most lupus nephritis flares).[61] However, a spot ratio can be a helpful screening test for the presence of proteinuria, and is useful in differentiating nephrotic from non-nephrotic range proteinuria.[62] A urine dipstick should not be used for the quantification of proteinuria because it reflects protein concentrations and varies depending on the volume of the sample. Many experts currently recommend calculation of the protein: creatinine ratio from a 12- or 24-hour urine collection as the gold standard of proteinuria assessment.[63]

### Measurement of Renal Function

Although easy to measure, serum creatinine is a fairly insensitive indicator of early decline in glomerular filtration rate (GFR). Creatinine is freely filtered across the glomerulus and is also secreted by the proximal tubule. While GFR falls, the rise in serum creatinine is counteracted by increased tubular creatinine secretion. In addition, hemodynamic changes, such as those caused by treatment with angiotensin-converting enzyme inhibitors or nonsteroidal anti-inflammatory drugs, are a common cause of changes in serum creatinine levels in the absence of progression of underlying renal disease. However, trending serum creatinine during a period of time is a reasonable method by which to follow a patient's renal function. Many clinicians prefer to utilize equations that estimate GFR, such as the Modification of Diet in Renal Disease (MDRD) or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equations. The CKD-EPI equation is more accurate than the MDRD equation in patients with higher GFRs. Whichever method is chosen, the detection of changes in renal function during a period of time is more important than the absolute level when following lupus nephritis patients in clinical practice.

### Renal Biopsy

When a patient with SLE has clinical or laboratory features that suggest the presence of nephritis, a renal biopsy should be performed to confirm the diagnosis, evaluate the degree of disease activity, and determine an appropriate course of treatment. The importance of renal biopsy was emphasized in the ACR's guidance document for the case identification, treatment, and monitoring of lupus nephritis.[64] According to this document, biopsy is highly recommended for patients who meet any of the following criteria: (1) increasing serum creatinine without compelling alternative causes, (2) confirmed proteinuria of greater than or equal to 1 g/24 hours, (3) proteinuria greater than 0.5 g/24 hours plus hematuria, or (4) proteinuria greater than 0.5 g/24 hours plus cellular casts. Proteinuria can be measured by a 24-hour collection or spot protein to creatinine ratio.

Before renal biopsy, ultrasonography is recommended to assess kidney size and structure and to rule out renal vein thrombosis. Kidney size of less than 75% of normal is a relative contraindication to biopsy.[65] SLE glomerulonephritis is classified by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) into six categories on the basis of light microscopic, immunofluorescent, and electron micrographic findings[66] (Table 80-4 and Figure 80-7).

An individual biopsy might exhibit just one of the ISN/RPS pathologic classes or a combination of classes. Class I is characterized by normal-appearing glomeruli on light microscopy and mesangial immune deposits on immunofluorescence. Class II is characterized by mesangial proliferation on light microscopy and mesangial deposits on immunofluorescence. Class III and IV lupus nephritis lesions are highly inflammatory and are characterized by immune complex deposition in the subendothelial space. They have traditionally been described as "proliferative" because of the presence of proliferating endocapillary cells within the glomeruli. They are believed to be inter-related lesions that

**TABLE 80-4** International Society of Nephrology/Renal Pathology Society Classification of Lupus Nephritis

| WHO Type | |
|---|---|
| Class I | **Minimal Mesangial Lupus Nephritis**<br>Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence |
| Class II | **Mesangial Proliferative Nephritis**<br>Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits<br>Few isolated subepithelial or subendothelial deposits may be visible by immunofluorescence or electron microscopy, but not by light microscopy |
| Class III | **Focal Lupus Nephritis**<br>Active or inactive focal, segmental, or global endocapillary or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations |
| Class IV | **Diffuse Lupus Nephritis**<br>Active or inactive diffuse, segmental, or global endocapillary or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is subdivided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits, but with little or no glomerular proliferation |
| Class V | **Membranous Lupus Nephritis**<br>Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations<br>Class V nephritis may occur in combination with class III or class IV, in which case both are diagnosed<br>Class V nephritis may show advanced sclerotic lesions |
| Class VI | **Advanced Sclerotic Lupus Nephritis**<br>≥90% of glomeruli globally sclerosed without residual activity |

WHO, World Health Organization.

Modified from Weening JJ, et al: The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 15:241, 2004.

differ in the distribution of endocapillary immune complex deposition. Class III denotes that less than 50% of glomeruli are involved, and class IV denotes that 50% or more of glomeruli are involved. Class IV lesions are subcategorized according to whether most glomeruli show focal (<50% of the glomerular tuft) or global (≥50% of the glomerular tuft) involvement. These lesions are further described as active (A), chronic (C), or a mixture of the two (A/C). Both active and chronic lesions are taken into consideration when determining whether the designation of class III or class IV is most appropriate. Thick subendothelial immune deposits form classic "wire loop" lesions. Sometimes, wire loop lesions are present in the absence of cellular proliferation. Class V lupus nephritis is characterized by immune complex deposition in the subepithelial space, resulting in widespread thickened capillary loops. These findings are similar to those observed in idiopathic membranous nephritis. However, the presence of concomitant mesangial deposits, C1q deposition on immunofluorescence, or tubuloreticular inclusion bodies would favor the diagnosis of lupus. This lesion is commonly manifested clinically as nephrotic range proteinuria. Class V nephritis may occur in a pure histopathologic form or in combination with features of class III or class IV nephritis. Class VI nephritis is defined by the presence of more than 90% globally sclerotic glomeruli. In addition to the type of glomerular pathology, the ISN/RPS classification system dictates that tubulointerstitial disease and/or vascular disease should be noted on the diagnostic line. However, it is important to note that the class of lupus nephritis is based entirely on the glomerular pathology.

Immunofluorescence studies are an important supplement to the findings on light microscopy. Immunofluorescence reveals the type and pattern of immune complex deposition. Lupus nephritis is characterized by a granular pattern of immunofluorescence along the glomerular basement membrane, mesangium, and/or tubular basement membranes. The characteristic findings of lupus nephritis are sometimes referred to as the "full-house" pattern, because IgG, IgM, IgA, C3, and C1q are all found in the deposits. Electron microscopy is useful in more precisely localizing the sites of immune complex deposition. The finding of tubuloreticular inclusion bodies within endothelial cells is strongly suggestive of the diagnosis of lupus nephritis. However, because tubuloreticular inclusion bodies are associated with increased levels of interferon (IFN)-α, chronic viral infections such as hepatitis B/C and HIV must be ruled out.

Renal biopsy is especially important because urinary parameters such as hematuria and the degree of proteinuria imperfectly predict the underlying renal pathology.[67,68] For example, hematuria might be absent in patients with severe class IV nephritis, and proteinuria can be modest in patients with class V nephritis. A repeat renal biopsy may be indicated in certain clinical settings (e.g., if a patient is not responding appropriately to therapy, if a patient unexpectedly worsens after having achieved a good response to therapy, or to determine whether residual proteinuria after a course of treatment is the result of ongoing active lupus nephritis vs. glomerulosclerosis). Repeat renal biopsy can be useful in detecting class transformation that occurs in 15% to 50% of lupus nephritis patients during the course of their disease. Class transformation can occur spontaneously or as a result of treatment.

## Outcome

Each ISN/RPS histopathologic class portends a distinct renal prognosis. Patients with class I and class II nephritis have an excellent renal prognosis and do not require any specific therapy. In contrast, the long-term renal prognosis of class III or class IV nephritis is poor in the absence of immunosuppressive therapy. Although the long-term renal prognosis of class V nephritis is more favorable than that of class III or class IV nephritis, class V patients are more likely to suffer from morbid complications of the nephrotic syndrome, including cardiovascular disease, thromboembolic disease, and hyperlipidemia. Several epidemiologic studies

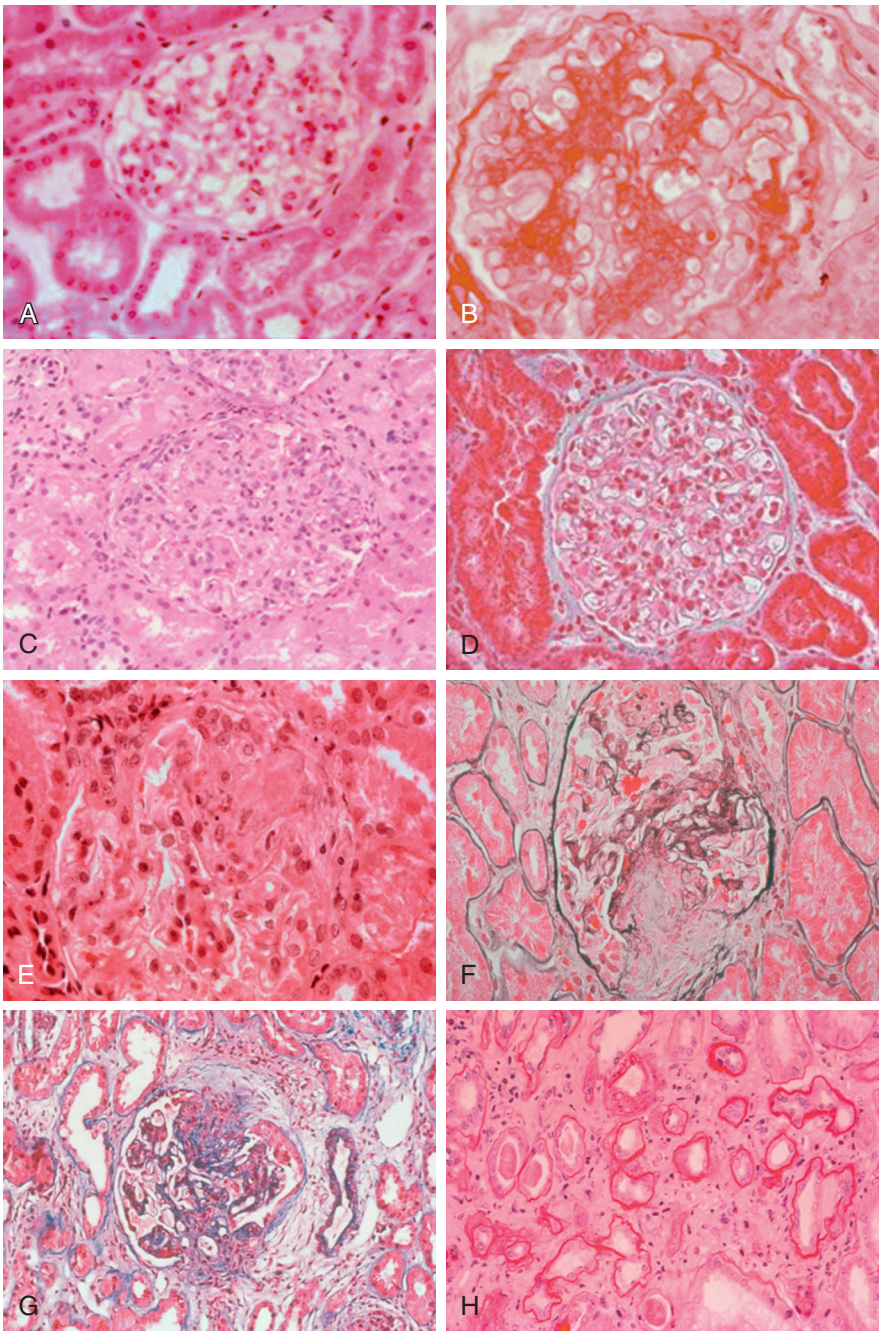



**Figure 80-7** **A** through **D**, World Health Organization types of lupus. (See Table 80-4 for a detailed description of histologic findings.) **A**, Normal glomerulus (type I). **B**, Mesangial proliferative (type II). **C**, Proliferative nephritis. Dramatic increase in mesangial and endocapillary cellularity produces a lobular appearance of the glomerular tufts and compromises the patency of most capillary loops. When less than 50% of glomeruli are involved, nephritis is denoted as focal (type III). When more than 50% of glomeruli are involved, nephritis is denoted as diffuse (type IV). **D**, Membranous nephropathy (type V). In membranous lupus nephropathy, the capillary walls of the glomerular tuft are prominent and widely patent, resembling "stiff" structures with decreased compliance. **E** through **H**, High-risk histologic features suggesting severe nephritis. **E**, Fibrinoid necrosis with karyorrhexis in a patient with focal proliferative glomerulonephritis. **F** and **G**, Cellular crescents with layers of proliferative endothelial cells and monocytes lining Bowman's capsule along with a predominantly mononuclear interstitial infiltrate. **H**, Severe interstitial fibrosis and tubular atrophy. Note the thickening of the tubular basement membranes and tubular epithelial degeneration with separation of residual tubules caused by deposition of collagenous connective tissue among tubules.

have defined demographic, clinical, and histopathologic factors associated with renal outcome in patients with lupus nephritis. Studies have shown that African-Americans and Hispanics/Latinos generally experience a worse renal prognosis than white and Chinese populations. The reasons for this disparity most likely involve a combination of genetic and socioeconomic factors.

## Pleuropulmonary Involvement

Pleuropulmonary manifestations of SLE are diverse and can involve any aspect of the lung (Table 80-5).

### Pleuritis

Pleuritis will develop in as many as 50% of patients with SLE. Clinically apparent pleural effusions are typically small, bilateral, and exudative.[69] Pleuritis is commonly manifested by pleuritic chest pain, but pleural effusions may be asymptomatic and detected on routine chest radiography performed for another purpose. Massive pleural effusions requiring pleurocentesis and/or pleurodesis are uncommon but have been reported.[70] The presence of pleuritis usually corresponds to active SLE in other organ systems.[71] Thoracoscopic evaluation has demonstrated nodules on the visceral pleura with immunoglobulin deposits detected on immunofluorescence. The differential diagnosis of pleural effusions in a patient with SLE includes infection, malignancy, and heart failure. In addition, pleural effusions are a common feature of drug-induced lupus. In the absence of infection, high levels of serum C-reactive protein (CRP) have been found to correlate well with the presence of pleuritis and other forms of serositis in SLE.[71,72]

**TABLE 80-5** Pleuropulmonary Manifestations of Systemic Lupus Erythematosus

| Manifestation | Key Features |
|---|---|
| Pleuritis | May occur with or without effusion<br>May correlate with elevated serum C-reactive protein |
| Pleural effusion | May be asymptomatic<br>Usually small, bilateral, exudative<br>Common feature of drug-induced lupus<br>Must exclude infection, malignancy, heart failure |
| Acute pneumonitis | Severe respiratory illness with fever, cough, pulmonary infiltrates, hypoxemia<br>Pleural effusion may be present<br>High mortality rate<br>Bronchoscopy with bronchoalveolar lavage might be necessary to exclude infection |
| Chronic interstitial lung disease | May develop after acute pneumonitis or in a more insidious fashion<br>Presents as dyspnea on exertion, pleuritic chest pain, nonproductive cough<br>High-resolution computed tomography is more sensitive than chest radiograph in detecting disease<br>Must exclude infection, pulmonary edema, malignancy |
| Diffuse alveolar hemorrhage | Presents as dyspnea and cough, alveolar infiltrates, fall in blood hemoglobin level<br>Hemoptysis may not be present<br>Diffusion capacity of carbon monoxide typically increased<br>Bronchoscopy with bronchoalveolar lavage confirms the diagnosis and excludes infection<br>High mortality rate |
| Pulmonary arterial hypertension | Presents as dyspnea on exertion, fatigue, chest pain, nonproductive cough<br>Diagnosis should be confirmed with right heart catheterization<br>Exclude secondary causes of pulmonary hypertension, including thromboembolic disease |
| Shrinking lung syndrome | Dyspnea, low lung volumes, elevation of hemidiaphragms in the absence of lung parenchymal involvement |

### Lupus Pneumonitis

Acute lupus pneumonitis is a rare manifestation of SLE that presents as a severe, acute respiratory illness with fever, cough, pulmonary infiltrates, and hypoxemia. Chest radiography usually reveals bilateral, lower lobe, acinar infiltrates that often occur in conjunction with a pleural effusion. Histopathologic findings are nonspecific and include diffuse alveolar damage, inflammatory cell infiltrates, hyaline membranes, and alveolar hemorrhage.[73] Immunofluorescence studies have demonstrated granular deposits of IgG and C3 within the alveolar septa.[74] Because clinical and pathologic features of acute lupus pneumonitis are nonspecific, careful evaluation is critical to exclude other potential pulmonary processes such as infection. If routine blood and sputum cultures are nondiagnostic, bronchoscopy with bronchoalveolar lavage can be useful in detecting pulmonary pathogens. A "tree and bud" pattern on high-resolution CT (HRCT) may suggest the presence of an atypical pneumonia. Acute lupus pneumonitis is associated with significant morbidity and mortality. One series of 12 patients reported a mortality rate of 50%, with deaths from respiratory failure, opportunistic infection, and thromboembolic events. Three of the surviving patients progressed to chronic interstitial pneumonitis.[75]

### Chronic Interstitial Lung Disease

Chronic interstitial lung disease is a rare manifestation of SLE. It occurs more commonly in other connective tissue diseases such as systemic sclerosis, RA, and polymyositis/dermatomyositis. Interstitial lung disease in the setting of SLE can develop after one or more episodes of acute pneumonitis but can also occur in an insidious fashion.[75] Symptoms are similar to those seen in patients with idiopathic interstitial lung disease and include dyspnea on exertion, pleuritic chest pain, and chronic, nonproductive cough. The diagnosis of interstitial lung disease is often made on the basis of clinical-radiologic findings; lung biopsy is not routinely performed. Chest radiography results might be normal early in the disease but can show reticular opacities. Pulmonary function studies show a restrictive pattern with reduction in total lung capacity and reduction in the diffusion capacity of carbon monoxide (DLCO). HRCT is more sensitive than chest radiography in detecting

interstitial lung disease and in distinguishing reversible lesions (ground-glass opacities) from irreversible fibrotic lesions. Nonspecific interstitial pneumonia (NSIP) and usual interstitial pneumonia (UIP) are the most common patterns detected on histopathology and HRCT. Before making the diagnosis of interstitial lung disease, it is important to exclude infection, pulmonary edema, and malignancy.

### Diffuse Alveolar Hemorrhage

Diffuse alveolar hemorrhage (DAH) is a life-threatening manifestation of SLE that occurs in less than 2% of patients. It is characterized by acute or subacute onset of dyspnea and cough in the setting of new alveolar infiltrates on chest radiography and a fall in blood hemoglobin level. Similar to other causes of DAH, hemoptysis is not universally present. Although most patients are too ill to undergo formal pulmonary function tests, the DLCO is typically increased in the setting of DAH because of the presence of extravascular hemoglobin within the alveoli. Bronchoscopy with bronchoalveolar lavage (BAL) is important in ruling out infection and confirming the diagnosis. Characteristic findings include visualization of blood in the airways and serosanguineous BAL fluid that does not clear with continued lavage. Hemosiderin-laden macrophages may be present in the BAL fluid. Various histopathologic patterns have been described in lupus DAH, including bland pulmonary hemorrhage, capillaritis, diffuse alveolar damage, and vasculitis of small arterioles and small muscular pulmonary arteries. DAH usually occurs in the setting of serologically and clinically active SLE, and lupus nephritis is the most common concurrent SLE manifestation. However, DAH occasionally may be the initial manifestation of SLE. Mechanical ventilation is often required,[76] and infectious complications are common. Despite aggressive therapy, the mortality rate from DAH continues to be 50%.

### Pulmonary Hypertension

Pulmonary hypertension (PH) is a rare, devastating complication of SLE that is defined as a mean pulmonary artery pressure greater than 25 mm Hg at rest on right heart catheterization. PH can be secondary to a variety of disorders, such as pulmonary disease, leading to hypoxemia, chronic thromboembolic disease, pulmonary veno-occlusive disease, or left-sided heart disease. Pulmonary arterial hypertension (PAH) is a subtype of PH that is defined by a normal pulmonary capillary wedge pressure and elevated pulmonary capillary resistance on right heart catheterization. Symptoms of PH include dyspnea on exertion, fatigue, chest pain, and nonproductive cough. Physical examination findings may include a pronounced second pulmonary heart sound, a left parasternal lift, and signs of a volume-overloaded state. Chest radiography and HRCT are important in excluding lupus pneumonitis. Chest radiography may show cardiomegaly and a prominent pulmonary artery segment. The electrocardiogram often shows right axis deviation. Pulmonary function studies demonstrate a reduction in DLCO. Although transthoracic Doppler echocardiography is an adequate screening test for PH, the diagnosis should be confirmed by catheterization of the right side of the heart. Similar to interstitial lung disease, PAH more commonly occurs in association with scleroderma and MCTD.

In a patient with SLE who has been diagnosed with PH, an evaluation must be performed for secondary causes. Ventilation and perfusion (V/Q) lung scan and/or helical CT are useful in excluding chronic thromboembolic disease. Echocardiography can rule out left heart failure and intracardiac shunting. A sleep study can be useful in ruling out obstructive sleep apnea. An evaluation for interstitial lung disease is necessary. Some studies suggest that PAH occurs more commonly in patients with Raynaud's phenomenon. Autopsy findings suggest that the pathogenesis of PAH in the setting of SLE is likely to be multifactorial, including both primary vascular and inflammatory/immune-mediated mechanisms. Macrophages and lymphocytes are present within the vascular plexiform lesions. In addition, IgG and complement are deposited within the pulmonary artery walls.

### Other

Shrinking lung syndrome occurs in a small subset of patients with SLE and should be considered when evaluating a patient with SLE who has unexplained dyspnea and pleuritic chest pain.[77,78] The syndrome is characterized by decreased lung volumes in the setting of normal lung parenchyma. The cause of the disorder remains controversial. Diaphragmatic myopathy, abnormal chest wall expansion, phrenic neuropathy, and pleural inflammation/fibrosis have been reported as possible factors. The prognosis of this syndrome seems to be good, and progressive respiratory failure is uncommon.

Although symptomatic bronchiolar disease is uncommon in SLE, abnormalities in pulmonary function studies have been reported in as many as two-thirds of patients with SLE.[79] One study of nonsmoking patients with SLE found that 24% of patients had pulmonary function studies consistent with small airway disease. Rare case reports of bronchiolitis obliterans organizing pneumonia (BOOP) in the setting of SLE have been described.[80]

Last, a syndrome known as "acute reversible hypoxemia" has been observed in patients with active SLE. This syndrome is characterized by hypoxemia and an abnormal alveolar-arterial $pO_2$ gradient in the absence of parenchymal lung disease. Pulmonary vascular endothelial cell injury and leukoagglutination secondary to complement activation is thought to be one potential mechanism

## Cardiovascular Involvement

Cardiovascular disease is a frequent complication of SLE and may involve the pericardium, myocardium, valves, and coronary arteries.

### Pericarditis

Pericarditis, with or without an effusion, is the most common cardiac manifestation of SLE, occurring in more than 50% of patients with SLE at some point during the course of their disease.[81] Pericardial effusions are usually small and asymptomatic and typically are detected on echocardiography performed for another indication. Consistent with this

observation, necropsy studies have shown that histopathologic evidence of pericarditis is much more common than clinically symptomatic disease during life.[82] Symptomatic pericarditis classically presents as sharp, precordial chest pain that is improved when the patient is in the upright position. A pericardial rub and tachycardia may be detected on cardiac auscultation. The electrocardiogram demonstrates diffuse ST segment elevation. Similar to pleuritis, pericarditis usually occurs in the setting of active SLE in other organ systems. Although rare, SLE pericarditis complicated by large effusions and tamponade physiology has been reported. Purulent effusions necessitating pericardiocentesis have also been described, but rarely. The differential diagnosis of precordial chest pain in a patient with SLE includes costochondritis, gastroesophageal reflux disease, pulmonary embolism, myocardial ischemia, pleuritis, pneumonitis, and pulmonary hypertension.

### Myocarditis

Myocarditis, an uncommon manifestation of SLE, should be suspected in a patient presenting with various combinations of the following clinical features: unexplained heart failure or cardiomegaly, unexplained tachycardia, and unexplained electrocardiographic abnormalities. Echocardiography can confirm the presence of systolic or diastolic dysfunction and/or global hypokinesis. If myocarditis is suspected, an endomyocardial biopsy may be helpful in confirming the diagnosis and excluding other causes of cardiomyopathy such as hydroxychloroquine toxicity. The distinguishing pathologic finding of hydroxychloroquine toxicity is myocyte vacuolization in the absence of active myocarditis. Histopathologic findings of SLE myocarditis include perivascular and interstitial mononuclear cell infiltration and sometimes fibrosis and scar.

### Valvular Abnormalities

Several valvular abnormalities have been described in patients with SLE, including Libman-Sacks endocarditis (also known as "atypical verrucous endocarditis"), valvular thickening, valvular regurgitation, and valvular stenosis. One transesophageal echocardiographic (TEE) study demonstrated a prevalence of valvular abnormalities of 61% in patients with SLE compared with 9% of controls, with vegetations present in 43% of patients with SLE compared with none of the controls.[83] Valvular thickening with a predilection for the mitral and aortic valves was the most common abnormality, occurring in 50% of patients with SLE. Valvular regurgitation and stenosis were detected in 25% and 4% of patients, respectively.[83] In this study, the presence and progression of valvular disease were not associated with SLE disease activity or treatment. During a follow-up period of as long as 5 years, some valvular abnormalities resolved and some new lesions occurred. The combined incidence of stroke, peripheral embolism, congestive heart failure, infective endocarditis, need for valve replacement, and death was 22% among patients with valvular disease compared with 15% in patients without valvular disease.[83]

Libman-Sacks endocarditis has been recognized in multiple pathologic studies as a characteristic valvular abnormality in SLE. Libman-Sacks verrucae typically appear as pea-sized, flat or raised, granular lesions that occur most commonly on the ventricular aspects of the mitral valve posterior leaflet.[84] The verrucae often extend onto the adjacent left ventricular mural endocardium and may lead to adherence of the leaflet and chordae tendineae to the ventricular mural endocardium, resulting in valvular regurgitation. All four valves may be involved, but recent studies suggest a predominance of left-sided lesions. The lesions are frequently clinically silent because they are typically found on the undersurface of valve leaflets, surrounded by fibrous tissue. Histologically, two types of verrucae have been described: (1) active lesions consisting of fibrin clumps with infiltrating lymphocytes and plasma cells, and (2) healed lesions consisting of dense vascularized fibrous tissue with or without calcification. Combinations of active and healed lesions also occur. Verrucae typically do not contain polymorphonuclear cells; thus the presence of such cells should prompt consideration of infectious endocarditis. Immunopathologic studies have demonstrated immunoglobulin and complement deposition in a granular pattern at the base of the valve, along the valve leaflet, and within the verruca itself. Cardiac murmurs are frequently heard in patients with SLE. They may simply result from high-flow states such as fever and anemia, or they may reflect cardiac pathology such as mitral valve prolapse or infective endocarditis. When a new murmur is evaluated, a transthoracic echocardiogram (TTE) is an appropriate first test. However, TEE should be used in the event of a nondiagnostic TTE or in a patient with suspected thromboembolic events. TEE has been shown to be superior to TTE for detection of Libman-Sacks endocarditis.[85] Although thromboembolic events are believed to be rare complications of Libman-Sacks endocarditis, one study showed that valvular heart disease detected on TTE was associated with the presence of cerebral infarcts on MRI.[86] Systematic reviews have suggested that the presence of valvular abnormalities in SLE are associated with anti-phospholipid antibody[87,88]. One study of 1656 patients with SLE determined that the odds of valvular heart disease in patients with SLE with anti-phospholipid antibodies was three times higher than in SLE patients without anti-phospholipid antibodies. The risk for valvular heart disease was greatest for patients with lupus anticoagulant or IgG anticardiolipin antibodies.[88]

### Coronary Artery Disease

Both intramural and extramural coronary artery disease is increased in patients with SLE. Necropsy studies have demonstrated fibrous intimal proliferation of small intramural coronary arteries and obstruction of these arteries with hyaline material.[89] These lesions are similar to those observed in pathologic studies of renal and central nervous system tissue in patients with SLE. The large epicardial coronary arteries may be obstructed because of arterial emboli, in situ thrombosis, vasculitis, or atherosclerotic disease. True coronary artery vasculitis is exceedingly rare. In contrast, atherosclerotic disease is a well-recognized complication of long-standing SLE.[90] Autopsy studies have demonstrated atherosclerosis in 25% to 40% of patients with SLE. One epidemiologic study demonstrated that young women with SLE have a 50-fold higher risk of myocardial infarction compared with age-matched controls.[91] A

multicenter inception cohort determined that male sex and older age at SLE diagnosis were significantly associated with the presence of atherosclerotic disease.[92] Although patients with SLE are more likely to have classic atherosclerotic risk factors such as hypertension and exposure to glucocorticoids, these risk factors alone do not fully account for the increased risk of atherosclerosis seen in patients with SLE.[93] Although the precise mechanisms of accelerated atherosclerosis in SLE are not completely understood, it is likely that an interplay between traditional cardiovascular risk factors and autoimmune related factors is important. One example of a nontraditional, autoimmune-related factor is pro-inflammatory high-density lipoprotein (piHDL) cholesterol. In the setting of inflammation, HDL can become pro-inflammatory rather than anti-inflammatory. One study demonstrated a piHDL prevalence of 45% in women with SLE compared with 20% of female patients with RA and 4% of control women.[94] In another study, piHDL was positively associated with measures of subclinical atherosclerosis.[95]A recent prospective study of 210 women with SLE was undertaken to try to develop a risk score that could be utilized to predict the presence and progression of carotid plaque.[96] As a result, the Predictors of Risk for Elevated Flares, Damage Progression, and Increased Cardiovascular Disease in Patients with SLE (PREDICTS) panel was developed. This panel comprises four novel biomarkers (piHDL function, leptin levels, soluble TNF-like weak inducer of apoptosis [TWEAK levels], homocysteine levels), and two traditional cardiovascular risk factors (age ≥48 years, history of diabetes). A high score that used the PREDICTS panel was associated with a 28-fold increased odds of baseline carotid plaque and plaque progression in women with SLE. It remains to be determined how risk profiles such as this can be utilized in clinical practice to aid in selection of preventative treatments for patients with SLE at high risk for atherosclerosis.

The possibility of coronary artery disease must be considered in any SLE patient who is seen with chest pain and/or shortness of breath, and one should have a low threshold for a functional evaluation with a cardiac stress test. Cardiac catheterization might also be necessary for diagnosis and therapeutic intervention. It is important to evaluate these patients for the presence of anti-phospholipid antibodies because coronary artery thrombosis may be a manifestation of the anti-phospholipid antibody syndrome. Evaluation for and treatment of modifiable risk factors such as obesity, smoking, hypertension, and hyperlipidemia are important in mitigating the development and progression of atherosclerotic disease.

## Neuropsychiatric Involvement

### General Considerations

Neuropsychiatric lupus (NPSLE) consists of a broad range of neurologic and psychiatric manifestations that can involve any aspect of the central or peripheral nervous system. With the intention of improving the terminology and classification of NPSLE, an ACR subcommittee categorized NPSLE into 19 distinct syndromes encompassing the central and peripheral nervous systems (CNS and PNS)[97] (Table 80-6). The extent of this classification system underscores the complexity of NPSLE. CNS disorders range from diffuse processes such as acute confusional state, headache, psychosis, and mood disorders, to more focal processes such as seizures, myelopathy, and chorea. It is notable that the ACR classification system has removed the cryptic term "lupus cerebritis" from the vernacular. Although the ACR case definitions are very helpful in providing a framework in which to study NPSLE, accurate attribution of neuropsychiatric manifestations remains challenging. It is often difficult to distinguish whether neuropsychiatric symptoms are to the result of active SLE or other factors such as infection, metabolic abnormalities, severe hypertension, adverse effects of medications, or independent neurologic or psychiatric problems. No laboratory or imaging study is sufficiently sensitive or specific to confirm the diagnosis of neuropsychiatric SLE. Instead, the diagnosis is made on a case-by-case basis and is based on a thorough clinical evaluation that is corroborated by findings (or lack thereof) on brain imaging, serologic testing, lumbar puncture, and neuropsychiatric assessment. Correct attribution to SLE or to alternative causes is essential in ensuring that appropriate treatment is administered.

**TABLE 80-6** American College of Rheumatology Classification of Neuropsychiatric Syndromes in Systemic Lupus Erythematosus

| Central Nervous System | Peripheral Nervous System |
|---|---|
| Aseptic meningitis | Guillain-Barré syndrome |
| Cerebrovascular disease | Autonomic disorder |
| Demyelinating syndrome | Mononeuropathy, single/multiplex |
| Headache | Myasthenia gravis |
| Movement disorder | Cranial neuropathy |
| Myelopathy | Plexopathy |
| Seizure | Polyneuropathy |
| Acute confusional state | |
| Anxiety disorder | |
| Cognitive dysfunction | |
| Mood disorder | |
| Psychosis | |

See reference 97.

### Pathogenesis

Multiple pathogenic mechanisms are undoubtedly involved in the various NPSLE syndromes, but in most cases, the precise pathogenesis is unknown. In addition, mechanisms are likely to vary depending upon whether the manifestation involves the CNS or the PNS. The underlying mechanisms of CNS manifestations can be grouped into two broad categories: primary injury to the vasculature and primary injury to the brain parenchyma. A combination of the two categories can also occur. Vascular injury can take several forms, including (1) damage to both large and small vessels via thromboembolic events, often as a consequence of anti-phospholipid antibodies; (2) a bland vasculopathy of small vessels characterized by vascular hyalinization,

perivascular inflammation, and endothelial proliferation; and (3) atherosclerotic lesions. In contrast, true vasculitis of cerebral blood vessels with an inflammatory infiltrate within the vessel walls is exceedingly rare. Primary injury to brain parenchyma can occur through the action of autoantibodies, cytokines, chemokines, and infiltrating cells, often in the setting of a breached blood-brain barrier. Several autoantibody subtypes are believed to play a role in some CNS manifestations of SLE. In addition to their well-established role in thrombotic events, leading to cerebral ischemia, anti-phospholipid antibodies might bind directly to neuronal cells, leading to neuronal cell damage. Anti-phospholipid antibodies have been associated with diffuse cognitive impairment, even in the absence of an ischemic event. Antiribosomal P antibodies have been associated with the CNS manifestations of psychosis, depression, and mood disorders in some studies. Although antiribosomal P antibodies are detected in only approximately 14% of patients with SLE, the specificity of these antibodies for SLE is thought to be greater than 90%.[98] Finally, a subset of anti-DNA antibodies that cross-reacts with the *N*-methyl-D-aspartate receptor (NMDAR) might be involved in cognitive dysfunction in SLE. Binding of these antibodies to neuronal cells leads to apoptotic, non-inflammatory cell death. Histopathologic studies on post-mortem human brain tissue have demonstrated multiple CNS abnormalities, including large and small multifocal infarctions, hemorrhage, bland small vessel vasculopathy, cortical atrophy, brain edema, and demyelination.

### Approach to Diagnosis

The diagnostic evaluation of a patient with potential NPSLE is tailored to the presenting neuropsychiatric manifestation. Depending on the manifestation, consultation with a neurologist and/or a psychiatrist is advised. The most important first step is exclusion of an alternative explanation for the neuropsychiatric symptoms/signs. Lumbar puncture with cerebrospinal fluid (CSF) examination is useful for exclusion of an infectious origin. Mild lymphocytic pleocytosis and elevated CSF protein are sometimes observed in CNS lupus but are not uniformly present. CSF findings are not sensitive or specific enough to confirm a diagnosis of neuropsychiatric SLE. It is important to recognize that infection is a common cause of CNS symptoms in patients with SLE who are hospitalized with altered mental status,[99] and must be rigorously ruled out. Progressive multifocal leukoencephalopathy (PML) is a rare infection that also merits consideration in a patient with SLE who is seen with symptoms of CNS dysfunction. PML occurs more frequently in patients with SLE than in patients with other rheumatic diseases, even in the absence of significant immunosuppressive therapy.[100,101] Thus, in an SLE patient with unexplained new neurologic symptoms, polymerase chain reaction (PCR) of the CSF for the presence of JC virus should be performed. In situations in which PCR is negative, a brain biopsy might be needed to confirm the diagnosis. Electromyography and nerve conduction studies are important in the setting of suspected peripheral neuropathy. Electroencephalogram (EEG) is necessary in the evaluation of seizure. Neuropsychological testing may be helpful in the setting of suspected cognitive dysfunction.

MRI is the preferred imaging modality in patients with suspected NPSLE. The most commonly noted abnormalities are small, hyperintense, T2-weighted, focal white matter lesions located in the periventricular and subcortical white matter of the frontoparietal regions of the brain. However, these findings are nonspecific and can be observed in other disease processes such as atherosclerotic vascular disease and multiple sclerosis. Other common MRI findings include cortical atrophy, ventricular dilation, cerebral edema, diffuse white matter abnormalities, gray matter abnormalities, infarction, leukoencephalopathy, and hemorrhage.[102] MRI is especially useful in detecting the presence of infarcts, hemorrhage, and myelopathy, and sometimes can help to exclude infectious conditions such as brain abscess.[102] MRI is most likely to show abnormalities in the setting of focal neurologic deficits, seizures, chronic cognitive dysfunction, and anti-phospholipid antibody–mediated disease and is less likely to show abnormalities in the setting of headache, acute confusional state, and psychiatric syndromes. A study of 74 patients with new-onset neuropsychiatric lupus found normal MRI results in 42% of patients.[103] Given the wide variety of potential MRI findings, it is critical that MRI findings (or lack thereof) are interpreted in the context of the individual patient case.

Estimates of the prevalence of NPSLE have varied widely in the literature, largely depending on the extent to which headache and/or mild cognitive abnormalities have been included in the analysis. However, the preponderance of these studies suggests that CNS manifestations predominate versus PNS manifestations. Several of the more common syndromes and associated differential diagnoses are described in the following paragraphs.

### Selected Neuropsychiatric Lupus Syndromes

Headaches are reported in more than 50% of patients with SLE, but attribution of the headache to SLE is extremely difficult. Both migrainous and tension-type headaches have been described. One meta-analysis[104] determined that the prevalence of primary headache syndromes was not different between SLE and control patients, and that headache was not related to SLE disease activity. The evaluation of headache in an SLE patient should be similar to that in a non-SLE patient and should be directed by the presence or absence of worrisome features such as fever, meningismus, altered mental status, and focal neurologic signs.

Cognitive dysfunction, manifested primarily by deficits in thinking, memory, and concentration, is increasingly recognized in patients with SLE. Some have estimated a prevalence of as much as 80%, although serious cognitive impairment is much less common. Some studies suggest that cognitive dysfunction may be associated with the presence of anti-phospholipid antibodies, but a causal relationship has not been definitively established. Documentation of the presence and extent of cognitive dysfunction via neuropsychiatric testing can be useful in establishing a baseline in a particular patient that can be followed during a period of time, particularly when a therapeutic intervention is under consideration.

Psychiatric disorders such as psychosis, depression, and anxiety can occur in SLE, and consultation with a psychiatrist is highly recommended in the evaluation of patients

with these symptoms. A patient with SLE who is seen with psychosis represents a distinct diagnostic and therapeutic challenge. The differential diagnosis includes CNS infection, primary schizophrenia, systemic metabolic abnormalities, and psychosis occurring as a side effect of glucocorticoid therapy or illicit drugs. Steroid-induced psychosis is dose dependent and typically occurs within the first 2 weeks of treatment initiation.

Although rare, demyelinating syndromes such as optic neuritis and myelitis can occur as part of the spectrum of NPSLE. Optic neuritis is characterized by pain with eye movement, central visual field loss, and a waxing and waning course.[105] Optic neuritis should be differentiated from ischemic optic neuropathy, which typically presents with acute, painless loss of vision and lack of significant improvement in vision with time.[106] Myelitis is characterized by the onset of bilateral lower extremity paresthesia, numbness, and weakness that can rapidly progress to involve the upper limbs and the muscles of respiration. A sensory level is usually noted, and autonomic involvement of the bowel and bladder is common. Bandlike pain or discomfort around the abdomen is a characteristic symptom. It is important to differentiate myelitis from other causes of myelopathy, including infection, structural spinal cord abnormalities, and vascular insult to the spinal cord. Spinal cord MRI is the critical first test. CSF examination is important to rule out infection.

The combination of optic neuritis and myelitis presents a special clinical challenge because this combination of features can occur in the setting of SLE, multiple sclerosis (MS), or neuromyelitis optica (NMO).[107] Complicating matters, the clinical presentation of anti-phospholipid antibody syndrome can mimic demyelinating disease. A thorough clinical history, MRI, CSF analysis, and serologic testing are helpful in distinguishing these entities. NPSLE and MS can have identical white matter lesions on brain MRI. Spinal cord lesions may differ in that MS spinal cord lesions usually span an area measuring less than two spinal segments, but SLE lesions are often longitudinally extensive with involvement of more than three spinal cord segments. Although oligoclonal bands may be present in the CSF of both SLE and MS patients, the absence of oligoclonal bands and the presence of a pleocytosis significantly lessen the likelihood of MS. Although a positive ANA has been described in as many as 27% of patients with MS,[108] more specific subserologies such as anti-dsDNA and anti-Sm favor the diagnosis of SLE. NMO is an autoimmune disease of the CNS characterized by episodes of optic neuritis, myelitis, or both. In the majority of patients, NMO is a relapsing disease that occurs unpredictably.[109] The diagnosis of NMO includes the presence of optic neuritis and myelitis in conjunction with two of the following three supportive criteria: presence of the NMO IgG antibody (antibody to aquaporin 4), absence of brain lesions diagnostic of MS, and longitudinally extensive myelitis on MRI.[109] Patients with the NMO antibody with some features of the disease who do not fulfill all of these diagnostic criteria are sometimes described as having "NMO spectrum disorder." The NMO antibody is present in as many as 80% of patients with NMO and has a specificity of greater than 90% for the diagnosis of NMO. SLE and NMO may occur concurrently in a given patient.

Patients with SLE are at increased risk of stroke; ischemic stroke is more common than intracerebral hemorrhage.[110] Studies have demonstrated an association between the presence of anti-phospholipid antibodies (aPL) and/or valvular heart disease and the risk of stroke.[81] Brain MRI is a critical test in the diagnosis of ischemic or hemorrhagic stroke, and magnetic resonance angiography (MRA) can detect vessel aneurysms. Echocardiography, carotid ultrasound, and electrocardiography are important diagnostic tests in the setting of suspected thromboembolic cerebrovascular disease.

A potential mimic of CNS lupus is posterior reversible encephalopathy syndrome (PRES). This syndrome was first described in a case series of 15 patients in 1996.[111] Two of the original 15 patients were known to have SLE. PRES is a clinical-radiologic syndrome characterized by headache, decreased consciousness, visual changes, and seizures in the context of posterior cerebral white matter edema. It has been associated with a variety of conditions, including hypertension, eclampsia, renal disease, immunosuppressive therapies, post–solid organ transplantation, and autoimmune disease. When PRES occurs in the setting of active SLE, it is unclear whether PRES is a manifestation of CNS lupus, or whether it is a complication of a concomitant condition such as immunosuppressive therapy, renal disease, or hypertension.

SLE is also associated with peripheral neuropathy. One large study of more than 2000 patients with SLE demonstrated a 6% prevalence of peripheral neuropathy, with 67% of the neuropathies attributable to SLE.[112] The most common type of neuropathy was a sensory or sensorimotor axonal polyneuropathy. Of the patients, 17% had biopsy-proven small-fiber neuropathy. This is an increasingly recognized subtype of peripheral neuropathy that is not included in the 1999 ACR case definitions for NPSLE. Small-fiber neuropathies affect unmyelinated C fibers, with symptoms of burning pain and paresthesias. Nerve conduction studies are normal. The diagnosis is confirmed by a skin biopsy that demonstrates decreased intraepidermal nerve fiber density. A devastating, large-fiber vasculitic neuropathy leading to mononeuritis multiplex can also develop in patients with SLE. Autonomic neuropathies, cranial neuropathies, inflammatory demyelinating polyneuropathies, and abnormalities of the neuromuscular junction resembling myasthenia gravis have also been described. When encountering a SLE patient with peripheral neuropathy, it is essential for the physician to consider and rule out non-SLE etiologies such as infections (e.g., HIV, syphilis, Lyme disease, leprosy), malignancy, endocrine disorders (e.g., diabetes mellitus, hypothyroidism), toxins (e.g., alcohol, heavy metals), vitamin deficiency (e.g., vitamins $B_{12}$, $B_6$), and drugs (e.g., hydroxychloroquine, colchicine, vitamin $B_6$ excess).

## Gastrointestinal Involvement

SLE can involve any part of the gastrointestinal system. Dysphagia is noted in as many as 13% of patients, and manometric studies have detected abnormalities of esophageal motility.[113] Decreased peristalsis is most commonly observed in the upper one-third of the esophagus. In contrast to scleroderma, involvement of the lower esophageal

sphincter is rare in SLE.[114] A variety of potential pathogenic mechanisms have been described, including muscle atrophy, inflammation of esophageal muscle, and ischemic or vasculitic damage to the Auerbach plexus.

Abdominal pain, sometimes accompanied by nausea and vomiting, has been reported in as many as 40% of patients with SLE and can be to the result of SLE-related causes, medication side effects, and non–SLE-related causes such as infection.[115] When evaluating an SLE patient with abdominal pain, it is critical for the physician to rule out non-SLE conditions. It is important to note that when patients are treated with glucocorticoids and/or other immunosuppressives, clinical signs of an acute condition in the abdomen, such as rebound tenderness, can be masked. Thus delay in diagnosis is common. SLE-related causes of abdominal pain may include peritonitis, pancreatitis, mesenteric vasculitis, and intestinal pseudo-obstruction. Although autopsy studies have revealed evidence of peritoneal inflammation in as many as 72% of patients with SLE,[115] the presence of ascites is rare. If a patient with SLE is seen with abdominal pain and ascites, paracentesis is warranted to rule out infection. Peritonitis can also occur in the setting of mesenteric ischemia, bowel infarction, and pancreatitis. Thus abdominal imaging is an important part of the initial evaluation.

Pancreatitis caused by SLE is uncommon and usually is associated with active SLE in other organs. When considering the possible diagnosis of pancreatitis, it is important to note that elevated serum amylase may be misleading—it has been observed in patients with SLE in the absence of pancreatitis.[116] Although glucocorticoids and azathioprine have been associated with the development of pancreatitis in nonpatients with SLE, these medications do not seem to play a major role in the development of pancreatitis in patients with SLE.[117] It is important to rule out non-SLE causes of pancreatitis, such as biliary disease, alcohol consumption, and hypertriglyceridemia.

Mesenteric vasculitis is a very rare manifestation of SLE. Patients with this condition can be seen with a range of symptoms, from cramping, bloating, and anorexia, to an acute condition in the abdomen with diarrhea and gastrointestinal hemorrhage. Accurate diagnosis and prompt treatment are essential to prevent the potential catastrophic complications of necrotic bowel, perforation, and sepsis. Abdominal radiography may show distention of bowel loops, thickening and thumbprinting of the bowel wall, and/or free air in the abdomen. Ultrasonography may be useful in demonstrating bowel wall edema and thickening. Abdominal CT is thought to be the most useful imaging modality for the early diagnosis of mesenteric ischemia and can demonstrate prominence of mesenteric vessels with a palisade pattern supplying dilated bowel loops, ascites, and bowel wall thickening with a double-halo sign.[118] Gastroscopy and colonoscopy can sometimes reveal findings of ischemia and ulceration. Because lupus mesenteric vasculitis typically involves the small vessels (arterioles and venules) of the bowel submucosa, mesenteric angiography is usually nondiagnostic. However, angiography can be helpful in ruling out larger vessel causes of mesenteric ischemia, such as polyarteritis nodosa, atherosclerotic disease, or thrombosis resulting from anti-phospholipid antibody disease.

Liver test abnormalities have been described in as many as 60% of patients with SLE at some point during the course of their illness, but clinically significant liver disease is rarely a direct manifestation of SLE.[119] For this reason, the presence of liver disease should prompt a search for non-SLE causes, including medications such as nonsteroidal anti-inflammatory drugs, methotrexate, and azathioprine. Abnormal liver enzymes may be caused by hepatic steatosis as a result of obesity, concomitant diabetes mellitus, or treatment with corticosteroids. Infections such as viral hepatitis, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) must also be excluded. Once medications and infections have been ruled out as possible culprits, persistent liver test abnormalities should prompt an investigation with an abdominal ultrasound and possibly a liver biopsy. Lupus hepatitis is believed to be a distinct entity from autoimmune hepatitis.[120] Lupus hepatitis is typically characterized by the presence of lobular inflammation with a paucity of lymphoid infiltrates. These findings contrast with those of autoimmune hepatitis, in which periportal (interface) inflammation and dense lymphoid infiltrates predominate. Although ANA is frequently seen in both disorders, anti–smooth muscle and anti-LKM antibodies are more frequently noted in autoimmune hepatitis than in lupus hepatitis. Rarely, nodular regenerative hyperplasia complicates SLE. This disorder causes diffuse nodularity of the liver with little fibrosis and can result in portal hypertension. Nodular regenerative hyperplasia is also associated with the presence of anti-phospholipid antibodies in some patients.[121] Vascular disorders of the liver such as Budd-Chiari syndrome, hepatic veno-occlusive disease, and hepatic infarction have been described, especially in the setting of anti-phospholipid antibodies.

Other rare gastrointestinal manifestations of SLE include intestinal pseudo-obstruction and protein-losing enteropathy. Intestinal pseudo-obstruction is characterized by decreased intestinal motility caused by dysfunction of the visceral smooth muscle or enteric nervous system.[122] The small bowel is more frequently involved than the large bowel. Presenting symptoms include abdominal pain, nausea, vomiting, and abdominal distention. Patients with protein-losing enteropathy experience abdominal pain, profound pitting edema, and diarrhea in the context of hypoalbuminemia. Other causes of hypoalbuminemia, such as nephrotic syndrome from renal disease, must be excluded.

## Occular Involvement

SLE can affect the eye in a variety of ways. The most common ocular manifestation is keratoconjunctivitis sicca (KCS), which can occur in the presence or absence of secondary Sjögren's syndrome.[123] Retinal abnormalities can be detected on ophthalmoscopic examination as retinal hemorrhages, vasculitic-appearing lesions, cotton wool spots, and hard exudates. SLE retinopathy is believed to be an immune complex–mediated vasculopathy and/or the result of microthrombotic events. The presence of retinal abnormalities has been shown to correlate with lupus nephritis, CNS lupus, and the presence of anti-phospholipid antibodies.[124] Episcleritis and scleritis can occur in SLE. Uveitis is extremely rare. Discoid lupus can involve the lower eyelid and conjunctiva. Glucocorticoids and antimalarial agents, two medications commonly used for the treatment of SLE, can affect the eye. Posterior subcapsular cataracts, elevated

intraocular pressure, and central serous maculopathy are well-described complications of glucocorticoid therapy, and maculopathy is a rare but serious complication of the use of hydroxychloroquine and chloroquine. The risk of retinal toxicity is low if the daily dose of chloroquine is kept below 3 mg/kg of ideal body weight and the daily dose of hydroxychloroquine is kept at or below 6.5 mg/kg of ideal body weight.

## Hematologic Involvement

Hematologic involvement is common in SLE; all three blood cell lines can be affected. When evaluating a patient with the hematologic abnormalities as described later, it is always necessary to consider the potential of myelosuppression from medications such as methotrexate, azathioprine, mycophenolate mofetil, and cyclophosphamide. In addition, glucocorticoids are a common cause of lymphopenia and leukocytosis secondary to neutrophilia.

### Anemia

Anemia of chronic disease (ACD) is the most common anemia in SLE. It is a normochromic, normocytic anemia characterized by the presence of low serum iron, low transferrin, and normal to increased serum ferritin. ACD can coexist with anemias resulting from other processes. Autoimmune hemolytic anemia (AIHA) should be suspected in the setting of the following laboratory abnormalities: increased serum unconjugated bilirubin, increased lactate dehydrogenase (LDH), increased reticulocyte count, and reduced serum haptoglobin. The direct Coombs test is typically positive and usually is mediated by warm-reacting IgG anti-erythrocyte antibodies. The peripheral blood smear demonstrates spherocytosis. Some reports have suggested an association between AIHA and the presence of anticardiolipin antibodies.[125,126] A positive direct Coombs test can occur without hemolysis. AIHA may be the presenting manifestation of SLE, or it may predate full-blown SLE by many years.

Microangiopathic hemolytic anemia (MAHA), characterized by the presence of schistocytes on peripheral blood smear, should prompt consideration of thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP-HUS). TTP is a syndrome consisting of MAHA, thrombocytopenia, fever, neurologic symptoms, and renal involvement, and may be associated with SLE. Because MAHA, thrombocytopenia, neurologic symptoms, and renal involvement can also occur in catastrophic anti-phospholipid antibody syndrome (CAPS), anti-phospholipid antibodies should always be measured as part of the evaluation. Blood loss, renal insufficiency, pure red cell aplasia, and medication-induced myelotoxicity are additional potential causes of anemia in patients with SLE.

### Leukopenia

Leukopenia occurs in approximately 50% of patients with SLE and can occur secondary to lymphopenia and/or neutropenia. One study of 158 newly diagnosed, clinically active patients with SLE demonstrated that 75% of patients had lymphocyte counts lower than 1500 cells/μL, and that lymphopenia eventually developed in 93% of patients.[127] The presence of lymphocytoxic antibodies in some patients with SLE correlates with lymphopenia and with disease exacerbation.[128] Lymphopenia may be a side effect of treatment with glucocorticoids or other immunosuppressive agents. Neutropenia resulting from SLE can result from immune-mediated destruction or marrow suppression.

### Thrombocytopenia

Mild thrombocytopenia is noted in as many as 50% of patients with SLE, but severe thrombocytopenia can also occur. Thrombocytopenia can be the result of immune-mediated platelet destruction similar to immune thrombocytopenic purpura (ITP). The platelet IIb/IIIa antigen is the primary target. Thrombocytopenia can also be caused by a consumptive process such as TTP or splenomegaly. Antithrombopoietin antibodies have been found in the sera of some patients with SLE and have been correlated with lower platelet counts.[129] Chronic, low-level thrombocytopenia is a characteristic feature of the anti-phospholipid antibody syndrome. Similar to AIHA, isolated ITP may predate the development of complete SLE by several years.[130]

## Lymphadenopathy and Splenomegaly

Lymphadenopathy commonly occurs in association with active SLE and is characterized by the presence of enlarged, soft, nontender lymph nodes. Lymphadenopathy can be focal or generalized; the cervical, axillary, and inguinal regions are typically involved. Lymph node histopathology demonstrates reactive hyperplasia and varying degrees of coagulative necrosis. The presence of hematoxylin bodies is specific for SLE. Histologic features of Castleman's disease have been reported.[131] The differential diagnosis of lymphadenopathy in a patient with SLE includes infection and/or a lymphoproliferative process; lymph node biopsy is sometimes required for diagnosis. Splenomegaly can be observed in patients with SLE and may be associated with hepatomegaly. Histopathologic studies demonstrate periarterial fibrosis (onion-skin lesions). Splenic atrophy and functional asplenism have also been reported.[132]

# DIAGNOSIS

Establishing the diagnosis of SLE can be challenging because of its heterogeneous disease manifestations and waxing and waning clinical course. No clinical manifestation or laboratory test can serve as a definitive diagnostic test. Instead, SLE is diagnosed on the basis of a constellation of characteristic symptoms, signs, and laboratory findings in the appropriate clinical context. Although the ACR classification criteria (see Table 80-1) cannot always be relied upon for diagnostic purposes in individual patients, they serve as useful reminders of the wide variety of clinical features that can be seen in SLE.

## Serologic Tests

Serologic tests play an important role in the diagnosis of SLE. SLE is the prototypic systemic humoral autoimmune disease. As such, it is characterized by production of a

**TABLE 80-7** Autoantibodies and Clinical Significance in Systemic Lupus Erythematosus

| Autoantibody | Prevalence in SLE (%) | Clinical Associations |
|---|---|---|
| **Anti-nuclear Antibody** | | |
| Anti-dsDNA | 60 | 95% specificity for SLE; fluctuates with disease activity; associated with glomerulonephritis |
| Anti-Smith | 20-30 | 99% specificity for SLE; associated with anti-U1RNP antibodies |
| Anti-U1RNP | 30 | Antibody associated with mixed connective tissue disease and lower frequency of glomerulonephritis |
| Anti-Ro/SS-A | 30 | Associated with Sjögren's syndrome, photosensitivity, SCLE, neonatal lupus, congenital heart block |
| Anti-La/SS-B | 20 | Associated with Sjögren's syndrome, SCLE, neonatal lupus, congenital heart block, anti-Ro/SS-A |
| Anti-histone | 70 | Also associated with drug-induced lupus |
| Anti-phospholipid | 30 | Associated with arterial and venous thrombosis, pregnancy morbidity |

SCLE, Subacute cutaneous lupus erythematosus; SLE, systemic lupus erythematosus.

wide variety of autoantibodies, which often provide important diagnostic information[133] (Table 80-7). Studies using antigen array technologies have demonstrated the presence of over 100 different autoantibodies in patients with SLE. The hallmark serologic feature is the presence of ANAs, as reflected by a positive ANA test. The gold standard method for detecting ANA is indirect immunofluorescence by using a human epithelial cell tumor line (HEp2 cell line). With this method, the ANA test is highly sensitive; it is positive in more than 95% of people with SLE. Because of a desire for automation and cost savings, some laboratories are utilizing the enzyme-linked immunosorbent assay (ELISA) or multiplex assays as the method of testing for ANA. However, the ELISA and multiplex methods are less accurate than the immunofluorescence method, resulting in a higher false-negative rate. Positive ANA tests also occur in many other autoimmune diseases, including RA, scleroderma, polymyositis, and autoimmune thyroiditis, among others. ANAs are also detectable in low titers (<1 : 80) in many people without autoimmune disease, especially in the elderly.[134] Therefore a positive test is not sufficient to establish the diagnosis of SLE. On the other hand, a negative test can be helpful in ruling out SLE. Although ANA$^-$ SLE has been reported, it is very rare with the immunofluorescence method of testing. In those rare instances, other tests (e.g., anti-Ro/SS-A) usually confirm the presence of lupus-associated autoantibodies.[135]

Once it has been established that ANAs are present, it is important to determine which particular nuclear antigens may be the target of the autoantibodies because some of these antigen-specific responses provide great diagnostic specificity. The most important of these tests is the test for anti-dsDNA antibodies. Anti-dsDNA antibodies are present in no more than 50% to 60% of patients with SLE, so their absence does not exclude the possibility of SLE. However, the presence of these antibodies is highly specific for SLE and therefore can be very helpful in establishing a definitive diagnosis. Similarly, antibodies to the Sm antigen have great specificity for SLE, but these antibodies are present in even fewer patients with SLE (~30%). The Sm antigen is a component of extractable nuclear antigens (ENAs), a term that refers to a heterogeneous mixture of non-DNA nuclear antigens that can be "extracted" from cells in the laboratory. These antigens are primarily ribonucleoproteins that can be divided into two major subsets based on their susceptibility to digestion by ribonuclease. The ribonuclease-sensitive antigens are designated RNP. Ribonuclease-resistant antigens are designated Sm (because these antibodies were first detected in a patient named Smith). Unlike anti-Sm, anti-RNP is not specific for SLE. However, high titers of anti-RNP antibodies can be helpful in supporting the diagnosis of MCTD.[136]

Numerous other autoantibodies can be found in patients with SLE. Antibodies to cytoplasmic antigens, such as Ro and La (SS-A and SS-B), can be found in some patients with SLE. Although these autoantibodies lack both sensitivity and specificity for SLE, they sometimes are associated with distinct clinical syndromes. The best example of such a relationship involves the presence of anti-Ro antibodies in more than 90% of cases of neonatal lupus.[137,138] Anti-Ro antibodies are also seen with increased frequency in patients with SCLE.[139] Other autoantibodies may be directed against cell surface molecules or against circulating proteins. For example, antibodies to blood components can be responsible for hemolytic anemia, neutropenia, or thrombocytopenia, and antibodies to phospholipids and phospholipid binding proteins can be detected in some patients with SLE with or without anti-phospholipid syndrome (see Chapter 82). It should be noted that rheumatoid factor (anti-IgG) can be found in 15% to 20% of people with SLE, whether or not joint disease is present.[140] ACPA can also be present.

Complement consumption arising from immune complex disease may lead to hypocomplementemia in patients with SLE.[141,142] Because hypocomplementemia is rare in other diseases, its presence in a patient with SLE can provide valuable supportive evidence for the diagnosis. Moreover, because hypocomplementemia most likely reflects complement activation by immune complexes, its presence is often a sign of active disease. However, hereditary complement deficiencies may be found in patients with SLE (C1q, C2, C4), so absence of a particular complement component does not always reflect consumption.[143–145] For this reason, it is often necessary to measure more than one complement component (e.g., C3 and C4) before concluding that hypocomplementemia is to the result of active disease.

The utility of serologic tests in assessing disease activity and predicting disease flares remains a topic of controversy. In the absence of a hereditary complement deficiency, hypocomplementemia is a reliable indicator that the disease is active, but normal complement levels do not rule out active

disease. Titers of anti-dsDNA antibodies correlate with disease activity in some patients, but not in others. One recent study attempted to resolve the long-standing debate about the prognostic value of changes in lupus serology.[146] In this study, patients with clinically quiescent lupus underwent monthly monitoring for levels of anti-dsDNA, C3a, C3, C4, and CH50 to identify patients with serologically active, clinically quiescent disease. These patients were then randomly assigned to treatment with corticosteroids or placebo to determine whether treatment of serologically active disease could prevent impending clinical flares. The results were equivocal. Some patients with serologically active disease flared, and some flares were apparently prevented. However, in most control subjects, serologic deterioration was not followed by a clinical flare, and most of the flares that occurred in the original patient population that had been monitored were not preceded by serologic deterioration. Thus there remains no substitute for knowledge of a particular patient's disease pattern or whether there is an association between clinical and serologic manifestations in that patient.

## DIFFERENTIAL DIAGNOSIS

Because of the involvement of multiple organ systems and the lack of specificity of symptoms and/or signs, many systemic diseases can mimic SLE. Thus, before a diagnosis of SLE is established, a comprehensive search for infectious, malignant, and other autoimmune diseases must be undertaken.

Several viral infections can produce symptoms and signs that are present in SLE. In addition, many viral illnesses are associated with the production of autoantibodies. A careful patient history with serologic testing for the potential pathogen should help to secure the correct diagnosis. Patients with classic parvovirus B19 are seen with fever, rash, symmetric inflammatory polyarthritis, and cytopenias. Furthermore, the presence of ANA, anti-dsDNA, and hypocomplementemia has been observed in a few cases. Cytomegalovirus and EBV can mimic SLE in that patients often are seen with fatigue, cytopenias, abdominal pain, and liver test abnormalities. Patients with acute HIV infection typically are seen with fever, diffuse lymphadenopathy, and oral ulcers. Inflammatory arthritis and positive autoantibodies can develop in patients with hepatitis B and C.

Malignancy, particularly non-Hodgkin's lymphoma, can manifest with constitutional symptoms, joint pain, cytopenias (including autoimmune hemolytic anemia), lymphadenopathy, rash, and a positive ANA. Physicians must be particularly alert to the possibility of malignancy in older patients seen with new lupus-like syndromes. It is important to ensure that patients undergo appropriate malignancy screening tests.

Other autoimmune diseases such as RA, dermatomyositis, and Still's disease often share similar clinical features with SLE. Differentiating between these disorders might be difficult in early phases of the disease. Patients with RA and SLE may develop a symmetric inflammatory arthritis with a predilection for the wrists and small joints of the hands. ANA and rheumatoid factor (RF) may be elevated in both disorders, although ACPA suggests RA, and anti-dsDNA or anti-Sm suggests SLE. The photosensitive, erythematous rashes of dermatomyositis and SLE can appear clinically and histopathologically identical. A careful patient history and supporting serologies will aid in making the correct diagnosis. Physicians must also consider MCTD when evaluating a patient for possible SLE. MCTD is a syndrome characterized by a high-titer anti-RNP antibody in conjunction with clinical features that are often present in SLE, scleroderma, and/or polymyositis. Patients frequently are seen with puffy, swollen hands and Raynaud's phenomenon. In contrast to SLE, an erosive arthritis that looks very similar to RA can develop in patients with MCTD.

Careful evaluation for drug-induced lupus should be undertaken in every new patient in whom the diagnosis of SLE is suspected. This is especially important in an older person who is seen with a lupus-like syndrome. Arthralgia, myalgia, fever, and serositis are common manifestations. A wide variety of drugs have been implicated in the development of drug-induced lupus; minocycline, procainamide, hydralazine, isoniazid, IFN-α, and TNF inhibitors are well-known culprits. Hydrochlorothiazide is associated with SCLE. All of these drugs may cause a positive ANA. Minocycline is occasionally associated with anti-dsDNA antibodies and perinuclear-staining anti-neutrophil cytoplasmic antibodies (P-ANCA), and TNF inhibitors can cause positive anti-dsDNA antibodies. Anti-histone antibodies are present in more than 95% of cases of drug-induced lupus, with the exception of those cases caused by minocycline. However, anti-histone antibodies cannot be used to confirm a diagnosis of drug-induced lupus because up to 80% of idiopathic patients with SLE will also produce anti-histone antibodies.

## NEONATAL LUPUS

Neonatal lupus is a passively acquired autoimmune disease of neonates that results from transplacental passage of maternal anti-SS-A and/or anti-SS-B antibodies.[132] It can occur in mothers with SLE, in those with Sjögren's syndrome, or in patients in whom an autoimmune disease has not been diagnosed. Neonatal lupus can involve multiple organ systems, including heart, skin, liver, and the hematologic system; the most severe complications are congenital complete heart block and cardiomyopathy.[147,148] The term *neonatal lupus* stems from early observations that the skin lesions in affected newborns were similar to the lesions of SCLE.

Congenital complete heart block is associated with a neonatal mortality rate as high as 20%, and most patients will eventually require a permanent pacemaker. This complication occurs in as many as 2% of babies born to mothers who are positive for anti-Ro/SS-A and/or anti-LA/SS-B antibodies. Thus maternal antibodies are essential but not sufficient to cause disease. Once a woman has given birth to a baby with complete heart block, the risk for recurrence in a subsequent pregnancy is approximately 17%. Once a woman has given birth to a baby with cutaneous features of neonatal lupus (without heart block), the risk for heart block in a subsequent pregnancy is 18%.[149] Evidence from in vitro studies suggests that during fetal development, fetal cardiocytes undergo apoptosis that results in expression of Ro/SS-A and La/SS-B on the cell surface. Binding of anti-Ro/SS-A and/or anti-La/SS-B to fetal cardiocytes leads

to inflammatory injury and subsequently to fibrosis of the atrioventricular (AV) node and surrounding tissue. The sinoatrial (SA) node may also be involved. Prospective studies have shown that the vulnerable period for the fetal heart is between 16 and 24 weeks of gestation. Thus it is recommended that mothers with anti-Ro/SS-A and/or anti-La/SS-B antibodies undergo monitoring with fetal echocardiography beginning at 16 weeks of gestation. The hope has been that detection of early stages of heart block (first-degree and second-degree block) might allow for treatment that would prevent progression to third-degree heart block. Currently, the treatment of choice is maternal administration of a fluorinated glucocorticoid such as dexamethasone. Fluorinated glucocorticoids are preferred because of their ability to cross the placenta and enter the fetal circulation. However, treatment of incomplete fetal heart block remains controversial because the benefits have not been clearly delineated, and glucocorticoids have been associated with several fetal side effects such as intrauterine growth retardation, oligohydramnios, and adrenal suppression. Complete heart block is irreversible even with treatment, and first- or second-degree heart block may or may not reverse with treatment. Complicating matters, complete heart block can occur in the absence of preceding first- or second-degree block. In addition to conduction blocks, structural cardiac abnormalities have been observed in the setting of neonatal lupus, including, but not limited to, patent ductus arteriosus, ventricular septal defect, atrial septal defect, and patent foramen ovale. Myocarditis and pericarditis have also been described.

Rash, a common manifestation of neonatal lupus, consists of erythematous, annular lesions that resemble the annular subtype of SCLE. The rash typically occurs on the scalp, face, trunk, and extremities with a predilection for the periorbital area; it often develops after exposure of the newborn to ultraviolet light. Lesions typically occur within the first 4 to 6 weeks of life but may be present at birth. The rash is self-limiting and does not necessitate treatment. Lesions tend to resolve by 6 months of age, at which time maternal anti-Ro/SS-A and/or anti-La/SS-B antibodies are no longer present in the baby's circulation. Lesions develop in approximately 25% of babies born to anti-SSA/SS-B positive mothers. Less common manifestations of neonatal lupus include hepatic, hematologic, and neurologic involvement.[150] Hepatic manifestations include asymptomatic elevation of liver function tests, hepatitis, hepatomegaly, cholestasis, and cirrhosis. Hematologic manifestations include thrombocytopenia, autoimmune hemolytic anemia, and leukopenia. Neurologic complications, including myelopathy, seizures, and aseptic meningitis, have been reported.


Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 81



# Treatment of Systemic Lupus Erythematosus

George Bertsias • Antonis Fanouriakis • Dimitrios T. Boumpas

**KEY POINTS**

- The treatment goal in systemic lupus erythematosus (SLE) is remission with a decrease in organ damage.
- Drug toxicity, especially from glucocorticoids, should be carefully considered and minimized as much as possible.
- Hydroxychloroquine (HCQ) maintenance is recommended for all patients with SLE who do not have contraindications to the use of this agent.
- SLE with severe, major organ involvement is usually managed with high-dose glucocorticoids and pulses of intravenous cyclophosphamide (CYC).
- In moderately severe lupus nephritis (LN), mycophenolate mofetil (MMF) is effective and has a better toxicity profile than CYC.
- Either azathioprine (AZA) or MMF is recommended for maintenance of renal response in moderate to severe LN.
- Belimumab (anti-Blys mAb) may be useful when standard therapies have failed Rituximab (anti-CD20 mAb) has been used by some clinicians in refractory cases.
- Family counseling and planning are important elements for the successful completion of pregnancies in women with SLE.

## GENERAL TREATMENT STRATEGY

**KEY POINTS**

- Treatment of moderate or severe systemic lupus erythematosus (SLE) requires induction immunosuppressive therapy followed by less intense maintenance therapy.
- Patients with SLE experience poor quality of life.
- Patients favor an active and collaborative role in decision making.

Systemic lupus erythematosus (SLE) activity usually follows the flare pattern, which is characterized by a relapsing-remitting course. However, an equal number of patients have continuously active disease, and only a few have long periods of disease quiescence.[1,2] Despite improvements in overall survival (85% to 90% during the first 10 years), some patients with SLE are still at risk for premature death.[3] Persistent inflammation inevitably results in irreversible major organ damage, which is linked to decreased quality of life and increased mortality.[4,5] Accordingly, therapeutic strategies should aim at reducing overall burden of systemic inflammation. Achieving these goals requires (1) accurate assessment of disease activity and flares, (2) stratification of patients according to severity of target organ involvement, (3) use of safe and effective drugs to induce remission promptly and prevent flares, and (4) prevention and management of disease and treatment-related comorbidities.[6]

In general, patients with mild lupus manifestations (skin, joint, and mucosal involvement) are treated with anti-malarials or disease-modifying anti-rheumatic drugs (DMARDs), alone or in combination with low-dose oral glucocorticoids (GCs). Severe SLE with major organ involvement requires an initial period of intensive immunosuppressive therapy (induction therapy) to control aberrant immunologic activity and halt tissue injury, followed by a longer period of less intensive and less toxic maintenance therapy, to consolidate remission and prevent flares. Immunosuppressive therapy helps reduce the use of GC, thus reducing their deleterious effects.

Patients with SLE experience poor quality of life, which is only in part associated with disease activity and organ damage. Important contributors include fatigue, fibromyalgia, depression, and cognitive dysfunction.[7,8] Treating physicians should regularly address these issues and engage symptomatic or remedial therapies as indicated. The realization that a significant proportion of patients have disease- and treatment-related comorbidities has shifted the attention of physicians to adjunct therapies and primary prevention strategies such as renoprotective and cardiovascular disease risk reduction measures.

Health professionals are increasingly encouraged to involve patients in treatment decisions, recognizing them as experts with a unique knowledge of their own health and their preferences for treatments, health states, and outcomes.[9] This approach may be particularly challenging in patients with severe lupus, who might benefit from cytotoxic therapy which is associated with significant toxicity.[10] Therefore, physicians should provide patients with balanced information to ensure accurate perception of the risks and benefits of prescribed medications.[11]

## DRUGS USED TO TREAT SYSTEMIC LUPUS ERYTHEMATOSUS

**KEY POINTS**

- The prescribed doses of GCs for long-term use should be as low as possible with the goal of no more than the prednisone equivalent of 7.5 mg/day.

**Anti-malarials are recommended for all patients with SLE because of their effectiveness in achieving remission in severe lupus and in reducing the risk of flare-ups and of damage accrual.**

**AZA is effective in mild to moderate SLE, including LN.**

**High-dose GCs with pulses of intravenous CYC are recommended for severe SLE with major organ involvement.**

**MMF is equally effective and has a better toxicity profile than CYC in moderately severe proliferative LN (PLN).**

**Calcineurin inhibitors can be used in refractory LN.**

**Belimumab is approved for active patients with SLE who are receiving standard therapy.**

**Rituximab has been used by some clinicians in cases refractory to immunosuppressive treatment.**

**Novel biologic therapies are currently being tested in SLE.**

## Glucocorticoids

GCs exert broad inhibitory effects on immune responses mediated by T and B cells, as well as on the effector functions of monocytes and neutrophils. On the basis of these effects and their rapid onset of action, GCs have been remarkably efficacious in managing acute SLE manifestations.

Low-dose GC therapy (≤7.5 mg prednisone equivalent per day) is generally used when other initial therapies (anti-malarials) are not tolerated or are inadequate to control disease activity. In moderate to severe disease, GCs are used as either single or background therapy in combination with immunosuppressive agents, at doses of 0.5 to 1 mg/kg prednisone equivalent in a single dose, usually in the morning. When combined with immunosuppressive agents, the GC dose should rarely exceed 0.5 to 0.6 mg/kg prednisone due to concerns for infections and other toxicities. Tapering of GC dose starts after the first 4 to 6 weeks of therapy, targeting a dose of 0.125 to 0.25 mg/kg given daily or every other day at 2 to 3 months. Concomitant use of immunosuppressive agents facilitates tapering and decreases cumulative GC toxicity.

Although two controlled studies[12,13] have suggested that short-term GC therapy (30 mg for 2 weeks, then quick tapering) reduces the risk of severe flare in patients with clinically stable but serologically active SLE, this practice may result in overtreating patients with higher cumulative GC doses. For these patients, vigilant monitoring for objective signs of disease exacerbation is recommended.

In severe, rapidly progressing disease or when doses greater than 0.6 mg/kg/day prednisone equivalent are required to control disease activity, GC pulse therapy may be introduced.[14,15] Intravenous (IV) pulses of methylprednisolone (MP) (500 to 1000 mg daily for 1 to 3 consecutive days) are used, although there is no strong evidence to support a survival advantage. In addition to expediting remission, IV MP pulses may allow for the use of lower GC doses during the induction period.

GC toxicity may involve early (mood effects, acne, myalgias, infections), later (metabolic), and late-onset (osteoporosis, avascular bone necrosis, cataract, cardiovascular disease) adverse events.[16] Although confounded by increased systemic disease activity, most effects are considered to be dependent on the cumulative dose and duration and, to a lesser extent, route of administration. Chronic exposure to GC doses higher than 6 to 7.5 mg/day of prednisone equivalent is clearly associated with an increased risk for irreversible organ damage.[17,18] Tailoring GC use on the basis of individual patient profile (disease activity, risk for toxicity) and prompt initiation of primary prevention measures (osteoporotic prophylaxis) is of paramount importance.

## Anti-malarials and DMARD Therapy

### Hydroxychloroquine

Anti-malarial drugs, mainly chloroquine and hydroxychloroquine (HCQ), are commonly prescribed to patients with SLE with skin and joint manifestations but are increasingly identified as an adjuvant treatment for achieving remission in severe lupus. Use of anti-malarials resulted in a greater than 50% reduction in SLE disease activity of all types and to a moderate reduction in severe flares and GC dose.[19] In a randomized controlled trial (RCT) in stable active patients with SLE on HCQ treatment, those who achieved blood HCQ levels greater than or equal to 1000 ng/mL had a tendency for reduced SLE flares (20.5% vs. 35.1%) during a 7-month period.[20] Beneficial effects on the lipid profile and subclinical atherosclerosis markers have also been described.[21] Importantly, prospective observational studies have reported an inverse association between use of HCQ and accrual of irreversible organ damage.[4,19] Although these findings may be confounded by milder forms of disease usually present in patients with SLE who are prescribed anti-malarials, the presumptive mode of the action of anti-malarials by inhibition of innate immunity pathways provides a plausible explanation for their multifold beneficial effects.[22] Furthermore, the use of HCQ has been associated with a decreased incidence of diabetes[23] and improved outcomes in patients with anti-phospholipid antibodies/anti-phospholipid syndrome (APS).[24,25] HCQ is well tolerated, with low rates of mild gastrointestinal and skin adverse events. Retinal toxicity is uncommon (estimated at 0.1% in patients who received HCQ for more than 10 years[19]), but routine ophthalmologic evaluation is recommended (annual screening during the first 5 years of usage is recommended for individuals who are treated with high HCQ doses (>6.5 mg/kg), for those treated more than 5 years, or for those who have other complicating factors).[26]

### Methotrexate

Methotrexate (MTX) has been used as a steroid-sparing treatment in mild to moderate SLE, particularly for articular and cutaneous manifestations of SLE.[27,28] MTX is administered once weekly either orally or parenterally. A systematic literature review reported significant reduction of the SLE Disease Activity Index (SLEDAI) among MTX-treated patients when compared with controls (odds ratio [OR], 0.44), followed by significant reduction in the average dose of corticosteroids (OR, 0.34).[29] Concomitant administration of folic acid (1 to 2 mg/day, is recommended to minimize toxicity (Table 81-1; see also Chapter 61).

**TABLE 81-1** Recommended Therapeutic Drug Monitoring in Systemic Lupus Erythematosus

| Drug | Dosage | Dose Adjustment | Toxicities Requiring Monitoring | Baseline Evaluation | Laboratory Monitoring |
|---|---|---|---|---|---|
| Azathioprine | 50-200 mg/day in 1-3 doses with food | ↓ 25% if eGFR 10-30 mL/min; ↓ 50% if eGFR < 10 mL/min | Myelosuppression, hepatotoxicity, lymphoproliferative diseases | CBC, platelets, Cr, AST or ALT | CBC and platelets every 2 wk, with changes in dosage; baseline tests every 1-3 mo |
| Mycophenolate mofetil | 1-3 g/day in 2 divided doses with food | Maximum 1 g/day if eGFR < 25 mL/min | Myelosuppression, hematotoxicity, infection | CBC, platelet, Cr, AST or ALT | CBCs and platelets every 1-2 wk with changes in dosage; baseline tests every 1-3 mo |
| Cyclophosphamide | 50-150 mg/day in a single dose with breakfast. Increase fluid intake (at least 3 L water/day), empty bladder before bedtime* | ↓ 25% if eGFR 25-50 mL/min; ↓ 30%-50% if eGFR < 25 mL/min; ↓ 25% if serum Bil 3.1-5 mg/dL or transaminases >3 times ULN | Myelosuppression, hemorrhagic cystitis, myeloproliferative disease, malignancies | CBC, platelet, Cr, AST or ALT, urinalysis | CBC with differential every 1-2 wk, with changes in dosage and then every 1-3 mo; keep WBC > 4000/mm$^3$ with dose adjustment; urinalysis, AST or ALT every 3 mo; urinalysis every 6-12 mo following cessation |
| Methotrexate | 7.5-25 mg/wk in 1-3 doses with food or milk/water | ↓ 50% if eGFR 10-50 mL/min; avoid use if eGFR < 10 mL/min; avoid use in hepatic dysfunction (serum Bil 3.1-5 mg/dL or transaminases >3 times ULN) | Myelosuppression, hepatic fibrosis, pneumonitis | CXR, hepatitis B/C serology in high-risk patients, AST or ALT, Alb, ALP, Cr | CBC with platelet, AST, Alb, Cr every 1-3 mo |
| Cyclosporin A | 100-400 mg/day in 2 doses at the same time every day with meal or between meals | Avoid in impaired renal function | Renal insufficiency, anemia, hypertension | CBC, Cr, uric acid, AST or ALT, Alb, ALP, blood pressure | Cr every 2 wk until dose is stable, then monthly; CBC, potassium, AST or ALT, Alb, and ALP every 1-3 mo; drug levels only with doses >3 mg/kg/day |
| Tacrolimus | 1-4 mg/day in 2 doses at the same time every day | Cautious use in liver or renal insufficiency | Renal insufficiency, neurotoxicity, malignancy, infections, hyperkalemia | Cr, potassium, AST or ALT, glucose, blood pressure | Baseline tests once a week for the first 3-4 wk, then every 1-3 mo; monitor drug trough levels |
| Leflunomide | 100 mg/day in a single dose for 3 days, then 10-20 mg/day | Avoid in hepatic dysfunction (serum Bil 3.1-5 mg/dL or transaminases >3 times ULN) | Myelosuppression, hepatotoxicity, fetal toxicity | CBC, Cr, AST or ALT, Alb, ALP | CBC, AST or ALT, Alb, and ALP monthly for 6 mo, then every 1-3 mo; monthly monitoring if MTX coadministered |
| Rituximab† | 1000 mg on day 1 and 15 | None | HBV reactivation (rare) | CBC, Cr, AST or ALT, HBV serology (high-risk patients), TST | CBC and platelets |

*See Table 81-3 for IV dosing for Cyclophosphamide.

†Note that placebo controlled studies have failed to demonstrate efficacy in controlled clinical trials.

Alb, Serum albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; Bil, bilirubin; CBC, complete blood cell count; Cr, serum creatinine; CXR, chest x-ray; eGFR, estimated glomerular filtration rate; HBV, hepatitis B; LFTs, liver function tests; MTX, methotrexate; TST, tuberculin skin testing; ULN, upper limit of normal; WBC, white blood cell count.

### Leflunomide

Leflunomide has been used in patients with SLE with mild to moderate disease activity, as well as in lupus nephritis (LN) refractory or intolerant to standard immunosuppressive therapy.[30,31] The drug has an elimination half-life of 14 to 18 days and is contraindicated in patients who are trying to become or are pregnant because of its teratogenicity (see also Chapter 61). As a result of this long half-life, if a patient has ever taken leflunomide, she should have leflunomide levels checked before conception.

## Cytotoxic Therapy

### Cyclophosphamide

**Pharmacology and Route of Administration.** Cyclophosphamide (CYC) is an alkylating agent that depletes lymphocytes and reduces the production of autoantibodies in lupus (Table 81-2). Both oral and IV administration of CYC result in similar plasma concentrations, and the serum half-life is approximately 6 hours. CYC is metabolized to various active metabolites by cytochrome P450 in the liver or other

**TABLE 81-2** Indications for Immunosuppressive Therapy in Systemic Lupus Erythematosus

| |
|---|
| **General Indications** |
| Involvement of major organs or extensive involvement of nonmajor organs (skin) refractory to other agents, or both |
| Failure to respond to or inability to taper corticosteroids to acceptable doses for long-term use |
| **Specific Organ Involvement** |
| **Renal** |
| Proliferative or membranous nephritis (nephritic or nephritic syndrome), or both |
| **Hematologic** |
| Severe thrombocytopenia (platelets <20,000/mm$^3$) |
| Thrombotic thrombocytopenic purpura-like syndrome |
| Severe hemolytic or aplastic anemia, or immune neutropenia not responding to glucocorticoids |
| **Pulmonary** |
| Lupus pneumonitis or alveolar hemorrhage, or both |
| **Cardiac** |
| Myocarditis with depressed left ventricular function, pericarditis with impending tamponade |
| **Gastrointestinal** |
| Abdominal vasculitis |
| **Nervous System** |
| Transverse myelitis, cerebritis, optic neuritis, psychosis refractory to corticosteroids, mononeuritis multiplex, severe peripheral neuropathy |

tissues. Drugs that induce hepatic microsomal enzymes (barbiturates, alcohol, phenytoin, rifampin) may accelerate the metabolism of CYC into its active metabolites and thus increase its pharmacologic and toxic effects. Conversely, drugs that inhibit the hepatic microsomal enzymes (antimalarials, tricyclic anti-depressants, and allopurinol) may slow the conversion of CYC to active metabolites. Approximately 20% of the drug is excreted by the kidneys, and dose modification is necessary for patients with renal impairment.

The National Institutes of Health (NIH) trials demonstrated equivalent efficacy yet lower toxicity with monthly IV (0.5 to 1 g/m$^2$) versus oral regimens, which led to the predominant use of IV CYC in clinical practice (Table 81-3). Reversible myelotoxicity is common and dose related. After pulse therapy, the nadir of the patient's lymphocyte count occurs on days 7 to 10 and that of granulocyte count on days 10 to 14. The dose should be adjusted to keep the patient's white blood cell count higher than 3000 cells/mm$^3$. A prompt recovery from granulocytopenia usually occurs after 21 to 28 days. Thrombocytopenia is rare in CYC monotherapy. Reversible alopecia and nausea are common, whereas infections (especially herpes zoster), gonadal toxicity, and malignancy (including bladder toxicity and carcinoma) are less frequent although much more serious adverse

**TABLE 81-3** National Institutes of Health Protocol for Administration and Monitoring of Pulse Intravenous Cyclophosphamide Therapy

| |
|---|
| Estimate GFR by standard methods (Cockcroft-Gault or the Modification of Diet in Renal Disease formula) |
| Calculate body surface area (m$^2$): BSA = ✓ Height (cm) × Weight (kg)/3600 |
| Cyclophosphamide (Cytoxan) (CYC) dosing and administration:<br>Initial dose 0.75 g/m$^2$ (0.5 g/m$^2$ if GFR less than one-third of expected normal)<br>Administer CYC in 150 mL normal saline IV during 30-60 min (alternative: equivalent dose of pulse CYC may be taken orally in highly motivated and compliant patients)<br>Determine WBC at days 10 and 14 after each CYC pulse (patient should delay prednisone until after blood tests drawn to avoid transient steroid-induced leukocytosis)<br>Adjust subsequent doses of CYC to maximum dose of 1 g/m$^2$ to keep nadir WBC >1500/mm$^3$. If WBC nadir falls <1500/mm$^3$, decrease next dose by 25% |
| Repeat IV CYC pulses monthly (every 3 wk in extremely aggressive disease) for another 6 mo (total 7 pulses), then quarterly for 1 year after remission is achieved (inactive urine sediment, proteinuria <1 g/day, normalization of complement [and ideally anti-dsDNA], and minimal or no extrarenal lupus activity) |
| Protection of urine bladder against CYC-induced hemorrhagic cystitis<br>Diuresis with 5% dextrose and 0.45% saline (2 L at 250 mL/hr). Frequent voiding; continue high-dose oral fluids for 24 hr. Patients return to clinic if they cannot sustain an adequate fluid intake<br>Consider mesna (each dose 20% of total CYC dose) intravenously or orally at 0, 2, 4, and 6 hr after CYC dosing. Mesna is especially important to use when sustained diuresis may be difficult to achieve or if pulse CYC is administered in an outpatient setting<br>If anticipating difficulty with sustaining diuresis (e.g., severe nephrotic syndrome) or with voiding (e.g., neurogenic bladder), insert a 3-way urinary catheter with continuous bladder flushing with standard antibiotic irrigating solution (e.g., 3 L) or normal saline for 24 hr to minimize risk of hemorrhagic cystitis |
| Antiemetics (usually administered orally)<br>Dexamethasone (10 mg single dose) *plus*<br>Serotonin receptor antagonists: granisetron 1 mg with CYC dose (usually repeat dose in 12 hr); ondansetron 8 mg three times daily for 1-2 days |
| Monitor fluid balance during hydration. Use diuresis if patient develops progressive fluid accumulation |
| Complications of pulse CYC<br>Expected: nausea and vomiting (central effect of CYC) mostly controlled by serotonin receptor antagonists; transient hair thinning (rarely severe at CYC doses ≤1 g/m$^2$)<br>Common: significant infection diathesis only if leukopenia not carefully controlled; modest increase in herpes zoster (very low risk of dissemination); infertility (male and female); amenorrhea proportional to age of the patient during treatment and to the cumulative dose of CYC. In females at high risk for persistent amenorrhea, consider using leuprolide, 3.75 mg, subcutaneously 2 wk before each dose of CYC |

CYC, Cyclophosphamide; GFR, glomerular filtration rate; IV, intravenous; WBC, white blood cell count.

events (see Comorbidities in Systemic Lupus Erythematosus and Women's Health Issues later in the text).

**Use in Lupus Nephritis.** RCTs with long-term follow-up have shown that intermittent pulse IV CYC therapy is effective for moderate to severe proliferative lupus nephritis (PLN).[32, 33] CYC may retard progressive renal scarring, preserve renal function, and reduce the risk for the development of end-stage renal disease (ESRD).[34] Following induction therapy, a maintenance regimen is necessary to decrease the risk of flares.[35] A combination of pulse therapy with IV CYC and IV MP improves renal outcomes without increasing toxicity.[36,37] The authors propose that severe PLN (with acute reduction in glomerular filtration rate [GFR] and/or adverse renal biopsy findings) should be treated with 7 monthly pulses of IV CYC (0.5 to 1 g/m$^2$), followed by quarterly pulses for at least 1 year after remission. In most severe cases, monthly pulses of IV MP may be given during the induction period.

In the Euro-Lupus Nephritis Trial that studied white patients with milder forms of LN, low-dose IV CYC (6 fortnightly pulses at a fixed dose of 500 mg each in combination with three daily doses of 750 mg of IV MP), followed by azathioprine (AZA) for maintenance had comparable efficacy and less toxicity than high-dose IV CYC (8 pulses).[38] Mean patient survival rate at 10 years was 92% for both groups; ESRD and doubling of Cr rates did not differ between the two groups.[33] Therefore, low-dose IV CYC may be an alternative option for white patients with moderately severe LN. The regimen has also been used in nonwhite patients but further studies will be required to determine its efficacy.[39]

Austin and colleagues[40] compared cyclosporin A (CsA), IV CYC (0.5 to 1 g/m$^2$ × 6 bimonthly doses), and GC alone in 42 patients with lupus membranous nephropathy (LMN). All patients received alternate-day oral prednisone (1 mg/kg every other day for 8 weeks, then tapered). At 1 year, the cumulative probability of remission was 27% with prednisone, 60% with IV CYC, and 83% with CsA. Rates of nephrotic syndrome relapse were 10 times higher in patients treated with CsA versus those treated with IV CYC. Thus, although IV CYC and CsA are equally effective as induction therapy in LMN, patients treated with CsA may require maintenance therapy (with lower doses of CsA, or AZA, or MMF) to prevent relapses.

**Use in Extrarenal Disease.** CYC has demonstrated efficacy in life-threatening or refractory extrarenal lupus manifestations such as severe thrombocytopenia (platelet count <20,000/mm$^3$), neurologic disease, abdominal vasculitis, acute pneumonitis/alveolar hemorrhage, and extensive skin disease.[41,42] Barile-Fabris and colleagues[43] have reported the superiority of IV CYC versus IV MP for severe nonthrombotic neurologic SLE (seizures, peripheral neuropathy, optic neuritis, transverse myelitis, brain stem disease, coma, internuclear ophthalmoplegia).

## Anti-metabolites

### Azathioprine

AZA inhibits the conversion of purine bases such as inosine to adenine and guanine ribonucleotides. AZA (2 to 2.5 mg/kg/day) is safe in the long term with no significant increase in the risk for infection, whereas it is associated with a marginally increased risk for malignancy. Gastrointestinal complaints are frequent and cause 15% to 30% of patients to discontinue the drug within 6 months. Mild liver enzyme elevation may occur, but severe liver injury is rare. Reversible, dose-related bone marrow toxicity is also common; leukopenia is encountered in 4.5% and thrombocytopenia in 2% (see Table 81-1) of patients, respectively. AZA toxicity has been associated with genetic polymorphisms resulting in decreased thiopurine methyltransferase (TPMT) activity and impaired ability to detoxify intermediate metabolites.[44] Genetic testing is available for *TPMT* variant alleles so that the starting dosages of AZA can be adjusted accordingly (see also Chapter 62). Concomitant use of allopurinol, which inhibits xanthine oxidase (the enzyme that metabolizes AZA) substantially increases toxicity and should be avoided.

Patients with manifestations such as mild PLN, thrombocytopenia with platelet count in the range of 20 to 50 × 10$^3$/mm$^3$, and serositis may respond to AZA, usually in combination with moderate to high GC doses (see Table 81-2). In a controlled study, European patients with PLN were randomly assigned to receive pulse IV CYC plus prednisone versus IV MP plus AZA plus a tapering dose of prednisone.[27] Both groups received maintenance therapy with AZA plus prednisone after the second year. After a median follow-up of 9.6 years, there were no significant differences between the two groups in rates of sustained doubling of Cr, ESRD, or mortality.[45] However, renal relapses occurred more often in the AZA group (hazard risk [HR], 4.5). Repeat renal biopsies showed that progression of chronic lesions was more effectively halted by CYC than by AZA.[46] Thus AZA should be used only in mild LN or in patients strongly opposed to or who cannot tolerate CYC or MMF. AZA has also been used in combination with GC in pure LMN, although approximately one out of three patients may experience relapse during follow-up.[47]

AZA is efficacious for maintenance of response in SLE.[33,48,49] Two RCTs have compared AZA versus MMF as maintenance regimens in PLN. In the MAINTAIN trial, which included European patients who received induction with low-dose IV CYC, both agents were equally efficacious in terms of time-to-renal flare, severe flares, renal remission, and doubling of Cr.[50] Repeat kidney biopsies failed to reveal histologic differences between the two groups.[51] In contrast, the Aspreva Lupus Management Study (ALMS), which included a larger number of patients with multiethnic backgrounds who were induced with either IV CYC or MMF, reported increased renal flares in the AZA versus the MMF group (see mycophenolate mofetil in the next section).[52] To this end, both agents can be used as maintenance therapy[53] on the basis of availability, clinical experience, and potential for pregnancy, because MMF is associated with an increased risk of spontaneous abortion and fetal malformation.

### Mycophenolate Mofetil

**Pharmacology.** Mycophenolate is a prodrug of mycophenolic acid (MPA), a potent inhibitor of inosine monophosphate dehydrogenase that is indispensable for the de novo synthesis of guanosine nucleotides. It is orally administered

either as mycophenolate mofetil (MMF) or as enteric-coated mycophenolate sodium (eMPA). A 720 mg dose of eMPA is roughly equivalent to a 1 g dose of MMF. The lack of any salvage nucleotide synthesis pathway in lymphocytes renders them a selective target for MMF. MMF has excellent oral bioavailability, with peak levels occurring within 1 to 2 hours after administration, and a half-life of 17 hours. Although therapeutic drug monitoring to guide MMF dosing has been proposed in renal transplantation, validation of therapeutic MPA monitoring in LN is still required.[54] Antacids and cholestyramine decrease the bioavailability of MMF, and coadministration with AZA should be avoided. MMF is teratogenic and should not be administered during pregnancy.

**Use in Lupus Nephritis.**

Induction Therapy. Several RCTs have demonstrated equal or even superior efficacy of MMF versus CYC in inducing renal response in PLN. The ALMS, one of the largest and most racially diverse RCT in LN, failed to demonstrate the superiority of MMF (2 to 3 g/day) versus monthly pulses of IV CYC (0.5 to 1 g/m$^2$), both combined with oral prednisone (maximum starting dose, 60 mg/day).[55] At 6 months, response rates were 56% for patients treated with MMF and 53% for patients treated with IV CYC. Post-hoc analysis suggested superiority of MMF in black and Hispanic patients.[56] Meta-analyses of the previously mentioned and other RCTs have concluded that MMF is as effective as CYC for induction treatment of PLN and is associated with lower risks of leukopenia, amenorrhea, and alopecia.[57,58] MMF may therefore be considered as first-line treatment in moderately severe PLN, especially when gonadal toxicity is an issue.

Preliminary evidence suggests that MMF is efficacious in severe forms of PLN, such as with impaired renal function or with crescents in renal glomeruli.[59,60] However, further studies with long-term follow-up are needed to determine whether MMF is equally effective as CYC in averting ESRD in LN.[61]

A pooled analysis of 84 patients with pure LMN who participated in two RCTs has demonstrated comparable anti-proteinuric effects and nephrotic syndrome remission rates in MMF- and IV CYC–treated patients.[62] Consequently, and based on its more favorable toxicity profile compared with CYC, MMF is considered as first-line treatment of LMN.

Maintenance Therapy. Meta-analysis of RCTs comparing MMF versus AZA as maintenance therapy in PLN[48,50,52] showed comparable rates of renal remission, doubling of Cr, and renal remission during a 3-year period.[53] However, treatment with AZA was associated with increased risk for renal relapses (relative risk [RR] 1.45) and leukopenia (RR, 7.1). To this end, both MMF and AZA can be used for maintenance of response in PLN on the basis of availability and potential for pregnancy. Early (before 18 to 24 months) discontinuation of MMF or switching to other immunosuppressant is associated with increased risk for renal flare.[63]

**Use in Extrarenal Lupus.** MMF has been successful in cases of refractory skin and blood manifestations in SLE.[64] Post-hoc analysis of the ALMS data showed that MMF was equally efficacious with IV CYC pulse therapy on controlling extrarenal disease activity, particularly in the general, mucocutaneous, musculoskeletal, and hematologic domains.[65] While awaiting additional data to define the efficacy of MMF in extrarenal lupus, the drug may be used in patients with moderately severe lupus who are intolerant or have not adequately responded to AZA.

## Calcineurin Inhibitors

### Cyclosporin A

**Pharmacology.** CsA, a fungus-derived calcineurin inhibitor that deactivates T cells, also reduces antigen presentation and autoantibody production by lupus B cells. Following oral administration, peak serum concentration occurs within 1 to 8 hours. Drug concentration is measured in whole blood, but this is rarely necessary in autoimmune diseases, unless CsA is used in doses greater than or equal to 3 mg/kg/day. Clinical response occurs 1 to 2 months after treatment initiation. Numerous drugs interact with CsA, leading to reduced drug concentrations (rifampin, phenytoin, phenobarbital, nafcillin) or increased CsA concentrations (erythromycin, clarithromycin, azoles, calcium channel blockers, amiodarone, allopurinol, colchicine). Other drugs may augment CsA's nephrotoxic effects (NSAIDs, aminoglycosides, quinolones, angiotensin-converting enzyme [ACE] inhibitors, amphotericin B). Common adverse effects include gastrointestinal complaints, hirsutism, gingival hyperplasia, and mild elevation in serum alkaline phosphatase levels. Tremor, paresthesias, electrolyte disturbances (hyperkalemia and hypomagnesemia), and hyperuricemia may also occur. Hypertension occurs in nearly 20% of patients receiving CsA and is controlled by either reduction of the dose or anti-hypertensive treatment. A significant adverse effect is nephrotoxicity, which is reversible after adjustment of the dose or drug discontinuation. Physicians should avoid use of CsA in patients with impaired renal function (see Table 81-1).

**Use in Proliferative Lupus Nephritis.** CsA, used in combination with GC, has demonstrated efficacy in PLN, including refractory cases.[66] In the CYCLOFA-LUNE trial, 40 patients with newly diagnosed PLN and mild renal insufficiency received sequential induction-maintenance therapy with either CYC (oral boluses 10 mg/kg) or CsA (4 to 5 mg/kg/day, then tapered), both combined with oral GC.[67] At 9 months, 76% of the CYC group and 68% of the CsA group achieved renal response, and after median follow-up of 7.7 years, the two groups had comparable rates of ESRD, adverse events, mean Cr, proteinuria, and Systemic Lupus International Collaborating Clinics (SLICC) damage score.[68] Moroni and colleagues[49] have also demonstrated comparable efficacy of AZA versus CsA as maintenance therapies in patients with diffuse PLN and preserved renal function. Together, CsA can be used for the induction and maintenance treatment of PLN, although it is not preferred as a first-line option because of its safety profile.

**Use in Membranous Lupus Nephropathy.** Balow and Austin[40] compared treatment with prednisone alone or in combination with CsA or pulses of IV CYC in the treatment of membranous lupus nephropathy (MLN). Remission rates at 1 year were 27% for prednisone, 60% for IV CYC, and 83% for CsA. After treatment discontinuation, however, rates of nephrotic syndrome relapse were 10-fold higher in the CsA than in the IV CYC group, suggesting

that, at a minimum, patients treated with CsA may require maintenance therapy to prevent relapses in MLN.

**Use in Extrarenal Lupus.** Uncontrolled studies of short duration have shown improvement in disease activity, anti-double-stranded (ds)DNA titers, and cytopenias, with modest GC reduction in patients with SLE who received low-dose CsA.[69,70] The British Isles Lupus Assessment Group (BILAG) group compared the steroid-sparing effect of CsA (2.5 to 3.5 mg/kg/day) versus AZA (2 to 2.5 mg/kg/day) in moderately severe lupus.[71] Although nearly 50% of the patients failed to respond, the mean reduction in prednisolone dose at 12 months was 9.5 mg in the CsA group and 10.2 mg in the AZA group. The two groups performed similarly with regard to disease activity, response to treatment, flares, damage accrual, and quality-of-life measures. Thus CsA may be used as an alternative steroid-sparing agent to AZA in patients with SLE but with close monitoring of the renal function and blood pressure.

### Tacrolimus

Tacrolimus (TAC) is a 10 to 100 times more potent calcineurin inhibitor than CsA. Dose-dependent reversible nephrotoxicity and blood pressure elevation have been reported, although less often than with CsA. Other adverse effects include cardiomyopathy in children, anxiety, seizures, delirium and tremor, diabetes, and hyperlipidemia. In patients with skin and musculoskeletal lupus, TAC (1 to 3 mg/day for 1 year) resulted in significant reduction in disease activity and the dose of GC.[72]

Controlled studies have demonstrated beneficial effects of TAC in PLN and MLN, including cases of refractory disease.[73] In Asian patients with LN with relatively preserved renal function, TAC exhibited fast anti-proteinuric action and at least equal efficacy with MMF or IV CYC in inducing renal response at 6 to 12 months.[74-76] In Chinese patients with class III-IV (81%) or pure class V (19%) LN, prednisolone (0.6 mg/kg/day) was administered with either TAC (0.06 to 0.1 mg/kg/day) or MMF (2 to 3 g/day).[77] At 6 months, complete renal response was achieved by 59% of patients in the MMF and 62% of patients in the TAC group. During maintenance with AZA, proteinuric and nephritic flares developed in 24% of patients and 18% of patients in the MMF group, and 35% and 27% in the TAC group, respectively.[77] Long-term maintenance treatment with TAC has also been used, although hypertension and/or metabolic adverse events may develop in a considerable proportion of patients.[78] Further studies in non-Asian patients are eagerly awaited.

TAC has been used in combination with MMF to treat severe or resistant LN.[79-81] A multicenter RCT in Chinese patients with new-onset active LN compared the combination of TAC (4 mg/day) and MMF (1.0 g/day) versus monthly pulses IV CYC (0.5 to 1.0 g/m$^2$) for 6 months. Both regimens were combined with GCs. Complete renal response rates were 46% with the MMF/TAC combination versus 26% with IV CYC.[82] These results need cautious interpretation because of the fast anti-proteinuric effect of TAC and the lack of long-term safety and efficacy data. Nonetheless, such "multitarget" treatment might represent an option for LN patients with incomplete response to standard immunosuppressive treatment.

## Biologic Therapies

### Belimumab

The B lymphocyte stimulator protein (BLyS, also known as B cell activation factor [BAFF]) and the proliferation-inducing ligand (APRIL) are growth factors important for B cell survival and maturation. BLyS binds to three different B cell receptors, namely, the transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI), the B cell maturation antigen (BCMA), and the BAFF receptor, whereas APRIL signals through TACI and BCMA.[90]

Belimumab is a fully human anti-BLyS monoclonal antibody. Two phase III placebo-controlled RCTs (BLISS-52,[91] BLISS-76[92]) evaluated two different doses of belimumab (1 mg/kg, 10 mg/kg; dosed IV on days 0, 14, 28, and then every 28 days) on top of standard therapy in more than 1500 serologically positive (anti-nuclear antibody [ANA] ≥1:80 and/or anti-dsDNA ≥30 IU/mL) patients with SLE, using the composite SLE Responder Index (SRI) as the primary endpoint. Enrolled patients had moderately active SLE (average SLEDAI, 9.7), 57% had involvement of at least three different organs (excluding active severe renal or CNS lupus), and 25% were receiving treatment with GC plus anti-malarials plus one immunosuppressive agent (MTX, AZA, or MMF). Belimumab treatment resulted in a modest, although significant, reduction in disease activity (pooled results from the two RCTs: 50.6% in patients treated with 10 mg/kg belimumab vs. 30.8% in placebo met the study endpoint.).[93] Most improved were the musculoskeletal (arthritis), mucocutaneous (alopecia, ulcers, malar rash), and immunologic domains, and also skin vasculitis. Belimumab-treated patients exhibited significantly reduced risk for severe SLE flares (HR, 0.74), modest reductions in GC dose, and improvements in fatigue and health-related quality of life. Although patients with active severe LN were excluded from the trials, a significant anti-proteinuric effect was observed with belimumab.[94] Post-hoc analysis showed that drug effectiveness was higher in patients who had SLEDAI scores greater than or equal to 10 or serologic activity (low C3/C4 and/or high anti-dsDNA), or those who were on GCs upon enrollment.[95] Long-term follow-up data suggest that belimumab is generally well tolerated, with no significant increase in risk for serious infections, although infusion reactions can be encountered especially during the first three infusions.[96] These data opened the way for approval of this agent for the treatment of patients with active nonrenal/non-CNS SLE despite standard therapy (Table 81-4).

### Rituximab

Rituximab (RTX) is a chimeric mouse-human monoclonal antibody that targets the cell membrane protein CD20, which is expressed in all developmental stages of B cells, except for the hematopoietic stem cell and the plasma cell. Its mechanism of action involves cytotoxicity through complement activation, antibody-dependent cell-mediated cytotoxicity, and induction of apoptosis. RTX is well tolerated, with mild infusion reactions the most common adverse event. These reactions are usually prevented by premedication with antihistamines and GCs. Mild infections

**TABLE 81-4** Candidate Patients for Treatment with Belimumab

| |
|---|
| Active SLE (SLEDAI ≥8) in spite of standard-of-care therapy, including GCs, anti-malarials, and/or DMARDs/immunosuppressives |
| Higher efficacy in patients with SLEDAI ≥10 or with concomitant serologic activity (high anti-dsDNA and/or low serum C3/C4) |
| Conventional treatment should have been uptitrated as tolerated |
| Belimumab is not indicated for the management of active LN or NPSLE |
| High risk for disease flares |
| Young-onset disease (≤25 years old); persistent disease activity, history of renal, CNS involvement, or skin vasculitis; organ damage (SDI >0); serologic activity, previous SLE flares, no treatment with anti-malarials |
| Failure to taper GC to <7.5 mg/day prednisone equivalent |
| Intolerance to DMARDs/immunosuppressives |

GC, Glucocorticoid, CNS, central nervous system, DMARDs, disease-modifying anti-rheumatic drugs; LN, lupus nephritis; NPLSE; neuropsychiatric systemic lupus erythematosus.; SLE, system lupus erythematosus; SLEDAI, SLE Disease Activity Index.

are common (occurring in as many as 20% of patients), but the overall risk for serious or opportunistic infections is not significantly increased. Following the report of a few cases of progressive multifocal leukoencephalopathy (PML) in RTX-treated patients with rheumatoid arthritis (RA) and SLE, the U.S. Food and Drug Administration (FDA) has issued an alert about this association. While open label studies suggest efficacy in lupus, controlled clinical trials have so far failed to show statistically significant clinical benefit.

**Use in Extrarenal Lupus.** In the Exploratory Phase II/III SLE Evaluation of Rituximab (EXPLORER) trial, patients with active moderate to severe extrarenal SLE were randomly assigned to receive RTX (two biweekly infusions of 1000 mg at baseline and at 6 months) or placebo, in combination with background immunosuppression (AZA, MMF, MTX) and prednisone (0.5 to 1 mg/kg/day).[83] At week 52, no difference was observed between patients treated with RTX and placebo in major or partial clinical responses. However, high background therapies could have masked RTX effects. Conversely, evidence from several uncontrolled studies, including more than 1200 patients with SLE with refractory-to-immunosuppressive therapy nonrenal manifestations, suggests the efficacy of RTX, which is mainly used as add-on therapy to GCs, other immunosuppressive agents, or both.[84] Complete or partial responses were noted in 64% to 91% of patients within 3 to 9 months, particularly in the musculoskeletal (arthritis), hematology (autoimmune hemolytic anemia, thrombocytopenia) and immunology domains. Relapses occurred in 25% to 85% of patients after 3 to 18 months, and most appeared to be successfully re-treated with RTX in these observational reports.[84]

**Use in Lupus Nephritis.** The LUNAR trial evaluated RTX versus placebo, both in combination with MMF (3 g/day), in 144 patients with active PLN.[85] By week 52, there was no significant difference between the two groups in terms of renal response. Similar to the EXPLORER trial, high background treatment with MMF could have masked RTX superiority versus placebo. In contrast, observational data from 300 patients with active LN, in spite of treatment with GCs plus immunosuppressives (CYC, MMF, or AZA), supports RTX efficacy.[86] After an average of 60 weeks, rates of complete and partial renal response were approximately 40% and 34%, respectively, with lower responses in mixed class V + III-IV or pure class V LN. In a nonrandomized study, patients with active PLN received induction treatment with GCs plus either RTX (1000 mg on days 0 and 14), or MMF (2 to 2.5 g/day) or low-dose IV CYC (3 g), followed by maintenance with AZA, MMF, or CsA.[87] Renal response rates at 12 months were comparable between the RTX, MMF, and IV CYC groups (71%, 53%, and 65%, respectively). The same group treated PLN patients seen with severe proteinuric flare with oral CYC (1 to 2 mg/kg/day for 3 months) or RTX, followed by maintenance with MMF or AZA.[88] At 3 months, resolution of proteinuria (<0.5 g/24 hr) occurred in 20% of RTX-treated versus 7% of CYC-treated patients. At 12 months, the respective figures were 90% versus 71%.[88] Although the previously mentioned studies are small and nonrandomized, they provide further evidence for the efficacy of RTX in the treatment of refractory/relapsing LN. Efforts to combine RTX, MMF, and pulses of IV MP with no oral GCs as induction treatment of LN are currently under investigation.[89]

## Biologic Therapies Under Development

### Ocrelizumab

The BELONG trial evaluated ocrelizumab (humanized anti-CD20 monoclonal antibody) versus placebo, both in combination with either MMF (as much as 3 g/day) or IV CYC (Euro-Lupus protocol) in PLN.[97] The study was prematurely terminated because of increased rates of serious infections in ocrelizumab-treated patients.

### Epratuzumab

Epratuzumab is a recombinant anti-CD22 monoclonal antibody that modulates B cell function.[81] Positive results were announced for phase IIb trials in moderate to severe SLE.[98,99] Patients who received epratuzumab 2400 mg cumulative dose had significant improvements in disease activity compared with placebo. Incidence of serious adverse events and infusion reactions was similar between the epratuzumab and placebo groups.[99]

### Atacicept

Atacicept (TACI-Ig) is a recombinant fusion protein of the extra-cellular domain of TACI and the human IgG1.Fc domain. TACI mediates signals from both BLyS and APRIL, thus affecting memory B cells, plasma cells, and immunoglobulin production. Two phase II/III RCTs of atacicept in LN (administered in combination with MMF)[100] and in moderately severe SLE,[101] were prematurely terminated because of cases of fatal infections.

### Blisibimod

Blisibimod selectively inhibits both soluble BAFF and membrane-bound BAFF. In active patients with SLE with

positive ANA and/or anti-dsDNA antibodies, blisibimod, 200 mg once-weekly, demonstrated efficacy versus placebo,[102] with reductions in flares, dose of GCs, proteinuria levels, and improvements in serology. The drug was generally well tolerated, with the exception of some injection site reactions. Further studies will be required to further define its efficacy and safety in SLE.

### Abatacept

Abatacept (CTLA4-Ig) antagonizes CD28-mediated T cell activation and is currently approved for the treatment of RA and juvenile idiopathic arthritis. A phase IIb trial of abatacept in combination with GC and background immunosuppressive therapy in patients with SLE failed to meet its primary endpoint (prevention of flares).[103] Two RCTs have evaluated abatacept in combination with MMF[104] or low-dose IV CYC[39] as induction treatment of PLN. The results of both trials were negative, although data reanalysis by using alternative renal response criteria suggested the superiority of abatacept versus placebo.[105] Further studies will be required to define the usefulness of the drug in LN.

### Interferon Inhibition

Type I interferon (IFN-α) has been implicated in lupus pathogenesis through breakdown of immune tolerance. Two phase IIb trials targeting IFN-α with sifalimumab (anti–IFN-α mAb) or anifrolumab (anti–IFN-α receptor subunit 1 mAb) yielded encouraging results. In patients with seropositive moderate to severe SLE, despite standard-of-care treatment, sifalimumab treatment resulted in a higher proportion of SRI responses compared with placebo.[106] Anifrolumab caused increased and more sustained target suppression of the IFN-α gene signature in Japanese patients with SLE compared with sifalimumab.[107]

### Anti-IL-6 Therapy

Tocilizumab a humanized monoclonal antibody against the α-chain of the IL-6 receptor, was tested in 16 patients with moderately active SLE.[108] Reduction in disease activity, acute phase reactants, and anti-dsDNA levels occurred. A phase II study failed to demonstrate the efficacy of sirukumab (anti-IL-6 mAb) versus placebo in patients with active PLN despite immunosuppression.[109] Conversely, administration of PF-04236921, a fully human anti-IL-6 mAb, in active SLE resulted in a higher proportion of SRI responders and reduced frequency of severe flares compared with placebo.[110] Phase III trials will determine the role of IL-6 blockade in the treatment of SLE.

## Other Therapies

### Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) exerts immunosuppressive effects by interaction with anti-idiotypic antibodies, interference with complement and cytokines, cytolysis of target cells, induction of apoptosis through Fc receptors, and modulation of co-stimulatory molecules.[111] IVIG (400 mg/kg for 5 consecutive days, monthly for 18 months) was as effective as pulse IV CYC (1 g/m$^2$ every 2 months for 6 months, then every 3 months for 1 year) as maintenance therapy of PLN.[112] A meta-analysis of 13 studies concluded that treatment with IVIG resulted in significant reduction in the SLE disease activity scores and improvements in serum complement levels.[113] Improvements in cytopenias, especially immune thrombocytopenia and hemolytic anemia, proteinuria, arthritis, fever, arthralgia, mood changes, and psychosis have been reported.[114] Common adverse events include fever, myalgia, headache, and arthralgia; less common are aseptic meningitis, nephropathy in patients who receive sucrose-containing preparations, and thromboembolic complications in older patients with atherosclerotic risk factors. The drug is contraindicated in patients with severe IgA deficiency.

### Synthetic Tolerogens

Tolerogenic peptides aim to restore immune tolerance in lupus. Abetimus sodium (LJP-394) reduces anti-dsDNA antibodies by binding and clearance of soluble anti-dsDNA antibodies and B cell receptor cross-linking on dsDNA-specific B cells.[115] A phase III placebo-controlled trial in LN patients failed to meet its primary endpoint (prolongation of time to renal flare).[116] The spliceosomal peptide P140 (lupuzor) has, however, demonstrated benefit in SLE.[117] In a phase IIb study, patients with SLEDAI greater than or equal to 6 were randomly assigned to receive lupuzor or placebo in addition to standard of care.[118] At 24 weeks, SRI response rates were significantly higher with lupuzor than placebo (53% vs. 36%), attributed to improvements in arthritis, rash, alopecia, mucosal ulcers, and serology.

## MANAGEMENT OF SPECIFIC SYSTEMIC LUPUS ERYTHEMATOSUS MANIFESTATIONS AND TREATMENT ALGORITHMS

**KEY POINTS**

- **Sun exposure prophylaxis, topical glucocorticoids or calcineurin inhibitors, and systemic anti-malarials are recommended for skin manifestations.**
- **In moderately severe PLN, MMF or low-dose intravenous CYC is recommended; high-dose intravenous CYC is preferred in cases of severe PLN.**
- **Maintenance therapy in LN involves AZA or MMF.**
- **Glucocorticoids and/or immunosuppressives are recommended for neuropsychiatric events thought to reflect an immune/inflammatory process.**
- **Anti-platelet/anti-coagulation therapy is recommended in APS.**

European League against Rheumatism (EULAR) and ACR have developed recommendations covering the most important aspects in the diagnosis, management, and monitoring of LN[119,120] and other SLE manifestations.[121-123] In the following text we discuss the management of mucocutaneous, joint, renal, hematologic, and neuropsychiatric manifestations in patients with SLE.

## Mucocutaneous and Joint Disease

Mild malar rash and other photosensitive rashes usually respond to prophylaxis from sun exposure, but the use of protective clothing and sunscreens with high sun protection factor cannot be overemphasized. Topical GCs reduce redness and scaling. In patients with discoid lupus erythematosus (DLE), high-potency topical fluocinonide, 0.05%, was more effective than low-potency hydrocortisone, 1% (response rates 27% vs. 10%, respectively).[124] Topical calcineurin inhibitors (tacrolimus, pimecrolimus) are a useful alternative, especially for the face, because they cause less atrophic and rosacea-like effects than topical steroids.[125]

In skin disease, systemic anti-malarials, if not already prescribed, can be used alone or in combination with oral GCs (as much as 20 mg/day). HCQ was equally effective and had more favorable safety profile than the oral retinoid acitretin in DLE patients.[126] The combination of quinacrine and HCQ has also been used.[127] MTX has shown efficacy in refractory subacute cutaneous lupus erythematosus (CLE) and DLE.[128] Alternative choices are retinoids, dapsone, MMF, and IV CYC.[42,129,130] Thalidomide and IVIG are reserved for severe recalcitrant CLE cases because of potential serious neurotoxicity and high cost, respectively (Figure 81-1).[131,132]

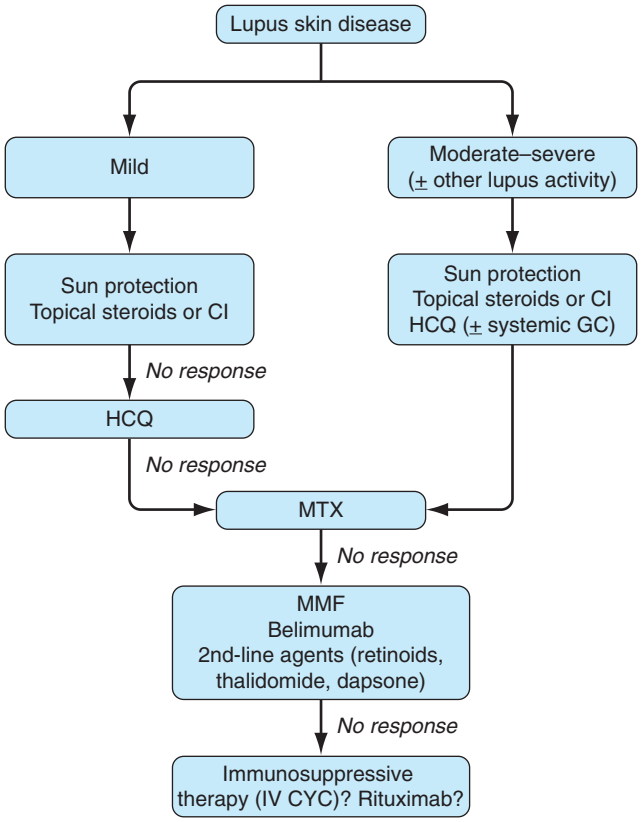



**Figure 81-1** Suggested algorithm for the management of cutaneous manifestations in systemic lupus erythematosus. See text for details. CI, Calcineurin inhibitors; GC, glucocorticoids; HCQ, hydroxychloroquine; IV CYC, pulse intravenous cyclophosphamide; MMF, mycophenolate mofetil; MTX, methotrexate. *(Modified from Kuhn A, Ruland V, Bonsmann G: Cutaneous lupus erythematosus: update of therapeutic options: part II. J Am Acad Dermatol 65:e195–e213, 2010; and Kuhn A, Ruland V, Bonsmann G: Cutaneous lupus erythematosus: update of therapeutic options: part I. J Am Acad Dermatol 65:e179–e193, 2010.)*

In mild lupus arthritis, initial therapy is anti-malarials. In persistent or aggressive disease, utilization of DMARDs is advocated, particularly MTX,[29] whereas leflunomide is used less often. Patients with inadequate response to DMARDs may benefit from belimumab treatment. RTX can be used in severe lupus arthritis or in overlap SLE-RA cases that are refractory to DMARDs.

## Lupus Nephritis

### Induction Therapy

Current therapeutic strategies in LN include an initial induction phase, aimed at substantially improving disease activity (or even attaining remission), followed by a maintenance phase, in which the goal is to maximize the therapeutic effect and consolidate the response. Risk stratification, according to renal pathology and patient demographic, clinical, and laboratory characteristics, enables the identification of patients at risk for renal dysfunction, ESRD, or both, who may benefit from aggressive cytotoxic therapy (Table 81-5).[133,134] Figure 81-2 and Table 81-6 summarize the treatment recommendations for LN.[119]

### Treatment of Renal Flares

Approximately 30% to 50% of patients with moderate to severe PLN will relapse after achieving partial or complete remission.[135,136] Nephritic flares are characterized by active urine sediment and reproducible increases of serum creatinine (Cr) (≥30% increase) and may adversely affect renal prognosis[137]; proteinuric flares without significant changes in renal function have a more benign prognosis. Risk factors for progression to ESRD after a nephritic flare are patients with marked loss of renal function (Cr >2 mg/dL) at the time of response, partial response to therapy, and high chronicity and activity indices at renal biopsy. Mild to moderate flares (stable Cr, subnephrotic proteinuria) may be treated with GC in combination with AZA or MMF. Calcineurin inhibitors (alone or added to existing immunosuppressive therapy) have also demonstrated efficacy. For severe nephritic flares, (re-)institution of cytotoxic therapy with monthly pulses of IV CYC and IV MP is the authors' preferred approach, with MMF considered as an alternative agent. RTX has also been successfully used.[88]

## Central Nervous System Disease

Neuropsychiatric SLE (NPSLE) represents a clinical challenge because of the often-ambiguous attribution of neuropsychiatric syndromes to SLE per se and the paucity of controlled trials (Figure 81-3).[122] Immunosuppressive therapy (GCs alone or in combination with AZA for mild to moderate cases, or IV CYC for severe ones) is advocated for neuropsychiatric events thought to reflect an immune/inflammatory process (particularly acute confusional state, aseptic meningitis, myelitis, optic neuritis, refractory seizure disorder, peripheral neuropathies, or psychosis) or when they occur in the context of active generalized lupus, following exclusion of non–SLE-related causes.[43,138] Antiplatelet or anti-coagulation therapy is recommended for events related to anti-phospholipid antibodies (APAs),

**TABLE 81-5** Definitions of Lupus Nephritis Severity

| | |
|---|---|
| **Proliferative Nephritis** | |
| Mild | 1. Class III nephritis without severe histologic features (crescents, fibrinoid necrosis)*; low chronicity index (≤3); normal renal function; non-nephrotic range proteinuria |
| Moderately severe | 1. Mild disease as defined above with partial or no response after the initial induction therapy, or delayed remission (>12 mo), *or*<br>2. Focal proliferative nephritis with adverse histologic features or reproducible Cr increase ≥30%, *or*<br>3. Class IV nephritis without adverse histologic features |
| Severe | 1. Moderately severe as defined above, but not remitting after 6-12 mo of therapy, *or*<br>2. Proliferative disease with impaired renal function and fibrinoid necrosis or crescents in >25% of glomeruli, *or*<br>3. Mixed membranous and proliferative nephritis, *or*<br>4. Proliferative nephritis with high chronicity alone (chronicity index >4) or in combination with high activity (chronicity index >3 and activity index >10), *or*<br>5. Rapidly progressive glomerulonephritis (doubling of Cr within 2-3 mo) |
| **Membranous Nephropathy** | |
| Mild | 1. Non-nephrotic range proteinuria with normal renal function |
| Moderate | 2. Nephrotic range proteinuria with normal renal function at presentation |
| Severe | 3. Nephrotic range proteinuria with impaired renal function at presentation (≥30% increase in Cr) |

*Concomitant therapy with corticosteroids or immunosuppressive drugs, or both, may modify urinary sediment and histologic findings and should be taken into consideration.
Cr, Serum creatinine.

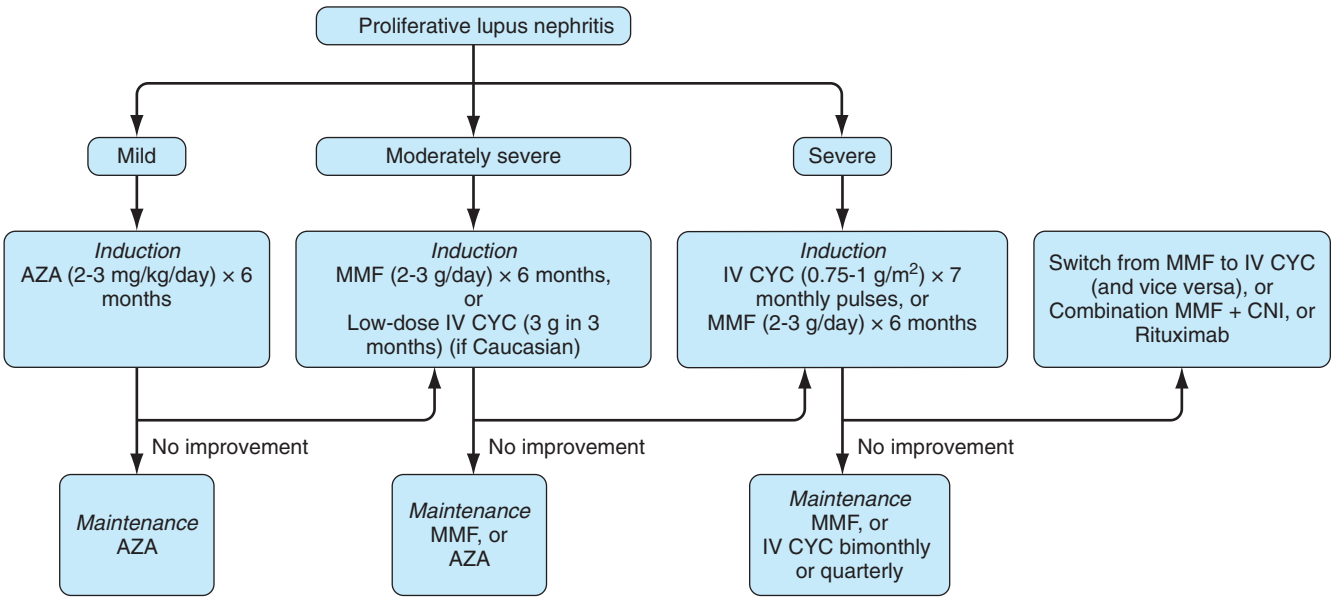



**Figure 81-2** Recommended treatment of proliferative lupus nephritis. Improvement (reduction in proteinuria, stabilization or increase in glomerular filtration rate) is assessed within the first 3 to 4 months after treatment initiation. All induction regimens include pulse intravenous methylprednisolone (IV MP) (1 g/pulse × 3), followed by oral prednisone (0.5-0.6 mg/kg for the first 4 weeks of induction, then tapered). See also Table 81-6. AZA, Azathioprine; CNI, calcineurin inhibitors; IV CYC, intravenous cyclophosphamide; MMF, mycophenolate mofetil.

especially for thrombotic cerebrovascular disease.[139,140] In severe NPSLE refractory to cytotoxic therapy, RTX can be efficacious.[141] Memantine (an N-methyl-D-aspartate [NMDA] receptor antagonist) failed to improve cognitive performance in patients with SLE.[142] Immunosuppressive therapy is usually not warranted for cognitive dysfunction in SLE, which typically follows a benign course.

## Hematologic Disease

Peripheral cytopenias are common but usually mild in SLE. A thorough clinical and laboratory evaluation is necessary to exclude offending drugs or other secondary causes. Mild cytopenias require no specific therapy other than regular monitoring. In more severe cases (platelet count < $50 \times 10^3$/mm$^3$ or active bleeding, neutrophil count <1000/mm$^3$), GCs are the mainstay of treatment. Steroid-sparing agents (AZA and CsA) can be added during steroid tapering. Resistant life-threatening cytopenias may require potent immunosuppressive or biologic therapy. The investigation and management of cytopenias in patients with SLE is outlined in Table 81-7.

## Presence of APAs/APS

APAs (anti-cardiolipin [aCL], anti–$\beta_2$-glycoprotein I [anti-$\beta_2$GPI], and lupus anti-coagulant [LAC]) are encountered in 30% to 40% of SLE and confer increased risk for

**TABLE 81-6** Treatment of Lupus Nephritis

| |
|---|
| **Class I-II LN** |
| Renin-angiotensin-aldosterone system blockade is recommended. |
| GCs (prednisone, 0.5-1 mg/kg/day for 4 to 6 weeks, then tapered): in cases of proteinuria >1 g/day, active urine sediment, histologic features of podocytopathy, or extrarenal disease activity. Three consecutive daily pulses of IV MP may expedite remission and allow for the use of lower GC doses (0.5 mg/kg/day). |
| Consider immunosuppressive therapy (AZA, 1-2 mg/kg/day) to decrease cumulative steroid dose. |
| **Class III-IV (± V) LN** |
| **Initial (Induction) Treatment**<br>Pulses IV MP (750-1000 mg × 3 days), followed by oral prednisone, 0.5 mg/kg/day; higher starting dose may be used if there is significant extrarenal lupus activity |
| Immunosuppressive treatment<br>AZA (2-2.5 mg/kg/day): consider only in white patients with mild disease<br>Low-dose IV CYC (500 mg × 6 every two weeks doses): in white patients with moderately severe disease<br>MMF (3 g/day; or equivalent dose of eMPA): in moderately severe disease; second-line in severe disease; preferable in black or Hispanic patients<br>High-dose IV CYC (0.75-1 g/m$^2$ × 7 monthly pulses): in severe disease |
| **Subsequent (Maintenance) Treatment**<br>Patients who improve (reduction in proteinuria with stabilization or increase in GFR) on induction treatment may continue with maintenance treatment |
| Gradual tapering of GCs: 7.5 to 15 mg of prednisone equivalent on alternate days |
| Immunosuppressive treatment<br>AZA (2 mg/kg/day): preferred if induction with IV CYC or pregnancy contemplation<br>MMF (2 g/day): preferred if induction with MMF or in severe LN<br>IV CYC (quarterly pulses for 1 year after response): in severe LN |
| Duration of maintenance treatment: 3 years; early (before 18-24 months after renal response) tapering or withdrawal of immunosuppressive treatment is associated with increased risk for flares |
| **Class V LN** |
| Renin-angiotensin-aldosterone system blockade is recommended. |
| Consider immunosuppressive therapy in cases of proteinuria >1 g/day (particularly if nephrotic-range proteinuria), reduced GFR. |
| **Initial (Induction) Treatment**<br>Oral prednisone, 0.5 mg/kg/day; consider initial pulses IV MP (750-1000 mg ×3 days) in severe cases |
| Immunosuppressive treatment<br>AZA (2-2.5 mg/kg/day): consider in mild disease<br>CNIs (cyclosporine A, tacrolimus): in mild or moderately severe disease |
| MMF (3 g/day; or equivalent dose of eMPA): in moderately severe disease or severe disease |
| High-dose IV CYC (0.75-1 g/m$^2$ × 7 monthly pulses): in moderately severe or severe disease |
| **Subsequent (Maintenance) Treatment**<br>Patients who improve (reduction in proteinuria with stabilization or increase in GFR) on induction treatment may continue with maintenance treatment |
| Gradual tapering of GCs: 7.5-15 mg of prednisone equivalent on alternate days |
| Immunosuppressive treatment<br>AZA (2 mg/kg/day): preferred if induction with IV CYC or pregnancy contemplation<br>CNIs (cyclosporine A, tacrolimus): in mild or moderately severe disease; careful consideration of risks for hypertension, nephrotoxicity or metabolic adverse effects<br>MMF (2 g/day): preferred if induction with MMF; in moderately severe or severe disease |
| **Therapeutic Goals and Refractory Disease** |
| Early response to therapy at 6 months (defined as normalization of Cr level and reduction in proteinuria by at least 50% or to less than 1 g/day) is a predictor of good long-term renal outcome.[196,197] |
| Failure to achieve improvement in renal function and proteinuria after the initial 3 to 4 months, or response after 6 to 12 months of therapy should precipitate decisions about treatment switching/intensification. |
| Microscopic hematuria or non-nephrotic proteinuria may not clear for several months, and complete renal response (defined as proteinuria <0.5 g/day with normal or near-normal [±10%] GFR) may occur at an average of 1.5 to 2 years after therapy.[198] |
| Discontinuing or switching immunosuppressive therapy because of lack of achieving complete renal response before completing at least 2 years of treatment is not justified unless there is definite disease worsening (reproducible increase in Cr and/or increase in proteinuria). |

AZA, Azathioprine; Cr, serum creatinine; CYC, cyclophosphamide; eMPA, enteric-coated mycophenolate sodium; IV, intravenous; MP, methylprednisolone; MMF, mycophenolate mofetil.

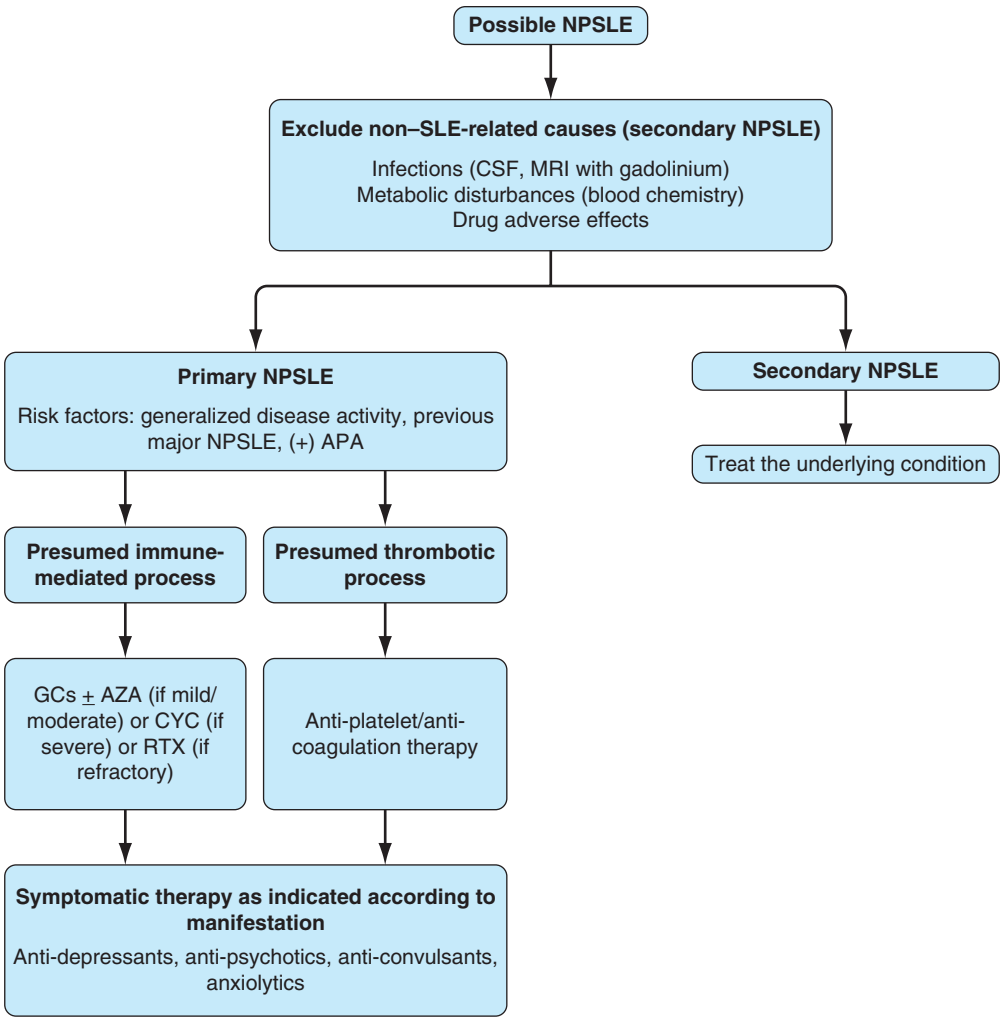



**Figure 81-3** Assessment and management of patients with systemic lupus erythematosus who are seen with neuropsychiatric manifestations. APA, Anti-phospholipid antibodies; AZA, azathioprine; CSF, cerebrospinal fluid analysis; CYC, cyclophosphamide; GC, glucocorticoids; MRI, magnetic resonance imaging of brain and/or spine that includes T1, T2, FLAIR, and diffuse-weighed imaging protocols; NPLSE, neuropsychiatric systemic lupus erythematosus; RTX, rituximab.

thrombotic events. The combination of vascular thrombosis or obstetric morbidity, or both, and persistently positive APAs measured at least 12 weeks apart, defines APS.[143] The principles of APS treatment (anti-platelet or anti-coagulation, target INR) do not differ between primary APS and SLE-APS and are described in detail in Chapter 82.

A distinct type of small-vessel vaso-occlusive nephropathy with histologic features of thrombotic microangiopathy and chronic vascular lesions, termed *APS nephropathy*, should be suspected in APA-positive patients with SLE with hypertension, proteinuria (usually subnephrotic range), hematuria, and renal impairment.[144] These patients may benefit from anti-platelet or anti-thrombotic therapy, but development of ESRD is common.[145] Aberrant activation of the mammalian target of rapamycin (mTOR) pathway has been documented in APS nephropathy and APS-related renal vascular lesions superimposed on inflammatory LN, suggesting that agents targeting this pathway may prove useful for these patients.[146]

## REFRACTORY DISEASE, PREGNANCY, AND CHILDREN

**KEY POINTS**

- **Pregnancy outcome in women with SLE is optimal when the disease is quiescent for at least 6 months before conception.**
- **Continuation of HCQ during pregnancy is associated with improved maternal and fetal outcomes.**
- **The risks of treatment during pregnancy must be weighed against the risks of an untreated SLE flare.**

### Treatment of Refractory Systemic Lupus Erythematosus

IV CYC in combination with IV MP has long been considered the treatment of choice for most patients with severe, life-threatening lupus. MMF may rescue a few refractory patients with SLE, including subacute CLE[130] and LN cases

**TABLE 81-7** Management of Hematologic Manifestations in SLE[84,199-201]

| |
|---|
| Exclude offending drugs or other secondary causes |
| History of drug exposure, infections, traveling to endemic regions |
| Clinical examination: signs of infection, splenomegaly, lymphadenopathy, skin hemorrhagic lesions |
| Peripheral blood smear examination (morphology of cells; rule-outs: hematologic malignancy, myelodysplastic syndrome, microangiopathic hemolytic anemia [MAHA] / thrombotic thrombopenic purpura [TTP] |
| Workup for anemia (ferritin, TIBC, folic acid, vitamin $B_{12}$, reticulocyte index) and hemolysis (reticulocyte index, LDH, bilirubin, Coombs IgG, haptoglobulin) |
| Workup for infections (as indicated by history and clinical examination) |
| High-yield indications for bone marrow examination: age >60 years, serum iron >90 µg/dL, mean corpuscular volume (MCV) >90 fL, serum monoclonal band, pancytopenia, history of exposure to myelotoxic drugs, absolute neutrophil count (ANC) <500/µL (on repeated tests), peripheral blood smear findings suggestive of bone marrow disorder |
| Immunosuppressive treatment |
| Mild cytopenias require no specific therapy other than regular monitoring |
| Consider immunosuppressive treatment if: autoimmune hemolytic anemia (AIHA) with hemoglobin <10 g/dL, leukopenia with white blood cell count <2500/µL or ANC <1000/µL, thrombocytopenia with platelet count <50-70 ×1000/µL |
| First-line treatment: (1) GCs (as much as 1 mg/kg/day prednisone equivalent); consider pulses IV MP (500-1000 mg × 3 days) to allow for lower oral GC (0.5 mg/kg/day); (2) AZA (2 mg/kg/day) or CsA as steroid-sparing agent (careful monitoring of white blood cell count) |
| Second-line treatment (refractory/relapsing disease): (1) IV CYC (AIHA, thrombocytopenia), (2) rituximab (AIHA, thrombocytopenia, leucopenia), (3) IVIG (short-term recovery of platelets), (4) CsA (neutropenia, thrombocytopenia) |
| Third-line treatment: danazol, MMF, plasma exchange (severe MAHA/TTP-like) |
| Additional or supportive treatment |
| Red blood cells transfusion: defer until hemoglobin <7 g/dL |
| Recombinant erythropoietin: if co-existing chronic kidney disease (stage 4-6) or anemia of chronic disease |
| Prophylaxis or treatment with broad-spectrum antibiotics (as recommended) |
| Recombinant G-CSF: only if ANC <500/µL and sepsis; use at lowest possible doses and until ANC >1000/µL |
| TPO-receptor agonists (romiplostin, eltrombopag): safe and efficacious in immune thrombocytopenia; require repeated administration; can be used when splenectomy is not favored or is contraindicated |
| Splenectomy: in selected patients with severe refractory AIHA or thrombocytopenia, when there is no lupus activity in other major organs; prophylactic measures for avoidance of infectious complications; post-splenectomy immunosuppressive treatment is usually required |

AZA, Azathioprine; Cr, serum creatinine; CsA, cyclosporin A; CYC, cyclophosphamide; GC, glucocorticoids; Ig, immunoglobulin; IV, intravenous; LDH, lactate dehydrogenase; MMF, mycophenolate mofetil; TIBC, total iron-binding capacity.

with inadequate response to CYC.[147] Calcineurin inhibitors have also shown efficacy in resistant-to-standard immunosuppressive therapy lupus,[148] particularly LN, where benefits of combination therapy with MMF have been illustrated.[79,80] Belimumab is an option for patients with active mucocutaneous and musculoskeletal manifestations that do not respond to standard of care treatment and/or who relapse upon tapering of GC.[93] Mounting evidence supports the beneficial effects of RTX (alone or in combination with IV CYC) in refractory SLE.[88,149] Higher response rates have been reported for skin, blood, CNS, and renal manifestations. For selected patients with CNS involvement, autoimmune thrombocytopenia, or APS, IVIG may be considered as an adjunct therapy. Synchronized therapy with plasmapheresis and pulse IV CYC has been used in severe PLN cases.[150] High-dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT) remains an experimental procedure to be considered in critically ill patients with SLE.[151]

**TABLE 81-8** Approach to the Management of Pregnancy in Systemic Lupus Erythematosus

| |
|---|
| Planning of pregnancy |
| Ensure that lupus is inactive for at least 6 months |
| Reassure patient: small risk for major flare |
| Discourage pregnancy if Cr >2 mg/dL |
| Drugs allowed during pregnancy: GCs, HCQ, AZA, CsA |
| Determine APAs and other antibodies that may be of relevance (anti-SSA, anti-SSB) |
| Obtain baseline serology and chemistry tests (Cr, Alb, uric acid, anti-dsDNA, C3/C4) |
| Be aware of the small risk for CHB, especially in women with both anti-SSA and anti-SSB antibodies or with a prior episode of CHB. In such cases, may monitor for CHB between 18 and 24 weeks of gestation |
| Monitor closely blood pressure and proteinuria. Should this develop, differentiate between active nephritis and pre-eclampsia |
| Presence of generalized lupus activity, active urine sediment, and low serum complement are in favor of lupus nephritis |
| For patients with APS, consider combined heparin and aspirin to reduce risk for pregnancy loss and thrombosis. Patients with APA may be treated with aspirin, although there are no adequate data to support its use |

Alb, Serum albumin; APA, anti-phospholipid antibodies; APS, anti-phospholipid antibodies/anti-phospholipid syndrome; AZA, azathioprine; CHB, congenital heart block; Cr, serum creatinine; CsA, cyclosporin A; dsDNA, double-stranded DNA; GCs, glucocorticoids; HCQ, hydroxychloroquine.

## Treatment of Lupus in Pregnancy

Pregnancy may increase disease activity and precipitate the appearance of flares (13% to 74%), which are usually mild. Pregnancies in patients with lupus, especially in patients with a history of LN or positive APA, are considered high risk for maternal and fetal complications, including pre-eclampsia, pregnancy loss, and pre-term birth.[152] The management of a pregnant woman with an SLE flare is challenging and should be dealt with on a multidisciplinary basis (Table 81-8).

Neonatal lupus occurs in some babies born to mothers with anti-SSA/Ro or anti-SSB/La antibodies. The most serious complication is neonatal complete heart block (CHB), which occurs in as many as 2% of such pregnancies and carries a 20% to 30% mortality risk. The risk of recurrence in a mother who has already borne a child with CHB ranges from 14% to 19%.[153] Therefore all women with SLE

should be screened for anti-Ro/La antibodies. The most vulnerable period for the development of conduction disturbances is between weeks 18 and 26 of gestation, during which anti-Ro/La–positive patients should be evaluated with weekly fetal Doppler echocardiography for the prompt identification of conduction anomalies, mainly PR interval prolongation. If CHB develops, no modality has been shown to reverse it. In contrast, in pregnant women with anti-Ro antibodies, first-degree block may be reversed by fluorinated GCs such as dexamethasone (4 mg/day) initiated at the time of diagnosis and continued throughout pregnancy.[154] HCQ has been associated with reduced risk for recurrences of cardiac neonatal lupus,[155] contrary to IVIG which has demonstrated no beneficial effect.[156,157]

Regarding breastfeeding, the American Academy of Pediatrics (AAP) states that nursing is permissible for women receiving GC, but the interval between dose and nursing should be at least 4 hours if the prednisone dosage is greater than 20 mg/day. Anti-malarials may be continued during lactation, whereas AZA is not recommended because of possible risk for immunosuppression, carcinogenicity, and growth restriction of the child. MTX, MMF, CsA, leflunomide, and CYC are also contraindicated.

## COMORBIDITIES

**KEY POINTS**

- **In a patient with SLE with possible infection, the clinical syndrome, history of epidemiologic exposures, and degree of immunosuppression should be considered.**
- **Immunizations are safe and effective in stable, inactive patients with SLE. Live vaccines are contraindicated in patients taking immunosuppressive drugs or high-dose glucocorticoids.**
- **Hemodialysis may be preferred versus peritoneal dialysis in end-stage renal disease. Patients should be evaluated for renal transplantation.**
- **Strict adherence to general population guidelines for primary prevention of cardiovascular disease is recommended.**
- **Cervical dysplasia is increased in women with lupus. Human papillomvirus vaccination should be considered, similarly to the general population.**

### Infections and Immunizations

#### Risk Factors and General Management

Infections account for 20% to 55% of all deaths in patients with SLE. A broad spectrum of infections has been reported, including bacterial, mycobacterial, viral, fungal, and parasitic infections. The respiratory system, urinary tract, and the CNS are the most commonly involved sites.[158] Risk factors include increased clinical or serologic lupus activity, or both, at baseline; major organ involvement (especially renal and lung involvement); lymphopenia; persistent neutropenia (<1000/mm$^3$); hypoalbuminemia (especially for severe CNS infections); high dose of GC (each increase of 10 mg/day prednisone is associated with an 11-fold increased risk for serious infection); and prior (within the last 6 months) use of immunosuppressive drugs (especially AZA and CYC).[159]

The evaluation of a patient with lupus who receives immunosuppressive therapy and is seen with symptoms or signs suggestive of infection is complicated by the fact that infections can mimic a lupus flare (Figure 81-4). Findings that favor the diagnosis of infection include the presence of shaking chills, leukocytosis and/or neutrophilia (especially in the absence of steroid therapy), increased numbers of band forms or metamyelocytes on peripheral blood smear, and concomitant immunosuppressive therapy.[160,161] SLE fever is favored by the presence of leukopenia (not explained by cytotoxic therapy), normal or only slightly increased C-reactive protein, low C3/C4, and elevated anti-DNA antibodies. If fever fails to resolve in a patient receiving prednisone, 20 to 40 mg daily, it is likely that that fever is to the result of infection. Elevated serum procalcitonin levels have been reported to be predictive of bacterial or mycotic infections, although their diagnostic utility in patients with systemic autoimmune diseases has been questioned.[162] Pending microbiology results, anti-microbial therapy (including broad-spectrum antibiotics in suspected nosocomial infection) is recommended to reduce adverse outcomes.

#### Immunizations

Although vaccination may hypothetically induce polyclonal cell activation in lupus precipitating a flare, it is felt to be safe.[163] Vaccines, however, should not be given to patients with active disease. Inactivated live vaccines (measles, mumps, rubella, polio, varicella-zoster virus (VZV), and vaccinia [smallpox]) are contraindicated in patients taking immunosuppressive drugs or prednisone at a dose greater than 20 mg/day. Influenza vaccine is safe and effective, although seroprotection and seroconversion rates are lower than those in healthy controls; booster vaccination does not increase antibody titers in annually vaccinated patients. Pneumococcal vaccine is also safe, but the resultant antibody titers may be decreased in patients with SLE; use of GC may contribute to blunted antibody responses.[164] Protective immune response can be achieved safely in patients with SLE with both tetanus toxoid and *Haemophilus influenzae* type B in addition to pneumococcus.

According to the Advisory Committee on Immunization Practices (ACIP) guidelines and EULAR guidelines,[165] individuals older than 60 years should be vaccinated with a single dose of VZV vaccine. This is a live-attenuated vaccine and should be administered at least 14 days before starting immunosuppressive therapy, or it should be deferred for at least 1 month after discontinuation of high-dose GC or intensive immunosuppressive therapy. Hepatitis B immunization with recombinant vaccine seems to be efficacious and safe in inactive patients with SLE treated with low-dose GC and not receiving immunosuppressive drugs.[166]

### Chronic Kidney Disease and End-Stage Renal Disease

#### Risk Factors and Dialysis

Approximately 10% to 20% of patients with SLE will develop ESRD. Clinical predictors are abnormal Cr values at presentation, delay in treatment initiation, failure

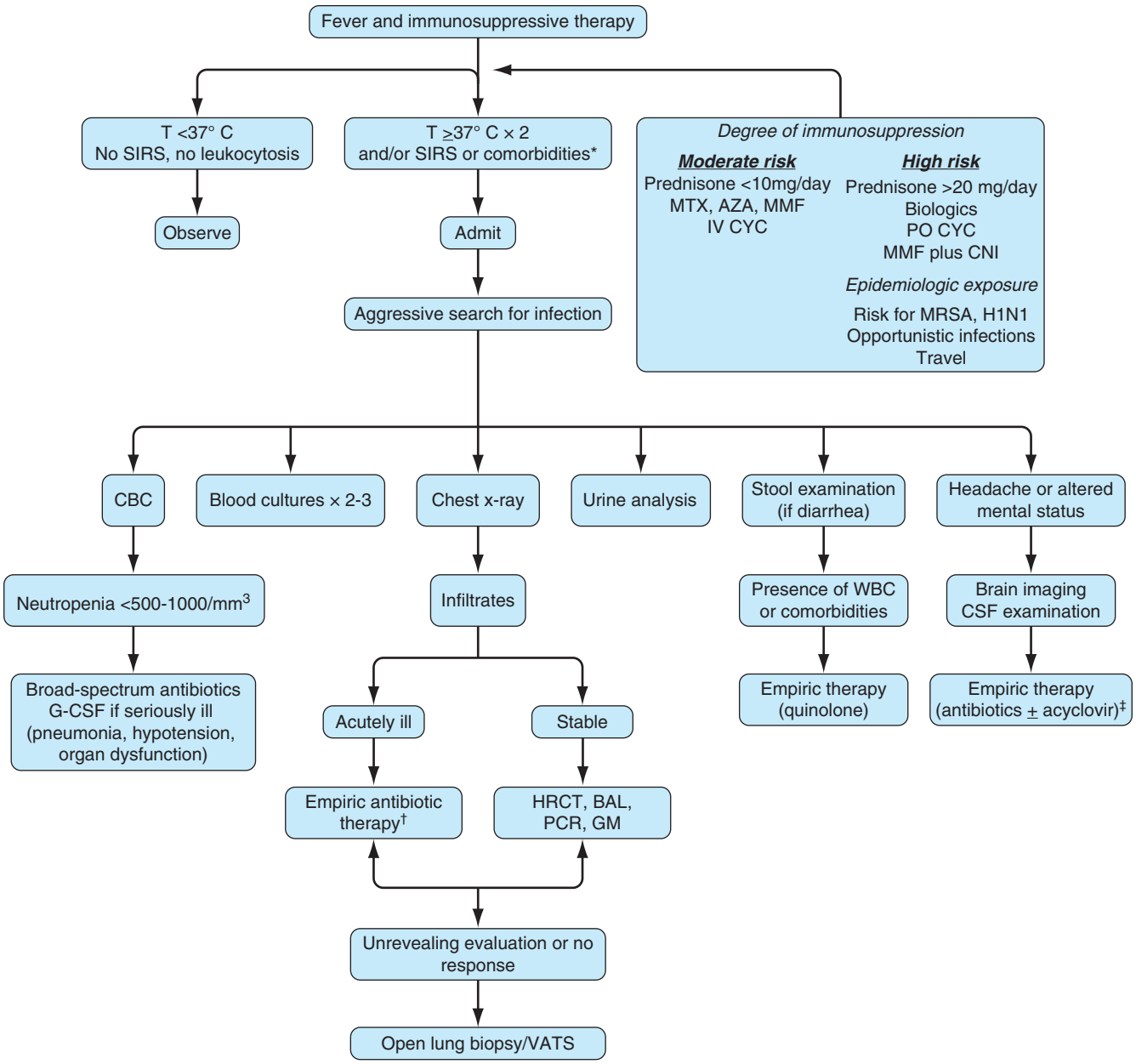



**Figure 81-4** Initial assessment and management of patients with systemic lupus erythematosus who receive immunosuppressive therapy and are seen with fever or other symptoms and signs suggestive of infection. *Systemic inflammatory response syndrome (SIRS): T ≥38° C or <36° C, tachycardia (heart rate >90/min), tachypnea (respiratory rate >20/min), white blood cells (WBC) >12,000/mm$^3$; comorbidities: age older than 65 years, diabetes, chronic cardiopulmonary disease. †Consider empiric therapy for *Pneumocystis* pneumonia in severe hypoxemia or diffuse pulmonary infiltrates. ‡Consider tuberculosis and other opportunistic central nervous system (CNS) infections. AZA, Azathioprine; BAL, bronchoalveolar lavage; CBC, complete blood count; CSF, cerebrospinal fluid; G-CSF, granulocyte colony-stimulating factor; GM, galactomannan; H1N1, influenza H1N1; HRCT, high-resolution chest tomography; IV CYC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; MRSA, methicillin-resistant *Staphylococcus aureus*; MTX, methotrexate; PCR, polymerase chain reaction; T, temperature; VATS, video-assisted thoracoscopy. *(Modified from Papadimitraki ED, Bertsias K, Chamilos MD, Boumpas DT: Systemic lupus erythematosus: cytotoxic drugs. In Tsokos G, Buyon JP, Koike T, Lahita RG, editors:* Systemic lupus erythematosus*, ed 5, St. Louis, 2010, Elsevier, pp 1083–1108.)*

to achieve remission, and systolic hypertension.[133,167,168] Patients with rapid deterioration of renal function are more likely to have a reversible physiologic (dehydration, infection, acute tubular necrosis) or pathologic (crescentic glomerulonephritis) component accounting for their renal insufficiency. In these patients, immunosuppressive therapy (pulse of IV MP and IV CYC 0.4 to 0.5 g/m$^2$, administered 8 to 10 hours before dialysis) may continue during dialysis.

The 5-year survival rate of patients with SLE on dialysis approximates 80% to 90%, comparable with that in non-SLE dialysis patients.[169] Hemodialysis may be the first choice of renal replacement therapy, especially for patients who are still on immunosuppressive therapy, due to increased rates of infectious complications (most commonly peritonitis) in patients on chronic ambulatory peritoneal dialysis (CAPD). Irrespective of the dialysis mode, most patients experience a decline in lupus activity. Discontinuation of

cytotoxic therapy may be considered in patients with steadily rising Cr to greater than or equal to 5 mg/dL with inactive urine sediment, renal biopsy showing exclusively scarring and atrophy, or contracted renal size. Judicious use of GCs and immunosuppressive drugs is essential to minimize the risk for septic complications. Cardiovascular morbidity and mortality are also increased in patients with ESRD, underscoring the need for tight control of atherosclerotic risk factors.[170]

### Renal Transplantation

Renal transplantation is a viable alternative for lupus patients with ESRD. Graft and patient survival rates are generally comparable with those in other patient groups.[171] Results are superior with living compared to cadaveric donor transplantation. Pre-emptive transplantation should be considered because it is associated with superior graft and patient outcomes. Nevertheless, a period of at least 3 months on dialysis may allow some patients to recover adequate renal function for significant time periods.

There are no prospective studies comparing various immunosuppressive regimens after renal transplantation in SLE. Calcineurin inhibitors are generally included in the induction phase (6 to 12 months) to prevent acute transplant rejection.[172] Due to potential nephrotoxicity and adverse metabolic effects, their use should be minimized during the maintenance phase and other immunosuppressive agents (MMF, AZA) should preferentially be employed. Recurrence of LN (usually mild mesangial proliferative disease) in the renal allograft is rare (3%) and not a major cause of graft loss. Risk factors for recurrence include black race, female gender, age younger than 33 years, and living-donor transplantation.[173,174] APS has been associated with post-transplant renal thrombosis and poor graft outcome and warrants anti-coagulation therapy.[175]

## Cardiovascular Morbidity

Traditional risk factors fail to fully account for the increased risk for atherosclerotic CVD in patients with SLE.[176] Biomarkers such as pro-inflammatory high-density lipoprotein (HDL) or high-sensitivity C-reactive protein are elevated in lupus patients and correlate with carotid intima media thickness (CIMT), a surrogate marker for subclinical atherosclerosis.[177,178]

Tight control of CVD risk factors is recommended for patients with SLE. The importance of smoking cessation cannot be overemphasized. Hypertension should be controlled below 130/80 mm Hg; for most cases, ACE inhibitors or angiotensin receptor blockers (ARBs), or both, are the initial treatments of choice. For dyslipidemia, two recent RCTs in adult and pediatric SLE failed to show benefits of atorvastatin in CIMT and coronary artery calcium.[179,180] The relatively small sample size and duration may have partly contributed to the negative results. Accordingly, lipid-lowering therapy in lupus should follow the guidelines for the general population.[181] Aggressive control of dyslipidemia (target LDL-C <100 mg/dL and triglycerides <150 mg/dL) is recommended for patients with multiple risk factors, especially those with at least moderate lupus or on chronic GC therapy.

## Osteoporosis

Ongoing disease activity, premature menopause caused by use of immunosuppressive drugs, relative vitamin D deficiency (associated with sun avoidance), and the use of systematic GCs all contribute to reduced bone mineral density (BMD) in SLE.[182] Quality indicators suggest that (1) all patients with SLE receiving prednisone greater than or equal to 7.5 mg/day for 3 or more months should undergo BMD testing unless already on anti-resorptive or anabolic therapy and receive osteoporosis prophylaxis with calcium and vitamin D supplements, and (2) patients receiving prednisone greater than or equal to 7.5 mg/day for 1 or more months and having a central T score of −2.5 or less or a history of fragility fracture should be treated with an anti-resorptive or anabolic agent unless contraindications exist.[183] This prednisone dosage is higher than the guideline proposed by the ACR for prevention of steroid-induced osteoporosis (5 mg/day) in the general population. The authors recommend osteoporosis prophylaxis with daily prednisone doses of greater than or equal to 5 mg for postmenopausal women and greater than or equal to 7.5 mg for pre-menopausal women.

## Malignancy in Lupus

Patients with SLE have a marginally increased risk for overall cancer development (standardized incidence ratio [SIR], 1.14).[184] Certain types of malignancies occur more commonly in patients with SLE, in particular non-Hodgkin's lymphoma (NHL), cervical and lung cancer, and some rare types (e.g., hepatobiliary tract, anal canal, and vagina/vulva cancer).[185] Both immunosuppressive therapy and the disease *per se* have been implicated for this increased risk.

NHL is associated with SLE (SIR, 3.6-4.3).[184,186] The most common subtype is diffuse large B cell lymphoma. Hodgkin's lymphoma (HL) is also more frequent (SIR, 3.2).[187, 188] A high index of suspicion is necessary for early detection of lymphoma, followed by aggressive investigation and a low threshold for lymph node biopsy.

Cervical dysplasia is increased in women with SLE (pooled OR, 8.7[189]) as a result of impaired clearance of human papillomavirus (HPV) resulting from exposure to immunosuppressive agents. Screening cervical cytology with HPV testing is recommended for all female patients with SLE; for women with detectable HPV DNA, subsequent screening with cervical cytology should be performed every 6 months. For the remaining patients, annual rescreening is sufficient. EULAR recommends HPV vaccination for women with SLE until the age of 25 years, similar to the general population.[165] Increased risk for lung cancer may be smaller than previously believed, yet higher in smokers (SIR, 1.16-1.30).[184,190] Patients with SLE have a slightly reduced risk of certain nonhematologic cancers (breast, ovarian, endometrial, and prostate: SIRs, 0.66 to 0.77).[184]

## Emergencies in Patients with Lupus

Patients with SLE may visit the emergency department (ED) for complications related to lupus itself or its therapy, or for unrelated reasons. Critical questions confronting the clinician are (1) whether the event is related to lupus and

(2) whether in the presence of lupus the management should differ. SLE-related emergencies frequently occur when disease is active. Poor compliance, low education level, severity of the underlying disease, and higher damage scores are risk factors for hospitalization.

Patients with SLE may also develop life-threatening organ dysfunction, related to disease activity or immunosuppressive therapy, severe enough to require admission to the intensive care unit (ICU). Infections are the leading cause of admission (45% to 61%). Overall mortality is high, although it has declined during the past decade (from 47% to 24%).[191] An aggressive search for infection and empiric anti-microbial therapy are crucial. When it is not clear whether the underlying process is related to infection or active disease, empiric anti-microbial therapy together with high-dose GC (0.5 to 1 mg/kg/day) may suffice. Once infection has been ruled out, pulse steroids or CYC, or both, may be used. Agents such as IVIG, plasmapheresis, and RTX may be considered as adjuvant therapy in life-threatening disease or in cases of deterioration or inadequate response following 1 to 2 weeks of therapy.

## WOMEN'S HEALTH ISSUES

**KEY POINTS**

**Gonadotropin-releasing hormone analogues decrease rates of gonadal failure in young women with severe SLE treated with CYC.**

**Contraceptive methods, including hormone agents, are generally safe for most patients with SLE.**

Premature ovarian failure is an age- and dose-dependent adverse effect of CYC therapy, whereas male gonadal toxicity may be observed with as little as 7 g cumulative CYC dose. Strategies to preserve fertility in post-pubertal SLE women include hormonal contraceptives, gonadotropin-releasing hormone (GnRH) antagonists, and embryo and oocyte cryopreservation. Individual patient preferences should be considered. Some authors recommend a GnRH antagonist–based protocol in CYC-treated female patients to protect against premature ovarian failure. In men, administration of testosterone and sperm banking represent valid strategies for preservation of testicular function and/or fertility (see Table 81-3).[192]

Hormonal contraceptive methods are generally safe for most patients with SLE with stable, inactive disease. Two RCTs found no significant increase in flares in patients without severe disease activity at study entry, and a systematic review concluded that benefits of use outweigh potential risks for most contraceptive methods.[193] However, women with APAs should avoid combined hormonal methods because of increased risk for thrombosis. Coexisting conditions such as severe thrombocytopenia, atherosclerosis, hypertension, and venous thrombosis could also make certain contraceptives less advisable.

Women with SLE may experience lower urinary tract symptoms, often accompanied by voiding dysfunction (small bladder capacity, low urinary flow rate).[194] The approach to investigation and management of recurrent urinary tract infections is similar to that in the general population; control of disease activity may prevent bladder dysfunction. Lupus mastitis represents a rare form of lupus panniculitis that occurs in 2% of women with SLE and may clinically mimic breast carcinoma.[195] GC treatment is generally effective, but refractory forms may necessitate surgical excision.

## CONCLUSION

This past decade has witnessed major advances in defining risk factors and phenotypes, elucidating pathogenesis, and optimizing treatment. Recognition of adjuvant-like factors that promote the production of type I interferon via Toll-like receptors offers new targets for therapy in addition to those targeting B and T cells. Exploration of the genetic and environmental factors that determine susceptibility to disease may eventually lead to the identification of individuals at risk and elucidate the primary events that cause autoimmunity. In contrast to monogenic diseases, the expansion of personalized medicine in lupus awaits a more complete description of predisposition. Genome-wide, next-generation sequencing efforts now underway will provide within the next few years a more comprehensive description of the relations between genome sequence variation and clinical phenotypes.

Meanwhile, new drugs have been added to the armamentarium and new therapeutic strategies are aimed at inducing prompt remission with more intense therapy and prevention of flares with less toxic therapies. There is finally a consensus that what is more important is a strategy aiming at remission and its maintenance with the treatment that best fits the patient. With the approval of the first biologic agent, the disease is finally showing signs of yielding to more targeted therapy and unraveling its heterogeneity and complexity. Most important, it has become clearer than ever that optimal long-term outcome requires not only treatment of the disease flares but also management of comorbidities. To this end, lupus highlights the need for a multidisciplinary approach and superb internal medicine skills.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 82

# Anti-phospholipid Syndrome



Doruk Erkan • Jane E. Salmon • Michael D. Lockshin

**KEY POINTS**

- **Anti-phospholipid antibodies (aPLs) are a family of autoantibodies directed against phospholipid-binding plasma proteins, most commonly β2-glycoprotein 1.**
- **The clinical manifestations range from asymptomatic to arterial/venous thrombosis to catastrophic anti-phospholipid syndrome (APS).**
- **Pregnancy losses typically occur after 10 weeks' gestation (fetal loss), but earlier losses also occur.**
- **Diagnosis should be made in the presence of characteristic clinical manifestations and persistently positive aPLs measured at least 12 weeks apart.**
- **Prevention of secondary thrombosis lacks a risk-stratified approach; the effectiveness of high-intensity anticoagulation is not supported by prospective controlled studies.**
- **A common strategy to prevent fetal loss in aPL-positive patients with a history of pregnancy morbidities is low-dose aspirin and heparin.**
- **Primary thrombosis prevention in persistently aPL-positive persons requires a risk-stratified approach; elimination of reversible thrombosis risk factors and prophylaxis during high-risk periods are crucial. The effectiveness of aspirin is not supported by prospective controlled studies.**
- **Patients with catastrophic APS usually receive a combination of anticoagulation, corticosteroids, intravenous immunoglobulin, and plasma exchange.**

Diagnosis of the anti-phospholipid syndrome (APS) requires that a patient have both a clinical event (thrombosis or pregnancy morbidity) and the persistent presence of the anti-phospholipid antibody (aPL), documented by a solid-phase serum assay (anti-cardiolipin antibody [aCL] or anti–β-2-glycoprotein I antibody [aβ2GPI] IgG or IgM), a coagulation assay (inhibitor of phospholipid-dependent clotting—the lupus anticoagulant test), or both. Preliminary (Sapporo) classification criteria for APS,[1] revised in 2004,[2] are listed in Table 82-1.

Certain factors are not included as criteria but may be helpful in the diagnosis of individual patients. Some of these factors include IgA aCL or aβ2GPI, valvular heart disease, aPL nephropathy, thrombocytopenia, and livedo reticularis (Table 82-2). These factors are rare, nonstandardized, or nonspecific phenomena that are too unreliable for use in clinical studies, but they occur in a sufficient number of patients to support a suspected diagnosis.

APS can occur as an isolated diagnosis (primary APS), or it can be associated with systemic lupus erythematosus (SLE) or another rheumatic disease. Transient aPL positivity, but probably not the syndrome, can be induced by drugs and infection.[3]

## EPIDEMIOLOGY

Low-titer, usually transient, aCL occurs in up to 10% of normal blood donors[4,5]; however, persistent moderate- to high-titer aCL/aβ2GPI or a positive lupus anticoagulant test occurs in less than 1%. The prevalence of positive aPL tests increases with age. Ten percent to 40% of patients with SLE[5] and approximately 20% of patients with rheumatoid arthritis[6] have positive aPL tests; however, the incidence of APS is relatively low.

Based on a limited number of uncontrolled and non–risk-stratified studies, aPL-positive patients who are asymptomatic (i.e., have no history of vascular or pregnancy events) have a 0% to 4% annual risk of thrombosis, whereas patients with other autoimmune diseases such as SLE are at the higher end of the range.[7,8] The aPL profile (low vs. high risk for thrombosis) and patients' clinical characteristics (e.g., the presence or absence of other acquired or genetic thrombosis risk factors) influence the individual risk of thrombosis.[9]

Although determining the frequency of a high-risk aPL profile in general population patients (with no autoimmune diseases) who have aPL-related clinical outcomes is difficult because of a lack of robust data, the best estimates for the overall aPL frequency is 6% for pregnancy morbidity, 14% for stroke, 17% for young age (<50 years), 11% for myocardial infarction, and 10% for deep vein thrombosis.[10,11] Twenty percent of women who have experienced three or more consecutive fetal losses[12] and 14% of patients with recurrent venous thromboembolic disease have aPL.[13]

## ETIOLOGY

The main antigen to which aPLs bind is not a phospholipid but rather a phospholipid-binding plasma protein, namely, β2-glycoprotein 1 (β2-GP1) apolipoprotein H. β2-GP1 is normally present in serum at a concentration of 200 mg/mL, is a member of the complement control protein family, and has five repeating domains and several alleles. An octapeptide in the fifth domain and critical cysteine bonds are necessary for both phospholipid binding and antigenicity[14]; a first-domain site activates platelets.[15,16] In vivo, β2-GP1 binds to phosphatidylserine on activated or apoptotic cell

**TABLE 82-1** Revised Sapporo Classification Criteria for Anti-phospholipid Syndrome

| |
|---|
| **Clinical Criteria** |
| 1. Vascular thrombosis*<br>One or more clinical episodes† of arterial, venous, or small vessel thrombosis‡ in any tissue or organ |
| 2. Pregnancy morbidity<br>(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, *or*<br>(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia, severe pre-eclampsia, or recognized features of placental insufficiency§, *or*<br>(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded |
| **Laboratory Criteria** |
| 1. Lupus anticoagulant present in plasma on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis |
| 2. Anti-cardiolipin antibody of IgG or IgM isotype in serum or plasma, present in medium or high titer (>40 IgG phospholipid [GPL] or IgM phospholipid [MPL], or >99th percentile), on two or more occasions at least 12 weeks apart, measured by a standardized ELISA |
| 3. Anti–β-2-glycoprotein I antibody of IgG or IgM isotype in serum or plasma (in titer >99th percentile) present on two or more occasions at least 12 weeks apart, measured by a standardized ELISA |
| Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met. Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive anti-phospholipid antibody test and the clinical manifestation. In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c in this table. |

*Coexisting inherited or acquired factors for thrombosis are not reasons to exclude patients from APS trials. However, two subgroups of patients with APS should be recognized by the presence and absence of additional risk factors for thrombosis. Indicative (but not exhaustive) cases include age (>55 years in men and >65 years in women), the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated low-density lipoprotein or low high-density lipoprotein cholesterol, cigarette smoking, family history of premature cardiovascular disease, body mass index >30 kg/m$^2$, microalbuminuria, and estimated glomerular filtration rate <60 mL/min), inherited thrombophilias, oral contraceptive use, nephritic syndrome, malignancy, immobilization, and surgery. Thus patients who fulfill these criteria should be stratified according to contributing causes of thrombosis.

†A thrombotic episode in the past can be considered a clinical criterion, provided that thrombosis is proved by appropriate diagnostic means and that no alternative diagnosis or cause of thrombosis is found.

‡Superficial venous thrombosis is not included in the clinical criteria.

§Generally accepted features of placental insufficiency include an abnormal or nonreassuring fetal surveillance test (e.g., a nonreactive nonstress test) suggestive of fetal hypoxemia, an abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia (e.g., absent end-diastolic flow in the umbilical artery), oligohydramnios (e.g., amniotic fluid index ≤5 cm), or a postnatal birth weight less than the 10th percentile for gestational age.

Investigators are strongly advised to classify patients with APS who are participating in studies into one of the following categories: I, more than one laboratory criteria present (any combination); IIa, only lupus anticoagulant present; IIb, only anti-cardiolipin antibody present; IIc, only anti–β-2-glycoprotein I antibody present.

APS, Anti-phospholipid syndrome; ELISA, enzyme-linked immunosorbent assay.

From Miyakis S, Lockshin MD, Atsumi T, et al: International consensus statement on an update of the classification criteria for definite anti-phospholipid syndrome. *J Thromb Haemost* 4:295–306, 2006.

**TABLE 82-2** Other Features Suggesting the Presence of Anti-phospholipid Antibodies

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|---|
| **Clinical** |
| Livedo reticularis |
| Thrombocytopenia (usually 50,000-100,000 platelets/mm$^3$) |
| Autoimmune hemolytic anemia |
| Cardiac valve disease (vegetations or thickening) |
| Multiple sclerosis–like syndrome, chorea, or other myelopathy |
| **Laboratory** |
| IgA anticardiolipin antibody |
| IgA anti–β-2-glycoprotein I |

membranes, including those of trophoblasts, platelets, and endothelial cells. Under physiologic conditions, β2-GP1 may function in the elimination of apoptotic cells[17] and as a natural anticoagulant.[18] Other, less relevant antigens targeted by aPLs are prothrombin, annexin V, protein C, protein S, high- and low-molecular-weight kininogens, tissue plasminogen activator, factor VII, factor XI, factor XII, complement component C4, and complement factor H.[19]

In experimental animal models, passive or active immunization with viral peptides,[20] bacterial peptides,[21] and heterologous β2-GP1[22] induces polyclonal aPLs and clinical events associated with APS. These data suggest that pathologic autoimmune aPL is induced in susceptible humans by infection via molecular mimicry. aPLs are able to upregulate the expression of Toll-like receptor 7 on plasmacytoid dendritic cells, sensitizing these cells to ligand, and thereby perpetuating and amplifying autoimmune responses.[23]

However, infection-induced aPLs (syphilitic and nonsyphilitic *Treponema*, *Borrelia burgdorferi*, human immunodeficiency virus, *Leptospira*, or parasites) are usually β2-GP1 independent and bind phospholipids directly.[24] Drugs (e.g., chlorpromazine, procainamide, quinidine, and phenytoin) and malignancies (e.g., lymphoproliferative disorders) also can induce β2-GP1–independent aPLs. Conversely, autoimmune aPLs bind β2-GP1 or other phospholipid-binding plasma proteins, which in turn bind negatively charged phospholipids such as cardiolipin (β2-GP1–dependent aPLs).

Low levels of aPL may be present normally; one of the functions of normal aPL may be to participate in the physiologic removal of oxidized lipids.

## PATHOGENESIS

Thrombosis is induced by aPLs through multiple mechanisms. Potential pathogenic pathways are illustrated in Figure 82-1. The "two-hit" model of thrombosis associated

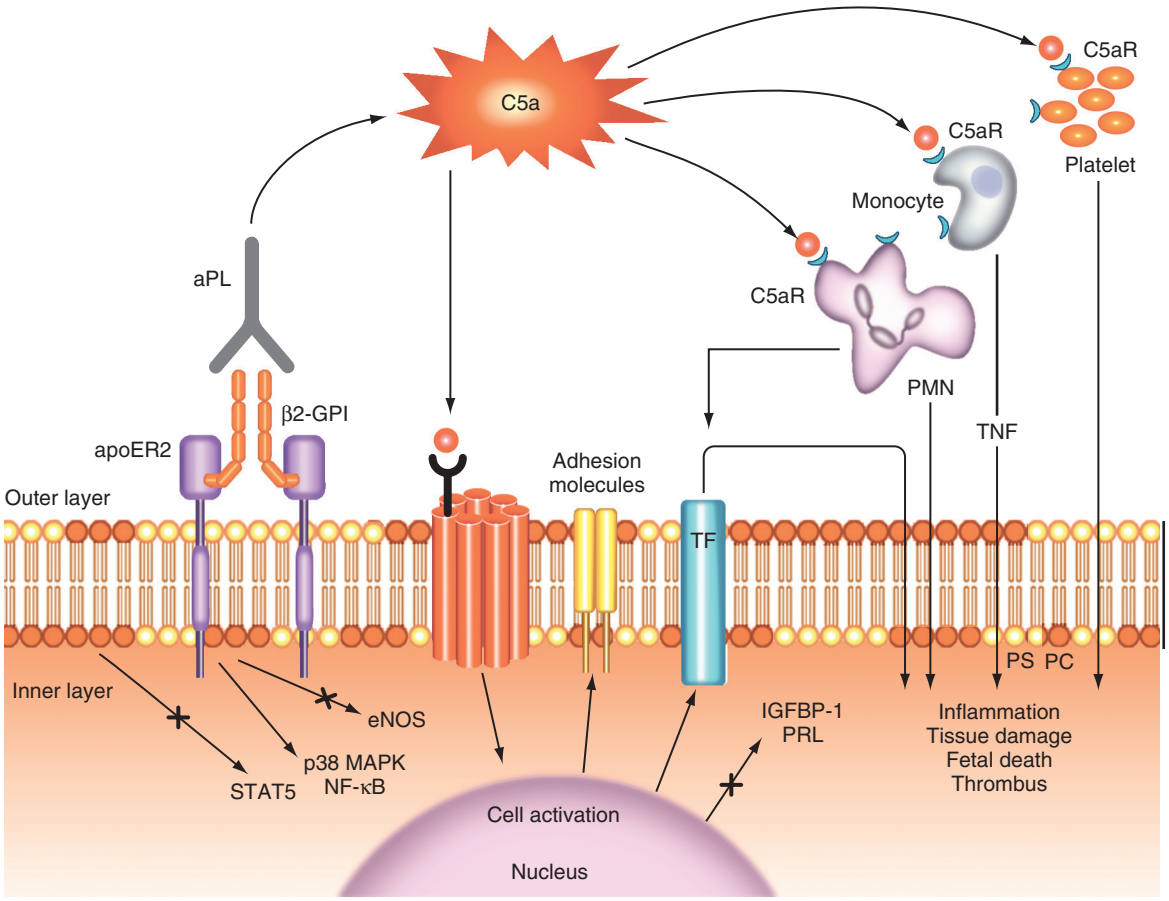



**Figure 82-1** Proposed mechanism of anti-phospholipid antibody (aPL)-related thrombosis and placental injury. The negatively charged phospholipid phosphatidylserine (PS, *yellow circles*) migrates from the inner to the outer cell membrane during activation or apoptosis of platelets and endothelial cells, and it is normally present on trophoblasts. The neutral phospholipid phosphatidylcholine (PC, *red circles*) is the major constituent of the outer layer of inactivated cells. Dimeric β2-glycoprotein 1 (β2-GP1) then binds to PS (probably via β2-GP1 surface receptors such as apoER2′, annexin A2, or a Toll-like receptor), and aPL binds to β2-GP1, activating the classic complement pathway and leading to the generation of C5a, which induces (1) expression of adhesion molecules (e.g., intra-cellular adhesion molecule-1 and tissue factor [TF]), and (2) activation of monocytes, polymorphonuclear (PMN) cells, and platelets, resulting in the release of pro-inflammatory mediators (e.g., TNF and vascular endothelial growth factor receptor-1) and initiation of the proadhesive and prothrombotic state. Cross-linking apoER2′ receptors antagonizes endothelial nitric oxide synthase (eNOS), blocking its phosphorylation and leading to increased leukocyte adhesion and thrombosis. Both nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (p38 MAPK) play a role in the intra-cellular signaling cascade. Anti-phospholipid antibodies also downregulate the expression of trophoblast signal transducer and activator of transcription 5 (STAT5), reducing the endometrial stromal cell production of prolactin (PRL) and insulin growth factor binding protein-1 (IGFBP-1).

with aPL proposes that the initiating "first hit" disrupts the endothelium, and the "second hit" potentiates thrombosis.[25] The process begins with activation or apoptosis of platelets, endothelial cells, or trophoblasts, during which phosphatidylserine (a negatively charged phospholipid) migrates from the inner to the normally electrically neutral outer cell membrane. Circulating β2-GP1 binds to phosphatidylserine, and then aPL binds to a β2-GP1 dimer.[26]

The aPL–β2-GP1 dimer binding activates the complement cascade extra-cellularly; initiates an intra-cellular signaling cascade, probably through the C5a and β2-GP1 surface receptors; and recruits and activates inflammatory effector cells, including monocytes, neutrophils, and platelets, leading to the release of pro-inflammatory products (e.g., TNF, oxidants, and proteases) and the induction of a prothrombotic phenotype.[25-27] The putative receptor of β2-GP1 binding protein that transduces signals from the cell membrane to the nucleus is not yet identified and may vary among cells. The following candidates have been suggested: apoER2 (a member of the low-density lipoprotein receptor superfamily), annexin A2, and a Toll-like receptor.[28-30] Both nuclear factor-κB and p38 mitogen-activated protein kinase play a role in the intra-cellular signaling cascade.[31,32]

In addition, through downregulation of the signal transducer and activator of transcription 5 (STAT5), aPLs inhibit the production of placental prolactin and insulin-like growth factor binding protein-1,[33] and they adversely affect the formation of a trophoblast syncytium, trophoblast migration, invasion, and placental apoptosis—all processes that are required for the normal establishment of placental development.

Other possible contributory mechanisms of aPL-mediated thrombosis include inhibition of coagulation cascade reactions catalyzed by phospholipids (e.g., activation of circulating procoagulant proteins and inhibition of protein C and S activation), oxidative stress, impaired function of

endothelial nitric acid synthase, induction of tissue factor (a physiologic initiator of coagulation) expression and activation on monocytes, reduction of fibrinolysis, and disruption of the annexin V anticoagulant shield.[25]

In experimental animal models, aPLs cause fetal resorption and increase the size and duration of trauma-induced venous and arterial thrombi.[34,35] Inhibiting complement activation prevents experimental aPL-induced fetal death and angiogenic dysregulation–associated abnormal placental development and pre-eclampsia; C5 knockout mice carry pregnancies normally despite having aPLs,[36] implying that a complement-mediated effector mechanism is an absolute requirement for fetal death to occur. Inflammation in association with activation of complement and production of inflammatory mediators such as TNF influence placental development and vascular remodeling.[37] A role for local thrombosis is less clear. Complement activation is also required for experimental thrombosis.[36,38] In addition, aPL-induced reduction of heparin-binding epidermal growth factor–like growth factor leads to defective placentation.[39]

Because high-level aPLs may persist for years in asymptomatic persons, it is likely that vascular injury, endothelial cell activation, or both immediately precede the occurrence of thrombosis in persons bearing the antibody (second-hit hypothesis). Of note, at least 50% of patients with APS who have vascular factors possess other acquired thrombosis risk factors at the time their events occur.[40,41]

Persons congenitally lacking β2-GP1,[42] as well as β2-GP1 knockout mice, appear normal. Although mice lacking β2-GP1 are fertile, the success of early pregnancy is moderately compromised; β2-GP1 is believed necessary for optimal implantation and placental morphogenesis.[43] In certain mouse models of lupus, deletion of β2-GPI potentiates autoimmunity.[44] β2-GP1 polymorphisms influence the generation of aPLs in individuals, but they have only a weak relationship to the occurrence of APS.[45] A cluster of 50 upregulated genes may have an effect on the occurrence of thrombosis in aPL-positive persons.[46]

## CLINICAL FEATURES

Clinical manifestations range from asymptomatic aPL positivity (no history of vascular or pregnancy events) to catastrophic APS (multiple thromboses occurring over days). Thus patients should not be evaluated and managed as if they have a single disease.

### Vascular Occlusion

APS affects all organ systems. Its principal manifestations are venous or arterial thromboses and pregnancy loss (see Table 82-1). Except for their severity, the youth of affected patients, and the unusual anatomic locations (Budd-Chiari syndrome; sagittal sinus and upper extremity thromboses), venous thromboses in APS do not differ clinically from thromboses attributable to other causes. Catastrophic APS can overlap with other thrombotic microangiopathies. Similarly, arterial thromboses differ from non–aPL-associated thromboses only by their recurrent nature, unusual locations, and occurrence in young patients. Deep vein thrombosis and stroke are the most common clinical manifestations of APS. Renal thrombotic microangiopathy, glomerular capillary endothelial cell injury, and thrombosis of renal vessels cause proteinuria without cells in the urine or hypocomplementemia and may lead to severe hypertension, renal failure, or both.[47]

### Pregnancy Morbidity

Pregnancy losses in patients with aPLs typically occur after 10 weeks' gestation (fetal loss), although earlier losses also occur. These pre-embryonic or embryonic pregnancy losses (<10 weeks' gestation) are commonly due to chromosomal and other genetic defects. Pregnancy in women with APS is often normal until the second trimester, when fetal growth slows and amniotic fluid volume decreases. Patients with APS may experience severe, early pre-eclampsia or HELLP (*H*emolysis, *E*levated *L*iver enzymes, *L*ow *P*latelets) syndrome.

In a prospective, multicenter study (PROMISSE), the primary predictor of adverse pregnancy outcome after 12 weeks' gestation in aPL-associated pregnancies was lupus anticoagulant.[48] Prior thrombosis and SLE were also risk factors of adverse pregnancy outcomes. Prior pregnancy loss did not predict outcomes, but previous studies suggested that prior late pregnancy losses predict future losses, independent of the aPL profile.

### Miscellaneous and Noncriteria Manifestations

Many patients have livedo reticularis or thrombocytopenia (Figure 82-2), although these conditions are not specific for APS. The incidence of cardiac valve disease (vegetations, thickening, or both), which is a late manifestation, along with its pathogenesis in persons with APS, are unknown. Recent studies suggest that APS does not add to the risk of atherosclerosis imparted by SLE.[49] Pulmonary hypertension may develop as a result of recurrent pulmonary embolism or small vessel thrombosis; rarely, aPL-positive patients may present with diffuse pulmonary hemorrhage. Some patients experience nonfocal neurologic symptoms such as lack of concentration, forgetfulness, and dizzy spells. Multiple small, hyperintense lesions seen on MRI, primarily in the periventricular white matter, do not correlate well with clinical symptoms. Rarely, high-affinity antiprothrombin

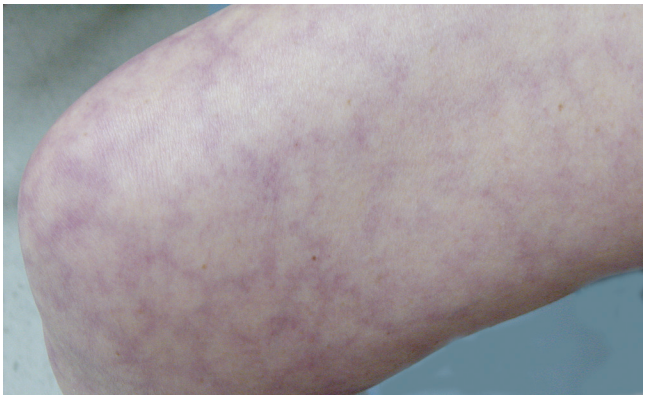


**Figure 82-2** Livedo reticularis in a person with anti-phospholipid syndrome.

antibodies may cause hemorrhage by depleting prothrombin (lupus anticoagulant hypoprothrombinemia syndrome).[50]

### Catastrophic Anti-phospholipid Syndrome

Catastrophic APS is a rare, abrupt, life-threatening complication. It consists of multiple thromboses of medium and small arteries occurring (despite apparently adequate anticoagulation) over a period of days and causing stroke; cardiac, hepatic, adrenal, renal, and intestinal infarction; and peripheral gangrene.[51] In a review of 220 patients with catastrophic APS, the main clinical manifestations included renal involvement in 154 patients (70%), pulmonary involvement in 146 patients (66%), cerebral involvement in 133 patients (60%), cardiac involvement in 115 patients (52%), and cutaneous involvement in 104 patients (47%).[52] Acute adrenal failure may be the initial clinical event. Proposed formal criteria for this syndrome are shown in Table 82-3.[51] Patients often have moderate thrombocytopenia and other features of thrombotic microangiopathies[53]; erythrocytes are less fragmented than in the hemolytic uremic syndrome or thrombotic thrombocytopenic purpura, and fibrin split products are not strikingly elevated. Renal failure and pulmonary hemorrhage may occur. Tissue biopsy specimens show non-inflammatory vascular occlusion.

**TABLE 82-3** Preliminary Criteria for the Classification of Catastrophic Anti-phospholipid Syndrome

| |
|---|
| 1. Evidence of involvement of three or more organs, systems, or tissues* |
| 2. Development of manifestations simultaneously or in less than 1 week |
| 3. Confirmation by histopathology of small vessel occlusion in at least one organ or tissue† |
| 4. Laboratory confirmation of the presence of anti-phospholipid antibody (lupus anticoagulant or anti-cardiolipin or anti–β-2-glycoprotein I antibodies)‡ |
| **Definite Catastrophic APS**<br>All four criteria |
| **Probable Catastrophic APS**<br>Criteria 2 through 4 and two organs, systems, or tissues involved |
| Criteria 1 through 3, except no confirmation 6 weeks apart as a result of the early death of the patient not tested before the catastrophic episode |
| Criteria 1, 2, and 4 |
| Criteria 1, 3, and 4 and development of a third event more than 1 week but less than 1 month after the first, despite anticoagulation |

*Usually, clinical evidence of vessel occlusions, confirmed by imaging techniques when appropriate. Renal involvement is defined by a 50% rise in the serum creatinine level, severe systemic hypertension, proteinuria, or some combination of these.

†For histopathologic confirmation, significant evidence of thrombosis must be present, although vasculitis occasionally may coexist.

‡If the patient has not been diagnosed previously with APS, laboratory confirmation requires that the presence of anti-phospholipid antibody be detected on two or more occasions at least 6 weeks apart (not necessarily at the time of the event), according to proposed preliminary criteria for the classification of APS.

APS, Anti-phospholipid syndrome.

From Asherson RA, Cervera R, de Groot PG, et al: Catastrophic anti-phospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus* 12:530–534, 2003.

## DIAGNOSIS AND DIAGNOSTIC TESTS

### Laboratory Studies

The diagnosis of APS requires a positive lupus anticoagulant test or a moderate- to high-titer aCL or anti–β2-GP1 IgG or IgM test in patients with characteristic clinical manifestations. Patients with negative lupus anticoagulant tests, aCL, and aβ2GPI should be tested for IgA aCL and aβ2GPI when there is a high suspicion for APS. Positive aPL results require a repeat test after 12 or more weeks to exclude a transient, clinically unimportant antibody. The diagnosis of APS should be questioned if less than 12 weeks or more than 5 years separate the positive aPL test from the clinical manifestation.[2]

The lupus anticoagulant test is a more specific but less sensitive predictor of thromboses than is the anti-cardiolipin test; it correlates better with aPL-related clinical events.[54] Lupus anticoagulant is the strongest aPL test for prediction of pregnancy complications; the predictive power of aCL is due to its association with lupus anticoagulant.[48] However, both false-positive and false-negative lupus anticoagulant test results may occur in patients who have undergone anticoagulation with heparin or warfarin. Documentation of a lupus anticoagulant requires a four-step process: (1) demonstration of a prolonged phospholipid-dependent coagulation screening test, such as activated partial thromboplastin time or dilute Russell viper venom time (however, low-level abnormalities are not clearly linked to APS); (2) failure to correct the prolonged screening test by mixing the patient's plasma with normal platelet-poor plasma, demonstrating the presence of an inhibitor; (3) shortening or correction of the prolonged screening test by the addition of excess phospholipid, demonstrating phospholipid dependency; and (4) exclusion of other inhibitors.[55] Approximately 80% of patients with lupus anticoagulant have anti-cardiolipin, and 20% of patients positive for anti-cardiolipin have lupus anticoagulant.[56]

The aCL enzyme-linked immunosorbent assay (ELISA) is sensitive but not specific for the diagnosis of APS.[57] Although the widely available ELISA test for IgG and IgM aCL is standardized, considerable variability exists among commercial laboratories that perform the test, especially for the IgA isotype.[58] Low-titer aCL or aβ2GPI, transient aPLs, and antibody to noncardiolipin phospholipids (phosphatidylserine and phosphatidylethanolamine) have no or less proven relationship to APS. ELISA tests other than aCL and aβ2GPI are neither standardized nor widely accepted as predictors of clinical illness; they are not part of the Updated APS Classification Criteria.[2]

A false-positive test for syphilis does not fulfill the laboratory criteria, but it should alert physicians to order the aPL tests previously described, especially in patients with a history of aPL-related clinical manifestations.

Whether to test persons with venous occlusive disease or recurrent fetal loss simultaneously for protein C, protein S, and antithrombin III deficiency or for the factor V Leiden and prothrombin mutations is a matter of economics and clinical likelihood; such testing is advisable when feasible. It is useful to test persons with arterial occlusive disease for hyperhomocysteinemia.

Anti-nuclear and anti-DNA antibodies occur in approximately 45% of patients clinically diagnosed as having

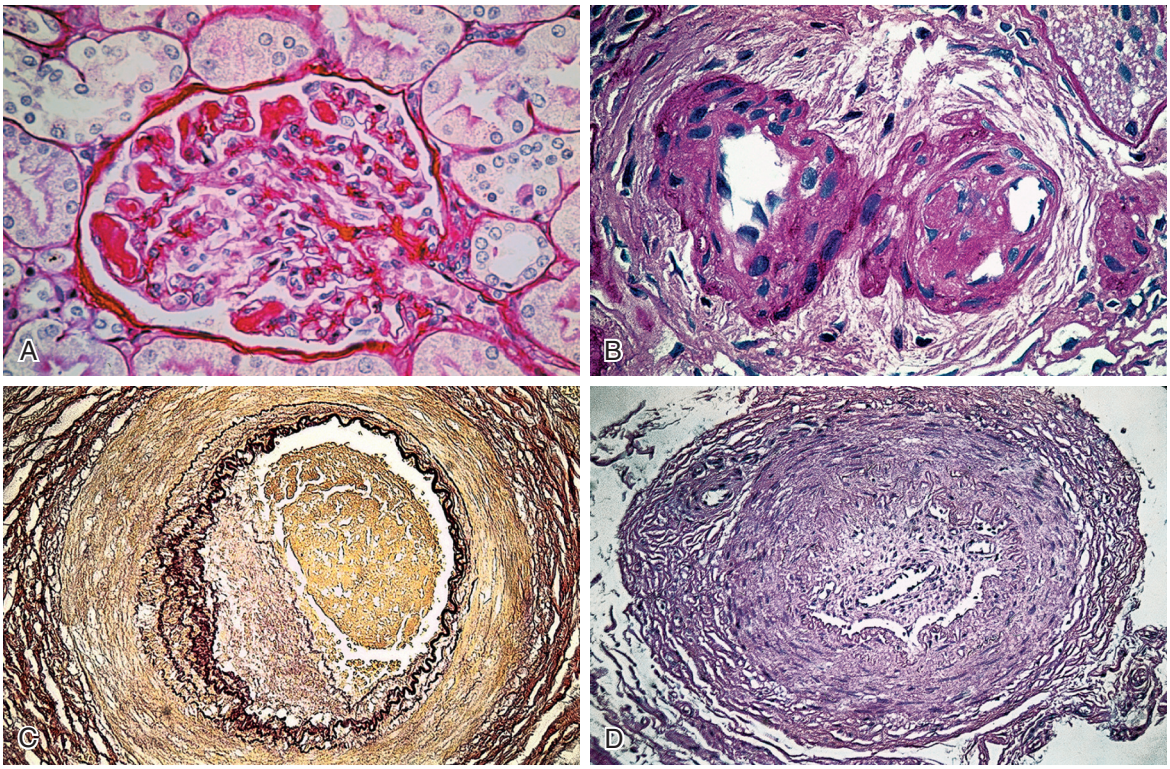



**Figure 82-3** Renal thrombotic microangiopathy in anti-phospholipid syndrome (APS). **A,** A kidney biopsy from a 35-year-old woman with primary APS, microhematuria, and non-nephrotic proteinuria. The glomerulus contains microthrombi and occluding capillary lumina, and endothelial swelling is evident. **B,** The same patient's small renal artery contains organized thrombus, with recanalization and arteriosclerosis (periodic acid–Schiff, ×100). **C,** An autopsy specimen from a 45-year-old man with primary APS. Note the thrombus in various stages of organization, intact elastic lamina with focal reduplication, and medial thickening (elastic Verhoeff stain, ×100). **D,** The same patient's medium-sized peripheral artery. Note the organized thrombus with recanalization, severe fibrointimal thickening, medial hypertrophy, and extreme stenosis of the lumen (hematoxylin and eosin, ×75). *(Courtesy Dr. Surya V. Seshan.)*

primary APS without an accompanying illness[59]; these antibodies do not mandate the additional diagnosis of SLE if the patient has no clinical indicators of SLE. Thrombocytopenia in APS is usually modest (>50,000/mm$^3$); proteinuria and renal insufficiency occur in patients with thrombotic microangiopathy. Pathologic examination demonstrates small artery and glomerular thrombi and recanalization (Figure 82-3). Hypocomplementemia, erythrocyte casts, and pyuria are not characteristic of thrombotic microangiopathy and, when present, imply lupus glomerulonephritis. Erythrocyte sedimentation rate, hemoglobin, and leukocyte count are usually normal in patients with uncomplicated primary APS, except during acute thrombosis. Prothrombin fragment 1 + 2 and other markers of coagulation activation do not predict impending thrombosis.

## Imaging Studies

MRI shows vascular occlusion and infarction consistent with clinical symptoms, with no special characteristics (other than multiple, otherwise unexplained cerebral infarctions in a young person). Multiple small, hyperintense white matter lesions are common and do not unequivocally imply brain infarction. Occlusions usually occur in vessels below the resolution limits of angiography; hence, angiography or magnetic resonance angiography is not indicated unless clinical findings suggest medium- or large-vessel disease. Echocardiography or cardiac MRI may show severe Libman-Sacks endocarditis and intracardiac thrombi.[60]

## Pathology

Skin, renal, and other tissues show thrombotic occlusion of all caliber arteries and veins, acute and chronic endothelial injury and its sequelae, and recanalization in late lesions. Uteroplacental insufficiency was once thought to be due to thrombosis or spiral artery vasculopathy (atherosis, intimal thickening, fibrinoid necrosis, and absence of physiologic changes in the spiral arteries).[61] Consistent with the importance of inflammation in murine models of APS, recent findings demonstrate inflammatory infiltrates, particularly macrophages, and suggest that inflammation contributes to placental injury in patients.[62] The finding of necrotizing vasculitis suggests concomitant lupus or other connective tissue disease. No other diagnostic immunofluorescence or electron microscopic findings have been reported.

## Differential Diagnosis

Infection-induced aCL is usually transient and is more commonly related to IgM than to IgG.[63] Transient aPLs or low-titer aCLs are inconclusive for diagnosis. Research laboratories can distinguish autoimmune from infection-induced aPLs by determining the antibody's β2-GP1

dependence. In a patient who has lupus or lupus-like disease, livedo reticularis, or long-standing thrombocytopenia and who has a persistently positive aPL test in addition to APS-related symptoms, it is usually unnecessary to exclude other diagnoses.

Because the prevalence of aPL-positive ELISA tests increases with age, and because the differential diagnosis of vascular occlusion is broader than it is in young adults, particular care is necessary in diagnosing APS in patients older than 60 years. Sustained high-titer aCL IgG, livedo reticularis, thrombocytopenia, coexisting rheumatic disease, and absence of other causes support a diagnosis of APS.

Heritable deficiency of protein C, protein S, and antithrombin III and the presence of the factor V Leiden (A506G), prothrombin (G20210A), and methylene tetrahydrofolate reductase (MTHFR, C677T) mutations are less common causes of fetal loss.[64] Attribution of pregnancy loss to APS is most certain when no coexisting plausible explanation is known, when the loss occurs after demonstration of a fetal heartbeat (10 weeks), when a significant aPL profile is repeatedly positive before and after pregnancy, and when the placenta shows vasculopathy and infarction. A single pregnancy loss before 10 weeks' gestation in a patient with a low-positive anti-cardiolipin test is more likely to be attributable to fetal chromosomal abnormalities, infection, or maternal hormonal or anatomic abnormalities.

Independent coagulopathies may further increase thrombotic risk in patients with aPLs. These and other acquired thrombotic risk factors (hypertension, diabetes, nephrotic syndrome, venous insufficiency, and immobility) are alternative causes of thromboembolic disease. Arterial occlusion occurs in patients with thrombotic thrombocytopenic purpura, infected or sterile emboli of cardiac or vascular origin, septicemia, hyperhomocysteinemia, myxoma, Takayasu's arteritis, polyarteritis nodosa, and severe Raynaud's disease. The relationship of Sneddon's syndrome (stroke and livedo reticularis, with or without aPLs) to APS is uncertain.

Few conditions mimic or overlap catastrophic APS. Among them are sepsis, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, heparin-induced thrombocytopenia, and disseminated embolization from myxoma, atrial thrombus, or atherosclerotic plaque. "Definite" and "probable" catastrophic APS have been defined based on the preliminary classification criteria[51]; however, in a real-world setting, some aPL-positive patients with multiple organ thromboses and/or thrombotic microangiopathies do not fulfill these criteria. Previous APS diagnosis and/or persistent clinically significant aPL positivity is of great importance for catastrophic anti-phospholipid syndrome (CAPS) diagnosis; however, almost half of the patients who experience CAPS do not have a history of aPL positivity.[53] Small vessel occlusions occurring in rapid succession suggest disseminated intravascular coagulation. Severe cerebral and renal disease suggests thrombotic thrombocytopenic purpura, whereas renal failure and hemolysis suggest hemolytic uremic syndrome. Anti-phospholipid antibodies are rarely present in patients with the alternative diagnoses. Acute adrenal insufficiency is highly suggestive of catastrophic APS and Waterhouse-Friderichsen syndrome.

**TABLE 82-4** Treatment Recommendations for Persons Persistently Positive for Anti-phospholipid Antibody

| Clinical Features | Recommendation |
|---|---|
| Asymptomatic | No treatment* |
| Venous thrombosis | Warfarin INR 2.5 indefinitely |
| Arterial thrombosis | Warfarin INR 2.5 indefinitely |
| Recurrent thrombosis | Warfarin INR 3-4 ± low-dose aspirin |
| Pregnancy: | |
| First pregnancy | No treatment† |
| Single pregnancy loss at <10 wk | No treatment† |
| ≥1 Fetal or ≥3 (pre)-embryonic losses, no thrombosis | Prophylactic heparin‡ + low-dose aspirin throughout pregnancy, discontinue 6-12 wk postpartum |
| Thrombosis regardless of pregnancy history | Therapeutic heparin§ or low-dose aspirin throughout pregnancy, warfarin postpartum |
| Valve nodules or deformity | No known effective treatment; full anticoagulation if emboli or intracardiac thrombi demonstrated |
| Thrombocytopenia >50,000/mm$^3$ | No treatment |
| Thrombocytopenia <50,000/mm$^3$ | Prednisone, IVIG |
| Catastrophic anti-phospholipid syndrome | Anticoagulation + corticosteroids + IVIG or plasmapheresis |

*Aspirin (81 mg/day) may be considered in high-risk patients with multiple non-aPL cardiovascular risk factors.
†Aspirin (81 mg/day) may be considered.
‡Enoxaparin 0.5 mg/kg subcutaneously once daily.
§Enoxaparin 1 mg/kg subcutaneously twice daily or 1.5 mg/kg subcutaneously once daily.
INR, International normalized ratio; IVIG, intravenous immunoglobulin.

## TREATMENT

### Thrombosis

Treatment recommendations are summarized in Table 82-4. Anticoagulation with heparin is the treatment for acute thrombosis in patients with APS. Warfarin, occasionally in association with low-dose aspirin, is used for secondary thrombosis prophylaxis. Two randomized, controlled trials demonstrated that moderate doses of warfarin (international normalized ratio [INR], 2 to 3) and high-intensity warfarin (INR, 3 to 4) are equally protective against recurrence in patients with APS after the first thrombosis.[65,66] The intensity of anticoagulation for aPL-related arterial thrombosis is still a matter for debate because in both studies, patients with arterial events constituted less than half of the study population. Although patients with APS who have arterial thrombosis and who are at high risk for recurrence may require high-intensity anticoagulation, in the absence of risk-stratified studies, *high risk* has no consensus definition and currently is based solely on clinical judgment.

Aspirin is the standard of care after an ischemic stroke or a transient ischemic attack to prevent a recurrence in aPL-negative patients. Although most aPL-positive patients

with ischemic strokes receive warfarin, the Antiphospholipid Antibody in Stroke Study (APASS) concluded that for selected aPL-positive patients who do not have atrial fibrillation or high-grade stenosis, aspirin and warfarin (target INR, 2.2) are equivalent in terms of both efficacy and major bleeding complications.[67] The APASS results probably do not apply to conventionally defined APS because the average age of study participants was much higher than that of the average APS population; in addition, the aPL determination was performed only once at study entry, and the titer cutoff for assigning a patient to the positive aCL group was very low. However, aspirin is an option for older aPL-positive patients who have a single low-titer anti-cardiolipin test and who have only had one stroke.

Some patients require larger than expected doses of both heparin and warfarin to achieve therapeutic anticoagulation. Uncommonly, positive lupus anticoagulant tests cause the INR to be unreliable.[68] Patients with constantly fluctuating INR levels and/or recurrent events despite therapeutic INR may be monitored by the measurement of anti–factor Xa activity or another appropriate assay.

For well-anticoagulated patients who continue to have thromboses, the addition of aspirin (81 to 325 mg/day) can be considered. Experimental evidence (with in vitro and/or animal models) and clinical evidence (in lupus patients) suggest that hydroxychloroquine may decrease the incidence of thrombosis; similarly, experimental evidence indicates that statins can interfere with aPL-mediated thrombosis.[69] Controlled studies are needed to determine the effectiveness of hydroxychloroquine and statins in patients with APS. Corticosteroids have no established role in the treatment of APS but are used for rheumatic symptoms in patients with accompanying systemic autoimmune illness. However, high doses of corticosteroids usually are given empirically to patients who have severe thrombocytopenia, hemolytic anemia, and catastrophic APS.

No controlled studies in patients with APS have been published for clopidogrel, pentoxifylline, aspirin-dipyridamole, argatroban, hirudin, and other new anticoagulant agents. Given lack of data, a recent Task Force on APS Treatment Trends recommended that oral direct thrombin or anti–Factor Xa inhibitors can be considered in patients with APC who have a first venous thrombosis only when there is known vitamin K antagonist allergy/intolerance or poor anticoagulant control. No data recommend use of these new agents in patients with APS who have recurrent venous thrombosis while undergoing therapeutic anticoagulation or who have APS-related arterial thrombosis.[70] Neither hirudin nor fondaparinux inactivates complement, and neither drug protects mice with experimental APS against pregnancy loss, so they may not be effective in human disease. Clinical experience suggests that thrombolytic agents for acute thrombosis are not helpful because reocclusion occurs rapidly.

Currently available data, although retrospective and not risk stratified, indicate that lifelong anticoagulation of patients with APS who have had vascular events is appropriate.[71] However, given recent recognition that many thrombotic events have recognizable triggers, a Task Force on Prevention and Management of Thrombosis recommended that in cases of a first venous event, a low-risk aPL profile, and a known transient precipitating factor, anticoagulation could be limited to 3 to 6 months.[72]

## Pregnancy Morbidity

Heparin is indicated at the diagnosis of pregnancy in an aPL-positive woman who has had prior pregnancy losses attributable to APS. Because warfarin is teratogenic, only unfractionated or low-molecular-weight heparin is used for the treatment of affected pregnancies in the United States; in other countries, converting to warfarin after the first trimester may be considered acceptable.[73] Low-molecular-weight heparin is the treatment of choice because of the decreased risk of thrombocytopenia and osteoporosis.

Patients with prior fetal losses at later than 10 gestational weeks should be treated with prophylactic heparin (enoxaparin, 30 to 40 mg subcutaneously once daily), together with low-dose aspirin; this regimen increases the fetal survival rate from 50% (untreated) to 80%.[74,75] Women who have had prior thromboses must undergo full anticoagulation (enoxaparin, 1 mg/kg subcutaneously twice daily or 1.5 mg/kg subcutaneously once daily) throughout pregnancy because the risk of new thrombosis markedly increases both during pregnancy and during the postpartum period. Even with treatment, prematurity and fetal growth restriction may occur. Clopidogrel and newer anti-thrombotic agents have not been cleared for use in pregnancy. Intravenous immunoglobulin (IVIG) and hydroxychloroquine can be considered in patients who do not respond to heparin treatment.

In aPL-positive women with prior thrombosis, warfarin is changed to heparin or low-molecular-weight heparin before conception, if possible, or at the first missed menstrual period. In aPL-positive women without prior thrombosis, if heparin is indicated during pregnancy, treatment begins after confirmation of pregnancy, continues until 48 hours before anticipated delivery (to allow epidural anesthesia), and resumes for 8 to 12 weeks during the postpartum period. Some physicians recommend the initiation of heparin before conception; no clinical trial supports this recommendation, however, and the risk associated with longer-duration heparin therapy is considerable. Patients in most published series received both low-dose aspirin and heparin, but the benefit of adding aspirin is unknown.

Because of the risk of postpartum thrombosis, it is prudent to continue anticoagulation for 8 to 12 weeks during the postpartum period and then discontinue it by tapering the doses. If desired, conversion from heparin to warfarin may be accomplished after the first or second postpartum week. Breastfeeding is permissible with both heparin and warfarin.

No studies unequivocally justify the treatment of women with aPLs during a first pregnancy, women with only very early losses, or women whose aPL titers are low or transient. Nonetheless, it is common to offer such patients low-dose aspirin.

## Asymptomatic Anti-phospholipid Antibody–Positive Persons

The persistence of aPLs for decades without occurrence of clinical events is well documented. The probability that the

syndrome will eventually develop in an asymptomatic person who is incidentally found to have aPLs is likely low.[7] Anticoagulation is not indicated for the prophylactic treatment of asymptomatic aPL-positive persons. For persons with moderate- to high-titer persistent aPLs, education about the meaning of abnormal tests is appropriate, as is a discussion of warning signs to be reported. Elimination of reversible thrombosis risk factors and prophylaxis during high-risk periods, such as surgical procedures, are crucial.

A recent meta-analysis demonstrated that the risk of first thrombosis is decreased by administration of low-dose aspirin among asymptomatic persons with aPL and patients with SLE or obstetrical APS; however, no significant risk reduction was observed when considering only prospective studies or those with the best methodologic quality.[76] In the only randomized, double-blind, placebo-controlled trial, low-dose aspirin (81 mg) appeared to be no better than placebo in preventing first thrombotic episodes in persistently asymptomatic aPL-positive patients; the incidence rate of first thrombosis was relatively low.[9]

Although drugs that induce lupus (e.g., hydralazine and phenytoin) may also induce aPLs, they may be prescribed for patients with aPLs if no alternatives are available. Drugs that promote thrombosis (e.g., estrogen and oral contraceptives that contain estrogen) are not currently deemed safe, even for asymptomatic women serendipitously known to have high-titer antibodies. This advice does not translate to a recommendation to test all healthy women before prescribing such medications, but it does suggest that special attention and further evaluation should be provided to persons with family histories or clinical suggestions of rheumatic disease, livedo reticularis, biologic false-positive tests for syphilis, or borderline thrombocytopenia. No reliable information is available regarding the safety of progestin-only contraception, "morning after" contraception, or the use of raloxifene, bromocriptine, or leuprolide in patients with APS. A small retrospective review of women undergoing artificial reproductive technology (in vitro fertilization) procedures demonstrated no thrombotic events.[77]

### Anti-phospholipid Antibody–Positive Persons with Ambiguous or Noncriteria Events

Some patients with positive aPL tests have clinical events of ambiguous meaning (e.g., dizzy or confusional episodes, nonspecific visual disturbances, and very early pregnancy loss). No consensus has been reached regarding the treatment of such persons. Because full anticoagulation carries high risk, many physicians prescribe low-dose (81 mg) aspirin, hydroxychloroquine, or both daily. No published data support or repudiate this recommendation.

Based on the presumed pathogenesis, some physicians prescribe anticoagulation for patients with noncriteria manifestations of aPL such as livedo reticularis, thrombocytopenia, leg ulcers, thrombotic microangiopathy, or valvulopathy. The efficacy of anticoagulation is not known in these conditions. One small, descriptive, cross-sectional study provides evidence that B cell depletion with rituximab is well tolerated and can be effective for refractory thrombocytopenia and skin ulcers in aPL-positive patients.[78] An open-label phase IIa descriptive pilot study assessing the effectiveness and safety of rituximab in patients with noncriteria and/or anticoagulation-resistant manifestations of aPL demonstrated that rituximab, even without inducing substantial change in aPL profiles, may be effective in controlling some noncriteria manifestations of aPL.[79]

### Catastrophic Anti-phospholipid Syndrome

The onset of catastrophic APS is usually sudden and immediately life threatening. Early diagnosis can be a challenge, especially in patients with no history of APS. However, early diagnosis is critical because, in contrast to other causes of multiple organ dysfunction syndrome, appropriate therapy includes anticoagulation and corticosteroids in combination with repeated plasma exchange, IVIG, and, in desperate situations, other modalities such as cyclophosphamide or rituximab. A case report described improvement of post–kidney transplant thrombotic microangiopathy in a patient with APS who was treated with eculizumab (terminal complement inhibitor).[80] In another patient with catastrophic APS who was resistant to anticoagulation, immunosuppression, plasmapheresis, and rituximab, eculizumab successfully blocked complement activity, aborted progressive thrombosis, and reversed thrombocytopenia.[81]

### Anti-phospholipid Antibody–Negative Persons with a Clinical Event

In patients clinically suspected of having APS but who have normal findings of aCL, lupus anticoagulant, and anti-β2-GP1 tests, alternative causes of clotting must be sought. Even among patients with concomitant rheumatic disease, APS may not be the cause of recurrent thromboembolism or pregnancy loss. Patients with SLE experience emboli from SLE-related cardiac valvular disease, vasculitis, or atheroma. Other patients have factor V Leiden or some other procoagulant mutation. Recurrent pregnancy losses may be caused by chromosomal abnormalities, uterine infection, diabetes, hypertension, or non-aPL coagulopathy. The concept of "seronegative" APS is extremely controversial.

## OUTCOME

Pulmonary hypertension, neurologic involvement, myocardial ischemia, nephropathy, gangrene of extremities, and catastrophic APS are associated with a worse prognosis. During long-term follow-up, serious morbidity and disability occur in an unpredictable proportion of patients with primary APS who experience major vascular events and in those who have delays in diagnosis and treatment. Thus the long-term functional outcome of patients with primary APS is poor; at 10 years, permanent organ damage develops in one third of patients, and one fifth are unable to perform everyday activities.[82]

Long-term outcomes of children born of women with APS are not known. In many patients with long-standing APS, the development of severe cardiac valvular disease necessitates valve replacement, and rare patients experience renal failure as a result of thrombotic microangiopathy. Immediate thrombosis may cause loss of a transplanted kidney or other organ; aPL positivity correlates with poor graft survival after renal transplantation in patients with SLE.[83]

Serious perioperative complications may occur despite prophylaxis in aPL-positive patients because they are at additional risk for thrombosis when undergoing surgical procedures. Thus perioperative strategies should be clearly identified before any surgical procedure is performed. In addition, pharmacologic and physical antithrombosis interventions should be vigorously employed, periods without anticoagulation should be kept to an absolute minimum, intravascular manipulation for access and monitoring should be minimized, and any deviation from a normal course should be considered a potential disease-related event.[84]


The references for this chapter can also be found on ExpertConsult.com.

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PART 12 SCLERODERMA, INFLAMMATORY MYOPATHIES, AND OVERLAP SYNDROMES

CHAPTER 83



# Etiology and Pathogenesis of Scleroderma

John Varga

**KEY POINTS**

- Scleroderma/systemic sclerosis (SSc) has a complex pathogenesis and protean clinical manifestations that reflect the underlying early immune dysregulation and microangiopathy, and subsequent systemic fibrosis.
- Marked patient-to-patient variability exists in clinical and laboratory manifestations, disease course, and molecular signatures, suggesting the existence of distinct disease subsets.
- Vascular lesions in small blood vessels occur early and progress to obliterative vasculopathy that causes tissue hypoxia, oxidative stress, and vascular complications.
- Immune dysregulation manifested by autoantibodies, evidence of innate immune activation, and an "interferon signature" is prominent in SSc, but its role as a primary factor in pathogenesis has not been established.
- Genetic association studies implicate the human leukocyte antigen (HLA) and other immunoregulatory genes that are also associated with systemic lupus erythematosus.
- Fibrosis is associated with sustained mesenchymal cell activation by growth factors, cytokines, chemokines, hypoxia and reactive oxygen species, and aberrant reactivation of developmental pathways.

Scleroderma or systemic sclerosis (SSc) is an uncommon disease of unknown cause and complex pathogenesis. The hallmarks of SSc are (1) autoimmunity, (2) inflammation, (3) functional and structural alterations in small blood vessels, and (4) widespread interstitial and vascular fibrosis affecting the skin and internal organs. This unique constellation of distinct but related pathophysiologic features, illustrated in Figure 83-1, accounts for the characteristic clinical manifestations of SSc. In its early stage, SSc may be dominated by clinical manifestations reflecting inflammation and vascular injury; in advanced stages of SSc, fibrosis and vascular insufficiency underlie the prominent clinical manifestations. However, there is enormous patient-to-patient variability in the presence, severity, and progression of these features. Recent advances in cell and molecular biology, mouse genetic engineering, genetics, and functional genomics have identified a large number of molecules, signaling pathways, and cell types implicated in the pathogenesis of SSc. The following sequence represents a plausible view of SSc pathogenesis: environmental triggers and epigenetic modifications in a genetically susceptible individual set off a cascade of events with early vascular injury, immune cell activation, autoimmunity, oxidative stress, and mesenchymal cell activation, resulting in excessive matrix accumulation and progressive tissue damage. Over time, chronic vascular insufficiency and widespread fibrosis cause failure of vital organs, accounting for substantial morbidity and mortality.

## ETIOLOGY

Neither the cause of SSc nor the precise contribution of genetic susceptibility is fully understood. Evidence indicates that infectious agents, environmental toxins, and drugs, as well as microchimerism, might be potential triggers.

### Genetic Risk: Family Studies

Familial clustering of a disease is considered to be evidence of inherited disease susceptibility, but such clustering might be explained by shared environmental exposures, shared genetic background, or the interaction between genes and environment.[1] The risk of SSc is considerably increased among first-degree relatives of persons with SSc compared with the general population. In one study, the relative risk of SSc among first-degree relatives of persons with SSc was 13, thus identifying a family history as the strongest known risk factor for SSc.[2] In a twin study of SSc only a relatively small disease concordance rate (4.7%) was found, although concordance for anti-nuclear antibody positivity was 90% for monozygotic (identical) twins and 40% for dizygotic (fraternal) twins.[3] Raynaud's disease and pulmonary fibrosis show increased prevalence in pedigrees of patients with SSc.[4] Moreover, up to 36% of first-degree family members of patients with SSc have autoimmune conditions, with the most common being hypothyroidism, hyperthyroidism, rheumatoid arthritis, and systemic lupus erythematosus (SLE).

### Genetic Association Studies

Genetic association studies using candidate gene approaches and, more recently, genome-wide association (GWA) studies have been performed in large multinational patient

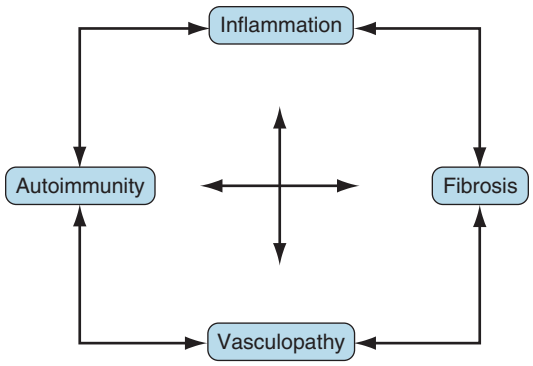



**Figure 83-1** The pathophysiologic quartet underlying systemic sclerosis. Patients with systemic sclerosis display evidence of inflammation, autoimmunity, vasculopathy, and fibrosis. Autoimmunity and vasculopathy generally precede the onset and contribute to the progression of fibrosis. Vascular obliteration and interstitial fibrosis perpetuate and further exacerbate chronic autoimmunity and inflammation. *(Courtesy Kathleen Kelley.)*

cohorts. The major histocompatibility complex is the dominant genetic region implicated in autoimmune disease, including SSc. The interpretation of human leukocyte antigen (HLA) associations is complicated by the extensive linkage disequilibrium of risk haplotypes. It has long been known that specific HLA alleles are associated with SSc and specific autoantibodies. For instance, a case-control study revealed strong associations with HLA DRB1*1104, DQA1*0501, and DQB1*0301 haplotypes.[5] Candidate gene approaches typically look for single nucleotide polymorphisms [SNPs]), the most common form of DNA variation. Non-HLA susceptibility loci associated with SSc include the protein tyrosine phosphatase nonreceptor 22 (PTPN22), which has been associated with SLE, myasthenia gravis, vitiligo, and Addison's disease; IL-1β and NLRP1, an inflammasome scaffold that promotes pro-IL-1β maturation and processing; and interferon regulatory factor 5 (IRF5), a transcription factor that mediates type I interferon (IFN) induction and is associated with SLE, as well as SSc and interstitial lung disease. The association of IRF5 with SSc is particularly interesting in light of the role of IFN type I in immune responses. Table 83-1 summarizes SSc susceptibility genes identified to date, although there will undoubtedly be many more discovered. In addition to classic SNPs, informative genetic polymorphisms in SSc also include copy number variations, rare allelic variants, and epigenetic changes.

## Other Candidate Genes and Genome-wide Association Studies

Thus far, candidate gene approaches focusing on genes involved in vascular homeostasis or extra-cellular matrix (ECM) remodeling have not yielded robust associations with SSc. Several GWA studies in large and ethnically diverse populations are currently under way. These unbiased approaches have an advantage over candidate gene studies in that they are driven by discovery rather than *a priori* hypotheses. The first large-scale SSc GWA study analyzed 300,000 SNPs in a cohort of 2296 cases and 5014 healthy control subjects.[6] Statistically significant associations were found with SNPs in the HLA region, as well as with *IRF5*, *TNFAIP3*, and *CD247*, a gene implicated in T cell signaling and also associated with susceptibility to SLE. The results of genetic association studies to date can be summarized as follows: (1) Genetic variants associated with SSc susceptibility are involved in innate and adaptive immune responses, and (2) they are largely shared with SLE and other autoimmune diseases. It is worth noting that important associations can be missed by GWA studies using current technologies. Moreover, despite the wealth of emerging information, the genetic associations discovered to date are of relatively modest magnitude, with odds ratios that are generally less than 1.5. This finding points to the potential importance of gene-gene interactions (epistasis), particularly for genes within the same molecular pathways. Even more important are the contributions of gene-environment interactions. A current challenge in SSc research is to delineate how genes shared among diverse autoimmune conditions contribute to disease-specific phenotypes. Shedding light on this important problem will require large collaborative studies involving phenotypically well-characterized populations of varied ethnic background and meta-analyses.

**TABLE 83-1** Selected Systemic Sclerosis Susceptibility Genes

| Locus | Chromosome | Associated SSc Subset | Potential Pathogenic Mechanism |
|---|---|---|---|
| *HLA* | 6 | Various | Antigen presentation |
| *PTPN22* | 1p3.2 | Topo1$^+$ positive | T and B cell activation |
| *NLRP1* | 17p13.2 | dcSSc, pulmonary fibrosis | Inflammasome component, IL-1β processing |
| *IRF5* | 7q32 | dcSSc | Transcription factor required for induction of type I interferon |
| *STAT4* | 2q32.3 | lcSSc, ACA | Transcription factor for IL-12 and IL-23 |
| *BANK1* | 4q24 | dcSSc | Scaffolding protein involved in B cell signaling |
| *TNFSF4* | 1q25 | SSc | T cell co-stimulation |
| *T-bet* | 17.q21.32 | SSc | Transcription factor for Th1 T cell polarization |

Based on published candidate gene studies and genome-wide association studies as of 2015.

ACA, Anti-centromere antibody; dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; SSc, systemic sclerosis; Th1, T helper 1.

## Epigenetic Factors

The relatively modest contribution of genetic factors to disease susceptibility in SSc has drawn attention to the role of epigenetic modifications in pathogenesis. Epigenetic modifications, often induced by environmental exposures, can alter gene expression without changes in the genetic code (see Chapter 22). Three key forms of epigenetic

modifications are DNA methylation, histone modifications, and expression of noncoding (long and micro) RNAs.

A genome-wide DNA methylation analysis in SSc revealed a large number of differentially methylated cytosine-phosphatidyl-guanine (CpG) sites in SSc fibroblasts.[7] Pathway analysis of genes that were abnormally methylated in SSc implicated genes involved with ECM-receptor interaction and focal adhesion. Another recent study using a candidate gene approach demonstrated that the antifibrotic transcription Friend leukemia integration 1 (Fli1) is repressed at the epigenetic level in explanted SSc fibroblasts because of promoter methylation, resulting in gene silencing.[8] Other reports evaluating SSc T cells showed a generalized reduction of DNA methylation and of methylation-regulating genes such as *DNMT1*, as well as increased DNA methylation of FOXP3, the transcription factor controlling regulatory T cell (Treg) differentiation. Moreover, SSc fibroblasts showed elevated levels of methylation-regulating genes and global DNA hypermethylation, accompanied by transcriptional silencing of genes involved in negative regulators of Wnt signaling such as DKK1, WIF, and SFRP1.[9-11]

Post-translational modifications of nucleosome histones, including acetylation/deacetylation and methylation, also can profoundly influence gene expression and have been implicated in SSc. For instance, the levels of the acetyltransferase p300 are elevated in SSc fibroblasts, and p300 promotes fibrotic responses by enhancing stimulation of collagen gene transcription.[12]

MicroRNAs (miRNAs) are a large family of 18 to 23 nucleotide noncoding RNAs that function as intra-cellular regulators of gene expression. Of particular interest are miR-21 and miR-29, which are aberrantly expressed in SSc. miR-21, which is elevated in SSc fibroblasts, suppresses expression of antifibrotic Smad7, thereby promoting expression of profibrotic genes.[13] In contrast, miR-29, which is reduced in SSc fibroblasts and is suppressed by fibrotic stimuli, has inhibitory effects on fibrotic gene expression.[14,15] miRNAs also can be detected in the circulation and exert biologic activities when they are incorporated into microvesicles. Although the full spectrum and mechanism of action of miRNAs that are aberrantly regulated in SSc remain to be investigated, these noncoding regulatory RNAs may serve as both biomarkers and therapeutic targets in SSc.

## INFECTIOUS AGENTS AND VIRUSES

Along with exposure to certain environmental and occupational agents and drugs, viruses such as Epstein-Barr virus (EBV), human cytomegalovirus (hCMV), and parvovirus B19 have been implicated in SSc. Patients with SSc have anti-hCMV antibodies directed against the UL83 and UL94 protein epitopes on hCMV. Anti-UL94 antibodies can induce endothelial cell apoptosis and fibroblast activation, suggesting a direct role for anti-viral antibodies in tissue damage. Antitopoisomerase-I can cross-react with hCMV-derived proteins, implicating molecular mimicry as a mechanism linking hCMV infection and SSc.[16] CMV is implicated in the pathogenesis of allograft vasculopathy, a complication of organ transplantation characterized by vascular neointima formation and smooth muscle cell proliferation strikingly reminiscent of the obliterative proliferative vasculopathy seen in SSc. In dermal fibroblasts, hCMV stimulates synthesis of the profibrotic growth factors in vitro.[17] In a recent study, genetic material from EBV were identified within dermal fibroblasts and microvessels in the skin of patients with SSc. Moreover, EBV induces Toll-like receptor (TLR)-mediated cellular responses in SSc fibroblasts, suggesting a potential role for EBV reactivation in vascular injury and fibrosis in SSc.

## ENVIRONMENTAL EXPOSURES, DIETARY SUPPLEMENTS, DRUGS, AND RADIATION

Although reports of putative geographic clustering suggest a role for shared environmental exposures, careful investigations have generally failed to substantiate apparent clusters of SSc. On the other hand, well-documented epidemic outbreaks of SSc-like illnesses with acute onset and chronic course have been reported. One such illness, called the *toxic oil syndrome*, was linked to the ingestion of contaminated rapeseed cooking oils.[18] Dietary supplements containing L-tryptophan were implicated in an explosive outbreak of the eosinophilia-myalgia syndrome (EMS) in 1989.[19] The EMS epidemic subsided after the ban on L-tryptophan, but sporadic cases of EMS following the ingestion of L-tryptophan and other food supplements continue to be reported. Although scleroderma-like skin and organ fibrosis was a prominent manifestation of these apparently de novo toxicoepidemic syndromes, the associated clinical, histopathologic, and laboratory features clearly differentiate them from SSc.[20]

The frequency of SSc appears to be increased among men with occupational exposure to silica dust. This observation was recently confirmed by a meta-analysis of 16 observational studies, with risk estimates as high as 15.[21] Other environmental exposures occasionally linked with SSc include polyvinyl chloride, toluene, xylene, trichloroethylene, and organic solvents. Additional reports alleged an association between SSc and environmental exposures to pesticides, hair dyes, and industrial fumes. Although exposure to cigarette smoke is known to increase the risk of multiple autoimmune diseases, no evidence to date implicates it as a risk factor for SSc.

Certain drugs have been implicated in SSc-like illnesses. The best studied is the anticancer drug bleomycin, which induces skin and lung fibrosis in the mouse (discussed in a subsequent section). Other drugs potentially implicated include pentazocine, docetaxel and paclitaxel, and cocaine. The use of appetite suppressants has been linked to the development of pulmonary arterial hypertension (PAH). The occurrence of SSc in women after cosmetic breast augmentation with silicone implants raised significant concern regarding a possible causal association.[22] Subsequent large-scale epidemiologic surveys and a meta-analysis, however, failed to confirm an increased risk of SSc or of other connective tissue diseases among women with silicone breast implants.[23] Radiation treatment for malignant neoplasms has been linked with the onset of de novo SSc, as well as exacerbation of tissue fibrosis in patients with pre-existing SSc.[24] Some of the environmental agents and drugs that have been linked with the development of SSc are listed in Table 83-2.

**TABLE 83-2** Environmental Agents and Drugs Implicated in Scleroderma-like Syndromes

| |
|---|
| **Chemicals** |
| Silica |
| Heavy metals |
| Mercury |
| Organic chemicals |
| Vinyl chloride |
| Benzene |
| Toluene |
| Trichloroethylene |
| **Drugs** |
| Bleomycin |
| Pentazocine |
| Taxol |
| Cocaine |
| **Dietary Supplement/Appetite Suppressants** |
| L-tryptophan (contamination) |
| Mazindol |
| Fenfluramine |
| Diethylpropion |

## MICROCHIMERISM

Healthy women may harbor circulating, and occasionally tissue-resident, stem cells of fetal origin that persist for many years after pregnancy, a condition called *microchimerism*. In some studies it was found that the number of circulating fetal cells is elevated in women with SSc compared with healthy women.[25] The persistence of fetal cells in women with SSc might be linked to the development of the disease through a graft-versus-host–like response triggered by the fetal cells or through a maternal (auto)immune response against the fetal cells.

## PATHOLOGY

### General Features

The pathologic hallmarks of SSc are a non-inflammatory proliferative/obliterative vasculopathy affecting small arteries and arterioles in multiple vascular beds, loss of capillaries (rarefication), and fibrosis that is most prominent in the skin, lungs, and heart.[26] Inflammatory cell accumulation is generally absent in long-standing SSc but may be prominent in patients with early-stage disease. Infiltrates are located predominantly around blood vessels and are composed primarily of $CD4^+$ T lymphocytes, dendritic cells (DCs), and monocytes/macrophages.

### Vascular Pathology

Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc. Histopathologic evidence of vascular damage can be detected in involved and uninvolved skin and is present before fibrosis.[27] Raynaud's phenomenon typically precedes other manifestations of SSc. Additional clinical signs indicative of vasculopathy include mucocutaneous telangiectasia; nailfold capillary changes (giant capillaries, hemorrhages, and avascular areas); PAH; digital tip pitting and ischemic ulcers; gastric antral vascular ectasia (also called *watermelon stomach*); and scleroderma renal crisis.

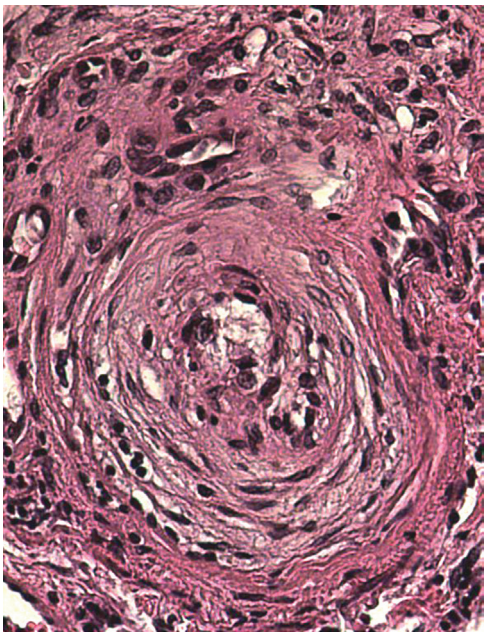


**Figure 83-2** Histologic appearance of systemic sclerosis vasculopathy. A pulmonary arteriole showing extensive medial hypertrophy and intimal thickening, leading to narrowing of the vascular lumen.

The most characteristic histopathologic finding in the small and medium-sized arteries is bland intimal proliferation (Figure 83-2). Intimal hypertrophy, a finding that SSc shares with chronic allograft arteriopathy, is thought to result from proliferation and migration of myointimal cells and local accumulation of collagen and other ECM components.[28] The vascular basement membranes are thickened and reduplicated. These changes are most prominent in the heart, lungs, kidneys, and gastrointestinal tract. A systematic survey of SSc skin biopsies revealed a marked reduction in the number of capillaries (rarefaction) and loss of vascular endothelial cadherin, a molecule required for vascular tube formation.[29] Remarkably, this study showed that clinical improvement after high-dose immunosuppressive therapy was accompanied by capillary regeneration in the skin.

Impaired fibrinolysis, increased levels of von Willebrand factor, and ongoing platelet aggregation are prominent and contribute to a procoagulant milieu. Endothelial cell injury itself causes further platelet aggregation, release of platelet-derived growth factor (PDGF) and endothelin-1 (ET-1), and endothelial cell apoptosis.[30] Vasculitic lesions and immune complex deposition in the vessel walls are uncommon. In later stages of SSc, extensive fibrin deposition and perivascular fibrosis cause progressive luminal occlusion, and eventually there is a striking paucity of small blood vessels and capillaries in lesional tissue.[31] Loss of vascular supply leads to chronic tissue hypoxia, which directly contributes to fibrosis. Widespread proliferative/obliterative vasculopathy of small and medium-sized arteries and capillary rarefaction are the pathologic hallmarks of all forms of SSc. In patients with SSc-associated PAH, pulmonary arteriolar intimal proliferation and evidence of veno-occlusive disease are prominent. In contrast to idiopathic PAH, plexogenic arteriopathy is uncommon in SSc-associated PAH.[32]

### Tissue Fibrosis

Fibrosis is characterized by excessive accumulation of fibrillar collagens, fibronectin, elastin, proteoglycans, cartilage oligomeric matrix protein, and other structural components of the ECM. Matrix deposition causes disruption and, ultimately, complete obliteration of tissue architecture. Fibrosis is most prominent in the lungs, gastrointestinal tract, heart, tendon sheath, and perifascicular tissue surrounding skeletal muscle. Histopathologic examination of these organs shows homogeneous and relatively acellular connective tissue with thick, densely packed, and hyalinized collagen bundles.

## ORGAN-SPECIFIC PATHOLOGIC FINDINGS

### Skin

Skin fibrosis, the hallmark of SSc and other scleroderma-like syndromes, is accompanied by marked expansion of the dermis with obliteration of the hair follicles, sweat glands, and sebaceous glands and other skin appendages. Collagen fiber accumulation is most prominent in the reticular (deep) dermis and progressively involves the subjacent adipose layer with entrapment of fat cells (adipocytes). Early-stage SSc skin biopsies reveal dermal edema and sometimes striking perivascular cellular infiltrates composed of T lymphocytes and monocytes (Figure 83-3). Less commonly, mast cells and eosinophils may also be detected.[33,34] The proportion of α-smooth muscle actin–positive myofibroblasts, a mesenchymal cell type that is intermediate between fibroblasts and contractile smooth muscle cells and plays a major role in fibrogenesis, is increased in the lesional skin.[35]

With disease progression, the skin undergoes atrophy with loss of epidermal-dermal ridges and effacement of the rete pegs reminiscent of the changes seen in aging skin. The fibrotic dermis is largely acellular and contains dense accumulation of compact hyalinized collagen bundles, fibronectin, hyaluronic acid, and other structural proteins. Sweat glands and eccrine glands atrophy with loss of periglandular adipose tissue. The intradermal adipose layer interposed between the dermis and muscle is obliterated. In a double-blind study of SSc skin biopsies, the histologic grade of skin fibrosis was found to closely correlate with the extent of clinical skin involvement.[36] Reduction in the number of dermal lymphatic vessels can be marked and contributes to interstitial fluid accumulation and skin edema.[37] Paucity of dermal capillaries, also known as rarefaction, is associated with chronic tissue hypoxia, which increases expression of angiogenic factors such as vascular endothelial growth factor (VEGF). Evidence of tissue hypoxia is found in clinically uninvolved, apparently "normal" skin.[38]

Biochemical analysis shows that the collagens in the fibrotic dermis are normal, and relative proportions of the main fibrillar collagens (type I and type III) are comparable with those of normal skin.[39] In contrast, the minor nonfibrillar type VII collagen, which is normally restricted to the dermal-epidermal basement membrane zone, is abundant throughout the lesional dermis. Enzymes mediating post-translational collagen and elastin modification such as lysyl oxidases (LOXL2) and lysyl hydroxylase (PLOD2) are elevated, resulting in an increase in aldehyde-derived collagen cross-links, which may account for the dense sclerotic nature of the fibrotic dermis.[40]

Genome-wide expression profiling of lesional skin using microarray technology provides an increasingly clearer understanding of the activation events that underlie fibrosis. Results from several studies reveal altered gene expression patterns in SSc skin biopsy specimens compared with healthy control subjects. Remarkably, clinically involved and uninvolved skin samples appear to be indistinguishable in terms of their gene expression profiles. Many genes involved in cellular immunity and inflammation, ECM homeostasis, and the transforming growth factor-β

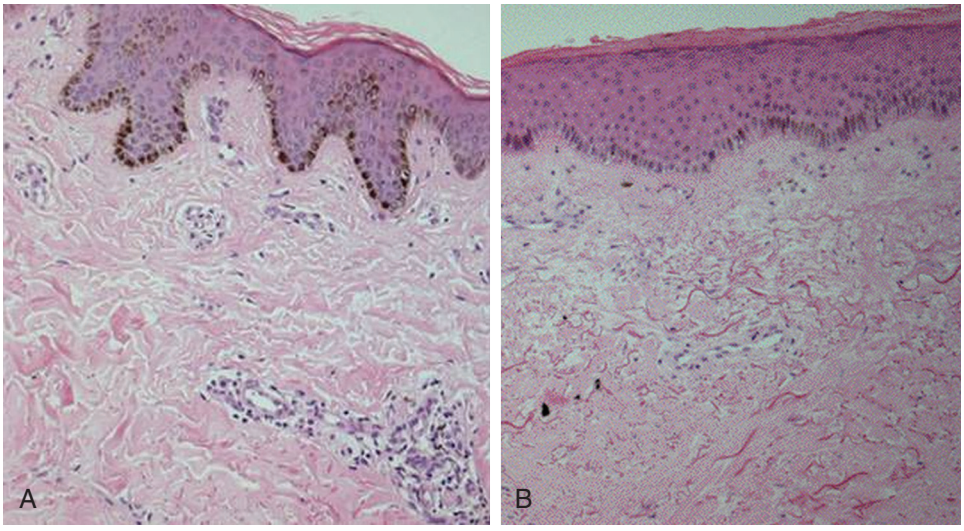



**Figure 83-3** Histology of skin in diffuse cutaneous systemic sclerosis. Perivascular infiltration is present in the dermis with inflammatory cells of multiple lineages. Microvascular endothelial cell activation and increased extra-cellular matrix deposition are also seen **(A).** Later, regression of the inflammatory features occurs. Secondary structures within the skin such as hair follicles and sebaceous and sweat glands are reduced, and rete pegs are flattened **(B).** Hematoxylin and eosin stain, original magnification ×40.

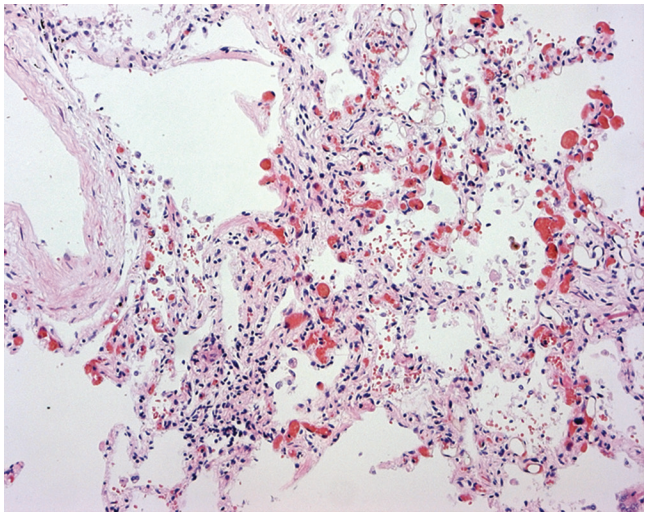


**Figure 83-4** Histologic appearance of the lung. Evidence of interstitial lung disease is common in systemic sclerosis. Although substantial variation exists in the appearance of lung histology, consistent features include reduction in air spaces and thickening of alveolar walls with matrix deposition. Inflammatory cell infiltrates may be prominent. A lung biopsy is rarely required for diagnosis.

(TGF-β), CCN2, IL-13, and Wnt signaling pathways show elevated expression in SSc skin.[41,42] Skin biopsy specimens from different patients with SSc show marked heterogeneity at the level of their "molecular signature," with distinct and reproducible patterns that define "intrinsic subsets."[43]

## Lungs

In early-stage SSc, the lungs show patchy infiltration of the alveolar walls with lymphocytes, plasma cells, macrophages, and eosinophils (Figure 83-4). At this stage, high-resolution computed tomography scanning of the lungs may show a ground-glass opacification pattern. With progression, interstitial lung fibrosis and vascular damage develop, often coexisting within the same lesions. Intimal thickening of the pulmonary arteries, best seen with elastin stain, underlies PAH and at autopsy is often found to be associated with multiple pulmonary emboli and myocardial fibrosis.

Lung fibrosis in SSc is characterized by expansion of the alveolar interstitium as a result of the accumulation of collagens and other connective tissue proteins. The typical histologic pattern of SSc-associated interstitial lung disease is nonspecific interstitial pneumonitis, which is characterized by mild to moderate interstitial inflammation, type II pneumocyte hyperplasia, and fairly uniform distribution of fibrosis.[44] Less commonly, SSc is associated with the usual interstitial pneumonia pattern that is characterized by scattered fibroblastic foci and patchy distribution of fibrosis and has a worse prognosis. Progressive alveolar septal thickening ultimately results in obliteration of the air spaces and honeycombing, as well as consequent loss of pulmonary blood vessels. This process impairs gas exchange and contributes to worsening of pulmonary hypertension. Extensive pulmonary fibrosis also may predispose to primary lung carcinoma.

## Gastrointestinal Tract

The gastrointestinal tract is virtually always affected in persons with SSc, and prominent pathologic changes are seen at any level from the mouth to the rectum. The esophagus is prominently involved, with fibrosis in the lamina propria, submucosa, and muscular layers and characteristic vascular lesions.[45] Replacement of the normal intestinal architecture results in disordered peristaltic activity, gastroesophageal reflux, gastroparesis and small bowel dysmotility, pseudo-obstruction, and bacterial overgrowth. Chronic gastroesophageal reflux is complicated by esophageal inflammation, ulcerations, stricture formation, and recurrent microaspiration. In up to one-third of patients with SSc who have severe gastroesophageal reflux, Barrett's esophagus develops, which is characterized by metaplasia of the normal squamous lining of the esophagus into columnar epithelium.[46] Because Barrett's metaplasia is a premalignant lesion associated with a greater than 30-fold increased risk of adenocarcinoma, patients with Barrett's esophagus need ongoing monitoring for dysplasia and adenocarcinoma.

## Kidneys

In the kidneys of a person with SSc, vascular lesions predominate, whereas glomerulonephritis is rare except in patients with overlap syndromes. Chronic renal ischemia is associated with shrunken glomeruli and other ischemic changes. Patients with acute scleroderma renal crisis have histopathologic changes indistinguishable from those in other forms of thrombotic microangiopathies associated with malignant hypertension.[47] Small interlobular and arcuate renal arteries show reduplication of elastic lamina, marked intimal proliferation (onion skinning), and fibrinoid necrosis of the arteriolar walls.[48] Of note, however, similar pathologic changes have also been reported in patients with SSc who do not experience a renal crisis. Intimal thickening leads to severe narrowing and total obliteration of the lumen and underlies microangiopathic hemolysis. Tubular changes such as flattening and degeneration of tubular cells occur as a result of vascular insufficiency. The clinical picture of scleroderma renal crisis may resemble thrombotic thrombocytopenic purpura. However, scleroderma renal crisis is not associated with reduced to absent levels or activity of von Willebrand factor–cleaving protease *(ADAMTS13)*. Figure 83-5 shows the characteristic histologic features associated with scleroderma renal crisis.

## Heart

At autopsy, evidence of cardiac involvement is found in up to 80% of patients with SSc.[49] Modest pericardial effusions are common, and occasionally, fibrosis and constrictive pericarditis may occur. Microvascular changes are prominent in the endocardium. A characteristic pathologic finding is myocardial contraction band necrosis, which is thought to reflect repeated episodes of ischemia-reperfusion as a result of "myocardial Raynaud's phenomenon."[50] Significant interstitial and perivascular fibrosis in the heart may occur in the absence of clinically evident heart involvement. Skeletal

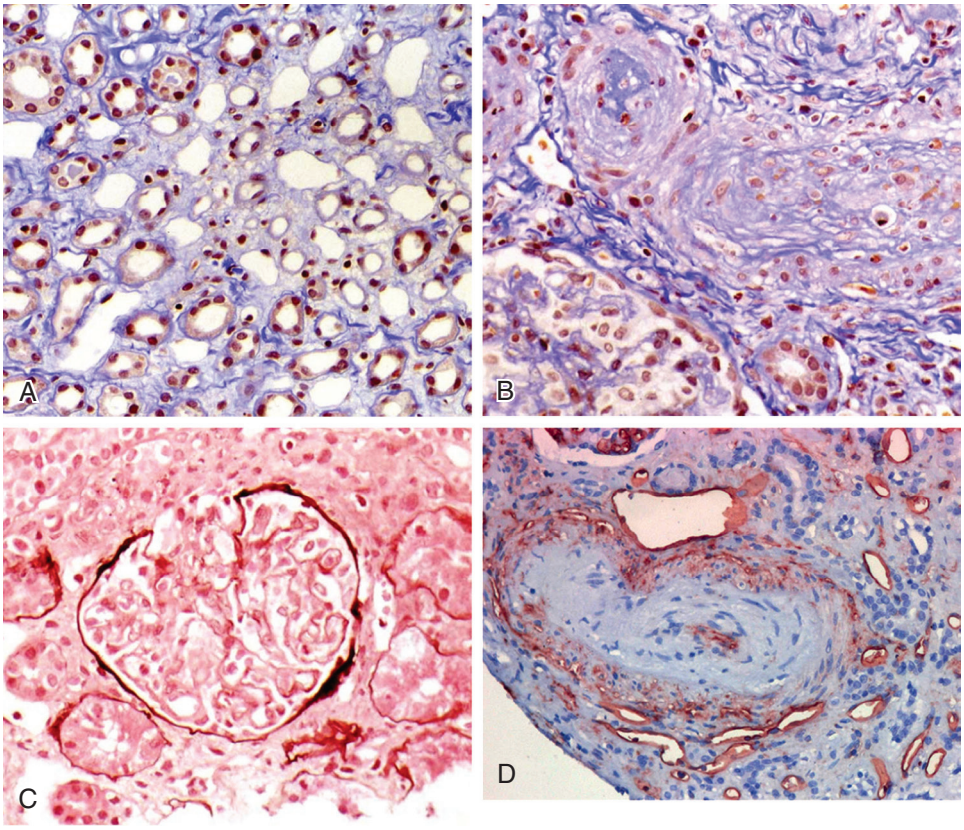



**Figure 83-5** Histologic appearance of scleroderma renal crisis. Characteristic histologic findings include interstitial fibrosis **(A)** and occlusion of intrarenal arteries with neointima formation, fibrinoid necrosis of the vessel wall, and reduplication of the internal elastic lamina **(B).** The glomeruli are shrunken and lack inflammatory cells or proliferative changes **(C).** Intravascular thrombosis resembling the changes of thrombotic thrombocytopenic purpura may be present **(D).**

muscle myositis in SSc is occasionally accompanied by acute myocarditis.[49]

### Pathologic Findings in Other Organs

Fibrosis of the thyroid glands is common, with broad bands of fibrous tissue in the thyroid gland and atrophy and obliteration of the follicles in the absence of inflammation. Abnormal thyroid function tests and anti-thyroid antibodies are common. Erectile dysfunction is frequent and may be a presenting manifestation of the disease in men. Pathologic examination shows extensive proliferative/obliterative changes in the penile blood vessels.[51] Fibrosis of the salivary and lacrimal glands in the absence of inflammation can occur and may be associated with Sjögren's syndrome. Synovial biopsies show fibrosis and characteristic vascular changes in the small arterioles.[52]

## ANIMAL MODELS OF SCLERODERMA

Animal models are indispensable research tools to facilitate the understanding of complex human diseases. Such models are used to identify the cellular and molecular components of the pathologic process, discover potential therapeutic targets, and develop and evaluate novel treatment strategies. Several animal models of SSc exist, although none fully reproduces the four cardinal features of the disease: obliterative and proliferative microangiopathy, autoimmunity, inflammation, and chronic multiorgan fibrosis.[53] However, as illustrated in Figure 83-6, particular animal models recapitulate selected features or stages of SSc. Broadly speaking, mouse models can be divided into four types: (1) naturally occurring, in which spontaneous mutations are associated with a genetically transmitted scleroderma-like phenotype such as tight skin (Tsk1/$^{+}$ mouse); (2) experimentally induced in which a scleroderma phenotype is elicited by chemical exposures or by manipulation of the immune system (bleomycin or angiotensin II–induced skin and lung fibrosis); (3) transplantation of HLA-mismatched bone marrow cells, resulting in a sclerodermatous graft-versus-host–like disease; and (4) genetic manipulations giving rise to engineered mouse strains with heritable scleroderma-like traits.

### Heritable Animal Models of Scleroderma

The Tsk1/$^{+}$ mutant is characterized by diffuse skin tightness and tethering to the underlying subcutaneous tissue. Whereas homozygous Tsk1 mice die in utero at 8 to 10 days of gestation, heterozygous (Tsk1/$^{+}$) mice survive and develop tight skin. In contrast to SSc, Tsk1/$^{+}$ mice show prominent subcutaneous hyperplasia but a relatively unremarkable dermis.[54] Furthermore, the lungs of Tsk1/$^{+}$ mice show emphysematous changes rather than fibrosis, and

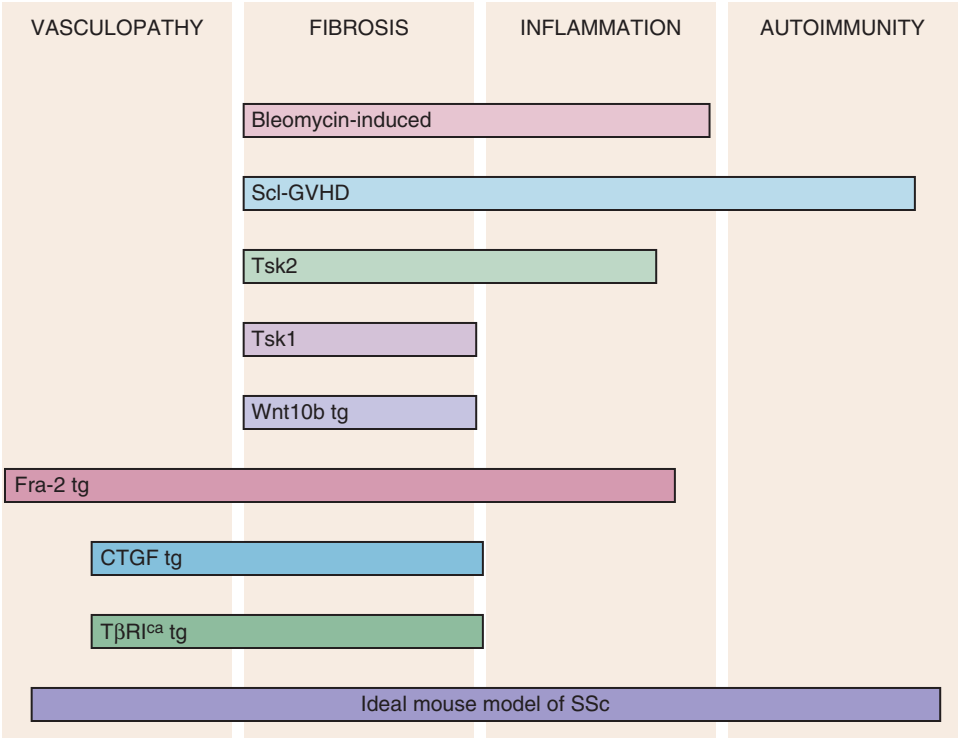



**Figure 83-6** Animal models of systemic sclerosis. Mouse models recapitulate selected pathophysiologic features of systemic sclerosis (SSc). Shown are the principal disease processes of SSc (inflammation, autoimmunity, microangiopathy, and fibrosis) and the extent to which they are represented in commonly used mouse models. CTGF, Connective tissue growth factor; GVHD, graft-versus-host disease; tg, transgenic. *(Modified from Distler JH, Distler O, Beyer C, Schett G: Animal models of systemic sclerosis: prospects and limitations.* Arthritis Rheum *62:2831–2844, 2010.)*

microangiopathy has not been reported. The Tsk1 mutation is an intragenic tandem duplication of the fibrillin-1 gene, giving rise to an abnormally large 450 kDa protein.[55] Fibrillin-1 is a widely distributed microfibril-associated ECM protein that modulates the latency and activation of TGF-β.[56] The accumulation of the abnormally large mutant fibrillin-1 might destabilize the matrix[57] or perturb the homeostatic control of TGF-β latency; however, the precise mechanisms linking the Tsk1/$^{+}$ fibrillin mutation to cutaneous hyperplasia are unknown. No corresponding fibrillin-1 mutations have been demonstrated in patients with SSc. Mutations throughout the fibrillin-1 gene cause Marfan's syndrome, characterized by bone overgrowth, ocular lens displacement, and aortic dilatation, associated with increased activity of TGF-β in multiple tissues. Patients with stiff skin syndrome, a rare Mendelian autosomal dominant condition with a childhood onset of skin fibrosis, have heterozygous mutations in the RGD domain of fibrillin-1. Genetically engineered mice harboring analogous fibrillin-1 mutations develop diffuse skin fibrosis associated with increased TGF-β signaling and activation of plasmacytoid DCs (pDCs) in the skin.[58]

The Tsk2 mouse strain spontaneously develops scleroderma-like skin changes by 4 weeks of age. In contrast to Tsk1/$^{+}$ mice, Tsk2/$^{+}$ mice have a thickened dermis infiltrated with mononuclear inflammatory cells and show evidence of autoimmunity. The Tsk2 phenotype, originally induced in normal mice by ethyl nitrosourea, is due to a missense mutation in the amino-terminal end of the *COL3A1* gene—mutations that previously have been associated with Ehlers-Danlos syndrome.[59]

## Inducible Animal Models of Scleroderma

Chronic skin and lung fibrosis (Figure 83-7) can be induced in BALB/c or C57 mice by subcutaneous injections of bleomycin, angiotensin II, oxidative agents such as hydroxyl radicals or hypochlorite, and ligands of TLRs. The sequence of histopathologic changes elicited by these agents recapitulates those seen in SSc: early mononuclear cell infiltration and macrophage activation, expression of cytokines such as TGF-β, IL-4, IL-6, IL-13, and chemokines such as monocyte chemotactic protein-1 (MCP-1), followed by progressive dermal fibrosis with excessive collagen deposition and accumulation of α-smooth muscle actin–positive myofibroblasts and disappearance of the intradermal white adipose tissue.[60,61] Although microangiopathic changes or autoantibodies are not prominent and skin fibrosis remains limited in extent and duration in chemically induced mouse models of scleroderma, in light of their reproducibility, relative strain independence, and ease of induction, these mouse models remain indispensable for investigating specific cell types and transdifferentiations, molecules, and pathways in the development and treatment of fibrosis. Subcutaneous injection of constitutively active TGF-β receptor I is sufficient to induce local dermal fibrosis. Transplantation of bone marrow or spleen cells into minor histocompatibility locus–mismatched recipient mice results in chronic sclerodermatous graft-versus-host–like disease with interstitial and perivascular fibrosis in the skin and lung, as well as autoimmunity.[62] Skin fibrosis is preceded by mononuclear cell and macrophage activation with elevated TGF-β and chemokine expression.

### Genetic Manipulations in Mice Giving Rise to Scleroderma-like Phenotypes

Mouse strains with genetic gain-of-function or loss-of-function modifications resulting in spontaneous scleroderma-like phenotypes have been created. These transgenic, knockin, and knockout mice provide robust novel experimental tools in scleroderma research. Mouse strains with constitutive or inducible expression of TGF-β signaling in fibroblasts recapitulate key clinical, histologic, and biochemical features of SSc and provide support for the role of perturbed TGF-β signaling in pathogenesis.[63,64] Other promising transgenic models of SSc include mice that overexpress CTGF, PDGF receptor (PDGFR)-α, Wnt10b, and Fra-2 and mice null for caveolin-1, uPAR, and Fli-1.[65] Scleroderma-like fibrotic, vascular, and calcific changes in the skin, and in some cases in the lungs, develop spontaneously in these mice. Other genetically engineered mice show increased sensitivity to the induction of fibrosis. Examples include *T-bet* null and *VEGF* null mice. These emerging mouse models provide new, exciting research opportunities for the discovery of molecules and pathways that underlie the pathogenetic features of SSc.

## PATHOGENESIS

### Integrated Overview

The pathogenesis of SSc is complex, and as previously discussed, existing animal models only imperfectly capture its diverse pathologic and clinical attributes and natural history. A comprehensive paradigm for SSc pathogenesis must encompass and integrate the following cardinal pathomechanisms: vascular damage, inflammation with activated innate and adaptive immunity, and fibroblast activation resulting in generalized tissue fibrosis. Evidence exists for each pathomechanism in patients with SSc; however, the contribution of individual processes to the disease manifestations varies from one patient to another. As illustrated in Figure 83-1, complex and dynamic interplay between these distinct processes is responsible for initiating, amplifying, and sustaining tissue damage underlying the symptoms and signs of SSc.[66]

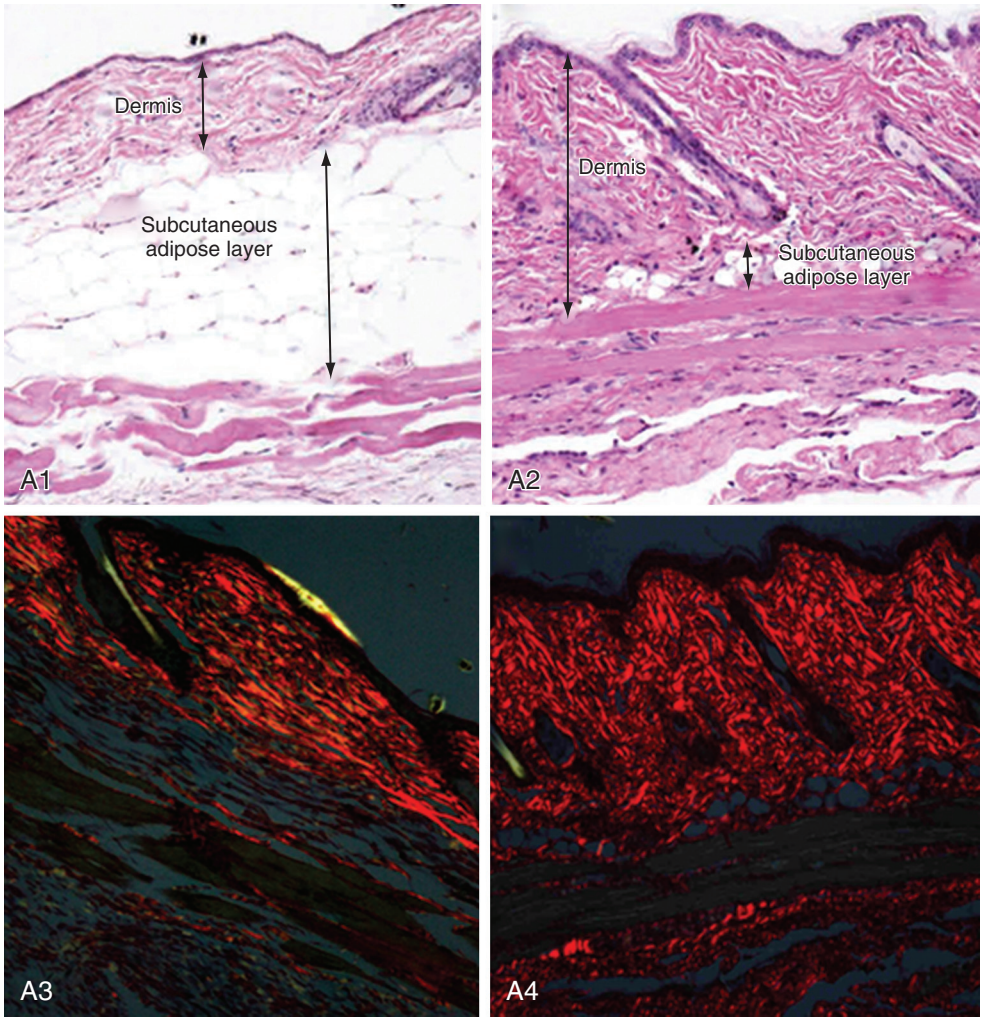



**Figure 83-7** Bleomycin-induced mouse model of systemic sclerosis. Mice received a subcutaneous injection of phosphate-buffered saline solution **(A1, A3)** or bleomycin **(A2, A4)** for 28 days. **A1-A4,** Skin changes. **A1** and **A2,** Hematoxylin and eosin stain; **A3** and **A4,** Sirius red stain. Note dermal fibrosis and loss of the subcutaneous adipose layer **(A2)** and increased collagen accumulation **(A4)**.

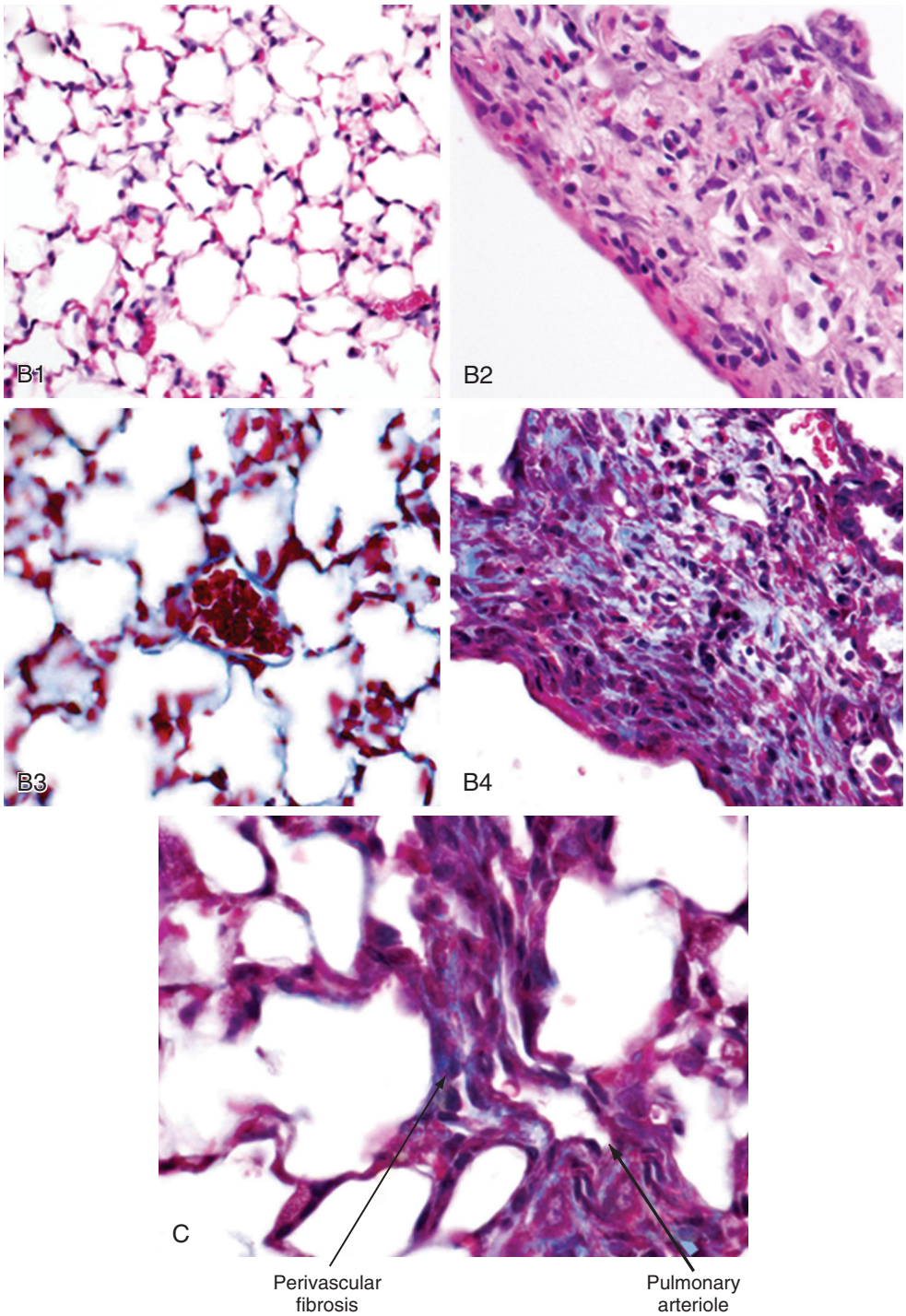



**Figure 83-7, cont'd** **B1-B4,** Lung changes. **B1** and **B2,** Hematoxylin and eosin stain; **B3** and **B4,** trichrome stain. Note dense lung fibrosis obliterating the alveolar parenchyma **(B2, B4). C,** Perivascular fibrosis in pulmonary arteriole. *(Courtesy D. Melichian.)*

## Vasculopathy

Vascular injury is an initiating proximal event in SSc (see Figure 83-5). Evidence of vascular involvement is early and widespread, and its progression over time is associated with significant clinical sequelae.[67] The presence of nail-fold microvascular changes in a person with Raynaud's phenomenon identifies an elevated risk of progression to SSc, indicating that microvasculopathy precedes other clinical manifestations of the disease.

## Vascular Injury and the Activated Endothelium

The initial vascular insult in SSc might be triggered by circulating factors such as (unidentified) cytotoxic molecules or T cell–derived proteolytic granzymes.[68] Other potential causes include anti-endothelial cell and endothelial cell receptor antibodies, vasculotropic viruses, inflammatory cytokines, reactive oxygen species (ROS), and other forms of environmental stress. Vascular injury in SSc causes activation of endothelial cels, with increased expression of

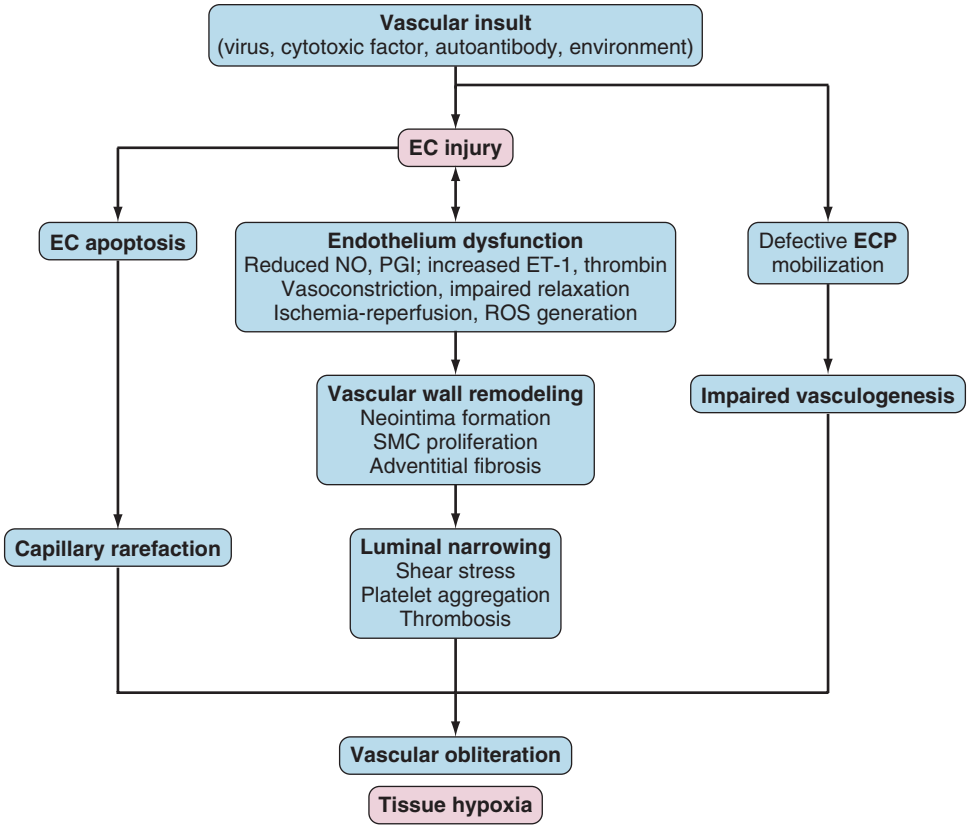



**Figure 83-8** Pathogenesis of vasculopathy. An initial vascular insult results in endothelial cell (EC) injury and activation, with reversible functional changes, increased expression of adhesion molecules, and enhanced leukocyte diapedesis resulting in perivascular inflammation. Damaged endothelium promotes platelet aggregation and thrombin release and shows impaired production of vasodilators such as nitric oxide (NO), increased production of vasoconstrictors such as endothelin-1 (ET-1), and release of reactive oxygen species (ROS). Consequent vasoconstriction and defective vasodilation aggravate vascular damage, leading to irreversible and progressive vascular wall remodeling, luminal occlusion, platelet aggregation, in situ thrombosis, and tissue hypoxia. Loss of blood vessels may be further compounded by insufficient vasculogenesis. ECP, Endothelial cell progenitor; PGI, prostaglandin I; SMC, smooth muscle cell.

vascular endothelial cell adhesion molecule-1 and E-selectin and altered secretion of vasoactive mediators, followed by platelet activation and activation of the thrombotic and fibrinolytic cascades.[67] Evidence also shows that activated endothelial cells in the injured arterioles and capillaries transdifferentiate into mesenchymal cells via *endothelial-mesenchymal transition* driven by TGF-β and Notch and are associated with loss of endothelial markers such as CD31 and acquisition of mesenchymal markers such as α–smooth muscle actin. Platelet activation is prominent in SSc and is associated with release of thromboxane $A_2$, PDGF, and TGF-β and activation of thrombin, which promote coagulation, potentiate vasoconstriction, and directly stimulate fibroblast activation and myofibroblast transdifferentiation. Structural cells in the walls of small blood vessels called pericytes show marked hyperplasia in lesional skin and express the surface marker Thy-1 (CD90) and receptors for PDGF.[69,70]

Prominent functional abnormalities of the vascular endothelium include impaired production of and responsiveness to endothelium-derived vasodilators such as nitric oxide, thrombomodulin, calcitonin gene-related peptide, and prostacyclins. Additionally, dysregulation of sensory and sympathetic nerves results in decreased release of vasodilator neuropeptides and increased expression of vasoconstrictors such as α2-adrenoreceptors on vascular smooth muscle cells. The ensuing imbalance of vasodilators and vasoconstrictors impairs blood flow responses. Recurrent episodes of ischemia-reperfusion promote oxidative stress with the generation of $H_2O_2$ and other oxygen free radicals. Damaged microvessels show increased vascular permeability, and injured endothelial cells promote and transendothelial leukocyte migration. Platelets are exposed to subendothelial structures, which further aggravates platelet aggregation. Activated endothelial cells release the extremely potent vasoconstrictor ET-1, which promotes leukocyte adhesion, vascular smooth muscle cell proliferation, and fibroblast activation (Figure 83-8). Levels of ET-1 are elevated in lesional tissue, in the blood, and in bronchoalveolar lavage fluids in persons with SSc.[71]

## Vascular Damage and Failure of Compensatory Vascular Repair

In medium-sized and larger vessels, a combination of intimal and medial hypertrophy and adventitial fibrosis causes progressive luminal narrowing. Together with endothelial cell apoptosis and thrombosis, the process culminates in obliterative vasculopathy with the characteristic paucity of blood vessels seen by angiography in late-stage SSc. Loss of

microvasculature results in reduced blood flow and chronic tissue hypoxia, which induces hypoxia-inducible factor-1 (HIF-1)–α dependent genes such as VEGF and its receptors.

The balance between angiogenic and antiangiogenic factors is fundamentally dysregulated in SSc. Plasma levels of the angiogenesis inhibitor endostatin, a degradation product of type XVIII collagen, are elevated.[72] Other investigators found elevated levels of the angiogenic factors such as VEGF, basic fibroblast growth factor, IL-8, CXCL-12, and PDGF. Furthermore, levels of VEGF and its receptors was also found to be elevated in lesional tissue.[73,74] It is unclear why, in the face of tissue hypoxia and elevated angiogenic factors, there is a failure of compensatory growth of new blood vessels via angiogenesis or vasculogenesis in SSc, resulting in progressive loss of blood vessels. An intriguing possibility is that VEGF premessenger RNA (pre-mRNA) undergoes alternate splicing to generate an antiangiogenic isoform VEGF, called $VEGF_{165b}$. $VEGF_{165b}$ is overexpressed in the skin in patients with SSc, suggesting that an alternate splicing event causing a switch from angiogenic to antiangiogenic VEGF isoform plays a crucial role in the failure to mount an appropriate compensatory angiogenic response to hypoxia in SSc.

Vasculogenesis, or de novo blood vessel formation, is a process critically dependent on bone marrow–derived circulating endothelial progenitor cells (EPCs) and their differentiation into mature endothelial cells. Recent studies implicate defective vasculogenesis as an explanation for loss of blood vessels in SSc. A reduction in the number of $CD34^+$ EPCs and their impaired differentiation into mature endothelial cells has been documented in patients with SSc.[75-77] Because EPCs are required for physiologic vasculogenesis in ischemic tissues, defective progenitor cell mobilization or function compromises the vascular repair. Whether the reduction in circulating EPCs in SSc is due to diminished release from the bone marrow as a result of fibrosis or "exhaustion," destruction in the peripheral circulation, or some other process remains unresolved.

## Hypoxia

The widespread microangiopathy and resultant capillary loss in SSc leads to diminished blood flow and consequent hypoxia of affected tissues. With the onset of fibrosis, excessive ECM accumulation increases diffusion distance from blood vessels to cells, further aggravating tissue hypoxia.[78] Hypoxia is itself a potent inducer of the basic helix-loop-helix transcription factors HIF-1α and HIF-1β, which, under normoxic conditions, are undetectable as a result of rapid and efficient proteosomal degradation mediated by the von Hippel–Lindau tumor suppressor. In hypoxic cells, the von Hippel–Lindau protein is unable to bind to its target, and therefore HIF-1α is protected from degradation and translocates into the nucleus, where it binds to hypoxia-responsive DNA elements and induces the transcription of hypoxia-regulated genes involved in erythropoiesis, angiogenesis, and glucose metabolism. Hypoxia is also a potent in vitro and in vivo stimulus for genes involved in ECM, including collagens, prolyl hydroxylases, and lysyl oxidase, and directly stimulates epithelial cell–myofibroblast differentiation.[79,80] These and other hypoxia-induced profibrogenic responses are partially mediated by autocrine TGF-β. Compelling experimental support for the role of hypoxia in fibrosis is provided by a recent study, which shows increased fibrosis and Wnt-β-catenin signaling in mice with myeloid cell-specific deletion of VEGF, culminating in exacerbation of fibrosis.[81]

A state of tissue hypoxia in SSc has been documented by noninvasive means, and cutaneous oxygen concentrations inversely correlate with skin thickness. With the use of a needle electrode inserted into the dermis, patients with SSc were found to have significantly reduced levels of $pO_2$ compared with control subjects (23.7 mm Hg vs. 33.6 mm Hg). Interestingly, HIF-1α was not detectable in the lesional skin.

## Oxidative Stress and Reactive Oxygen Species

Oxidative stress results from an imbalance between the generation of chemically reactive oxygen-derived molecules called ROS and antioxidant defense mechanisms. Substantial evidence indicates enhanced oxidative stress and elevated ROS generation in patients with SSc and supports their role in the pathogenesis of SSc.[82,83] In SSc, ROS are generated from the endothelium in response to episodes of ischemia-perfusion and from fibroblasts exposed to mediators such as TGF-β, PDGF, angiotensin II, and ET-1, or in response to stimulatory anti-PDGFR antibodies. Moreover, explanted SSc dermal fibroblasts and monocytes spontaneously produce ROS.[84] Although several cellular enzymes, as well as mitochondria, can generate ROS, in SSc the membrane-associated nicotinamide adenine dinucleotide phosphate oxidase Nox4 appears to play a prominent role. Hydrogen peroxide and other oxygen free radicals can stimulate collagen synthesis, myofibroblast differentiation, and secretion and activation of TGF-β and Wnt-β-catenin pathways, as well as induce cellular senescence.

# INFLAMMATION AND IMMUNE DYSREGULATION IN THE PATHOGENESIS OF SYSTEMIC SCLEROSIS

Recent discoveries from genetic and genomic studies, along with the application of high throughput cellular phenotyping technologies, have prompted a resurgence of interest in the role of immune dysregulation in SSc. Persons with SSc might be genetically predisposed to develop dysregulated innate and adaptive immune responses. Lesional skin and lungs show early and prominent perivascular accumulation of mononuclear cells, macrophages, and pDCs, whereas circulating T cells, B cells, monocytes, and DCs show evidence of activation and polarization. The characteristic "type I IFN signature" prominent in SLE and other autoimmune diseases is also seen in SSc. Autoimmunity manifested by the presence of highly specific and mutually exclusive autoantibodies is prominent throughout the course of the disease (Figure 83-9). The strong genetic associations between SSc and the class II HLA locus, as well as numerous genes related to B and T cell function and innate immunity, provide strong support for an immunologic basis of SSc. Ongoing therapeutic trials are evaluating the efficacy of global (myeloablative) and selective immunomodulatory interventions in SSc and suggest that the immune system contributes to progressive disease.

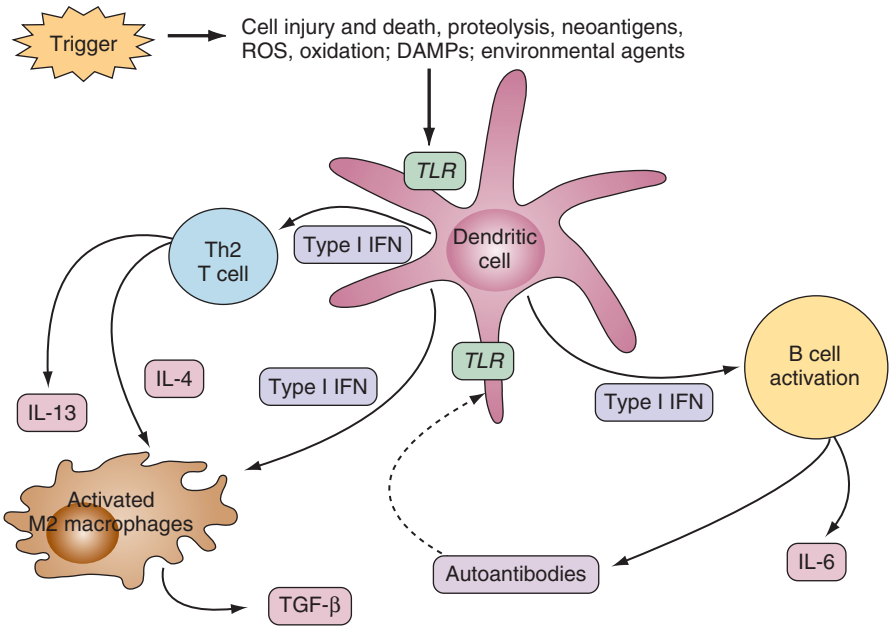



**Figure 83-9** Complex immune dysregulation in scleroderma. An inciting event such as infection, oxidative damage, necrotic/apoptotic cell debris, or environmental toxins causes activation of dendritic cells, possibly via Toll-like receptors (TLRs). Activated dendritic cells produce type I interferon (IFN), which causes T helper (Th)2 T cell polarization, monocyte differentiation to an alternately activated (M2) phenotype, and B cell activation with plasma cell maturation and production of autoantibodies. Autoantibodies form immune complexes that in turn further induce type I IFN production via TLR signaling. Th2-polarized T cells and M2 macrophages secrete profibrotic chemokines and cytokines, inducing fibroblast activation. Additional T cell subsets such as regulatory T cells and Th17 also may be involved. DAMPs, Damage-associated molecular patterns; ROS, reactive oxygen species; TGF-β, transforming growth factor-β.

## Cellular Effectors of Dysregulated Immunity: T Cells, B Cells, and Monocytes/Macrophages

### T Cell Subsets and Activation

T cell activation is evident in SSc lesional tissues, as well as in peripheral blood, and appears to play a direct role in tissue injury. In the early stage of the disease, activated CD4 and CD8 T lymphocytes, monocytes/macrophages, and B cells, along with eosinophils, mast cells, and natural killer cells, are observed in a perivascular distribution in the lesional skin, lungs, and other affected organs and can be detected in these tissues before the appearance of fibrosis.[85] In situ hybridization studies show that fibroblasts with prominent procollagen gene expression in early-stage SSc skin are adjacent to inflammatory cells, suggesting that they or their soluble products play a direct role in fibroblast activation.[86] The extent of lymphocytic tissue infiltration correlates with the severity and progression of skin fibrosis.

Tissue-infiltrating T cells are predominantly $CD3^+$ and $CD4^+$, express markers of activation (CD69, CD45, HLA-DR, and IL-2R), and display restricted T cell receptor signatures, suggesting oligoclonal T cell expansion in response to an as-yet unidentified antigen.[87] In the lungs, a predominance of $CD8^+$ and γ/δ T cells is observed.[88] Currently it is not known if T cells in lesional tissue are activated nonspecifically (by cytokines or chemokines) or specifically in response to antigen. Evidence of T cell activation in SSc is also detected in the peripheral blood, with elevated serum levels of IL-2 and IL-2R and chemokines receptors, spontaneous cytokine secretion, and increased expression of leukocyte function-associated antigen 1 (LFA-1) and α1 integrins, which enable T cells to adhere directly to endothelium and fibroblasts. At the same time, vascular endothelial cells express intercellular adhesion molecule-1 (ICAM-1), E-selectin, and other adhesion molecules that facilitate leukocyte diapedesis. Peripheral blood lymphocytes show the "IFN signature" defined by the induction of type I IFN-inducible genes.[89-91]

### T Helper 1/T Helper 2 Cytokine Balance and Polarized Immune Responses

A skewed balance between T helper (Th)1 and Th2 cytokines is a characteristic of fibrosis. Th2 cells secrete abundant IL-4, IL-5, and IL-13, but only low levels of the hallmark Th1 cytokine IFN-γ. Th2 cytokines directly stimulate collagen synthesis, myofibroblast transdifferentiation, and production of TGF-β, a potent stimulus for ECM accumulation, whereas IFN-γ blocks these responses and exerts antifibrotic effects. The Th2 skewing of the immune response thus creates a profibrotic environment.

Support for the significance of a Th2-polarized immune response in fibrosis comes from animal studies, which show that in vitro Th2-polarized T cells induce fibrosis when they are passively transferred in mice. Moreover, in mice lacking the T-bet transcription factor, which directs Th1 differentiation, exaggerated skin fibrosis develops in response to bleomycin.[92,93]

Patients with SSc display an altered Th1/Th2 cytokine balance with a predominant Th2 profile. For example, IFN-γ serum levels and in vitro IFN-γ production by peripheral blood monocytes are reduced in persons with SSc. Microarray analysis of SSc peripheral blood leukocytes

showed elevated expression of GATA3, which controls Th2 polarization. Cloned $CD4^+$ T cells from SSc skin biopsies secrete IL-4 but not IFN-γ.[94] T cell lines generated from SSc skin biopsies or alveolar fluid produce high levels of Th2 cytokines, and the Th2 predominance predicts accelerated decline in lung function.[95,96] Proteomic and DNA microarray analysis also demonstrated a Th2 cytokine predominance in SSc bronchoalveolar lavage fluids and $CD8^+$ lymphocytes. Furthermore, a longitudinal study of patients with SSc showed that skin improvement over time was associated with a decline in serum levels of Th2 cytokines and a concomitant increase in IL-12, a Th1 cytokine.[97]

### Other T Cell Subsets in SSc

Th17 lymphocytes are implicated in the pathogenesis of rheumatoid arthritis and other inflammatory diseases. Patients with SSc have increased numbers of IL-17–producing $CD4^+$ T cells in both the peripheral blood and bronchoalveolar lavage fluid.[98] The presence of a Th1, Th2, or Th17 predominant immune response might be useful in identifying clinical phenotypes in SSc.

Suppressor Tregs are important in controlling autoimmunity. One study found that although the frequency of peripheral blood Tregs was increased in patients with SSc, they showed impaired suppressive activity and defective secretion of TGF-β and IL-10.[99] Other studies demonstrated reduced numbers of $Foxp3^+$ $CD4^+$ T cells in the lesional skin compared with other inflammatory skin diseases.

## Monocytes and Macrophages

Bone marrow–derived phagocytic monocytes, and the macrophages they give rise to, regulate both innate immunity and tissue repair by virtue of their ability to secrete paracrine regulators of cell function, and they play a fundamental role in vascular injury and fibrosis in SSc β.[100]

Monocytes are a major source of cytokines and chemokines, including IL-1, TNF, MCP-1, PDGF, and TGF-β, all of which are important in inflammatory and fibroproliferative processes. Additionally, monocytes produce collagenases and other matrix-degrading enzymes, as well as their inhibitors. In early-stage SSc, macrophages are prominent in the lesional skin and express Siglec-1 and AIF-1, which are markers of IFN-induced activation. Macrophages undergo polarization in response to specific stimuli, with the alternatively activated (M2) macrophage phenotype typically induced in response to IL-4 and TGF-β.

In patients with SSc, circulating monocytes express elevated levels of CCL18 and CD163, an M2 monocyte/macrophage marker. Serum levels of soluble CD163 are also elevated and correlate with disease severity. The number of macrophages expressing CD163 and CD204 is also elevated in the lesional skin. Another study showed that multiple macrophage activation markers are elevated in SSc lungs and correlate with the severity and progression of interstitial lung disease. In animal models of fibrosis, macrophages expressing the scavenger macrophage receptor with collagenous structure (MARCO) accumulate in affected organs. In some cases, evidence of local mast cell and eosinophil activation and degranulation are prominent in lesional skin.

## Dendritic Cells

DCs bearing CD11c surface markers are potent antigen-presenting cells that reside in the skin and other tissues. DCs bridge innate and adaptive immunity, and by modulating T cell, B cell, and monocyte/macrophage function, they shape the immune response (see Figure 83-9). pDCs are primarily known as the major source of type I IFN and play a fundamental role in innate anti-viral immunity. pDCs can also secrete fibrogenic cytokines, including TGF-β, suggesting that they participate in fibrogenesis independent of their T cell–stimulatory properties. TLR7 and TLR9 on pDCs contribute to the pathogenesis of SLE by recognizing endogenous DNA and RNA in the form of immune complexes, leading to production of type I IFN. Recent studies show that $CD11c^+$ DCs accumulate in lesional tissue in animal models of fibrosis and in patients with SSc. As an example, $BDCA2^+$ pDCs were found to be abundant in the skin from patients with early diffuse cutaneous (dc)SSc and were shown to secrete CXCL4, also known as platelet factor 4.[101] Prominent accumulation of $CD317^+$ pDCs secreting type I IFN and IL-6 in lesional dermis from transgenic mice harboring the stiff skin syndrome fibrillin-1 mutation was reported. In this model, selective pDC depletion abolished skin fibrosis.[58] In SSc, activated pDCs might directly or indirectly modulate activation of resident fibroblasts. Moreover, peripheral blood monocyte–derived DCs in SSc show enhanced ex vivo activation upon incubation with SSc sera containing topoisomerase-1 antibodies and enhanced responses to TLR2/3 ligands with increased secretion of IL-6 and IL-10.

# HUMORAL AUTOIMMUNITY AND B CELLS

## Autoantibodies in Scleroderma: Pathogenetic Considerations

Humoral autoimmunity, manifested by serum autoantibodies with multiple antigenic specificities, can be detected in virtually all patients. The presence of SSc autoantibodies assists with diagnosis and classification, as well as in predicting organ-specific complications and clinical course. In contrast, a direct pathogenic role of SSc autoantibodies in tissue damage has not been conclusively established. Autoantibodies in SSc tend to be highly specific and mutually exclusive and are strongly associated with particular disease phenotypes and immunogenetic backgrounds. Some studies suggest that the levels of autoantibodies, particularly anti-DNA topoisomerase I, might correlate with the extent of skin and lung fibrosis and fluctuate with disease activity.

Various hypotheses exist to explain the generation of autoantibodies in SSc. Self-antigens such as DNA topoisomerase I might, in the presence of ROS, undergo proteolytic cleavage, exposing cryptic epitopes that break immune tolerance.[102] Other potential mechanisms include molecular mimicry as a consequence of viral infection, chronic B cell hyper-reactivity, and altered expression or subcellular localization of potential autoantigenic peptides. Alternately, SSc autoantibodies might be produced by B cells activated by endogenous TLR ligands such as nucleic acid–containing cellular debris.

Recent studies in SSc report the presence of functional antibodies that are directed against ECM components, cell surface receptors, fibroblasts, and endothelial cells. Stimulatory autoantibodies can induce target cell activation or apoptosis in vitro.[103,104] In one study, autoantibodies against PDGF receptors were detected in patients with SSc and induced ROS generation and myofibroblast differentiation in cultured fibroblasts. It is not known if these autoantibodies precede, or are consequent to, fibrosis, and their direct pathogenic role in SSc remains to be established.

### B Cells in Scleroderma

Recent reports and clues from genetic studies provide evidence for a potential direct role for B cells in the pathogenesis of SSc. B cells have multiple immune regulatory functions in addition to the generation of antibodies. These functions include antigen presentation, cytokine production, lymphoid organogenesis, and T cell differentiation. Candidate gene and GWA studies in SSc have implicated several B cell genes, including *BANK1, BLK, CSK*, and *PTPN22*, all of which are involved in regulating B cell signaling. Although B cells were found in SSc-associated interstitial lung disease biopsy specimens,[105] they are not generally prominent in lesional skin. Nevertheless, microarrays demonstrate molecular signatures indicative of B cell activation in SSc skin biopsy specimens. Patients with SSc display intrinsic B cell abnormalities, with elevated numbers of naïve B cells, reduced numbers of plasma cells, and increased expression of activation markers CD95 and CD86, as well as CD19, a signaling cell surface receptor that regulates intrinsic and antigen receptor–induced B cell responses.[106] Transgenic mice that overexpress CD19 develop spontaneous autoimmunity and high titers of antitopoisomerase I antibodies. Chronic B cell activation in SSc may not only account for autoantibody production but also may contribute directly to fibrosis, because activated B cells secrete TGF-β and IL-6, which directly stimulate fibroblasts. Levels of BAFF (B cell survival factor B cell activating factor belonging to the TNF family) are elevated in the serum and in lesional skin, as is the expression of the BAFF receptor on B cells. B cell depletion in the mouse attenuates scleroderma, and small clinical trials of B cell depletion in SSc have reported clinical improvement.

### Type I Interferon Signature and Innate Immune Signaling: Similarities to Systemic Lupus Erythematosus

Stimulation of pDCs by ligands of TLR3, TLR7, and TLR9 results in robust secretion of type I IFN. Accordingly, the presence of IFN or IFN-dependent cellular responses suggests ongoing TLR-mediated innate immune signaling. Type I IFNs (α and β) are themselves potent modulators of innate immunity. Elevated expression of IFN-regulated genes (the "IFN signature") in peripheral blood cells, indicative of their exposure to IFN in the circulation or in the periphery, was first described in patients with SLE.[107] Circulating immune complexes containing antibodies to nucleic acids serve as endogenous TLR ligands that stimulate type I IFN secretion by pDCs and macrophages. Similar to SLE, a prominent IFN signature is detected in leukocytes from patients with SSc. Indeed, a recent study reported that the most highly expressed genes in SSc peripheral blood leukocytes were IFN-regulated.[90] Moreover, in normal leukocytes, incubation with SSc sera induced secretion of IFN, indicative of TLR activation by nucleic acid–containing immune complexes in the serum.[108] By identifying a role for TLRs and type I IFNs in SSc, these observations, along with immunogenetic studies, reveal important similarities between SLE and SSc. The reasons why these two related autoimmune diseases display largely nonoverlapping clinical phenotypes remain elusive. The direct pathogenetic role of type I IFN in SSc microangiopathy and fibrosis and the mechanisms responsible remain to be established.

## FIBROSIS

Fibrosis, characterized by replacement of normal tissue architecture with stiff paucicellular connective tissue, is the pathologic hallmark of SSc and represents a form of aberrant wound healing. Fibrosis is the end result of a complex series of vascular and immune-mediated responses to injury in a genetically predisposed individual. As illustrated in Figure 83-10, injured or activated vascular and immune cells produce soluble mediators, autoantibodies, and ROS that in turn induce and propagate mesenchymal cell activation and differentiation in a variety of tissue, culminating in excessive and ultimately irreversible ECM accumulation.[109]

### Extra-cellular Matrix

Connective tissue consists of a cellular compartment of resident fibroblasts and myofibroblasts and infiltrating cells embedded in an ECM composed of large structural proteins such as collagens, proteoglycans, elastin, microfibrils, and adhesive proteins. The ECM also serves as a reservoir sequestering growth factors and matricellular proteins that control the differentiation, function, and survival of mesenchymal cells. Excessive ECM accumulation in fibrosis results from overproduction by fibroblasts in response to autocrine and paracrine signals such as cytokines and chemokines; hypoxia and ROS; signals from the surrounding ECM; or via cell-cell interactions (Table 83-3). Impaired ECM degradation and turnover, as well as expansion of the tissue reservoir of ECM-producing mesenchymal cells, also play roles.

### Regulation of Collagen Synthesis

The collagens belong to a family of more than two dozen structural proteins with critical roles in organ development, growth, and differentiation. The skin, bones, and tendons are composed largely of type I collagen, the most abundant protein in mammals, with smaller amounts of associated type III collagen. Type II collagen is found mainly in articular cartilage, whereas type IV collagen is found in the basement membranes. The fibrillar collagens consist of three α chains wound into a characteristic triple helix. This structure is made possible by the presence of a glycine at every third residue of repeating Gly-X-Y sequence, where X is frequently a proline and Y is frequently a hydroxyproline. During their biosynthesis, fibrillar collagens undergo

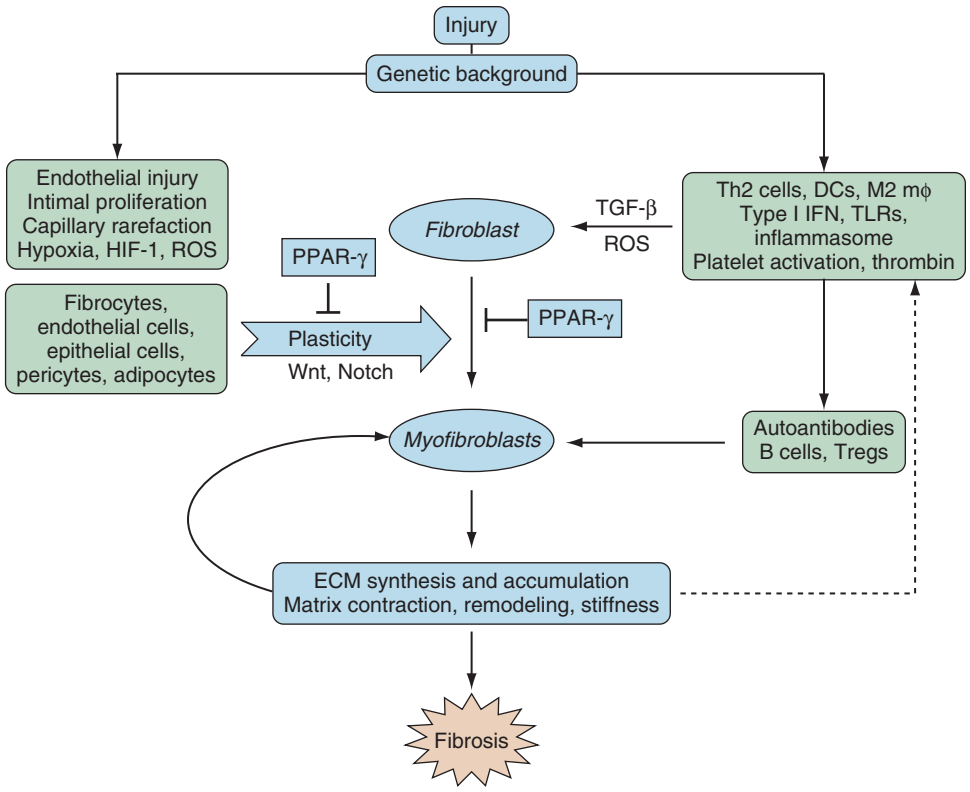



**Figure 83-10** Pathogenesis of fibrosis. Fibrosis is the end result of immune dysregulation and vascular damage and hypoxia. Injury causes vascular damage, perivascular inflammation, and innate immune signaling, with oxidative stress, secretion of inflammatory and fibrogenic cytokines and chemokines, autoantibodies, fibroblast activation, and myofibroblast accumulation. Circulating mesenchymal progenitor cells traffic to and accumulate within the lesional tissue and transdifferentiate into fibrotic fibroblasts, accelerating matrix accumulation. Tissue hypoxia, matrix remodeling, and stiffness further contribute to fibroblast activation, which causes disruption of tissue architecture and organ failure. DCs, Dendritic cells; ECM, extracellular matrix; HIF-1, hypoxia-inducible factor-1; IFN, interferon; PPAR-γ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; Th2, T helper 2; TLRs, Toll-like receptors.

extensive enzymatic modifications inside the cell followed by additional extra-cellular maturation and assembly. Covalent cross-linking stabilizes the collagen fiber network in the extra-cellular space.

Environmental cues allow fibroblasts to respond to dynamic tissue requirements during development and wound healing. Type I collagen synthesis is regulated by cytokines, growth factors, ROS, hypoxia, and cues provided by the ECM (Table 83-3). The genes encoding the various collagens harbor cis-acting regulatory elements recognized by DNA-binding transcription factors such as Sp1, Ets1, Smad2/3, Egr-1, and CCAAT-binding factor (CBF) that stimulate collagen gene transcription, as well as Sp3, C/EBP, YB1, c-Krox, and Fli-1 that suppress collagen gene transcription.[109] These transcription factors interact with one another and with non–DNA-binding cofactors, scaffold proteins, chromatin-modifying enzymes such as p300/CBP, PCAF, and histone deacetylases; their DNA-binding activities and protein-protein interactions and post-translational modifications are also controlled by extra-cellular cues. Enzymes that modify chromatin structure, causing it to unwind, enhance DNA-binding factor access to cognate cis-acting regulatory sequences and facilitate transcription. Alterations in the levels, activities, or interactions among the various transcription factors, cofactors, and chromatin-modifying enzymes contribute to persistent fibroblast activation in fibrosis.

## Effector Cells of Fibrosis: Fibroblasts

Fibroblasts are versatile spindle-shaped cells distributed in different parenchymal tissues that are capable of both ECM synthesis and resorption. Although unstimulated fibroblasts are biosynthetically quiescent, under the influence of appropriate extra-cellular cues they secrete ECM macromolecules and proteolytic enzymes, growth factors, cytokines and chemokines; adhere to and contract connective tissue; and transdifferentiate into myofibroblasts. Together, these biosynthetic, pro-inflammatory, contractile, and adhesive functions enable fibroblasts to execute effective wound healing. In contrast to physiologic ECM maintenance where the fibroblast repair program is self-limited, pathologic fibrosis is characterized by uncontrolled fibroblast activation that results in exaggerated and persistent ECM accumulation and remodeling. Recent DNA microarray studies reveal that fibroblasts derived from diverse anatomic locations retain markedly different patterns of gene expression.[110] This apparent "positional memory" of fibroblasts is governed by genetic imprinting by the homeobox (HOX) family transcription factors.

**TABLE 83-3** Extra-cellular Factors Implicated Fibrosis in Systemic Sclerosis

| Signal | Primary Cellular Source |
|---|---|
| TGF-β | Inflammatory cells (macrophages, T cells), platelets, fibroblasts |
| PDGF | Platelets, macrophages, fibroblasts, endothelial cells |
| CTGF/CCN2 | Fibroblasts |
| IGF-1 | Fibroblasts |
| IL-1α | Keratinocytes, macrophages |
| IL-4, IL-13 | Th2 lymphocytes, mast cells |
| IL-6 | Macrophages, B cells, T cells, fibroblasts |
| Chemokines (MCP-1, MCP-3) | Neutrophils, epithelial cells, endothelial cells, fibroblasts |
| Fibroblast growth factor | Fibroblasts |
| ET-1 | Endothelial cells |
| Wnt ligands | Developmental pathway aberrantly reactivated |
| Notch/Jagged | Developmental pathway aberrantly reactivated |
| Hypoxia | Hypoxic underperfused tissue |
| ROS | Generated from activated macrophages, fibroblasts, endothelial cells |

CTGF, Connective tissue growth factor; ET-1, endothelin-1; IGF-1, insulin-like growth factor-1; MCP, monocyte chemotactic protein; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SSc, systemic sclerosis; TGF-β, transforming growth factor-β.

## Effector Cells of Fibrosis: Myofibroblasts, Pericytes, Endothelial Cells, and Cellular Plasticity

In fibrosis, the tissue reservoir of activated matrix-modifying mesenchymal cells is expanded not only by proliferation of resident fibroblasts but also by transdifferentiation of other cell types, as well as by the influx of bone marrow–derived progenitor cells. Myofibroblasts, characterized by the cytoskeletal protein α–smooth muscle actin, are specialized cells that arise from resident fibroblasts, as well as epithelial and endothelial cells, adipocytes, and fibrocytes. Myofibroblasts synthesize collagens, tissue inhibitor of metalloproteinases (TIMPs), and other components of the ECM and are a major source of TGF-β during the fibrotic response. In normal wound healing, transient myofibroblast accumulation is detected in early granulation tissue, followed by their disappearance via apoptosis. The removal of myofibroblasts is a crucial step in normal wound healing. In contrast, in pathologic fibrogenesis, myofibroblasts persist in lesional tissue, resulting in stiff and contracted ECM characteristic of a chronic scar.

Residing within the walls of small blood vessels, pericytes are mesenchymal cells that are in intimate contact with the underlying endothelium and regulate vascular homeostasis. In patients with SSc, a marked increase in the microvascular pericyte compartment and increased expression of PDGF receptors have been reported. Activated pericytes can transdifferentiate into collagen-producing fibroblasts and myofibroblasts, thus linking microvascular injury and fibrosis.

Under certain conditions, epithelial cells can also undergo phenotypic transformation to fibroblasts. Epithelial-mesenchymal transition (EMT) plays a vital role during vertebrate embryonic development. Upon injury, epithelial cells lose characteristic markers and cell-cell adhesion and acquire fibroblast markers such as α–smooth muscle actin. Pathologic EMT is prominent in cancer and has also been implicated in renal fibrosis and idiopathic pulmonary fibrosis.[111] Similar to epithelial cells, upon fibrotic injury, vascular endothelial cells, mesothelial cells, and intradermal adipocytes can undergo a transition to myofibroblasts. These processes have been demonstrated in various forms of experimental fibrosis and might be implicated in SSc.

## Fibrocytes and Monocyte-Derived Mesenchymal Progenitor Cells

Fibrocytes are bone marrow–derived $CD34^+$ mesenchymal progenitor cells, normally present in small numbers in the peripheral blood, that can present antigen and synthesize collagen.[112] They express $CD14^+$ (a monocyte marker), as well as chemokine receptors (CCR3, CCR5, and CXCR4), which allows them to accumulate in specific tissues. The role for circulating fibrocytes and their tissue trafficking in the pathogenesis of fibrosis was established in animal models using neutralizing antibodies and in CXCR4-deficient mice. Mice with accelerated senescence have an increased number of circulating fibrocytes and show increased sensitivity to bleomycin-induced fibrosis. It is thought that in lesional tissue, fibrocytes undergo differentiation into activated myofibroblasts and contribute to the progression of fibrosis. Other studies have identified monocyte-derived multipotent mesenchymal progenitor cells in peripheral blood. Despite these experimental observations, the role of fibrocytes and other bone marrow–derived progenitor cells in SSc-associated fibrosis remains speculative.

# MOLECULAR DETERMINANTS OF FIBROSIS: TRANSFORMING GROWTH FACTOR-β

The expression of ECM genes is normally tightly controlled by paracrine/autocrine mediators, cell-cell contact, hypoxia, and contact with the surrounding ECM. Of the multitude of cytokines implicated in SSc (see Table 83-3), TGF-β is considered to be the master regulator of both physiologic fibrogenesis (wound healing and tissue repair) and pathologic fibrosis.[113]

A member of a large cytokine superfamily that also includes activin and bone morphogenetic proteins, TGF-β is secreted by platelets, monocytes/macrophages, DCs, and fibroblasts, and most cell types express surface receptors for TGF-β. TGF-β plays essential roles in tissue repair, angiogenesis, and immune regulation, and its dysregulation is implicated in cancer, fibrosis, and autoimmunity. Most cell types secrete TGF-β as a latent complex that is sequestered within the ECM and upon injury is converted to its biologically active form, which is capable of triggering cellular responses. Sequestration and activation of latent TGF-β is controlled in part by fibrillin-1 and is mediated by integrins, thrombospondins, αvβ6 integrin, and proteolytic enzymes. Because of its fundamental role in orchestrating fibrotic responses, TGF-β is considered a potential therapeutic target in SSc.[114]

**TABLE 83-4** Fibrogenic Activities of Transforming Growth Factor-β Relevant to Systemic Sclerosis

| |
|---|
| Recruits monocytes/macrophages |
| Stimulates synthesis of collagens, fibronectin, proteoglycans, elastin, tissue inhibitor of metalloproteinases; inhibits matrix metalloproteinases |
| Stimulates fibroblast proliferation, chemotaxis |
| Induces fibrogenic cytokine production: connective tissue growth factor; autoinduction; stimulates production of endothelin-1 |
| Stimulates generation of reactive oxygen species from Nox4 and mitochondria |
| Stimulates expression of TGF-β and PDGF surface receptors |
| Promotes fibroblast-myofibroblast differentiation, monocyte-fibrocyte differentiation |
| Promotes epithelial-mesenchymal and endothelial-mesenchymal transition; suppresses adipogenesis |
| Inhibits fibroblast and myofibroblast apoptosis |

PDGF, Platelet-derived growth factor.

## Cellular Signaling by Transforming Growth Factor-β: Canonical Smad Pathways

The type of response elicited by TGF-β is specific for the target cell and is highly context dependent. In mesenchymal cells, TGF-β acts as a potent inducer of fibrillar collagen synthesis and fibroblast proliferation, migration, and adhesion, and it suppresses the production of matrix-degrading metalloproteinases (Table 83-4). In endothelial and epithelial cells and adipocytes, TGF-β drives transdifferentiation into myofibroblasts. Binding of activated TGF-β to the type II TGF-β surface receptor triggers an intra-cellular signal transduction cascade that leads to the target gene expression. The evolutionarily conserved canonical TGF-β signal transduction pathway involves phosphorylation of the transmembrane type I TGF-β receptor, a serine-threonine kinase that in turn phosphorylates cytosolic Smads. Phosphorylated Smad2/3 form heterocomplexes with Smad4 and translocate into the nucleus, where they bind to consensus Smad-binding elements (SBEs) and recruit transcriptional cofactors such as the histone acetylase p300/CBP. Conserved SBE sequences are found in many TGF-β–inducible genes, including type I collagens, plasminogen activator inhibitor–1, α–smooth muscle actin, and connective tissue growth factor (CTGF). Signal transduction through the Smad pathway is tightly controlled by endogenous inhibitors such as Smad7 and bone morphogenetic protein and activin membrane-bound inhibitor.

## Noncanonical Transforming Growth Factor-β Signaling

Alternative non-Smad pathways also play a role in TGF-β signaling. Non-Smad TGF-β signaling involves protein kinases (c-Abl, ERK1/2, p38 and JNK, focal adhesion kinase [FAK], and TGF-β activated kinase [TAK1]); lipid kinases such as phosphatidylinositol 3′ kinase and its downstream target Akt; and the calcium-dependent phosphatase calcineurin. c-Abl is particularly relevant to SSc because this nonreceptor tyrosine kinase implicated in chronic myelogenous leukemia mediates profibrotic TGF-β and PDGF signal and is activated in SSc fibroblasts.[115] Imatinib, a small molecule inhibitor of c-Abl, reversed abnormal ECM gene expression in SSc fibroblasts and prevented skin fibrosis in mouse models of scleroderma.[116]

# CYTOKINES, GROWTH FACTORS, CHEMOKINES, AND LIPID MEDIATORS

Multiple paracrine/autocrine mediators of ECM accumulation and mesenchymal cell function show aberrant expression or activity in SSc. In addition to TGF-β, soluble mediators such as CTGF, PDGF, IL-4, IL-6, IL-13, adenosine, prostaglandin F2α, and lysophosphatidic acid (LPA1) play prominent roles in the pathogenesis of fibrosis and represent potential therapeutic targets.

## Connective Tissue Growth Factor/CCN2

CTGF is a cysteine-rich 40 kDa matricellular growth factor implicated in angiogenesis, wound healing, and development. Although it is undetectable in normal adults, CTGF is markedly elevated in fibrotic conditions. In SSc, serum CTGF levels correlate with the extent of skin and pulmonary fibrosis. The expression of CTGF can be induced in normal fibroblasts by TGF-β, IL-4, and VEGF, whereas TNF and prostacylins block stimulation. Transgenic mice overexpressing CTGF develop scleroderma-like diffuse skin fibrosis and microvascular disease.[117] Whereas CTGF exerts multiple profibrotic effects in normal fibroblasts, the cellular CTGF receptors and the mechanism of action underlying CTGF profibrotic responses remain incompletely characterized.

## Platelet-Derived Growth Factor

Platelet-derived growth factors (PDGFs) are disulfide-bonded heterodimeric proteins that act mainly on stromal cells and play key roles in wound healing. Originally isolated from platelets, PDGF isoforms are also secreted from macrophages, endothelial cells, and fibroblasts. By signaling via the α and β transmembrane receptors, PDGF acts as a potent fibroblast mitogen and chemoattractant and induces ROS generation and synthesis of collagen, fibronectin, and proteoglycans, as well as the secretion of TGF-β1, MCP-1, and IL-6. In transgenic mice expressing a constitutively active PDGF-α receptor, fibrosis develops in the skin and multiple organs.[118] Lesional skin fibroblasts from patients with SSc show elevated PDGF and PDGF-β receptor expression,[119] and PDGF levels are increased in SSc bronchoalveolar lavage fluid. Some patients with SSc appear to have functional circulating antibodies to the PDGF receptor that can induce fibroblast activation and ROS generation; however, they have also been detected in patients with graft-versus-host disease, and their significance in pathogenesis remains to be established.

## Developmental Pathways: Wnt and Notch

Wnt and Notch are involved in embryonic developmental pathways that appear to be deregulated in fibrosis and SSc. The Wnts comprise a family of poorly soluble glycoproteins with dual roles in cell-cell adhesion and transcriptional

regulation. The Wnts have essential roles in morphogenesis, stem cell homeostasis, and cell fate determination, and abnormal Wnt signaling is implicated in cancer, as well as rheumatoid arthritis and osteoarthritis, osteoporosis, PAH, and aging. Intra-cellular Wnt signaling is mediated via both canonical (β-catenin) and noncanonical pathways, with extensive cross-talk with TGF-β signaling. Transgenic mice overexpressing Wnt10b or a constitutively active mutant β-catenin develop exuberant wound healing and tissue fibrosis.[120] Lungs from patients with idiopathic pulmonary fibrosis show increased nuclear β-catenin accumulation at fibrotic foci. Genomewide transcriptome analysis of SSc skin biopsies shows elevated expression of Wnt ligands, receptors, and targets. Constitutive Wnt-β-catenin signaling in SSc appears to be due to epigenetic silencing of endogenous Wnt inhibitors such as DKK1.

Notch, the transmembrane receptor for Jagged, has a fundamental role in embryonic development, wound healing, and tissue repair. Notch signaling regulates endothelial cell and fibroblast responses, including myofibroblast differentiation. Notch signaling was markedly enhanced in the skin and lungs in a mouse model of scleroderma. ADAM-17 is a proteinase induced by TGF-β and ROS that initiates Notch signal transduction, and its activity is elevated in SSc skin biopsies.[121]

## Interleukins

Members of the IL-1 family are secreted from multiple cell types. A recent study showed that SSc epidermal keratinocytes secreted IL-1α, and their co-culture with normal skin fibroblasts resulted in fibroblast activation and myofibroblast differentiation that was mediated by IL-1α. The Th2 cytokine IL-4 stimulates fibroblast proliferation, chemotaxis, collagen synthesis, and production of TGF-β, CTGF, and TIMP. Serum levels of IL-4 are elevated in SSc, and the number of IL-4–producing T cells is increased in both peripheral blood and skin. IL-6, which is produced by monocytes, B and T cells, fibroblasts, and endothelial cells, directly stimulates collagen and TIMP-1 synthesis in fibroblasts and promotes a Th2-polarized immune response. The biologic activities of IL-6 are mediated via the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) intra-cellular signaling pathway that is shared with other cytokines. Serum levels of IL-6 are elevated in SSc and correlate with the severity of skin involvement. Another Th2 cytokine, IL-13, is implicated in asthma and a variety of fibrotic conditions. IL-13 has profibrotic effects that are both indirect via stimulation of TGF-β production by macrophages and direct via stimulation of fibroblast proliferation and collagen synthesis. Serum levels of IL-13 are elevated in persons with SSc.

## Chemokines

Chemokines represent a superfamily of more than 40 low-molecular-weight soluble mediators that were originally identified based on their chemotactic effects on leukocytes but also have a broad range of cellular targets and biologic activities, including regulation of angiogenesis, wound healing, and fibrosis. The CC chemokine MCP-1 stimulates collagen production directly, as well as through induction of endogenous TGF-β. Serum and bronchoalveolar lavage fluid levels of MCP-1, along with those of macrophage inflammatory protein (MIP)-1α, IL-8 (CXCL8), and CCL18, are elevated in persons with SSc and correlate with the severity of skin fibrosis. Mononuclear cells and dermal fibroblasts from patients with SSc spontaneously produce these chemokines, and lesional SSc fibroblasts show constitutively high expression of the MCP-1 receptor CCR2. Because MCP-1 drives a Th2 response, the MCP-1–CCR2 axis is thought to play a major role in the pathogenesis of SSc by amplifying collagen stimulation and promoting Th2 cytokine production. Significantly, MCP-1 null mice are resistant to the development of fibrosis induced by bleomycin.[122] A global survey of chemokine expression in SSc skin revealed enhanced levels of MCP-1, CCL19, CXCL13, and MCP-3, particularly in patients with early-stage disease.[123]

Additional chemokines overexpressed in lesional tissue or serum in patients with SSc or in animal models of scleroderma include regulated upon activation, normal T cell expressed and secreted (RANTES) and pulmonary and activation-regulated chemokine (PARC; CC chemokines), as well as MIP-2 and fractalkine (CXC chemokines). Insulin-like growth factor binding protein-1 (IGFBP-1) stimulates collagen synthesis and fibroblast proliferation and induces TGF-β. Patients with SSc have elevated levels of IGF-1 in the bronchoalveolar lavage fluid. Expression of IGFBP-3 is markedly elevated in SSc fibroblasts. Adenovirus-mediated overexpression of IGFBP-5 caused chronic scleroderma-like fibrosis in mice.[124]

## Angiotensin II

Angiotensin II is a vasoconstricting peptide hormone generated via proteolytic cleavage of angiotensin I by the angiotensin-converting enzyme in endothelial cells. The renin-angiotensin system is implicated in pathologic fibrosis in the heart, liver, lung, and kidneys. Serum levels of angiotensin II are elevated in patients with difuse SSc (dcSSc), and many patients have functional antibodies targeting the angiotensin II type 1 receptor. Moreover, angiotensin II is elevated in SSc dermal fibroblasts. In cultured fibroblasts, angiotensin II promotes collagen synthesis and TGF-β production. Chronic subcutaneous administration of angiotensin II induces scleroderma-like skin fibrosis and inflammation in mice, whereas treatment with an inhibitor of the angiotensin II type I receptor ameliorates fibrosis in experimental mouse models.

## Bioactive Lipids

Bioactive lipids are potent modulators of fibroblast function and tissue repair. Although some prostanoids inhibit fibrotic responses, prostaglandin F ($PGF_{2\alpha}$) stimulates collagen production and fibroblast proliferation and is elevated in patients with pulmonary fibrosis and can induce fibrotic responses.[125] Mice with targeted deletion of the PGF receptor are protected from bleomycin-induced pulmonary fibrosis. LPA, generated from membrane phospholipids via hydrolysis, exerts multiple biologic activities through G protein–coupled transmembrane receptors. LPA was shown to induce fibroblast chemotaxis and CTGF

production. A recent study indicated that LPA induces αvβ6 integrin-mediated TGF-β activation in epithelial cells, contributing to sustained autocrine TGF-β signaling.[126] LPA levels are elevated in the lungs of patients with pulmonary fibrosis. Moreover, LPA1 knockout mice are protected from bleomycin-induced skin and lung fibrosis.

### Regulation of Fibroblast Function via Innate Immune Signaling: Toll-like Receptors and the Inflammasome

The presence of a type I IFN signature in SSc leukocytes, and genetic studies showing an association of innate immunity genes with SSc, support the role of TLR-mediated innate immune responses in pathogenesis. All members of the TLR family are expressed by normal fibroblasts. Activation of TLR4 by its ligand LPS plays a critical role in liver fibrosis, with sensitization to TGF-β as the underlying mechanism. In SSc, tissue fibroblast TLRs are activated by endogenous TLR ligands. These so-called *damage-associated molecular patterns* are generated by tissue injury, autoimmunity, and oxidative stress. Endogenous TLR ligands potentially implicated in SSc belong to three categories: ECM-derived molecules such as hyaluronan and its small-molecular-weight degradation products, tenascin C, alternatively spliced extra domain A (EDA) fibronectin ($Fn^{EDA}$), and biglycan; cellular stress proteins (alarmins) such as HMGB1 and Hsp60; and nucleic acids and immune complexes released from damaged or necrotic cells. Levels of TLR3 and TLR4 are elevated in SSc skin and lung biopsies and correlate with disease progression. The TLR3 ligand poly(I:C) causes dramatic induction of type I IFN, IL-6, and other inflammatory cytokines, as well as ECM molecules, in normal fibroblasts. The damage-associated molecules $Fn^{EDA}$ and tenascin C are alternatively spliced isoforms on normal ECM proteins that are generated during tissue injury, accumulate to high levels in lesional SSc skin and lung, and function as endogenous TLR4 ligands to elicit profibrotic responses.[127] These observations suggest that in SSc, fibroblast TLR signaling initiated by damage-associated endogenous TLR ligands in SSc converts self-limited regenerative tissue repair into an aberrant and intractable fibrotic scar.

Intra-cellular innate immune sensors include NOD-like receptors (NLRs), retinoic acid inducible gene I (RIG-I), and NALP3. They respond to cytosolic nucleic acids, damage-associated endogenous molecules, oxidative stress, and environmental signals such as silica, bleomycin, and gadolinium. Once activated, these sensors facilitate inflammasome assembly with activation of caspase-1 and secretion of proIL-1β and IL-18. *NLRP1* is identified as a susceptibility gene for SSc and associated pulmonary fibrosis. Inflammasome activation and IL-1β play an important role in experimental mouse fibrosis and might be involved in SSc as well.[128]

### Negative Regulation of Extra-cellular Matrix Accumulation

To prevent excessive scarring after injury, redundant biologic mechanisms have evolved. Fibroblasts are equipped with endogenous molecules that repress ECM gene expression and TGF-β stimulation. For example, Smad7 is an inhibitory Smad that blocks TGF-β signal transduction by accelerating ubiquitin-mediated TGF-β receptor degradation. Functional impairment of Smad7 was demonstrated in SSc fibroblasts. Other cell-intrinsic endogenous repressors of collagen synthesis include the transcription factors Sp3, Fli-1, p53, Ras, Nrf2, and the nuclear hormone receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ), and their impaired expression, induction, or function might contribute to the failure to limit fibroblast activation in SSc.

### Interferon-γ

IFN-γ, the prototypical Th1 cytokine, is a major negative regulator of fibroblast activation. IFN-γ represses collagen gene expression and abrogates stimulation induced by TGF-β.[129] IFN-γ is also a potent inhibitor of fibroblast proliferation, fibroblast-mediated matrix contraction, and myofibroblast transdifferentiation. Significantly, some studies have shown that fibroblasts from patients with SSc are relatively resistant to the inhibitory effects of IFN-γ. Clinical trials of IFN-γ in SSc have demonstrated a modest and inconsistent improvement in skin fibrosis.

### Peroxisome Proliferator-Activated Receptor-γ

PPAR-γ is an intra-cellular molecule that modulates TGF-β signaling and mesenchymal cell plasticity and is functionally associated with fibrosis. Originally identified as a key regulator of adipogenesis, glucose homeostasis, and lipid metabolism, PPAR-γ has a dual function as both a nuclear hormone receptor and ligand-inducible transcription factor. Multiple lipid moieties and electrophilic prostanoids such as 15d-prostaglandin $J_2$ (15d-$PGJ_2$) can serve as endogenous ligands for PPAR-γ. The thiazolidinedione class of insulin-sensitizing drugs, including rosiglitazone and pioglitazone, are potent pharmacologic PPAR-γ agonists. PPAR-γ modulates vascular and immune processes, and abnormal PPAR-γ function is implicated in lipodystrophy, atherosclerosis, PAH, and inflammatory diseases. In fibroblasts, 15d-$PGJ_2$ or pharmacologic PPAR-γ ligands abrogated TGF-β–induced collagen production, myofibroblast transdifferentiation, EMT, and other Smad3-dependent responses. Both the expression and the activity of PPAR-γ are impaired in patients with dcSSc.[130] Of note, multiple factors in SSc pathogenesis including TGF-β, Wnt ligands, IL-13, hypoxia, LPA, and CTGF potently inhibit PPAR-γ function.

## SCLERODERMA FIBROBLAST

Fibroblasts explanted from SSc skin or lungs display an abnormal activated phenotype that persists during their serial passage in vitro, indicating autonomous alteration in cell function. The "SSc phenotype" is characterized by enhanced ECM synthesis, secretion of profibrotic cytokines and chemokines, insensitivity to IFN-γ and other inhibitory signals, and resistance to apoptosis. Moreover, SSc fibroblasts show spontaneous myofibroblast differentiation, in part because of constitutive activation of the FAK focal adhesion kinase. It remains unsettled whether the activated

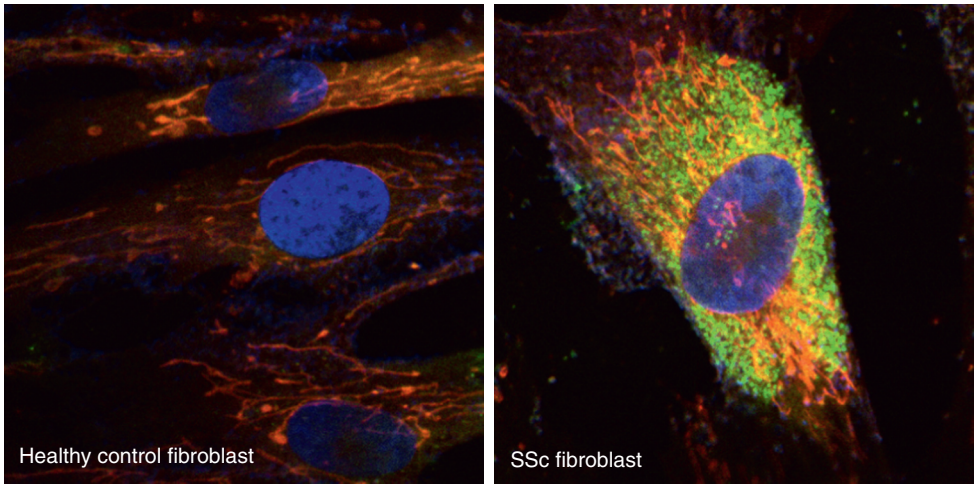



**Figure 83-11** Spontaneous reactive oxygen species (ROS) production by systemic sclerosis (SSc) skin fibroblasts. Skin fibroblasts from healthy control subjects and patients with SSc were incubated in media with MitoSOX Red and CellROX Green probes to measure mitochondrial or cytosolic ROS generation. Nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI; *blue color*). Note the marked increase in both cytosolic *(red)* and mitochondrial *(green)* ROS accumulation in SSc fibroblasts. Representative confocal images. *(Courtesy Jun Wei, PhD.)*

SSc fibroblast phenotype represents an enduring intrinsic cell-autonomous abnormality or activation by exogenous stimuli in the fibrotic milieu.

Numerous molecules involved in intra-cellular signal transduction are abnormally expressed or constitutively activated in SSc fibroblasts. The list includes protein kinase C, Smad3, FAK, Akt, SIRT1, Egr-1, p300, and c-Abl. Elevated expression of Bcl-2 and Akt in SSc fibroblasts may play a role in their relative resistance to apoptosis. Spontaneous ROS generation contributes to the sustained activation of SSc fibroblasts in a self-amplifying loop (Figure 83-11). Because most characteristics of the SSc fibroblasts can be induced by treating normal fibroblasts with TGF-β, the SSc phenotype could contribute to autocrine TGF-β signaling. The levels of TGF-β receptors are elevated on SSc fibroblasts, which might sensitize these cells to endogenously produced TGF-β or to low levels of environmental TGF-β. Furthermore, SSc fibroblasts express thrombospondin and αvβ3 integrins, which activate latent TGF-β at the cell surface. Consistent with the autocrine TGF-β hypothesis, SSc fibroblasts show constitutive TGF-β signaling with elevated Smad3 activation and interaction with p300/CBP. Other studies demonstrate defective expression or function of endogenous suppressors of TGF-β signaling and ECM production such as Smad7, Fli1, adiponectin, and NF4a1, suggesting that failure to terminate fibroblast activation may represent a fundamental defect in SSc.

Autocrine TGF-β activation of fibroblasts cannot fully account for all of the phenotypic hallmarks of SSc fibroblasts, indicating that both Smad-independent TGF-β signaling mechanisms, as well as non-TGF-β–mediated activation events, underlie the induction or maintenance of the SSc phenotype. The autonomous SSc phenotype also could result from abnormal integrin-mediated signaling from the surrounding ECM. Moreover, epigenetic alterations in SSc fibroblasts are associated with persistent and heritable fibroblast dysfunction. For example, silencing the *FLI1* gene, an important endogenous negative regulator of collagen gene expression, by DNA methylation or chromatin histone deacetylation suppresses its expression in fibroblasts from lesional skin, which increases collagen synthesis.

## CONCLUSION

Novel insights from genetic association studies, whole genome-scale analysis of altered gene expression and the role of epigenetic modifications, and phenotyping of circulating and tissue-resident cell types have identified fundamental alterations in immune, wound healing, and developmental responses that underlie the clinical manifestations of SSc. At the same time, these unbiased high-throughput studies also highlight the surprising degree of molecular heterogeneity that is a hallmark of SSc. Of interest, the genes associated with increased risk in SSc are predominantly those that are involved in inflammation, innate immunity, and immune cell signaling and show pervasive overlap with genes associated with SLE, psoriasis, and other autoimmune conditions. These observations suggest that immune dysregulation likely plays a primary and fundamental role in the pathogenesis of SSc.

Nonetheless, it remains unknown why autoimmune diseases with significant shared genetic risk factors, such as SSc and SLE, show such diverse and nonoverlapping clinical and pathologic manifestations, natural histories, responses to immunomodulatory therapies, and disease outcomes. The precise environmental exposures important in SSc, and how they elicit epigenetic modifications that contribute to the persistence of autoimmune and fibrotic processes, are beginning to be characterized. These insights are likely to make significant contributions toward an improved understanding of disease pathogenesis, identification of novel biomarkers of diseases activity and subtype, and discovery and validation of novel therapeutic targets. Although no effective disease-modifying therapies are currently approved for SSc, an improved understanding of pathogenesis is opening the door for the evaluation of novel and selective immunomodulatory, antifibrotic agents and epigenetic modifiers that are precision-targeted for the treatment of disease subsets in SSc.

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CHAPTER 84



# Clinical Features and Treatment of Scleroderma

Fredrick M. Wigley • Francesco Boin

**KEY POINTS**

- **Systemic sclerosis is a multisystem connective tissue disease affecting the skin and internal organs.**
- **The disease process is characterized by chronic inflammation with variable degrees of collagen accumulation (fibrosis) in affected tissues and obliterative vasculopathy of the peripheral and visceral vasculature.**
- **Scleroderma can be associated with high morbidity and mortality in certain subsets, particularly when the lungs, heart, gastrointestinal tract, and kidneys are involved.**
- **Effective management of organ-specific disease manifestations is recommended, although no disease-modifying therapy is yet available.**

Some people believe that the first description of systemic sclerosis (scleroderma) was put forth in 1753 by Cario Curzio (Naples, Italy).[1] However, a careful review of the reported case suggests that the diagnosis was really *scleroedema* because of the distribution of the skin changes and the fact that the 17-year-old female improved with bloodletting, warm milk, and small doses of quicksilver. In 1836, Fantonetti (1791-l877), a Milanese physician, became the first person to use the word *scleroderma* to designate a skin disease in an adult. However, it is likely that this patient too had scleroedema. The first convincing case of scleroderma was reported in 1842; several acceptable cases were then published before 1847, when Gintrac used the term *sclerodermie*, establishing this condition as a specific clinical entity.[2] By 1860, numerous cases had been reported, and articles reviewing the disease were published. Maurice Raynaud (1834-1881) described a patient with *sclerodermie* and cold-induced "asphyxie locale," which was the first description of Raynaud's phenomenon (RP) in a person with scleroderma. Sir William Osler described scleroderma while at Johns Hopkins Hospital between 1891 and 1897.[3] Osler recognized the systemic nature of the disease and was so impressed with the severity of scleroderma that he wrote:

> In its more aggravated forms, diffuse scleroderma is one of the most terrible of all human ills. Like Tithonus, to "whither slowly," and like him to be "beaten down and marred and wasted" until one is literally a mummy, encased in an ever-shrinking, slowly contracting skin of steel, is a fate not pictured in any tragedy, ancient or modern.

Matsui (Japan, 1924) emphasized the importance of visceral involvement as part of scleroderma based on several autopsies. Goetz[4] (Capetown, 1945) further confirmed the multisystem involvement and suggested that the disease be named *progressive systemic sclerosis*.

The concept of subtypes of scleroderma was introduced in 1964, when Winterbauer[5] reported cases with the CRST (calcinosis, Raynaud's phenomenon, sclerodactyly, and telangiectasia) syndrome. A similar group of patients was reported in 1920, and the condition was named after the authors—the Thiberge-Weissenbach syndrome. Velayos and colleagues[6] recognized that esophageal dysmotility was common in these patients, and for this reason the term "CREST syndrome" was introduced.

In 1969, 58 autopsy cases of scleroderma were compared with matched control subjects.[7] The organs found to be frequently and significantly affected by this disease were the skin, gastrointestinal (GI) tract, lungs, kidneys, skeletal muscle, and pericardium. This report first described the systemic nature of scleroderma vascular disease with findings of both kidney and lung arterial changes. In 1979, Rodnan et al.[8] introduced a clinical method to evaluate the extent of skin disease. Steen and Medsger with others conducted extensive surveys of large populations of patients with scleroderma, defining the clinical course and specific subtypes of disease. In the 1970s, an expert subcommittee established diagnostic criteria, and Leroy and colleagues suggested the classification of two major subsets of disease defined by skin involvement: *limited* and *diffuse*. Later, work by several investigators led to the recognition that scleroderma has an autoimmune basis and that the occurrence of specific autoantibodies is associated with subtypes of disease and is useful in predicting disease course. In 2001, Leroy and Medsger[9] proposed that the presence of RP, abnormal nail-fold capillaries, and positive serologic findings for scleroderma-specific autoantibodies would be adequate for the diagnosis of *early* disease. New classification criteria have recently been proposed by a collaborative effort between the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), significantly improving the sensitivity and specificity of the 1980 ACR criteria, particularly for patients with early/limited disease.[10]

Scientific work in the modern era has revealed details regarding the pathogenesis of the disease and has led to the recognition that scleroderma is a complex polygenetic autoimmune disease associated with a unique disease process involving tissue fibrosis. Although a drug with clear disease-modifying properties and the ability to fully control the

underlying disease process has yet to be discovered, major progress has been made in managing specific organ disease. The discovery that an angiotensin-converting enzyme (ACE) inhibitor could reverse the scleroderma renal crisis in the 1970s has changed the course of kidney disease in people with scleroderma and has improved the survival of patients. Current therapies for GI, cardiac, pulmonary, vascular, and interstitial lung disease (ILD) have improved quality of life and survival.

## EPIDEMIOLOGY

### Incidence and Prevalence

Scleroderma is a rare disease with an estimated incidence of approximately 18 to 20 cases per million population per year and a prevalence of 100 to 300 cases per million population. Reported rates vary based on the method of ascertainment, the population under study, and the definition of disease. Scleroderma is found in all races and in various geographic areas, but the prevalence and severity of disease vary among different racial and ethnic groups. The prevalence of scleroderma appears to be greater in the United States, where it has been estimated at 24.2 per 100,000, than in Europe, where recent studies in Iceland, England, France, and Greece have estimated rates ranging from 7.1 to 15.8 per 100,000.[11] In Australia, estimates are similar to those in the United States, whereas in Asia (i.e., Japan and Taiwan), lower prevalences (3.8 to 5.6 per 100,000) similar to those in Europe have been reported.[12,13] The highest scleroderma prevalence is reported among Choctaw Native Americans, in whom the disease appears to be more severe than in other populations.[14] Several surveys in the United States show that African-Americans have a higher age-specific incidence rate and experience more severe disease than do white persons.[15] Occurrence is not different from urban to rural areas. Occasional reports have described unusually large numbers of cases of scleroderma observed in restricted geographic regions, suggesting nonrandom distribution of the disease. For example, in a rural area in the province of Rome, a geographic cluster of scleroderma and disease with related features was reported, suggesting prevalence 1000 times greater than expected.[16] Likewise, a study in the United Kingdom reported a higher prevalence of scleroderma in three geographic areas clustered near airports compared with other areas of the United Kingdom.[17]

The average age at onset is between 35 and 50 years, and the disease is more common among women (3 to 7:1 female-to-male ratio). Disease onset in elderly individuals is well described; it is uncommon for the disease to become manifest before the age of 25 years. Older age at onset is associated with increased risk for multisystem disease and, in particular, pulmonary arterial hypertension (PAH). A progressive increase in the incidence of scleroderma has been noted with increasing age. During the period from 1963 to 1982, in Pittsburgh, Pennsylvania, the highest rates of scleroderma were observed between the ages of 45 and 54 years in black women and between the ages of 55 and 64 years in white women.[18] Younger age at disease diagnosis among black women compared with white women was reported in Detroit, Michigan.[11]

### Survival

Mortality among patients with scleroderma is high, with most deaths being attributed directly to disease manifestations.[19] In fact, the prognosis in scleroderma is highly variable, and survival is influenced by disease subtype, degree of internal organ involvement, and comorbid conditions. Factors associated with poor prognosis include diffuse skin disease, the presence of pulmonary disease (particularly PAH), renal or cardiac involvement, severe GI failure, multisystem disease, older age at disease onset, and the presence of anemia. A worse disease course and higher mortality have also been reported in African-American patients.[20,21] The standardized mortality rate (SMR) is the measure used to assess the relative mortality of a disease in comparison with the general population. Surveys of patients with scleroderma report an SMR from 1.46 to 7.1. A survey found that among 284 patients with scleroderma who died, the median disease duration as estimated from the onset of RP until death was 7.1 years for patients with diffuse skin disease and 15.0 years for patients with limited skin disease. Among non–scleroderma-related causes of death are, as expected, infection, malignancy, and cardiovascular events. Premature atherosclerosis has been implicated as the cause of early death in other inflammatory autoimmune diseases. Recent studies indicate that scleroderma also is independently associated with a higher risk of developing complications from macroscopic coronary artery or cerebrovascular involvement beyond that expected in the general population.[22-24]

Several studies suggest that the main cause of death has changed over time. Improved survival in recent years is thought to be to the result of more effective treatments for specific organ-based complications. It seems clear that the natural history of scleroderma renal complications has changed as a result of the use of ACE inhibitors for renal crisis. Historically, patients with scleroderma renal crisis had a 1-year survival rate of less than 15%.[25] Recent case-control studies conducted after the introduction of ACE inhibitors suggest a 1-year survival rate that is greater than an 85%. Pharmacologic prevention and treatment of ILD and PAH are thought to have an impact. Cohort studies have demonstrated improved overall survival among patients with scleroderma. At one scleroderma center, the 10-year survival rate from the time of disease diagnosis improved from 54% in the 1972-1981 group to 66% in the 1982-1991 group.[26] In another survey it was found that the 5-year survival rate among patients with diffuse cutaneous scleroderma improved from 69% in 1990-1993 to 84% in 2000-2003 ($P = 0.018$), whereas 5-year survival among patients with limited cutaneous scleroderma remained unchanged at 93% and 91%, respectively.[27]

### Environmental Exposures

Geographic clustering of scleroderma cases suggests that environmental exposure may be responsible for the disease, but definitive proof is still lacking. Epidemics of scleroderma-like illness after exposure to dietary (L-tryptophan or adulterated rapeseed oil), occupational (silica), or pharmacologic (e.g., bleomycin and gadolinium-based contrast media) toxins support the idea that environmental exposure can trigger a fibrotic disease. Typical scleroderma is found among

coal miners and workers exposed to silica, particularly male workers. A meta-analysis of the risk of silica exposure found that the combined estimator of relative risk was 1.03 (95% confidence interval [CI], 0.74 to 1.44) in females and 3.02 (95% CI, 1.24 to 7.35) in males.[28] Although occupational exposure to solvents (e.g., paint thinner or removers, mineral spirits, trichloroethylene, trichloroethane, perchloroethane, gasoline, aliphatic hydrocarbons, halogenated hydrocarbons, and BTX solvents containing benzene, toluene, or xylene) or polyvinyl chloride is reported to be associated with scleroderma, the role of these agents in causing disease is controversial and remains unproven. Likewise, case reports have suggested that the silicone present in breast implants has triggered scleroderma, but large epidemiologic surveys have not found a greater incidence of scleroderma than expected in women with breast implants or exposure to silicone. Drugs implicated as potentially causative for systemic sclerosis (SSc)-like illnesses include bleomycin, pentazocine, and cocaine. Interestingly, cigarette smoking is not associated with an increased risk for scleroderma.[29] Actually, a study conducted in Canada indicated that smoking was associated with a decreased severity of scleroderma skin disease, although the mechanisms for this finding are unclear.[30]

## CLINICAL FEATURES

### Diagnostic Criteria

A subcommittee of the ACR established the "preliminary" diagnostic criteria in 1980 based on a consensus of experts who evaluated a multicenter survey of patients with scleroderma compared with other patient groups (Table 84-1).[31] The purpose of these criteria was to provide diagnostic certainty and consistency for research and other documentary exercises. Consequently, despite a high specificity, their sensitivity was poor, and patients with early stage scleroderma and 20% of those with limited cutaneous disease did not meet these criteria.[9] Since then, knowledge about the prognostic relevance of scleroderma-specific autoantibodies has significantly improved, and the use of nail-fold capillaroscopy has become a widely accepted, reliable diagnostic tool, supporting the recommendation by some experts to revise the 1980 classification criteria to be more inclusive of patients with early disease manifestations.[32,33] For this reason, the ACR and EULAR established a joint committee to define new classification criteria, which were published in 2013 (Table 84-2).[10] The single finding of thickening of the skin proximal to the metacarpophalangeal (MCP) joints of the hands remains a criterion that alone is sufficient to classify a patient as having scleroderma. If skin thickening is found only distal to the MCP joints, then a point system is applied based on the presence of seven other specific manifestations. These manifestations include skin thickening of the fingers, fingertip lesions, telangiectasias, abnormal nail-fold capillaries, PAH and/or ILD, RP, and scleroderma-related autoantibodies. Patients with a combined score of 9 or more points are classified as having scleroderma. The new criteria have a specificity of 0.91 and a sensitivity of 0.92, which overall represent a much better performance compared with the previous criteria. Nonetheless, patients without skin thickening but with the presence of RP, abnormal nail-fold capillaries, and positive scleroderma-specific autoantibodies still do not meet the 2013 ACR/EULAR criteria. Large longitudinal surveys confirm that in a high percentage of patients with this "early" phenotype, scleroderma develops within a 2- to 4-year period of follow-up.[32,34] Therefore, although classification criteria are useful in defining cases for research purposes, the clinician needs to take into account subtle features of the disease to make an early diagnosis and institute prompt management. Most experts would accept the finding of three of the five features of the CREST syndrome (*c*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysfunction, *s*clerodactyly, and *t*elangiectasias) to make a clinical diagnosis of scleroderma. In clinical practice, one needs to recognize that although some patients do not experience skin changes, they nonetheless have systemic disease (systemic sclerosis sine scleroderma), and that many patients will present with only partial expression of the disease (e.g., RP with nail-fold changes only), with well-defined disease developing later. Experts argue that patients who have partial expression, especially when a specific scleroderma-related autoantibody is present, should be considered to have a diagnosis of scleroderma and be treated accordingly.

**TABLE 84-1** Establishing a Diagnosis of Scleroderma

| |
|---|
| **1980 ACR Criteria** |
| Must have (a) or two of (b), (c), or (d): |
| a. Proximal SSc (proximal to MCPs/MTPs) |
| b. Digital pits |
| c. Sclerodactyly |
| d. Pulmonary fibrosis (chest radiograph; HRCT) |
| **CREST Criteria** |
| Must have three of the five features: |
| a. Calcinosis |
| b. Raynaud's phenomenon |
| c. Esophageal dysmotility |
| d. Sclerodactyly |
| e. Telangiectasias |
| **Minor Criteria**[*†] |
| Must have all three: |
| a. Definite Raynaud's phenomenon |
| b. Abnormal capillary loops |
| c. Specific scleroderma autoantibody |

*Some experts continue to classify patients with minor criteria only as undifferentiated connective disease with scleroderma features.

†Proximal areas include the face. Autoantibody includes anticentromere, antitopoisomerase 1 (Scl-70), and anti-RNA polymerase III.

ACR, American College of Rheumatology; CREST, calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, telangiectasias; HRCT, high-resolution computed tomography; MCPs, metacarpophalangeal joints; MTPs, metatarsophalangeal joints; SSc, systemic sclerosis.

### Classification and Clinical Subsets

The scleroderma disease process is complex and its clinical expression is very heterogeneous, such that the disease is expressed in several distinct clinical phenotypes. Classifying patients into subtypes is useful both for investigative purposes and for clinical practice. A specific subtype can define increased risk for internal organ involvement and overall prognosis. The traditional way to classify patients is by the

**TABLE 84-2** American College of Rheumatology/European League against Rheumatism Classification Criteria for the Classification of Systemic Sclerosis

| Item | Sub-item(s) | Weight/Score |
|---|---|---|
| Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints *(sufficient criterion)* | — | 9 |
| Skin thickening of the fingers *(only count the higher score)* | Puffy fingers<br>Sclerodactyly of the fingers (distal to the MCPs but proximal to the proximal interphalangeal joints) | 2<br>4 |
| Fingertip lesions *(only count the higher score)* | Digital tip ulcers<br>Fingertip pitting scars | 2<br>3 |
| Telangiectasia | — | 2 |
| Abnormal nailfold capillaries | — | 2 |
| Pulmonary arterial hypertension and/or interstitial lung disease *(maximum score 2)* | Pulmonary arterial hypertension<br>Interstitial lung disease | 2<br>2 |
| Raynaud's phenomenon | — | 3 |
| SSc-related autoantibodies *(maximum score is 3)* | ACA<br>Scl-70<br>RNA Pol | 3 |

*The total score is determined by adding the maximum weight (score) in each category. Patients with a total score of ≥9 are classified as having definite SSc.

ACA, Anticentromere; MCPs, metacarpophalangeal joints; RNA Pol, anti-RNA polymerase III; Scl-70, antitopoisomerase 1; SSc, systemic sclerosis.

Modified from van den Hoogen F, Khanna D, Fransen J, et al: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 65:2737–2747, 2013.

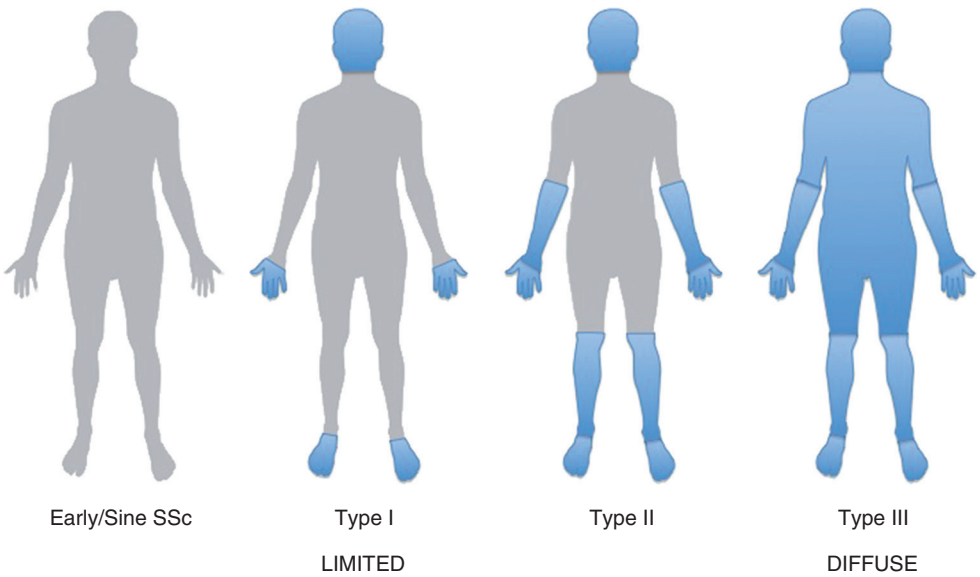



**Figure 84-1** Classification of scleroderma and clinical subsets. Most experts classify scleroderma into two major groups: limited and diffuse. Limited includes patients with no skin changes (early scleroderma or systemic sclerosis "sine" scleroderma), and type I (fibrosis limited to the digits) or type II (fibrosis up to the elbows and the knees). Diffuse or type III have more proximal limb or truncal involvement (the face is excluded). SSc, Systemic sclerosis.

degree of skin thickening as determined by physical examination. The skin is scored by pinching a fold and deciding whether it is abnormally thickened because of the scleroderma process. A committee of experts decided by consensus that two major groups of patients can be identified based on the distribution of skin changes and associated clinical and laboratory outcomes (Figure 84-1).[35] Patients are considered to have *diffuse skin disease* if skin changes are found proximal to the elbows and/or knees or on the trunk, excluding the face. These patients tend to have higher risk of multisystem disease and poor survival. Patients are considered to have *limited disease* if skin changes occur distal to the elbows and/or knees and not on the trunk. Facial skin thickening can be present in the limited group. Some clinicians argue that the term *CREST syndrome* should be eliminated and that these patients should be classified in the

limited skin group. Others believe that the CREST syndrome is a unique subtype within the broader group of limited skin diseases with a distinct disease course.

Another less popular classification system divides patients into three groups based on skin changes: limited (fingers only), intermediate (skin on the elbows or knees), and diffuse (skin above the elbows and/or knees and/or trunk). Studies using this classification have found that the intermediate group had distinct clinical outcomes, including an intermediate survival statistic between limited (best) and diffuse (worse).[36] A subtype of disease with absence of skin fibrosis has been recognized and is referred to as *scleroderma sine scleroderma*. Serologic studies have shown that the presence of specific autoantibodies predicts clinical features of the disease, suggesting that the classification might be based on the autoantibody type (see the "Natural History of Disease" section). Patients with early or partial expression of the disease (e.g., RP alone with nail-fold capillary loop abnormalities) are often diagnosed as having undifferentiated connective tissue disease. Clear features of scleroderma will develop in about 20% of these patients, usually within 2 to 4 years of follow-up.[34]

Patients with scleroderma often have features of another rheumatic or autoimmune disease. These patients are considered to have an *overlap syndrome*. The most common overlap syndromes are polyarthritis, myositis, sicca complex, and hypothyroidism. Less common are primary biliary cirrhosis, autoimmune hepatitis, vasculitis, rheumatoid arthritis, and anti-neutrophil cytoplasm antibody (ANCA)-associated pauci-immune glomerulonephritis. Mixed connective tissue disease is an overlap syndrome with features of scleroderma, polymyositis, lupus-like rashes, and rheumatoid-like polyarthritis.

### Natural History of Disease

The natural history of scleroderma varies a great deal depending on the specific subtype of disease established in a patient. Scleroderma tends to be a chronic monophasic illness, unlike systemic lupus erythematosus, which is characterized by quiescence interrupted by sudden disease flares. The pattern of the disease and the degree or type of internal organ involvement are variable but generally can be predicted by the extent of skin affected. Internal organ disease is more severe in patients with the diffuse skin type than in those with limited scleroderma. The disease course is usually indolent in patients with sclerodactyly alone. In these patients, years pass from onset of RP before obvious organ dysfunction manifests, although explosive multisystem disease can emerge rapidly over several months in patients with widespread skin changes. Likewise, when patients with diffuse skin disease experience skin improvement, new internal organ disease is generally uncommon. The degree of skin thickening varies in extent and progression over time, with some cases stopping after several months with relatively mild disease and others progressing over years to involve most of the body surface (see the Skin Involvement section).

Patients classified in the *limited scleroderma* group may have intermediate involvement of skin extending over the hands and forearms or lower legs but not over the trunk. These patients appear to have an intermediate disease course that is not as severe as that of patients with widespread skin disease. The intermediate group has decreased survival compared with patients who only have sclerodactyly. Although the degree of skin disease provides a rough guide by which to predict disease course, many exceptions have been noted. Variability is associated with other clinically defined factors, including age, gender, and racial/ethnic background. Late age at onset increases the risks for PAH and poor survival. Males may have a more aggressive disease course than do females. African-American or Native-American background predicts a worse disease course and worse multisystem disease.[11,20,21]

## GENERAL PRINCIPLES OF DISEASE EVALUATION

### Measuring Disease Activity and Severity

One of the challenges in studying or caring for patients with scleroderma consists of determining and measuring disease activity, severity, and damage. These measures are not independent of each other and thus cannot be considered in isolation. Activity is a measure of ongoing disease that is dynamic and reversible; it is a direct reflection of ongoing biologic processes such as autoimmune-mediated inflammation or progressive tissue fibrosis. Severity is a measure of the impact of damage on the whole person or a specific organ at any given time. In the extreme, severity is a measure of irreversible end-stage damage. It is important to distinguish reversible disease activity from irreversible damage because although both can cause clinical distress, intervention differs for the two. Patients with scleroderma accumulate irreversible damage over time, and thus the measure of activity is best performed early, when disease has the potential of being reversed.

Measurement of changes in skin thickness (a modified Rodnan skin score) is used as a surrogate measure of disease severity and a predictor of the extent of organ involvement and overall prognosis (see the Skin Involvement section).[37] It is also used as a quantifiable, easily obtainable, and valid score that changes over time in response to therapy. Thus the skin score is now frequently used in clinical trials as a major outcome measure. In patients with diffuse skin disease, improvement in the skin score is associated with a better clinical outcome. A core set of items covering 11 domains (skin, musculoskeletal, cardiac, pulmonary, renal, GI, health-related quality of life measures, global health, RP, digital ulcers, and the biomarkers erythrocyte sedimentation rate and C-reactive protein) is now considered to provide the minimum clinically relevant treatment effect values, as defined by consensus of experts in the field.[38] More detailed scales for specific organs are also in use, as well as other composite measures that include functional testing and clinical outcomes. For example, a skin assessment tool has been developed that combines the modified Rodnan skin score with a visual analogue scale (VAS) score of the patient's assessment of skin activity, a VAS score of the physician's assessment of skin activity, and measures obtained by a durometer.[39] A GI scale is in use to define mild to severe GI disease.[40] Tools to assess severity and outcomes in PAH as related to scleroderma include pulmonary arterial pressures and cardiac function as measured by

right heart catheterization and echocardiography (ECHO), exercise testing as measured by the 6-minute walk and oxygen saturation, New York Heart Association functional class, severity of dyspnea on a VAS, and measures of quality of life and function obtained by the Short Form 36 and the Health Assessment Questionnaire and Disability Index (HAQ-DI) and physician global scale. Other outcome measures that are considered valid include the Raynaud's condition score for severity of Raynaud's phenomenon, forced vital capacity, and Mahler's dyspnea index to monitor ILD, serum creatinine, blood pressure, and complete blood cell count during a scleroderma renal crisis, as well as serum creatine phosphokinase to monitor muscle disease.

A scleroderma severity scale has been developed by Medsger et al.[41] to assess disease severity status in individual patients both at a given time and longitudinally (Table 84-3). This scale is based on scoring that ranges from 0 (normal) to 4 (end-stage) for each organ system involved in scleroderma, including a general measure, along with measures of the peripheral vascular system, skin, joints and tendons, GI tract, lung, heart, and kidney. This severity scale is now used by many experts to define the status of a patient in clinical trials and in research that investigates risk for clinical outcomes.

The HAQ-DI is a self-administered tool initially developed for rheumatoid arthritis to measure functional impairment; it has been validated and used in people with scleroderma as well, in whom it correlates with objective physical signs and reflects the disease course. Higher degrees of impairment are found by HAQ scores in patients with scleroderma who have diffuse skin disease, higher skin scores, abnormal hand function, presence of myopathy or friction rubs, and joint pain.[42] Change in the HAQ score occurs with changes in skin involvement and progressive organ disease.[43] The HAQ is useful in evaluating patients with RP and correlates with the severity and presence of digital ulcers. VAS instruments that assess disease in various domains specific for scleroderma are added to the HAQ to focus on scleroderma-specific issues, including measures of RP and digital ulcers. In addition, the Short Form 36 survey, a simple self-report tool composed of 36 items in 8 domains, is used to assess health-related quality of life.

Developing an activity scale is much more challenging than establishing severity measures. Attempts include evaluating changes in skin score, defining evidence of active lung disease, and documenting congestive heart failure or ongoing scleroderma renal crisis. A composite score generated as a physician global assessment has been used to measure disease activity. This simple global score has some merit, but it is influenced, along with disease activity, by organ severity and irreversible damage.[44] A European study group developed the Valentini disease activity index, which is currently used in clinical studies.[45] This index provides more of an organ-specific assessment than a measure of global activity. The Medsger disease severity scale has the potential to measure disease activity if it is used in studies with serial observations, when individual organ scales can be combined into a composite score. A significant body of

**TABLE 84-3** Medsger Systemic Sclerosis Severity Scale*

| Organ System | 0 (Normal) | 1 (Mild) | 2 (Moderate) | 3 (Severe) | 4 (End Stage) |
|---|---|---|---|---|---|
| General | Wt loss <5%; Hct 37%+; Hb 12.3+ g/dL | Wt loss 5%-10%; Hct 33%-37%; Hb 11.0-12.2 g/dL | Wt loss 10%-15%; Hct 29%-33%; Hb 9.7-10.9 g/dL | Wt loss 15%-20%; Hct 25%-29%; Hb 8.3-9.6 g/dL | Wt loss 20+%; Hct 25%; Hb <8.3 g/dL |
| Peripheral vascular | No RP; RP not requiring vasodilators | RP requiring vasodilators | Digital pitting scars | Digital tip ulcerations | Digital gangrene |
| Skin | TSS 0 | TSS 1-14 | TSS 15-29 | TSS 30-39 | TSS 40+ |
| Joint/tendon | FTP 0-0.9 cm | FTP 1.0-1.9 cm | FTP 2.0-3.9 cm | FTP 4.0-4.9 cm | FTP 5.0+ cm |
| Muscle | Normal proximal muscle strength | Proximal weakness, mild | Proximal weakness, moderate | Proximal weakness, severe | Ambulation aids required |
| Gastrointestinal tract | Normal esophagogram; normal small bowel series | Distal esophageal hypoperistalsis; small bowel series abnormal | Antibiotics required for bacterial overgrowth | Malabsorption syndrome; episodes of pseudo-obstruction | Hyperalimentation required |
| Lung | DLCO 80+%; FVC 80+%; no fibrosis on radiograph; sPAP <35 mm Hg | DLCO 70%-79%; FVC 70%-79%; basilar rales; fibrosis on radiograph; sPAP 35-49 mm Hg | DLCO 50%-69%; FVC 50%-69%; sPAP 50-64 mm Hg | DLCO <50%; FVC <50%; sPAP 65+ mm Hg | Oxygen required |
| Heart | ECG normal; LVEF 50+% | ECG conduction defect; LVEF 45%-49% | ECG arrhythmia; LVEF 40%-44% | ECG arrhythmia requiring therapy; LVEF 30%-40% | CHF; LVEF <30% |
| Kidney | No history of SRC with serum creatinine <1.3 mg/dL | History of SRC with serum creatinine <1.5 mg/dL | History of SRC with serum creatinine 1.5-2.4 mg/dL | History of SRC with serum creatinine 2.5-5.0 mg/dL | History of SRC with serum creatinine >5.0 mg/dL or dialysis required |

*If two items are included for a severity grade, only one is required for the patient to be scored as having disease of that severity level.

CHF, Congestive heart failure; DLCO, diffusing capacity for carbon monoxide, % predicted; ECG, electrocardiogram; FTP, fingertip-to-palm distance in flexion; FVC, forced vital capacity, % predicted; Hb, hemoglobin; Hct, hematocrit; LVEF, left ventricular ejection fraction; RP, Raynaud's phenomenon; sPAP, estimated pulmonary artery pressure by Doppler ECHO; SRC, scleroderma renal crisis; TSS, total skin score; Wt, weight.

Modified from Medsger TA Jr, Bombardieri S, Czirjak L, et al: Assessment of disease severity and prognosis. *Clin Exp Rheumatol* 21(3 Suppl 29):S51, 2003.

research has been devoted to define more precise measures of disease activity and prognosis, including quantification of serologic markers of inflammation, immune activation, and circulating cellular subsets, but none has developed into a widely accepted, clinically useful tool. Currently there is no "gold standard" to measure disease activity in scleroderma, and defining biomarkers or other measures of disease activity remains a major challenge.

## Autoantibodies

Measurement of the autoantibodies present in scleroderma can be helpful in determining the clinical features and prognosis of the disease, in that specific scleroderma-related autoantibodies have been established as strong predictors of disease outcome and the pattern of organ complications (Table 84-4).[46] The three most frequently observed types of scleroderma-specific autoantibodies are anticentromere, antitopoisomerase I, and anti-RNA polymerase III antibodies.

Patients with anticentromere antibodies tend to be older white women. Skin disease usually is limited and most often involves just the fingers. The disease course is indolent, and often the diagnosis is delayed until anticentromere antibodies are discovered or late organ disease becomes evident. Patients frequently have features of the CREST syndrome. Subcutaneous calcinosis is a late manifestation, appearing in areas such as small clusters on the fingers and along the forearm and anterior lower leg. RP can become severe and is associated with increased risk for macrovascular disease. Higher risk for digital gangrene and amputation is noted in the anticentromere-positive group. ILD is less common, but progressive pulmonary vascular disease with PAH and right heart failure occur in a significant proportion of patients. Overall, however, anticentromere antibodies generally are predictors of a favorable prognosis. The presence of anticentromere antibodies can also be seen in patients with primary Sjögren's syndrome who do not have scleroderma.

Antitopoisomerase I antibodies are seen in 30% of African-American patients and are correlated with a poor prognosis and higher scleroderma-related mortality. ILD is highly associated with antitopoisomerase, independent of the degree of skin disease. Patients usually have diffuse skin disease and are at risk for rapid skin changes and renal crisis, usually in the first few years from disease onset. RP may be the first symptom, followed in the first years of disease by skin changes and joint-tendon involvement leading to contractures, particularly in the fingers and elbows. The degree of skin thickening varies a great deal in these patients, but usually the level of severity of skin involvement is established in the first 1 to 3 years of disease.

Anti-RNA polymerase III antibodies are associated with rapid and aggressive diffuse skin disease and renal involvement. These patients have the worst cutaneous involvement, with rapid widespread skin disease associated with signs and symptoms of deep tissue fibrosis involving joints, tendons, and muscles. Flexion contractures of the fingers, wrist, elbows, shoulders, hips, knees, and ankles are complications that can occur within a few months of onset of disease activity. Friction rubs are common. A "fibrosing" myopathy without inflammation, along with skin and joint disease, leads to loss of strength and flexibility. Significant disability occurs in early stage disease. Anti-RNA polymerase III–positive patients are relatively protected from the development of ILD or pulmonary vascular disease and are not likely to have severe GI disease, although a small study has shown an association with gastric antral vascular ectasia.[47] Approximately 25% to 40% of patents have a high risk for developing scleroderma renal disease with hypertensive crisis in subjects positive for this autoantibody. Risk is greatest in the early years, when skin is rapidly changing. Heart disease can be a late complication, but major disability from skin and deep tissue disease is the most worrisome long-term problem in this subtype of scleroderma. Studies conducted in different countries have consistently found an association between scleroderma and underlying malignancy in subjects positive for anti-RNA polymerase III.[48-50] The detection of cancer and the onset of scleroderma have been described in close temporal relationship, indicating the importance of appropriate screening for underlying neoplastic disease in this subset of patients.

Other antinucleolar antibodies are found among subgroups of patients with scleroderma and are associated with specific clinical phenotypes and clinical outcomes. Of these, anti-Th/To antibodies and anti-PM/Scl antibodies are associated with limited skin disease, whereas anti-U3-RNP (fibrillarin) antibodies are associated with diffuse disease. Patients with anti-Th/To antibodies are at increased risk for development of severe ILD and pulmonary hypertension. Anti-U3-RNP is another predictor of a less favorable prognosis with a higher frequency of internal organ involvement, including ILD, PAH, and renal crisis. It is frequently present in African-Americans and is associated with diffuse

**TABLE 84-4** Autoantibodies and Associated Phenotypes in Scleroderma

| Antigen | Subtype | Clinical Phenotype |
|---|---|---|
| Topoisomerase 1 (Scl-70) | Diffuse | Pulmonary fibrosis, cardiac involvement |
| Centromere (protein B, C) | Limited | Severe digital ischemia, PAH, sicca syndrome, calcinosis |
| RNA polymerase III | Diffuse | Severe skin disease, tendon rubs, cancer, GAVE, renal crisis (±sine scleroderma) |
| U3-RNP (fibrillarin) | Diffuse or limited | Primary PAH; esophageal, cardiac, and renal involvement; muscular disease |
| Th/To | Limited | Pulmonary fibrosis, rare renal crisis, lower GI dysfunction |
| B23 | Diffuse or limited | PAH, lung disease |
| Cardiolipin, $\beta_2$GPI | Limited | PAH, digital loss |
| PM/Scl | Overlap | Myositis, pulmonary fibrosis, acro-osteolysis |
| U1-RNP | Overlap | SLE, inflammatory arthritis, pulmonary fibrosis |

GAVE, Gastric antral vascular ectasia; GI, gastrointestinal; GPI, glycoprotein I; PAH, pulmonary arterial hypertension; RNP, ribonucleoprotein particle; SLE, systemic lupus erythematosus.

Modified from Boin F, Rosen A: Autoimmunity in systemic sclerosis: current concepts. *Curr Rheumatol Rep* 9:165–172, 2007.

skin disease and poor prognosis. Hyperpigmented and hypopigmented skin changes are common, and contractures of large joints associated with inflammatory or fibrotic muscle disease lead to significant morbidity. Cardiac disease is often subclinical until late-stage disease, when both right and left heart failure can occur.

Anti-PM/Scl, anti-Ku, and anti-U1-RNP antibodies are seen mainly in patients with overlap syndromes. The presence of anti-Pm/Scl is associated with acute onset of weakness as a result of inflammatory muscle disease and a higher risk for ILD. Anti-Ku positivity is strongly associated with muscle and joint involvement. Anti-U1-RNP is seen in patients with mixed connective tissue disease and is common among African-Americans with limited scleroderma. Polyarthritis, myositis, and lupus with skin or renal involvement are common complications. A subset can experience diffuse skin disease, and risk for late onset of PAH is increased overall. Although uncommon, anti-B23 autoantibody is associated with PAH. Up to 11% of patients with scleroderma can test negative for anti-nuclear antibodies (ANAs).

## CLINICAL MANIFESTATIONS

### General Principles

Several basic principles can be of help in managing patients with scleroderma. The first principle is that scleroderma encompasses subsets of disease, each with a unique clinical phenotype; thus carefully determining the specific subtype of the patient sets the scene for deciding the most appropriate therapeutic approach. Clinical problems and disease activity in scleroderma are highly variable in expression, even within a given subtype. This variability is thought to be a result of different susceptibility to, or risk for, systemic complications. It is also due to the presence of a dynamic biologic process unique to scleroderma. Therefore, patients move through stages of the disease process with levels of disease activity that vary with time and with a possible shift in nature. Signs of classic inflammatory events are often present during the initial phase of the disease, but later, an indolent subclinical fibrotic process is dominant and gradually causes organ damage.

Although the skin may be the site of the most dramatic physical findings, scleroderma is a multisystem disease, and the physician must search for early organ-based complications, including cardiopulmonary, GI, renal, or musculoskeletal involvement. Understanding the shortcomings of current investigations, not getting locked into "traditional" therapy, focusing on an organ-specific approach, and defining the disease subtype and level of disease activity are most important in establishing optimal management. Although no good-quality studies have been conducted to prove the effectiveness of this approach, it seems reasonable to use combination therapy with rapid control of the inflammatory immune process early on, followed by maintenance immunosuppression for an extended time. The antiinflammatory program is then coupled with organ-specific therapy (see later sections). The missing component in this comprehensive approach is the availability of a direct antifibrotic agent able to prevent the progression of fibrosis once the inflammatory phase is under control. Obviously, early intervention is most important for success; once irreversible tissue or organ damage is noted, supportive care becomes the main and often the only option. More recently, myelo- or immune-ablative therapeutic protocols with or without autologous hematopoietic stem-cell transplantation have shown the ability to achieve rapid control of skin disease and stabilization of lung function.[51-53] However, long-term data are not yet available to define how lasting these effects are, and the treatment mortality associated with these approaches remains significant.

### Raynaud's Phenomenon

**KEY POINTS**

- **RP is common in the general population and usually presents with a benign clinical course.**
- **In scleroderma, RP is more clinically symptomatic and can be associated with manifestations of digital ischemia.**
- **Abnormal vasomotor regulation and progressive endothelial and structural vessel disease occur in RP associated with scleroderma.**
- **Treatment for RP must include control of excessive vasoreactivity, modification of structural vascular disease, and prevention of microthrombotic events.**

RP is an exaggerated vascular response of the digital arterial circulation triggered by cold ambient temperature and emotional stress. The diagnosis of RP is based on a history of excessive cold sensitivity and recurrent events of sharply demarcated pallor and/or cyanosis of the skin of the digits (Figure 84-2). Blanching reflects digital arterial vasospasm, and cyanosis occurs as a result of the deoxygenation of sluggish venous blood flow. Some skin blushing (redness) may follow as a result of reactive hyperemia after regular blood flow has been restored. RP occurs in 3% to 15% of the general population. It is more common among females (3 to 4:1) and is likely to begin before age 20 years. During cold exposure (particularly during shifting temperatures and winter months), RP attacks increase in frequency and



**Figure 84-2** Active Raynaud's phenomenon with well-demarcated pallor at the fingertips in a patient with scleroderma.

intensity. RP can be categorized clinically into primary and secondary forms (Figure 84-3). Primary RP occurs when no disease process is associated with recurrent vasospastic events. Distinguishing primary RP from that associated with an underlying disorder is frequently challenging. Young age at onset (<20 years), symmetric manifestations of symptoms, mild to moderate severity, no association with digital ulceration or tissue gangrene, normal nail-fold capillary examination, and a negative ANA titer are all indicative of primary RP.[54] Secondary RP occurs in a variety of settings, including connective tissues disorders and other rheumatic conditions, occupational trauma (e.g., hypothenar hammer syndrome), the use of certain drugs (e.g., antimigraine agents, ergotamine derivatives, and bleomycin), increased blood viscosity, and compressive or obstructive vascular disease (e.g., thoracic outlet syndrome, atherosclerosis, and thromboangiitis obliterans).

Nail-fold capillaroscopy is the tool most commonly used at the bedside to distinguish patients with primary RP from those with scleroderma or another rheumatic disease. Maricq and LeRoy[55] first described the abnormal pattern of nail-fold capillary vessels seen in scleroderma. Sulli and colleagues[56] provided further evidence regarding the utility of assessing patterns of nail-fold capillary abnormalities to monitor progression toward more severe disease manifestations. The simplest method of detection is to coat the skin

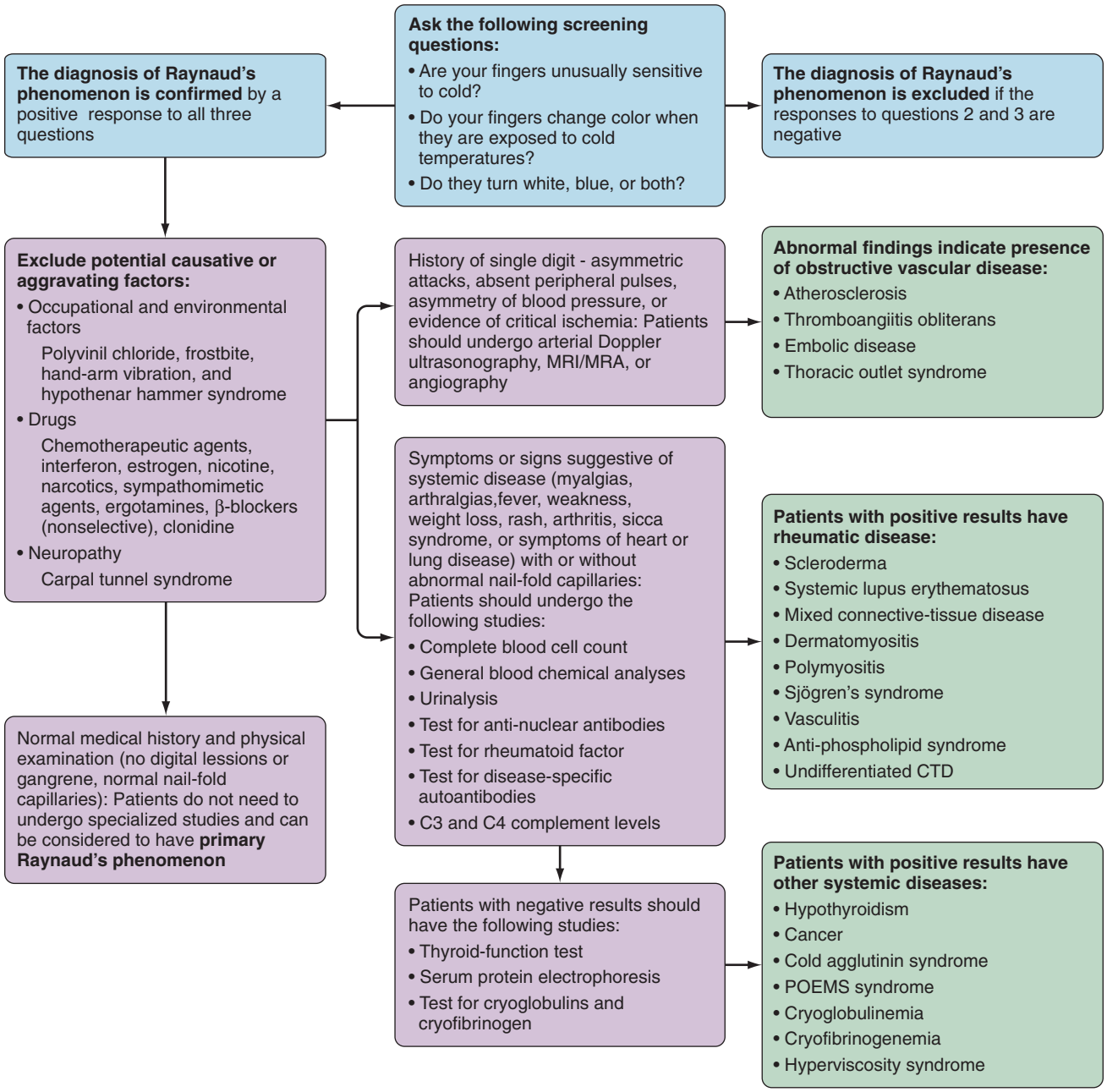



**Figure 84-3** Approach to diagnosis of Raynaud's phenomenon. CTD, Connective tissue disease; MRA, magnetic resonance angiography; POEMS, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes.

of the nail fold with immersion oil and then view the area using a bifocal dissecting microscope or a handheld device such as an ophthalmoscope set at 20 or 40 diopters. Portable dermatoscopes can also be used to perform a rapid and effective examination of the nail-fold area.[57] A computerized nail-fold videocapillaroscopy technique is used in specialty centers. This technique provides enhanced digital images that can assess local blood flow and follow the disease course.[58] Although patients with primary RP may have normal, thin, palisading nail-fold capillary loops (Figure 84-4), in secondary RP, capillary loop dilation/enlargements and dropouts represent the salient features. Patterns of capillary abnormalities appear to correlate with the course of systemic disease manifestations. Capillary dilation (giant capillaries), microhemorrhages, and some disorganization of the capillary network are typical of early disease; dropouts, avascular areas, and signs of neoangiogenesis with bizarre architectural distortion manifest at later stages of scleroderma.[59,60] Clinical features of scleroderma will develop in approximately 20% to 30% of patients with RP and abnormal nail-fold capillary changes, usually within a 2- to 3-year period.[34] Patients presenting with RP, nail-fold capillary changes, and the presence of a scleroderma-related autoantibody have a 70% to 80% chance of developing scleroderma within 2 to 3 years from presentation. Therefore, capillaroscopy represents an important standard tool that can be used to examine patients presenting with RP.

In scleroderma, RP and digital ischemia are the clinical manifestations of both fixed structural vascular disease and abnormal regulation of local vasomotor control. Cold-induced vasoconstriction of peripheral blood vessels normally occurs via sympathetic stimulation. Abnormal thermoregulation is associated with a nonvasculitic vasculopathy characterized by endothelial dysfunction and a fibrotic proliferation, which produces an increase in collagen content of the intima of small and medium vessels and causes loss of vessel flexibility and obliteration of their lumina. Digital pitting with loss of fingertip tissue and small, painful, superficial ulcerations are very common and usually are noted as a consequence of disease affecting the small arteries and arterioles of the skin (Figure 84-5). Large, deep ulceration of the distal portion of the finger is a consequence of larger vessel (e.g., digital artery) occlusion associated with severe vasospasm. The latter event usually presents as a sharp demarcation of the distal digit with intense, localized pain resulting from ischemia. Failure to reverse these events may lead to loss of the entire digit or limb as a result of deep tissue infarction.

Although a number of methods may be used to objectively score attacks of RP, no test is considered practical or reproducible enough to replace the clinical criteria for diagnosis and management. Patients with an active RP attack will present with coolness of the involved distal fingertips and/or toes, which can be associated with a line of demarcation of skin pallor or cyanosis. In later stages of severe RP, if the condition is not treated, one may find signs of critical digital ischemia resulting in painful and unremitting digital ulcerations. Such findings warrant immediate medical

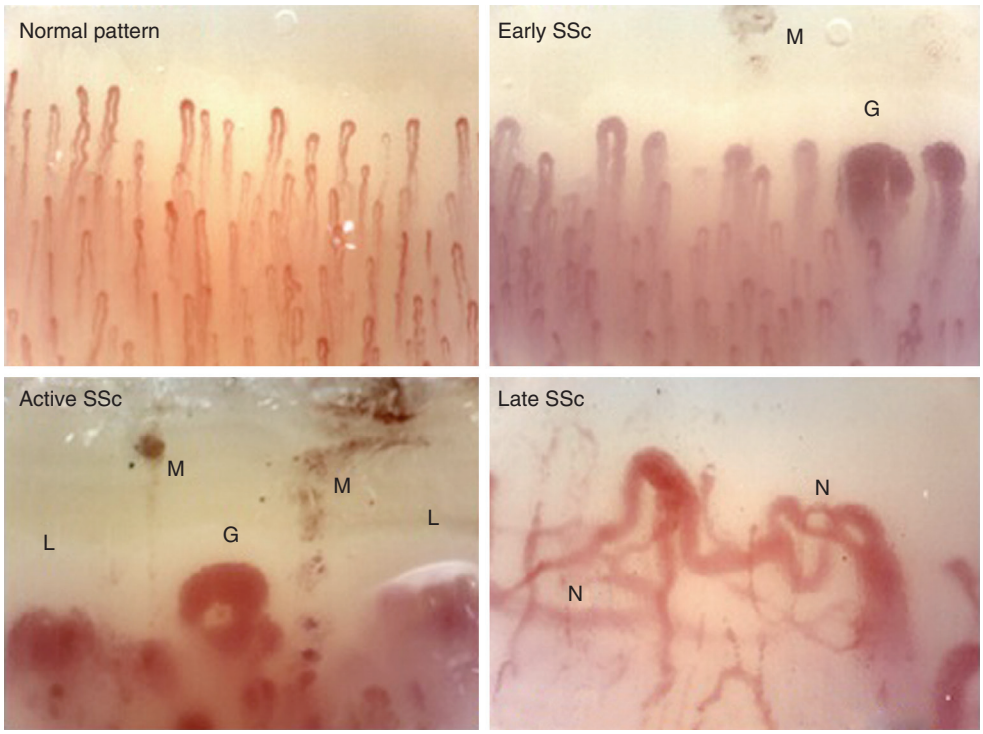



**Figure 84-4** Patterns of nail-fold capillary abnormalities assessed by video capillaroscopy in patients with scleroderma. *Top right,* "Early pattern" shows the presence of few enlarged/giant capillaries, few capillary hemorrhages, and no evident loss or distortion of capillaries. *Bottom left,* "Active pattern" presents with frequent dilated capillary loops, frequent microhemorrhages, moderate loss of capillaries, and mild disorganization of the capillary architecture. *Bottom right,* "Late pattern" is characterized by severe loss of capillaries with avascular areas, ramified/bushy capillaries (neovascularization), and disorganization of the normal capillary architecture. G, Giant capillaries; L, loss of capillaries; M, microhemorrhages; N, neoangiogenesis; SSc, systemic sclerosis. *(Courtesy Professor Maurizio Cutolo.)*




**Figure 84-5** Scleroderma and Raynaud's phenomenon can be associated with digital ulcerations and severe digital ischemia. **A**, Traumatic ulcers over the proximal interphalangeal joints. **B** and **C**, Ischemic digital ulcerations as a result of small arterial disease. **D**, Digital gangrene as a result of macrovascular disease.

treatment. Several methods may be used to assess the severity of RP. Traditionally, the patient is asked to use a diary to record the frequency and duration of attacks during days of usual activity. Raynaud's condition score is a patient-based measure that takes into account the impact of RP on the patient, including pain, discomfort, and effect on daily function. Other laboratory-based measures, including laser Doppler, thermography, and plethysmography methods, are used in an attempt to obtain objective data.

## Treatment of Raynaud's Phenomenon and Digital Ischemia

The primary goal in the management of RP is prevention of digital ischemia through the use of nonpharmacologic and pharmacologic measures (Figure 84-6). In the setting of acute digital ischemia, rapid intervention using both treatment modalities is required. The primary and most important nonpharmacologic therapy for prevention is avoidance of cold ambient temperatures, particularly transitioning from a warm or hot environment to a cold one. A warm ambient temperature reduces the frequency and severity of RP. All patients with RP should understand that clothing should be layered and loose-fitting, with the goal of maintaining a warm core body temperature, not just warmth of the affected extremities. Patients who have an acute ischemic event are best treated with rest in a warm environment (at home or in the hospital), insulated from cold temperatures. Other potential therapies include minimizing emotional distress (reducing sympathetic tone) and avoiding aggravating factors such as smoking, sympathomimetic drugs (e.g., preparations for the common cold), migraine headache medications (e.g., serotonin agonists), and nonselective β-blockers (e.g., propranolol). Although behavioral therapies (including biofeedback, autogenic training, and classical conditioning) are reported to be helpful, their benefit is controversial, and they play no role in the management of acute ischemia related to scleroderma.[61] In fact, biofeedback alone is not helpful for primary RP.

Drug therapy for RP in scleroderma includes the use of a variety of oral or systemic vasodilators. Calcium channel blockers are considered to be first-line therapeutic agents in the treatment of RP. This class of medication works primarily by inducing arterial vasodilation through direct inhibition of contraction of vessel smooth muscle cells; however, these agents provide additional benefits by reducing oxidative stress and inhibiting platelet activation.[62,63] Most published studies evaluating the efficacy of calcium channel

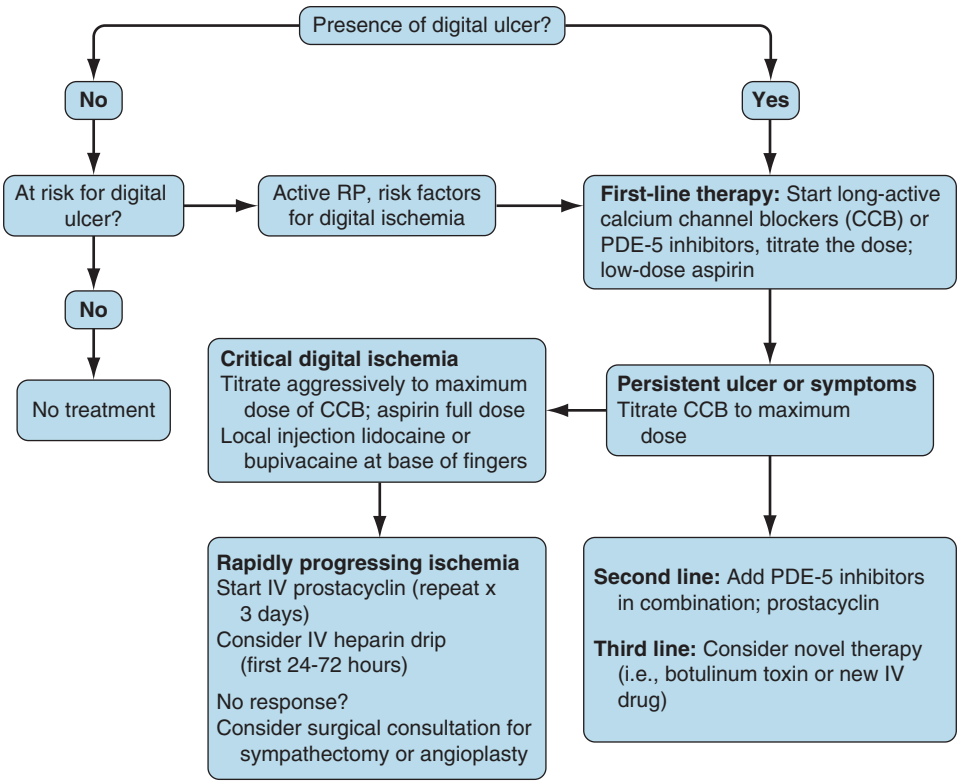



**Figure 84-6** Approach to drug treatment of Raynaud's phenomenon (RP) and acute digital ischemia. CCB, Calcium channel blockers; IV, intravenous; PDE-5, phosphodiesterase-5; SSRI, selective serotonin reuptake inhibitor.

blockers in RP have used dihydropyridines (e.g., nifedipine, amlodipine, nisoldipine, isradipine, and felodipine), and few have looked at nondihydropyridines. The calcium channel blockers appear to be more efficacious in primary than in secondary RP.[64,65] A meta-analysis of 17 studies evaluating the efficacy of short- and long-acting formulations of calcium channel blockers reported a 33% reduction in attack severity and a near 50% reduction in the number of attacks per week.[66] The current recommendation is to use an extended-release formulation of a calcium channel blocker for treatment of nonurgent RP. The dose should be titrated to clinical efficacy, but common adverse effects (e.g., hypotension, headache, and pedal edema) should be monitored. For urgent cases of RP, a short-acting formulation of the medication is preferred, but titration should be carefully monitored. Although calcium channel blockers are the agents most likely to be effective, a host of other vasodilators are used, including nitrates, phosphodiesterase-5 inhibitors (e.g., sildenafil), intravenous (IV) prostaglandins, and sympatholytic agents (e.g., prazosin).

Among other agents being tested in the treatment of RP are drugs that enhance nitric oxide availability—serotonin uptake blockers (e.g., fluoxetine), Rho-kinase inhibitors, local injection of botulinum toxin (Botox), antioxidants (acetylcysteine), angiotensin receptor blockers, and selective $\alpha$2-adrenergic receptor antagonists. A combination of these agents, if tolerated, can be used in refractory cases. Various formulations of topical and oral nitroglycerin are used.[67,68] Although some degree of efficacy is obtained, use of nitroglycerin is limited by substantial adverse effects, including headache, dizziness, and local skin irritation. The cyclic guanine monophosphate phosphodiesterase-5 inhibitors are thought to be effective in the treatment of RP by sustaining the vasodilatory effect of nitric oxide.[69,70] Studies using phosphodiesterase inhibitors have reported variable effects, and the ideal drug and dosing have not yet been defined. Despite the theoretical advantage of ACE inhibitors, a study of quinapril showed that these medications did not affect the occurrence of digital ulcers or the frequency or severity of RP episodes.[71] Thus, although ACEs are critical for the treatment of a scleroderma renal crisis (see the Renal Involvement section), they do not have a major role in the management of RP.

New emphasis is being placed on the prevention of scleroderma vascular disease through the use of immunosuppressive and vasoprotective drugs. HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors (statins) can modify progression of vascular injury and prevent vascular ischemia through different pleiotropic functions, such as by improvement of endothelial dysfunction, reduction of clotting, and introduction of some antiinflammatory effects.[72,73] Data suggest that statins may increase the number of endothelial progenitor cells and improve vascular remodeling after injury.[74,75] In a randomized, placebo-controlled study of patients with scleroderma who had secondary RP, statin use significantly decreased the number of digital ulcers that formed.[76]

IV infusions of vasodilating prostaglandins (e.g., alprostadil, epoprostenol, iloprost, and treprostinil) reduce the severity and frequency of RP attacks and are most

helpful during periods of sustained critical ischemia. These prostaglandins are efficacious in the treatment of RP because of their strong vasodilating effect, inhibition of platelet aggregation, and enhancement of vascular function through other mechanisms. IV prostacyclins can be given intermittently to patients with scleroderma during winter months or throughout the year, or they can be used acutely during a vascular crisis. Oral prostaglandins have variable absorption and are not yet available.

Endothelin-1 is strongly implicated in the pathogenesis of RP and the vascular disease of scleroderma.[77] In a randomized, placebo-controlled, double-blind multicenter study, bosentan, an endothelin-1 receptor blocker, showed efficacy in preventing new digital ulcers, along with improved hand function, but it did not improve healing of existing ulcers compared with placebo.[78]

Sympathectomy is a viable option for patients with RP who do not respond to medical therapy and should be considered as an acute intervention during a critical ischemic event. Localized digital sympathectomy with lysis of fibrosis around the vessel is effective for acute ischemia and for the most part has replaced cervical sympathectomy.[79,80] Responses to sympathectomy are not sustained over time, suggesting limited long-term benefit. In fact, RP attacks eventually recur, and medical therapy is needed to prevent new events. Injections of botulinum toxin A to the hands and fingers have shown favorable effects both in managing acute digital ischemia and in controlling chronic vasospastic activity.[81] Large, randomized, controlled trials are not yet available to confirm the validity of initial reports.

Careful assessment for correctable macrovascular disease should be performed when acute digital ischemia occurs, particularly when the entire finger is demarcated or when the event involves a lower extremity. In this setting, appropriate studies such as arterial Doppler ultrasound or angiographic imaging are warranted. If macrovascular disease is present, vascular surgery may help alleviate the occlusive process. Preferential involvement of the ulnar artery has been reported in patients with scleroderma.[82]

Patients with critical digital ischemia should be hospitalized to reduce vasospastic activity, maintain warmth, and permit rapid initiation of vasodilator therapy. IV vasodilating prostaglandins can be infused to maximize vasodilation. Anti-platelet therapy with low-dose aspirin may be useful, but its benefit is unproven in the acute setting. Heparinization may be considered during acute ischemic crises of the digits, but chronic anticoagulation in scleroderma is not recommended. Fibrinolysis to manage acute occlusion of larger arterial vessels can be considered, but this practice has not been studied for routine use in scleroderma-related critical digital ischemia. Chemical sympathectomy of the affected digit, performed by local infiltration with lidocaine or bupivacaine, may provide immediate relief. For refractory cases, a surgical approach to digital sympathectomy is used.

Ischemic digital lesions should be treated with topical antibiotics and daily cleansing with soap and water. Débridement procedures should be performed very cautiously because tissue trauma may extend the injury as a result of the avascular nature of the digital tissue. Digital lesions that progress to dry gangrene should be permitted to undergo autoamputation. Surgical amputation is best offered only in cases of intractable pain or deep tissue infection.

## Skin Involvement

The most overt clinical manifestation of scleroderma is skin disease. The degree of involvement can vary among patients, and involvement can change in severity and distribution over time in the same individual. Almost every patient with scleroderma presents with skin thickening and hardening due to increased collagen and extra-cellular matrix deposition in the dermis. The distribution of skin changes is characteristic, with more frequent and intense involvement of fingers, hands, forearms, distal legs, feet, and the face, as well as, to a lesser degree, the proximal limbs and anterior trunk. Sparing of the midback is typical. Scleroderma is classically subdivided into *limited* and *diffuse* cutaneous forms (Figure 84-7). Limited scleroderma is defined by skin thickening that is restricted to the face and limbs distal to the elbows and knees. Commonly, in this form of the disease, only the fingers and the face are involved. In contrast, diffuse cutaneous involvement is characterized by widespread skin thickening, including proximal limbs and truncal areas. Proximal skin involvement defines the diffuse cutaneous subset but may be absent in early stages of disease when signs of skin thickening are not prominent, other disease manifestations (e.g., RP and nail-fold capillary abnormalities) are more apparent, and evidence of internal organ involvement is still lacking.

Traditionally, patients are clustered into limited and diffuse skin subsets, but evidence suggests the existence of an intermediate group of patients. Each subset of disease defined by the degree of skin thickening has a unique pattern of disease manifestation and risk for specific clinical outcomes. Therefore, expression of skin disease can be used as a predictor or a "clinical biomarker" of the disease course. The variable degree of skin involvement can be quantified by physical examination. The most widely accepted scoring system (the modified Rodnan skin score) is applied by pinching the skin in 17 different body areas and subjectively averaging the thickness of each specific site from 0 (normal) to 3 (very thick; Figure 84-8). The skin score (maximum, 51) is a useful clinical measurement tool that can be used to quantify the severity of skin disease.[37] Although the overall extent of skin involvement as measured by the skin score can predict certain clinical outcomes, it does not define the quality of the skin process nor the level of disease activity at any single point in time. Therefore, it is important to follow up on the skin score over time and measure changes sequentially to monitor disease progression.

Cutaneous involvement in scleroderma begins with clinical signs of inflammation, which is called the *edematous phase* because it is characterized by nonpitting edema of affected body areas. In patients with limited scleroderma, this event is mild and is restricted to the digits; however, in the diffuse form of the disease, cutaneous swelling and edema can be widespread and so impressive in the limbs that it mimics a fluid overload state such as congestive heart failure. Edema can also cause local tissue compression. For example, upon involvement of the wrist area, patients with scleroderma are not infrequently diagnosed with carpal tunnel syndrome (especially at disease onset) to explain hand and wrist discomfort. In association with edema, signs and symptoms of inflammation are common. Erythema of the skin and intense pruritus and pain (see Figure 84-7C)




**Figure 84-7** Skin involvement in scleroderma is subdivided into "limited" and "diffuse" cutaneous forms. **A**, Sclerodactyly in limited cutaneous disease. **B**, Truncal changes in diffuse cutaneous disease. **C**, Inflammatory signs in early active skin disease. **D**, Finger contracture in the chronic fibrotic phase of skin involvement in scleroderma.

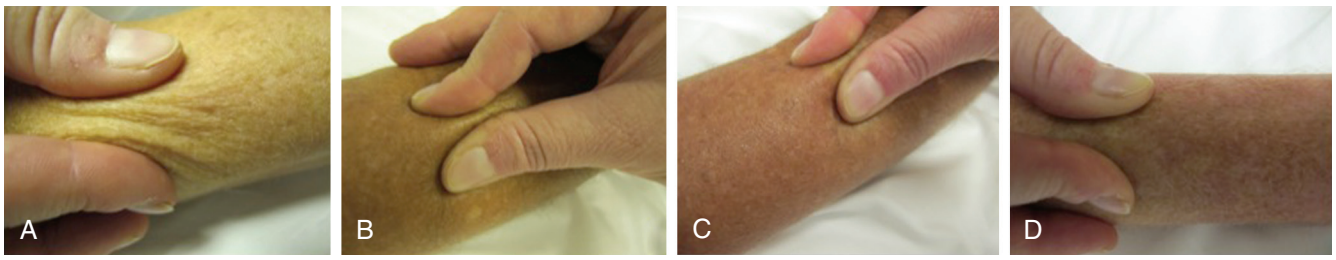



**Figure 84-8** Method used to semi-quantify skin thickness in scleroderma. The modified Rodnan skin score is obtained by clinical palpation of 17 different body areas (fingers, hands, forearms, upper arms, chest, abdomen, thighs, lower legs, and feet) and subjective averaging of the thickness of each specific site: **A,** 0 = normal; **B,** 1 = mild; **C,** 2 = moderate; and **D,** 3 = severe. The maximum score is 51.

are characteristic of advancing active diffuse skin disease. This pain has a neuropathic quality with a reported "pins and needles" sensation. The disease process leads to loss of skin appendages, as well as decreased hair growth and loss of sweat and exocrine glands; thus the skin surface becomes dry and uncomfortable. Small papules can be seen in areas of trauma as the result of scratching, giving the surface a cobblestone texture. The edematous phase continues for several weeks but eventually gives way to a fibrotic stage, with protracted activity that may last months or years.

During the fibrotic phase, acute inflammation is clinically less obvious, and deposition in the dermis of excessive collagen and other extra-cellular material thickens the skin, making it inflexible and causing further loss of skin appendages. Fibrosis extends beyond the dermis into the deeper layers with loss of subcutaneous adipose tissue

(lipodystrophy). In late stages of the disease, skin actually thins with atrophy and has a non-inflammatory bound-down appearance. Deeper tissue fibrosis causes permanent contractures around joints or may involve underlying muscle, causing a myopathy (see Figure 84-7D). Patients with diffuse cutaneous scleroderma experience the most dramatic widespread skin changes; those with limited skin disease may note only puffy fingers and digital thickening typical of sclerodactyly. A masked facies, small oral and orbital apertures, and vertical furrowing of the perioral skin are consequences of skin and soft tissue fibrosis. In some patients, gum atrophy and facial skin tightening make the front teeth appear more prominent. Flexion contractures of fingers, wrists, and elbows often appear as a result of dermal sclerosis and fibrosis with atrophy of underlying tissues. Skin ulcers can develop as a complication of avascular fibrotic or damaged thinned skin and are very common at sites of trauma, such as over the digital MCP or proximal interphalangeal (PIP) joints or at the tip of the elbows, particularly when joint contractures are present at these sites (see Figure 84-5A). Ulcerations may be noted as a result of underlying vascular disease and tissue ischemia (see Treatment of Vascular Disease section). Ankle or lower extremity ulcers occur rarely as a result of macrovascular occlusive disease or comorbid conditions (venous disease). Areas of hypopigmentation (vitiligo-like) and/or patches of depigmentation with preserved perifollicular pigmentation (a "salt-and-pepper" appearance) can develop, typically on the face, arms, and trunk (Figure 84-9A). General tanning of the skin may be seen, even in the absence of sun exposure.

Active skin involvement might persist for the first 12 to 18 months of the disease, with no further clinical signs of inflammation or progressive skin fibrosis seen after this interval. During this later stage, the skin begins to repair and can return to normal texture or, in areas most severely affected (e.g., the fingers and hands), it can become thin and atrophic. During this recovery phase, new robust hair growth is seen, particularly on the forearms, and itching and pain disappear, consistent with spontaneous resolution of disease activity. After years from disease onset, the skin rarely relapses again into an active phase and gradually can recover normal texture and color. Patients who, over time, experience the establishment of a phenotype that is limited to the fingers (plus or minus facial changes) do not undergo conversion to the diffuse form of the disease.

Telangiectasias are erythematous matted skin lesions of vascular origin; for this reason, they blanch upon application of local pressure. Telangiectasias are composed of vasodilated postcapillary venules without evidence of inflammation and resemble the type of lesions seen in Osler-Weber-Rendu disease (hereditary hemorrhagic telangiectasia). Telangiectasias develop primarily on the fingers, hands, face, and mucous membranes, but they also may be found on the limbs and trunk (Figure 84-9B). They tend to become more numerous over time in both limited and diffuse types of skin disease and are more obvious in white patients with limited scleroderma. The biologic mechanism leading to the development of telangiectasias in scleroderma is thought to be related to the underlying chronic tissue hypoxia that stimulates abnormal secretion of vascular growth factors (e.g., vascular endothelial growth factor). Thus the development of telangiectasias may indicate ongoing vascular injury and abnormal vascular repair or angiogenesis. The total burden of telangiectasias is associated with increased risk of pulmonary hypertension, suggesting that they are clinical biomarkers of systemic vascular disease. Nail-fold capillary abnormalities can be observed after immersion oil is applied to the skin surface at the base of the fingernails, and direct visualization is performed using an ophthalmoscope or by videocapillaroscopy. In early scleroderma, dilated capillary loops (giant capillaries) and microhemorrhages may be seen. At later stages, the nail-fold capillaries are attenuated and disorganized (see Figure 84-4).

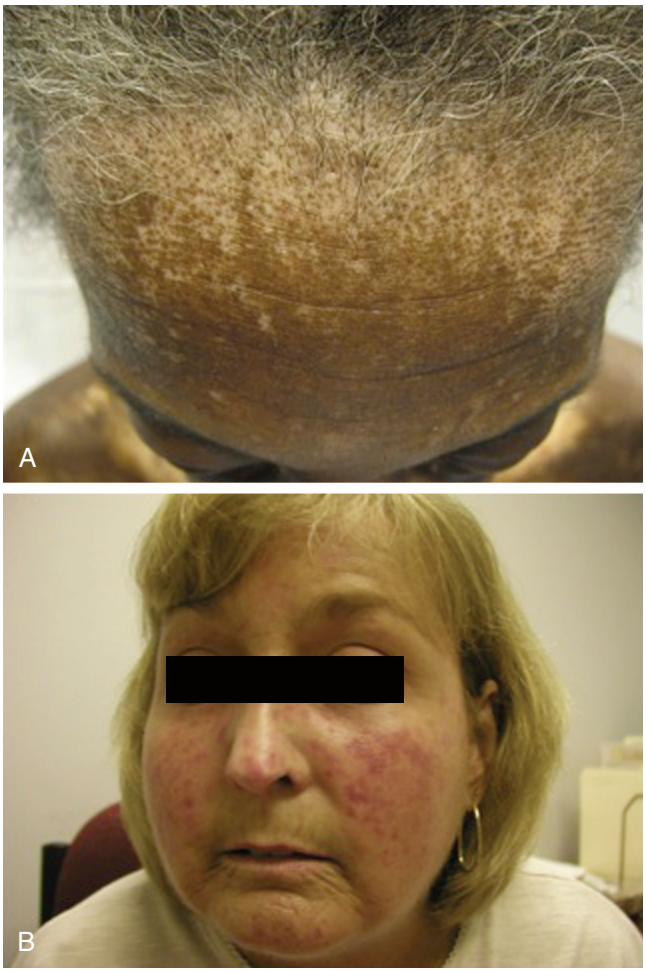



**Figure 84-9** Skin manifestations in patients with scleroderma. **A**, Depigmentation with preserved perifollicular pigmentation ("salt-and-pepper") appearance of the forehead. **B**, Large facial telangiectasias.

Despite evidence of active inflammation during the early edematous phase of scleroderma skin involvement, administration of systemic corticosteroids has not uniformly shown efficacy in stopping disease progression. A variety of other immunomodulatory drugs and newer agents with potential "disease-modifying properties" (i.e., antifibrotic properties) have been used to control the skin disease, but to date, none has proved uniformly successful. In the early active stage of diffuse scleroderma, pruritus can be one of the most distressing symptoms. Antihistamines, analgesics, or tricyclic anti-depressants (e.g., doxepin) are often used but usually provide only partial benefit. Dryness of the skin surface results from damage to the exocrine structures caused

by decreased or absent natural oil (sebum) production. This dryness can worsen pruritus, leading to skin trauma as a result of repetitive scratching. Ulceration and secondary tissue infection also may result. The best approach to treatment is characterized by frequent topical application of an emollient preparation, periodic cleansing with soap and water, and use of topical antibiotics for any traumatic skin ulceration. Physical therapy is important to prevent severe skin and joint contractures and to help patients with activities of daily living.

## Gastrointestinal Involvement

**KEY POINTS**

- **Manifestations of gut dysmotility are universally present in patients with scleroderma and can affect any segment of the GI tract.**
- **Involvement of the upper GI tract is more common than the lower GI tract and can present with severe symptoms.**
- **Dysfunction and failure of the lower GI tract are associated with poor prognosis.**

Almost every patient with scleroderma has symptoms of GI disease, ranging from mild gastroesophageal reflux disease (GERD) to severe bowel dysfunction, which can be life threatening. Virtually any segment of the GI tract can be affected (Table 84-5).

**TABLE 84-5** Gastrointestinal Manifestations of Scleroderma

| Site | Manifestation | Management |
|---|---|---|
| Oropharynx | Perioral tight skin<br>Decreased oral aperture<br>Periodontitis, gum disease<br>Dry mouth<br>Swallowing difficulties<br>Coughing, aspiration | Regular dental and periodontal care<br><br>Artificial saliva<br>Targeted swallowing exercises and rehabilitation |
| Esophagus | Acid reflux (heartburn)<br>Dysphagia<br><br>Strictures<br>Barrett's esophagus | Lifestyle modifications<br>Proton pump inhibitors<br>Prokinetics<br>Endoscopic treatments and procedures |
| Stomach | Gastroparesis, dyspepsia<br>Gastric antral vascular ectasia | Prokinetics<br>Proton pump inhibitors, iron replacement<br>Endoscopic laser or cryotherapy<br>Transfusions<br>Surgery |
| Small and large intestine | Hypomotility, constipation<br><br>Bacterial overgrowth, diarrhea<br>Pseudo-obstruction<br>Pneumatosis intestinalis<br>Malabsorption<br>Colonic pseudodiverticula | Mild laxatives<br>Promotility agents<br>Rotational antibiotics<br>Octreotide<br>Avoidance of surgery<br>Enteral or parenteral nutrition support |
| Anorectum | Sphincter incompetence | Biofeedback, sacral nerve stimulation, surgery |

### Oropharynx

Patients may report that chewing is difficult because of decreased facial flexibility caused by skin and deeper tissue fibrosis. Perioral skin tightening can result in a decreased oral aperture and the inability to open the mouth wide enough for proper dental care or to bite into large solid foods such as an apple (Figure 84-10). Dry membranes resulting from decreased saliva production can lead to difficult mastication. In some patients, periodontal disease and gum regression cause loosening of teeth, which further affects chewing capacity.

Upper pharyngeal function is usually normal, but in a subset of patients a myopathy may develop that causes weakness of pharyngeal muscles, mimicking a neuromuscular disease.[83] This manifestation is characterized by pharyngeal dysfunction with problems initiating swallowing and frequent coughing as a result of laryngeal penetration, and it can be associated with increased risk for aspiration of foods or liquids. Although few pathologic studies of the upper pharyngeal structures have examined scleroderma, both myositis and fibrosis are known to occur.

### Esophagus

Dysphagia resulting from esophageal disease is the most common GI symptom, occurring in approximately 90% of patients. Heartburn, regurgitation, and dysphagia for pills and solids (more than for liquids) are the most common symptoms, but atypical retrosternal pain (particularly at night), periodic cough, a sense of food "sticking" in the esophagus, and nausea can result from esophageal dysfunction. Esophageal involvement in scleroderma is characterized by loss of normal smooth muscle function, especially in the lower two-thirds of the esophagus, and hypotonia of the lower esophageal sphincter (Figure 84-11A). Functional studies of esophageal motility suggest that neural dysfunction is common in patients with scleroderma and that it may precede myopathic dysfunction and histologic changes in smooth muscle layers.[84] Manometric evaluation has identified the presence of esophageal hypomotility in areas that later did not show any histologic abnormality of the smooth muscle. Other studies have shown esophageal smooth muscle activity in response to pharmacologic stimulation (methacholine challenge) early in the disease, but not in patients with more advanced scleroderma. The cause of these abnormalities is unknown, but clear evidence indicates that neural dysfunction precedes muscle disease.

Tissue fibrosis is often evoked as the cause of esophageal disease in scleroderma. However, pathologic studies demonstrate atrophy of the smooth muscle layers of the distal esophagus in the absence of significant fibrosis. Ischemia of the esophagus in scleroderma is suggested by functional studies that show impaired esophageal blood flow after exposure to cold temperatures and institution of rewarming protocols. Non-inflammatory intimal layer hyperplasia in the arterioles of the GI tract has been described. Inflammatory infiltrates usually are not present in the smooth muscle unless severe transmural esophagitis is present, which suggests that inflammation is not a cardinal feature of the pathogenesis of esophageal smooth muscle lesions in scleroderma.




**Figure 84-10** Oral manifestations. **A**, Perioral skin tightening with decreased oral aperture, furrowing around the lips, and dry membranes. **B**, Periodontal disease with regression of gum and loosening of teeth. **C** and **D**, Telangiectasias on the lips and tongue.

The severity of esophageal symptoms may not accurately reflect the seriousness of the underlying disease. Therefore, every patient with scleroderma should be fully evaluated for esophageal involvement. If untreated, GI reflux may lead to esophagitis, bleeding, esophageal strictures, or precancerous lesions such as Barrett's esophagus. Asymptomatic patients without abnormal laboratory test results (e.g., anemia or positive evidence of GI bleeding) do not require specific interventions. Patients with mild GERD can be treated empirically with proton pump inhibitors and then can be followed up clinically if they become symptom free with normal laboratory data. When more severe symptoms are present (e.g., GERD with dysphagia), or when treatment with proton pump inhibitors fails, an upper GI endoscopy is necessary to fully assess the anatomy of the esophagus and the extent of related disease. Direct endoscopy is appropriate to rule out Barrett's esophagus, stricture, or a site of uncontrolled esophagitis or bleeding. Barium swallow and cine-esophagography are sensitive tests for esophageal strictures. However, direct measurement of esophageal motility via esophageal manometry may be needed if the cause of symptoms such as atypical chest pain is unclear. Although complications from Barrett's esophagus (e.g., esophageal cancer) are uncommon, periodic repeat endoscopy to reassess status is recommended, even if symptoms are under therapeutic control.

It is critical that patients alter their eating behavior to complement medication therapy. Patients often experience better results by eating more frequent smaller meals, avoiding food intake for several hours before bedtime, moving the main meal toward midday, taking a walk after meals to help gastroesophageal emptying, and eliminating foods that aggravate symptoms (e.g., spicy sauces and caffeinated or carbonated beverages). Bedtime is often a time of severe reflux. The occurrence of reflux can be improved by avoiding filling the stomach at least 2 to 3 hours before bedtime and by elevating the head and trunk during sleep. Treatment of reflux by suppression of gastric acid with antacids or histamine-2 is helpful but overall disappointing in patients with scleroderma. Conversely, proton pump inhibitors (e.g., omeprazole and lansoprazole) can be very effective. Usually, long-term daily treatment is required. Higher doses may be used periodically for breakthrough symptoms. If symptoms are not controlled with recommended medication dosages, then a formal 24-hour ambulatory pH study may be necessary to determine whether persistent symptoms are due to uncontrolled acid reflux. A prokinetic drug (e.g., metoclopramide, domperidone, or erythromycin) should be added when symptoms of dysphagia or endoscopic findings of esophagitis are present despite the use of effective acid suppression. These medications tend to be effective during early disease but are less likely to help later in the presence

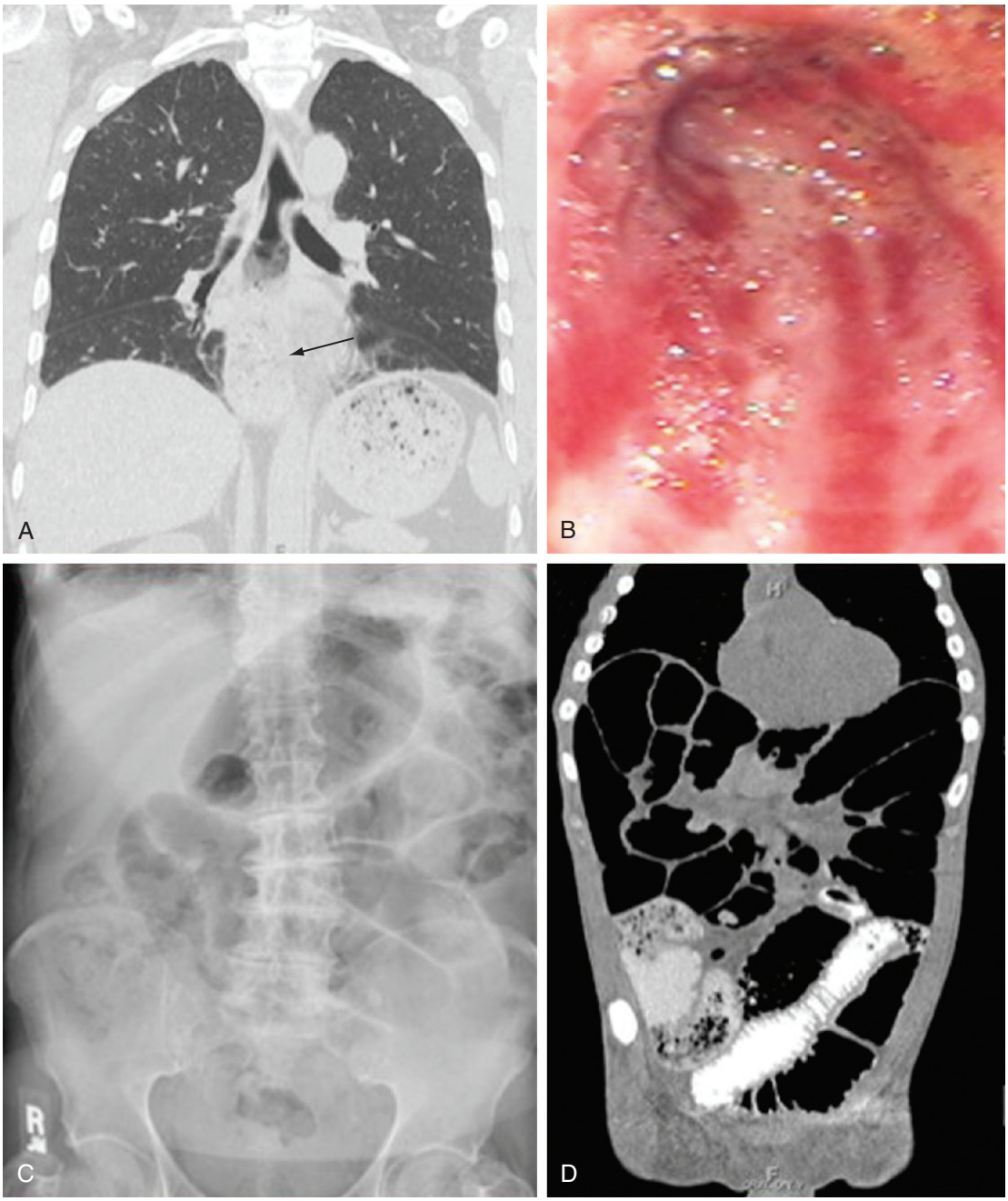



**Figure 84-11** Gastrointestinal manifestations in scleroderma. **A**, Chest computed tomography (CT) (sagittal view) showing severe esophageal dysmotility with dilation and *(arrow)* retention of gastric content. **B**, Upper endoscopy: gastric antral vascular ectasias presenting as "watermelon" stomach. **C** and **D**, Plain abdominal radiograph and abdominal CT: small intestinal dysmotility with pseudo-obstruction, pneumatosis cystoides intestinalis.

of advanced esophageal dysfunction. Many patients respond to relatively low doses, for example, at bedtime alone. Given the increased risk of neurologic complications with the use of metoclopramide, many clinicians recommend domperidone as a relatively safer alternative when long-term prokinetic treatment is needed. Domperidone should not be administered to subjects with pre-existing QT prolongation or in association with other drugs inhibiting CYP3A4 because of its arrhythmogenic potential.[85] Prucalopride, a selective high-affinity serotonin receptor agonist commonly used for constipation, has shown some benefit in subjects with GERD.[86]

### Stomach

The degree of delayed gastric emptying (gastroparesis) is underappreciated in scleroderma, and gastroparesis often causes early satiety, aggravation of GERD symptoms, anorexia, abdominal pain, a sensation of bloating, or nausea. Frequently, weight loss in patients with scleroderma is a consequence of poor caloric intake as a result of lack of appetite from poor gastric function. Prokinetics are used to improve gastric emptying and related symptoms, but results are generally disappointing. Gastritis or gastric ulcers can occur. Dilation of the microvasculature in the gastric mucosa

is found in a subset of patients. This manifestation, also called *gastric antral vascular ectasia* (GAVE), is thought to be caused by an abnormal angiogenesis, leading to bizarre dilation of microvessels and arterial-venous (A-V) malformations similar to the telangiectasias seen in the skin, lips, and oral mucous membranes. In general, these lesions are asymptomatic, but they can be responsible for occult GI bleeding. Extensive clusters of A-V malformations lead to the presence of longitudinal red stripes in the inner lining of the stomach, converging to the pylorus and described on endoscopy as "watermelon stomach," based on their appearance (Figure 84-11B). Endoscopic therapy with laser photocoagulation or cryotherapy can be used to ablate bleeding vessels. Rarely, bleeding associated with GAVE is refractory and requires multiple transfusions, repeated laser or cryotherapy, or even gastric surgery (e.g., antral or gastric resection).

### Lower Gastrointestinal Tract

Bloating, abdominal distention, diarrhea, and constipation are common complaints caused by dysmotility of the small and large bowel. Functional and pathologic studies again suggest that microvascular disease, neural dysfunction, smooth muscle atrophy, and tissue fibrosis similar to that seen in the esophagus are causing the bowel disease. Most cases present in similar fashion to irritable bowel syndrome. Constipation due to sluggish or atonic bowel function can result in repeated bouts of diarrhea with malabsorption, progressive loss of weight, and severe malnutrition. Diarrhea is thought to be caused by bacterial overgrowth as a consequence of bowel dysfunction, but other causes have been considered, including decreased mesenteric blood flow or pancreatic insufficiency. Recurrent episodes of pseudo-obstruction constitute one of the most serious intestinal problems in scleroderma. These episodes are sometimes mistaken for surgical emergencies. Pseudo-obstruction is a manifestation of profound loss of bowel smooth muscle function, causing severe dysmotility and segments of luminal dilation. Pneumatosis cystoides intestinalis sometimes complicates scleroderma of the bowel when gas leaks into the diseased intestinal wall and tracks into the mesentery of the gut or the peritoneal cavity, mimicking a bowel perforation (Figure 84-11C and D). Asymptomatic wide-mouthed diverticula, also resulting from fibrosis and atrophy of the bowel wall, are pathognomonic of scleroderma. Volvulus, stricture, and perforation are uncommon complications of severe bowel involvement.

Management of the lower GI tract includes avoiding a constipation-diarrhea cycle through adequate fiber ingestion, use of stool softener (docusate), or, if constipation is severe, periodic doses of osmotic laxatives (polyethylene glycol). Lubiprostone is helpful if chronic constipation predominates. Novel compounds are now available for chronic, refractory constipation such as prucalopride and linaclotide, but their efficacy in scleroderma has not yet been formally tested. Some experts also support the use of pyridostigmine in patients with scleroderma who have severe and persistent lower GI motility. Other prokinetic drugs are less effective for treatment of the lower GI tract. Octreotide is reported to help patients with severe lower GI hypomotility and pseudo-obstruction. In cases with bloating, recurrent bouts of diarrhea, or episodes of pseudo-obstruction, the use of cyclic antibiotics and/or probiotics is helpful. In some cases of intractable diarrhea, the use of cholestyramine is beneficial. Total parenteral nutrition may become necessary in patients with severe scleroderma-related bowel disease without response to other medical therapy. The presence of intestinal failure is a poor prognostic complication. Fecal incontinence can result in scleroderma caused by dysfunction of both internal (atrophy and fibrosis) and external (weakness) anal sphincters. Successful treatment includes firming stool texture, performing exercises to strengthen the pelvic muscles, providing biofeedback methods, and conducting surgical repair of aggravating factors such as rectal prolapse or severe hemorrhoids.

## Pulmonary Involvement

**KEY POINTS**

- **Lung disease is a major cause of morbidity and mortality in patients with scleroderma.**
- **Pulmonary fibrosis occurs in both limited and diffuse subsets of scleroderma, with a variable disease course in terms of severity and outcome.**
- **Nonwhite and antitopoisomerase 1–positive patients generally have the worst prognosis.**
- **Pulmonary function testing (spirometry and diffusing capacity) is helpful for screening and monitoring of ILD.**
- **The degree of lung fibrosis on high-resolution computed tomography predicts outcome.**
- **Current treatment for scleroderma-related ILD is limited to immunosuppression.**
- **Risk factors for pulmonary arterial hypertension include late onset of scleroderma, limited phenotype, and the presence of numerous telangiectasias.**

Pulmonary involvement is found in most patients with scleroderma. ILD and pulmonary hypertension are recognized as the most common lung complications and are now regarded as the major cause of death in scleroderma. In combination, it is estimated that they are responsible for 60% of scleroderma-related deaths.[26] They are present in many patients at the same time, but one process may dominate over the other. Lung disease can occur without symptoms, or it can cause progressive respiratory failure and severe limitation of the quality of life. Additional pulmonary complications such as chronic aspiration, pleural disease, spontaneous pneumothorax, neuromuscular weakness, drug-induced pneumonitis, and lung cancer need to be considered in the differential diagnosis of a patient with scleroderma who presents with respiratory symptoms.

### Interstitial Lung Disease

ILD, the most common lung manifestation, becomes clinically significant in up to 60% of patients with diffuse cutaneous scleroderma and in 20% of patients with limited skin disease. Higher risk of developing severe progressive ILD has been observed in patients with diffuse skin involvement, African-Americans, Native-Americans, and those

positive for antitopoisomerase 1 (Scl-70), anti-U3-RNP, or anti-Th/To antibodies. Data from a recent study of patients with active lung disease show that patients with limited scleroderma and those with diffuse scleroderma were indistinguishable with regard to their baseline pulmonary functions, although patients with limited skin disease presented with more extensive pulmonary fibrosis, perhaps reflecting a delay in diagnosis and more advanced progression of lung disease before study entry.[87]

ILD typically manifests with declining lung volumes and increased parenchymal fibrosis with reticular interstitial thickening that is greatest at the lung bases. Pathologic studies have shown that the most common histologic pattern of fibrosing alveolitis in scleroderma is nonspecific interstitial pneumonia (NSIP), as opposed to usual interstitial pneumonia (UIP), which is the common presentation of idiopathic pulmonary fibrosis. A mixed pattern of NSIP and UIP can also be found. The histopathologic classification does not appear to predict outcome, which is related to a greater degree to baseline disease severity and functional respiratory measures.[88] For this reason, a surgical lung biopsy is not required in the setting of patients with scleroderma who are experiencing a typical disease course. Although ILD is usually characterized by a functional restrictive ventilatory defect with reduced gas exchange, at later stages of the disease, some degree of airway obstruction can be found in association with honeycombing of lung parenchyma and bronchiectasis. Pleural reactions are not common in scleroderma. Rare cases of spontaneous pneumothorax, adult respiratory distress syndrome, and pulmonary hemorrhage have been reported. Aspiration as a result of GERD, secondary infection, and heart failure can complicate the course of scleroderma lung disease. Some investigators consider GERD associated with chronic aspiration to be a potential inciting factor for the development of ILD in scleroderma.[89]

During earlier stages of ILD, the underlying active fibrosing alveolitis may be totally asymptomatic and may not be detected by conventional chest radiographs. The most common symptoms of scleroderma lung disease are dyspnea (initially upon exertion) and fatigue. Chest pain is not typical, and nonproductive cough is usually a late complication, particularly in association with the presence of traction bronchiectasis. Coughing in patients with scleroderma often is not the result of a primary lung problem but rather is a manifestation of GERD with associated laryngeal irritation. The most characteristic finding of ILD on physical examination is bilateral fine inspiratory crackles (i.e., "Velcro" rales) at the lung bases. Pulmonary function testing (PFT) is the method most commonly used to detect ILD (Figure 84-12). The earliest functional abnormality is a reduction in the single breath diffusion capacity of carbon monoxide (DLCO). Although a decline in DLCO correlates with the severity of pulmonary fibrosis as measured by HRCT and predicts worse outcome, it is not a very specific measure and also can be abnormal in the context of

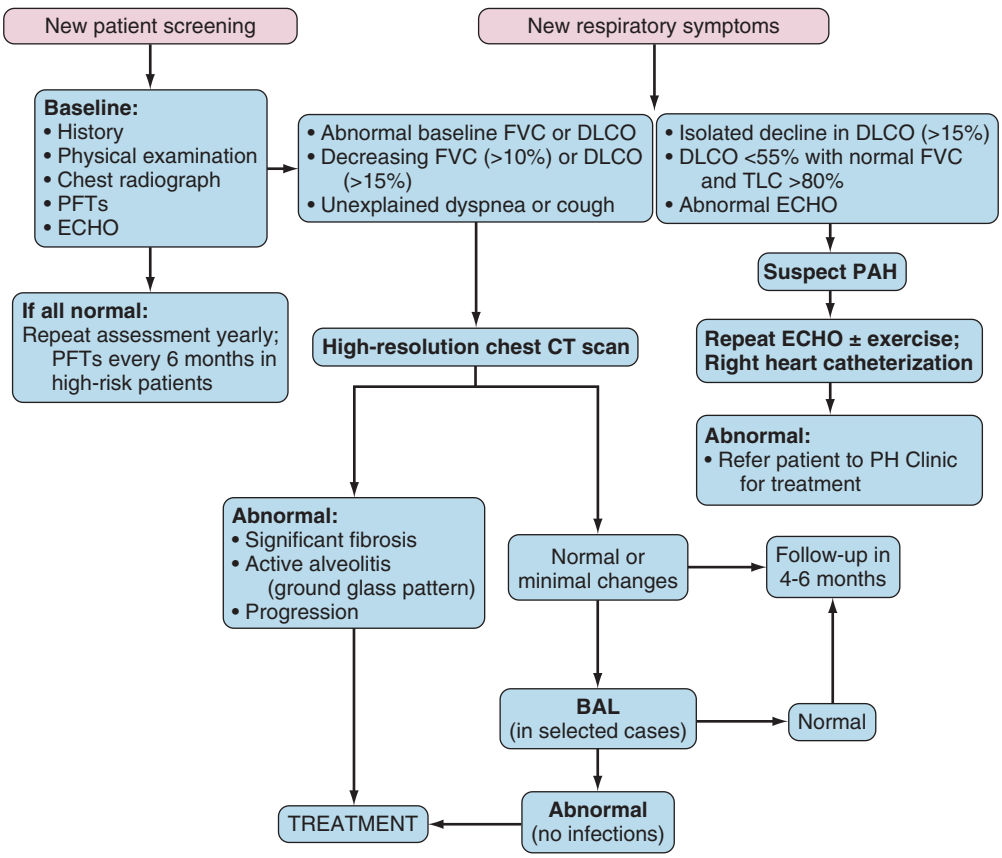



**Figure 84-12** Approach to lung disease in scleroderma. This approach is recommended for all patients with scleroderma who have both new-onset and long-standing disease. BAL, Bronchoalveolar lavage; CT, computed tomography; DLCO, diffusing capacity; ECHO, echocardiography; FVC, forced vital capacity; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PFT, pulmonary function test; TLC, total lung capacity.

pulmonary vascular disease and underlying chronic obstructive disease/emphysema. A restrictive ventilatory defect is identified by a depression of the forced vital capacity (FVC) and the total lung capacity. The most reliable tool for monitoring ILD over time is serial measurements of FVC. A decline in FVC of more than 10% from baseline is often indicative of disease activity and is associated with increased mortality. The course of lung disease may remain stable or exhibit worsening after variable intervals (months or years) of unchanged lung function. In some cases, progression is invariable and rapidly leads to end-stage lung disease. Patients presenting with a normal FVC during early stages of scleroderma have a better lung prognosis, and an early reduction in FVC is likely to be associated with further progression of lung involvement, usually within the first 2 to 4 years from disease onset. The presence of ILD predicts increased mortality. One study found that patients with severe ILD have a 9-year survival rate of approximately 30%, whereas patients with no severe organ involvement have a 72% survival rate.[26] The most rapid decline in FVC is thought to occur within the initial 3 years from disease onset, but progression of ILD can occur in later years, so it is recommended that respiratory status be monitored on a regular basis and that pulmonary function be tested yearly.

HRCT is more accurate than chest radiography in evaluating diffuse lung disease; it is a well-established, sensitive, and noninvasive method of detecting and characterizing ILD in persons with scleroderma. The extent of pulmonary fibrosis on HRCT correlates closely with PFT abnormalities and can be used to predict prognosis.[90] Recent evidence shows that the degree of HRCT changes coupled with the level of lung function deficit can be used to generate a simple staging system (limited vs. extensive disease) with good prognostic value.[91] An FVC of less than 70% or a disease extent according to HRCT of greater than 20% predicts ILD progression and higher mortality. HRCT features of fibrosis are present in 55% to 65% of all patients with scleroderma and in almost every patient with abnormal pulmonary function tests. The earliest and most common abnormality on HRCT is usually found in the posterior and basilar portion of the lungs in the form of increased subpleural lung attenuations in the absence of distinct architectural distortion. With progression of the disease, increased "ground glass" opacities are seen, but it has been well established that these changes alone are not always indicative of active inflammation, nor can they predict progression itself. Subsequent detection of a fine reticular pattern often precedes overt pulmonary fibrosis manifested by parenchymal distortion, reticular intralobular interstitial thickening, traction bronchiectasis and bronchiolectasis, and, in the late stages, honeycomb and cystic air spaces (Figure 84-13). When reticular interstitial abnormalities are present on HRCT, regression of disease rarely, if ever, occurs.

Pathologic studies of patients with scleroderma who have early lung disease have shown that lung fibrosis is preceded by the presence of a mixed interstitial inflammatory infiltrate spilling into the alveolar spaces (alveolitis). It has been assumed that measuring abnormal levels of inflammatory cells in the bronchoalveolar lavage (BAL) would allow identification of patients with active lung disease who are at risk for progression and would potentially benefit from immunosuppressive therapy. Published data have shown that an abnormal BAL cell count, in particular with increased granulocytes (neutrophils and eosinophils), is associated with more severe lung disease and mortality in patients with scleroderma-related ILD.[92] However, the value of BAL cytology in predicting lung disease progression or response to treatment has not been uniformly confirmed. Few observational studies have indicated that the presence of BAL granulocytic alveolitis was associated with significant deterioration of lung function tests over time in patients who were not receiving immunosuppressive therapy.[93] In contrast, other studies did not find a clear association between BAL cellular abnormalities and clinical outcomes.[94] At present, evidence to recommend routine clinical use of BAL cytology to predict outcome in patients with scleroderma and ILD is still insufficient. Other biomarkers have been investigated to monitor the activity and progression of ILD more accurately, but they are not yet available for routine clinical use. Markers of alveolar cell damage such as KL-6 or surfactant proteins A (SP-A) and D (SP-D) are increased in the serum of patients with scleroderma who have active ILD and predict survival.[95,96] Increased levels of pro-inflammatory molecules such as CCL-18 or IL-6 are also associated with declining lung function and worse outcome.[97,98]

Early detection and prompt therapeutic intervention remain essential in preventing progression of lung disease. Current treatment of scleroderma-related ILD is most often directed against the immune response and the inflammatory process mediating lung injury and tissue fibrosis. A randomized placebo-controlled study comparing 1 year of oral cyclophosphamide (CYC) versus placebo suggests some modest benefit of the active drug (improvement in FVC).[87] This benefit was sustained at 18 months but was completely lost after 2 years' follow-up, suggesting that a sequential or maintenance immunosuppressive approach may be indicated to retain the clinical response. Other studies suggest that use of IV monthly CYC followed by azathioprine (AZA) as maintenance therapy provides benefits. Several small retrospective studies have shown moderate benefit from mycophenolate mofetil (MMF) with improvement in or stabilization of lung function, suggesting that this drug may prove to be a safe and effective treatment in patients with scleroderma and a possible alternative to CYC. Although more studies are needed to confirm the benefit of immunosuppressive therapy in scleroderma-associated ILD, newer drugs are under investigation, including targeted biologic immunotherapies and antifibrotic molecules (see section on Therapeutic Approach for Disease Modification). More aggressive immunosuppressive protocols such as autologous or allogeneic hematopoietic stem cell transplantation, preceded by myeloablative or immunoablative conditioning regimens, are under investigation, but their treatment toxicity remains a concern.

It remains very important to pursue careful selection of patients and to treat only those with evidence of active lung disease. In fact, in most cases, no or minimal progression to severe disease is seen despite some evidence of underlying pulmonary fibrosis. Selection of patients who require treatment is best performed with serial studies and careful assessment of both pulmonary function tests and HRCT scans.

Lung transplantation is an option in patients with severe ILD who are not responsive to medical therapy. Carefully

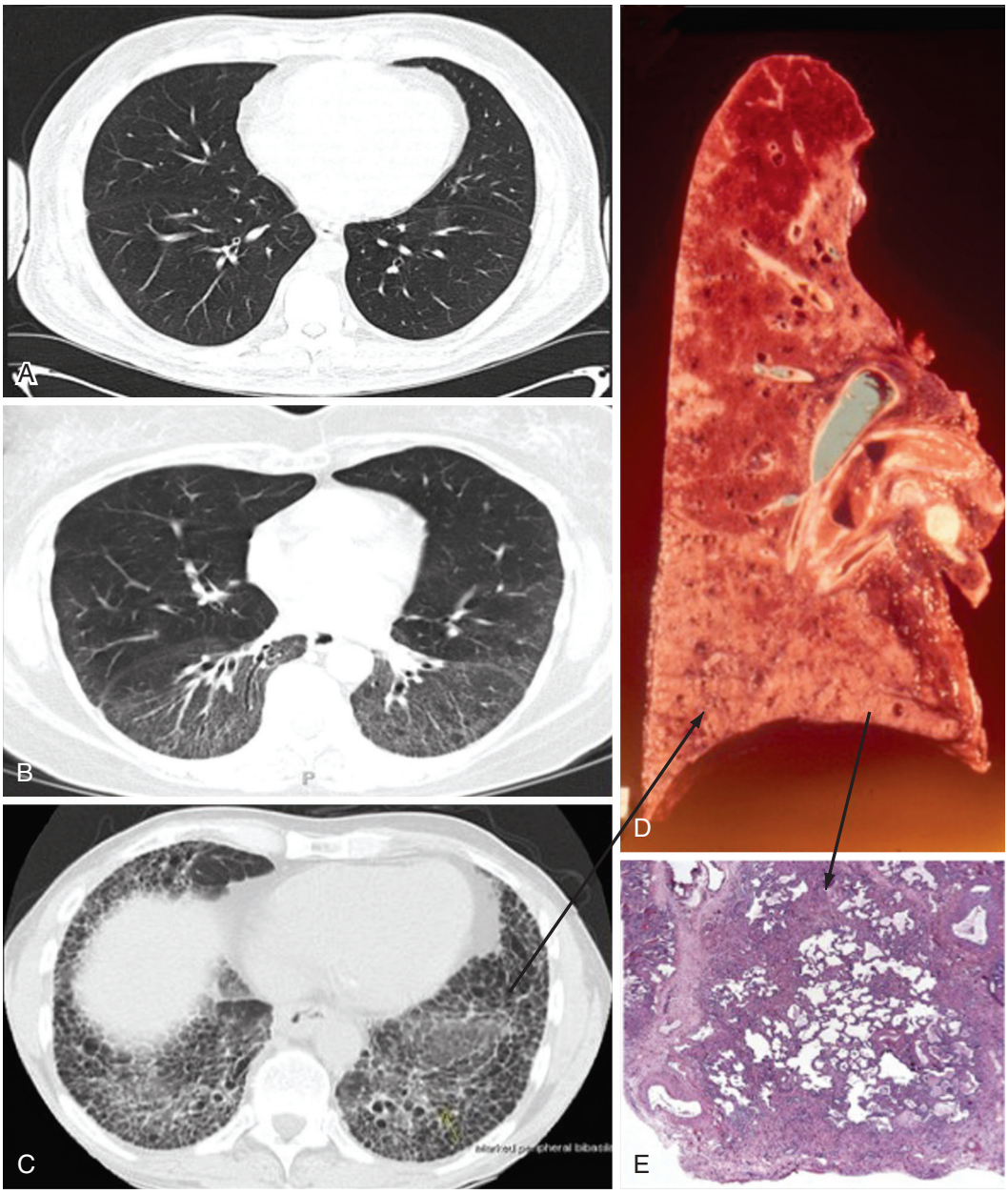



**Figure 84-13** Scleroderma-related interstitial lung disease: high-resolution chest computed tomography scan showing **(A)** normal lung, **(B)** active alveolar inflammation ("ground glass" opacification), and (**C)** end-stage lung disease with honeycombing. **D**, Gross pathology. **E**, Histology showing fibrosing alveolitis.

selected patients with scleroderma who undergo lung transplantation have similar morbidity and mortality as patients undergoing transplantation for idiopathic lung disease.[99,100] A retrospective survey of 47 patients with scleroderma who underwent lung transplantation found that 1- and 3-year survival rates were 68% and 46%, respectively.

### Pulmonary Hypertension

Pulmonary arterial vascular disease is a common manifestation of scleroderma; when associated with PAH, it represents a difficult clinical challenge with life-threatening consequences. The pulmonary vascular process can be indolent and remain clinically silent, or it may lead to respiratory distress as a result of severe PAH and associated right heart failure. Typical symptoms associated with clinically manifested PAH include dyspnea upon exertion, fatigue, and, less commonly, chest pain or syncope. Physical examination may be normal during early stages of PAH, but as the disease progresses, a systolic murmur of tricuspid regurgitation, a loud pulmonic component, the S2, an S3 gallop, and signs of right heart failure (e.g., right-sided parasternal heave, prominent and elevated jugular venous pulse, hepatomegaly, and signs of fluid overload with peripheral edema) are seen. Later in the disease, patients become dyspneic with little activity, have a resting tachycardia, and may appear cyanotic. Sudden syncope or death can occur as a result of hypoxia and congestive heart failure.

PAH is usually a late complication of scleroderma, typically manifesting after 10 years from disease onset, particularly in patients with limited cutaneous involvement. However, it is becoming more evident that in some patients this condition may develop early and remain undetected. A study of a French scleroderma cohort identified a substantial proportion of patients in whom PAH actually developed within 5 years from disease onset.[101] Considering the potentially devastating consequences of PAH, early diagnosis is very important. Simple bedside examination is not very sensitive, particularly during early stages, and thus it is recommended that every patient with scleroderma be screened using objective testing such as electrocardiography (ECG), ECHO, and pulmonary function testing (Figure 84-14). Proper initial workup also should aim at excluding chronic thromboembolic disease and the possibility of underlying obstructive sleep apnea. The ECG can be normal, but as disease progresses, signs of right-sided heart strain with right ventricular hypertrophy and right axis deviation can be observed. Detection by ECHO of increased right ventricular systolic pressure (RVSP) is useful, but values need to be confirmed by right heart catheterization (RHC), given the possibility of false-positive or false-negative results. When the ECHO estimates an RVSP greater than 45 mm Hg about 95% of the time, this finding will be confirmed by RHC.[102] Some clinicians use the tricuspid gradient as a measure of pulmonary artery pressure. Additional noninvasive screening includes pulmonary function testing. A low diffusing capacity (DLCO) without evidence of obstructive or restrictive lung disease (low FVC) suggests a defect in gas exchange as a result of pulmonary vascular disease or PAH. DLCO may progressively decline for years before the diagnosis of PAH is established.[103] Increased serum N-terminal pro-brain natriuretic peptide (NT-proBNP) may indicate the presence of PAH and right heart strain, even when levels are modestly elevated (>395 pg/mL).[104] The diagnosis of PAH should be confirmed by RHC, providing direct measurement of pulmonary hemodynamics. PAH is defined as resting mean pulmonary arterial pressure equal to or greater than 25 mm Hg, with normal pulmonary capillary wedge pressure equal to or less than 15 mm Hg. In addition to confirming abnormal hemodynamic values, the RHC can help in differentiating true PAH from pulmonary venous hypertension as a result of left heart failure or pulmonary

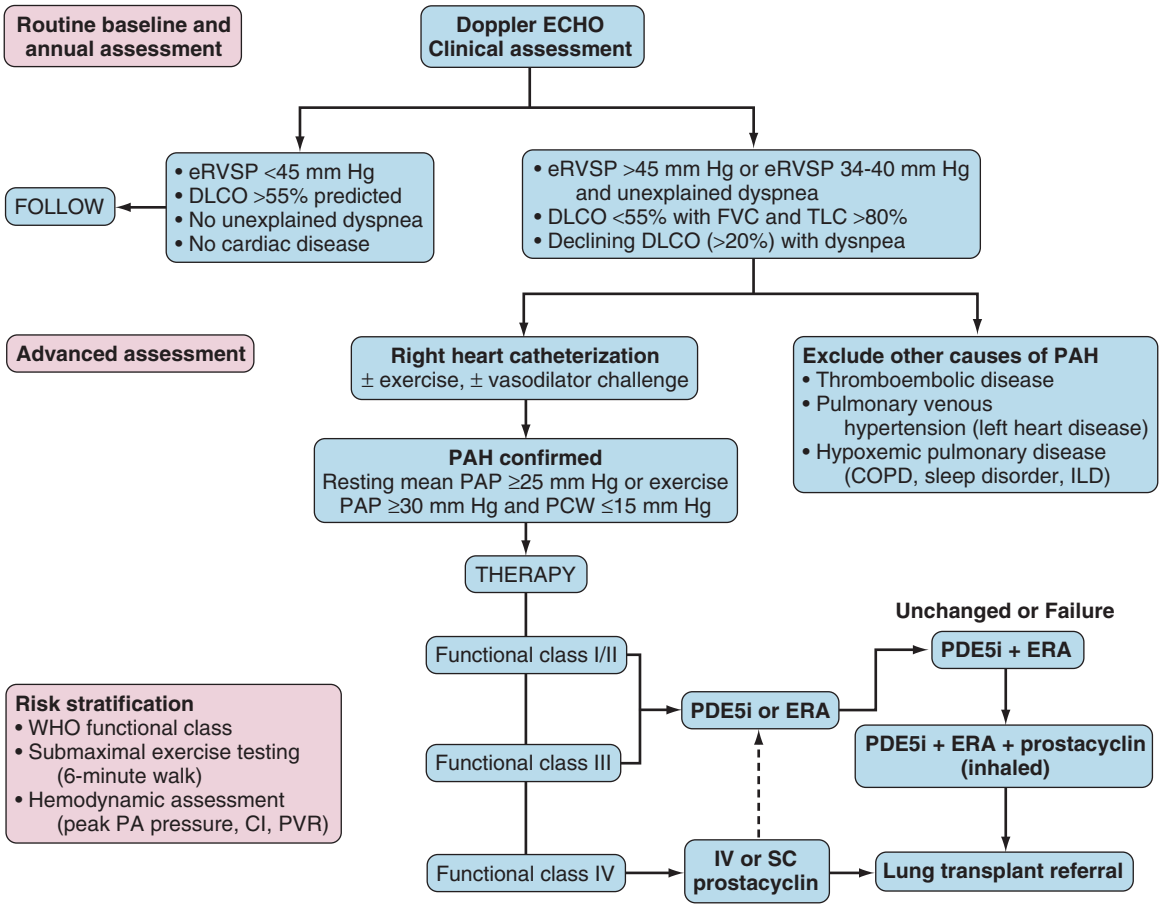



**Figure 84-14** Approach to scleroderma-associated pulmonary arterial hypertension (PAH). This approach is recommended for all patients with scleroderma who have new-onset disease or especially long-standing disease with limited scleroderma. CI, Cardiac index; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity; ECHO, echocardiography; ERA, endothelin receptor antagonist; eRVSP, estimated right ventricular systolic pressure; FVC, forced vital capacity; ILD, interstitial lung disease; IV, intravenous; PA, pulmonary artery systolic pressure; PAP, pulmonary artery pressure; PCW, capillary wedge pressure; PDE5i, phosphodiesterase-5 inhibitor; PVR, pulmonary vascular resistance; SC, subcutaneous; TLC, total lung capacity on pulmonary function testing. Functional class: World Health Organization (WHO) functional classification of disease severity in PAH according to level of function and associated symptoms. Increasing WHO functional class reflects more severe symptoms and greater restriction in activity.

occlusive disease. The 6-minute walk test can provide baseline functional information but cannot accurately assess the severity of PAH. Because baseline hemodynamic parameters do not correlate well with the clinical course of PAH in patients with scleroderma, new cardiopulmonary dynamic testing such as exercise stress ECHO and exercise during RHC have been introduced, but their role in diagnosing or monitoring PAH has not yet been fully established.

Based on the definition and the tools used to obtain measurements, the prevalence of PAH in patients with scleroderma has been reported with variability. Using ECHO as a diagnostic tool, the prevalence of PAH has been estimated at between 30% and 50% of all studied patients, and investigations using confirmatory RHC have detected a prevalence of 8% to 12%.[105,106] Clinically, scleroderma-related pulmonary vascular disease can present in three different ways. In some patients, it manifests and progresses as severe isolated PAH in the absence of other significant lung disease. In other patients, it can be associated with mild or severe pulmonary fibrosis that is believed to drive vascular disease and PAH by causing chronic hypoxia coupled with destruction of lung parenchyma and underlying vasculature. Association of ILD and PAH usually entails a worse prognosis. In a third group of patients, pulmonary vascular disease presents with dyspnea upon exertion, isolated decline of the DLCO with no significant signs of ILD (i.e., low lung volumes), and a normal resting ECHO and/or RHC. These patients are considered to have microvascular lung disease and may be at high risk of developing overt PAH. Increased risk of developing PAH has been associated with limited skin disease, late age at onset of scleroderma, the presence of numerous telangiectasias, low DLCO, and the presence of anti-U3-RNP antibody. Some studies suggest that the presence of anticentromere, anti-B23, and anti–$\beta_2$-glycoprotein I antibodies also increases the risk for PAH.

PAH has a major impact on quality of life and survival of affected patients. The natural course of the disease is characterized by progression of hemodynamic impairment, resulting in right heart failure and leading to a poor outcome. Despite the availability of newer targeted therapies, the median survival in patients with scleroderma who have PAH ranges between 1 and 3 years.[107,108] A progressive rapid increase in pulmonary pressure is more likely to occur in patients with limited skin disease and late age at the onset of scleroderma.[109] This more aggressive course is associated with high mortality. Survival in patients with ILD-associated PAH is significantly worse (a 3-year survival of 46%) than in patients with PAH alone (a 3-year survival of 64%).[110] This difference is possibly explained by the presence of underlying comorbidities in scleroderma, and in particular of biventricular heart (myocardial) involvement. A 10-fold increase in NT-proBNP blood levels is associated with higher mortality.[111,112] ECHO findings can help predict prognosis. For example, the degree of tricuspid annular plane systolic excursion (TAPSE) reflects right ventricular function. A TAPSE of less than 1.8 cm is associated with decreased survival in patients with PAH.[113] A recent survey identified that pulmonary vascular resistance, stroke volume index, and pulmonary arterial capacitance were strong predictors of survival.[114] This finding suggests that right ventricular dysfunction is associated with poorer outcome. Male gender and higher New York Heart Association functional class also have a negative impact on prognosis. It is suggested that survival has improved with current modes of therapy. One survey found a 2-year survival rate of 47% in historical control subjects as opposed to a rate of 71% among patients treated with modern therapy.[115]

All patients with PAH are encouraged to continue an active lifestyle as tolerated. Formal educational and exercise programs can improve their quality of life. Conventional therapy includes diuretic therapy (loop and potassium-sparing diuretics) and supplemental oxygen therapy if needed. Anticoagulation is used if there is no defined risk for bleeding. Although calcium channel blockers may help patients with evidence of acute vasodilative response during hemodynamic testing, this finding is uncommon in scleroderma. In recent years, a number of targeted agents have become available for the treatment of PAH. Many clinical trials testing these drugs in primary PAH have included about 20% of patients with connective tissue disease, primarily scleroderma. Specific therapies include prostaglandin derivatives (e.g., epoprostenol, treprostinil, beraprost, and iloprost), endothelin receptor antagonists (e.g., bosentan, sitaxsentan, ambrisentan, and macitentan), phosphodiesterase-5 inhibitors (e.g., sildenafil, tadalafil, and vardenafil), and a soluble guanylate-cyclase stimulator (riociguat). Inhaled prostaglandins can be an option in severe cases. These drugs can improve hemodynamics, exercise tolerance, and quality of life. No evidence from clinical trials exists to suggest that any one of these agents is superior to another, but evidence suggests that prostacyclin therapy is the most effective for severe PAH. Most clinicians believe that early intervention is most important because it leads to better outcomes and that combination therapy may be helpful (see Figure 84-14). The use of prostaglandin therapy should be considered immediately in people with more severe PAH. Decisions regarding the preferred agents to use and how to deliver them are based mostly on personal preferences and the clinical status of the individual patient. For patients with mild to moderate disease (functional class I or II), treatment with a single oral agent is usually initiated (i.e., sildenafil, bosentan, or ambrisentan). Our preference is to use sildenafil, given its favorable adverse effect profile. Patients presenting with more severe disease (class III or IV) and those who do not respond to monotherapy are usually treated with IV or inhaled prostaglandins alone or in combination with one or two oral agents. Sildenafil and bosentan can be used in combination, even if concern arises about their interaction and increased hepatotoxicity.[116,117] Lung or heart-lung transplantation for PAH remains the treatment of last resort when medical therapy fails. Patients with scleroderma who are carefully selected for transplantation have an outcome that is similar to patients with other causes of lung disease. PAH in scleroderma is a progressive disease for which no specific or single therapy may suffice. Therefore, careful longitudinal monitoring is important. Noninvasive testing with periodic clinical assessment with the 6-minute walk time, repeat pulmonary function testing, and ECHO studies are helpful. When a major therapeutic decision is needed, such as switching to or adding a new therapy, a repeat RHC may be indicated. Further improvement in survival of patients with scleroderma who have PAH may result from future therapeutic interventions targeting the underlying inflammatory process and the dysregulated

endothelial proliferation that mediates pulmonary vascular remodeling and consequent failure of the right heart.

## Cardiac Involvement

The clinical manifestations of heart disease are highly variable, ranging from clinically silent cardiac involvement to frank heart failure. The reported prevalence of heart disease varies from 10% to more than 50%, depending on the diagnostic method used, but in general it tends to be underestimated. Symptoms such as dyspnea, chest pain, or palpitations are commonly thought to be the result of more obvious organ involvement such as the lungs or the upper GI tract, and the contribution of heart disease often is not appreciated until later stages. Cardiac disease can occur in both diffuse and limited subtypes of scleroderma and can manifest as a primary heart problem or in association with other organ failure. When clinically evident, heart disease entails an overall poor prognosis and predicts shortened survival.[118] Along with pulmonary fibrosis and PAH, cardiac disease accounts for the majority of deaths in scleroderma. One study found that 25% of deaths could be directly related to heart disease (mostly heart failure and arrhythmias).[19] In a large meta-analysis, cardiac involvement was associated with increased mortality (hazard ratio, 2.8; 95% CI, 2.1 to 3.8) after adjustments for age and gender.[119] A survey comparing 129 patients with scleroderma who had a left ventricle ejection fraction (LVEF) of less than 55% with 256 subjects who had a normal LVEF demonstrated that male gender, age, digital ulcerations, myositis, and no use of calcium channel blockers were independent factors associated with left ventricular dysfunction.[120]

Cardiac disease in scleroderma can be characterized by involvement of the endocardium, myocardium, and pericardium, separately or concomitantly. As a consequence, pericardial effusion, auricular and/or ventricular arrhythmias, conduction disease, valvular regurgitation, myocardial ischemia, myocardial hypertrophy, and heart failure are all reported. Clinically overt pericarditis is uncommon, but asymptomatic and hemodynamically benign pericardial effusions are frequently detected by ECHO. In a controlled study, significant pericardial effusion was found in about 15% of patients compared with 4% of control subjects.[121] Pathologic studies have shown that some degree of pericardial involvement is detectable in 33% to 77% of patients with scleroderma, usually with evidence of a fibrinous pericarditis with adhesions and chronic inflammatory cell infiltrates.[122] Hemodynamic compromise resulting from tamponade physiology is rare but may require acute intervention with antiinflammatory medication, pericardiocentesis, or creation of a pericardial window for slow decompression. The presence of a large pericardial effusion, even if it is asymptomatic, is a poor prognostic sign, particularly if it is associated with PAH or renal disease.

Focal myocardial fibrosis is the hallmark of established primary heart involvement in scleroderma. Studies have provided controversial data regarding increased risk for atherosclerotic coronary artery disease. Although pathologic studies and an analysis of coronary angiograms have found little involvement of coronary arteries in patients with scleroderma, epidemiologic surveys seem to indicate an increased risk of atherosclerosis in this disease, similar to other rheumatic conditions such as rheumatoid arthritis or systemic lupus erythematosus.[7,24,123] Therefore, typical angina symptoms should make one consider atherosclerosis of the coronary vessels or another process. Atypical chest pain can also be caused by musculoskeletal problems, esophageal reflux disease, or PAH mimicking cardiac disease. The fibrotic lesions in the hearts of patients with scleroderma are patchy, involve the myocardium of both ventricles, and usually are accompanied by evidence of microvascular disease with concentric intimal hypertrophy associated with fibrinoid necrosis of the intramural coronary arteries and arterioles.[124] This phenomenon results in reduced coronary flow reserve even with normal epicardial coronary arteries and in the absence of clinically manifested cardiac dysfunction.[125] Vasospasm and associated myocardial perfusion defects are demonstrated to occur at rest, with exercise, and after exposure to cold temperatures.[126] These findings suggest that myocardial fibrosis may be associated with reversible vasospasm of the microvascular coronary circulation and that vasodilating agents such as calcium channel blockers may have the capacity to improve coronary flow and prevent further cardiac damage.[127] Patients with angina-like chest pain may need to undergo angiographic studies because nuclear perfusion studies are likely to be abnormal as a result of microvascular disease.

ECG and Holter monitoring often show some heart conduction defect or asymptomatic arrhythmia, which are thought to be consequences of myocardial fibrosis. Premature ventricular contractions are the most common abnormality, but sinus node dysfunction, first-degree heart block, supraventricular arrhythmias (supraventricular tachycardia and atrial fibrillation), and ventricular arrhythmias (ventricular tachycardia) are also observed. Complete heart block is uncommon. Scleroderma-related syncope is an ominous manifestation of late-stage PAH or an important arrhythmia. When an arrhythmia is suspected, a Holter monitoring study should be ordered promptly. More extensive evaluation with electrophysiologic studies is indicated in serious cases because a pacemaker or ablative therapy can help in the management of these life-threatening complications. In subjects with documented advanced cardiomyopathy and significant ejection fraction depression, placement of an automatic implantable cardioverter defibrillator device should be considered to prevent sudden cardiac death.[128]

Transthoracic ECHO (TTE), a widely used type of noninvasive cardiac testing, is a sensitive tool to detect the presence of cardiac disease. TTE can provide estimates of elevated right ventricular (RV) and pulmonary artery pressures, as well as evidence of right heart dysfunction such as atrial and ventricular dilation or septal wall motion abnormalities. A depressed left ventricular (LV) contractility is not frequently detected in patients with scleroderma with the use of conventional TTE, but relaxation abnormalities can be found in up to 40% of cases.[129] LV diastolic dysfunction is detected, especially in patients with diffuse scleroderma, and it may occur in the absence of systemic hypertension (independent of renal disease) and in association with pulmonary vascular disease. Although TTE is very helpful in monitoring underlying cardiac abnormalities later in the disease process, it lacks some sensitivity during preclinical stages of heart involvement. Novel diagnostic methods such as tissue Doppler ECHO, with or without

strain imaging, and MRI that have been applied more recently to study myocardial disease in scleroderma have shown the ability to detect abnormalities earlier than conventional ECHO and with greater accuracy.[130,131] Provocative exercise testing during ECHO can be used to detect underlying diastolic dysfunction and pulmonary hypertension not previously recognized by resting studies.

Cardiac evaluation has been improved by the systematic measurement of brain natriuretic peptide (BNP) or its prohormone NT-proBNP. Blood levels of NT-proBNP reflect both RV and LV volume or pressure overload. It can be a useful test and a prognostic marker to follow up on right or left heart failure in patients with scleroderma.[112,132] No formal studies have been conducted to measure serum levels of troponin in relationship to scleroderma cardiac involvement. However, this test can help identify patients with ongoing myocardial injury.[133]

Patients with diffuse cutaneous scleroderma and skeletal muscle inflammation (polymyositis) are particularly prone to the development of severe cardiomyopathy and to have a particularly poor prognosis. Acute myocarditis can in fact occur in association with inflammatory muscle disease and can present as sudden-onset heart failure. Prompt institution of immunosuppressive treatment is indicated in these cases. A cardiac MRI and/or an endomyocardial biopsy may be helpful tools to differentiate overt myocarditis from the myocardial fibrosis commonly present in patients with scleroderma. It is not clear whether antiinflammatory treatment is indicated for small, stable, and asymptomatic pericardial effusions. In selected cases, NSAIDS or colchicine may be helpful. The potential cardiotoxicity of colchicine may be a limitation for its use in patients with scleroderma who have myocardial involvement.

## Renal Involvement

**KEY POINTS**

- **Scleroderma renal crisis (SRC) is a life-threatening condition that occurs in 5% to 10% of patients with scleroderma.**
- **Risk factors for SRC include early diffuse skin disease, use of corticosteroids, and the presence of anti-RNA polymerase III antibodies.**
- **Early pharmacologic intervention with ACE inhibitors is crucial to control and possibly reverse the disease process.**

Renal involvement in scleroderma is classically characterized by the abrupt onset of very high blood pressure (malignant hypertension), elevated plasma renin, and rising serum creatinine reflective of acute renal failure, along with a constellation of symptoms and clinical manifestations such as headaches, malaise, hypertensive retinopathy, encephalopathy, and pulmonary edema, usually referred to as *scleroderma renal crisis* (SRC). Renal disease in scleroderma can also be asymptomatic, and despite the high prevalence of histopathologic renovascular lesions typical of scleroderma vasculopathy, clinically important renal dysfunction, independent of SRC, is seen in only a minority of patients.[134] Although SRC is the most recognized renal complication, abnormal renal function can be explained by factors other than intrinsic scleroderma renal disease such as medication adverse effects, comorbid conditions, or associated heart, GI, or lung disease. One survey suggested that mild unexplained proteinuria without loss of renal function or evidence of glomerular disease is a common sign of renal disease in scleroderma.[135] A superimposed inflammatory kidney disease can also occur. Several cases of pauci-immune necrotizing crescentic glomerulonephritis associated with myeloperoxidase-specific ANCAs have been reported in patients with scleroderma who have a presentation mimicking SRC.[136]

SRC needs to be considered in patients with scleroderma who have sudden and severe elevation of blood pressure, with or without renal failure; in patients with sudden renal failure, with or without hypertension; and in patients with sudden-onset microangiopathic hemolytic anemia, with or without hypertension or renal failure. Nonmalignant hypertension alone without signs of renal dysfunction or mild urine abnormalities is unlikely to be due to SRC. SRC occurs in 5% to 10% of patients, mostly in those with diffuse scleroderma. It is usually encountered within the first 2 to 4 years from disease onset. The estimated median duration of scleroderma at the time of SRC diagnosis is 8 months.[137,138] Occurrence in later disease is uncommon.[108] Patients at greatest risk of developing SRC are those with diffuse cutaneous scleroderma, particularly those who have the rapidly progressive skin disease. SRC is a rare complication in patients with limited scleroderma (approximately 1% to 2%). African-Americans and patients with diffuse skin disease who are treated with a high dose of corticosteroid (>40 mg/day of prednisone) or low-dose cyclosporine are at increased risk of developing an SRC. A long-term low dose of prednisone may also be a risk factor for SRC. For this reason, it is recommended that use of corticosteroids be avoided or that doses less than 15 mg daily be used if necessary. Renal crisis is also associated with a positive ANA (speckled pattern) anti-U3-RNP and usually is not seen in patients with anticentromere antibodies. Antibodies to RNA polymerase III were found in about 60% of patients with SRC in one survey.[137] Although antitopoisomerase I antibodies are prevalent in patients with diffuse skin disease, no association between their presence and SRC has been reported. Nonmalignant hypertension, abnormal urinalysis, and isolated increases in plasma renin activity are not predictors of an SRC.[139]

Patients experiencing SRC present with typical signs of malignant hypertension, including headache, altered vision, signs of congestive heart failure, florid pulmonary edema, confusion, or neurologic signs as a result of hypertensive encephalopathy in the setting of an abnormally high blood pressure, usually greater than 150/90 mm Hg. Hypertensive encephalopathy is characterized by acute or subacute onset of lethargy, fatigue, confusion, headaches, visual disturbances (including blindness from hypertensive retinopathy), seizures, or cerebral hemorrhage. In about 10% of cases, SRC occurs with normal blood pressure. New-onset anemia, asymptomatic pericardial effusion, and cardiac disease may precede an SRC. Laboratory testing at onset may show normal or high creatinine, modest to absent proteinuria, and/or microscopic hematuria. Renal insufficiency usually is not the presenting problem in an SRC but rather is a late complication of untreated disease. However, a rapid rise in serum creatinine can occur over

several days with a fairly benign urinalysis—less than 2 g of protein excretion in 24 hours—and a mild hematuria with some granular casts. A microangiopathic hemolytic anemia and thrombocytopenia can accompany or precede an SRC and may be the only finding in a normotensive patient. This presentation with normotensive SRC usually entails a worse prognosis. Distinguishing SRC-associated thrombotic microangiopathy from idiopathic thrombotic thrombocytopenic purpura may be difficult but is important because treatments differ. Accurate assays for ADAMTS (a disintegrin and metalloproteinase [ADAM family] with thrombospondin-1 domains)-13 activity can be helpful because its level should be normal in scleroderma.

Poorer outcome is more frequent among males and among patients presenting with creatinine greater than 3 mg/dL (265 mmol/L). Approximately two-thirds of patients with SRC will require renal support with dialysis, but about half will recover function and be able to discontinue therapy, usually within several weeks, and most before 6 months.[137] However, cases of recovery from up to 24 months of dialysis support have been reported, distinguishing SRC from other causes of renal failure. The renal crisis truly mimics malignant hypertension, with rapidly progressive renal failure as a result of microvascular disease, vasospasm, and tissue ischemia. The characteristic lesion of vascular disease such as intimal hyperplasia or luminal narrowing involving in particular the renal arcuate arteries is detected even in patients without SRC. Although the renal disease is now well described, the pathogenesis of the renal crisis remains poorly understood. It is assumed that intrinsic renal vessel disease is complicated by an intense vasospasm triggering high levels of plasma renin, which results in malignant hypertension.

Before the discovery of ACE inhibitors, hypertensive renal crisis almost uniformly had a fatal outcome. In contrast, patients treated with ACE inhibitors have a good outcome 60% of the time, and death or end-stage renal disease is now much less common. The mortality rate at 1 year associated with SRC has decreased from 76% to less than 15%. However, despite aggressive anti-hypertensive therapy, the 5-year survival of patients with SRC remains only 65%. Although ACE inhibitors are standard therapy for SRC, it remains unclear whether other related drugs such as angiotensin receptor blockers (ARBs) can be effective in treating or preventing a crisis. Endothelin and other mediators of vascular disease are implicated in the pathogenesis of SRC, and thus interference with endothelin function by a specific inhibitor may offer a novel therapeutic approach. A vasodilating prostaglandin (prostacyclin) may also provide rapid control of blood pressure while improving renal blood flow. The overall outcome in SRC remains unsatisfactory in terms of acute disease outcome, morbidity, and the need for renal transplantation or long-term renal support with dialysis. Currently no evidence supports the prophylactic use of ACE inhibitors in patients with scleroderma who are at risk for SRC. Several studies have actually shown that use of an ACE inhibitor before the onset of SRC was associated with worse outcome, including higher mortality.[137,138,140]

Any newly developed hypertension in a patient with scleroderma should be urgently evaluated because the key to successful therapy is early detection and rapid intervention (Figure 84-15). Patients with SRC should be hospitalized and should immediately start taking an ACE inhibitor. Captopril, a rapid-acting ACE inhibitor, should be started to allow dose increases until systolic blood pressure is decreased by 20 mm Hg within 24 hours while avoiding hypotension. Treatment continues with an ACE inhibitor even if creatinine continues to rise. When full doses of an ACE inhibitor are not controlling blood pressure, other anti-hypertensive medications may be added, including calcium channel blockers, endothelin inhibitors, and

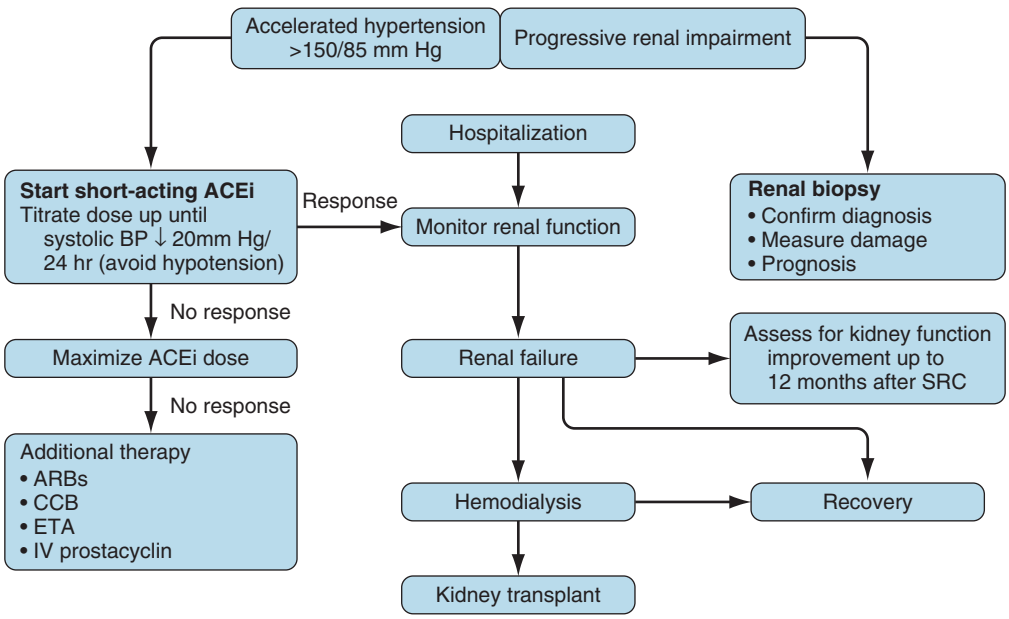



**Figure 84-15** Management of scleroderma renal crisis. ACEi, Angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CCB, calcium channel blocker; ETA, endothelin receptor antagonist; IV, intravenous; SRC, scleroderma renal crisis.

prostacyclin or an ARB. Some patients continue to have progressive renal failure despite attaining control of blood pressure. Other causes of renal disease always need to be considered, especially in patients with limited scleroderma and in those with a high level of proteinuria or abnormal urinalysis such as red blood cell casts. A renal biopsy should be performed to confirm the diagnosis and to gain insight into the prognosis by determining the degree of renal damage. Detection of other forms of glomerular inflammation via a kidney biopsy is important because it will dictate a different specific treatment approach.

Successful renal transplantation has been performed in patients with scleroderma, providing an overall survival benefit compared with long-term dialysis. Renal allograft survival at 3 years is about 60%, which is comparable with the rate observed in systemic lupus erythematosus. It is not recommended that renal transplantation be pursued until it is clear that recovery will not occur spontaneously. This determination can be made in part by assessing the degree of damage on renal biopsy and by waiting at least 6 months (some investigators suggest 2 years) after recovery from the acute crisis. Recurrence of SRC is uncommon (5%) after a renal transplant.

## Musculoskeletal Involvement

Musculoskeletal symptoms are almost always present in scleroderma, usually are multifactorial in origin, and often are the initial symptoms of the disease. The most common symptoms are nonspecific pain, stiffness, and diffuse muscular discomfort that mimics a flu-like syndrome. Impaired hand function, characterized by decreased hand mobility, reduced dexterity, and decreased grip force, is in particular a major source of difficulty in performing activities of daily living. The degree and type of musculoskeletal disease vary a great deal and are influenced by the duration of disease, the level of overall disease activity, and the subtype of skin involvement. For example, in early diffuse scleroderma, pain that is often widespread in areas of skin inflammation and advancing fibrosis can involve joint structures, tendons, subcutaneous tissue, and underlying muscle. In later stage diffuse disease, joint contractures and muscle atrophy are often associated with pain and loss of function, causing significant disability. In patients with limited scleroderma, puffy fingers and loss of hand function and grip may be the only musculoskeletal symptoms throughout the disease course. Although histologic evidence suggests that synovitis occurs, arthralgias, pain, and stiffness without signs of frank arthritis constitute the usual clinical picture. However, overlap syndromes of rheumatoid-like polyarthritis or inflammatory muscle disease can dominate the clinical picture in an individual patient.

In the early edematous phase of diffuse scleroderma, patients often are diagnosed with carpal tunnel syndrome as a result of soft tissue swelling and inflammation in the hand and wrist area. Erosive arthritis with joint space narrowing can be seen, but soft tissue swelling, periarticular osteopenia, and contractures of the joint are more common. Distal bone resorption, osteolysis, and periarticular calcinosis are found in the fingers of patients with later stage diffuse scleroderma. Contractures of the PIP and MCP joints are most common, and rarely, contractures of the distal interphalangeal joint occur. Patients with diffuse skin disease experience contractures of large joints, including wrists, elbows, shoulders, hips, knees, and ankles. These contractures are the hallmark of severe scleroderma and are associated with fibrosis and ankylosis of the joint, resulting from disease in the overlying skin, fascia, joint capsule, and tendons.

Tendon friction rubs can be felt as a coarse crepitus over joints or over the forearm or lower leg with adjacent joint movement. These rubs are thought to be a result of low-grade tenosynovitis, local edema, and fibrosis of the tendon sheath, fascia, and joint structures. Friction rubs are seen primarily in patients with diffuse skin disease; when present, they are an indicator of a poor overall prognosis. They are found in about 15% to 30% of patients and are more common in patients with diffuse skin disease and in patients with antitoposiomerase, anti-RNA polymerase, or anti-U3-RNP antibodies.[141]

In relatively small observational studies, the prevalence of signs of joint inflammation in scleroderma is estimated to be about two-thirds of patients.[142,143] A large multicenter survey reported the frequencies of synovitis, tendon friction rubs, and joint contractures to be 16%, 11%, and 31%, respectively.[144] Synovitis, tendon friction rubs, and joint contractures are more likely to occur together and are more prevalent in patients with the diffuse cutaneous subset. These manifestations also tend to be associated with severe vascular, muscular, renal, and interstitial lung involvement. Synovitis, joint contracture, and tendon friction rubs are associated with more severe disease and with systemic inflammation.

Erosive arthritis is commonly associated with periarticular calcinosis (Figure 84-16A) and can be seen with significant bone loss or osteolysis. A radiologic survey of 120 patients found abnormalities including erosion (21%), joint space narrowing (28%), arthritis (defined by concomitant erosion and joint space narrowing; 18%), radiologic demineralization (23%), acro-osteolysis (22%), flexion contracture (27%), and calcinosis (23%).[144] Radiologic studies using ultrasonography and MRI confirm that hand involvement is striking, with articular, bone, and soft tissue changes.[145,146] Resorption of distal phalanges was significantly associated with digital ulcers and extraarticular calcification; flexion contracture was associated with the diffuse cutaneous form and with high HAQ disability scores.[147] Calcinosis is most often seen in patients with digital ulcers but was similarly observed in patients with the diffuse or limited cutaneous subtype. Calcinosis is often seen in the subcutaneous tissue in areas of trauma such as the extensor surfaces of the forearms, elbows, or patellae. Unlike the more diffuse calcinosis seen in patients with myositis, calcium deposits in scleroderma tend to be found in clusters around joints and sites of trauma. These deposits can restrict joint motion and may be associated with acute inflammation similar to a gouty arthritis, or the deposits can rupture through the skin, weeping a thick, white material. Once the skin is broken, secondary infection can occur.

Erosive polyarthritis, seemingly specific for scleroderma, or seropositive rheumatoid arthritis can occur in patients with limited or diffuse skin disease. However, the prevalence of scleroderma with rheumatoid arthritis overlap is low (1% to 2%), with an estimated incidence of 5%.[144,148,149]




**Figure 84-16** **A**, Hands of a patient with scleroderma showing reabsorption of distal phalanges corresponding to radiographic evidence of (**B** and **C**) acro-osteolysis, calcinosis, and joint erosions.

This finding is confirmed by the low frequency of anti-citrullinated protein antibodies found in patients with scleroderma and arthritis. When an inflammatory arthritis is present, traditional medications used in rheumatoid arthritis can be helpful. One case series demonstrated that the TNF inhibitor etanercept appeared to be efficacious in improving active inflammatory joint disease in a subset of patients with scleroderma, and it was generally safe and well tolerated.

A sense of weakness in the muscles of the hands, arms, and legs is very common (80%) and can be subtle or profound. One study found measurable weakness in 30% of patients, with proximal more than distal strength being compromised. Myopathy in a person with scleroderma is often multifactorial, but patients with myopathy are at increased risk for poor outcome and decreased survival.[120] Weakness is often caused by muscle atrophy as a result of the inflexibility due to joint disease and fibrotic skin with lack of usual mobility and exercise. It also can occur because of malnutrition resulting from scleroderma-related bowel disease. Muscle weakness in scleroderma may be a result of direct muscle disease. In people with diffuse scleroderma, fibrosis can extend into the striated muscle, causing muscle atrophy and clinical weakness. This phenomenon has been called a *fibrosing myopathy*. It presents in the setting of severe diffuse skin disease with joint contractures and mild elevation of creatine phosphokinase (CPK). EMG demonstrates nonirritable myopathy, and a muscle biopsy shows little inflammation, fibrosis, and type 2 muscle atrophy. The fibrosing myopathy does not respond to traditional anti-inflammatory medications, including corticosteroids. This type of muscle disease is associated with cardiomyopathy, heart failure, and arrhythmia that can be severe and irreversible.[150]

Approximately 5% to 10% of patients with scleroderma have an inflammatory muscle disease that can follow the same course as polymyositis and other forms of idiopathic inflammatory myopathy. Patients present with rapid proximal muscle weakness associated with high CPK. EMG shows irritable myopathy, and muscle biopsy is typical of an inflammatory myositis. In cases with severe polymyositis, myocarditis can occur with heart failure. High levels of anti-PM/Scl-100 antibodies are found in about 4% of patients with SSc, often in association with acute inflammatory muscle disease. Patients with muscle weakness should be fully evaluated for underlying causes with the use of laboratory measures of muscle enzymes, EMG studies, MRI, and muscle biopsy. These studies will help differentiate inflammatory muscle disease from other forms of myopathy.

## Endocrine Involvement

Endocrine dysfunction can complicate the course and management of patients with scleroderma. The most common endocrine problem associated with scleroderma is thyroid disease, and the frequency of other endocrinopathies is similar to that of the general population. Thyroid disease is reported in 10% to 15% of patients with scleroderma.[151,152] A case-control study found a 10 to 14 times increased risk of hypothyroidism.[153] Evidence for both fibrosis of the thyroid and autoimmune injury exists. Patients with

features of the CREST syndrome are more likely to have hypothyroidism than are those with diffuse skin disease. An autoimmune basis for thyroid disease is suggested by one series, which found Hashimoto's thyroiditis in 6% and Graves' disease in 3% of patients.[154] In a survey of 719 patients with scleroderma, it was found that 273 (38%) had at least one other autoimmune condition; the most frequent was autoimmune thyroid disease.[155] Thyroid autoantibodies are commonly found in patients with scleroderma who have hypothyroidism. In addition, the association of CREST syndrome with autoimmune hypothyroidism and primary biliary cirrhosis has been reported, suggesting multiple autoimmune targets in these patients. Thyroid disease may not be recognized in patients who have subclinical disease or symptoms associated with chronic multisystem disease. It is recommended that thyroid function be monitored periodically, especially in patients with limited scleroderma who have CREST syndrome and in those with long-standing disease.

## Other Associated Manifestations

It must be recognized that several less common and often forgotten complications may require attention in patients who have scleroderma. Awareness of these associated problems can improve quality of life and prevent undue distress. Bone disease can occur for a variety of reasons. Most patients with scleroderma are women who are near or in menopause and therefore are at risk for osteoporosis and its complications. This risk is increased by the chronic inflammatory nature of the disease, disuse, and several commonly used medications, such as corticosteroids or proton pump inhibitors. Malabsorption and lack of sun exposure may lead to vitamin D deficiency, and bowel dysfunction can limit calcium intake. Avascular necrosis of carpal bones of the wrist is reported and is thought to be a result of scleroderma peripheral vascular disease. In addition, osteolysis or bony resorption of the tips or tufts of the fingers, middle phalanges, distal radius, and ulna bones (less commonly, the distal clavicle, ribs, mandible, and distal toes) may occur (Figure 84-16B and C). Acro-osteolysis is fairly common, presenting with shortening of the finger or pain of the fingertip. Osteolysis is thought to be a manifestation of peripheral vascular disease and poor nutritional blood flow on affected bones. Neurologic complications can be a significant unrecognized problem. The CNS is generally spared in scleroderma, but unilateral or bilateral trigeminal neuralgia is known to occur in a subset of patients. Peripheral neuropathy occurs in a higher percentage of patients than expected as a result of multiple causes, including nutritional deficiency from GI disease, entrapment neuropathy, vasculitis, or vasculopathy of small vessels typical of scleroderma vascular disease. Carpal tunnel syndrome can complicate diffuse scleroderma and may occur in patients with associated inflammatory joint disease.

Dry eyes (keratoconjunctivitis sicca) and/or mucous membranes (xerostomia) occur in about 25% of patients. Dry eyes may occur as the result of severe facial periorbital skin fibrosis, which inhibits full closure of the eyelids. Topical artificial tears should be used frequently in these cases. Facial changes alter oral aperture and decrease facial flexibility. Vertical lines around the lips and thinning of the lips along with pinching of the nose affect self-esteem, especially in younger women. A decreased oral aperture prevents daily oral hygiene and dental procedures from being performed easily. Loosening of the teeth happens because of loss of the normal periodontal ligament attaching the tooth to the alveolar bone. Decreased saliva and difficulty performing usual dental hygiene can lead to significant periodontal disease. Treatment of oral manifestations requires early referral for experienced dental care and frequent cleaning with fluoride treatment. Pilocarpine and cevimeline are agonists for the muscarinic cholinergic receptors that can increase saliva and improve symptoms of xerostomia.

Most data suggest normal fertility in scleroderma, but during pregnancy, risk for hypertension, SRC, or premature fetal loss is increased. Sexual performance is often affected, particularly in women with diffuse disease. Decreased sexual satisfaction is associated with painful inflexible joints, dry vaginal membranes, and general fatigue. Erectile dysfunction and associated impotence have been reported in more than 80% of men with scleroderma as a result of local fibrosis of the corporeal smooth muscle and scleroderma microvascular disease. Psychogenic and medication-induced sexual dysfunction must be considered.

Several causes of lower extremity lesions can occur, including skin fibrosis, stasis dermatitis, lipodermatosclerosis, panniculitis, subcutaneous calcinosis, and leg ulcers. Leg ulcers may result from traumatic breakdown of atrophic fibrotic skin, ischemic ulcers as a result of scleroderma vascular disease, vasculitis, and occlusive vascular events resulting from an associated hypercoagulable state. Livedoid vasculopathy is a rare complication causing skin ulcerations on the lower leg and foot as the result of small vessel vasculitis or small vessel thrombosis.

## Psychosocial Aspects

Scleroderma is a disease that can alter virtually every aspect of the patient's life. Although scleroderma is often mild in its expression, the patient is now faced with a life crisis and must deal with a rare chronic disease that is complex and not completely understood by the medical community. Patients learn that scleroderma is potentially a life-threatening disease that alters physical capacity and can be disfiguring. At the onset of the disease, patients are often confused, anxious, and frightened. Fear and misunderstanding become a major source of distress. Studies of psychosocial adjustment support the conclusion that a substantial proportion of patients with scleroderma have difficulty adapting to the disease and have reduced quality of life measures. Although little evidence suggests that scleroderma directly affects the CNS with a direct organic cause for altered mental status, a complex array of medications used to treat the disease and its complications may have some influence on mood and the sense of well-being.

Psychosocial aspects of the disease do not appear to be consistently influenced by age, gender, ethnicity, education, or marital status. However, some data suggest that being disabled with low financial income leads to higher psychological distress. Evidence suggests that depression in patients with scleroderma is common and is related more to the patient's personality, degree of pain, and level of social

support than to actual disease severity. Disease-related disability, as measured by the HAQ-DI, is a more important predictor of adjustment problems than are disease symptoms related to severity. Pain and fatigue are common in scleroderma. Together with depressive symptoms, they are the most significant determinants of physical functioning and social adjustment—two important components of health-related quality of life—in patients with limited and diffuse disease subtypes.

Personality traits influence the degree of psychological adjustment. Patients with scleroderma who describe themselves as anxious, worried, tense, or detail-oriented are more likely to be depressed than are patients who describe themselves as agreeable or extraverted and outgoing. Disease unpredictability and lack of control cause increased distress. High self-reported uncertainty is related to poor adjustment. Thus, increasing the patient's understanding of the disease and expectations through education may reduce uncertainty and improve quality of life. Body image dissatisfaction is a significant concern. Patients are distressed by disease-related changes in visible parts of their body, especially the fingers and hands. A survey found that tight skin and facial changes were not as distressful as hand deformity with finger contractions and hand dysfunction. Sexual function is affected in both men and women with scleroderma. A significant proportion of men experience erectile dysfunction. Women with scleroderma, particularly those with diffuse skin disease, have high levels of sexual impairment compared with women who have other chronic diseases for which sexual function has received greater attention. Both women with scleroderma and women with other chronic diseases may have a decreased sexual drive during the disease course. The impact of sexual function on quality of life is not well studied, but it exists and should be appropriately addressed as part of comprehensive care.

Psychological factors with demonstrated relevance to scleroderma include pain, depression, and distress about disfigurement, physical function, and social function. Although these dimensions of quality of life are interrelated, pain, depression, and distress about disfigurement are common and may respond to psychological intervention. Such intervention begins with providing compassionate support beyond just ordering medications. It is important to spend time in a comfortable setting educating patients about scleroderma, providing a clear understanding of the degree of their disease, and explaining to them what they need to do and what can be done for relief. Having insight into social support and life circumstances, including financial distress, work environment, and family structure, provides a framework for helping to decrease external distress. For example, a family conference held to explain the reality of the health situation or the needs of the patient can reduce home tension and can clarify the patient's capacity in their family role. An adjustment in the work environment (e.g., permission to use a space heater) can make a difference. Clearly, effective social support improves quality of life. Follow-up visits that provide time to discuss issues of coping and social support are most important. Recognizing the personality of patients while addressing their specific concepts is helpful. Treating underlying depression and especially providing effective management of disease-related pain help to improve quality of life and reduce social and psychological distress. Body image dissatisfaction is a significant concern in women with scleroderma and should be assessed routinely. Early identification and treatment of body image dissatisfaction may help to prevent depression and psychosocial impairment in this population. The possibility of premature death from the disease is a major cause of fear and needs to be addressed with the patient and the family. Most often, life expectancy is not influenced by the disease, yet patients fear death because they have scleroderma. When a patient is facing death from severe disease, appropriate honest and sensitive support must be provided.

## TREATMENT

### Immunotherapy

Scleroderma is thought to be an inflammatory connective tissue disorder triggered by an autoimmune process.[87] Nonselective immunosuppressive treatments are usually used in scleroderma to treat specific organ manifestations such as early progressing skin disease, active ILD, and underlying inflammatory joint or muscle disease (Table 84-6). CYC has shown some modest efficacy in scleroderma-related ILD in a randomized placebo-controlled trial.[87] Current therapeutic protocols use CYC as a daily oral regimen or as monthly IV therapy until control of disease is achieved. The CYC is often followed by maintenance treatment with AZA or MMF, agents that have anti-proliferative effects on inflammatory cells and in particular on activated T and B lymphocytes.[156] Uncontrolled studies suggest benefit of MMF in both ILD and active scleroderma skin disease.[157-160] Most physicians are now using MMF as the preferred first agent for ILD and skin disease because of its potential benefit and safer profile compared with CYC. Evidence supporting the use of AZA as a primary agent to treat scleroderma-related ILD is weak and remains limited to small retrospective studies. Two open-label trials support its role as maintenance therapy after primary CYC immunosuppression.[161-163]

Methotrexate (MTX) is frequently used in patients with scleroderma to treat associated inflammatory arthritis and myositis. MTX efficacy in treating skin disease and lung function was investigated in two randomized placebo-controlled trials and showed only a small benefit on skin scores after 6 months (with weekly intramuscular injections) or after 12 months (with weekly oral therapy), respectively.[164,165] Based on the findings of these studies, many experts use MTX for active skin disease. However, other experts prefer using MMF as an initial agent for active skin disease because uncontrolled studies have shown greater benefit.

Selective immunotherapy has also been used in scleroderma (see Table 84-5). T lymphocyte–directed treatments such as cyclosporin A, sirolimus (rapamycin), and antithymocyte globulin have shown some benefit.[166,167] However, these studies generally were small and were limited by drug toxicity. The use of extracorporeal photoimmunotherapy or photopheresis has been reported with modest improvement in skin disease but lack of any efficacy on internal organ manifestations.[168] This approach is not currently supported. Preliminary studies of rituximab, a chimeric IgG1 monoclonal antibody directed against CD20, a surface molecule

**TABLE 84-6** Immunotherapeutic Treatments in Scleroderma

| Therapeutic Category | Drug | Clinical Studies (Reference) |
|---|---|---|
| Nonselective immunotherapy | Cyclophosphamide | Tashkin 2006[87]<br>Hoyles 2006[156]<br>Nadashkevich 2006[189]<br>Tashkin 2007[190] |
| | Mycophenolate mofetil | Derk 2009[157]<br>Liossis 2006[158]<br>Nihtyanova 2007[159]<br>Gerbino 2008[191]<br>Zamora 2008[192]<br>Le 2011[160] |
| | Azathioprine | Dheda 2004[193]<br>Nadashkevich 2006[189]<br>Paone 2007[194]<br>Berezne 2008[163] |
| | Methotrexate | van den Hoogen 1996[164]<br>Pope 2001[165] |
| T cell–targeted immunotherapy | Cyclosporin A | Clements 1993[167]<br>Filaci 1999[195]<br>Morton 2000[166] |
| | Anti-thymocyte globulin | Matteson 1996[196]<br>Stratton 2001[197] |
| | Extracorporeal photopheresis | Rook 1993[198]<br>Krasagakis 1998[199]<br>Knobler 2006[168] |
| | Sirolimus (rapamycin) | Su 2009[200] |
| B cell–targeted immunotherapy | Rituximab | Lafyatis 2009[172]<br>Smith 2010[169]<br>Daoussis 2010[201]<br>Moazedi-Fuerst 2014[170]<br>Jordan 2015[171] |
| Intravenous immunoglobulins | IVIG | Levy 2000[202]<br>Amital 2003[203]<br>Levy 2004[173]<br>Ihn 2007[204]<br>Nacci 2007[174] |
| Biologic immunotherapy | TNF inhibitors<br>Etanercept<br>Infliximab | Lam 2007[205]<br>Denton 2009[206] |
| Antifibrotic therapy | CAT-192 (anti–TGF-β ab)<br>Imatinib mesylate | Denton 2007[181]<br>Gordon 2009[207]<br>Pope 2011[183] |
| Cell-based immunotherapy | Autologous HSCT | Binks 2001[175]<br>McSweeney 2002[208]<br>Farge 2004[176]<br>Nash 2007[209]<br>Oyama 2007[177]<br>Vonk 2008[51]<br>Burt 2011[52]<br>van Laar 2014[53] |

HSCT, Hematopoietic stem cell transplantation; IVIG, intravenous immunoglobulin; TGF-β, transforming growth factor-β.

expressed on early pre-B and mature B cells, have shown some promising results, with improvement of skin fibrosis and stabilization of lung function.[169-172] This clinical benefit awaits confirmation in larger, controlled trials. Administration of IV immunoglobulin (IVIG) to patients with scleroderma has been reported in small open-label studies with some evidence that it can improve skin fibrosis or joint disease.[173,174]

Studies of cell-based immunotherapy using autologous hematopoietic stem cell transplantation (HSCT) preceded by myeloablative or nonmyeloablative conditioning regimens have recently been completed.[52,53] In the Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trial and the American Scleroderma Stem Cell versus Immune Suppression Trial (ASSIST), patients with scleroderma were randomized to receive HSCT or pulsed IV doses of CYC administered monthly. Overall, these investigations have confirmed previous observations that HSCT is effective in rapidly controlling skin disease, stabilizing lung function, and improving quality of life as assessed by patient-reported outcome measures.[175-177] HSCT also shows a better long-term event-free and overall survival compared with CYC. Despite these positive results, the high treatment-related morbidity and mortality (10.1% in ASTIS) associated with high-dose immunosuppression and the evidence that the disease tends to relapse in a substantial subset of treated patient, are still preventing consideration of this approach as standard of care for patients with scleroderma. Stratification of subjects based on disease severity and selection of appropriate candidates remain crucial for a safe and effective use of HSCT. Nonmyeloablative (immunoablative) conditioning regimens without HSCT have demonstrated similar results.[178]

## Treatment of Fibrosis

To date, no drugs have proved able to reverse the fibrotic disease process in scleroderma, but several potential agents are being used off-label or are under investigation. Their use is still based on in vitro data, animal studies, or case series because formal well-designed controlled trials are still missing.

D-Penicillamine is a chelating agent that blocks collagen cross-linking and thus has the potential to exert antifibrotic effects. Several case reports and retrospective reviews have suggested that D-penicillamine is beneficial.[179] A double-blind, randomized clinical trial comparing the efficacy of low-dose versus high-dose D-penicillamine in early diffuse scleroderma found no significant difference between skin scores of the two groups at 24 months.[180] Given the potential toxicity of D-penicillamine, its lack of effectiveness in the only controlled trial, and the long duration before benefit was achieved in several uncontrolled surveys, the drug has fallen out of favor and generally is not used.

Transforming growth factor (TGF)-β is a cytokine known to promote fibroblast proliferation and differentiation, in addition to upregulation of collagen and extra-cellular matrix synthesis. Therefore, it represents an appealing target to specifically control progression of collagen and extra-cellular deposition in tissues. A human recombinant neutralizing TGF-β antibody was studied compared with placebo in a relatively small phase I/II trial in patients with early diffuse scleroderma.[181] No clinical benefit was observed when skin score or lung function testing parameters were evaluated. Although the results of this study were negative, it does not negate the idea that a more potent neutralizing antibody might be helpful. A new study is ongoing.

Imatinib, dasatinib, and nilotinib are small molecules that inhibit the tyrosine kinase activity of the abl-kinases and platelet-derived growth factor receptors, thus

interfering with important profibrotic pathways activated in scleroderma. In addition, dasatinib inhibits Src kinases, which are also involved in fibroblast differentiation and secretory function.[182] Several small uncontrolled trials with tyrosine kinase inhibitors have been reported with no indication of benefit and frequent adverse events including fluid retention, nausea, fatigue, and elevation of creatine kinase.[183,184] Therefore, although a potent antifibrotic effect has been shown by tyrosine kinase inhibitors in vitro or in animal models, clinical studies to date have not provided solid evidence for their use in treating skin and systemic fibrosis.

Halofuginone, a plant-derived alkaloid with antifibrotic properties via inhibition of TGF-β and T cell activation, is known to reduce collagen production in animal models. Only limited use of topical halofuginone therapy in patients with scleroderma skin disease is reported, with some encouraging results.[185] Rosiglitazone is an agonist of peroxisome proliferative-activator receptor-γ and has been shown to suppress in vitro fibroblast production of collagen and to alleviate fibrosis in the bleomycin-induced scleroderma mouse model.[186] Pirfenidone (5-methyl-1-phenylpyridin-2[1H]-one) is a novel agent with antifibrotic and antiinflammatory properties. In a phase III randomized controlled trial, compared with placebo, pirfenidone has shown significant benefit (decreased disease progression) and a relatively mild adverse-effect profile in patients with idiopathic pulmonary fibrosis.[187] A clinical trial is ongoing in scleroderma-related ILD. Neutralizing antibodies against connective tissue growth factor (CTGF) can effectively suppress the development of skin fibrosis in animal models, and anti-CTGF therapy is now under consideration to treat scleroderma.[188]

New understanding of molecular pathways that play a unique role in fibrosis is emerging that may provide new targets for treatment. This understanding includes insight into fibroblast-myofibroblast differentiation, epithelial-mesenchymal transition, and fibroblast interactions with extra-cellular matrix via cellular integrins.

### Treatment of Vascular Disease

Although scleroderma is generally considered a fibrosing disorder, it is well recognized that underlying vasculopathy plays a fundamental role in its pathogenesis and associated tissue injury. In RP, small and medium-sized peripheral blood vessels involved in tissue nutrition and body thermoregulation are affected. The pathologic and clinical consequences of scleroderma vascular disease are not limited to the skin but are widespread and are found in all involved organs. Currently, no guidelines have been put forth for a general treatment of scleroderma vascular disease. Therefore, pharmacologic therapies are focused on the treatment of organ-specific disease such as scleroderma renal crisis, PAH, and RP (see related sections). Although conventional treatment strategies with nonspecific vasodilator agents are still routinely used, novel agents targeting specific biologic processes involved in scleroderma vascular disease (i.e., prostacyclin, endothelin antagonists, statins, and phosphodiesterase inhibitors) are now available with evidence of broader beneficial effects (see the sections on Raynaud's Phenomenon and Pulmonary Hypertension).

## SUMMARY OF CURRENT PRACTICAL RECOMMENDATIONS FOR TREATMENT

The key to current management of scleroderma is to not isolate treatment to one problem that seems to be dominant but to appreciate the complexity and dynamics of this multisystem disease (Table 84-7). It is clear that use of just one drug is not effective in managing scleroderma and that long-term therapy is required with possible adjustments based on specific clinical circumstances. A combination therapy approach that attempts to treat the immune response, the vascular disease, and the underlying tissue fibrosis is recommended.

Patients with severe RP should be taking vasodilators and anti-platelet therapy with aspirin. Vasodilator treatment should start with a calcium channel blocker, with a

**TABLE 84-7** Current Recommendations for Treatment of Scleroderma

| Manifestation | Primary Therapy | Alternative/ Second-Line Therapy |
|---|---|---|
| Raynaud's phenomenon | Vasodilators (CCB or PDE5 inhibitors)<br>Anti-platelet | PDE5 inhibitors, prostacyclin, endothelin antagonists |
| Hypertensive renal disease | ACE inhibitors | ARBs, CCB, prostacyclin, renal transplant (wait at least 24 mo) |
| GI involvement | **Upper GI**<br>Dental/periodontal care, lifestyle modifications, proton pump inhibitors, prokinetics<br>**Lower GI**<br>Probiotics, rotational antibiotics | EGD to treat stenosis and/or GAVE<br><br>Total parenteral nutrition |
| Skin | Mycophenolate mofetil, cyclophosphamide | IVIG, ATG, research trial (severe cases) |
| Interstitial lung disease | Mycophenolate mofetil, cyclophosphamide | Research trial |
| Pulmonary arterial hypertension | PDE5 inhibitors, endothelin antagonists, prostacyclin, soluble guanylate-cyclase stimulators | Combination therapy, atrioseptostomy, lung transplant, research trial |
| Cardiac involvement | Heart failure therapy, diuretics, CCB | Immunosuppression (myocardial inflammation) |
| Joints | Prednisone, methotrexate, TNF inhibitors | IVIG (if contractures and rubs are present), PT/OT |
| Muscles | Prednisone, methotrexate, azathioprine | IVIG |
| Psychosocial | Anti-depressants, pain control, sleep control | Support group |

ACE, Angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; ATG, anti-thymocyte globulin; CCB, calcium channel blockers; EGD, esophagogastroduodenoscopy; GAVE, gastric antral vascular ectasia; GI, gastrointestinal; HSCT, hematopoietic stem cell transplantation; IVIG, intravenous immunoglobulin; PDE5, phosphodiesterase-5 inhibitor; PT/OT, physical therapy/occupational therapy.

second agent added if needed—this can be a phosphodiesterase inhibitor or intermittent IV prostacyclin (it is hoped that oral prostacyclin will be available soon). If digital ulcers are recurring, then the addition of an endothelin inhibitor or a statin may be indicated.

Patients with hypertensive renal disease should be treated urgently with an ACE inhibitor or, if needed, with additional ARBs or anti-hypertensive agents, including calcium channel blockers or a prostacyclin analogue. The use of ACE inhibitors to prevent a scleroderma renal crisis is not recommended.

All patients need periodic professional dental care, and if loss of saliva production is noted, the use of pilocarpine or cevimeline is recommended. Patients with upper GI disease should be treated with lifestyle changes, such as improving their eating habits; with proton pump inhibitors; and in cases of unremitting symptoms, with prokinetic drugs (e.g., metoclopramide and domperidone). If the lower intestinal tract is involved, then rotating antibiotics can improve episodes of pseudo-obstruction, diarrhea, or malabsorption.

Management of established, nonactive cutaneous involvement is based on supportive care with use of moisturizing agents to prevent excessive dryness. First-line therapy for active skin disease has not yet been formally established, but immunosuppressive therapy is recommended based on severity and level of disease activity. MMF is preferred by many clinicians for mild to moderate disease, although some persons still recommend MTX. Combination with IVIG or cyclophosphamide alone (daily oral doses or monthly IV administration) should be considered for severe, aggressive disease. More innovative and targeted therapies are needed. These therapies may include immune cell depleting protocols (i.e., rituximab and anti-thymocyte globulin) or biologic agents (i.e., tocilizumab, abatacept, and belimumab), which are currently under evaluation. Immunoablation or myeloablation with or without hematopoietic stem cell rescue may be an option in selected patients who are not responsive to conventional treatment. Participation of patients in new clinical trials should be pursued.

Active ILD should be treated with immunosuppression. MMF is now commonly used as initial therapy. CYC (daily oral or monthly IV) also can be used as a first-line agent, but its use should be limited to 6 to 12 months, followed by maintenance (several years) with a secondary immunosuppressive drug (i.e., MMF or AZA). High-dose immunosuppression (HSCT) or novel therapies (e.g., tocilizumab and rituximab) should be considered in refractory cases. Lung transplant is a viable option for patients with end-stage lung disease.

Pulmonary vascular disease and PAH are best treated with vasoactive drugs, including endothelin-1 inhibitors, phosphodiesterase inhibitors, prostacyclin analogues, or soluble guanylate-cyclase stimulators given alone or in combination. The role of anticoagulation or immunosuppressive agents is not yet defined. Treatment of associated heart failure is most important.

Cardiac disease should be treated with vasoactive drugs such as calcium channel blockers, other anti-hypertensive agents, diuretics, and antiarrhythmic medications. Immunosuppression should be used if inflammatory muscle or pericardial disease is present.

Distinct manifestations of inflammatory arthritis should be treated in a similar fashion to the therapeutic approach used in rheumatoid arthritis. However, a fibrosing process causing a nonerosive arthropathy in association with friction rubs and joint contractures is best treated with the same medications used for active scleroderma skin disease.

Treatment for muscle disease will vary depending on the presence of inflammatory myositis or a nonirritable fibrosing myopathy. The former is treated with corticosteroids, MTX, or other immunosuppressive agents effective for immune-mediated polymyositis. The fibrosing muscular process is best treated with the same approach used for scleroderma skin disease.

All patients need emotional and physical support. Scleroderma is a painful disease, and managing pain is essential for reducing depression and improving quality of life. Managing pain may require the use of sleep aids (e.g., lorazepam or zolpidem) and/or intermittent use of narcotics. Physical and occupational therapy is most important early in the course of the disease to reduce pain and improve activities of daily living. Family counseling helps the patient cope by providing a clear understanding of what is needed at home. Adjustment of the work environment (e.g., limiting time at work, adjusting room temperature, and changing work assignments) or helping patients obtain disability support is also important. Open communication between the physician and the patient provides an essential element of care.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 85

# Inflammatory Diseases of Muscle and Other Myopathies



Kanneboyina Nagaraju • Heather S. Gladue • Ingrid E. Lundberg

**KEY POINTS**

- Myopathies are a heterogeneous group of muscle diseases characterized by symmetric proximal muscle weakness and frequent involvement of other organs.
- Myopathies are often accompanied by elevated levels of serum muscle enzymes and abnormal electromyograms.
- Histology shows varying degrees of inflammation and muscle fiber degeneration and regeneration.
- Some patients have autoantibodies that bind to molecules involved in protein synthesis, and these antibodies are often associated with distinct clinical phenotypes.
- Corticosteroids and cytotoxic drugs are common therapies for inflammatory muscle disease.

Inflammatory muscle diseases are a heterogeneous group of systemic autoimmune rheumatic disorders characterized by chronic muscle weakness, muscle fatigue, and mononuclear cell infiltration into skeletal muscle. These disorders were described in the literature more than a century ago as generalized muscle disorders affecting principally the trunk and proximal limb muscles, with or without skin involvement.[1-5] It was also recognized that these diseases can range from acute and even fatal to slow, progressive, chronic, insidious conditions, with patterns of relapse and remission. Steiner's[6] summary of myositis cases in 1903 made a clear distinction between idiopathic polymyositis (PM) and other forms of myositis caused by bacteria and parasites,[6] and Stertz[7] in 1916 first reported an association between dermatomyositis (DM) and internal malignancy.[7] At about the same time, Batten[8] described the first case of DM with classic histologic features in a child.

Since the 1940s, it has been recognized that PM may occur in the absence of cutaneous lesions, muscle pain, or constitutional symptoms. It may present in an acute, subacute, or chronic insidious form, with some fraction of cases showing systemic features or involvement of organs and tissues.[9] The differential diagnosis has been described independently by several investigators, and the most chronic form was differentiated from an adult variety of muscular dystrophy.[10-12] Banker and Victor[13] noted that DM in children was different and involved a greater degree of vascular inflammation and thrombosis (systemic angiopathy). The first, and still widely used, classification scheme and set of diagnostic criteria for myositis were proposed by Bohan and Peter in 1975.[14,15] They include PM and DM but not the later described subset known as inclusion body myositis (IBM). IBM was later defined by the presence of distinct histopathologic changes, including vacuoles and nuclear and cytoplasmic inclusions, as well as by distinct clinical features, including resistance to glucocorticoids.[16,17]

## EPIDEMIOLOGY

The actual annual incidence of inflammatory myopathy is currently unknown. Because these diseases are so rare, no large-scale epidemiologic studies have been reported. However, several retrospective studies have reported an annual incidence of less than 10 per million individuals[18-22] (Table 85-1). This number may be an overestimate, given that the Peter and Bohan diagnostic criteria used in these studies did not distinguish IBM as a separate disease entity. The prevalence of IBM has been estimated to be 10.7 per million in the United States, 9.3 per million in Australia, and 4.9 per million in the Netherlands.[23-25] The age-adjusted prevalence of IBM for those older than 50 years was reported as 16 to 35 per million.[24,25] In some geographic areas, IBM appears to be the most common acquired progressive myopathy, representing 16% to 28% of all inflammatory myopathies.[25] However, there may be referral biases in these studies. The incidence-prevalence studies need to be interpreted cautiously, given that most have not reported confidence intervals for their rates.

The incidence of the various myopathies varies according to ethnicity, age, and gender. Some studies have reported that the incidence of PM is higher in black patients than in white patients.[18] Idiopathic inflammatory myopathies (IIMs) can occur in any age group, from early childhood to late in adult life. The onset of PM is usually in the late teens or older: the mean patient age at onset is 50 to 60 years. DM shows two peaks: 5 to 15 years and 45 to 65 years. IBM is commonly seen in individuals older than 50 years and is rare in younger adults. Some studies have reported gender-specific incidence rates. For example, in the case of PM and DM, females are more commonly affected than males (ratio >2:1), whereas in IBM, the converse is true (again, >2:1 ratio).

Inflammatory myopathies can occur in association with other autoimmune connective tissue diseases such as scleroderma, systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome, mixed connective tissue disease, polyarteritis nodosa, and sarcoidosis. Significant proportions of all myositis patients (11% to 40%) have an associated connective tissue disease.[23,26,27] Several studies have also confirmed an association between malignancies and inflammatory myopathies. A 2-decade-long retrospective study of myositis patients revealed that myositis in

**TABLE 85-1** Incidence of Inflammatory Myopathies

| Country | Study Dates | Incidence (million/yr) | Reference |
|---|---|---|---|
| United States | 1963-1982 | 5.5 | 18 |
| United States | 1947-1968 | 5.0 | 20 |
| Australia | 1989-1991 | 7.4 | 21 |
| Sweden | 1984-1993 | 7.6 | 22 |
| Israel | 1960-1976 | 2.1 | 246 |

approximately 12% (37/309) of the patients was associated with malignant disease. A majority (81%) (30/37) of the patients studied had DM, and the remainder (19%) had PM. Overall, the frequency of malignancies has varied widely (4% to 42%) in different studies,[19,28] but in general, the incidence of malignancy is higher in DM patients than in IBM or PM patients.[29] It is difficult to determine the relative risks for a particular malignancy because a variety of malignancies are associated with myositis, and only small numbers of individual malignancies have been reported in any one study.

## ETIOLOGY OF MYOSITIS

### Genetic Risk Factors

An association with immune response genes and occasional reports of familial clustering of myositis support the role of genetic factors in these diseases.[30-35] Polymorphisms in class I and II human leukocyte antigen (HLA) genes are known genetic risk factors for several autoimmune diseases that include myositis, but the mechanisms for these associations remain unclear. One possibility is that because the gene products influence T cell repertoire development, tolerance, and immune responses to foreign agents, certain polymorphisms are selected on the basis of environmental triggers. It appears that haplotypes HLA-DRB1*0301 and HLA-DQA1*0501 are the strongest known genetic risk factors for all forms of myositis in whites. However, different phenotypes have additional HLA risk and protective factors.[35,36] In African-American patients neither DRB1*0301 nor DQA1*0501 is strongly associated with myositis. Instead, the HLA-DRB1*08 allele shows the highest general risk for myositis, whereas the HLA-DRB1*14 allele is strongly protective in African-American patients. The HLA-B8/DR3/DR52/DQ2 haplotype is found in a significant proportion of IBM patients.[37] The risk and protection conferred by HLA associations differ significantly among different ethnic and serologic groups. For example, in some populations (e.g., Koreans, Mesoamericans), there is no association with HLA genes.[33] Further, HLA-DRB1*0301, which is a risk factor in whites, is a protective factor in the Japanese population.[38] The HLA-DRB1*0301, HLA-DQA1*0501, and HLA-DQB1*0201 alleles are strongly associated with myositis-specific antibodies in PM patients.[39] Mechanistic data supporting the role of HLA molecules in disease pathogenesis are, unfortunately, lacking at present. Some studies reported that maternally derived chimeric cells are present in the peripheral blood and muscle tissues of juvenile DM patients, suggesting that HLA alleles control the occurrence of chimerism and explain the HLA association found in these disorders.[40,41] Like other autoimmune disease conditions, myositis is a complex multigenic disorder involving other non-HLA immune response genes (e.g., cytokines and receptors, including TNF, IL-1, and TNFR-1); complement components (e.g., C4, C2); immunoglobulin (Ig) heavy-chain allotypes; and T cell receptors.[42] Polymorphisms in some non-HLA genes, such as signal transducer and activator of transcription 4 gene (STAT4) and C8orf13–BLK, appear to contribute prominently in Asian populations.[43,44] The exact contribution of the genetic component in these disorders is currently unknown, in part because of their rarity, the small number of subjects in any single cohort, and the heterogeneity seen in the disease phenotype. A recent genome-wide association study (GWAS) of DM patients (adult and juvenile) has confirmed the MHC as the major genetic region associated with DM. Furthermore, DM also shares non-MHC genetic features with other autoimmune diseases, suggesting the presence of additional novel risk loci.[45] International collaborative efforts are currently under way to identify novel genetic and environmental risk factors in myositis.

### Environmental Risk Factors

The temporal association of myositis onset and environmental agents in certain individuals suggests that specific exposures in the context of certain genetic backgrounds can initiate muscle inflammation. Common environmental agents implicated in myositis include infectious organisms, such as viruses and bacteria, and noninfectious agents, such as drugs and food supplements (Table 85-2). For example, enteroviruses (influenza, coxsackievirus, echoviruses) and retroviruses (human T-lymphotropic virus-I) are known to induce muscle inflammation. The myositis associated with enteroviruses usually occurs in children and is generally self-limited. A viral cause is strengthened by the presence of high-titer anti-viral antibodies and viral particles in patients' serum and tissue samples,[46,47] as well as the induction of muscle inflammation by enteroviruses in animal models. A more recent association was observed between a previous cytomegalovirus (CMV) infection and a subphenotype of PM and DM patients with high frequency of so-called *CD28-null T cells* (see later), indicating that some viral infections may influence the immune system and lead to chronic inflammation.[48] Similarly, some microorganisms, such as staphylococci, clostridia, and mycobacteria, are known to affect skeletal muscle and cause acute muscle inflammation, but there is no evidence that these organisms actually cause chronic, self-sustaining muscle inflammation.

Parasites such as *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Borrelia burgdorferi* have been implicated in the triggering of IIMs. The evidence in support of a parasitic cause includes the recovery of parasites from some myositis patients and their serologic response to the parasites; improvement in myositis symptoms after treatment with anti-parasitic drugs; a histologic picture of inflammation, including infiltration of macrophages and CD4 T cells; and induction of myositis after parasitic infection in animal models.[49-55]

Ultraviolet (UV) light irradiation is likely to be a risk factor for DM because epidemiologic data have

**TABLE 85-2** Possible Environmental Risk Factors

| |
|---|
| **Infectious Agents** |
| ***Viruses*** |
| Picornavirus family, enteroviruses |
| Polio, coxsackievirus types A and B, echoviruses |
| Retroviruses |
| HIV-1, HTLV-I |
| Parvovirus B19 |
| Hepatitis C virus |
| Hepatitis B virus |
| ***Bacteria*** |
| Staphylococci |
| Clostridia |
| Mycobacteria |
| ***Parasites*** |
| *Toxoplasma gondii* |
| *Trypanosoma cruzi* |
| *Borrelia burgdorferi* |
| **Noninfectious Agents** |
| ***Drugs*** |
| D-Penicillamine |
| Corticosteroids |
| Chloroquine |
| Statins |
| Atorvastatin, lovastatin, pravastatin, simvastatin |
| Lipid-lowering fibrates |
| Bezafibrate, clofibrate, gemfibrozil |
| L-Tryptophan |
| Biologic agents |
| Growth hormone, interferon-α, interleukin-2 |
| Vaccination for tetanus, BCG, diphtheria, hepatitis B, hepatitis A |
| Miscellaneous drugs |
| Local anesthesia, hydroxyurea, leuprolide acetate |
| ***Ultraviolet radiation exposure*** |
| ***Miscellaneous agents*** |
| Silicone breast implants, chronic graft-versus-host disease associated with bone marrow transplantation, collagen injection, silica exposure |

BCG, Bacille Calmette-Guérin; HTLV-I, human T-lymphotropic virus I.

demonstrated a latitude gradient of PM and DM, with the latter more frequent closer to the equator and the former more frequent in northern countries. The ratio between PM and DM is associated with a latitude gradient and is directly correlated with UV light irradiation. This observed correlation is particularly strong in a subset of DM patients with anti–Mi-2 autoantibodies, indicating that UV light may be an environmental risk factor for its development. The association between UV light exposure and the subtype of myositis suggests that UV light is an exogenous modifier that can influence the clinical and serological phenotype in myositis.[56,57]

Smoking is a risk factor for a subtype of myositis, the anti-Jo-1 positive, (one of the anti-synthetase syndromes—see the section on autoantibodies) and has a possible interaction with the HLA-DRB1*03 genotype.[58] Environmental and occupational agents such as dusts, gases, or fumes are also reported risk factors for patients with anti-synthetase syndrome.[59] These inhaled risk factors together with the high frequency of interstitial lung disease (ILD) as a manifestation of the anti-synthetase syndromes indicate a role for the lung as target of the immune reaction in this subset of myositis.

Malignancy is an additional risk factor for myositis, and there is a strong association between DM and malignancies. This early clinical observation has been confirmed in epidemiologic studies.[28,60] With regard to PM and IBM, the association with malignancy is less convincing. The increased risk of malignancy associated with DM has been established both at the time of DM diagnosis and more than 10 years after diagnosis. A subgroup of DM has been named *cancer-associated dermatomyositis* (CADM). *CADM* is often used to classify patients with DM with a detected cancer within 3 years before or after DM diagnosis, although the time span between the malignancy and DM varies between studies. No specific malignancy is associated with DM; rather, the most frequent malignancies are also the most common malignancies in the population.

The pathophysiologic mechanism for the association between malignancy and DM has not been clarified, but there could be several explanations. The strong association between malignancy and the onset of DM, the observation that removal of a tumor sometimes results in amelioration of muscle weakness, and the fact that tumor reappearance sometimes coincides with muscle weakness, suggests that DM could be a paramalignant phenomenon. That is, the development of myositis is a consequence of the malignancy (related to autoantigens), or the malignancy and DM share disease mechanisms. The molecular mechanisms underlying this unique association are currently unclear, however. A recent report has shed some light on the connection between DM and malignancy by showing that myositis-specific antigens are highly expressed in cancer tissues, as well as in regenerating muscle cells of myositis patients.[61,62] It has been proposed that in cancer-associated myositis, an autoimmune response directed against cancer cross-reacts with regenerating muscle cells, enabling a feed-forward loop of tissue damage and antigen selection.[63] This is further supported by the association between CADM and two newly identified myositis-specific autoantibodies (TIF1-γ and NXP2) in adult patients, (see the paragraph on myositis autoantibodies). For malignancies that develop during established disease, the potential explanations include the presence of chronic inflammation or prolonged immunosuppressive treatment, which could contribute to the development of malignancy.

A recent report notes a novel association of myositis with hypertension, diabetes, and ischemic heart disease. The prevalence of hypertension and diabetes in this population was 62% and 29%, respectively, considerably higher than the background prevalence of 9.4% and 4%. The authors of this report suggest that hypertension and ischemic heart disease are more likely to be present before the diagnosis of myositis, whereas hypertension and diabetes occur more frequently following the diagnosis of myositis in patients with DM than in patients with PM or IBM, suggesting that it is essential for the physician to perform a comprehensive assessment of vascular risk factors in these patients.[64] The same group also reported that patients with IIM are at a 75% increased risk for mortality, and cardiovascular diseases followed by infection and malignancy account for the most common causes of death.[65]

## Mimics of Myositis

A variety of factors induce the clinical and pathologic spectrum that mimics myositis in some individuals (see Table

85-2). A number of drugs are known to cause a myopathy that closely mimics myositis. For example, D-penicillamine causes clinically and histologically indistinguishable IIM.[67] Likewise, commonly used lipid-lowering drugs such as statins (e.g., atorvastatin, lovastatin) can cause a myopathy that resembles inflammatory myositis. These agents inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme involved in the conversion of HMG-CoA to mevalonic acid, thereby preventing the synthesis of bioactive sterol and nonsterol metabolic intermediates in the cholesterol synthetic pathway. The mechanism by which these drugs cause myopathy is not yet clear.[68-70] Patients with anti-HMGCR antibodies often have prominent myofiber necrosis with little or no muscle inflammation, also referred to as *necrotizing autoimmune myopathy* (NAM). It has been suggested that statins upregulate HMG-CoA autoantigen in regenerating muscle cells that, in turn, sustain an autoimmune response even after statin withdrawal, providing a mechanism for statin-induced immune-mediated necrotizing myopathy.[71] However, recent studies have indicated that the majority of NAM patients are statin-naïve, and anti-HMGR antibody titers correlate with muscle strength, suggesting a pathogenic role for these antibodies in NAM[72] (see details in Necrotizing Myopathies). Other drugs, such as hydroxyurea, can cause skin rashes that resemble DM.[73] TNF inhibitors have been associated with the onset of autoimmune diseases such as vasculitis and a lupus-like syndrome. Recent reports have suggested that TNF inhibitors in inflammatory arthritis patients either induce or exacerbate DM or anti-Jo-1-positive PM.[74,75,79,80] Some other reports have pointed to the vaccine adjuvant aluminum hydroxide as a cause of macrophagic myofasciitis. Histologic examination shows infiltration by macrophages and some $CD8^+$ T cells into the endomysium, perimysium, and epimysium, together with clinically elevated creatine kinase (CK) levels, muscle weakness, myalgias, fatigue, and arthralgias.[76] Despite some reports of vaccine-induced myositis, systematic investigation has failed to link any vaccine to myositis.[77]

## PATHOGENESIS

Significant advances have been made in our understanding of the pathogenesis of the human inflammatory myopathies.[78-83] It is generally thought that IIMs are autoimmune in origin because they are frequently associated with other autoimmune diseases (e.g., Hashimoto's thyroiditis) and collagen vascular diseases (e.g., scleroderma). Many patients exhibit an autoantibody response, including the presence of myositis-specific autoantibodies; some studies have provided evidence for lymphocyte-mediated muscle fiber injury, and a favorable response to immunosuppressive therapies in some patients supports an autoimmune cause of these disorders.

### Humoral Immune Response

More than 50% of all IIM patients have uniquely defined autoantibodies, some of which are specific to myositis, and some of which are merely associated with myositis. These are generally referred to as *myositis-specific autoantibodies* (MSAs) and *myositis-associated autoantibodies* (MAAs), respectively. MAAs include autoantibodies to various nuclear and cytoplasmic antigens. Anti-nuclear antibodies (ANAs) present in myositis are not particularly associated with any disease subgroup, whereas MSAs that are directed against antigens of the protein synthesis pathway (e.g., aminoacyl–transfer RNA [tRNA] synthetases and signal recognition particles) and nuclear components (e.g., nuclear helicase [Mi-2]) are often associated with distinct clinical disease groups and subgroups (e.g., tRNA synthetases with interstitial lung disease, Mi-2 with DM) (Table 85-3 and see Table 85-6).

Anti–histidyl-tRNA synthetase antibodies are the most frequent autoantibodies seen and are present in approximately 16% to 20% of myositis patients.[84-86] Antibodies against other aminoacyl-tRNA synthetases, such as threonyl-tRNA synthetase (PL-7), alanyl-tRNA synthetase (PL-12), isoleucyl-tRNA synthetase (OJ), glycyl-tRNA synthetase (EJ), asparaginyl-tRNA synthetase (KS), tyrosyl-tRNA synthetase (Ha), Phenylalanyl-tRNA synthetase (Zo) are found less frequently (1% to 5%). Anti–Mi-2 antibodies are strongly associated with DM,[87,88] with prominent features such as Gottron's papules, heliotrope rash, the V sign, and the shawl sign. Anti-TIF-1 gamma and anti-NXP2 are associated with CADM in adults and with JDM.[89,90,91] Anti-MDA5 antibodies are associated with clinically amyopathic DM and rapidly progressive interstitial lung disease in Asian population, whereas the clinical phenotype was less specific in Caucasians.[92]

**TABLE 85-3** Myositis-Specific Antibodies

| Autoantibodies | Clinical Disease/Features |
|---|---|
| Anti-synthetase autoantibodies* | More common in polymyositis than dermatomyositis; interstitial lung disease, arthritis, Raynaud's phenomenon, fevers, mechanic's hands |
| Signal recognition particle (SRP)† | Polymyositis; possible severe disease and cardiac involvement |
| Chromodomain helicase DNA binding proteins 3 and 4 (Mi-2α and β)‡ | Dermatomyositis |
| Anti-MDA5/Anti-CADM-140 | Dermatomyositis; mucocutaneous lesions; severe lung disease minimal muscle involvement |
| Anti-TIF1γ | Dermatomyositis; malignancy |
| Anti-nuclear matrix protein (NXP)-2/anti-MJ | Predominantly juvenile DM; joint contractures; calcinosis |
| Anti-HMG-CoA reductase | Statin-associated myopathy; necrotizing myopathy |

*Common anti-synthetase antibodies found in myositis are targeted to histidyl-tRNA synthetase (Jo-1), threonyl-tRNA synthetase (PL-7), alanyl-tRNA synthetase (PL-12), isoleucyl-tRNA synthetase (OJ), glycyl-tRNA synthetase (EJ), and asparaginyl-tRNA synthetase (KS), tyrosyl (Ha) and phenylalanyl (Zo).

†Autoantibodies commonly bind to a 54 kDa SRP protein in the U.S. patient population and 72, 54, and 9 kDa proteins in the Japanese population.

‡Targets a 240 kDa helicase protein that is part of the nucleosome remodeling deacetylase complex.

HMG-CoA, 3-Hydroxy-3-methylglutaryl-coenzyme A; MDA, melanoma differentiation associated gene 5; TIF, transcriptional intermediary factor 1 gamma; SRP, signal recognition particle.

An individual usually has only one MSA because MSAs are often mutually exclusive. The MSAs are most common in patients with other autoimmune diseases and are infrequent or absent in IBM patients and those with malignancies, muscular dystrophies, or other myopathies. These antibodies are sometimes present before the onset of clinical disease.[93] Recent studies have found that IBM patients have antibodies targeting muscle proteins, such as cytosolic 50-nucleotidase 1A (cN1A), found in one-third of IBM patients.[94,95]Another antibody reactivity found in patients with IBM-targeted desmin.[96] Other recently described autoantibodies include anti-cortactin antibodies present in patients with PM, DM, and NAM, but not with IBM.[97]

MAAs such as PM-Scl are frequently associated with a characteristic overlap syndrome that includes features of scleroderma.[98,99] This syndrome is characterized by mild muscle disease, prominent arthritis, and limited skin involvement; it frequently responds to therapy.[100] Some myositis patients also have other MAAs, such as anti-snRNP, anti-Ro/SSA, anti-Ku, and anti-PMS1. Antibodies recognizing an uncharacterized 56 kDa large nuclear ribonucleoprotein have been found in a majority of myositis patients (86%), and the antibody titer appears to vary with disease activity, suggesting its importance for our understanding of disease pathogenesis and its potential usefulness as a clinical disease marker.[101] Some of the MSAs show strong immunogenetic associations; for example, antibodies against aminoacyl-tRNA synthetases are associated with HLA-DQA1*0501, anti-Jo-1 with HLA-DRB1*03, anti-SRP with DR5, anti–Mi-2 with DR7, and anti–PM-Scl with DR3 suggesting a role of the adaptive immune system in the pathogenesis of these myositis subgroups, as a major role of the class II HLA molecules is to present antigens to T cells. Likewise HLA-DRB1 alleles (DRB1*11:01) have been found to be associated with an elevated risk for anti–HMGCR-positive statin-induced myopathy.[102] These antibodies serve as excellent clinical markers and can help diagnose and categorize these heterogeneous disorders into homogeneous subgroups.

## Cell-Mediated Immune Response

At the cellular level, there are distinct differences in the distribution and location of the various lymphocyte subsets in the muscle tissues in different IIMs. Two major patterns of inflammatory cell infiltrates are seen in muscle tissue. The first is a predominantly perivascular distribution (Figure 85-1A), often in perimysial areas (Figure 85-1C), and is largely made up of $CD4^+$ T cells, macrophages, and dendritic cells. Occasionally, B cells are present in some patients. This pattern is seen mainly in patients with DM who have skin rash, but occasionally in patients without a rash. The second pattern has a predominantly endomysial distribution (Figure 85-1B), with mononuclear inflammatory cells often surrounding and sometimes invading non-necrotic muscle fibers. These inflammatory cellular infiltrates are composed primarily of $CD8^+$ T cells and macrophages, but $CD4^+$ T cells and dendritic cells are also present. This pattern is generally seen in patients without skin rashes and often in those classified with PM or IBM. In some patients, the two patterns of inflammation are seen in the same biopsy. The two distinct locations and the varying compositions of the inflammatory cell populations in the two areas suggest two different pathogenic mechanisms—one that targets the blood vessels and one that targets the muscle fibers. Notable inflammation is also seen in other organs.

The vascular involvement in patients with DM is also manifested in the skin and can be seen clinically in the form of nailfold changes and changes in the gastrointestinal (GI) tract. The capillaries show clear hyperplasia, vacuolization, and necrosis, contributing to an ischemia that could cause

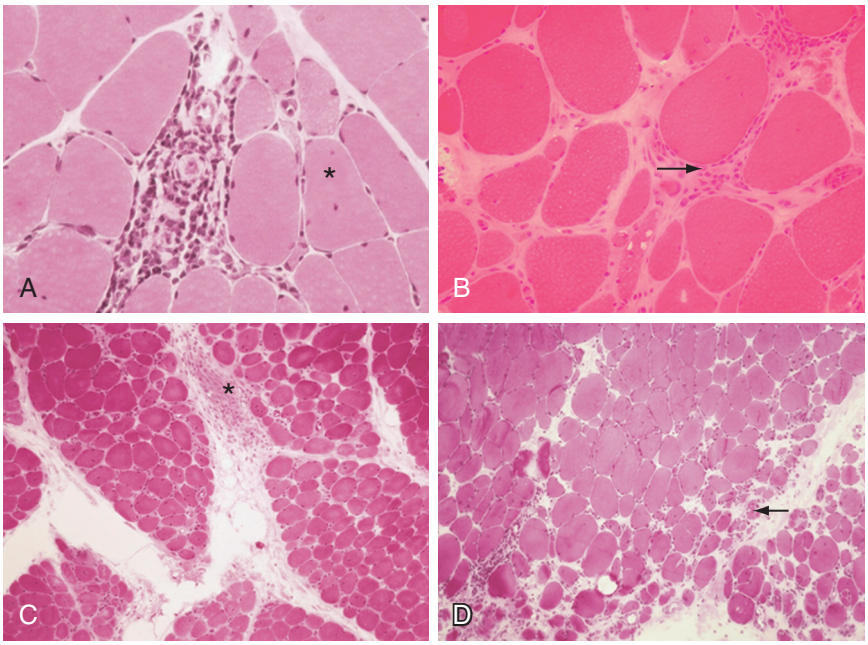



**Figure 85-1** Hematoxylin and eosin staining of a muscle biopsy showing perivascular inflammation. **A,** Variation in fiber size and central nucleation *(asterisk)*. **B,** Endomysial inflammation and increased fibrosis *(arrow)*. **C,** Perimysial inflammation *(asterisk)*. **D,** Perifascicular atrophy *(arrow)*. *(**B**, Courtesy Dr. Inger Nemmesmo. **D**, Courtesy Dr. Paul Plotz.)*

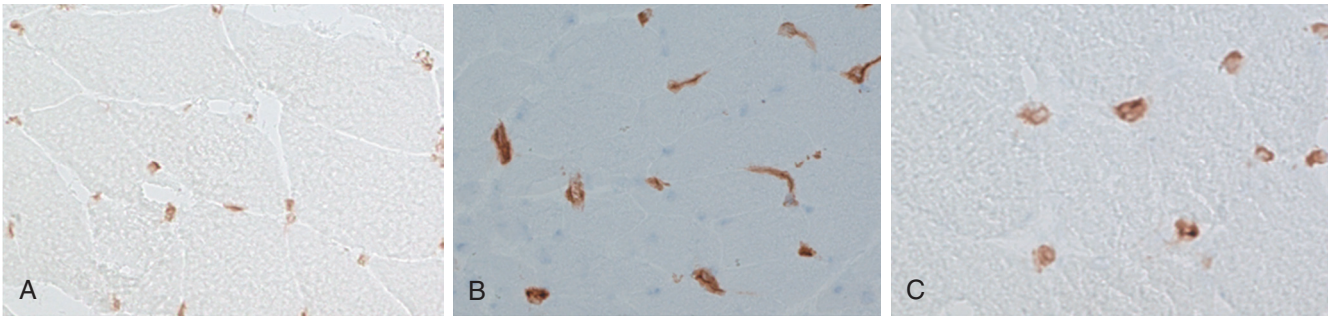



**Figure 85-2** Muscle biopsy staining with CD146 (Mel-CAM), an endothelial cell marker. Results are shown in normal **(A),** dermatomyositis **(B)**, and polymyositis **(C)** samples. Note the abnormal capillary size in both dermatomyositis and polymyositis.

fiber damage.[103,104] One of the earliest events in the pathogenesis of DM appears to be activation of the complement cascade. This activation leads to the subsequent deposition of complement components, which in turn results in the deposition of lytic membrane attack complexes in the endothelial cells and the eventual loss of capillaries from complement-mediated damage. The capillaries are abnormally thickened and enlarged and look like high endothelial venules, which are characteristics of vessels that facilitate lymphocyte trafficking (Figure 85-2). The capillaries also show signs of neovascularization.[105] This loss of capillaries results in some of the histopathologic features characteristic of this disease: capillary necrosis and loss, perivascular inflammation and ischemia (rarely seen), and perifascicular atrophy (a late feature; see Figure 85-1C and D). Recent studies also point to a role for type I interferon (IFN)-inducible genes in the pathogenesis of DM. Plasmacytoid dendritic cells produce type I IFN and induce expression of IFN-inducible proteins such as MxA and IFN-inducible gene 15 (ISG15) in the perifascicular myofibers and capillaries of DM biopsies, suggesting that injury to muscle fibers and capillaries occurs as a result of the intra-cellular overproduction of one or more type I IFN-inducible proteins in DM.[106,107] Although no direct comparison has been reported, the pathologic changes in juvenile and adult DM appear to be similar, except that all the basic pathologic features are more prominent in the childhood form (see later in this chapter). The factors that initiate complement activation in this disease are poorly understood. However, the consequences of complement-mediated damage are clearly visible in DM.[108] Recent studies have provided evidence that type I IFN-mediated innate immunity is linked to the expression of innate immune receptors such as Toll-like receptors (TLR) (e.g., TLR-3, TLR-7, and TLR-9.) Other studies have found that class I MHC-expressing immature muscle precursors can serve as a source of type I interferon, IFN-β, in myositis. In vitro experiments have indicated that TLR-3-induced IFN-β, in combination with IFN-γ, increases the expression of class I MHC molecules, suggesting a link between the innate and adaptive immune systems in myositis.[109]

The endomysial inflammatory aggregates contain a high percentage of T cells, particularly activated $CD8^+$ T cells, macrophages, and $CD4^+$ T cells, and few natural killer cells. Immunoelectron microscopic studies have provided evidence of the invasion, replacement, and probable destruction of non-necrotic muscle fibers by T cells and macrophages.[110] Infiltrating CTLs express perforin-containing granules, which are characteristically oriented toward the target muscle fiber, indicating that muscle fiber injury may be partially mediated by perforin-dependent cytotoxic mechanisms (Figure 85-3B and C).[111] In PM and IBM, there is evidence of clonal proliferation of $CD8^+$ T cells, both within the muscle and in the peripheral circulation.[112,113] T cell lines from patients demonstrate cytotoxicity against autologous myotubes,[114] suggesting that the muscle fiber injury in PM and IBM is mediated by CTLs. CTLs are known to mediate target cell damage by both the perforin–granzyme B and Fas-FasL pathways. The overexpression of anti-apoptotic molecules, such as Bcl-2, Fas-associated death domain–like IL-1 converting enzyme inhibitory protein (FLIP) and human inhibitor of apoptosis protein–like protein, in the skeletal muscle of myositis patients suggests that perforin–granzyme B–mediated cytolytic T lymphocyte cell (CTL) damage may play a predominant role in muscle fiber injury and dysfunction in myositis.[115-117]

In contrast to previous findings, recent studies have also shown accumulations of B cells, plasma cells, myeloid dendritic cells, late-activated macrophages expressing the 25F9 marker, as well as $CD8^+$ $CD28^-$ and $CD4^+$ $CD28^-$ T cells (TCR V[β]-expanded T cells) in the skeletal muscle and peripheral circulation of patients with PM, DM, and IBM. These $CD28^-$ cells and late-activated macrophages expressing 25F9 are thought to exhibit cytotoxic potential and produce pro-inflammatory cytokines in IIM skeletal muscle.[118,119] Another study has also explored the potential role of $FOXP3^+$ Treg cells in the pathology of myositis.[120]

On the basis of the data described, two different pathways have been proposed as major mediators of muscle damage and inflammation: one mediated through T lymphocytes (CTLs) directed against muscle fibers, predominating in PM and IBM, and the other directed against vessels, predominating in DM. However, the degree of inflammation is not consistently correlated with the severity of the structural changes in the muscle fibers or with the severity of the clinical disease,[121] suggesting that nonimmune processes also play a role in disease pathogenesis. A role for nonimmune processes is supported by the following observations. First, marked structural changes in the muscle fibers occur in the absence of any inflammatory cells.[122,123] Second, there is a lack of correlation between the degree of inflammation and the degree of muscle weakness.[124] Third,

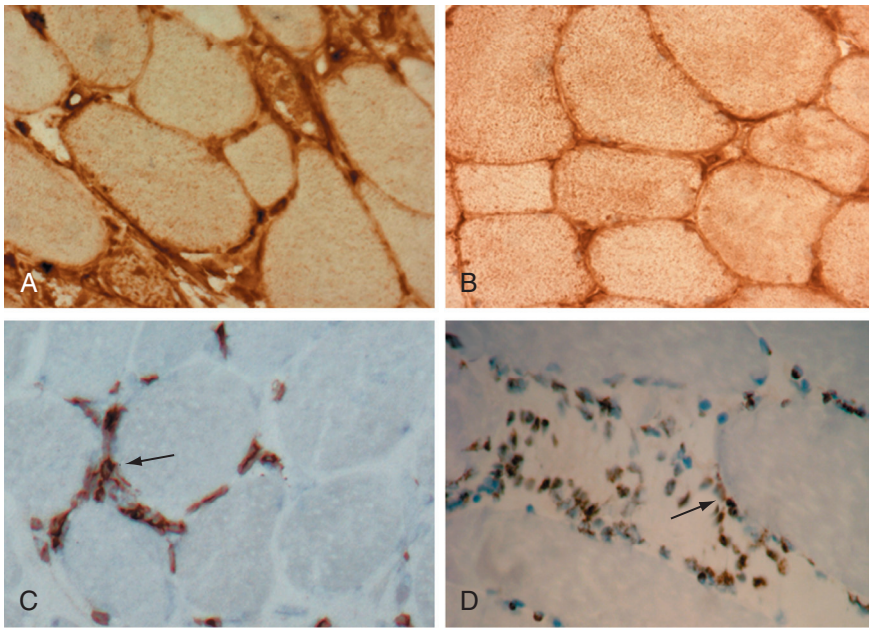



**Figure 85-3** Human leukocyte antigen (HLA)-ABC, $CD8^+$ T cell, and granzyme B staining of polymyositis biopsy. HLA expression is evident on muscle fibers, infiltrating cells, and endothelial cells **(A)**. HLA cell-surface and sarcoplasmic staining is shown on muscle fibers **(B)**, $CD8^+$ T cells (**C,** *arrow*), and granzyme B–positive cells **(D)** surrounding muscle fibers *(arrow)*.

some myositis patients do not respond even to powerful anti-inflammatory therapy.[125,126] Fourth, steroid treatment may eliminate inflammatory cells in myositis muscle tissue, but this removal alone may not substantially improve the clinical disease, suggesting that immunosuppressive therapies modulate disease activity but do not change other mediators of the disease process.[127] Finally, the clinical disease may progress when identifiable inflammation has subsided,[128] suggesting a role for nonimmune mechanisms in the pathogenesis of myositis. Thus the exact contribution of immune-mediated pathways to muscle damage is currently unknown.

## Class I Major Histocompatibility Complex Expression

Normal skeletal muscle cells do not constitutively express or display class I MHC molecules, although they can be induced to do so by pro-inflammatory cytokines such as IFN-γ or TNF[122,129-131] or by the alarmin HMGB1.[152] In contrast, in human IIMs, the early and widespread appearance of class I MHC in non-necrotic muscle cells is a striking feature, even in muscle cells distant from the lymphocytic infiltration.[122,123,132] Class I MHC staining is usually observed on the sarcolemma of muscle fibers, but some fibers also show staining in both the sarcolemma and the sarcoplasm (see Figure 85-3A and B). In some patients, the expression is restricted to a few clusters (often early in the disease), whereas in others, almost every fiber is positively stained, particularly in late-phase and treatment-resistant cases. Researchers have explored the biologic significance of these observations by generating a conditional transgenic mouse model overexpressing syngeneic mouse class I MHC. The overexpression of class I MHC molecules in the skeletal muscle of mice results in the development of clinical, biochemical, histologic, and immunologic features that resemble human myositis and provides a close model of the human disease. The disease in these mice is inflammatory, limited to skeletal muscle, self-sustaining, more severe in females, and often accompanied by MSAs.[133] Recent studies in this model further suggest that class I MHC overexpression leads to endoplasmic reticulum (ER) stress, muscle atrophy, and a decrease in the force generation capacity of skeletal muscle, suggesting a role for class I MHC muscle weakness in myositis.[134,135]

A number of observations in human myositis patients and in the mouse model of myositis suggest that class I MHC molecules mediate muscle fiber damage and dysfunction in the absence of lymphocytes. For instance, in human myositis, the induction of class I MHC antigen in muscle fibers occurs early, preceding inflammatory cell infiltration.[136,137] Class I MHC staining of human myositis biopsies shows both a cell surface and a sarcoplasmic reticulum pattern of internal reactivity, demonstrating that some of the class I MHC molecules may be retained in the ER of these fibers.[83,123,138] Persistent class I MHC overexpression in muscle fibers can exist in the absence of an inflammatory infiltrate.[128] The controlled induction of class I MHC in the mouse model is followed by muscle weakness before mononuclear cell infiltration.[133] Researchers have recently shown that in vivo gene transfer of class I MHC plasmids attenuates muscle regeneration and differentiation.[139] Together, these observations, and particularly the obvious retention of class I MHC within the cell in both human and murine disease, indicate that the muscle fiber damage seen in myositis may not be solely mediated by immune attack (e.g., CTLs, autoantibodies) but may also be mediated through nonimmunologic mechanisms such as the ER stress response and hypoxia. Activation of ER stress in cells is linked to the induction of different forms of cell death such as autophagy and apoptosis. The expression of molecules that facilitate autophagy (tumor necrosis factor-related apoptosis-inducing

ligand [TRAIL]) and apoptosis (TNF-like weak inducer of apoptosis [TWEAK]) were increased in myositis patients, providing a molecular basis for progressive muscle damage in myositis.[140,141]

In myositis, it appears that overexpression of class I MHC in myofibers initiates a series of cell-autonomous changes that contribute to myofiber pathology. Recent investigations have indicated that overexpression of class I MHC on muscle fibers results in the activation of the NF-κB and ER stress response pathways in human inflammatory myopathies and in the mouse model of myositis.[83,142] NF-κB can be activated within minutes by a variety of stimuli, including inflammatory cytokines such as TNF and IL-1, T cell activation signals, and stress inducers. It is likely that in human myositis, NF-κB activates both classic (pro-inflammatory cytokines) and nonclassic (ER stress response) pathways.[83,142-145] Furthermore, there is evidence that downstream target genes (e.g., class I MHC, intercellular adhesion molecule [ICAM], monocyte chemoattractant protein [MCP]-1) regulated by the NF-κB pathway are highly upregulated in myositis patients.[138,146,147] Recent studies have indicated that NF-κB p65 is activated both in human myositis biopsies and in the mouse model,[83,142,148,149] suggesting that this pathway may be directly involved in muscle fiber damage (Figure 85-4).

## Cytokines and Hypoxia

A number of other effector molecules produced in muscle tissue by inflammatory cells, endothelial cells, and muscle fibers are thought to play a role in the pathogenesis of myositis.[80] Most of the data assembled relate to cytokines, but some data related to chemokines are also available. The most consistently demonstrated cytokines in muscle tissue from patients with IIMs are cytokines with pro-inflammatory properties: IL-1α, IL-1β, TNF, and IFN-α. Recently, IL-10, IL-13, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), CCL3 (macrophage inflammatory protein [MIP-1α]), CCL4 (MIP-1β), CCL11 (eotaxin), IL-15, and IL-15Rα were also demonstrated to be significantly upregulated, and granulocyte colony-stimulating factor (G-CSF) to be downregulated in patients with IIMs when compared with unaffected individuals.[150,151] Also, DNA-binding high mobility group box 1 (HMGB1) was found to exhibit both extranuclear and extra-cellular patterns in the muscle tissue of patients with PM and DM. Stimulation with IFN-γ showed an increased HMGB1 expression in muscle nuclei and myoplasm. Exposure to HMGB1 induced a reversible upregulation of class I MHC in the muscle fibers and an irreversible decrease in $Ca^{2+}$ release from the sarcoplasmic reticulum during fatigue, suggesting a role for HMGB1 and class I MHC early in the pathogenesis of IIMs.[152] In addition to inducing the upregulation of class I and II MHC molecules on muscle fibers, cytokines can have a direct effect on muscle fiber function, as has been demonstrated for TNF.[153] The relative importance of the various cytokines and chemokines in patients with myositis is still uncertain, but these molecules are potential biomarker candidates in this disease, as has been indicated by a recent study showing that serum IL-6 production and the type I IFN gene signature in the peripheral blood are correlated with disease activity in patients with DM.[154] Recent studies provide evidence on how pro-inflammatory cytokines such as TNF decrease myogenic miRNAs (e.g., miR-1, miR-133a, miR-133b, and miR-206) and facilitate muscle degeneration in myositis.[155]

Microvessel involvement was first observed in DM but has also become evident in PM. The endothelial cells in both subsets show increased expression of adhesion molecules and pro-inflammatory cytokines such as IL-1α. This phenotype can be induced by tissue hypoxia, which may result from capillary loss and local tissue inflammation. Muscle tissue hypoxia can contribute to the clinical symptoms and muscle fatigue, and may be associated with disease mechanisms in inflammatory myopathies.[80] The expression of VEGF receptor in muscle fibers and HIF-2α reactivity in the endothelial cells of patients with PM and IBM support a role for hypoxia in these conditions. Patients with DM showed hypoxia-inducible factor (HIF)-1α and HIF-1β expression in endothelial cells, whereas expression of HIF-2α, erythropoietin receptor, VEGF, and VEGF-R were also observed on muscle fibers. These observations suggest that deprivation of blood supply by immune-mediated mechanisms could trigger the upregulation of hypoxia-related proteins as an adaptive response.[156] In

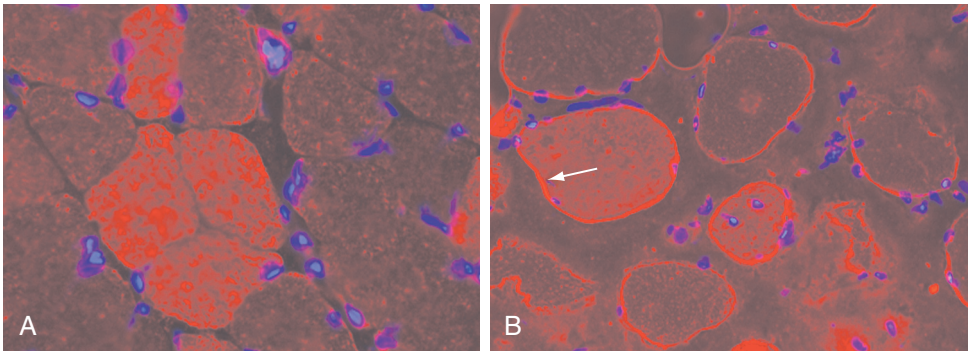



**Figure 85-4** Nuclear factor-κB (NF-κB) expression in biopsies of a normal individual and patient with myositis. Immunofluorescent staining with rabbit anti-NF-κB and anti–rabbit Texas red and counterstaining with 4, 6-diamino-2-phenylindole *(blue nuclei)*. Note the cytoplasmic expression of NF-κB in normal muscle **(A)** and a subsarcolemmal pattern in the myositis biopsy (**B,** *arrow*). *(From Nagaraju K, Casciola-Rosen L, Lundberg I, et al: Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and destruction.* Arthritis Rheum *52:1824–1835, 2005.)*

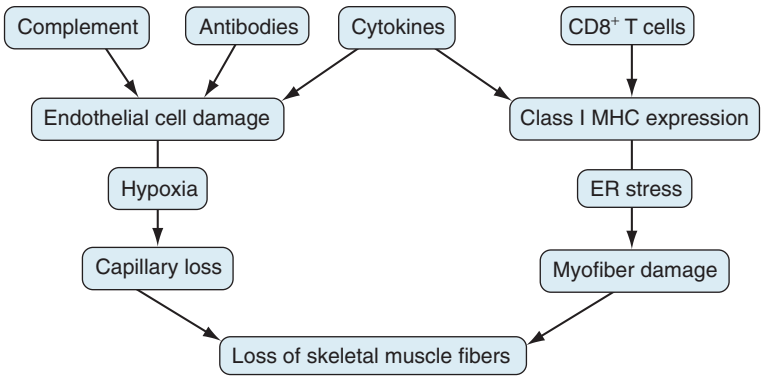



**Figure 85-5** Mechanisms of muscle fiber damage in myositis. ER, Endoplasmic reticulum; MHC, major histocompatibility complex.

addition, magnetic resonance spectroscopic analysis, before and after a workload, has demonstrated reduced levels of energy substrates that are important for muscle contraction, such as adenosine triphosphate and phosphocreatine, when compared with the levels in healthy individuals. This finding supports the hypothesis that an acquired metabolic disturbance occurs in chronic inflammatory myopathies, and that this disturbance can contribute to impaired muscle performance.

Microarrays have provided considerable new insight into DM. Early on, mRNA profiling in DM showed a predominance of type I IFN-responsive pathways, suggesting the possible persistence of an anti-viral response.[205] Particularly prevalent was the dramatic expression of the IFN-inducible MxA gene. This signature was confirmed and extended,[206] supporting an important role of the innate immune response, with prominent plasmacytoid dendritic cell infiltration in DM when compared with the other inflammatory myopathies.

### Proposed Mechanisms of Muscle Damage

Currently available data suggest that both immune (cell-mediated and humoral) and nonimmune (ER stress, hypoxia) mechanisms play a role in muscle fiber damage and dysfunction in myositis. Innate immune cell activation occurs as a result of injury to skeletal muscle, resulting in the release of damage-associated molecular patterns (DAMPs). TLR signaling activates various antigen-presenting cells (APC) in the muscle. These APCs then activate CD4 T cells and initiate autoantigen-specific T cell responses and autoantibody responses. Class I MHC overexpression on muscle also leads to the activation of CD8 T cell mediated cytotoxicity. DAMPs bind to surface or endogenous TLRs expressed in muscle-infiltrating immune cells, capillaries, and fibroblasts. TLR signaling activates NF-κB and inflammasomes, resulting in the production of pro-inflammatory cytokines, chemokines, and adhesion molecules. Cytokines such as TNF can directly induce the death of muscle cells and also effectively inhibit myogenesis through microRNAs. TLR signaling also leads to inflammasome activation (IL-1 secretion) and, potentially, to induction of pyroapoptosis in the affected muscle, whereas activation of TRAIL induces autophagy in the muscle. Unknown cytokines reduce the expression of rate-limiting enzymes of the purine nucleotide cycle and of AMPD1 in skeletal muscle. This acquired deficiency of APMD1 is likely to cause muscle weakness and fatigue in patients with myositis. In addition, class I MHC accumulation in the ER induces the ER stress response in muscle. Thus, several immune, nonimmune, and metabolic pathways directly and indirectly contribute to muscle weakness and damage in myositis. The relative contribution of each of these pathways to muscle fiber damage is presently unclear (Figure 85-5). Therefore, the use of specific drugs to inhibit these pathways, either alone or in combination, would help define their roles in myositis and potentially show them to be effective therapeutic agents.

## CLINICAL FEATURES

The inflammatory myopathies can occur as distinct disease entities, or they can coexist with some other rheumatic disease. This observation is true for all three subsets of myositis, but it is most often seen in PM and DM. The rheumatic diseases most often associated with inflammatory myopathies are systemic sclerosis, mixed connective tissue disease, Sjögren's syndrome, and SLE; however, rheumatoid arthritis may also be associated with inflammatory myopathies. IBM may be associated with Sjögren's syndrome, SLE, and other autoimmune diseases.[130,158] Because the clinical features of IBM differ somewhat from those of PM and DM, they are discussed separately, as are necrotizing myopathies.

### Polymyositis and Dermatomyositis

The predominant symptoms in patients with PM or DM are muscle weakness and low muscle endurance. The weakness is most pronounced in proximal muscle groups—typically in the neck, pelvic area, thigh, and shoulder muscles—with a symmetric distribution. Patients generally experience more problems with performing repetitive movements than with single-strength exercises, and they report difficulty walking uphill or upstairs, working with their arms above their shoulders, or rising from chairs. Also, distal muscles may be involved in PM and DM, affecting grip strength and health-related quality of life.[159] The onset of muscle weakness is often subacute, occurring during a few weeks, or it can be insidious, developing during several months. If

untreated, the muscle weakness slowly progresses, and in the most severe cases, the patients may become wheelchair-dependent. Problems with swallowing and nutrition can occur as a result of impaired contractility of the throat muscles, potentially leading to aspiration pneumonia. In rare cases, patients experience difficulty breathing because of weakness of the diaphragm or thoracic muscles, and they may require assisted ventilation. Other striated muscles may be involved, such as in the lower part of the esophagus (causing reflux problems) or the sphincter ani (causing incontinence).

### Skin

DM is characterized by the presence of certain types of rashes[160]; the same types are often seen in both children and adults. The most specific skin manifestations are Gottron's papules and the heliotrope rash (Figure 85-6). Gottron's papules are slightly elevated, violaceous, pink, or dusky red papules located over the dorsal side of the metacarpal or interphalangeal joints. These papules may also occur over the extensor side of the wrist, elbow, or knee joints. Gottron's papules are considered to be pathognomonic of DM. A macular rash (without papules) with the same distribution as Gottron's papules is called *Gottron's sign* (see Figure 85-6C and D). The heliotrope rash is a periorbital red or violaceous erythema of one or both eyelids, often with edema (see Figure 85-6B). Linear erythema overlying the extensor surfaces of joints is also relatively specific to DM (Figure 85-7A). Many patients with DM have photosensitive rashes, typically found on the face or scalp or over the neck (the so-called *V sign*), although this rash is not specific to DM (see Figure 85-7B and C). Another common rash in DM is located over the shoulders (shawl sign; Figure 85-7D) or over the hips (holster sign). Pruritus is common. Patients with DM often have skin lesions on their fingers, such as periungual erythema, nailfold telangiectasias, and cuticular overgrowth (Figure 85-8C), and this development can even lead to digital ulceration. Other less common skin manifestations are panniculitis, livedo reticularis, and nonscarring alopecia. Vasculitis may be seen in children with DM, but only rarely in adults.

In general, the skin rash is moderate, with local erythema. In rare cases, a severe, diffuse erythema (erythroderma) can occur, occasionally with vesiculobullous lesions or ulcers. The skin rash can precede the muscle symptoms by months or even years, and in some patients, the skin manifestations may be the only clinical sign of DM. This condition is often called *clinically amyopathic DM* or *DM sine myositis* (see following text). The pattern of the rash over the knuckles and dorsum of the hand is distinct in SLE and DM. The rash in DM generally spares the phalanges but affects the knuckles, whereas in SLE the rash is located over the phalanges but the knuckles are spared (see Figure 85-8A and B). However, no histopathologic skin features are specific for DM; most of the features are also seen in patients with SLE. Thus skin biopsy is rarely helpful in distinguishing between these two disorders. The cutaneous manifestations may fail to respond to immunosuppressive treatment, despite improvement in muscle symptoms. Thus it is possible that different molecular pathways or disease mechanisms cause the skin rash and the muscle inflammation.

Calcinosis, which can be severe, is found mainly in juvenile DM but is occasionally seen in adults. The calcinosis occurs predominantly in sites that have been subject to friction or trauma, such as the elbows or knees. Sometimes the calcinosis can be extensive and erupt, leading to ulcers. It is most often localized to the subcutaneous tissue but can also develop in the skin, fascia, or muscle and can be visualized by radiography, CT, or MRI. The calcinosis seems to progress as long as there is active inflammatory disease.

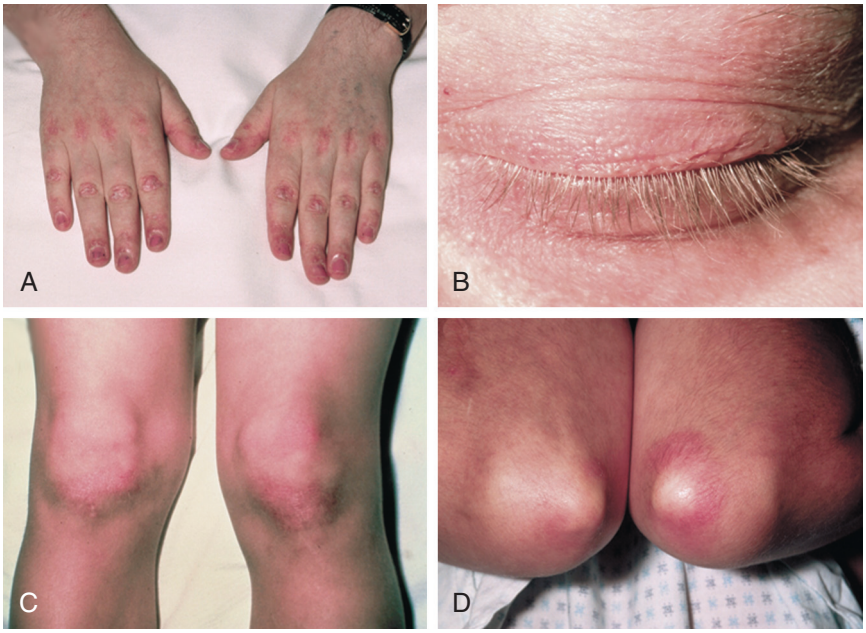



**Figure 85-6** Characteristic features of dermatomyositis skin changes. **A,** Gottron's papules. **B,** Heliotrope rash. **C,** Gottron's sign on knee and **D,** elbow. *(Courtesy Dr. Paul Plotz.)*




**Figure 85-7** Characteristic features of dermatomyositis skin changes. **A,** Linear erythema. **B,** Scalp rash. **C,** V-like sign. **D,** Shawl sign. *(Courtesy Dr. Paul Plotz.)*




**Figure 85-8** Erythematous rashes on the hand in dermatomyositis and systemic lupus erythematosus. **A,** Note the changes on the knuckles and dorsum of the hand in dermatomyositis (Gottron's sign). **B,** Rash is absent from the knuckles but present on the phalanges in lupus. **C,** Capillary nailfold changes in dermatomyositis. *(Courtesy Dr. Paul Plotz.)*

Also, once it has developed, it is often treatment resistant. Some data, however, suggest that the progress of calcinosis can be inhibited by effectively treating the inflammatory process in the skin and muscle.[161]

Another type of skin pathology seen in inflammatory myopathies is called *mechanic's hands*. This rash is often associated with the presence of anti-synthetase autoantibodies and can be seen in both PM and DM. The rash is a hyperkeratotic, scaling, fissuring of the fingers, particularly on the radial side of the index fingers (Figure 85-9).

## Lungs

Lung involvement is frequent in PM and DM and is a major risk factor for morbidity and mortality. Clinical symptoms such as dyspnea and cough are common. Lung involvement can be caused by weakness of the respiratory muscles or inflammation of the lung tissue (ILD). Weakness of the respiratory muscles may lead to restrictive lung disease, and involvement of the pharyngeal muscles is a risk factor for aspiration pneumonia. ILD, caused by inflammation in the small airways, is common in PM and DM and is often associated with anti-synthetase autoantibodies. It may be present in as many as 70% of patients when investigated with sensitive techniques such as high-resolution CT and measurement of pulmonary function and diffusion capacity.[162] In most cases, the changes are present at the time of diagnosis of myositis; they rarely develop after immunosuppressive treatment has started. In most cases, the ILD is mild and has a slowly progressive course. Anti-synthetase and anti-MDA5 antibodies are strongly associated with ILD, and thus serve as biomarkers for ILD. In some cases, improvement in lung function is seen with immunosuppressive treatment. The course and outcome vary depending on the histopathology, suggesting that different disease mechanisms cause ILD.

In general, the clinical course and histopathology of ILD in myositis are no different from those in idiopathic ILD. The most common histopathologic finding is nonspecific interstitial pneumonia, but other entities such

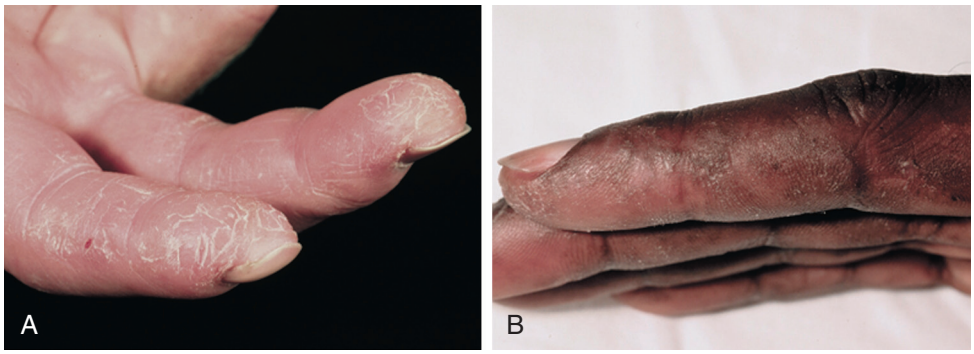



**Figure 85-9** Mechanic's hands in a white **(A)** and a black **(B)** patient. Note the characteristic skin changes on the lateral side of the fingers. *(Courtesy Dr. Paul Plotz.)*

as cryptogenic organizing pneumonia, bronchiolitis obliterans organizing pneumonia, diffuse alveolar damage, and usual interstitial pneumonia are also found. Some studies suggest that bronchiolitis obliterans organizing pneumonia responds favorably to corticosteroids, whereas histopathologic changes compatible with diffuse alveolar damage, usual interstitial pneumonia, or acute interstitial pneumonia respond poorly to corticosteroids or other immunosuppressive therapies and have a poor prognosis.

### Arthritis

Joint pain and arthritis are common in patients with PM or DM. The most common form of arthritis is a symmetric arthritis of the small joints of the hands and feet. This arthritis is typically nonerosive but can sometimes be erosive and destructive. Most frequently, arthritis is seen in patients with anti–Jo-1 antibodies and other anti-synthetase autoantibodies, but it is also seen in patients with overlapping syndromes of other rheumatic diseases.

### Heart

Cardiovascular disease is a risk factor for death among patients with PM and DM. However, clinically evident heart involvement is rare, perhaps indicating that cardiac involvement may be overlooked in these conditions. Subclinical manifestations are frequently discovered when patients with PM or DM are evaluated. The most frequently reported subclinical manifestations are conduction abnormalities and arrhythmias detected by electrocardiogram (ECG), and subclinical cardiomyopathies are common when cardiac MRI is performed, even in patients thought to be in remission.[163] The underlying pathophysiologic mechanisms that can lead to cardiac manifestations in patients with PM or DM are myocarditis and coronary artery disease, as well as involvement of the small vessels of the myocardium.

Examination with ECG is recommended in patients newly diagnosed with PM or DM. Serum tests such as CK-myocardial band (CK-MB) to detect cardiac involvement are unreliable in patients with inflammatory myopathies because CK-MB can be released from regenerating skeletal muscle fibers, a common feature in biopsies from patients with PM or DM. The CK-MB/total CK ratio may be greater than 3%, a threshold value that is used to define myocardial damage. A more specific marker for myocardial damage in myositis patients is increased serum levels of cardiac isoform troponin I. The other cardiac troponin isoforms, troponin C and troponin T, are less specific and are also expressed in adult skeletal muscle; increased serum levels have been reported in various muscle disorders.

### Gastrointestinal Tract

Difficulty swallowing is frequent in patients with inflammatory myopathies, particularly those with IBM. Muscle weakness occasionally becomes severe and causes problems with nutrition and aspiration pneumonia. The pathophysiology is related to weakness in the tongue, pharyngeal muscles, and sometimes lower esophagus. Reflux that requires special care is common, occurring in 15% to 50% of patients. Constipation, diarrhea, and stomach pain are common symptoms and may result from disturbed motility of the gut or GI tract inflammation. Vasculitis in the blood vessels of the GI tract is rare but may be complicated by intestinal bleeding.

### Anti-synthetase Syndrome

MSAs are strongly associated with clinical phenotypes and may thus be helpful to identify clinical subsets of myositis. The most common of the MSAs are the anti-synthetase autoantibodies directed against aminoacyl-tRNA synthetases (anti-Jo-1, anti-EJ, anti-PL-7, anti-PL-12, anti-KS, anti-OJ, anti-Ha, and anti-Zo).[164] A clinically distinct subset of myositis, often called anti-synthetase syndrome, has been identified in patients with anti-synthetase autoantibodies.[39,67] The most common of the anti-synthetase autoantibodies is anti–Jo-1, which is directed against histidyl-tRNA synthetase. This autoantibody is present in approximately 20% of patients with PM or DM, but is only rarely found in patients with IBM.[84] Anti-synthetase syndrome is characterized by the presence of anti-synthetase autoantibodies and a set of clinical features that includes myositis, ILD, Raynaud's phenomenon, nonerosive symmetric polyarthritis of the small joints, and mechanic's hands (see Figure 85-9). Notably, some of the anti-synthetase autoantibodies are mainly associated with ILD (anti-PL12), and these patients with ILD may be missed as having an anti-synthetase syndrome in the absence of muscle symptoms.

### Amyopathic Dermatomyositis

A subset of DM is clinically referred to as *amyopathic DM*. These patients have a skin rash, which is typical of DM, but no clinical signs of muscle involvement.[165] The proposed definition is based on a skin biopsy consistent with DM and a duration of 6 months or longer in the absence of clinical or laboratory evidence of myositis. Some of these patients do have subclinical myositis, based on MRI or biopsy findings at presentation; others manifest clinically overt myositis sometime later. Patients without clinically overt myositis, however, can develop extramuscular manifestations such as ILD, which can be severe. Amyopathic DM may be associated with malignancies, as is the case for classic DM. The frequency of this subset is uncertain, but some recent studies suggest that this form of DM may be more common than previously thought.

### Juvenile Dermatomyositis

The incidence of juvenile dermatomyositis (JDM) is between 1.7 and 3 per million children and is the most common IIM in children; PM and overlap myositis are much less common and have a poorer prognosis. Malignancy-associated myositis is very rare in children, accounting for less than 1% of all IIM.[166] Because JDM accounts for 85% of all cases of juvenile IIM, we will focus on it here. The disease onset has two peaks—age 6 and 11 years. JDM is more common in girls than in boys in Europe and North America; in Japan and Saudi Arabia, this difference is less prominent. The diagnosis is based on clinical and laboratory parameters, because most children do not undergo electromyelograms or muscle biopsies. The discovery of more myositis-specific antibodies means that they can currently be found in more than 70% of all children with JDM. Anti-TIF-1γ and anti-MJ antibodies are the most prevalent in JDM compared with adult DM.[167,168] The most common clinical manifestations at disease onset are muscle weakness, easy fatigability, skin rash, malaise, and, in some cases, fever.[161] The CK levels tend to be mildly elevated. The skin rash is often pathognomonic and similar to that in adult DM: the most typical skin manifestations are heliotrope discoloration of the upper eyelids, Gottron's papules, periungual erythema, and capillary loop abnormalities (dilated and tortuous with decreased capillary density). Calcinosis, cutaneous ulceration, and lipodystrophy are more common in juvenile cases than in adults. Calcinosis is seen in 30% to 70% of children with JDM, and the presence of anti-NXP2 autoantibodies increases the risk of calcinosis and is associated with more aggressive disease.[169] In addition, a delay in diagnosis, cardiac involvement, and a prolonged disease course increase the risk of calcinosis.[170] The calcinosis is most often located at sites exposed to trauma and can be seen in the skin, fascia, or muscles. In some children, the calcinosis becomes prominent and causes contractures and ulcerations. Vasculopathy that affects the GI tract, with ulceration, perforation, or hemorrhage, is rare but seems to be more common in children than in adults with DM. Because this vasculopathy can be a serious sign, screening for GI involvement should be included in the evaluation of patients with JDM. ILD is rarely seen in JDM.

Recently validated core set measures have allowed the Childhood Arthritis and Rheumatology Research Alliance (CARRA) to develop consensus guidelines for the treatment of JDM.[171,171] Daily prednisone starting at 2 mg/kg/d is considered first-line therapy, with intravenous (IV) methylprednisolone, methotrexate, and IVIG used as adjunct therapy for first-line treatment of severe JDM. Mycophenolate mofetil, tacrolimus, and rituximab are used for refractory disease, and IV Cytoxan is used for refractory life-threatening disease. The overall prognosis is variable. The mortality rate is estimated at 2% to 3%, with 24% to 40% of patients going into remission within 2 years (allowing cessation of immunosuppressive therapy). However, the majority of patients have a chronic course.[167,172-174] Side effects of immunosuppressive treatment such as growth failure are common.

## Inclusion Body Myositis

IBM is distinguished from PM and DM on the basis of both clinical and histopathologic features.[175,176] Sporadic IBM is a distinct entity from familial hereditary inclusion body myopathy, which shares some of the same clinical and histopathologic features but lacks signs of inflammation in muscle tissue. IBM was identified in the 1960s as a subset of inflammatory myopathies, distinct from PM, primarily on the basis of typical histopathologic features that include sarcoplasmic and nuclear inclusions and rimmed vacuoles.[16,17] A characteristic clinical phenotype was later identified, characterized by an insidious onset of muscle weakness (localized predominantly to the thigh muscles and finger flexors) during months to years and resistance to glucocorticoid treatment. IBM patients often have a history of frequent falling. Sporadic IBM cases are sometimes misdiagnosed as PM because the classic histopathologic changes (rimmed vacuoles and inclusions) may not be evident in early biopsies (Figure 85-10).

In contrast to PM and DM, IBM is more frequent in men than in women, and it is seen mostly in individuals older than 50 years. The onset is more insidious than that of PM or DM. Patients with IBM rarely have pain. The most frequent initial symptoms are difficulty climbing stairs and walking uphill and frequent falls as a result of weakness in the knee extensor muscles. Muscle weakness may become prominent, and even walking across a threshold may become a problem. Difficulty swallowing may also be an early clinical feature, reflecting the involvement of the pharyngeal muscles. The course is slowly progressive, leading to muscle atrophy that can be striking, particularly in the thigh and forearm muscles. Severe weakness may develop, and many patients become wheelchair-dependent. Extramuscular organ involvement is rare, although a subgroup of patients with IBM has sicca symptoms and may develop a secondary Sjögren's syndrome.[177] IBM is usually resistant to treatment with glucocorticoids and other immunosuppressive agents. Because of this resistance to treatment, some have questioned whether IBM is an autoimmune disease or a degenerative muscle disease supported by the abnormal accumulation of proteins such as amyloid-β in muscle fibers. This issue is still under debate and is the subject of ongoing research in several institutions around the world.

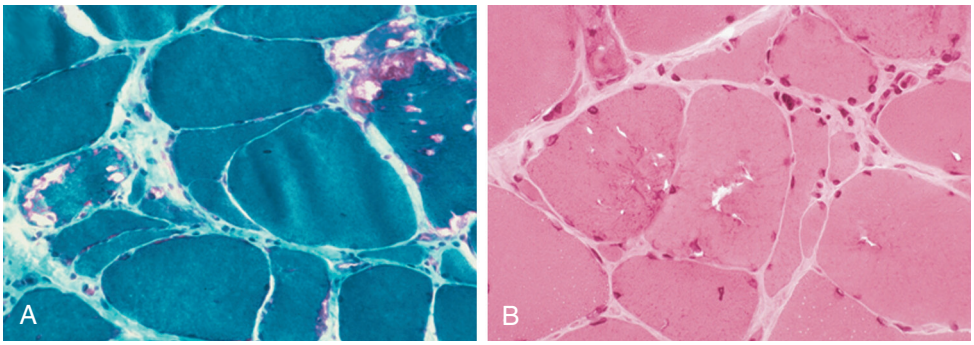



**Figure 85-10** Trichrome and hematoxylin and eosin staining of inclusion body myositis biopsy. Note the *red-rimmed* inclusions **(A)** and marked variation in muscle fiber size (**A** and **B**). *(Courtesy Dr. Paul Plotz.)*

### Necrotizing Myopathies

In the past decade, another subset of immune-mediated myositis has been identified and is defined by presence of autoantibodies; anti-SRP antibodies or anti-HMG-Co A reductase antibodies and by predominating muscle fiber necrosis without the classic lymphocytic infiltration in muscle biopsies seen in other IIM.[178,179] Patients who have antibodies to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) can be further divided into those who have a history of statin exposure and those who are statin-naïve (non–statin-induced necrotizing myositis). Presence of autoantibodies together with an upregulation of class I MHC in the muscle fibers and by deposition of complements on capillaries are suggestive of an autoimmune etiology of a necrotizing myopathy, but they can also be seen in anti-synthetase syndrome or DM.[180,181] A necrotizing muscle biopsy can also be seen in other nonautoimmune etiologies, including hypothyroidism, heritable myopathies, and toxic myopathies or cancer-associated myopathies.[182]

### Statin Myopathy

Statins (e.g., lovastatin, simvastatin) are lipid-lowering drugs that are known to cause necrotizing myopathy. Most cases of statin myopathy resolve with cessation of therapy. However, statins have also been linked to a more severe autoimmune myopathy that is associated with necrotizing myopathy on biopsy, severe progressive proximal muscle weakness with an elevation in CK, and autoantibodies to HMGCR. This antibody, however, is not found in most patients who are exposed to statins and have no autoimmune myopathy, even those with mild myalgias.[183] This form of statin myopathy requires immunosuppressive therapy. Other agents that are known to cause necrotizing myopathy include fibric acid derivatives (clofibrate, gemfibrozil), nicotinic acid, organophosphate poisoning, and ε-aminocaproic acid.

### Non–Statin-Induced Necrotizing Myopathy

Non–statin-induced necrotizing myopathy occurs in a subset of patients who are statin-naïve, develop a necrotizing myopathy, and have HMGCR antibodies. These patients tend to be younger, have high CK levels, and respond less well to immunosuppressive therapy.[184]

## Myositis Associated with Malignancies

An association between DM and malignancies was observed in several early case reports. The clinical implications of this association, irrespective of the pathophysiologic mechanisms involved, are that it is imperative to screen for tumors in patients with DM at the time of diagnosis and at relapse, particularly if the symptoms do not respond to conventional immunosuppressive treatment. Two myositis-specific autoantibodies are associated with DM and malignancies in adults: anti-TIF1gamma and anti-NXP2.[185,186] The types of malignancies vary and include not only hematologic malignancies such as lymphoma but also solid tumors such as lung, ovarian, breast, and colon cancer. For women, mammography and a gynecologic examination should be conducted as well. If any abnormalities are found, physicians should guide a more thorough investigation for malignancies. Ethnic backgrounds should be considered in screening, because cancer types vary according to race, with lung and nasopharyngeal cancers being more frequent in Asians than Caucasians.[187]

## CLASSIFICATION AND DIAGNOSTIC CRITERIA

At present, there are no prospectively validated diagnostic or classification criteria for myositis. Dividing diseases into homogeneous subsets serves several important functions, including allowing us to estimate disease incidence and prevalence, understand disease pathogenesis and natural history, and evaluate the patient's response to therapy and prognosis. More than three decades ago, Bohan and Peter[14,15] proposed a set of five criteria to facilitate the diagnosis of IIM patients (Table 85-4). They classified IIMs into five groups: primary idiopathic PM, primary idiopathic DM, IIMs associated with malignancy, childhood IIMs associated with vasculitis, and IIMs associated with collagen vascular diseases. The exclusion criteria were signs of central or peripheral neurologic disease, a family history of muscle disease (although familial myositis has been reported in dozens of cases), and symptoms and signs suggestive of muscular dystrophy, granulomatous myositis, infections (including trichinosis, schistosomiasis, trypanosomiasis, staphylococcal infection, and toxoplasmosis), drug-induced myopathy, toxic myopathy, rhabdomyolysis, metabolic disorders, endocrinopathies, myasthenia gravis, and myositis after viral infection (influenza or rubella). A weakness of

the Bohan and Peter classification is that it overdiagnoses patients with PM and loosely defines overlap syndromes.

Despite their several drawbacks, these criteria have served well in diagnosing and defining patients for research purposes for the past 3 decades. IBM was later recognized as a separate disease entity characterized by a slow onset and progression involving finger flexors or the quadriceps muscles that may be asymmetric.[175,176] It can occur as a stand-alone entity or with other connective tissue diseases, and patients are often resistant to steroid therapy. Other focal and diffuse forms of myositis, such as orbital myositis, focal nodular myositis, macrophagic myositis, and eosinophilic myositis, are relatively rare.

**TABLE 85-4** Bohan and Peter Criteria for Polymyositis and Dermatomyositis

| |
|---|
| Exclude all other myopathies |
| Symmetric proximal muscle weakness |
| Increase in serum muscle enzymes, such as CK, AST, ALT, aldolase, and LDH |
| Abnormal electromyographic findings, such as short, small, polyphasic motor units; fibrillations; positive sharp waves; insertional irritability; and bizarre high-frequency repetitive discharges |
| Abnormal muscle biopsy findings, such as mononuclear infiltration, regeneration, degeneration, and necrosis |
| Skin rashes, such as heliotrope rash, Gottron's sign, and Gottron's papules |

ALT, Alanine transaminase; AST, aspartate transaminase; CK, creatine kinase; LDH, lactate dehydrogenase.

Since Bohan and Peter proposed their classification criteria, advances in clinical research have led to the identification of certain autoantibodies that are strikingly associated with some clinical phenotypes of myositis (see Table 85-3). The identification of clinical features associated with MSAs and MAAs has led to the proposal of a serologic approach to complement the Bohan and Peter classification system. Others have suggested that the Bohan and Peter criteria be modified to add MSA as a criterion.[188] However, the inclusion of MSA has some limitations. These antibodies are not present in all patients, the immunoprecipitation techniques that are the "gold standard" for identifying these antibodies are available in only a few commercial laboratories, and the enzyme-linked immunosorbent assays often used can give false-positive or false-negative results.

Ongoing debate and dialogue within the scientific community about the nature of the diagnostic and classification criteria that could better define these disorders is extensive.[78,189,190] Some schemes emphasize a focus on histopathologic features, and others on autoantibody profiles. Certainly, the addition of autoantibody profiles, characteristic histopathologic and immunohistochemical features, and imaging techniques such as MRI would significantly strengthen the current criteria and better define these disorders (Table 85-5).

**TABLE 85-5** Clinical and Laboratory Features of Idiopathic Inflammatory Myopathy Subgroups

| Diagnostic Features | Dermatomyositis | Polymyositis | Inclusion Body Myositis | Necrotizing Myopathy |
|---|---|---|---|---|
| Clinical features | | | | |
| Age | Children and adults | Adults* | Adults >50 yr | Adults |
| Disease onset | Subacute | Subacute | Chronic | Subacute |
| Muscle weakness | Proximal | Proximal | Selective pattern† | Proximal |
| Symmetry | Symmetric | Symmetric | Asymmetric | Symmetric |
| Systemic features | Yes‡ | Yes‡ | Yes§ | Yes§ |
| Skin changes | Yes‖ | No | No | No |
| Calcinosis | Yes¶ | Rarely | No | No |
| Associated systemic autoimmune disease | Yes** | Yes** | Yes†† | No |
| Associated malignancy‡‡ | Yes | ? | ? | ?? |
| Laboratory features | | | | |
| Serum enzymes§§ | Normal to high | Normal to high | Normal to high | High |
| Abnormal EMG‖‖ | Yes | Yes | Yes | Yes |
| Abnormal muscle biopsy | Perifascicular atrophy, capillary depletion, patchy class I MHC expression and microinfarcts | $CD8^+$ T cell invasion of non-necrotic fibers and class I MHC expression on fibers | $CD8^+$ T cell invasion, MHC expression, vacuolated fibers, and tubulofilamentous inclusions in fibers | Necrotic and regenerating fibers, upregulation of class I MHC |

*Rarely in children.
†Early involvement of finger flexor, wrist flexor or wrist extensor weakness, and involvement of quadriceps femoris.
‡Some patients have dysphagia, synovitis, and interstitial lung disease.
§Some patients have dysphagia.
‖Gottron's sign and heliotrope rash.
¶Especially in children.
**Overlap with scleroderma, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and mixed connective tissue disease.
††Associated with Sjögren's syndrome but less frequently associated with other connective tissue diseases.
‡‡Dermatomyositis is more frequently associated with cancer than are polymyositis and inclusion body myositis and not overrepresented in polymyositis or inclusion body myositis.
§§Serum creatine kinase, aspartate transaminase, lactate dehydrogenase, and aldolase vary from normal to very high levels.
‖‖Myopathic motor unit potentials with spontaneous discharges in dermatomyositis, with and without spontaneous discharges in polymyositis, and mixed pattern of short- and long-duration motor unit potentials in inclusion body myositis.
EMG, Electromyogram; MHC, major histocompatibility complex; ?, association between PM/IBM and malignance is not clear; ??, necrotizing myopathy (other than autoimmune necrotizing myopathy) can be associated with cancer.

Recently, the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) supported a multidisciplinary collaboration, the International Myositis Classification Criteria Project (IMCCP). Retrospective case-control clinical and laboratory data were collected from more than 47 clinics worldwide and were both internally and externally validated. Two models were used: one used clinical evaluation of muscle and skin and laboratory evaluations, and the second model used these variables as well as muscle biopsy results. These models resulted in a high sensitivity and specificity and performed better than the existing Bohan and Peter classification. Moreover, they also performed well in classifying the more recent recognized subgroups, clinical amyopathic DM and the immune-mediated necrotizing myopathy.[192]

## PHYSICAL FINDINGS

Although most patients are first seen with muscle weakness or fatigue, the IIMs are systemic connective tissue diseases, and other organs are frequently involved; therefore, the physician should conduct a full clinical examination when patients are seen with muscle symptoms. This examination could also be helpful in distinguishing IIMs from noninflammatory myopathies.

The muscle problems reported by many patients consist of not only muscle weakness but also muscle fatigue or reduced muscle function. Thus, the evaluation must differentiate between strength and fatigue by evaluating muscle strength and testing repetitive movements for muscle fatigue. In the early phases, atrophy is usually not a pronounced phenomenon in PM or DM. In later phases, a moderate symmetric atrophy of proximal muscles may be present. Asymmetric atrophies indicate conditions other than inflammatory myopathies. More severe atrophy of the quadriceps muscles and the flexor muscles of the forearms, which may be asymmetric, often develops in patients with IBM, and deformities in the finger joints and difficulty making fists may also develop.

Muscle strength can be tested in various ways. The physician can quickly screen for weakness in proximal lower leg muscles by asking the patient to stand up from a sitting or squatting position without support. A more standardized test that is easy to perform in the clinic is the manual muscle test, with grading according to the Medical Research Council (MRC) scale. There are many variants of this test, but a short form, addressing eight muscles on the dominant side, is recommended as part of the disease activity score by the International Myositis Assessment and Clinical Studies (IMACS).[193] In many patients with PM or DM, muscle strength as assessed by the manual muscle test is good in most of the tested muscle groups. Typically, moderate muscle weakness is seen in the neck flexors and hip girdle muscles. Testing that involves a number of repetitions is often a more sensitive method of detecting muscle impairment. The Functional Index in Myositis-2 is a myositis-specific outcome measure that assesses a number of repetitions. With this test, proximal muscle groups are more involved than are distal muscles. This index is often used by physical therapists.[194] In patients with IBM, knee extensors and finger flexors are often weak.

The physician should also examine the skin to detect changes, including those in nailfolds and the scalp. Joints can be affected by arthritis, and heart and lung changes should be carefully looked for.

## LABORATORY FINDINGS

Laboratory evaluations are critical components of both diagnosis and patient management. Combinations of laboratory tests are generally used during patient evaluations. Because no laboratory test is highly specific for IIMs, the results of these tests are usually interpreted in the clinical context.

### Biochemical Tests

Measuring serum levels of muscle enzymes is an important part of the evaluation of patients with myositis. Elevated levels of muscle-derived serum enzymes reflect ongoing damage to the muscle parenchyma. These measurements help differentiate IIMs from conditions such as steroid myopathy and denervation, in which atrophy is a prominent feature.[195] Measurement of the serum CK level is traditionally the first step in the assessment of patients with IIM. CK exists as MM (skeletal muscle), MB (cardiac muscle), and BB (brain) isoforms in serum. In comparison with other serum muscle enzymes, CK appears to be a relatively specific and sensitive indicator of the degree of muscle fiber injury. However, the range varies significantly among patients, with levels being normal in some patients and elevated by several hundred-fold in others.

Generally, 80% to 90% of adult myositis patients show an increase in CK during the initial evaluation. However, a certain proportion of patients, especially those in advanced stages of the disease, show normal or relatively modest elevations in CK, in part because of a lack of muscle mass or the presence of inhibitors of CK activity.[196,197] Normal CK is relatively more common in DM than in PM. In the absence of CK elevation, it is usually easier to diagnose DM than PM because of the presence of skin rashes in the former. It is also known that CK levels are generally lower in patients with IBM than in those with PM or DM. Therefore, a normal CK level does not exclude a diagnosis of IIM, particularly IBM or DM.

Constantly elevated levels of CK are often a sign of inflammatory activity. A rise in CK level is generally correlated with overall disease activity during time, but not with strength or functional measures of disease activity.[198,199] CK measurements are usually not useful for monitoring disease exacerbations, and they should always be evaluated in the clinical context. CK levels may normalize without clinical improvement, or they may increase without clinical worsening; however, increasing levels point to a potential flare and warrant closer clinical evaluation. CK elevations are not specific for myositis because this enzyme is also elevated in other muscle diseases, including muscular dystrophies, rhabdomyolysis, hypothyroidism, and many drug-induced myopathies. The cardiac isoform troponin I has the highest specificity as an indicator of myocardial involvement and is the most reliable serum marker for detecting myocardial damage in patients with inflammatory muscle disease.[200]

Measurement of other serum muscle enzymes, including aldolase, aspartate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH), significantly improves the chance of diagnosing myositis, especially in patients with active disease and normal CK levels. Aldolase, LDH, and AST are better correlated with disease activity in patients with JDM. The main disadvantage of these enzymes is that they are also elevated in liver diseases; therefore, the muscle source needs to be identified before interpreting the data.[201]

The serum myoglobin level is a sensitive index of muscle fiber membrane integrity, and can therefore be used to assess the degree of disease activity. The advantage of the myoglobin assay is that it involves a nonenzymatic immunologic reaction. The disadvantages are that a significant range of serum myoglobin levels occurs in myositis patients because of circadian variation,[202] and the test is less readily available than CK for routine use.

## Histopathology

Muscle biopsy is the "gold standard" for the diagnosis of inflammatory myopathies and a critical component of the definitive diagnosis of IIMs.[203,204] For optimal biopsy results, it is important to select a muscle that is moderately weak. The histologic features can be grouped into general features that are common to all IIMs and specific features unique to a particular subgroup. The general features include necrosis, regeneration, degeneration, variation in fiber diameter, increase in connective tissue, and inflammation. The features specific to DM include loss of capillaries, alterations in the morphology of the capillaries, capillary necrosis with the deposition of complement products (e.g., membrane attack complex) on the vessel walls, and, rarely, muscle infarcts. Another specific histopathologic finding, albeit a late sign, is perifascicular atrophy. The infiltrates typically have a perivascular distribution. The inflammatory infiltrates are dominated by a high percentage of CD4 T cells and macrophages at the sites of inflammation, with B cells occasionally in evidence. Although perifascicular atrophy is the hallmark of the histologic changes seen in DM, it may not be visible if a biopsy is acquired early in the course of the disease. In early DM, the class I MHC expression is patchy, and the perifascicular areas are usually stained.

The features of PM include the presence of macrophages and activated $CD8^+$ T cells in muscle fibers and the expression of class I MHC molecules on muscle fibers. Mononuclear cell invasion around non-necrotic muscle fibers in endomysial areas is a characteristic feature of PM and IBM. The histologic features of IBM resemble those of PM, but they also include unique features such as red-rimmed vacuoles and inclusions (cytoplasmic or nuclear) and amyloid deposits.[176] An increased number of cytochrome-*c* oxidase–negative fibers can also be seen, but this change is not specific for IBM. In IBM, electron microscopy usually demonstrates 15 to 21 nm cytoplasmic and intranuclear tubulofilaments, which are not found in DM or PM. It is not uncommon to find biopsies that are negative for both rimmed vacuoles and tubulofilamentous inclusions. In this situation, if the suspicion for IBM is high, the diagnosis may be based on typical clinical features. Nonresponsiveness to immunosuppressive treatment further supports the diagnosis of IBM in an otherwise typical patient. Inflammation surrounding necrotic fibers is a feature of some muscular dystrophies (e.g., facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy type IIB, Duchenne's muscular dystrophy), in which it is secondary to muscle cell degeneration. The common and unique immunologic and histologic features of the various subgroups are listed in Tables 85-6 and 85-7, respectively.

# IMAGING

## Muscles

Ultrasonography, CT, and MRI are the three general imaging techniques used to evaluate skeletal muscle. MRI has emerged as the method of choice for the examination of soft tissue muscle abnormalities because it efficiently visualizes and quantifies inflammation, fat infiltration, calcification, and alterations in muscle size and localizes pathologic changes to specific muscle groups (Figure 85-11). MRI examinations can be done on large volumes and can be helpful guides for muscle biopsy sampling. MRI is a potential outcome tool to be used in the longitudinal analysis of responses to therapy and in clinical trials, although its sensitivity to changes has not been validated.[209-212]

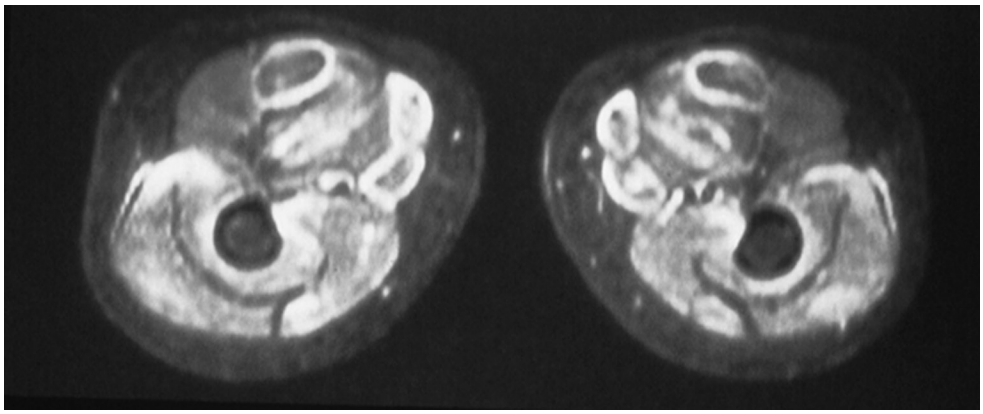


**Figure 85-11** Magnetic resonance images (short tau inversion recovery) of the thigh. Note the symmetric inflammation in the affected muscle, seen as bright areas relative to unaffected muscle.

**TABLE 85-6** Immunologic Features of Idiopathic Inflammatory Myopathies

| Feature | Dermatomyositis | Polymyositis | Inclusion Body Myositis | Necrotizing Myositis |
|---|---|---|---|---|
| B cell infiltration | + | ± | ± | - |
| T cell infiltration | + | + | + | - |
| $CD8^+$ T cell infiltration in non-necrotic fibers | ± | + | + | - |
| Vascular membrane attack complex | + | – | – | + |
| Immunoglobulin deposition on blood vessels | + | – | – | + |
| Class I MHC expression on muscle fibers | ±* | + | + | + |
| Cytokines and chemokines | + | + | + | |
| Cell adhesion molecules | + | + | + | |
| Anti-nuclear antibodies | + | + | +† | - |
| Anti-Jo 1 antibodies‡,§ | + | + | ± | - |
| Anti–signal recognition particle antibodies§ | ± | + | ± | + |
| Anti–Mi-2 antibodies‖ | + | ± | ± | - |
| Anti–PM-Scl antibodies¶ | + | + | – | - |
| Anti-HMGCR antibodies** | - | - | - | ± |
| Anti-MDA5 | + | - | - | - |
| Anti-SAE | + | - | - | - |
| Anti-TIF1γ | + (JDM) | - | - | - |
| Anti-TIF1γ | + (JDM) | | | |

*Mostly in perifascicular areas and necrotic fibers.
†Less frequently, but 20% higher than in normal population.
‡Frequency varies among ethnicities; more frequent in polymyositis (22%) than dermatomyositis (16%) or inclusion body myositis (5%). D. Not known
§Present only in a proportion of polymyositis (14%), dermatomyositis (5%), and inclusion body myositis (3%) patients.
‖Present only in a proportion of polymyositis (9%), dermatomyositis (21%), and inclusion body myositis (8%) patients.
¶Present only in a proportion of polymyositis (7%) and dermatomyositis (6%) patients.
**Either anti-SRP or HMGCR antibodies typically present.
JDM, Juvenile dermatomyositis; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MHC, major histocompatibility complex; SAE, small ubiquitin-like modifier activating enzyme; TIF, transcription intermediary factor 1.

**TABLE 85-7** Histologic Features of Idiopathic Inflammatory Myopathies

| Feature | Dermatomyositis | Polymyositis | Inclusion Body Myositis | Necrotizing Myopathy |
|---|---|---|---|---|
| Necrosis of muscle fibers | + | + | + | + |
| Variation in fiber diameter | + | + | + | |
| Regeneration of muscle fibers | + | + | + | + |
| Proliferation of connective tissue | + | + | + | |
| Infiltration of mononuclear cells* | + | + | + | |
| Perivascular and perimysial inflammation | + | ± | ± | |
| Endomysial inflammation | ± | + | + | |
| Perifascicular atrophy | + | – | – | |
| Abnormally dilated capillaries | + | ± | – | |
| Reduced capillary density | + | ± | – | |
| Deposition of complement on vessel walls | + | ± | – | + |
| Microinfarcts | + | – | – | |
| Invasion of non-necrotic fibers by cytotoxic T lymphocytes and macrophages | – | + | + | |
| Expression of class I MHC on muscle fibers | ± | + | + | + |
| Rimmed vacuoles with amyloid deposits and tubulofilaments† | – | – | + | |
| Angulated or atrophic and hypertrophic fibers | – | – | + | |
| Ragged red or cytochrome oxidase–negative fibers | – | – | + | |

*Inflammation is absent in a small proportion of polymyositis and dermatomyositis biopsies.
†Also seen in chronic neurogenic conditions and distal myopathies.

Ultrasonography can be used to detect abnormal vascularization, and rates of blood flow can be monitored effectively with color Doppler imaging. The usefulness of ultrasound in diagnosis or as a tool to monitor treatment in patients with IIM still needs to be determined. CT is the modality of choice for identifying calcifications in soft tissues (e.g., JDM), but it is not useful for detecting inflammatory changes in muscle tissue. Cross-sectional CT images allow quantification of muscle atrophy and fat replacement in deep muscles that may not be generally accessible to ultrasonography. A combination of MRI and P-31 MR spectroscopy examinations produces the most comprehensive and accurate evaluation of patients but has not been evaluated for use in clinical practice.[209]

### Lungs

Radiography and high-resolution CT of the lungs are important for detecting lung involvement and should be considered at the time of myositis diagnosis because the prevalence of ILD is high. In contrast, conventional radiography may not always be sensitive enough to detect ILD. These are also important tests for assessing the effects of immunosuppressive treatment.

## ELECTROMYOGRAPHY

Electromyogram (EMG) changes are usually nonspecific but are a useful indicator of myopathic changes. The major abnormalities include abnormal electrical irritability, a decrease in the mean duration of motor unit potentials or increase in the percentage of polyphasic motor unit potentials (short duration), and rapid firing of the motor unit potentials in relation to the level of activity. Later in the course of the disease, fibers are lost from some motor units, and recruitment is reduced. Abnormal electrical irritability in DM and PM involves increased insertional activity, trains of positive sharp waves, and fibrillation potentials. Spontaneous electrical activity is a reasonable measure of disease activity in DM and PM. Earlier studies indicated that EMG abnormalities correlate with alterations in muscle strength and serum muscle enzymes; however, there is an agreement that EMG is not very sensitive to change in muscle strength. The inflammation in IIMs is often patchy, and EMG is useful in determining which muscle should be sampled for biopsy. Because EMG can cause histopathologic changes that complicate the interpretation of the biopsy, it is best to perform EMG on one side and obtain the muscle biopsy from the same muscle on the contralateral side. Even for IBM, in which the disease is often asymmetric, this technique is useful as long as the contralateral muscle is also weak.

## PULMONARY FUNCTION TESTS

Pulmonary function tests are an important means of obtaining an objective assessment of respiratory involvement. Typically, patients demonstrate a restrictive ventilatory impairment, with decreased total lung capacity, functional residual capacity, residual volume, forced expiratory volume in 1 second ($FEV_1$), and forced vital capacity (FVC), but with a normal or elevated $FEV_1$/FVC ratio and reduced diffusing capacity for carbon monoxide. Pulmonary function tests are also important in estimating disease severity and response to therapy, in concert with radiographic examination.

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of IIMs and other myopathies is important because the clinical associations and response to therapeutic interventions differ significantly. A variety of myopathies closely mimic IIMs (Table 85-8).

### Dystrophic Myopathies

#### Dysferlinopathy

Genetic defects in the dysferlin gene result in limb-girdle muscular dystrophy type 2B and distal muscular dystrophy of the Miyoshi type. These diseases can appear in patients in their late teens or early 20s. The weakness in the limb-girdle type 2B phenotype first assumes a pelvifemoral

**TABLE 85-8** Differential Diagnosis of Inflammatory Myopathies

| Disease | Key Diagnostic Features |
|---|---|
| **Dystrophic Myopathies** | |
| Dysferlinopathy (Miyoshi myopathy and LGMD2B) | Mutations in dysferlin gene<br>Progressive proximal (LGMD2B) and distal (Miyoshi myopathy) muscle weakness<br>Onset in late teens to early 20s<br>Increased CK levels<br>Inflammation in muscle biopsy<br>Nonresponsiveness to steroids |
| Facioscapulohumeral muscular dystrophy | Partial deletion in D4Z4 repeats near chromosome 4q telomere at 4q35<br>Initial facial and shoulder girdle weakness progresses to pelvic girdle and extremities<br>Normal serum CK levels or modest elevation |
| Becker's dystrophy | Mutations in dystrophin gene<br>X-linked recessive disorders<br>Limb-girdle weakness and cardiomyopathy<br>High serum CK levels |
| Proximal myotonic myopathy | CCTG expansion in intron 1 of ZNF9 gene<br>Autosomal dominant<br>Proximal muscle weakness |

*Continued*

**TABLE 85-8** Differential Diagnosis of Inflammatory Myopathies—cont'd

| Disease | Key Diagnostic Features |
|---|---|
| Sarcoglycanopathy | Mutations in sarcoglycans (α, β, γ, and δ)<br>Limb-girdle weakness and cardiomyopathy<br>High serum CK levels |
| **Metabolic Myopathies** | |
| Acid maltase deficiency | Mutations in acid α-glucosidase<br>Proximal muscle weakness<br>Respiratory muscle involvement<br>Abnormal irritability on EMG<br>Increased serum CK levels |
| McArdle's disease | Mutations in myophosphorylase gene<br>Exercise intolerance<br>Fixed proximal muscle weakness<br>Increased serum CK levels |
| **Mitochondrial Myopathies** | Mutations in complex I-IV, complex V, and coenzyme Q10 genes<br>Myopathy with limb-girdle weakness<br>Exercise intolerance and fatigue<br>Increased serum CK levels |
| **Endocrine Myopathies** | |
| Cushing's syndrome | Insidious onset<br>Proximal muscle weakness<br>Normal serum CK, AST, and LDH levels |
| Thyrotoxic myopathy | Subacute onset of proximal muscle weakness<br>Normal serum CK levels or modest elevation<br>Respiratory muscle weakness |
| **Infectious Myopathies** | |
| HIV myopathy | Progressive myopathy<br>Proximal symmetric muscle weakness<br>Endomysial inflammation<br>Increased serum CK levels |
| **Parasitic Myopathies** | |
| Protozoal myopathy | Clinical features of idiopathic inflammatory myopathies<br>Focal or diffuse inflammation<br>Myocarditis<br>Increased serum CK levels |
| **Drug-Induced Myopathies** | |
| Zidovudine myopathy | Proximal muscle weakness<br>Increased serum lactate levels<br>Ragged red fibers and abnormal mitochondria in muscle<br>Improves with drug discontinuation |
| Statin myopathy | Necrotizing myopathy<br>Acute or subacute painful proximal myopathy<br>Increased serum CK levels |
| Corticosteroid myopathy | Proximal and distal weakness<br>Type 2 atrophy and vacuolar changes in muscle<br>Increased serum CK levels |
| D-Penicillamine, interferon-α, and procainamide-induced myopathy | Proximal muscle weakness and pain<br>Inflammation and necrosis in muscle<br>Skin changes<br>Increased serum CK levels |
| **Neuromuscular Diseases** | |
| Motoneuron disease | Upper and lower motoneuron signs<br>Asymmetric weakness with denervation atrophy<br>Fasciculations and fatigability<br>Fibrillations and enlarged motor unit potentials on EMG<br>Modest elevation in serum CK levels |
| Spinal muscular atrophy | Symmetric muscle weakness and atrophy<br>Neurogenic changes on EMG and biopsy<br>Normal serum CK levels |
| Myasthenia gravis | Abnormal weakness and fatigability<br>Decremental EMG response<br>Anti-acetylcholine receptor antibodies<br>Positive anti-cholinesterase drug test |

AST, Aspartate transaminase; CK, creatine kinase; EMG, electromyogram; HIV, human immunodeficiency virus; LDH, lactate dehydrogenase; LGMD, limb-girdle muscular dystrophy.

distribution: the quadriceps muscle is affected first, followed by weakness in the arms in the later stages of the disease. A relatively acute onset with elevated levels of serum muscle enzymes points to PM as a differential diagnosis. The weakness in the Miyoshi phenotype occurs predominantly in the gastrocnemius and soleus muscles, thereby affecting the patient's ability to walk on the toes. The weakness is slowly progressive, with loss of ambulation generally occurring in the fourth decade, but earlier in some cases. Serum CK levels are high during the active phase of the disease. In general, the muscle biopsy is dystrophic, with significant mononuclear cell infiltration and small sarcolemmal defects with thickened basal lamina structures over the defects.[214]

### Facioscapulohumeral Muscular Dystrophy

A partial deletion of the D4Z4 repeats near the chromosome 4q telomere at 4q35 leads to the facioscapulohumeral phenotype. The initial weakness usually affects the facial muscles, and the onset is insidious. Shoulder weakness is commonly seen because of the weakness of the scapular fixator muscles. The weakness is generally slowly progressive, with typical myopathic changes on the EMG such as brief, small-amplitude, polyphonic voluntary motor unit potentials. The presence of perivascular, endomysial, and perimysial inflammation is a common feature.[215] Serum CK levels are elevated and vary with patient age and sex.

### Dystrophinopathies

These X-linked recessive disorders are caused by mutations in the dystrophin gene. Milder forms of Becker's muscular dystrophy manifest as myalgias, muscle cramps, exercise intolerance, mild limb-girdle weakness, and quadriceps myopathy. The severe Becker's phenotype that is seen in patients younger than age 8 years is indistinguishable from the Duchenne's phenotype. An elevation in serum CK levels is seen in asymptomatic patients. In Becker's dystrophy, the number of necrotic and regenerating fibers is decreased when compared with the Duchenne phenotype, and the incidence of hypercontracted and central nuclei increases with age.[216]

### Proximal Myotonic Myopathy

CCTG expansion in intron 1 of the zinc finger transcription factor (ZNF9) gene results in type II myotonic dystrophy. The myotonia usually appears to be absent or minimal but is detectable by EMG. The weakness is mainly proximal, with minimal or no facial involvement. Smooth muscle, cardiac, and diaphragmatic involvement is common in this disease. First-degree heart block is the most common abnormality, and sudden death is well documented.[217] Muscle biopsies show nonspecific features such as central nuclei, sarcoplasmic masses, and atrophy of type I fibers.

### Sarcoglycanopathy

Mutations in sarcoglycans (α, β, γ, and δ) result in limb-girdle muscular dystrophy types IIC to IIF. Sarcoglycanopathies often start in childhood, with a median patient age of onset of 6 to 8 years. These diseases present initially as pelvic muscle weakness, including a waddling gait and difficulty performing common tasks such as getting up from the floor, climbing the stairs, and running. The trunk muscles are prominently affected, and upper extremity involvement usually follows lower extremity involvement. Distal muscles are generally spared until later in the disease process.[218] Dilated cardiomyopathy is often seen in these disorders, and muscle biopsies show marked regeneration and necrosis.

## Neuromuscular Disorders

### Motoneuron Diseases

These diseases, including amyotrophic lateral sclerosis (ALS), are progressive, degenerative disorders of motoneurons in the spinal cord, brain stem, and cerebral motor cortex that manifest clinically as amyotrophy and exaggerated reflexes. These diseases are characterized by a selective loss of function of upper or lower motoneurons, finally leading to a progressive loss of both types of motoneurons over time. EMG shows fibrillation and fasciculation potentials in the muscles of the lower and upper limbs or in the bulbar muscles. Muscle biopsies show the presence of denervation atrophy and secondary myopathic changes in chronically denervated muscles. IBM is the primary muscle disorder most likely to be confused with ALS, and muscle biopsy helps differentiate the two. Serum CK levels are slightly elevated, particularly in the early stages of the disease and in men who are physically active.

### Spinal Muscular Atrophy

Late-onset forms of spinal muscular atrophy (SMA) are characterized by progressive muscle weakness and atrophy and reduced tendon reflexes. EMG and muscle testing reveal neurogenic changes in the muscle. Typical muscle biopsy findings include small and large groups of atrophic fibers in the chronic and severe forms of SMA, respectively. Histochemical changes show fiber type grouping, indicating re-innervation. Serum CK levels are slightly increased in juvenile-onset cases and normal in other forms of SMA. EMG shows abnormal spontaneous electrical activity (fibrillations, positive sharp waves, fasciculations), suggesting ongoing denervation.

### Myasthenia Gravis

The clinical manifestations of myasthenia gravis include abnormal weakness that is worsened by repeated or sustained exertion and fatigability. Proximal muscles are usually more severely affected than are distal muscles. This is a generalized disease that exhibits external ocular muscle involvement, positive anti-cholinesterase drug tests, and a decremental EMG response. Patients are often positive for anti-acetylcholine receptor antibodies.

## Metabolic Myopathies

### Acid Maltase Deficiency

This autosomal recessive glycogen storage disease is caused by acid maltase gene mutations. The disease has infantile,

childhood, and adult variants. The infantile form manifests in the first few months after birth as a rapidly progressive weakness and hypotonia, with death occurring as a result of cardiorespiratory failure. The childhood form manifests as a myopathy in which the weakness is usually greater in the proximal than in the distal muscles; the disease progresses relatively slowly, and patients die of respiratory failure. The adult form presents in the 20s as a progressive myopathy that resembles PM or limb-girdle muscular dystrophy, with additional respiratory symptoms. Serum muscle enzymes (CK, AST, and LDH) are increased in all three forms of the disease, and EMG indicates myopathy in all three cases.

### McArdle's Disease

McArdle's disease is the most common of the nonlysosomal muscle glycogenoses. Exercise intolerance is the characteristic feature of this disease, and it often manifests as early fatigue, myalgia, and stiffness of exercising muscle that is relieved by resting. The EMG is normal in some patients and shows nonspecific myopathic changes in others. Forearm ischemic exercise testing shows virtually no increase in venous lactate in most patients. Serum CK levels, however, are variably elevated in these patients. Muscle biopsies show subsarcolemmal deposits of glycogen at the periphery of the fibers.

## Mitochondrial Myopathies

Mitochondrial diseases are heterogeneous and often present a diagnostic challenge. It has been suggested that myopathy is the result of mutations in mitochondrial DNA in the skeletal muscle.[219] The clinical course of the pure myopathy varies from rapidly progressive to almost reversible disease, with disease onset occurring from infancy through adulthood. The weakness is facioscapulohumeral and more proximal than distal, with involvement of the orbicularis and extraocular muscles. Patients often complain of exercise intolerance and fatigue and have recurrent episodes of myoglobinuria. Muscle biopsy plays a critical role in the diagnosis of these conditions, especially the use of special mitochondrial stains that detect succinate dehydrogenase, Cox staining, and Gomori trichrome staining.

## Endocrine Myopathies

### Cushing's Syndrome

Cushing's syndrome is an endogenous glucocorticoid excess disease that manifests as muscle weakness and wasting. Chronic corticosteroid treatment results in similar manifestations and significant loss of strength within a few weeks of treatment. Muscle biopsy shows increased vacuolations and glycogen accumulation in type II muscle. The onset of weakness is usually insidious. The weakness is primarily proximal, with more severe involvement in the legs than in the arms. These patients generally show normal serum muscle enzyme levels (CK, AST, and LDH). Muscle wasting can often be reversed if the glucocorticoid levels are returned to the normal range.

### Hyperthyroid and Hypothyroid Myopathy

Myopathic thyroid disease is characterized primarily by proximal muscle weakness and muscle wasting. When distal weakness occurs, it often follows proximal myopathy. Exercise intolerance, fatigue, and breathlessness are common complaints, and weakness of the respiratory muscles results in respiratory insufficiency and the need for ventilatory support. Patients often have difficulty rising from a sitting position or lifting their arms above their heads. Serum muscle enzymes (CK, AST, and ALT) are often normal or low in hyperthyroidism and elevated in hypothyroidism. EMG findings are variable, with short-duration motor unit potentials and increased polyphasic potentials in proximal muscles; fibrillations and fasciculations are uncommon.

## Infectious Myopathies

### Human Immunodeficiency Virus Myopathy

Neuromuscular manifestations are common in HIV–induced myopathy. The clinical features typically include a myopathy of subacute onset that progresses slowly. The myopathy often starts as proximal symmetric muscle weakness with or without muscle wasting, similar to that in IIMs. EMG shows spontaneous activity, with fibrillation potentials, positive sharp waves, and brief, low-amplitude polyphasic motor unit potentials.

### Human T-Lymphotropic Virus I Myopathy

Myositis associated with human T-lymphotropic virus I (HTLV-I) has been noted in certain areas of the world (e.g., Japan, Jamaica). In these patients, symptoms of PM and IBM occur either alone or in combination with tropical spastic paraparesis.[220] Typical features include weakness and increases in serum CK levels. Histologic findings include interstitial inflammation, muscle fiber necrosis in PM and endomysial inflammation, vacuoles, amyloid deposits, and tubulofilaments in IBM.

## Parasitic Myopathies

Diseases caused by various parasites—protozoa (e.g., toxoplasmosis, trypanosomiasis, sarcocystosis, malaria), cestodes (e.g., cysticercosis, echinococcosis, coenurosis, sparganosis), and nematodes (trichinellosis, toxocariasis, dracunculiasis)—can cause myositis. The clinical features include nonspecific complaints such as myalgia and focal swelling, as well as typical features of PM and DM. Each parasitic infection shows typical changes on muscle biopsy (e.g., the presence of tachyzoites and toxoplasma cysts along with perimysial and endomysial inflammation). A combination of muscle biopsy and serologic findings is useful in making a diagnosis.

## Drug-Induced Myopathies

D-penicillamine is known to induce clinical features reminiscent of DM. Recovery usually occurs after withdrawal of the drug. Agents such as IFN-α that are used to treat viral hepatitis and certain malignant tumors are also known to

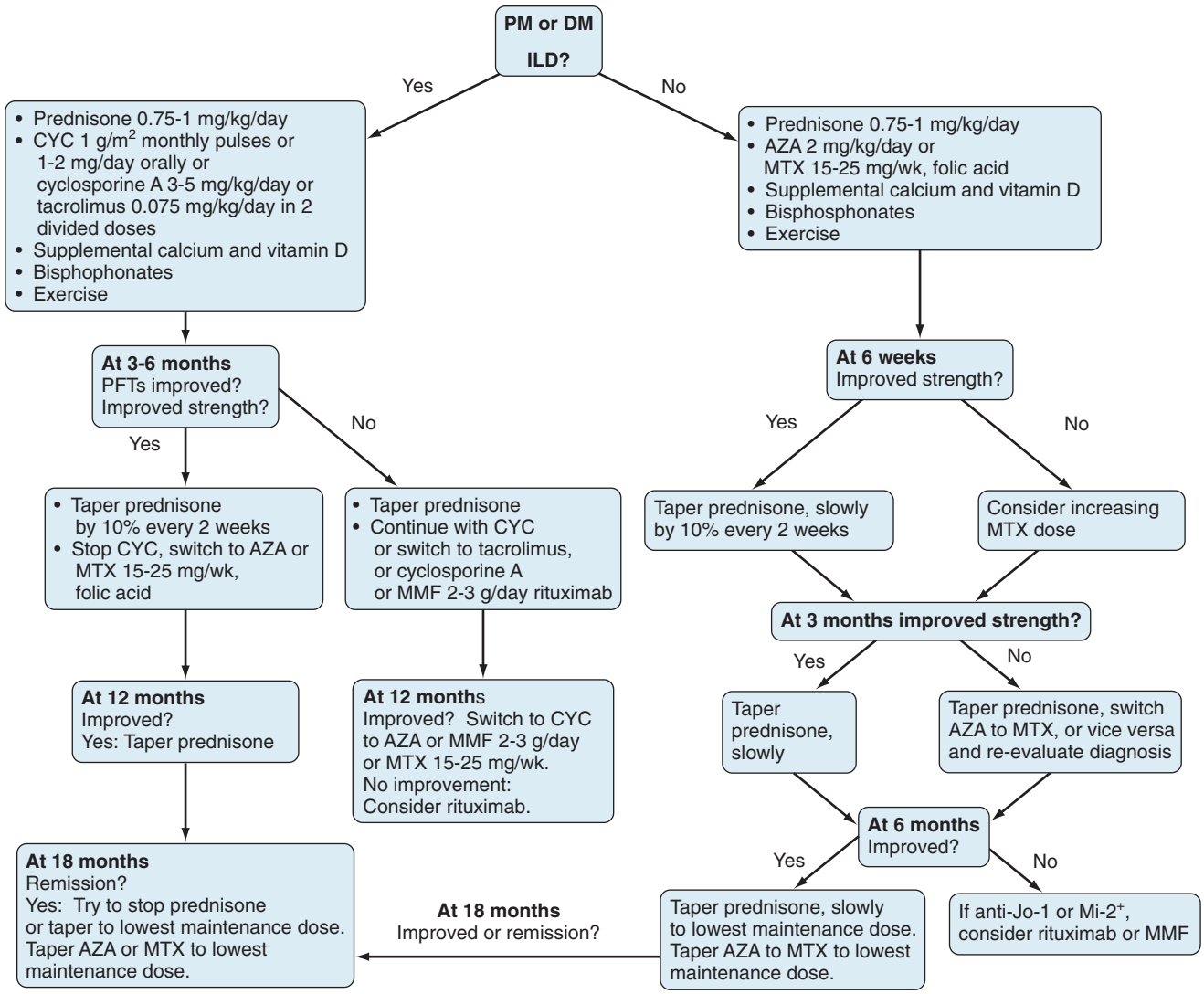



**Figure 85-12** Treatment algorithm for adult patients with polymyositis (PM) or dermatomyositis (DM). AZA, Azathioprine; CYC, cyclophosphamide; ILD, interstitial lung disease; IVIg, intravenous immunoglobulins; MMF, mycophenolate mofetil; MTX, methotrexate; PFT, pulmonary function test; SC, subcutaneous.

induce clinical features that resemble PM. Amphiphilic drugs such as chloroquine, hydroxychloroquine, and amiodarone are also known to induce cytoplasmic vacuoles, necrosis, and longitudinal branching of muscle fibers.[221] Drugs that affect microtubules, such as colchicine and vincristine, also induce myopathic changes, with the appearance of characteristic autophagic vacuoles in muscle fibers. Nucleoside analogues such as zidovudine are used to treat HIV because they act as false substrates for the viral reverse transcriptase. These drugs also cause myalgias, proximal muscle weakness, and fatigue and are sometimes associated with increased levels of serum CK. In these patients, zidovudine-induced mitochondrial myopathy may coexist along with HIV-induced T cell–mediated inflammatory myopathy.[222]

## MANAGEMENT AND OUTCOME

The recommended treatment for patients with PM or DM is based on a combination of pharmacologic therapy and physical exercise. The optimal pharmacologic treatment in PM and DM is unclear. Few controlled trials have been undertaken, so recommendations are based on clinical observations from case series. With these limitations in mind, a suggested outline of treatment for patients with PM or DM is depicted in Figure 85-12. In addition to providing treatment, it is important to give patients adequate information about their disease and its treatment. This educational component is best provided by a rheumatology team and patient support groups.

### Pharmacologic Treatment

The initial pharmacologic treatment in PM and DM is high-dose glucocorticoids: 0.75 to 1 (as much as 2) mg/kg body weight per day of prednisone for 4 to 12 weeks. Most experts recommend that glucocorticoid treatment be combined with another immunosuppressive drug to reduce the side effects of the glucocorticoid and to boost the immunosuppressive effect. The most frequently used

immunosuppressive agents are azathioprine and methotrexate. In the extension phase of one of the few double-blind, placebo-controlled trials that have been reported, the combination of azathioprine and glucocorticoids, as compared with prednisone alone, was associated with better functional ability and a lower requirement for prednisone after 1 and 3 years.[223,224] The recommended azathioprine dosage is 2 mg/kg per day. The dosing regimen for methotrexate is similar to that for rheumatoid arthritis—as much as 25 mg weekly, although there have been reports of higher doses. Pulmonary involvement related to myositis does not seem to be a contraindication for methotrexate.

The combination of methotrexate and azathioprine has proved to be successful in a few patients with refractory myositis in a prospective, randomized, open-label crossover study comparing two aggressive approaches. There are also newer reports that mycophenolate mofetil might be effective. In patients with ILD, cyclophosphamide could be of value. There are also a few reports that cyclosporine A or tacrolimus can be beneficial in these cases.[225,226] In treatment-resistant DM, a high dose of IVIG had a beneficial effect on muscle strength when compared with placebo; however, the therapeutic effect was temporary, and repeated infusions were required.[227] In patients with severe, rapidly progressive disease that might be life threatening, high-dose pulses of IV methylprednisolone have been reported to be beneficial. Pharmacologic treatment, including tapering of the corticosteroid dose, should be guided by clinical outcome measures. As discussed earlier, the most appropriate outcome measures are muscle endurance and muscle strength. Side effects of glucocorticoids in these high doses are frequent. Prophylaxis against osteoporosis is recommended with vitamin D and calcium and, when clinically indicated, bisphosphonates. Steroid myopathy is another possible consequence of glucocorticoid treatment that is particularly problematic in patients with inflammatory myopathies. There is no specific test to verify steroid myopathy, but in the absence of active clinical disease, steroid myopathy could contribute to muscle weakness. If steroid myopathy is suspected, tapering of the glucocorticoid dose with careful evaluation of the clinical response is recommended. Glucocorticoids may also cause hypokalemia, and if this is not corrected, it may be associated with muscle weakness and incorrectly interpreted as myositis activity. The depletion of B cells has recently emerged as a new strategy in autoimmune diseases. One approach is to use rituximab (monoclonal antibody against CD20). Although a large multicenter randomized clinical trial (RIM trial) in patients with refractory PM, DM, and JDM did not meet its endpoint, a post hoc analysis of the RIM trial indicate that presence of anti-Jo-1 or anti-Mi-2 autoantibodies may predict response to rituximab.[228,229,230] Use of TNF inhibitors has produced mixed results. For example, treatment with infliximab at 3 mg/kg resulted in improved muscle function in patients with juvenile DM. However, interpretation of the data from this trial was complicated by the fact that all of the patients were concomitantly treated with a tapering dose of prednisone.[231] A second, larger trial examining the use of infliximab in patients with PM, DM, or IBM found that only 30% of patients experienced an improvement in disease symptoms, whereas the remaining participants suffered from adverse side effects or worsening of the disease.[232] In addition, a pilot study of etanercept showed worsening of the rash associated with DM.[233]

IBM is usually nonresponsive to glucocorticoids. There are occasional case reports of "stabilization" for a period of months, but this condition probably reflects the natural history of the disease. Prolonged administration of glucocorticoids to patients with IBM may actually lead to worsening of clinical aspects of the disease, despite the improvement in CK levels and reduced T cell numbers in biopsies. Prednisone treatment also increases the number of amyloid-containing fibers.

A few small studies have shown some beneficial effect of methotrexate, anti–T lymphocyte globulin, or mycophenolate. However, an open-label trial comparing methotrexate with methotrexate with anti-T lymphocyte globulin showed deterioration in the methotrexate group.[234]

Anabolic steroids and oxandrolone may have some beneficial effects on muscle strength, although this benefit needs to be confirmed in larger studies. Most experts consider their use justified in combination with glucocorticoids for a limited period in patients who have inflammatory infiltrates on muscle biopsy, or in combination with a more aggressive immunosuppressive treatment (e.g., methotrexate, azathioprine) in patients with another connective tissue disease. Treatment of JDM is similar to adult IMM. (For details, please see the JDM clinical section.)

## Nonpharmacologic Treatment

With immunosuppressive treatment, approximately 75% of all patients improve, but few recover normal muscle function, even in the absence of muscle inflammation. In previous decades, patients with myositis were advised against exercise because of concerns over possible muscle damage and inflammation, but recent publications have demonstrated that combining exercise and immunosuppressive therapy is a safe approach and has clear beneficial effects on muscle strength and function.[235] Chronic inflammation leads to loss of skeletal muscle because of low levels of physical activity, negative effects of glucocorticoids, and circulating cytokines. In addition, in late phases of disease, patients with PM and DM may still experience muscle weakness, despite absence of muscle inflammation.[128] Exercise has proven safe and able to improve muscle performance and health-related quality of life in both early and late phase of disease. Emerging evidence suggests that exercise can even decrease muscle and systemic inflammation.[236] Similar to healthy individuals, exercise has a short-term benefit and needs to be implemented on a regular basis.[237-241]

In a pair of small studies of immunosuppressed myositis patients, personalized submaximal exercise programs improved[237] muscle function without causing any increase in inflammation. This improvement was true for patients who had individually tailored exercise regimens[242] and self-administered home exercise routines.[243] Examinations of biopsies both before and after exercise indicated that the patients showed improved muscle strength as the result of an increase in the proportion of slow twitch (type I) muscle fibers and a reduction in inflammation and fibrosis.[243,244] More evidence suggests that combining exercise and immunosuppressive therapy is a safe approach and can improve

performance without disease exacerbation, and exercise can decrease muscle and systemic inflammation.[231,245] The exercise regimen should be individualized and supervised by a physiotherapist to avoid the overuse of muscles, and it should combine aerobic and resistance exercise, starting approximately 4 weeks after initiation of immunosuppression.[237] Physical exercise is now recommended as a combination therapy with immunosuppressive treatment.

## Assessing Disease Activity and Outcome

The most important variable to measure in myositis patients is muscle performance or physical function. However, it is equally important to evaluate whether impaired muscle function reflects disease activity or irreversible muscle damage.

### Muscle Examination

**Manual Muscle Test.** There are several tools to measure muscle performance, but the method used most often in clinical practice and clinical trials is the manual muscle test with the MRC scale (see earlier). The drawback is that these tools measure muscle strength but not muscle endurance, which is often a major problem in PM or DM. Furthermore, they have not been validated in adults with inflammatory myopathies. Previously, the number of muscle groups tested using the manual muscle test varied, and different scales were used (5 or 10 grade). Recently, a consensus was reached to assess eight muscle groups on the dominant side using a 0- to 10-point scale, in which 0 indicates no muscle contraction, 5 indicates the ability to hold the test position without any added pressure, and 10 indicates the ability to hold the test position against strong pressure. The points between these scores are based on gradual increased resistance against the examiner's pressure. The eight muscle groups tested are neck flexors, shoulder abduction (deltoid middle), biceps brachii, wrist extensors, knee extensors (quadriceps), dorsiflexion of ankle, gluteus maximus, and gluteus medius. The score achieved varies between 0 and 80.

**Functional Index in Myositis.** The Functional Index in Myositis and its revised form, the Functional Index in Myositis-2, were developed as outcome measures for patients with PM or DM. This test measures the number of repetitions that can be performed in defined muscle groups.[194,199] It is a more sensitive method of measuring impaired muscle function in patients with PM or DM.[194] The drawback is that it takes longer to perform than the manual muscle test, and it may be difficult to use in everyday clinical practice. Preferably, the Functional Index in Myositis-2 is administered by a physiotherapist and can be combined with the manual muscle test.

### Extramuscular Involvement

In some patients, extramuscular symptoms predominate among the clinical features. These symptoms may require other assessment tools, such as those used to evaluate ILD. For monitoring the effects of treatment of ILD, high-resolution CT and pulmonary function tests are recommended.

**TABLE 85-9** Disease Activity Measure: Core Set

| |
|---|
| Physician's overall assessment of disease activity on a visual analogue scale (VAS) |
| Patient's or parent's overall assessment of disease activity (VAS) |
| Functional assessment (health assessment questionnaire) |
| Muscle strength testing (manual muscle test) |
| Serum levels of at least 2 of 4 muscle enzymes (CK, LDH, AST, ALT) |
| Extramuscular score (myositis disease activity assessment VAS [MYOACT] or myositis intention to treat activity index [MITAX]), in which disease activity in seven organ systems (general symptoms, skin, joints, gastrointestinal tract, pulmonary, heart, and muscles) is scored |

ALT, Alanine transaminase; AST, aspartate transaminase; CK, creatine kinase; LDH, lactate dehydrogenase.

### Disease Activity and Damage

It is also important to distinguish whether symptoms are caused by active inflammatory disease or are a consequence of organ damage. IMACS, an international collaboration, made a consensus recommendation that outcome measures for patients with myositis include tools that measure disease activity, damage, and health related quality of life. The IMACS network has developed one outcome measure to assess myositis disease activity and one to measure organ damage: the myositis disease activity assessment tool and the myositis damage index, respectively.[193] The disease activity outcome measure is a core set that consists of the six variables listed in Table 85-9. The damage index is recorded by the physician on the basis of the patient's history and covers several organ systems that can be affected in patients with inflammatory myopathies. To assess the impact on general health, the generic Short Form 36, a self-administered health-related quality of life questionnaire, is recommended. These outcome measures have been developed for clinical trials and research but can also be useful in clinical practice. More detailed information about these outcome measures can be found on the IMACS website: https://dir-apps.niehs.nih.gov/imacs.

IMACS has also reached a consensus on what constitutes improvement. Improvement is based on the disease activity core set and is defined as greater than 20% improvement in three of the six variables of the core set, with two or fewer of the variables (except the manual muscle test) worsening by less than 25%. However, this definition of improvement must be validated in longitudinal studies.



The full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 86

# Overlap Syndromes



Robert M. Bennett

**KEY POINTS**

- In their early stages most autoimmune connective tissue diseases (AICTD) are not clinically well differentiated; this stage of their evolution is called undifferentiated connective tissue disease (UCTD).
- About 50% of UCTDs remain undifferentiated.
- Important clues as to the eventual direction of differentiation can be obtained from nailfold capillary microscopy and the autoantibody profile in conjunction with regular clinical evaluations.
- All of the major AICTDs have varying degrees of overlap with each other; the propensity for this overlap is often correlated with a distinctive autoantibody profile.
- Mixed connective tissue disease (MCTD) is the prototypical overlap disease with features of lupus, rheumatoid arthritis, scleroderma, and inflammatory myositis. This overlap is not seen at the outset of MCTD; rather it often takes several years to develop.
- The most common presentation of MCTD is Raynaud's phenomenon.
- MCTD is most commonly associated with antibodies to U1-RNP; in general this antibody predicts lack of severe renal and CNS involvement.
- The major cause of death in MCTD is pulmonary hypertension, and all MCTD patients should have regular echocardiograms to determine its evolution.
- The management of overlap syndromes is based on the usual treatment of the constituent features of its clinical components (i.e., systemic lupus erythematosus, rheumatoid arthritis, scleroderma, and inflammatory muscle disease).

The clustering of symptoms and signs into readily recognizable groupings has an important historic precedence in the classification of disease. With the progress of knowledge, such groupings may become more precisely defined in terms of distinctive pathology, specific laboratory findings, and genetic associations. According to current nosology, there are six autoimmune connective tissue diseases (AICTDs):

- Systemic lupus erythematosus (SLE)
- Scleroderma (Scl)
- Polymyositis (PM)
- Dermatomyositis (DM)
- Rheumatoid arthritis (RA)
- Sjögren's syndrome

All six classic AICTDs are descriptive syndromes without a "gold standard" for diagnosis. The diagnosis of a diffuse connective tissue disorder (DCTD) is usually readily apparent without recourse to extensive investigations. However, in the early stages there are often common features such as Raynaud's phenomenon, arthralgias, myalgias, esophageal dysfunction, and positive tests for anti-nuclear antibodies (ANA). In such cases, the diagnosis is not always so obvious. These cases are often referred to as *undifferentiated connective tissue disease* (UCTD).[1] Approximately 35% of such patients have clinical overlap syndromes. whereas most differentiate into a clinical picture consistent with the traditional description of a DCTD. In some instances, one DCTD evolves into another DCTD with time.

The propensity for differentiation into a classic DCTD or the maintenance of an overlap state is often associated with distinctive serologic profiles and major histocompatibility (MHC) linkages. Although most rheumatologists generally feel more comfortable thinking in terms of the classic DCTD paradigms, a case can be advanced for the use of serologic profiles and human leukocyte antigen (HLA) typing to better understand the clinical features and prognoses. In this respect, a careful analysis of the overlap syndromes and their serologic associations has provided insights to understand the clinical heterogeneity of the AICTDs.[2] Numerous clinical correlations of autoantibodies have been reported and are summarized in Table 86-1.

## EPIDEMIOLOGY

The reported prevalence of AICTDs is variable and depends on the study methodology, nature of referral bias, and patient ethnicity.[3]. It is generally accepted that Sjögren's syndrome has the highest prevalence (0.5% to 3.6%), whereas SLE has a much lower prevalence of approximately 15 to 50 cases per 100,000 individuals. Scl, PM, and DM are relatively rare AICTDs, with a prevalence of less than 10 per 100,000 individuals. There is increasing realization that overlap syndromes of Scl and myositis are more common than the "pure" forms of the disease.[4] Two epidemiology studies examined the prevalence of mixed connective tissue disease (MCTD). In Japan, the point prevalence was 2.7 per 100,000,[5] and in Norway it was 3.8 per 100,000, with a yearly incidence of 2.1 per million.[6] The syndrome of MCTD usually occurs as an isolated finding, but there are reports of a familial occurrence. Unlike SLE, precipitation by sun exposure has not been described in patients with MCTD. Similarly, drug exposure has not been related to the onset of MCTD, although a transient appearance of antiribonuclear protein (RNP) antibodies has been seen at the initiation of procainamide therapy.[7] Vinyl chloride and

**TABLE 86-1** Correlations of Autoantibodies with Clinical Features

| Autoantigen | Clinical Associations |
|---|---|
| ACL/B2-glycoprotein | SLE, thrombosis, thrombocytopenia, miscarriages |
| Alpha enolase | Behçet's disease, RA, MCTD, Scl, Takayasu's |
| Angiotensin-converting enzyme 2 | AICTDs with vasculopathy |
| Anti-TIF1 (anti-p155/140) | Dermatomyositis and cancer |
| B2-glycoprotein independent ACL | MCTD (not associated with APL syndrome) |
| Centromere | Limited Scl, CREST, Raynaud's phenomenon, pulmonary hypertension, PBC |
| Citrullinated peptides | Rheumatoid arthritis |
| dsDNA | SLE, glomerulonephritis, vasculitis |
| Endothelial cell | Pulmonary hypertension, severe digital gangrene |
| Fibrillarin | Severe generalized Scl |
| Fodrin | Sjögren's syndrome, glaucoma, Moyamoya disease |
| Histones H1, H2A, H2B,H3, H4 | SLE, UCTD, RA, PBC, generalized morphea |
| hnRNP-A2 (also called RA-33) | MCTD, RA, erosive arthritis in SLE and Scl |
| hnRNP-I | Scl (early diffuse and limited) |
| Ku | Myositis overlap, primary pulmonary hypertension, Grave's disease |
| Malondialdehyde 5 5 | Amyopathic dermatomyositis and progressive lung disease |
| Myeloperoxidase | Eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome), pauci-immune glomerulonephritis |
| Nucleosome | SLE, Scl, MCTD |
| Platelet-derived growth factor (PDGF) | Diffuse and limited Scl |
| PM / Scl | Myositis overlap with arthritis, skin lesions, mechanic's hands |
| Proteasome | SLE, PM/DM, Sjögren's syndrome, multiple sclerosis |
| Proteins 3 | GPA, pulmonary capillaritis |
| Rheumatoid factor | RA, erosive arthritis, cryoglobulinemia, other systemic autoimmune diseases |
| Ribosomal P | Psychosis in SLE |
| RNA polymerases I and III | Scl (diffuse with renovascular hypertension.) |
| Ro/La | Sjögren's syndrome, SLE, congenital heart block, photosensitivity, PBC |
| Signal recognition particle—SRP | Myositis overlap (severe course with cardiac disease) |
| Sm snRNP | SLE |
| Synthetases (Jo-1, PL-7, PL-12) | Myositis overlap with arthritis and interstitial lung disease |
| Th/To | Limited Scl |
| Topoisomerase 1 (Scl-70) | Diffuse Scl with prominent organ involvement |
| U1 snRNP | MCTD, SLE, PM |
| U1-RNP (68 kDa peptide) | MCTD, Raynaud's phenomenon, pulmonary hypertension |
| U3 snRNP | Limited Scl |
| U5 snRNP | Myositis overlap |

AICTD, Autoimmune connective tissue diseases; APL, anti-phospholipid antibody; CREST, calcinosis, Raynaud's phenomena, esophageal dysmotility, sclerodactyly, and telangiectasia; DM, dermatomyositis; GPA, granulomatosis with polyangiitis; MCTD, mixed connective tissue disease; PBC, primary biliary cirrhosis; PM, polymyositis; RA, rheumatoid arthritis; ScL, scleroderma; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease.

silica are the only environmental agents associated thus far with MCTD.

## AUTOIMMUNITY IN OVERLAP SYNDROMES

Gene expression is mediated by a cascade of RNA functions that regulate, process, store, transport, and translate RNA transcripts. The RNA network that promotes this cascade depends on a large cohort of proteins that partner RNAs: so-called *ribonucleoproteins* (RNPs). Because the cytosol and nucleoplasm need to be densely packed (with its severe restriction on diffusion of macromolecules), partitioning of the eukaryote cell into functionally specialized compartments is essential for efficiency. This necessitates the association of RNA and protein into large RNP complexes, including ribosomes and spliceosomes.[8] There is compelling evidence that autoimmunity is often antigen driven by these RNA-associated components of subcellular particles.[6]

## Autoimmunity to Spliceosomal Components

Certain components of the spliceosome are common targets of autoimmunity in the AICTDs.[9] Furthermore, it appears that post-translational modifications of these molecules, as occurs during apoptosis, are often associated with increased immunogenicity.[10] Spliceosomes are complex nuclear particles made up of some 300 distinct proteins and five RNAs, which are involved in the processing of premessenger RNA into mature "spliced RNA."[11] Two major spliceosomal subunits are antigenic targets in autoimmunity: small nuclear ribonucleoprotein protein particles (snRNP) and heterogeneous nuclear RNP particles (hnRNP).

The snRNPs contain small RNA species ranging in size from 80 to 350 nucleotides, which are complexed with proteins.[12] These RNAs contain a high content of uridine, and are therefore called *U-RNAs*; five different U-RNAs were defined on the basis of immunoprecipitation (U1, U2, U4, U5, and U6). Autoantibodies to these complexes are mainly directed to the protein components. Anti-Sm antibodies precipitate five proteins with molecular weights of 28,000 (B'B), 16,000 (D), 13,000 (E), 12,000 (F) and 11,000 (G); five of these polypeptides are common to the U1-, U2-, U4-, U5-, and U6-RNAs. Anti-RNP antibodies precipitate three proteins with molecular weights of 68,000 (70K), 33,000 (A'), and 22,000 (C); these polypeptides are uniquely associated with U1-RNA (Figure 86-1). The clinical correlates distinctive of MCTD are associated with the 70 kDa specificity with an immunodominant epitope-embracing amino acid residue 125 flanked by important conformational residues at positions 119-126. On the other hand, SLE is associated with anti-Sm antibodies.

The heterogeneous nuclear RNP particles (hnRNP) are among the most abundant proteins in the eukaryotic cell nucleus. They contain pre-mRNA associated with 30 small proteins that are all structurally related and have molecular weights of 33 to 43 kDa. Nine hnRNP core proteins have been designated A1, A2, B1a, B1b, B1c, B2, C1, C2, and C3. An antibody termed *anti-RA33*, which targets the 33 kDa hnRNP-A2, is particularly interesting. It is found in approximately one third of sera from patients with RA, SLE, and MCTD.[13] Furthermore, it has associations with patient subsets of erosive arthritis in SLE, Scl, and MCTD, and predicts the eventual development of RA in patients with early polyarthritis.[14] Importantly, this association with anti-RA33 is not seen in Scl (sine erosions), PM, or overlaps of PM/Scl or PM/DM. The antigenic epitopes of hnRNP-A2 contain two RNA-binding regions at the N-terminal end and a glycine rich C-terminal region. Certain disease subsets target these two RNA-binding regions differently. For instance, RA and SLE sera preferentially react with the complete second RNA-binding domain, whereas MCTD sera target an epitope that spans both RNA-binding domains.

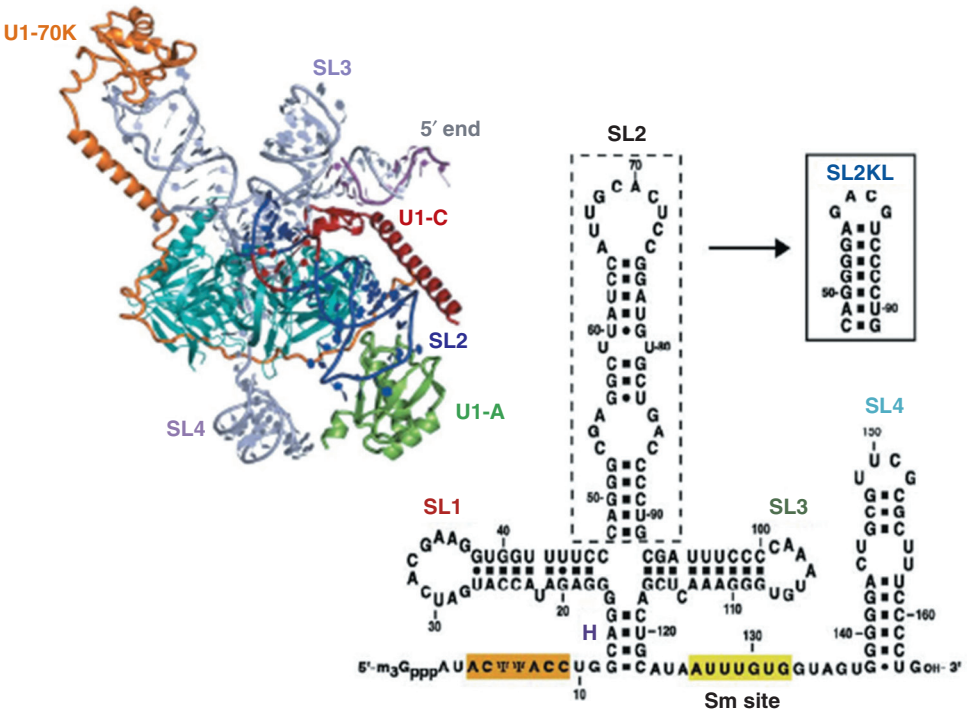



**Figure 86-1** Anatomy of the spliceosome. The spliceosome is made up of five small nuclear RNAs (snRNAs) complexed with proteins to form a small nuclear ribonucleoprotein particle (snRNP). This subcellular structure is responsible for splicing introns from pre-mRNA to form mRNA via a 59-splice recognition site. Antibodies to various spliceosomal constituents are a common feature of autoimmune rheumatic disorders with a tendency to associate with different clinical profiles (see Table 86-1). The U1 small nuclear RNP (U1 snRNP) particle of the spliceosome is composed of U1 RNA, RNP proteins (70 kDa, A, and C), and common Sm proteins (B'B, D, E, F, and G). The structure of U1 RNA consists of single-stranded RNA and double-stranded RNA called stem-loops I, II, III, and IV. An electron density map of the functional core of U1 snRNP at 5.5 A resolution has enabled the spatial visualization of the RNA and placement of the seven Sm proteins, U1-C, and U1-70K into the map. A striking feature is the amino (N)-terminal polypeptide of U1-70K, which extends over a distance of 180 A from its RNA-binding domain, wraps around the core domain consisting of the seven Sm proteins, and finally contacts U1-C, which is crucial for 59-splice-site recognition. *(Modified from Newman J: Structural studies of the spliceosome.* Curr Opin Struct Biol *20:82-89, 2010; and Pomeranz AD: Crystal structure of human spliceosomal U1 snRNP at 5.5 A resolution.* Nature *458:457-480, 2009.)*

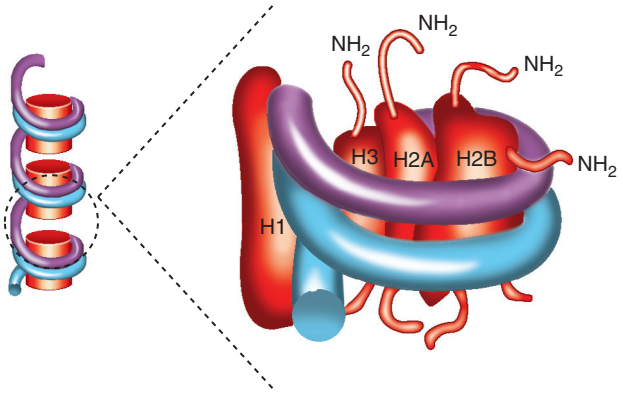



**Figure 86-2** Anatomy of the nucleosome. The nucleosome is the fundamental repeating unit of chromatin. The central part of the nucleosome is composed of a tetramer composed of two molecules of histones H3 and H4, flanked by two dimers of histones H2A and H2B. This central core is surrounded by two super-helical turns, consisting of 146 base pairs of histone-free DNA. Histone H1 is located at the point where DNA enters and exits the nucleosome. Antibodies to the nucleosome arise early in the evolution of systemic lupus erythematosus—before anti-DNA and anti-histone antibodies. Thus, the nucleosome is thought an important early autoantigen in the development of epitope spreading. Nucleosome antibodies are also found in scleroderma and mixed connective tissue disease. *(From Amoura Z, Koutouzov S, Piette C, et al: The role of nucleosomes in lupus.* Curr Opin Rheumatol *12:369-373, 2000.)*

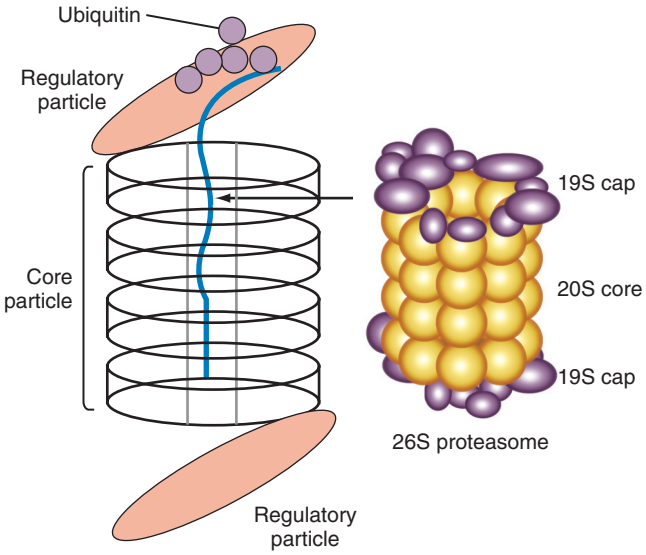



**Figure 86-3** Anatomy of the proteasome. Most proteins in the cytosol and nucleus are degraded via the proteasome-ubiquitin pathway. The 26S proteasome is a huge complex of 2.5 mega-dalton size, made up of approximately 35 different subunits. It contains a proteolytic core complex, the 20S proteasome, and one or two 19S regulatory complexes that associate with the termini of the barrel-shaped 20S core. The function of proteasomes is twofold: (1) to degrade intra-cellular proteins that have been tagged with ubiquitin, and (2) to generate antigenic peptides for presentation by the class I major histocompatibility complex molecules. Antibodies to proteasomal subunits have been reported in several autoimmune diseases (especially systemic lupus erythematosus and polymyositis/dermatomyositis) and elevated levels of proteasomes have been correlated with disease activity.

## Autoimmunity to Nucleosomal Components

Nucleosomes are the compact building blocks of chromatin and consist of an octamer of two copies of histones H2A, H2B, H3, and H4, wrapped around approximately 146 base pairs (bp) of DNA (Figure 86-2). During apoptosis, endonucleases cleave chromatin with the liberation of nucleosomal particles. Following the release into the cytoplasm, nucleosomes migrate to the surface of the dying cell[15] and thus become accessible to B cell receptors. The development of autoimmunity has been linked to defective phagocytosis of apoptotically released constituents.[15] Nucleosomal antibodies are directed to antigenic determinants on the intact nucleosome rather than its individual components, DNA, and histones. In a study of 496 patients with 13 different DCTD, and 100 patients with hepatitis C, antinucleosome antibodies were found in the sera of patients with SLE (71.7%), Scl (45.9%), and MCTD (45.0%).[16]

## Autoimmunity to Proteasomal Components

The 26S proteasome is a large subcellular particle involved in the degradation of proteins that have been tagged with ubiquitin, and the generation peptides for presentation by the class I MHC molecules (Figure 86-3). There is good evidence that it is the target of an autoimmune response in DCTD. Antibodies to proteasomal subunits have been reported in patients with autoimmune myositis, SLE, and primary Sjögren's syndrome. Circulating 20S proteasomes (c20S) subunits appear to have an association with disease activity in MCTD and SLE.[17]

## Generation of Autoimmunity

The antibody response to just one component of an intracellular structure, such as a spliceosome, will result in the uptake of the entire particle by antigen processing cells (APC). Thus all the proteins that make up the particle will be subject to antigen processing, with potential peptide presentation linked to their affinity to class II HLA antigens. Depending upon the polymorphisms of the individuals HLA molecules, there will be a diversification of the antibody response to include some of these other antigens. This process is called *epitope spreading* and is considered pivotal in the development of the linked antibody responses that are observed in different CTDs.[18] For instance, the induction of an immune response to one component of a U-RNP complex can induce a diversified auto-antibody response to other components of the complex.[19] In this way an immune response becomes modified with time, and this change has been associated with changes in the clinical picture.

The interaction of T cell receptors (TCRs) and peptides presented by HLA molecules are a critical event in the generation of autoimmunity. The 70 kDa anti-U1 RNP antibody responses are associated with the HLA DR4 and DR2 phenotype.[20] DNA sequencing of HLA-DB genes has revealed that DR2- and DR4-positive patients share a common set of amino acids in the beta chain at positions 26, 28, 30, 31, 32, 70, and 73, which form a pocket for antigen binding. It is hypothesized that these two HLA subtypes represent a critical genetic specificity for the presentation of antigenic peptides to their cognate T cell receptors.[21] The shared epitope on HLA-DR4/DR2 that is associated with an anti-U1-RNP response is different from the shared epitope associated with HLA-DR4/DR1 in RA patients.[22] The 70 kDa polypeptide has several different

epitopes. The most consistent sequence is KDK DRD RKR RSS RSR.[23] This region is preferentially targeted by MCTD sera, but not by SLE sera.[24] The autoimmune response to the spliceosome in these three disorders is characterized by differential degrees of epitope spreading. The widest range of antibodies, to both snRNP and hnRNP, is seen in SLE, a more restricted antispliceosomal antibody repertoire to snRNP and hnRNP is seen in MCTD, and in RA the antispliceosomal antibody repertoire is restricted to hnRNP.[25] In general, the autoimmune rheumatic diseases are characterized by the production of autoantibodies that recognize evolutionarily conserved molecules. The two main theories are apoptotic modification[26] and molecular mimicry.[27] Proteins modified during apoptosis can be presented to the immune system in ways that bypass tolerance to self-proteins.[12] Although rheumatic disease autoantigens are not unified by a common structure or function, they do have the common feature of becoming clustered and concentrated as blebs the surface of apoptotic cells. A population of small blebs contains fragmented endoplasmic reticulum and ribosomes, as well as the ribonucleoprotein Ro. A larger population of surface blebs contains nucleosomal DNA, Ro, La, and the small nuclear ribonucleoproteins.[28] During the process of apoptosis, several enzyme systems are up-regulated with resulting post-translational modifications of the cleaved proteins. These modifications, which include citrullination, phosphorylation, dephosphorylation, transglutamination, and conjugation to ubiquitin, render the molecules more antigenic. For instance, the U1-70K protein is cleaved by the enzyme caspase-3, converting it into a C-terminally truncated fragment that contains a major B cell epitope that is preferentially recognized by autoimmune sera.[29]

The initial stimulus for a first antibody response may be a non-self-protein possessing a peptide region that mimics a self-epitope: a so-called "molecular mimicry". Environmental stressors such as infection, toxins, drugs, and ultraviolet light may, under some circumstances, induce accelerated apoptosis. A critical limitation to molecular mimicry is the necessity for the antigenic sequence to undergo TCR recognition. T helper (Th) lymphocytes ($CD4^+$) usually recognize peptides of 12-16 amino acids in the context of class II HLA molecules. However, in some instances, smaller peptides may be recognized, which are more immunostimulatory than the parent ligand. Thus antigen recognition by T cells is highly degenerate and expands the potential for molecular mimicry because the universe of molecules containing a pentapeptide, for example, is many-fold greater than for 12 amino residue peptide. Once an immune response to one component of an immunogenic molecular complex has been elicited, other proteins/epitopes of the complex may become antigenic by the same process of epitope spreading.[18]

## Regulation of Autoimmunity

**KEY POINTS**

**Most patients with a UCTD have Raynaud's phenomenon in combination with unexplained arthralgias.**

**Approximately 35% of patients with a UCTD eventually manifested a defined CTD within 5 years**

**Nailfold capillary microscopy is useful in evaluating the potential for evolution into a defined CTD.**

**An increase in the ratio of Th17 cells to Tregs predicts the differentiation into a defined CTD.**

Once an autoimmune reaction becomes established, a counter-offensive is mounted by immuno-regulatory T cells (Tregs).[30] These cells, characterized by the presence of CD4, CD25, and Foxp3, develop in the thymus and are called *naturally occurring Tregs*. Another population of regulatory T cells develops in the periphery, and these are called *induced regulatory T cells* (iTregs); these are characterized by the presence of $CD4^+$ $CD25^+$ $Foxp3^+$ and the production of IL-10. In many autoimmune disorders, Th17 cells are pivotal in the initiation of the inflammatory response and tissue destruction. Th17 cells produce IL-17, which causes activation and migration of neutrophils; they also secrete IL-21 and IL-22. Lack of Th17 cells is associated with opportunistic infections. The ratio of Th17 cells to Tregs is commonly used as a marker of disease intensity or susceptibility to developing an autoimmune disorder (Figure 86-4).

## UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE

Rheumatologists frequently see patients with a weakly positive ANA and nonspecific symptoms such as arthralgias, fatigue, and cold sensitivity. The critical questions for such patients are "will they develop a connective tissue disease?" or "do they have fibromyalgia?" The answer to these questions is not always straightforward because fibromyalgia is not a diagnosis of exclusion.[31] It has a common comorbidity with the well-defined CTDs, and is often associated with cold-induced vasospasm.[32] An algorithm for diagnosing UCTDs is given in Figure 86-5. In the early stages of a CTD, there may be just one or two suspicious clinical and laboratory features, but a definitive diagnosis cannot always be made. In such cases a working diagnosis of UCTD may be appropriate.[33] Most patients with this UCTD have Raynaud's phenomena with or without an unexplained polyarthralgia, and a positive ANA with usually just a single autoantibody specificity, often anti-Ro and anti-RNP.[34] A 5-year follow-up study of 665 patients with UCTD reported a well-defined CTD (RA, 13.1%; Sjögren's syndrome, 6.8%; SLE, 4.2%; MCTD, 4.0%; Scl, 2.8%; systemic vasculitis, 3.3%; and PM/DM 0.5%) developed in only 34% of patients.[35] Certain combinations of features are predictive for the development of an established CTD: polyarthritis plus U1RNP antibodies predict MCTD, sicca symptoms plus anti–SS-A/SS-B antibodies predict Sjögren's syndrome, Raynaud's phenomenon plus a nucleolar ANA pattern predict Scl, polyarthritis plus high levels of rheumatoid factor predict RA and fever/serositis plus a homogeneous ANA pattern or anti-dsDNA antibodies predict progression into SLE (Table 86-2). In a follow-up study of 104 patients presenting with isolated Raynaud's phenomena, the presence of giant loops on capillaroscopy was a significant prognostic factor for the development of Scl with a hazard ratio of 2.64 (Figure 86-6).[36]

A shift in the balance between Th17 cells and Tregs is an important feature in the development of autoimmune

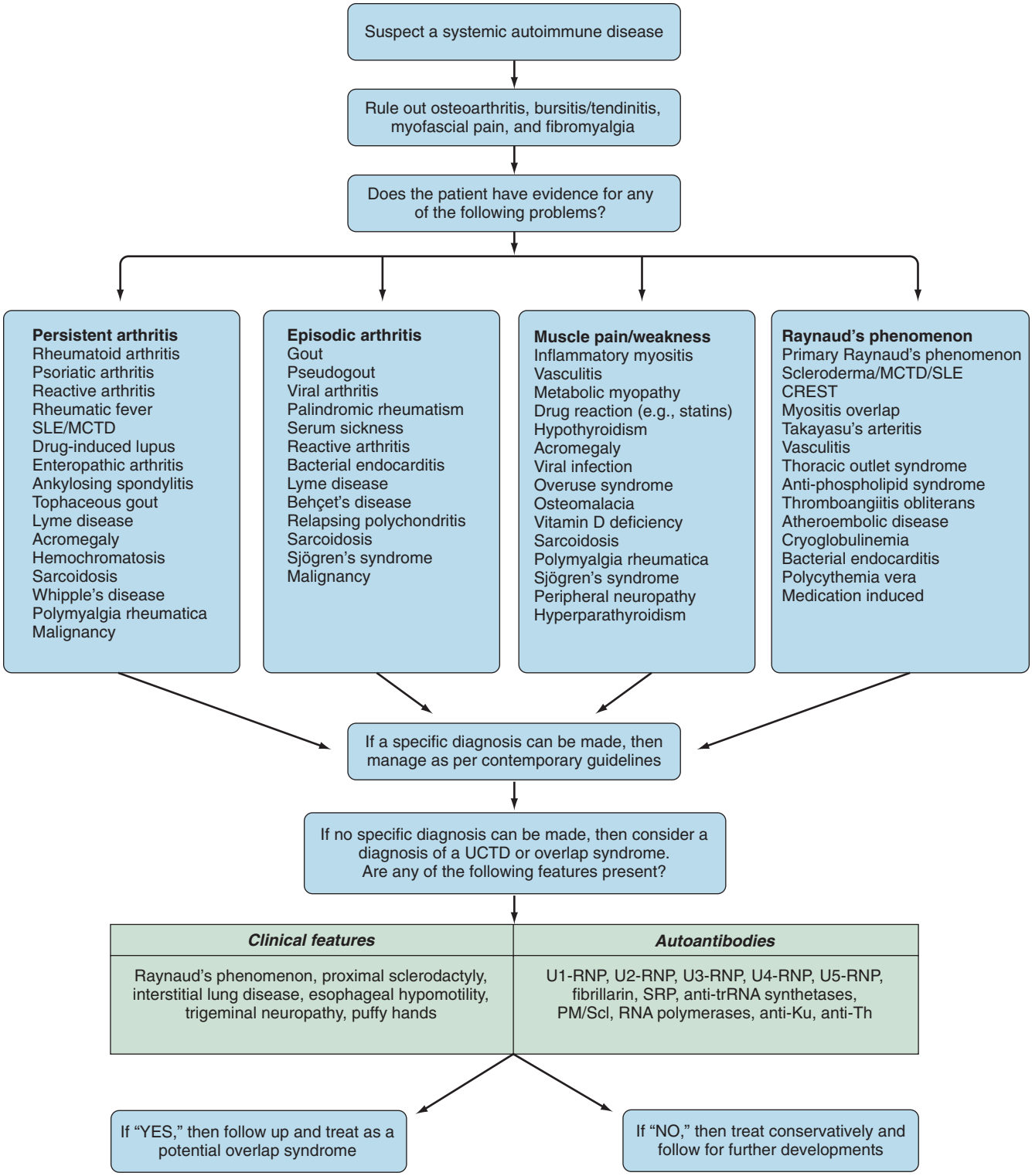



**Figure 86-4** Diagnostic algorithm for evaluating patients with undifferentiated connective tissue disease (UCTD). CREST, Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus.

disorders. In a 3-year follow-up of 51 patients with UCTDs, the ratios of Th17-cells to T cell regulatory subsets (either iTregs or Tregs) were evaluated with flow cytometry. Some 37.3% of the UCTD patients progressed to a systemic autoimmune disorder (SAID); the Th17/Treg ratio progressively increased from controls through UCTD patients, reaching the highest levels in patients in whom a defined systemic tissue disorder had developed (see Figure 86-4). This is a good example of how Tregs vary with disease expression and have the potential to be a prognostic marker.

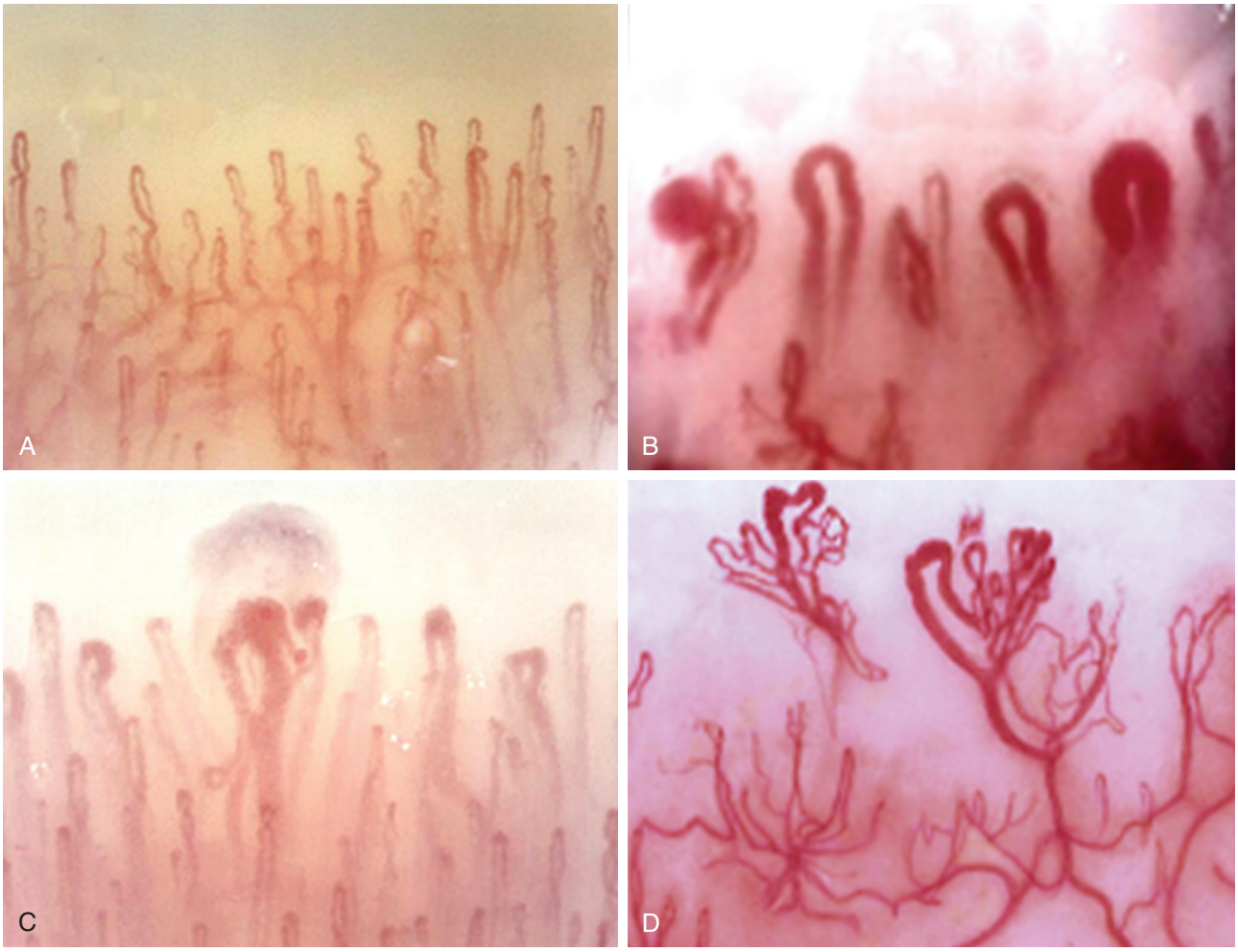



**Figure 86-5** Nailfold capillaroscopy in patients with systemic sclerosis and mixed connective tissue disease (MCTD). **A,** Normal capillaries. **B,** MCTD patient showing dilated and thickened capillary loops. **C,** Early scleroderma with irregular capillaries and mild dropout. **D,** Advanced scleroderma with capillary dropout and neoangiogenesis. *(Modified from Cutolo M. Capillaroscopy as an outcome measure for clinical trials on the peripheral vasculopathy in SSc—is it useful?* Int J Rheumatol *2010.)*

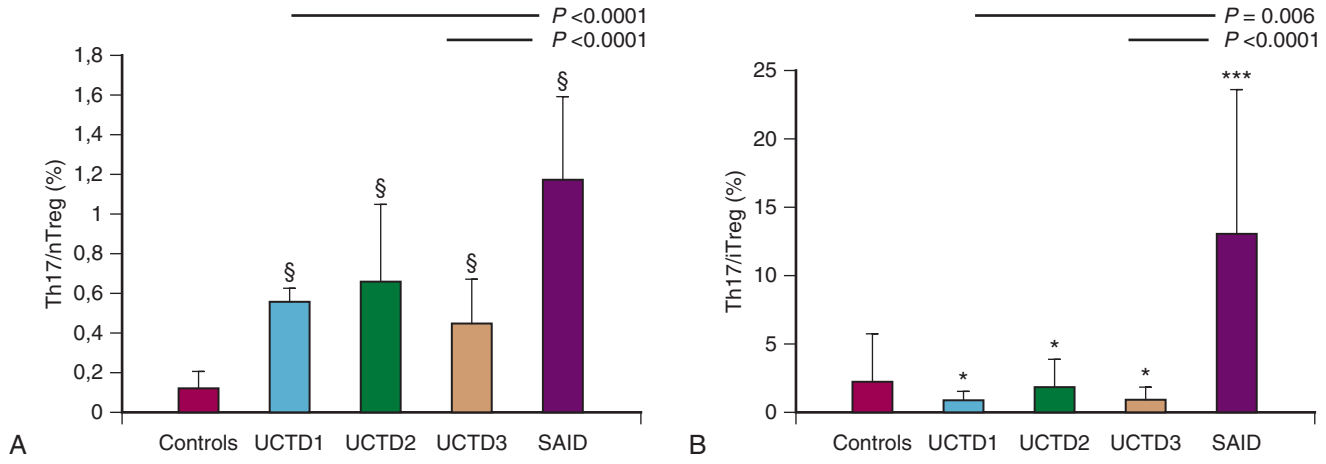



**Figure 86-6** The association of T helper (Th)17/natural Treg (nTreg) cells ratio in the evolution of undifferentiated connective tissue disease (UCTD) into differentiated systemic autoimmune disease (SAID). The ratio of Th cells producing interleukin 17 (Th17) to nTreg cells determines the propensity to develop an autoimmune disease as well as its intensity. Here it is seen that patients in various stages of UCTDs undifferentiated connective tissue diseases have a higher Th17/nTreg ratios than healthy controls but considerably lower than patients with SAID. **A,** Th17/nTreg (%) ratio in controls and patients with various stages of UCTDs and SAIDs. **B,** Th17/induced Treg (iTreg) (%) ratio in controls and patients with various stages of UCTD and SAIDs. UCTD1, UCTD2, UCTD3 (years of consecutive follow-ups). *(From Szodoray P, et al: Altered Th17 cells and Th17/regulatory T-cell ratios indicate the subsequent conversion from undifferentiated connective tissue disease to definitive systemic autoimmune disorders.* Hum Immunol *74:1510–1518, 2013.)*

**TABLE 86-2** Disorders Associated with Increased Fibrosis

| |
|---|
| **Localized** |
| Morphea |
| Scleredema |
| Scleromyxedema |
| Eosinophilic fasciitis |
| Peyronie's disease |
| Dupuytren's contracture |
| Pachydermoperiostitis |
| Idiopathic pulmonary fibrosis |
| Sclerosing cholangitis |
| Primary biliary cirrhosis |
| Cryptogenic fibrosis |
| **Systemic** |
| Scleroderma |
| Metastatic carcinoid |
| Retroperitoneal fibrosis |
| Graft versus host disease |
| Nephrogenic systemic fibrosis |
| Amyloidosis |

## SCLERODERMA OVERLAP SYNDROMES

**KEY POINTS**

- **CREST has a common overlap with primary biliary cirrhosis.**
- **Scl overlap syndromes include Scl variants such as CREST, myositis associated with sclerodactyly and mixed connective tissue disease (MCTD)**
- **Raynaud's phenomenon is often the first clinical feature of SSc overlaps and has to be distinguished from primary cold Raynaud's (i.e., cold induced vasospasm).**
- **The finding of thickened and dilated capillaries on nailfold microscopy and pathological autoantibodies (e.g., Scl-70, anticentromere, PM/Scl, and U1-RNP) are important clues as to the development of an overlap syndrome.**
- **CREST has a common overlap with primary biliary cirrhosis.**
- **Pulmonary fibrosis and pulmonary hypertension are the main causes of morbidity/mortality.**
- **Scl like disorders (e.g., eosinophilic fasciitis, scleromyxedema, nephrogenic fibrosis, scleredema) need to be considered in the differential diagnosis of Scl overlaps.**

Scl has a widespread heterogeneity of disease expression, ranging from a diffuse cutaneous disease, with a poor prognosis, to a limited cutaneous involvement, with a mostly good prognosis. Scl often has overlaps with one or more connective tissue disorders which, in turn, have associations with specific autoantibodies. In many cases, these overlaps occur in patients who do not have prominent skin involvement (sine Scl) or with the limited form of the disease: CREST (calcinosis, Raynaud's phenomena, esophageal dysmotility, sclerodactyly, and telangiectasia). In a German registry of 3240 Scl patients, 10% had an overlap syndrome. They were predominantly female (82.5%), with a mean age of 48 years, and developed musculoskeletal involvement more frequently (62.5%) than patients with limited Scl (32.2%) or diffuse Scl (43.3%).

Some 90% of patients with Scl have a positive ANA, and specific antibody profiles are associated with distinctive patterns of morbidity and mortality in Scl.[38] Rheumatoid factor is found in approximately 60% of patients with Scl; 35% have clinically evident synovitis.[39] Anti-citrullinated protein antibody (ACPA)is a predictor of erosive arthritis.[40] Erosive arthritis also has an association with anti-RA33; the Scl component in such overlap patients is often an incomplete form of CREST.[41] Anticentromere antibodies (ACA) are most commonly seen in patients with CREST. Less often there are overlapping features with Sjögren's syndrome, RA and primary biliary cirrhosis.[42] Patients with ACA antibodies generally have a milder form of Scl, but there is an increased prevalence of isolated pulmonary hypertension and Hodgkin's lymphoma.[43,44]

Anti-PM/Scl antibodies are associated with a myositis/Scl overlap. These antibodies are associated with improved survival compared with dcSSc alone (91% vs. 65% at 10 years), despite having more muscle involvement and pulmonary fibrosis.[45] A European review of 114 Scl overlap patients reported a 95% PM-Scl[46] antibody positivity with 80% having an inflammatory myositis. Anti-PM/Scl antibodies are reactive with several proteins of the nucleolar PM/Scl macromolecular complex.[47] The two major components are the PM/Scl-75 and PM/Scl-100 epitopes. Antibodies to PM-1α (the major component of PM/Scl-100) are positively associated with a younger age of onset, inflammatory myopathy, calcinosis, inflammatory arthritis, and overlap features; they are negatively associated with interstitial lung disease and gastrointestinal involvement; and they produce a pattern of nucleolar staining on indirect immunofluorescence.[48] Muscle biopsies in PM/Scl overlap patients are distinguished by a high frequency of necrotic muscle fibers.

Anti-U3 RNP antibodies are also associated with skeletal muscle involvement and pulmonary hypertension.[49] Anti-U11/U12 antibodies are only seen in approximately 3% of Scl patients, but are strongly predictive of lung fibrosis.[50] Antimitochondrial antibodies (AMA) have an association with primary biliary cirrhosis and the limited form of Scl.[51] Interestingly, AMA are often reactive with the anti-70 and 50 kDa antibodies seen in patients with diffuse Scl,[52] thus providing an autoimmune link between the two disorders.

Antipolymerase III antibodies occurring in recent onset Scl have been associated with a diagnosis of cancer (mainly breast).[53]

The occurrence of anti-Ku in patients with Scl is often associated with inflammatory myopathy and interstitial lung disease.[54]

Several nonscleroderma fibrotic disorders may be mistaken for an Scl overlap at initial presentation (see Table 86-2). Although these disorders may have some systemic involvement, they seldom exhibit overlap features with other AICTDs.[55,56]

Scleromyxedema is characterized by cutaneous mucinosis and is often associated with a gammopathy, usually immunoglobulin (Ig)M and light chains.[60] The mucinous skin lesions appear as waxy papules on the face, neck, and limbs. If the papules coalesce, the condition may be mistaken for Scl (Figure 86-7A). Systemic involvement may occur with dysphagia, proximal muscle weakness, and pulmonary, cardiac, and renal complications. It is difficult




**Figure 86-7** Four examples of fibrosing disorders. **A,** Scleromyxedema (mucinous skin lesions appear as waxy papules on the face). **B,** Eosinophilic fasciitis (induration of skin and subcutaneous tissues). **C,** Scleredema (diffuse subcutaneous edema with swelling over right trapezius). **D,** Nephrogenic systemic fibrosis (coalescence of indurated nodules with joint contracture). *(Modified from Boin F, Hummers LK: Scleroderma-like fibrosing disorders.* Rheum Dis Clin N Am *34:1-17, 2008.)*

to manage; corticosteroids are usually tried initially, in refractory cases some benefit has been reported for intravenous immunoglobulin and thalidomide.

Eosinophilic fasciitis presents with limited Scl, such as skin changes involving the extremities (Figure 86-7B). The correct diagnosis is suggested by finding a peripheral eosinophilia and a hypergammaglobulinemia.[58] The definitive diagnosis is established by a full thickness skin biopsy that shows a diffuse inflammation of the fascia. MRI can confirm the thickened fascia and guide biopsy (Figure 86-8).[59] Initial treatment is with corticosteroids (prednisone, 0.5-1.0 mg/kg) tapering according to the clinical response; some patients need to continue moderate-dose corticosteroids for as long as 2 years.[58] Methotrexate and mycophenolate has been used in refractory cases.

Scleredema is a cutaneous mucinosis that often starts with a febrile episode and resolves spontaneously.[61] More chronic scleredema has been associated with paraproteinemias, including multiple myeloma and diabetes mellitus. The dermis is thickened with increased collagen glycosylation, as in diabetic stiff skin syndrome. The face and neck are commonly involved and there is relative sparing of the hands and feet (see Figure 86-7C). Systemic organ involvement is rare, but a monoclonal gammopathy is sometimes seen. Such cases need to be worked up for lymphoma. Refractory cases have been helped by local radiotherapy.

Nephrogenic systemic fibrosis (NSF) is a fibrotic disorder that, in some patients, develops following exposure to gadolinium-containing contrast agents. Most patients have pre-existing renal disease.[57] Histologically, there is fibroblast proliferation, thickened collagen bundles, and deposits of mucin, similar to those observed in scleromyxedema. The clinical presentation is a rapid progression with confluence of initially focal areas of indurated skin (see Figure 86-7D). The face is usually spared, but joint contractures may occur at the elbows and knees, and systemic involvement with pulmonary and neurological symptoms can develop in refractory cases. NSF is usually nonresponsive to corticosteroids and immunosuppressive therapy.

## LUPUS OVERLAP SYNDROMES

**KEY POINTS**

**Except for an overlap of SLE and rheumatoid arthritis, so-called "rhupus," and an overlap with Sjögren's syndrome, lupus overlaps are relatively uncommon.**

**SLE and RA overlap is seen in about 10% of SLE patients. The typical clinical picture is that of classical SLE in association with a deforming and often erosive arthritis; patients are often RF and ACPA positive.**

**An overlap of SLE and Sjögren's syndrome occurs in about 25% of lupus patients. These patients more commonly have Raynaud's phenomena than are classical SLE patients, and are usually positive for anti-Ro/SS-A antibodies.**

Unlike Scl overlaps, well-defined overlap syndromes with SLE are unusual. They mostly consist of isolated case reports of SLE associated with specific organ damage (e.g., hepatitis,

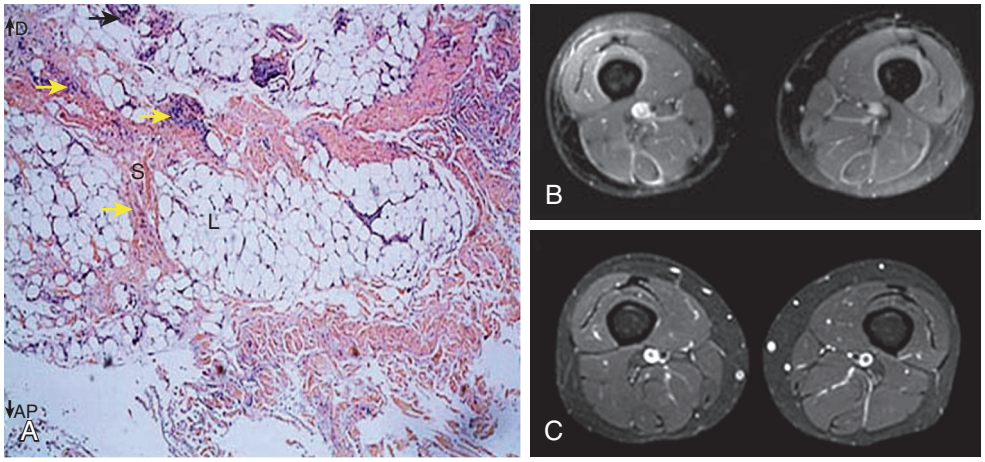



**Figure 86-8** The utility of magnetic resonance imaging in guiding the biopsy in a patient with eosinophilic fasciitis. **A,** Biopsy of quadriceps fascia (hematoxylin & eosin staining, magnification ×50). Deep dermis with fat lobules (L) and interlobular septas (S). Inflammatory cells composed of lymphocytes, mononuclear cells, and occasional eosinophilic cells, infiltrate the septa *(yellow arrows)*. **B,** Axial T2-weighted fat-suppressed MR images, showing thickening and signal hyperintensity of muscular fascias, involving both thighs. **C,** Axial T2-weighted fat-suppressed MR images (water IDEAL sequences) after corticosteroid treatment, showing normal findings, revealed complete remission. *(From Desvignes-Engelberet A, et al: From diagnosis to remission: place of MRI in eosinophilic fasciitis.* Clin Rheumatol *29:1461-1464, 2010; and modified from Pope JE: Other manifestations of mixed connective tissue disease.* Rheum Dis Clin N Am *31:519–533, 2005.)*

orchitis, and pancreatitis). The one exception is the overlap of RA and SLE, commonly referred to as "rhupus."[62] This overlap occurs in approximately 10% of SLE patients. The clinical picture is usually more consistent with SLE, with a deforming and often erosive arthropathy (Figure 86-9), accompanied by less severe renal involvement. Erosive arthritis in SLE is associated with the presence of ACPA.[63] This overlap must be distinguished from nonerosive Jaccoud's arthropathy, which is characterized by mild joint pain without synovitis, radiographs that show prominent ulnar deviation, swan neck deformities, and Z deformity of the thumb.

An overlap of SLE with Sjögren's syndrome (SS) occurs in some 10% to 25% of patients; typically in SLE patients aged 25 years or older.[64] The clinical picture is notable for a higher frequency of Raynaud's phenomena and a lower frequency of renal involvement, thrombocytopenia, and lymphadenopathy. The presence of anti-Ro/SS-A antibodies at the time of SLE diagnosis predicts the eventual development and SLE/SS overlap.[65]

## MYOSITIS OVERLAP SYNDROMES

| KEY POINTS |
|---|
| Myositis overlap syndromes are more common than the classic presentations of PM or DM. |
| Aminoacyl-tRNA synthetase (ARS) antibodies are associated with myositis, arthritis, and interstitial lung disease. |
| Arthritis and interstitial lung disease may antedate the appearance of myositis in patients with ARS. |
| Antibodies to synthetases, SRP, and nucleoporin tend to be associated with corticosteroid unresponsiveness. |
| Antibodies to U1RNP, PM-Scl, or Ku tend to be associated with corticosteroid responsiveness |
| Antibodies to TIF1-γ (p155/140) have been associated with an increased risk of myositis-associated malignancy. |

PM, DM and inclusion body myositis (IBM) and necrotizing autoimmune myositis (NAM) are the classic idiopathic inflammatory myopathies (IIM). Myositis-specific autoantibodies target proteins involved in key processes of protein synthesis and tend to be associated with distinctive clinical features within the spectrum of myositis.[66] These autoantibodies include the aminoacyl-tRNA synthetases (ARS), the Mi-2 helicase/histone deacetylase protein complex, and the signal recognition particle (SRP).[67] The ARS family of enzymes catalyze the transfer of a specific amino acid to its cognate transfer RNA. Antibodies against eight different ARS have been reported: Jo-1 (histidyl), PL-7 (threonyl), PL-12 (alanyl), OJ (isoleucyl), EJ (glycyl), KS (asparaginyl), Ha (tyrosyl) and Zo (phenylalanyl). The clinical features associated with ARS are often referred to as the "anti-synthetase syndrome:" a fluctuating inflammatory myopathy associated with fever, interstitial lung disease, Raynaud's syndrome, and mechanic's hands[68] (Figure 86-10). The arthritis of ARS may initially mimic RA with an inflammatory arthritis and nodules; erosions, however, do not occur.[69] Interstitial lung disease may be a presenting clinical feature of patients with ARS antibodies, with myopathy occurring much later. The association of myositis in patients with anti-U1-RNP antibodies is usually seen in the context of MCTD.[70] Antibodies to the SRP have been reported in 4% of patients with Scl/PM overlap; these patients usually have a severe, rapidly progressive myositis with prominent muscle fiber necrosis without much inflammatory cell infiltration.[71]

An association between DM and cancer has long been documented; this association has recently been linked to specific antibodies.[72] Breast, lung, and colorectal cancer are the most commonly associated cancers in Western countries, and nasopharyngeal carcinoma in China and Southeast Asia.[73] The patient's risk for cancer is highest during the first year after the diagnosis of the myositis, suggesting that the myopathy may occur as a paraneoplastic phenomenon. In this respect, the association of human transcriptional intermediary factor 1-gamma (TIF1-γ), formerly

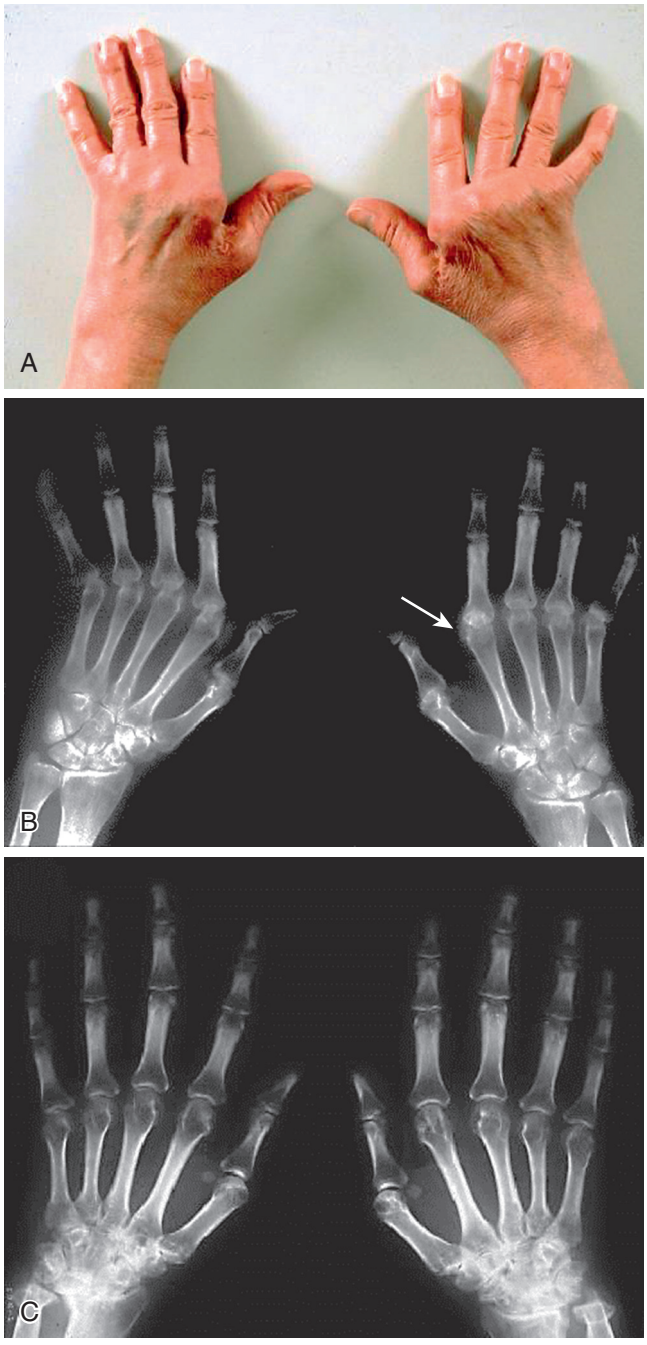



**Figure 86-9** Comparison of radiographic findings in a patient with rheumatoid arthritis (RA)/systemic lupus erythematosus (SLE) overlap (rhupus) and a lupus patient with Jaccoud's arthropathy. **A**, A 66-year-old woman was diagnosed with RA at 30 years and developed SLE at 37 years. The diagnosis of SLE was made based on the presence of leukopenia, positive anti-nuclear, anti-DNA, ribonuclear proteins, anti-Sm antibodies, and membranous-type lupus nephritis. Her rheumatoid factor was 1:320, and anticyclic citrullinated protein antibodies were 5.1 U/mL (normal <4.5). A recent radiograph of the patient's hands showed severe bilateral ulnar dislocation of the fingers without erosions in the metacarpophalangeal joints. The carpal bones were almost completely resorbed on the left side and fused on the right side. Diagnosis: rhupus. **B**, A 44-year-old female with a history of SLE starting at age 10 years, with malar rash, photosensitivity, leukopenia, oral ulcerations, alopecia, and a deforming arthropathy. The fingers' subluxations resemble rheumatoid arthritis but were completely reversible. Apart from some periarticular osteopenia, the radiographs are normal. In particular, there are no erosions. Diagnosis: Jaccoud's arthropathy *(From Nakamura J, et al: Radiographic features of rhupus arthropathy.* Intern Med *52:2837, 2013.)*



**Figure 86-10** Mechanic's hand in a patient with anti-synthetase syndrome. This patient with dermatomyositis presented with mechanics and (thickening and cracking of the skin, a result of hyperkeratosis), shortly followed by interstitial lung disease and arthritis. The presence of anti-Jo1 antibodies confirmed the suspicion of an anti-synthetase syndrome.

known as anti-p155/140, with idiopathic inflammatory myopathies is especially relevant.[74] TIF1-γ plays a key role in regulating the TGF-β signaling pathway, an essential intermediary in the development various types of cancer.

Overlap syndromes of inflammatory myopathy with SLE, Scl, MCTD, and Sjögren's syndrome are quite common.[75] Such overlaps, especially with Scl, have been reported as more common than the classic description of PM.[4] When clinical overlaps emerge, they are most commonly associated with specific autoantibodies: namely anti-PM-Scl, anti-Ku, U1 RNP, Jo-1, SRP.[76] A 2006 clinical and longitudinal study of 100 consecutive French-Canadian patients with idiopathic IIM concluded that the original Bohan and Peter classification of inflammatory myopathies should be abandoned, because 60% of patients with IIM were found to have overlap syndrome.[4] In this study, an overlap syndrome was based on the presence of an inflammatory myopathy as per the Bohan and Peter classification,[77] plus at least one clinical overlap feature (Table 86-3), or one of the following autoantibodies: synthetases, centromere, topo I, RNA-polymerases I or III, Th; U1RNP, U2RNP, U3RNP, U5RNP, Pm-Scl, Ku, SRP and nucleoporins (see Table 86-3). The distinction between classic PM/DM and an overlap syndrome was reported to be of prognostic/therapeutic significance, because classic PM nearly always manifested a chronic course, with 50% of patients initially unresponsive to corticosteroid therapy. Pure DM was almost always chronic, but most patients had an initial response to corticosteroids. On the other hand, patients with myositis overlap syndromes (usually with Scl features) were almost always responsive to corticosteroids (~90% response rate). When overlap patients were divided according to antibody subsets, anti-synthetase, SRP, and nucleoporin aAbs were markers for treatment-resistant myositis, whereas aAbs to U1RNP, PM-Scl, or Ku were markers for corticosteroid responsiveness. Patients with autoimmune myositis, especially DM are at risk for cancer,[78] and it has been problematic as to how far and how often one should pursue a

**TABLE 86-3** Suggested Classification for Inflammatory Myopathies

| Abbreviation | Description |
|---|---|
| PM | Pure polymyositis |
| DM | Pure dermatomyositis |
| OM | Overlap myositis: myositis with at least 1 clinical overlap feature and/or an overlap autoantibody |
| CAM | Cancer-associated myositis: with clinical paraneoplastic features and without an overlap autoantibody or anti-Mi-2 |
| **Definition of Polymyositis** | |
| 1) Symmetric proximal muscle weakness | |
| 2) Elevation of serum skeletal muscle enzymes | |
| 3) Electromyographic triad of short, small, polyphasic motor unit potentials; fibrillations, positive sharp waves and insertional irritability; and bizarre, high-frequency repetitive discharges | |
| 4) Muscle biopsy abnormalities of degeneration, regeneration, necrosis, phagocytosis, and an interstitial mononuclear infiltrate | |
| 5) Typical skin rash of DM, including the heliotrope rash, Gottron sign, and Gottron papules | |
| • *Definite myositis:* 4 criteria (without the rash) for PM, 3 or 4 criteria (plus the rash) for DM | |
| • *Probable myositis:* 3 criteria (without the rash) for PM, 2 criteria (plus the rash) for DM | |
| • *Possible myositis:* 2 criteria (without the rash) for PM, 1 criterion (plus the rash) for DM | |
| **Definition of Clinical Overlap Features** | |
| Inflammatory myopathy plus of the following clinical findings: polyarthritis, Raynaud's phenomenon, sclerodactyly, scleroderma proximal to the metacarpophalangeal joints, typical systemic sclerosis-type calcinosis in the fingers, lower esophageal or small-bowel hypomotility, diffusing capacity for carbon monoxide lower than 70% of the normal predicted value, interstitial lung disease on chest radiogram or computed tomography scan, discoid lupus, anti-native DNA antibodies plus hypocomplementemia, four or more of 11 American College of Rheumatology systemic lupus erythematosus criteria, anti-phospholipid syndrome | |
| **Definition of Overlap Autoantibodies** | |
| Anti-synthetases (Jo-1, PL-7, PL-12, OJ, EJ, KS), scleroderma (Scl)-associated autoantibodies (Scl-specific antibodies: centromeres, topo I, ribonucleic acid-polymerases I or III, Th; and antibodies associated with Scl in overlap: U1RNP, U2RNP, U3RNP, U5RNP, Pm-Scl, Ku), and other autoantibodies (SRP, nucleoporins) | |
| **Definition of Clinical Paraneoplastic Features** | |
| Cancer within 3 yrs. of myositis diagnosis, plus the absence of multiple clinical overlap features, plus if cancer was cured, myositis was cured as well | |

Modified from Troyanov Y, et al: Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. *Medicine (Baltimore)* 84(4):231-249, 2005.

malignancy work-up. It is now apparent that the finding of an antibody against 155 kDa and 140 kDa protein specificities (anti-155/140 antibody) signifies a significant risk for the co-occurrence of a malignancy and points to the need for a thorough cancer work-up.[79]

## MIXED CONNECTIVE TISSUE DISEASE

**KEY POINTS**

- **The clinical overlap features of MCTD (i.e., Scl, SLE and PM/DM) seldom occur concurrently, but develop sequentially over months or years.**
- **Raynaud's phenomenon is seen in nearly all patients with MCTD; if it is absent, the diagnosis should be reconsidered.**
- **About 25% of MCTD patients develop renal involvement—usually membranous glomerulonephritis. Proliferative glomerulonephritis is uncommon in MCTD.**
- **Serious CNS involvement is rare in MCTD; the most common neurologic findings are trigeminal neuropathy and sensorineural hearing loss.**
- **Pulmonary HTN is the most common cause of death in MCTD patients, and it should be screened for on an ongoing basis.**

MCTD was described by Sharp and colleagues in a 1972 paper reporting an overlap of SLE, Scl, and PM.[80] This was the first overlap syndrome defined in terms of a specific antibody, namely, antibodies to a ribonuclease-sensitive extractable nuclear antigen (ENA). During the last 38 years, many studies have explored the clinical correlates of this antibody system (now called *U1 RNP*).

### Serologic Features

The basic premise of the MCTD concept is that the presence of high-titer anti-U1 RNP antibodies modifies the expression of a DCTD in ways which are relevant to prognosis and treatment.[81] The first clue to diagnosing MCTD is usually a positive ANA with a high titer speckled pattern. The titer is often greater than 1:1000 and sometimes greater than 1:10,000. This finding should prompt the measurement of antibodies to U1-RNP, Sm, Ro, and La. It is also pertinent to note whether the serum contains antibodies to dsDNA and histones, because patients destined to follow a course most consistent with MCTD have sera with predominant U1-RNP reactivity. Antibodies to dsDNA, Sm, and Ro are occasionally seen as a transient phenomenon in patients with MCTD. But when they are found

consistently, as the predominant antibody system, the clinical picture is usually more consistent with classic SLE. Antibodies to the 70 kDa antigen, especially in its apoptotic form, are most closely associated with the clinical correlates of MCTD.[10]

## Clinical Features

### Diagnosis

MCTD is an overlap syndrome that embraces features of SLE, Scl, and PM/DM.[82] These overlap features seldom occur concurrently. It usually takes several years before enough overlapping features have appeared for one to be confident that MCTD is the most appropriate diagnosis.[83] The most common clinical associations with U1 RNP antibodies in the early phase of the disease are hand edema, arthralgia, Raynaud's phenomenon, inflammatory muscle disease, and sclerodactyly. No American College of Rheumatology criteria exist for diagnosis of MCTD, but a comparative study reported that two criteria sets, those of Alarcon-Segovia and Kahn, had the best sensitivity and specificity (62.5 % and 86.2%, respectively)[84] (Table 86-4). The sensitivity could be improved to 81.3% if the term "myalgia" was substituted for "myositis."[85] Some patients initially diagnosed with MCTD will evolve into a clinical picture most consistent with SLE or RA. In one long-term follow up more than half of the patients continued to satisfy criteria for MCTD.[86] A comparison of the clinical and serologic features of MCTD with SLE, RA, Scl, and PM/DM is given in Table 86-4.

### Early Symptoms

In the early stages, most patients destined to develop MCTD cannot be differentiated from patients with other classic AICTDs (Table 86-5). The assumption that a diagnosis of MCTD implies a simultaneous presence of features usually seen in SLE, Scl, and PM is erroneous. It is unusual to see such an overlap during the early course of MCTD, but with the progress of time the overlapping features usually occur sequentially. Early in the course of the disease, most patients complain of fatigue, poorly defined myalgias, arthralgias, and Raynaud's phenomenon. At this point in time a diagnosis of RA, SLE, or UCTD seems most appropriate.[1] If such a patient is found to have swollen hands and/or puffy fingers in association with a high-titer speckled ANA, they should be carefully followed up for the evolution of overlap features (see Table 86-3). A high titer of anti-RNP antibodies in a patient with UCTD is a powerful predictor for a later evolution into MCTD. Less commonly, there is an acute onset of MCTD, which gives little clue to the subsequent course; such presentations have included PM, acute arthritis, aseptic meningitis, digital gangrene, high fever, acute condition in the abdomen, and trigeminal neuropathy.[87]

**TABLE 86-4** Two Diagnostic Criteria for Mixed Connective Tissue Disease

| Alarcón-Segovia Criteria | Kahn Criteria |
|---|---|
| **A. Serologic Criteria**<br>Anti-RNP at hemagglutination titer of ≥1:1600 | **A. Serologic Criteria**<br>High-titer anti-RNP corresponding to a speckled ANA of ≥1:1200 titer |
| **B. Clinical Criteria**<br>1. Swollen hands<br>2. Synovitis<br>3. Myositis (biologically proven)<br>4. Raynaud's phenomenon<br>5. Acrosclerosis | **B. Clinical Criteria**<br>1. Swollen fingers<br>2. Synovitis<br>3. Myositis<br>4. Raynaud's phenomenon |
| **MCTD Present if:**<br>Criterion A is accompanied by three or more clinical criteria—one of which must include synovitis or myositis. | **MCTD Present if:**<br>Criterion A is accompanied by Raynaud's phenomenon and two or more of the three remaining clinical criteria. |

ANA, Anti-nuclear antibodies; MCTD, mixed connective tissue disease; RNP, ribonuclear protein.

From Bischoff L, Derk CT: Eosinophilic fasciitis: demographics, disease pattern and response to treatment: report of 12 cases and review of the literature. *Int J Dermatol* 47(1):29-35, 2008.

### Fever

Fever may be a prominent feature of MCTD in the absence of an obvious cause.[87] Fever of unknown origin has been the initial presenting symptom of MCTD; after careful evaluation, fever in patients with MCTD can usually be traced to a coexistent myositis, aseptic meningitis, serositis, lymph adenopathy, or intercurrent infection.[87]

### Bones and Joints

Joint pain and stiffness is an early symptom in nearly all patients in whom the MCTD syndrome develops. During the past 2 decades it has become increasingly apparent that joint involvement in MCTD is more common and more severe than in classic SLE.[88] An obvious arthritis, often with deformities commonly seen in RA (e.g., ulnar deviation and swan neck and boutonniere deformities),[89] eventually develops in approximately 60% of patients with MCTD (Figure 86-11). Radiographs usually show a characteristic absence of severe erosive changes, and they often resemble Jaccoud's arthropathy. Small marginal erosions, often with a well-demarcated edge, are the most



**Figure 86-11** Deforming arthritis in a patient with mixed connective tissue disease, characterized by MCP synovitis, ulnar deviation, and swan neck deformities. These findings are clinically indistinguishable from rheumatoid arthritis, including the presence of erosions. *(Courtesy Robert M. Bennett, MD.)*

**TABLE 86-5** Differential Features of Classic Autoimmune Connective Tissue Diseases

| System Involved | Clinical Feature | SLE | RA | Scl | PM | MCTD |
|---|---|---|---|---|---|---|
| **Joints** | *Arthralgias (nonspecific)* | 3 + | 4 + | 3 + | 1 + | 4 + |
| | *Erosive arthritis* | ± | 4 + | 1 + | ± | 2 + |
| | *Inflammatory arthritis* | 1 + | 4 + | 1 + | ± | 3 + |
| **Skin/mucous membranes** | *Butterfly rash* | 3 + | – | – | – | 1 + |
| | *Oral ulcers* | 2 + | – | ± | – | 1 + |
| | *Sclerodactyly* | ± | – | 4 + | – | 1+ |
| | *Nonacral thickening* | – | – | 3 + | – | – |
| | *Swollen fingers* | 1 + | – | ± | – | 2 + |
| | *CREST syndrome* | – | – | 3 + | – | ± |
| **Pulmonary** | *Pulmonary artery hypertension* | 2 + | ± | 1 + | 1 + | 3 + |
| | *Pulmonary Interstitial fibrosis* | 1 + | ± | 3 + | 2 + | 2 + |
| | *Pleurisy* | 3 + | 1 + | 1 + | – | 2 + |
| **Cardiac** | *Pericarditis* | 3 + | 1 + | 1 + | – | 3 + |
| | *Interventricular conduction defects* | 2 + | 1 + | 2 + | – | 1 + |
| | *Myocarditis* | 2 + | ± | 1 + | – | 2 + |
| **Neurologic** | *Seizures/psychosis* | 3 + | – | – | – | – |
| | *Aseptic meningitis* | 3 + | ± | – | – | 3 + |
| | *Peripheral neuropathy* | 2 + | 2 + | ± | – | 2 + |
| | *Sensineuronal hearing loss* | 1 + | ± | – | – | 2 + |
| | *Transverse myelitis* | 3 + | 1 + | – | – | 2 + |
| | *Trigeminal neuropathy* | 1 + | ± | 2 + | – | 3 + |
| **Vascular** | *Raynaud's phenomena* | 2 + | – | 4 + | 1 + | 4 + |
| | *Inflammatory vasculitis* | 2 + | 2 + | 1 + | 1 + | 1 + |
| | *Non-inflammatory vasculopathy* | 1 + | – | 4 + | 1 + | 3 + |
| | *Renovascular hypertension* | 1 + | – | 3 + | – | 3 + |
| **Renal** | *Membranous nephritis* | 3 + | – | – | – | 2 + |
| | *Diffuse proliferative glomerulonephritis* | 4 + | – | – | – | 1 + |
| **Muscle** | *Myalgias (nonspecific)* | 3 + | ± | 1 + | 4 + | 4 + |
| | *Inflammatory myopathy* | 1 + | 1 + | 1 + | 4 + | 3 + |
| **Gastrointestinal** | *Esophageal dysmotility* | 1 + | ± | 4 + | 1 + | 3+ |
| | *Bacterial overgrowth malabsorption* | 2 + | ± | 3 + | ± | 1 + |
| | *Colonic pseudo–diverticula* | ± | ± | 3 + | – | 2 + |

MCTD, Mixed connective tissue disease; PM, polynyositis; RA, rheumatoid arthritis; SCL, scleroderma; SLE, systemic lupus erythematosus.



**Figure 86-12** A radiograph of a patient with mixed connective tissue disease, showing two small, well-demarcated erosions *(arrows)* at the base of the second and third phalanxes. *(Courtesy Robert M. Bennett, MD.)*

characteristic radiologic feature in patients with severe joint disease[90] (Figure 86-12). However, a destructive arthritis, including an arthritis mutilans, is a well-established association[89] (Figure 86-13). Erosive disease typically starts approximately 5 years after the onset of MCTD and is usually associated with ACPA positivity.[40,91]

Flexor tenosynovitis, bone edema, and pericapsular inflammation reminiscent of a seronegative spondyloarthropathy develops in some patients. In a follow-up study of 201 MCTD patients, Szodoray found osteoporosis in 32.3%, whereas 18.9% had osteoporotic vertebral fractures (mainly those on long-term corticosteroids).[91]

## Skin and Mucous Membranes

Mucocutaneous changes develop in most patients with MCTD sometime during the course of the syndrome.[92] Raynaud's phenomenon is the commonest problem, and one of the earliest manifestations of MCTD.[93] It may be accompanied by puffy, swollen digits and, sometimes, total hand edema.[87] In some patients, skin changes commonly associated with classic SLE are prominent findings, particularly malar rash and discoid plaques (Figure 86-14). Other problems include buccal ulceration, sicca complex, orogenital ulceration, livedo vasculitis, subcutaneous nodules, and nasal septal perforation.

## Muscle

Myalgia is a common symptom in patients with MCTD syndrome. In most patients there is no demonstrable weakness, EMG abnormalities, or muscle enzyme changes. It is



**Figure 86-13** A radiograph of a patient with mixed connective tissue disease with a destructive arthritis and digital osteolysis. *(Courtesy Robert M. Bennett, MD.)*

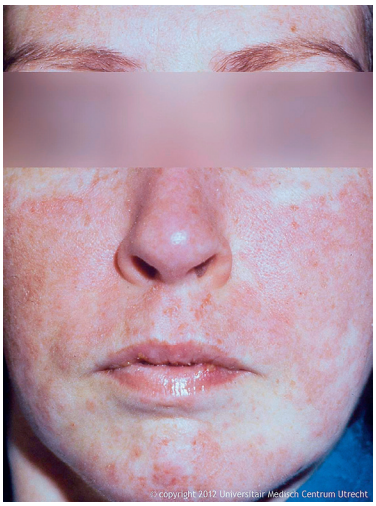



**Figure 86-14** A patient with mixed connective tissue disease showing facial changes typical of systemic lupus erythematosus. *(Copyright University Medical Center Utrecht, 2012.)*

often unclear whether the symptom represents a low-grade myositis, physical deconditioning, or an associated fibromyalgia syndrome. The inflammatory myopathy associated with MCTD is similar histologically to IIM, with features of both the vascular involvement as DM and cell-mediated changes of PM.[94] In most patients, myositis occurs as an acute flare against a background of general disease activity. Such patients usually respond well to a short course of high-dose corticosteroid therapy. Another scenario is that of a low-grade inflammatory myopathy, which is often insidious

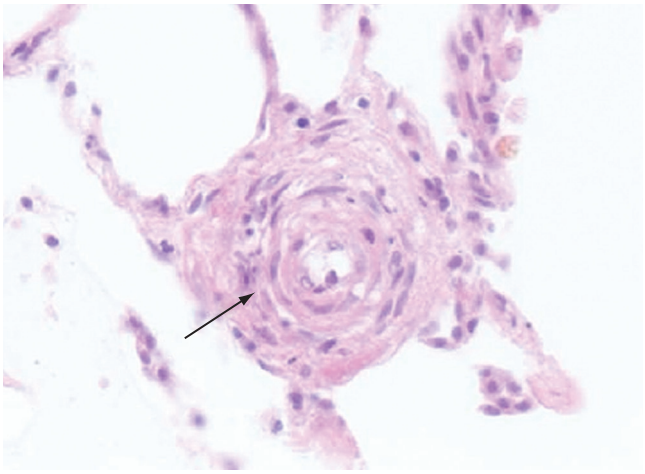


**Figure 86-15** Intimal hyperplasia and smooth muscle hypertrophy without accompanying inflammation are the characteristic features of the vasculopathy of mixed connective tissue disease. When it occurs in the lung, as shown here, it may give rise to severe pulmonary hypertension. (Note absence of pulmonary fibrosis.) The plexiform lesion *(arrow)* is a characteristic pathologic finding in this disease process. *(From Bull TM, et al: Pulmonary vascular manifestations of mixed connective tissue disease.* Rheum Dis Clin N Am *31:451–464, 2005.)*

in its onset. These patients often have a poor therapeutic response to corticosteroids. An impressive fever develops in some patients with PM associated with MCTD[87]; other patients may give a history of febrile myalgias that have been diagnosed as "flu."

## Blood Vessels

Raynaud's phenomenon is an early feature of nearly all patients who are eventually diagnosed with MCTD.[93] A bland intimal proliferation and medial hypertrophy that affects medium- and small-size vessels is the characteristic vascular lesion of MCTD[95] (Figure 86-15) and is also the characteristic pathology in pulmonary hypertension and renovascular crises. Both nailfold capillary microscopy[96] and color Doppler[97] are useful in distinguishing benign primary Raynaud's phenomenon from secondary involvement in MCTD and other AICTDs (see Figure 5). Fingernail capillaroscopy in MCTD patients shows the same pattern of capillary dilation and drop out that has been reported in Scl.[98,99] An angiographic study reported a high prevalence of medium-size vessel occlusions[100] (Figure 86-16). As in SLE, premature atherosclerosis is a problem in many MCTD patients.[101] Risk factors include dyslipidemia, anticardiolipin antibodies, endothelial cell antibodies, and persistent chronic inflammation.[102] Antibodies to heat shock protein (HSP)60 have a strong association with atherosclerotic disease in MCTD and provide a direct indication of endothelial cell injury.[103]

## Heart

All three layers of the heart may be involved in MCTD.[104] An abnormal electrocardiogram (EKG) is seen in approximately 20% of patients. The most common EKG changes are right ventricular hypertrophy, right atrial enlargement, and interventricular conduction defects. Pericarditis is the

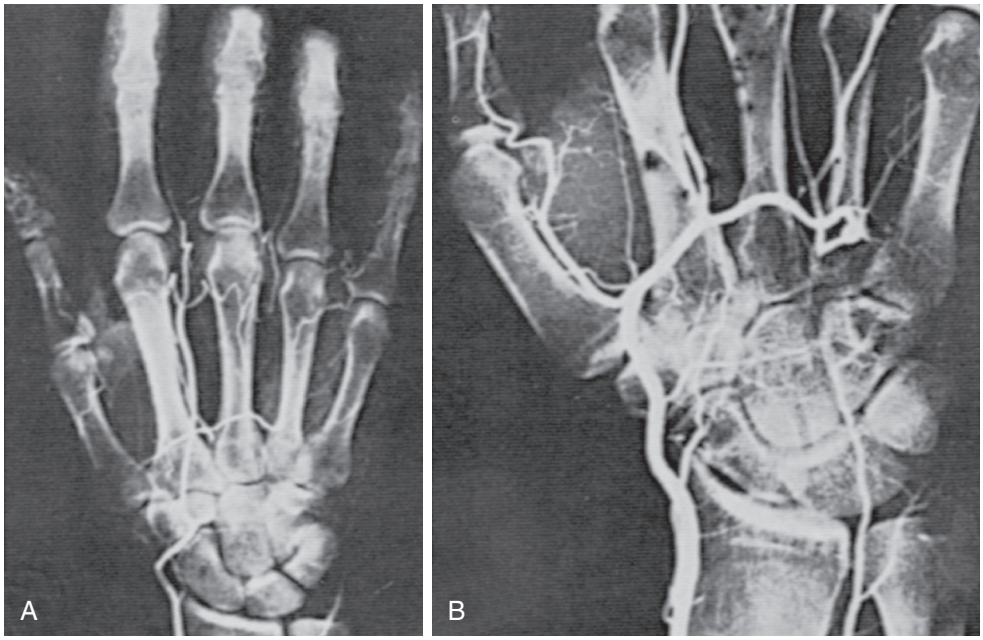



**Figure 86-16** **A**, Digital angiogram showing multiple arterial occlusions with collateral formation. **B,** Digital angiogram showing ulnar artery occlusions. *(From Peller JS, et al: Angiographic findings in mixed connective tissue disease: correlation with fingernail capillary photo-microscopy and digital photoplethysmography findings.* Arthritis Rheum *28:768, 1985. Reprinted with permission of the American College of Rheumatology.)*

most common clinical manifestation of cardiac involvement, reported in 10% to 30% of patients, whereas pericardial tamponade is rare. Involvement of the myocardium is increasingly recognized. In some patients, myocardial involvement is secondary to pulmonary hypertension (PAH); this occurs in approximately 20% of patients and is often asymptomatic in its early stages.[105] The early detection of pulmonary hypertension is increasingly important, because there are now more effective therapeutic options. PAH is probably underdiagnosed in its early stages. In a community rheumatology practice setting, an elevation of the estimated right ventricular systolic pressure (ERVSP), consistent with the diagnosis of PAH, was found in 13% of previously undiagnosed patients.[106] This diagnosis should be suspected in patients with increasing exertional dyspnea. Two-dimensional echocardiography with Doppler flow studies is the most useful screening test. A definitive diagnosis requires cardiac catheterization showing a mean resting pulmonary artery pressure greater than 25 mm Hg at rest. The development of pulmonary hypertension has been correlated with a nailfold capillary pattern similar to that seen in Scl, anti-endothelial cell antibodies, anti-cardiolipin antibodies, and anti-U1-RNP antibodies.[107] Regular electrocardiograms and echocardiographic evaluations are recommended for all MCTD patients, because subclinical disease is found in as many as 38% of patients.[104]

## Lung

Lung involvement occurs in as many as 75% of patients.[108] Early symptoms that should prompt further investigation are dry cough, dyspnea, and pleuritic chest pain.[109] PAH is prognostically the most severe form of pulmonary involvement in MCTD (Figure 86-17). In a Norwegian cohort of unselected MCTD patients, the overall prevalence of PAH was 3.4%; all those afflicted had elevated levels of N-terminal probrain natriuretic peptide (NT-proBNP).[110] Unlike Scl, in which pulmonary hypertension is often secondary to an interstitial pulmonary fibrosis, PAH in MCTD is usually caused by a bland intimal proliferation and medial hypertrophy of pulmonary arterioles[111] (see Figure 86-15). Interstitial lung disease (ILD), occurs in as many as 50% of patients. High-resolution computed tomography (HRCT) is the most sensitive test to determine the presence of ILD.[112] The most common HRCT findings are septal thickening and ground-glass opacities. Untreated ILD is usually progressive, with the development of severe pulmonary fibrosis in 25% of patients after 4 years of follow-up.[113]

## Kidney

In the initial description of MCTD, renal involvement was considered rare.[114] After 4 decades of observation, it is now evident that renal involvement occurs in approximately 25% of patients.[115] However, high titers of anti-U1 RNP antibodies are relatively protective against the development of diffuse proliferative glomerulonephritis, regardless of whether they occur in a setting of classic SLE or MCTD. When renal changes do develop in patients with MCTD, they usually take the form of a membranous glomerulonephritis.[116] This is often asymptomatic but may sometimes cause an overt nephrotic syndrome.[115] The development of diffuse proliferative glomerulonephritis or parenchymal interstitial disease has been rarely recorded in MCTD. There is increasing recognition that MCTD patients are at risk of developing a renovascular hypertensive crisis similar to the Scl kidney.

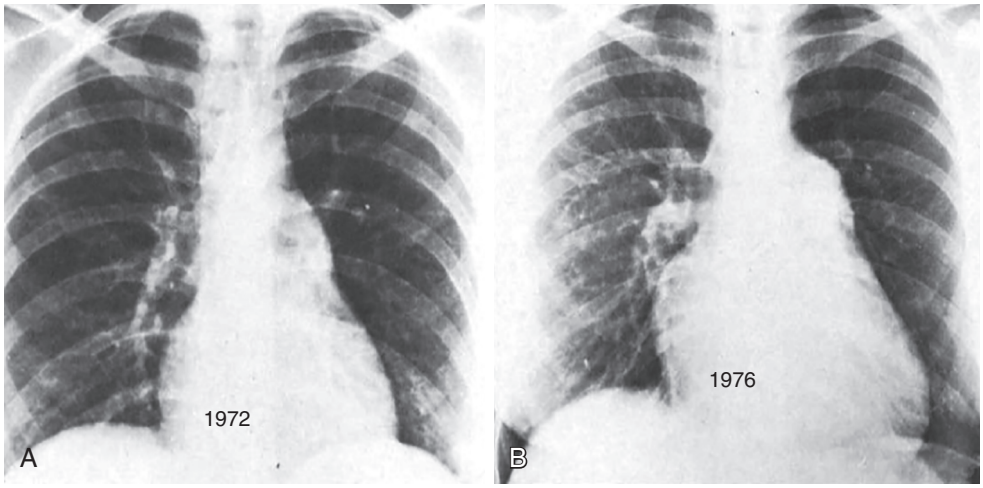



**Figure 86-17** A patient with mixed connective tissue disease with progressive pulmonary hypertension. In 1972, there was mild dilatation of the pulmonary trunk, but the heart and lungs were otherwise normal. By 1976, there was cardiac dilatation with increased enlargement of the pulmonary trunk and proximal pulmonary arteries. This picture is the typical appearance of severe pulmonary arterial hypertension. *(Courtesy Robert M. Bennett, MD.)*

### Gastrointestinal

Gastrointestinal involvement is a major feature of the overlap with Scl, occurring in approximately 60% to 80% of patients.[88] The most common abdominal problem in MCTD is disordered motility in the upper gastrointestinal tract. Histologically, this manifests as severe atrophy and loss of smooth muscle cells in the muscular layer of the lower esophagus, followed by fibrosis. Immunostaining is positive for anti-IgG and anti-C3 antibodies, but not for anti-IgM antibodies.[117] IgG immunoglobulins separated from the sera of MCTD patients were reactive with esophageal smooth muscle cells from non-CTD patients, suggesting an immune-mediated trigger for esophageal disease. There have been case reports of hemoperitoneum, hematobilia, duodenal bleeding, megacolon, pancreatitis, ascites, proteins loosing enteropathy, primary biliary cirrhosis, portal hypertension, pneumatosis intestinalis, and autoimmune hepatitis.[118] Abdominal pain in MCTD may result from bowel hypomotility, serositis, mesenteric vasculitis, colonic perforation, and pancreatitis. Malabsorption syndrome can occur secondarily to small bowel dilation with bacterial overgrowth. Liver involvement in the form of chronic active hepatitis and Budd-Chiari syndrome has been described. Pseudodiverticulae, identical to those seen in SCC, may be seen along the antimesenteric border of the colon.

### Nervous System

In keeping with Sharp's original description, CNS involvement has not been a conspicuous feature of MCTD. The most common problem is a trigeminal neuropathy.[119,120] In a review of 81 case of trigeminal neuropathy seen in a neurologic clinic, the most frequently associated CTDs were UCTD (47%), mixed connective tissue disease (26%), and Scl (19%). A sensorineural hearing loss has been reported in nearly 50% of MCTD patients.[121] In contrast to CNS involvement in classical SLE, frank psychosis and convulsions have rarely been reported in MCTD.[119] In a similar vein, cognitive function in MCTD is relatively unimpaired compared with SLE.[122] Headaches are a relatively common symptom; in the majority of patients they are vascular in origin with many of the components of classic migraine. In a subset of these patients, signs of meningeal irritation develop and examination of the cerebral spinal fluid reveals the changes of aseptic meningitis.[123] Aseptic meningitis in MCTD has also been described as a hypersensitivity reaction to nonsteroidal antiinflammatory drugs (NSAIDs), in particular sulindac and ibuprofen. There are isolated reports of transverse myelitis, cauda equina syndrome, cerebral hemorrhage, retinal vasculitis, optic neuropathy, progressive multi-focal leukoencephalopathy, cold-induced brain ischemia, myasthenia gravis, polyradiculopathy, demyelinating disorder, and peripheral neuropathy. Elevated CSF levels of anti-U1 RNP antibodies, with a predominance of anti-70 kDa antibodies, have been reported in both SLE and MCTD patients with diffuse central neuropsychiatric involvement.[124] Many patients with AICTDs have changes on brain MRI that are referred to as *unspecific bright objects* (UBO). In many instances UBOs occur in the absence of neurologic symptoms. However, there is a modest correlation between the density and positioning of UBOs; in MCTD these lesions tend to cluster at the corticomedullary junction and periventricular region.[125]

### Blood

Hematologic abnormalities are a common finding in MCTD. Anemia is found in 75% of patients, the usual profile is most consistent with the anemia of chronic inflammation.[88] A positive Coombs test is seen in approximately 60% of patients, but an overt hemolytic anemia is uncommon.[126] As in SLE a leukopenia affecting mainly the lymphocyte series is seen in approximately 75% of patients and tends to correlate with disease activity. Less common associations are thrombocytopenia, thrombotic thrombocytopenia purpura, and red cell aplasia. Hypocomplementemia has been

described in several studies[88]; it is not as prevalent as in classic SLE and has not been correlated with any particular clinical situation. Positive tests for rheumatoid factor (RF) have been found in approximately 50% of patients.[127] The presence of RF is associated with more severe degrees of arthritis, especially if anti-A2/RA33 are also present.[14] ACPAs occur in some 60% of MCTD patients and are strongly correlated with the presence of erosive disease.[63,91] Anti-cardiolipin antibodies and/or lupus anti-coagulants are a common occurrence and have been associated with vasculopathy and increased mortality.[128] Unlike the anti-cardiolipin antibodies found in SLE they are β2-glycoprotein independent and tend to be associated with thrombocytopenia rather than thrombotic events.[129]

### Pregnancy

Reports of maternal and fetal morbidity in MCTD are quite diverse.[130] In a comparison study of patients with MCTD and SLE, the fertility rates in patients with diseases were unaltered, whereas the parity and fetal wastage was increased in both.[131] Some studies have reported an exacerbation of MCTD during pregnancy and postpartum flares,[131] whereas others have not. Anti-endothelial antibodies have been linked to spontaneous abortion in MCTD.[132] A single case of neonatal "lupus" has been reported, suggesting a pathogenic role for the transplacental passage of anti-U1 RNP antibodies.[133]

### Juvenile Mixed Connective Tissue Disease

MCTD may first become apparent in childhood. The average age of onset in one report was 10.7 years.[134] Polyarthritis and Raynaud's phenomena are the most common presenting features. There tends to be a progression of organ involvement with 20% involvement at 5 years and 48% at ten years. Significant myocarditis, glomerulonephritis, thrombocytopenia, seizures, hemolytic uremic syndrome, an acute coronary syndrome, and aseptic meningitis have been described in isolated cases.

### Is Mixed Connective Tissue Disease a Distinct Clinical Entity?

Despite the 40 years that have passed since the initial description of MCTD, there remains some hesitation in designating it a "real disease." Ten years after the initial description, some researchers were asking "does MCTD exist?"[134a] or "MCTD—a decade of growing pains."[134b] After a further 10 years more strident titles appeared, including "MCTD—goodbye to all that"[134c] and "MCTD: fact or fiction"[134d] and "MCTD—33 years of uncertainty."[134e] One of the major areas of uncertainty was whether MCTD described an intermediate phenotype, which would eventually evolve into one of the better established CTDs, such as Scl or SLE. In 2012 Capelli et al. reported on 161 MCTD patients that had been followed up for a mean of 8 years. They found that the MCTD label was still appropriate in 75% of the initial cohort, with the conditions of 17% evolving into SCL, 9.1% into SLE, then 2.5%, in turn, into RA. They concluded that "MCTD is a distinct clinical entity, but a subgroup of patients may evolve into another CTD." By 2014 the concept of MCTD as a relevant entity was better established. A pathogenetic role for anti-U RNP antibodies was emerging, especially with regard to clinical features of Raynaud's phenomena and esophageal dysmotility and prognosis in regards to PAH.[135] Diseases as "diagnostic entities" are difficult to reliably authenticate. It is evident that the autoimmune connective tissue disorders cannot be designated as "diseases" on the basis of Koch's postulates." The overlaps of SLE and Scl described in this chapter attest to this conundrum. However, it is now evident that patients with persistently high levels of anti-U1RNP antibodies often manifest a clinical picture of Raynaud's phenomena, esophageal dysmotility, polyarthritis (sometimes erosive), puffy hands/fingers, and absence of severe renal or neuropsychiatric disease. On the other hand, interstitial lung disease and PAH (which is often fatal) are more likely to develop in these patients. When all is said and done, it is important to remain grounded and not let one's clinical judgment be swayed by esoteric pronouncements on whether your patient has a disease, an entity, or a syndrome.

## MANAGEMENT OF OVERLAP SYNDROMES

**KEY POINTS**

**The management of overlap syndromes has not been the subject of controlled trials.**

**Management is therefore based on an analysis of the clinical features and the application of management strategies used in the usual treatment presenting features in terms of inflammatory arthritis, Raynaud's phenomenon, inflammatory muscle disease, serositis, interstitial lung disease, pulmonary hypertension, and the gastrointestinal features of Scl.**

**By definition, the clinical features of an overlap syndrome will be diverse and can change over time. Thus a reappraisal of management strategies is needed at each patient encounter.**

The rational management of overlap CTDs is confounded by the absence of controlled trials. Recommendations for management are based on conventional treatments for SLE, PM/DM, RA, and Scl.[136] General guidelines to treat specific features of the overlap CTDs are given in Table 86-6. Almost all patients with CTDs experience Raynaud's phenomenon. Apart from advice on minimizing cold exposure, most patients should be given calcium blockers (e.g., nifedipine). The use of topical nitrates, endothelin antagonists (e.g., bosentan), phosphodiesterase-5 inhibitors (e.g., tadalafil), prostaglandin analogs (e.g., iloprost) should be considered in severe refractory cases.[137] Pulmonary hypertension is the main cause of death in MCTD, and patients should be evaluated at regular intervals for this complication; early intervention is the key to effective management. Recent advances in the treatment of pulmonary hypertension have led to reduced morbidity and mortality.[111] Overall, effective management requires anticoagulation and vasodilator therapy, such as calcium channel blockers or prostacyclin analogues. Long-term treatment with intravenous epoprostenol or prostacyclin improves exercise capacity, hemodynamics, and survival in many patients[138] as does therapy with inhaled iloprost.[139] There is evidence that

**TABLE 86-6** Guidelines for Managing Overlap Syndromes

| System Involved | *Problems* | Treatments |
|---|---|---|
| Non-specific | *Fatigue, arthralgias, myalgias* | NSAIDs, anti-malarials, low-dose prednisone (<10 mg/day)<br>Trial use of modafinil |
| | *Vascular headache* | Trial of propranolol and/or alternate-day aspirin, 350 mg<br>Symptomatic use of a triptan (e.g., sumatriptin, eletriptan) |
| Blood | *Autoimmune anemia and thrombocytopenia* | High dose steroids (prednisone, ~80 mg/day) with taper dependent on clinical course. Consider danazol, IVIG and immunosuppression in recalcitrant cases |
| | *Thrombotic thrombocytopenic purpura* | Immediate infusion of fresh frozen plasma. May require plasma exchange and transfusion of platelet depleted RBCs. Consider splenectomy in recalcitrant cases |
| Vascular | *Raynaud's phenomenon* | Keep warm, avoid finger trauma, avoid β-blockers, stop smoking. Dihydropyridine Ca channel blockers (e.g., nifedipine). Alpha sympatholytics (e.g., prazosin). Consider endothelin receptor antagonist (e.g., bosentan) in recalcitrant cases |
| | *Acute-onset digital gangrene* | Local chemical sympathectomy (infiltration of lidocaine at base of involved digit). Anti-coagulation. Topical nitrates. Consider hospitalization for intra-arterial prostacyclin. Start endothelin receptor antagonist therapy. Consider hyperbaric oxygen |
| Joints | *Arthritis* | NSAIDs, anti-malarials, methotrexate, consider TNF inhibition* |
| Pulmonary | *Asymptomatic pulmonary hypertension* | Trial of steroids and cyclophosphamide, low dose aspirin and ACE inhibitors. Consider endothelin receptor antagonist (oral bosentan) |
| | *Symptomatic pulmonary hypertension* | Intravenous prostacyclin, angiotensin-converting enzyme inhibitors, anti-coagulation. Endothelin receptor antagonist (oral bosentan). Sildenafil trial. Heart–lung transplantation |
| | *Pleurisy* | NSAID or short course of prednisone—about 20 mg/day |
| Neurological | *Trigeminal neuropathy* | No effective therapy for numbness. Trial of an anti-epileptic (e.g., gabapentin) or tricyclic anti-depressant (e.g., nortryptiline) for pain |
| | *Aseptic meningitis* | Discontinue NSAIDs.† Short course of high-dose prednisone, about 60 mg/day |
| Renal | *Membranous glomerulonephropathy* | Mild—no treatment required<br>Progressive proteinuria—trial of ACE inhibitor; trial of low dose aspirin combined with dipyridamole.<br>Severe—trial of prednisone, 15-60 mg/day plus intermittent pulse cyclophosphamide or daily chlorambucil or rituximab |
| | *Nephrotic syndrome* | Steroids alone seldom effective. Low dose aspirin combined with dipyridamole to prevent thrombotic complications. ACE inhibitor to reduce protein loss. Trial of prednisone, 15-60 mg/day plus monthly pulse cyclophosphamide or rituximab<br>May require dialysis/transplantation |
| | *Scleroderma-like renal crisis* | ACE inhibitor |
| Cardiac | *Myocarditis*<br>*Pericarditis* | Trial of steroids and cyclophosphamide‡; avoid digoxin§<br>NSAID or short course of prednisone—about 20 mg/day Tamponade will require percutaneous or surgical drainage |
| | *Incomplete heart block* | Avoid chloroquine‖ |
| Gastrointestinal | *Dysphagia* | Mild—no treatment<br>With reflux—proton pump inhibitor. Consider Nissen fundoplication<br>Severe—Calcium channel antagonist, alone or in combination with an anti-cholinergic agent |
| | *Intestinal dysmotility* | Prokinetic agents such as metoclopramide, and erythromycin. Small bowel bacterial overgrowth—tetracycline, erythromycin |
| | *Heartburn/Dyspepsia* | Raise head of bed, discontinue smoking, lose weight, and avoid caffeine. H2 antagonists, $H^+$ proton pump blockers. Metoclopramide trial. Consider *H. Pylori* infection in recalcitrant cases |
| Muscle | *Myositis* | Acute onset/severe—prednisone, 60-100 mg/day<br>Chronic/low grade—prednisone, 10-30 mg/day¶<br>Consider methotrexate and/or IVIG in recalcitrant cases |
| Osteoporosis | *Osteoporosis* | Ca/Vit D supplements, estrogen replacement, or raloxifene Biphosphonates.# Nasal calcitonin. Carboxyl truncated PTH analogs such as hPTH-(1-34) |

*Has been associated with flares in MCTD and SLE.
†Sulindac and ibuprofen have been associated with a hypersensitivity aseptic meningitis.
‡Cardiotoxic at high doses.
§Predisposes to ventricular arrhythmias.
‖Predisposes to complete heart block.
¶Remain alert for steroid myopathy, aseptic necrosis of bone, and accelerated osteoporosis.
#Cannot be used if esophagus is more than mildly involved.
ACE, Angiotensin-converting enzyme; Ca, calcium; PTH, parathyroid hormone; TCA, tricyclic anti-depressant; Vit, vitamin.

some patients respond to a regimen of intravenous cyclophosphamide and corticosteroids.[140] Bosentan, an oral endothelium-1 antagonist, has been reported to improve dyspnea and to slow PAH progression in MCTD.[141] In the largest study of bosentan to date, 173 patients with World Health Organization (WHO) functional class II PAH were evaluated for a period of median exposure of 51 months. At the end of bosentan treatment, 77.8% of patients were in WHO functional class I/II, with a 4-year event-free survival rate of 79.5%; 20% of patients discontinued treatment

because of adverse events (mostly worsening of PAH and elevated liver enzymes).

Many of the problems that cause morbidity in overlap syndromes are intermittent and responsive to corticosteroids (e.g., aseptic meningitis, myositis, pleurisy, pericarditis, and myocarditis). On the other hand, nephrotic syndrome, Raynaud's phenomenon, deforming arthropathy, acrosclerosis, and peripheral neuropathies are usually steroid resistant. Many of the Scl-like gastrointestinal problems can be managed according to the usual practice in Scl, such as management of renal crisis with angiotensin-converting enzyme (ACE) inhibitors, Raynaud's phenomenon with calcium channel blockers, and gastrointestinal reflux disease with proton pump inhibitors.[137] Fibrotic lung disease is notoriously resistant to corticosteroids and immunosuppressives. There is some evidence that a new class of drugs, tyrosine kinase inhibitors (e.g., imatinib) may be effective in some patients.[142]

In patients with steroid-resistant thrombocytopenia, refractory myositis or hemolytic anemia, it is worth considering the use of intravenous gammaglobulin and/or rituximab.[143,144] Rituximab has also proved beneficial in some patients with severe anti-synthetase syndrome.[145] In patients with refractory disease, the use of these agents combined with plasmapheresis should be considered.[146] Successful autologous peripheral blood stem cell transplantation has been reported in a patient with refractory myositis and MCTD.[147] During the long term, concern usually mounts over the total corticosteroid burden and the possibility of inducing an iatrogenic steroid myopathy, nosocomial infection, aseptic necrosis of bone, or accelerated osteoporosis. Routine evaluation of bone mineral density is warranted to detect early presymptomatic osteoporosis and initiation of therapy with anti-resorptive agents. Unless contraindicated, all patients should take supplementary calcium and vitamin D. In patients requiring long-term corticosteroids, it would seem reasonable to use anti-malarials[148] or methotrexate[149] in an attempt to minimize the cumulative steroid burden. Anti-malarials should be used with caution in overlap patients with a fascicular or bundle branch block, because of the risk of causing a complete heart block[150] or an idiosyncratic hepatitis.[151] One should also remember that anti-malarial cardiotoxicity can mimic a cardiomyopathy with a prolonged QT(C).[152] Digitalis is relatively contraindicated in patients with myocarditis, because of the risk of inducing ventricular arrhythmias. As in SLE, the TNF inhibitor etanercept has been reported to exacerbate MCTD.[153] Patients with severe hand deformities may be helped by soft tissue release operations and selected joint fusions.

The management of pregnancy presents several special problems in patients with and in overlap CTDs. Doria has provided the following general advice[154]:

- Patients should be correctly informed on the risk of becoming pregnant.
- Pregnancies should be planned when the disease is in remission, because it increases the probability of successful maternal and fetal outcome.
- Patients should be regularly monitored during gestation and postpartum by a multidisciplinary team to include a rheumatologist, obstetrician, and neonatologist.
- In the case of disease relapse, an adequate treatment, even aggressive if necessary, should be recommended, because active disease can be more detrimental for the fetus than drugs.

There is often a tendency to assume that all patients with overlap CTDs should be on long-term corticosteroids. This mistake is compounded by the assumption that all medical problems in these patients are related to their underlying overlap CTDs. For instance, apparent flares of discomfort and pain in overlap CTDs may be caused by myofascial pain syndrome or fibromyalgia, and are thus unresponsive to corticosteroids. Similarly, the feeling of malaise and easy fatigability may be related to a reactive depression or the fact that the patient has become deconditioned. Premature atherosclerosis is now well recognized as a cause of increased morbidity and mortality in AICTDs.[155] All patients with overlap syndromes need ongoing evaluation for cardiovascular risk factors and appropriate advice and therapy for hypertension and hyperlipidemia. The management of patients with overlap CTDs requires continuing reassessment of an ever-changing pattern of clinical problems, and a constant alertness to the development of iatrogenic disease. As with any disease of unknown etiology, effective management of patients with overlap CTDs presents a constant and ever-evolving challenge.

## OUTCOME

The prognosis for overlap syndromes is often better than for the classic AICTDs. For instance, Troyanov reported on the follow up of 100 patients with idiopathic inflammatory myopathy. It was found that the long-term course after treatment with prednisone, with a dose/duration that initially resulted in good symptomatic improvement, was very different. All patients with PM (100%) and most patients with DM (92%) progressed to chronic myositis, whereas persistent muscle disease developed in only 58% of overlap patients.[4] In general, patients with Scl overlap syndromes have a better prognosis than patients with "pure" Scl. The tendency for chronic disease to develop in myositis first overlap patients was more common in patients with anti-synthetase and nucleoporin antibodies, and less common in patients with antibodies to -U1RNP, Pm-Scl, or Ku. Antibodies to TIF1-γ (p155/140) proteins in myositis are a risk factor for the development of cancer.[156] There is unequivocal evidence that patients with high-titer U1 RNP antibodies have a low prevalence of serious renal disease and life-threatening neurologic problems; in this sense MCTD can be favorably compared with classic SLE. However, not all patients with MCTD have a favorable prognosis. A follow-up of 280 MCTD patients seen between 1979 and 2011 reported a total of 22 deaths: nine deaths from pulmonary arterial hypertension, seven deaths from cardiovascular events, three deaths from thrombotic thrombocytopenic purpura, and three deaths from infections (Figure 86-18). Anti-endothelial antibodies and anti-cardiolipin antibodies were associated with increased mortality. Deceased patients were somewhat younger than survivors (35.5 ± 10.4 vs. 41.8 ± 10.7 yrs).[157] It is evident that the course of overlap syndromes is unpredictable. Many patients do follow a relatively benign course, but major organ involvement ultimately dictates the morbidity and mortality of the disease.

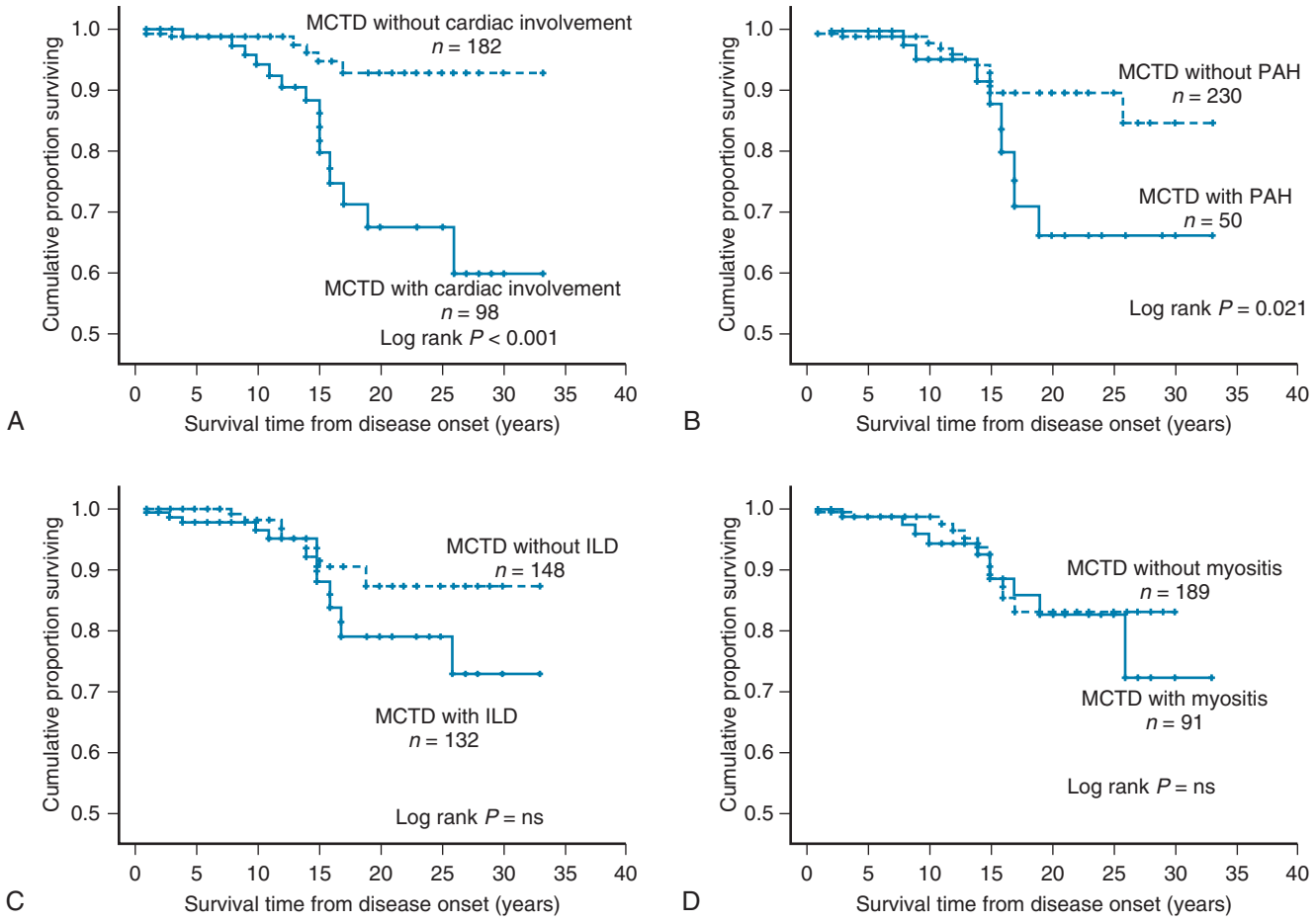



**Figure 86-18** Survival of patients with mixed connective tissue disease (MCTD) within various disease subgroups. **A,** Survival of patients with and without cardiovascular disease. **B,** Survival of patients with and without pulmonary arterial hypertension (PAH). **C,** Survival of patients with and without interstitial lung disease (ILD). **D,** Survival of patients with and without myositis. *(From Hajas A, et al: Clinical course, prognosis, and causes of death in mixed connective tissue disease.* J Rheumatol *40:1134–1142, 2013.)*

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PART 13 VASCULITIS

CHAPTER 87



# Classification and Epidemiology of Systemic Vasculitis

John H. Stone

**KEY POINTS**

- Vasculitis is a heterogeneous group of disorders linked by the primary finding of inflammation within blood vessel walls. Nearly 30 forms of systemic vasculitis are currently recognized.
- Different forms of vasculitis have widely divergent profiles with regard to age, sex, and ethnicity.
- Vasculitides are classified first by the size of blood vessel involved—small (capillaries and postcapillary venules), medium (muscular arteries and arterioles), or large (the aorta and its major branches).
- Considerations in the clinical diagnosis of vasculitis include patient demographics, organ tropism, presence or absence of granulomatous inflammation, participation (or not) of immune complexes, finding of autoantibodies, detection of infections associated with some vasculitides, and the identification of other probable causes (e.g., drug-induced vasculitis syndromes).
- Associations between genes and vasculitis have been recognized increasingly in recent years. Progress also has been made in the area of gene-environment interactions.

## CLASSIFICATION

Few disorders in medicine are more challenging to diagnose and treat than the systemic vasculitides. These heterogeneous disorders are linked by the common finding of destructive inflammation within the walls of blood vessels. Current nomenclature and classification schemes recognize nearly 30 primary forms of vasculitis and several major categories of secondary vasculitis (e.g., other rheumatologic diseases, malignancy, and infection; Table 87-1). A widely accepted nomenclature scheme known as the Chapel Hill Consensus Conference (CHCC) was published in 2013.[1] During the past half century, however, a number of other classification schemes have been devised.[2] None—including the CHCC—has been entirely satisfactory because understanding of these conditions continues to evolve. Any vasculitis classification scheme will change as new information emerges.

Current classification schemes are understood best in light of their nosologic predecessors. The first "modern" case of systemic vasculitis was recognized in the 1860s by Kussmaul and Maier.[3] That case, which involved medium-sized, muscular arteries, has served as the reference point for classifying many subsequently recognized forms of vasculitis. Because of the importance of that first report in the understanding and classification of vasculitis, the case is described in detail here.

### First Modern Case: "Periarteritis Nodosa"

In 1866, Kussmaul and Maier reported the case of a 27-year-old tailor who died during a month-long hospital stay.[3,4] Upon presentation, the patient was strong enough to climb two flights of stairs to the clinic, but "afterward felt so weak that he immediately had to go to bed." He reported numbness on the volar aspect of his thumb and the two neighboring fingers on the right hand. During the ensuing days, "the general weakness increased so rapidly that he was unable to leave the bed, [and] the feeling of numbness also appeared in the left hand." Muscle paralysis progressed quickly: "Before our eyes, a young man developed a general paralysis of the voluntary muscles … [He] had to be fed by attendants, and within a few weeks was robbed of the use of most of his muscles."[3,4]

The patient's weakness, caused by vasculitic neuropathy (mononeuritis multiplex), was accompanied by tachycardia, abdominal pains, and the appearance of cutaneous nodules over his trunk. His death was described as follows: "He was scarcely able to speak, lay with persistent severe abdominal and muscle pains, opisthotonically stretched, whimpering, and begged the doctors not to leave him…. Death occurred … at 2 o'clock in the morning." At autopsy, grossly visible nodules were present along the patient's medium-sized arteries. Kussmaul and Maier[3] suggested the name "periarteritis nodosa" for this disease because of the apparent localization of inflammation to the perivascular sheaths and outer layers of the arterial walls, leading to nodular thickening of the vessels. The name was later revised to polyarteritis nodosa (PAN), to reflect the widespread arterial involvement of this disease and the fact that inflammation in PAN generally extends through the entire thickness of the vessel wall.[5,6]

### Polyarteritis Nodosa as a Reference Point

Several features of PAN make it a logical reference point for the classification of inflammatory vascular disease, in

**TABLE 87-1** Names for Vasculitides Adopted by the 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides

| |
|---|
| **Large-Vessel Vasculitis** |
| Takayasu's arteritis |
| Giant cell arteritis |
| **Medium-Vessel Vasculitis** |
| Polyarteritis nodosa |
| Kawasaki's disease |
| **Small-Vessel Vasculitis** |
| **Anti-neutrophil Cytoplasmic Antibody–Associated Vasculitis** |
| Microscopic polyangiitis |
| Granulomatosis with polyangiitis |
| Eosinophilic granulomatosis with polyangiitis |
| **Immune Complex Small Vessel Vasculitis** |
| Anti-glomerular basement membrane disease |
| Cryoglobulinemic vasculitis |
| IgA vasculitis (Henoch-Schönlein purpura) |
| Hypocomplementemic urticarial vasculitis |
| **Variable Vessel Vasculitis** |
| Behçet's disease |
| Cogan's syndrome |
| **Single-Organ Vasculitis** |
| Cutaneous leukocytoclastic angiitis |
| Cutaneous arteritis |
| Primary central nervous system vasculitis |
| Isolated aortitis |
| **Vasculitis Associated with Systemic Disease** |
| Lupus vasculitis |
| Rheumatoid vasculitis |
| Sarcoid vasculitis |
| Others (e.g., $IgG_4$-related aortitis) |
| **Vasculitis Associated with Probable Etiology** |
| Hepatitis C virus–associated cryoglobulinemic vasculitis |
| Hepatitis B virus–associated vasculitis |
| Syphilis-associated aortitis |
| Drug-associated immune complex vasculitis |
| Drug-associated ANCA-associated vasculitis |
| Cancer-associated vasculitis |
| Others |

ANCA, Anti-neutrophil cytoplasmic antibody.

addition to the fact that it was the cause of the first "modern" vasculitis case. Other forms of vasculitis can usually be differentiated from PAN through their contrasts to one or more of the following PAN characteristics:

- The general confinement of PAN to medium-sized vessels* as opposed to capillaries and postcapillary venules (small vessels) and the aorta and its major branches (large vessels)
- The exclusive involvement of arteries by PAN, with sparing of veins
- The tendency of PAN to form microaneurysms
- The absence of lung involvement in PAN
- The lack of granulomatous inflammation in PAN
- The absence of autoantibodies in PAN (e.g., anti-neutrophil cytoplasmic antibodies [ANCAs], anti-glomerular basement membrane [anti-GBM] antibodies, or rheumatoid factor)
- The association of some cases of PAN with hepatitis B virus (HBV) infection

*Vessel size overlap in vasculitis syndromes is subsequently acknowledged and discussed.

**TABLE 87-2** Typical Clinical Manifestations of Large-, Medium-, and Small-Vessel Involvement by Vasculitis

| |
|---|
| **Large** |
| Limb claudication |
| Asymmetric blood pressures |
| Absence of pulses |
| Bruits |
| Aortic dilation |
| Renovascular hypertension |
| **Medium** |
| Cutaneous nodules |
| Ulcers |
| Livedo reticularis |
| Digital gangrene |
| Mononeuritis multiplex |
| Microaneurysms |
| Renovascular hypertension |
| **Small** |
| Purpura |
| Vesiculobullous lesions |
| Urticaria |
| Glomerulonephritis |
| Alveolar hemorrhage |
| Cutaneous extravascular necrotizing granulomas |
| Splinter hemorrhages |
| Uveitis/episcleritis/scleritis |

Constitutional symptoms: fever, weight loss, malaise, arthralgias/arthritis (common to vasculitides of all vessel sizes).

## Classification by Vessel Size

Because the causes of most forms of vasculitis are unknown, the most valid basis for classifying the vasculitides is the size of the predominant blood vessels involved. Under such classification schemes, the vasculitides are categorized initially by whether the vessels affected are large, medium, or small (see Table 87-1 and Figure 87-1). *Large* generally denotes the aorta and its major branches (and the corresponding vessels in the venous circulation in some forms of vasculitis, e.g., Behçet's disease).

*Medium* refers to vessels that are smaller than the major aortic branches yet still large enough to contain four elements: (1) an intima, (2) a continuous internal elastic lamina, (3) a muscular media, and (4) an adventitia. In clinical terms, medium-vessel vasculitis (see Table 87-1) is generally macrovascular (i.e., it involves vessels large enough to be observed in gross pathologic specimens or visualized by angiography). A useful way of thinking about this construct is that a large artery becomes a medium-sized artery when it penetrates a viscus. Thus, the renal artery is considered a large artery, but once it enters the kidney and separates into the smaller arcuate and interlobular arteries, these vessels are regarded as medium-sized arteries.

*Small-vessel* vasculitis, which incorporates all vessels below macroscopic disease, includes capillaries, postcapillary venules, and arterioles. Such vessels typically are all less than 500 μm in outer diameter. Because glomeruli may be viewed simply as differentiated capillaries, forms of vasculitis that cause glomerulonephritis are considered to be small-vessel vasculitides. Table 87-2 presents the typical clinical manifestations associated with small-, medium-, and large-vessel vasculitides.

Two points are relevant to a full understanding of the use of vessel size as the fundamental basis for classifying the

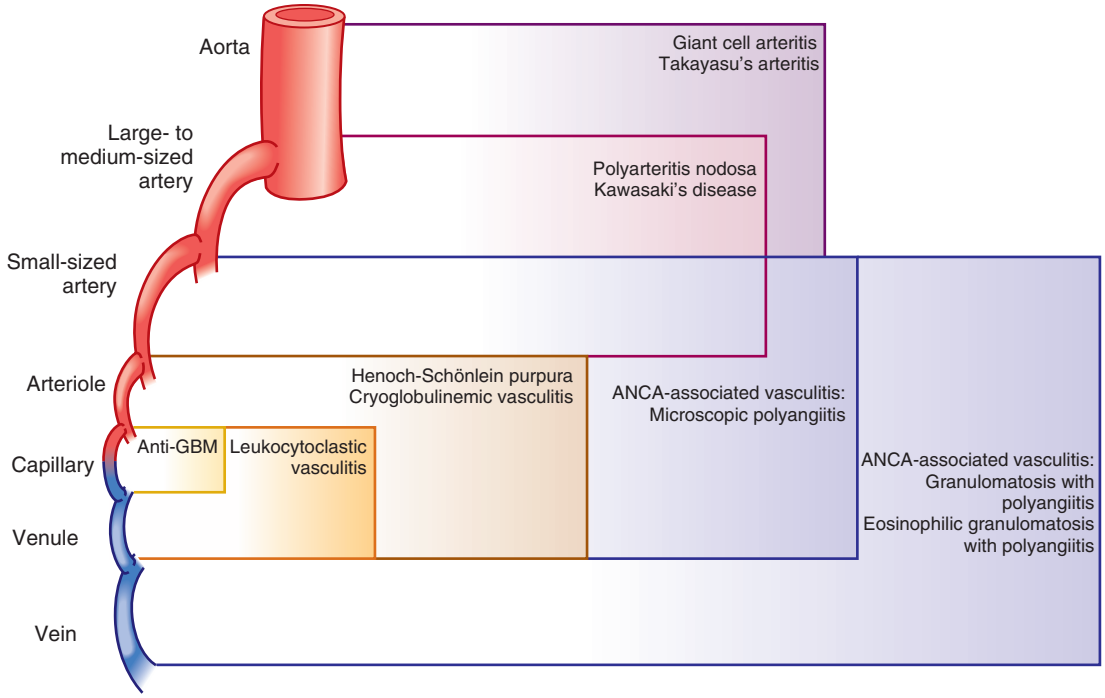



**Figure 87-1** Classification by blood vessel size. ANCA, Anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane.

vasculitides. The first is that although large-vessel vasculitides affect larger arteries more often than do medium- or small-vessel vasculitis, medium-vessel vasculitis affects primarily medium-sized arteries, and small-vessel vasculitis primarily affects small arteries and other small vessels, all three major categories of vasculitides (large, medium, and small) can affect arteries of any size. It is crucial to recognize that both medium-vessel and even large-vessel vasculitides can affect small arteries. One transparent example of this phenomenon occurs in giant cell arteritis, the prototypical "large-vessel" vasculitis: the clinical manifestation of this disease most feared by patients—anterior ischemic optic neuropathy—is caused by small branches of the posterior ciliary and retinal arteries.

The second but related point pertains to the concept that some vasculitides are not easily pigeonholed as being primary large, medium, or small-vessel diseases. In fact, the most current CHCC nomenclature scheme includes a category termed "variable-vessel vasculitides." Classic variable vessel vasculitides are Behçet's disease and Cogan's syndrome. Despite the possibility of vessel size overlap within individual cases and the existence of forms of vasculitis that routinely affect vessels of variable size, the categorization of a patient's vasculitis as primarily large, medium, or small vessel remains enormously useful in focusing the differential diagnosis and initiating plans for treatment.

## Additional Considerations in Classification

Many other considerations are important in the classification of vasculitis (Table 87-3): (1) the patient's demographic profile (see the Epidemiology section), (2) the disease's tropism for particular organs, (3) the presence or absence of granulomatous inflammation, (4) the participation of immune complexes in disease pathophysiology, (5) the finding of characteristic autoantibodies in the patients' serum (e.g., ANCAs, anti-GBM antibodies, or rheumatoid factor), and (6) the detection of certain infections known to cause specific forms of vasculitis.

**TABLE 87-3** Considerations in the Classifications of Systemic Vasculitis

| |
|---|
| Size of predominant blood vessels affected |
| Epidemiologic features<br>Age<br>Sex<br>Ethnic background |
| Pattern of organ involvement |
| Pathologic features<br>Granulomatous inflammation<br>Immune complex deposition versus pauci-immune histopathology<br>Linear staining along glomerular basement membrane |
| Presence of ANCA, anti-GBM antibodies, or rheumatoid factor in serum |
| Demonstration of a specific associated infection (hepatitis B or hepatitis C) |

ANCA, Anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane.

The organ tropisms of these disorders are illustrated by the following examples. Granulomatosis with polyangiitis (GPA) classically involves the kidneys, upper airways, and lungs. In contrast, IgA vasculitis/Henoch-Schönlein purpura (HSP) often affects the kidneys, but never the nose or sinuses and almost never the lungs. In contrast to both of these forms of vasculitis, Cogan's syndrome is defined by the simultaneous occurrence of ocular inflammation caused by a small-vessel vasculitis (most often interstitial keratitis) and sensorineural hearing loss (and, in 10% of cases, a large vessel vasculitis). The histopathologic findings in these

**TABLE 87-4** Forms of Vasculitis Associated with Granulomatous Inflammation

| |
|---|
| Giant cell arteritis |
| Takayasu's arteritis |
| Cogan's syndrome |
| Granulomatosis with polyangiitis |
| Eosinophilic granulomatosis with polyangiitis |
| Primary angiitis of the central nervous system* |
| Rheumatoid vasculitis |

*Sometimes granulomatous.

three disorders are equally distinctive, ranging from granulomatous inflammation of small to medium vessels (GPA) to IgA deposition in small vessels (HSP), to large vessel vasculitis centered on the adventitia (Cogan's syndrome).

The granulomatous features of some forms of vasculitis resemble chronic infections (e.g., infections caused by fungi or mycobacteria) or the inflammation induced by the presence of a foreign body. Granulomatous inflammation is more likely to be found in some organs (e.g., the lung) than in others (e.g., the kidney or skin). Some patients without evidence of granulomatous inflammation at early points in their courses later exhibit such features as their diseases unfold. Patients initially diagnosed with cutaneous leukocytoclastic angiitis or microscopic polyangiitis may be reclassified as having GPA if disease manifestations appear in new organs and granulomatous inflammation is found on biopsy specimens. Table 87-4 presents forms of vasculitis commonly associated with granulomatous inflammation.

Immune complexes are essential to the pathophysiology of some small- and medium- vessel vasculitides. Immune complex–mediated tissue injury does not produce a single unique clinical syndrome, but rather applies to many forms of vasculitis and overlaps with injuries caused by other immune mechanisms. Anti-GBM disease (Goodpasture's disease) is a unique form of immune complex disease in which the immune complexes form in situ rather than in the circulation.[7] Complexes of IgA1 are found in HSP. Immune complexes comprising IgG, IgM, complement components, and the hepatitis C virion characterize most cases of mixed cryoglobulinemia. HBV surface antigen/antibody complexes are present in the circulation and involved tissues of patients with HBV-associated vasculitis, a condition that often strongly resembles the "idiopathic" case of PAN described by Kussmaul and Maier. Rheumatoid factor and complement proteins are found within organs affected by rheumatoid vasculitis. The immune complex–mediated vasculitides are discussed further in Chapter 91.

In contrast, other small- and medium-vessel vasculitides such as GPA, microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) are disorders associated with "pauci-immune" inflammation. "Pauci-immune" refers not to a lack of immunologic involvement in these disorders but rather to the relative absence (compared with the conditions regarded as immune complex–mediated) of immunoglobulin or complement deposition within diseased tissues. Many (but not all) patients with pauci-immune forms of vasculitis have ANCAs in their serum. Three decades before the description of ANCAs, Godman and Churg[8] observed pathologic links between these three entities, noting that the disorders "group themselves into a compass, [ranging from] necrotizing and granulomatous processes with angiitis, through mixed forms, to vasculitis without granulomata."

ANCAs (see Chapter 91) are directed against antigens that reside within the primary granules of neutrophils and monocytes.[9] Two types of ANCAs seem to be relevant to vasculitis: (1) ANCAs directed against proteinase 3 (PR3), a serine protease found within the primary granules of neutrophils and monocytes; and (2) ANCAs directed against myeloperoxidase and another serine protease found within the same granules. Although rigorous serologic assays for these antibodies are helpful in diagnosis, evidence for a primary etiologic role of these antibodies in human forms of pauci-immune vasculitis is still lacking. In contrast, anti-GBM antibodies have been proven to play a major role in the pathogenesis of Goodpasture's disease.[10] In rheumatoid arthritis (RA), systemic rheumatoid vasculitis occurs only in patients who are rheumatoid factor positive. Rheumatoid factor is believed to play an essential role in the immune complex nature of that disease complication. Finally, although the causes of most forms of vasculitis are unknown, several infections have been linked definitively with specific forms of these diseases (e.g., HBV with some cases of PAN and hepatitis C with mixed cryoglobulinemia).

## Historical Efforts at Classification and Nomenclature

For decades after this initial description of vasculitis, most forms of systemic inflammatory vascular disease were termed *periarteritis nodosa* because of the index case reported by Kussmaul and Maier. In the 1900s, two major factors led to the recognition of new forms of vasculitis: (1) the use of microscopy in the evaluation of pathologic specimens became routine, and (2) horse serum and sulfonamides came to be used in the treatment of many medical conditions. These new therapies frequently induced small-vessel vasculitides on the basis of immune complex deposition. Moreover, the serum sickness or "hypersensitivity" phenomena could be observed readily on tissue biopsy specimens through the microscope. In some cases, the histopathologic findings of hypersensitivity reactions were confused with periarteritis nodosa. The gradual recognition that these syndromes represented departures from the PAN described by Kussmaul and Maier spurred interest in the first classification scheme for necrotizing angiitis.

### The Zeek Classification

In 1952, Zeek[11] identified five major categories of necrotizing angiitis. The Zeek classification included (1) hypersensitivity angiitis, (2) allergic granulomatous angiitis (Churg-Strauss syndrome), (3) rheumatic arteritis (vasculitis associated with fulminant rheumatic fever), (4) periarteritis nodosa, and (5) giant cell arteritis. This classification scheme omitted several forms of systemic vasculitis that were known but had not yet been described in the English medical literature (e.g., GPA and Takayasu's arteritis). The number of diseases included in vasculitis classification and nomenclature schemes has continued to grow through

several subsequent attempts at defining more comprehensive systems for cataloging these diseases.

### The American College of Rheumatology Criteria for the Classification of Vasculitides

In 1990 the American College of Rheumatology (ACR) performed a study designed to establish criteria for the classification of vasculitis.[12,13] The study was designed to identify features that distinguished one form of vasculitis from others. An important caveat is that the study was *not* designed to establish criteria for diagnosis but rather to facilitate research by permitting the inclusion of similar types of patients in studies. Patients with giant cell arteritis, Takayasu's arteritis, PAN, GPA, eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome), HSP, and hypersensitivity vasculitis were included in this study.[14-20] The findings of the ACR study remain useful for the purposes of the study's original intention—the assurance of uniform inclusion criteria for patients in research studies.

The passage of time and the development of new insights pertaining to vasculitis have shown the need for updates to the ACR classification criteria. First, because the study was performed before the days of reliable and widely available assays for ANCAs, ANCA positivity was not considered as a possible classification criterion. Second, the ACR classification criteria study did not include microscopic polyangiitis as a separate disease but rather lumped such patients under the heading of PAN.[17] Third, the study did not define classification criteria for rarer forms of vasculitis such as Cogan's syndrome and Behçet's disease. Classification criteria for Behçet's disease were formulated in 1990 in a separate conference.[21]

### The First and Second Chapel Hill Consensus Conferences on Nomenclature

The first Chapel Hill Consensus Conference, convened in 1992, reviewed the nomenclature of systemic vasculitides.[22] Formal diagnostic criteria were not attempted, but definitions were created for 10 forms of vasculitis. In addition to the seven forms of vasculitis included in the ACR study, microscopic polyangiitis, Kawasaki's disease, and "essential" cryoglobulinemic vasculitis were defined. The CHCC emphasized the important role that ANCAs play in the diagnosis of small- to medium-vessel vasculitis and distinguished microscopic polyangiitis from classic PAN. The conference defined classic PAN as necrotizing inflammation of medium-sized or small arteries without glomerulonephritis.[22] Microscopic polyangiitis was defined as a necrotizing vasculitis with few or no immune deposits that (1) affects small blood vessels (e.g., capillaries, venules, or arterioles), (2) often includes glomerulonephritis and pulmonary capillaritis, and (3) is often associated with either myeloperoxidase (MPO)- or PR3-ANCA.

In the years following the first CHCC, it became clear that hepatitis C plays a major role in 90% of cases that formerly were termed *essential mixed cryoglobulinemia*. Some cases of this syndrome are not associated with hepatitis C infections, however, and probably have some other infectious etiology. Similarly, with the availability of the HBV vaccine, increasingly fewer cases of PAN are associated with this infection. Most PAN cases have no known cause. As emphasized by Churg,[23] cases of PAN currently termed *idiopathic* do not represent a single entity but almost certainly include several different disorders. Some of these disorders, as indicated by low serum complement levels and measurable immune complexes in the blood, are mediated by immune complex deposition. Others seem to be independent of this mechanism.

## Sources of Confusion in Classification

Two forms of vasculitis, now termed *microscopic polyangiitis* and *cutaneous leukocytoclastic angiitis*, have been consistent sources of confusion in vasculitis nosology. In the current understanding of systemic vasculitides, these two conditions are separate entities. Microscopic polyangiitis affects capillaries, veins, and arteries (in contrast to PAN) and is associated with ANCAs, most often directed against MPO, in at least 70% of cases.[24] Davson and colleagues[25] remarked on two forms of "periarteritis nodosa" with differential effects on the kidney—one with a predilection for medium-sized, muscular arteries and the other with a predilection for the smaller vessels, including the glomeruli. Davson and colleagues[25] termed this latter form *microscopic periarteritis nodosa*. Swayed by human and animal models of hypersensitivity that showed small-vessel disease involving the kidneys, lungs, and other organs,[26,27] Zeek chose to group microscopic periarteritis nodosa under the heading of *hypersensitivity vasculitis*.[11]

During the next several decades, hypersensitivity vasculitis came to refer to an immune complex–mediated small vasculitis of the skin that spared internal organs and often followed drug exposures.[14] The first CHCC[22] in 1992 recommended eliminating the term *hypersensitivity* altogether because evidence for hypersensitivity is lacking in many cases. Participants in the CHCC preferred the term *cutaneous leukocytoclastic angiitis* because of the disorder's typical confinement to the skin and the usual predominant cell type—the neutrophil. Although cutaneous leukocytoclastic angiitis can mimic the skin features of microscopic polyangiitis, cutaneous leukocytoclastic angiitis does not involve the kidneys, lungs, peripheral nerves, and other internal organs and is not associated with ANCAs.

Although *cutaneous leukocytoclastic angiitis* may be preferable to *hypersensitivity vasculitis* in describing small vessel vasculitis confined to the skin, the term fails to acknowledge the few cases in which lymphocytic infiltrates predominate, and the nonspecific term "lymphocytic vasculitis" is used. Thus, the term *hypersensitivity vasculitis* continues to have utility, particularly when the inciting event (e.g., a drug or infection) is known. Hypersensitivity vasculitis is discussed further in Chapter 91.

## EPIDEMIOLOGY

Developing an accurate definition of the epidemiology of vasculitis involves the following challenges: (1) the uncommon nature of some forms of vasculitis; (2) the frequent difficulties in making the correct diagnosis of vasculitis (and in distinguishing one form of vasculitis from another); (3) the fact that the causes of most types of vasculitis remain unknown; and (4) historical uncertainty with regard to the

**TABLE 87-5** Epidemiology of Select Forms of Vasculitis

| Disease | INCIDENCE | | Age/Sex/Ethnic Predispositions |
|---|---|---|---|
| | United States | Elsewhere | |
| Giant cell arteritis | 240/1 million | 240/1 million (Scandinavia) | Age >50 yr, mean age 72 yr; females 3:1/Northern European ancestry |
| Takayasu's arteritis | 3/1 million | 200-300/1 million (India) | Age <40 yr; females 9:1/Asian |
| Behçet's disease | 3/1 million | 3000/1 million (Turkey) | Silk Route countries |
| Polyarteritis nodosa | 7/1 million | 7/1 million (Spain) | Slight male predominance |
| Kawasaki's disease | 100/1 million* | 900/1 million (Japan) | Children of Asian ancestry |
| Granulomatosis with polyangiitis | 4/1 million | 8.5/1 million (United Kingdom) | Whites ≫ blacks |
| Henoch-Schönlein purpura | In children: 135-180/1 million; in adults: 13/1 million | | Only 10% of cases occur in adults |

*Among children younger than 5 years.
From Gonzalez-Gay MA, Garcia-Porrua C: Epidemiology of the vasculitides. *Rheum Dis Clin North Am* 27:729–750, 2001.

classification of these conditions. Nevertheless, in recent years, the epidemiology of some forms of vasculitis has been defined with reasonable precision. Table 87-5 presents the major epidemiologic features of several forms of systemic vasculitis.

## Geography

The epidemiologic features of systemic vasculitis vary tremendously by geography. This variation may reflect genetics, differences in environmental exposures dictated by continent and latitude, and the prevalence of other disease risk factors. Although Behçet's disease is rare in North Americans (affecting only approximately 1 in 300,000), the condition is substantially more common among inhabitants of countries that border the ancient Silk Route.[21,28] Similarly, although Takayasu's arteritis is rare in the United States—on the order of three new cases per 1 million people per year—the disease is reportedly the most common cause of renal artery stenosis in India, where the incidence may be 200 to 300 per million per year. Several studies indicate that the prevalence of giant cell arteritis in Olmsted County, Minnesota, is similar to that of Scandinavian countries, with an annual incidence rate of approximately 240 cases for every 1 million persons older than 50 years.[29] The similar prevalence calculations across these countries probably reflect shared genetic risk factors for this condition, because many of the current inhabitants of Olmsted County are descended from Scandinavia and northern Europe. On the basis of 2010 U.S. Census data, the prevalence of giant cell arteritis in the United States is on the order of 200,000 cases.

## Age, Sex, and Ethnicity

Age is an important consideration in the epidemiology of vasculitis. Among patients with Kawasaki's disease, 80% are younger than 5 years.[30] In contrast, giant cell arteritis virtually never occurs in patients younger than 50 years, and the mean age of patients with that disease is 72 years. Age also may affect disease severity and outcome. In HSP, most cases in children (with 90% of all cases occurring in children) have self-limited courses that resolve within several weeks. In adults, HSP has a higher likelihood of chronicity and a greater likelihood of a poor renal outcome.[31]

The distribution of sex varies across many forms of vasculitis. Takayasu's arteritis has an overwhelming tendency to occur in women (a 9:1 female-to-male ratio), a fact that presently has no explanation. Pauci-immune forms of vasculitis such as GPA occur in men and women with approximately equal frequencies, but some evidence exists for a female predominance in patients with the limited form of the disease and a male predominance among patients with severe GPA.[32] Behçet's disease tends to be more severe in male patients, with a greater frequency of advanced ocular disease.

Some forms of vasculitis have striking variations in tendencies to affect specific ethnic groups. For example, giant cell arteritis and GPA occur with an overwhelming predominance in white persons,[33-35] whereas Takayasu's arteritis and Kawasaki's disease have higher incidences in persons of Asian ancestry.

## Genetics

Although genetic risk factors are undoubtedly important in the susceptibility to some forms of vasculitis, familial cases are rare (with the exception of giant cell arteritis, as subsequently discussed). The rarity of familial cases in vasculitis indicates that the genetics of these disorders are polygenic and complex. The strongest link between any single gene and vasculitis is the association of human leukocyte antigen (HLA)-B51 with Behçet's disease. In Behçet's disease, 80% of Asian patients have the *HLA-B51* gene.[28] The prevalence of *HLA-B51* is significantly higher among patients with Behçet's disease in Japan than among control subjects without the disease (55% vs. <15%). Among the sporadic cases of Behçet's disease involving white patients in the United States, however, *HLA-B51* occurs in less than 15% of cases. In addition to increasing the risk of disease susceptibility in some patients, *HLA-B51* increases disease severity. Patients with this gene are more likely to have posterior uveitis, central nervous system involvement, or other severe manifestations.

Reports of familial aggregation in giant cell arteritis are common. Genetic studies have indicated roles for class II HLA alleles such as HLA-DRB1*0401 and HLA-DRB1*0101, although the specific associations have varied from study to study.[36,37] Other work indicates that certain

TNF microsatellite polymorphisms may contribute to disease susceptibility.[38]

The greatest progress in understanding the relationship of genetics to systemic vasculitis has come in the area of rheumatoid vasculitis. The contribution of the "shared epitope" found on class II HLA molecules (DR4) to the development of RA has been appreciated for two decades.[39] Possession of the shared epitope is now known to substantially increase the risk of extraarticular manifestations in RA including vasculitis, at least in Northern European populations. A gene dosage effect for extraarticular RA with severe organ manifestations has been noted; patients with two copies of shared epitope alleles have a substantially higher risk of extraarticular disease manifestations, many of which are mediated by vasculitis.[40] One study reported an association between rheumatoid vasculitis and 0401/0404.[41]

In a case-control study of patients who had severe extraarticular RA compared with patients with RA who did not have extraarticular disease manifestations, the presence of two HLA-DRB1*04 alleles encoding the shared epitope was associated with extraarticular RA (odds ratio [OR], 1.79; 95% confidence interval [CI], 1.04 to 3.08) and rheumatoid vasculitis (OR, 2.44; 95% CI, 1.22 to 4.89).[42] In a meta-analysis of HLA-DRB1 genotyping studies of patients with rheumatoid vasculitis,[43] rheumatoid vasculitis was found to be associated with the genotypes 0401/0401, 0401/0404, and 0401/0101.

An association between rheumatoid vasculitis and class I HLA molecules also has been reported. An analysis of 159 patients with severe extraarticular RA (46 of whom had vasculitis) and 178 patients with RA who did not have extraarticular disease reported a strong association between the HLA-C3 allele and vasculitis.[44] Among patients with vasculitis, the allele frequency of HLA-C3 was 0.41 compared with 0.20 in patients with RA who did not have extraarticular disease ($P < 0.001$). The OR for vasculitis in patients with HLA-C3 was 4.15 (95% CI, 2.14 to 8.08).

The association between HLA-C3 and vasculitis was not due to linkage disequilibrium with HLA-DRB1, suggesting that these two genetic risk factors operate through different pathways. HLA-C3 was a strong predictor of vasculitis in patients lacking HLA-DRB1*04 shared epitope alleles, suggesting that *HLA-C* and *HLA-DR* genes influence the RA disease process through different pathways. Linkage disequilibrium with other genes in the major histocompatibility complex (MHC) could not be excluded in this study, however.

In GPA, the allele frequency of a functional polymorphism, 620W, in the intra-cellular tyrosine phosphatase gene *PTPN22* was found to be increased significantly among ANCA-positive patients compared with healthy control subjects.[45] Analyses of families with multiple autoimmune disorders have identified this allele as a risk factor for type 1 diabetes, seropositive RA, systemic lupus erythematosus, and autoimmune thyroid disease. In GPA, the allelic association was particularly strong among patients with generalized disease (i.e., vasculitis involving the kidney, lung, eye, and peripheral nervous system).

A genome-wide association study confirmed a modest genetic component to the pathogenesis of ANCA-associated vasculitis.[46] The study, performed in a discovery cohort of 1233 United Kingdom patients with ANCA-associated vasculitis and 5884 control subjects and replicated in 1454 Northern European case patients and 1666 control subjects, demonstrated genetic distinctions between GPA and microscopic polyangiitis that were associated with ANCA specificity. The strongest genetic associations were with the type of ANCA (anti-PR3 vs. anti-myeloperoxidase) rather than with the clinical diagnoses of GPA versus MPA. Anti-PR3 ANCA was associated with HLA-DP and the genes encoding α(1)-anti-trypsin *(SERPINA1)* and PR3 *(PRTN3)* ($P = 6.2 \times 10[-89]$, $P = 5.6 \times 10[-12]$, and $P = 2.6 \times 10[-7]$, respectively). Anti-myeloperoxidase ANCA was associated with HLA-DQ: $P = 2.1 \times 10(-8)$.

## Environment

Several environmental and occupational exposures have been linked to the development of vasculitis. Medications and certain infections have well-known associations with vasculitides. As examples, antibiotics such as the penicillins and cephalosporins are common causes of hypersensitivity vasculitis (see Chapter 91), and virtually any medication can trigger this syndrome. Hepatitis B and C have well-recognized links to PAN and mixed cryoglobulinemia, respectively. Moreover, both medications and infections can serve as triggers for HSP.

Cigarette smoking is the strongest environmental exposure risk for RA, and smoking increases the risk of severe forms of RA such as rheumatoid vasculitis. In a case-control study of patients with recent-onset RA, the interactions among smoking, shared epitope genes, and antibodies to citrullinated proteins were studied.[47] A dose-dependent relationship between smoking and the occurrence of anti-citrullinated protein antibody (ACPA) was found. The presence of shared epitope genes was a risk factor for RA only among patients who were positive for ACPA. A major gene-environment interaction between smoking and *HLA-DR* SE genes was evident in the large subgroup of patients who possessed ACPAs: The combination of smoking history and double copies of shared epitope alleles increased the risk of RA 21-fold. Smoking may trigger RA-specific immune reactions to citrullinated proteins in the context of shared epitope genes.

Associations have been reported, but not confirmed, between exposure to inhaled silica dust and some types of pauci-immune vasculitis.[48] Precise definitions of the relationships between exposures and vasculitis are complicated by difficulties in obtaining reliable measurements of the levels of such exposures, the likelihood of recall bias among patients who are diagnosed with vasculitis, and the choice of appropriate control groups.

The epidemiologic differences among individual types of vasculitis raise compelling questions about the etiologies of these diseases. Ultimately, better insights into the pathogenesis of these conditions should explain these epidemiologic differences and facilitate the development of more refined classification schemes.

## ADDITIONAL RESOURCES

The Johns Hopkins Vasculitis Center: http://www.hopkinsvasculitis.org/

The Cleveland Clinic Foundation Center for Vasculitis: www.clevelandclinic.org/arthritis/vasculitis/default.htm
The Vasculitis Foundation: www.vasculitisfoundation.org/
The Vasculitis Clinical Research Consortium: http://rarediseasesnetwork.epi.usf.edu/vcrc/
The National Institute of Allergy and Infectious Disease: http://www.niaid.nih.gov

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 88



# Giant Cell Arteritis, Polymyalgia Rheumatica, and Takayasu's Arteritis

David B. Hellmann

**KEY POINTS**

- Giant cell arteritis affects adults older than 50 years.
- The most common manifestations of giant cell arteritis are constitutional symptoms, headache, jaw claudication, and visual symptoms. Almost all untreated patients have an elevated erythrocyte sedimentation rate.
- The diagnosis of giant cell arteritis is usually confirmed by temporal artery biopsy.
- Early treatment of giant cell arteritis can prevent blindness.
- Polymyalgia rheumatica can occur by itself or with giant cell arteritis.
- Polymyalgia rheumatica responds to prednisone, 10 to 20 mg/day, whereas giant cell arteritis requires an initial dose of prednisone of approximately 60 mg/day.
- Takayasu's arteritis most frequently affects the aorta and its major branches in young women.

Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) are discussed together because they affect similar epidemiologic subsets of patients and often occur together in the same individual. Although GCA is a disease of older people and Takayasu's arteritis (TA) is a disease of younger people, their shared predilection for causing vasculitis of large arteries and their nearly identical histopathologic changes prompt their inclusion in the same chapter.

## GIANT CELL ARTERITIS AND POLYMYALGIA RHEUMATICA

### American College of Rheumatology Criteria

Classification criteria for the diagnosis of GCA have been proposed by the American College of Rheumatology (ACR) (Table 88-1).[1] Three classification schemes have been proposed for PMR (Table 88-2).[2-4] The provisional criteria for 2012 were developed in part to incorporate information from ultrasound evaluation.

### Definitions

#### Giant Cell Arteritis

GCA is the most common form of systemic vasculitis in adults.[5] The disease affects primarily the extracranial branches of the carotid artery in patients older than 50 years. The most feared complication of GCA is irreversible loss of vision. Because the cause of GCA is unknown, various names—including *temporal arteritis*, *cranial arteritis*, and *granulomatous arteritis*—have been used to highlight different salient features.[6] All the names for this disease have both merits and shortcomings. The designations *temporal arteritis* or *cranial arteritis*, for example, convey how frequently the temporal arteries or other cranial arteries are involved but fail to capture GCA's more widespread nature. Although the terms *granulomatous arteritis* and GCA recognize an important pathologic finding, this focus is undeserved because giant cells are absent in approximately half of the cases, and may be present in other forms of vasculitis. With no perfect name available, this chapter bows to convention and refers to this disease as *giant cell arteritis*.

### Polymyalgia Rheumatica

*Polymyalgia rheumatica*, a term suggested by Barber, is a syndrome characterized by aching in the proximal portions of the extremities and torso. Because no specific diagnostic tests or pathologic findings exist, PMR is defined by its clinical features. The features included in most definitions of PMR are as follows (1) aching and morning stiffness lasting half an hour or longer in the shoulder, hip girdle, neck, or a combination; (2) duration of these symptoms for 1 month or longer; (3) patient age older than 50 years; and (4) laboratory evidence of systemic inflammation such as an elevated erythrocyte sedimentation rate (ESR).[2] Some definitions also include a rapid response to small doses of glucocorticoids, such as prednisone 10 mg/day.[7] The presence of another specific disease other than GCA, such as rheumatoid arthritis (RA), chronic infection, polymyositis, or malignancy excludes the diagnosis of PMR.

### Epidemiology

The incidence of GCA varies widely in different populations, from less than 0.1 per 100,000 to 77 per 100,000 individuals aged 50 years and older.[5,8-12] The greatest risk factor for GCA is aging: the disease almost never occurs in individuals younger than 50 years, and its incidence rises steadily thereafter. The average age at diagnosis has risen during the past half century, from 74.7 years in the 1950s to 79.2 years at present.[13] Nationality, geography, and race are also important. The highest incidence is found in Scandinavians and in Americans of Scandinavian descent. The lowest incidence of GCA is reported in Japanese, northern



Supplemental images available on the accompanying ExpertConsult website.

**TABLE 88-1** American College of Rheumatology Classification Criteria for Giant Cell Arteritis

| Criterion | Definition |
|---|---|
| Age at disease onset ≥50 yr | Development of symptoms or findings beginning at age 50 yr or older |
| New headache | New onset or new type of localized pain in the head |
| Temporal artery abnormality | Temporal artery tenderness to palpation or decreased pulsation, unrelated to arteriosclerosis of cervical arteries |
| Elevated ESR | ESR ≥50 mm/hr by the Westergren method |
| Abnormal artery biopsy | Biopsy specimen with artery showing vasculitis, characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells |

*For purposes of classification, a patient with vasculitis is said to have giant cell (temporal) arteritis if at least three of these five criteria are present. The presence of any three or more criteria yields a sensitivity of 93.5% and a specificity of 91.2%.

ESR, Erythrocyte sedimentation rate.

From Hunder GG, Bloch DA, Michel BA, et al: The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 33:1125, 1990.

Indians, and African-Americans. In Western Europe, GCA is more common in the northern latitudes than the southern. The diagnosis of GCA has been increasing during the past 20 to 40 years, possibly because of greater physician awareness.[5,8] Some studies have reported seasonal variations and clustering of cases, with peaks approximately 7 years apart.[8,11] The prevalence of GCA in Olmsted County, Minnesota, home to many Scandinavian immigrants, is 200 per 100,000 population aged 50 years or older. Autopsy studies suggest that GCA may be more common than is clinically apparent. Östberg[14] found arteritis in 1.6% of 889 postmortem cases in which sections of the temporal artery and two transverse sections of the aorta were examined.

Genetic susceptibility to GCA was initially suggested by reports of GCA in families[15] and, more recently, by studies demonstrating an association with genes in the class II human leukocyte antigen (HLA) region.[16] Sixty percent of GCA patients have HLA-DRB1*04 haplotype variants, which have a common sequence motif in the second hypervariable region of the B1 molecule.[16] This motif differs from the one associated with RA.[16] The low prevalence of these alleles in African-Americans might explain why GCA is uncommon in this population. To date, GCA is the form of

**TABLE 88-2** Diagnostic* and Classification Criteria for Polymyalgia Rheumatica

| | | |
|---|---|---|
| **Diagnostic Criteria of Chuang and Colleagues (1982)** | | |
| Age 50 yr or older | | |
| Bilateral aching and stiffness for 1 month or more and involving two of the following areas: neck or torso, shoulders or proximal regions of the arms, and hips or proximal aspects of the thighs | | |
| ESR >40 mm/hr | | |
| Exclusion of all other diagnoses except giant cell arteritis | | |
| **Diagnostic Criteria of Healey (1984)** | | |
| Pain persisting for at least 1 mo and involving two of the following areas: neck, shoulders, and pelvic girdle | | |
| Morning stiffness lasting >1 hr | | |
| Rapid response to prednisone (≤20 mg/day) | | |
| Absence of other diseases capable of causing the musculoskeletal symptoms | | |
| Age older than 50 yr | | |
| ESR >40 mm/hr | | |
| **Classification Criteria of Dasgupta and Colleagues (2012)** | | |
| Age 50 years or older, bilateral shoulder aching, and abnormal C-reactive protein and/or ESR† | | |
| | **Points Without US (0-6)** | **Points With US‡ (0-8)** |
| Morning stiffness duration >45 min | 2 | 2 |
| Hip pain or limited range of motion | 1 | 1 |
| Absence of rheumatoid factor or anti-citrullinated protein antibody | 2 | 2 |
| Absence of other joint involvement | 1 | 1 |
| At least one shoulder with subdeltoid bursitis and/or biceps tenosynovitis and/or glenohumeral synovitis (either posterior or axillary) and at least one hip with synovitis and/or trochanteric bursitis | Not applicable | 1 |
| Both shoulders with subdeltoid bursitis, biceps tenosynovitis or glenohumeral synovitis | Not applicable | 1 |

*For each set of criteria, all the findings must be present for polymyalgia rheumatica to be diagnosed.

†A score of 4 or more is categorized as polymyalgia rheumatica in the algorithm without ultrasound (US), and a score of 5 or more is categorized as polymyalgia rheumatica in the algorithm with US.

‡Optional ultrasound criteria.

ESR, Erythrocyte sedimentation rate.

From Chuang T-Y, Hunder GG, Ilstrup DM, Kurland LT: Polymyalgia rheumatica: a 10-year epidemiologic and clinical study. *Ann Intern Med* 97:672, 1982; Healey LA: Long-term follow-up of polymyalgia rheumatica: evidence for synovitis. *Semin Arthritis Rheum* 13:322, 1984; and Dasgupta B, Cimmino MA, Kremers HM, et al: 2012 provisional classification criteria for polymyalgia rheumatica: a European League Against Rheumatism/American College of Rheumatology collaborative initiative. *Arthritis Rheum* 64:943–954, 2012. Reproduced with permission.

systemic vasculitis most closely associated with class II HLA genes. Susceptibility to GCA and PMR has also been associated with polymorphisms of genes for TNF, intercellular adhesion molecules, and interleukin (IL)-18.[5]

The existence of environmental risk factors has been suggested by the geographic clustering of GCA cases. Smoking increases the risk for GCA sixfold in women.[17] Circumstantial evidence links the development of GCA to a variety of infectious agents including *Mycoplasma pneumoniae*, varicella-zoster virus, parvovirus B19, and parainfluenza virus type I.[11,12] The results of examining temporal artery biopsy specimens with polymerase chain reaction methods to detect parvovirus B19 or herpesvirus DNA have been negative or inconsistent.[18] The reported association between GCA and *Chlamydia pneumoniae* infection has not withstood close scrutiny.[19] One study, published only in abstract form in 2012, identified a *Burkholderia* bacterium in 9 of 10 temporal arteries in GCA but in 0 of 11 controls.[20] Sex and health status also influence the development of GCA. Women are affected approximately twice as often as men.[11] Having diabetes reduces the risk of GCA by 50% in women.[2] Although patients with GCA have an increased risk of developing thoracic aortic aneurysms, they do not have overall higher mortality rates.[11]

PMR is two to three times more common than GCA.[5,9,10,21] In Olmsted County, Minnesota, 245 cases of PMR were diagnosed during the 22-year period from 1970 through 1991, providing an average annual incidence rate of 52.5 cases per 100,000 individuals aged 50 years or older.[21] The prevalence of PMR (active plus remitted cases) was approximately 600 per 100,000 individuals aged 50 years and older.[21] PMR is associated with the same *HLA-DR4* genes as GCA.[16,22]

## Etiology, Pathology, and Pathogenesis

The causes of GCA and PMR are unknown. Because pathologic studies have provided important clues to the pathogenesis, they are discussed first.

In GCA, inflammation is found most often in medium-sized muscular arteries that originate from the arch of the aorta.[11,14,23,24] The inflammation tends to affect the arteries in a segmental fashion (possibly leading to "skip lesions" within arteries), but long portions of arteries may be involved.[25] In patients who died during the active phase of GCA, the greatest frequency of severe involvement was noted in the superficial temporal arteries, vertebral arteries, and ophthalmic and posterior ciliary arteries.[26] The internal carotid, external carotid, and central retinal arteries were affected somewhat less frequently.[26] In other post-mortem studies, lesions were commonly found in the proximal and distal aorta and internal and external carotid, subclavian, brachial, and abdominal arteries.[14] Because GCA affects vessels with an internal elastic lamina and vasa vasorum, and because intracranial arteries lose these structures after penetrating the dura, it is not surprising that GCA rarely involves intracranial arteries.[26-28] In some patients with GCA, follow-up biopsy or autopsy surveys showed the persistence of mild chronic inflammation, even though symptoms had resolved.[2]

Early in the disease, collections of lymphocytes are confined to the region of the internal or external elastic lamina or adventitia. The inflammation may be limited to the vasa vasorum in some cases.[3] Intimal thickening with prominent cellular infiltration is a hallmark of more advanced cases. In heavily involved areas, all layers are affected (Figure 88-1). Transmural inflammation of portions of the arterial wall (including the elastic laminae) and granulomas containing multinucleated histiocytic and foreign body giant cells, histiocytes, lymphocytes (which are predominantly $CD4^+$ T cells), and some plasma cells and fibroblasts are found.[24,29,30] Eosinophils may be seen, but polymorphonuclear leukocytes are rare. Thrombosis may develop at sites of active inflammation; later, these areas may recanalize. The inflammatory process is usually most marked in the inner portion of the media adjacent to the internal elastic lamina. Fragmentation and disintegration of elastic fibers occur, closely associated with an accumulation of giant cells (Figure 88-2; see supplemental Figure 88-1 at ExpertConsult.com). However, giant cells are seen in only approximately half of routinely examined specimens; therefore, they are not required to make the diagnosis if other features are compatible. In contrast to some other forms of systemic vasculitis (e.g., polyarteritis nodosa, microscopic polyangiitis, granulomatosis with polyangiitis [GPA]), fibrinoid necrosis is rarely, if ever, observed in GCA.[3]

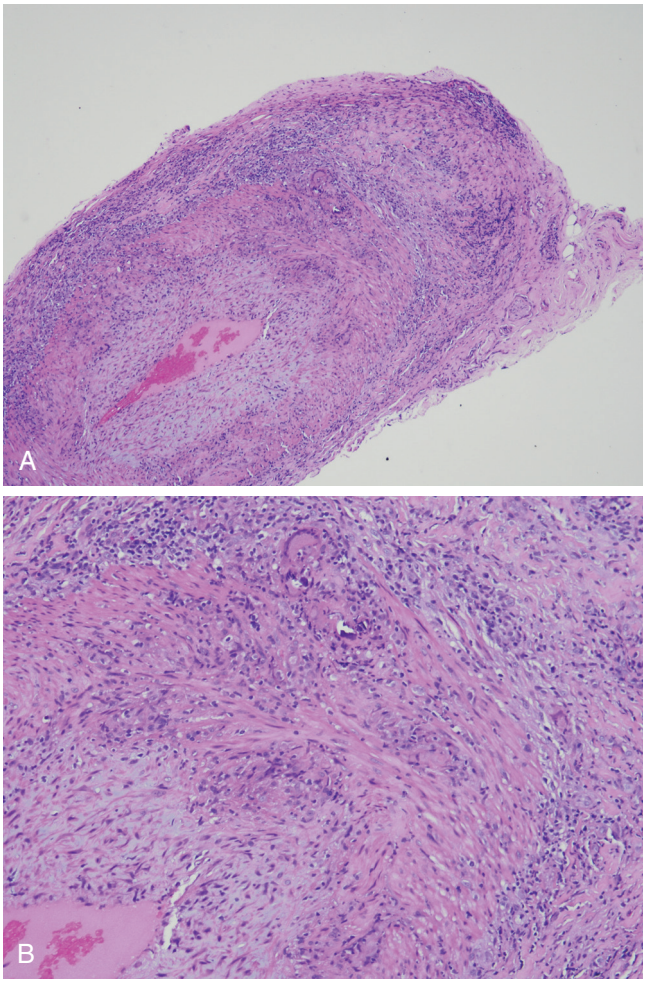
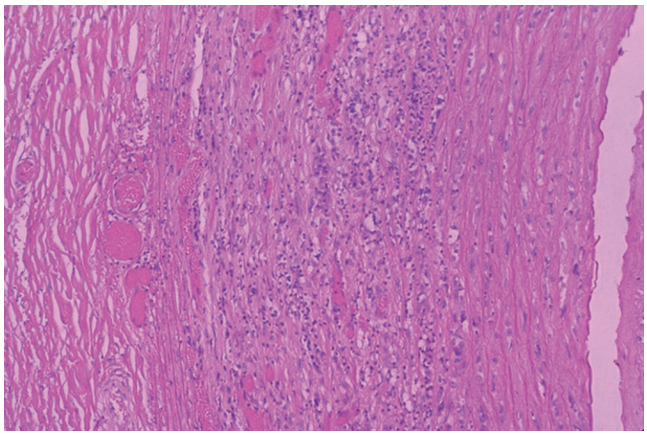



**Figure 88-1** Giant cell arteritis. **A,** Cross-section of a temporal artery showing transmural inflammation with mononuclear cells and giant cells (hematoxylin and eosin, ×10). **B,** Higher-power (×100) view demonstrating giant cells infiltrating the media. *(Courtesy Dr. Frederic Askin.)*



**Supplemental Figure 88-1** Aortitis in giant cell arteritis. Media of aorta is infiltrated with giant cells and mononuclear inflammatory cells (hematoxylin and eosin, ×40). *(Courtesy Dr. Gene Hunder.)*

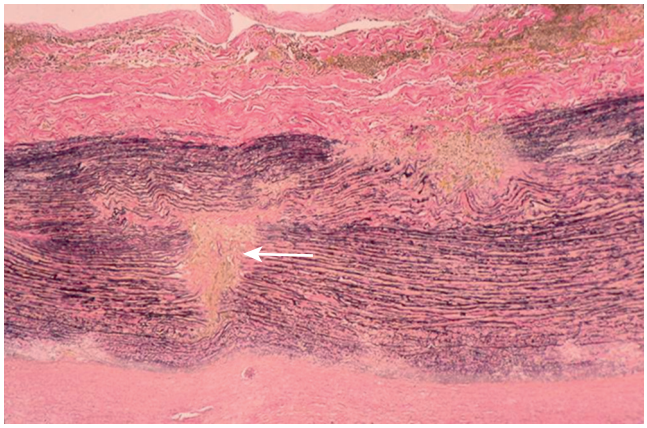


**Figure 88-2** Giant cell arteritis involving the proximal aorta in a patient who died of a ruptured ascending aorta. This section of the ascending aorta is distal to the ruptured portion and shows destruction of elastic fibers *(arrow)* (elastic van Gieson stain, ×64). Neighboring sections stained with hematoxylin and eosin showed infiltrations of mononuclear leukocytes in the areas of disrupted fibers.

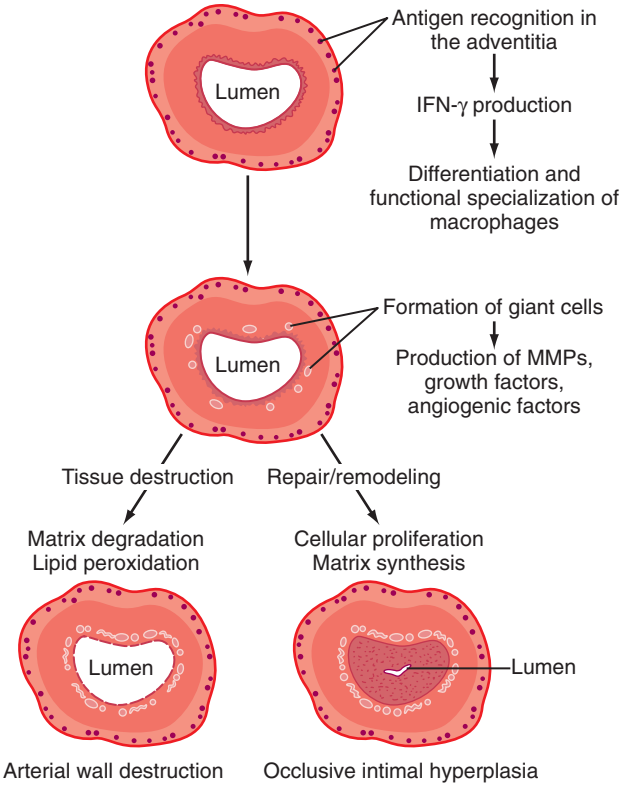



**Figure 88-3** Proposed model for the pathogenesis of giant cell arteritis. IFN, Interferon; MMPs, matrix metalloproteinases.

Immunohistochemical studies demonstrate inflammatory changes that are specific for each layer of the affected artery.[12, 24, 30-33] Dendritic cells, which can present antigen and activate T cells, are found in the adventitia, along with two separate lineages of $CD4^+$ T cells: (1) T helper (Th)1 cells that secrete interferon (IFN)-γ and IL-2 and (2) Th17 cells that secrete IL-17 family members including IL-17A.[34-36] The adventitial T cells show evidence of clonal expansion. The adventitia is also infiltrated with macrophages that secrete IL-1, IL-6, and transforming growth factor (TGF)-β. The media is populated mostly by macrophages that, in contrast to those in other layers, produce matrix metalloproteinases and oxygen-free radicals. Closer to the intima, the macrophages secrete nitric oxide and unite to form syncytia—the giant cells—which produce platelet-derived growth factor (PDGF) and substances that stimulate intimal proliferation.[24,37]

Although the gross appearance of arteries in patients with PMR is usually healthy, immunohistochemical studies of apparently uninvolved temporal arteries reveal increases of the same macrophage-related inflammatory cytokines found in GCA.[24] The T cell cytokine IFN-γ is abundantly expressed in GCA and is absent in arteries from patients who have only PMR. Pathologically, relatively little else has been found in the arteries of patients with PMR. Granulomatous myocarditis and hepatitis have been noted.[38] Muscle biopsies may be normal or show nonspecific type II muscle atrophy. However, a number of reports have shown the presence of lymphocytic synovitis in the knees, sternoclavicular joints, and shoulders and evidence of a similar reaction in sacroiliac joints.[39,40] Synovitis (mostly subclinical) was shown in bone scans demonstrating an increased uptake of technetium pertechnetate in the joints of 2 of 25 patients with PMR.[39] More sensitive studies that used MRI and ultrasonography have convincingly demonstrated that in PMR, the principal foci of inflammation are the bursae surrounding the shoulder more than the glenohumeral joint itself.[6] Sera from patients with GCA, PMR, or both demonstrate evidence of systemic inflammation, with increased levels of circulating immune complexes during active disease[41] and elevated levels of IL-6 and IL-1.[8]

These observations, together with the results from experiments in which temporal arteries from patients with GCA have been implanted into mice with severe combined immunodeficiency (SCID), suggest that GCA results from an adaptive immune response in the wall of an artery.[24,35,42,43] In this model, (Figure 88-3) the key initiating event is activation of dendritic cells located in the adventitia, the only arterial layer normally penetrated by the vasa vasorum. In large- and medium-sized arteries, immunohistochemical studies reveal that the dendritic cells in temporal arteries have a specific phenotype and express fascin and CD11c.[24,34] Dendritic cells express Toll-like receptors (TLRs) that normally serve as sentinels, monitoring for any immunologic breach of the adventitia.[44] In GCA, activation of TLRs on dendritic cells appears to be the initial triggering event.[34,44,45] Of the many different types of TLRs, TLR-2 and TLR-4 might be the most important in GCA. Although the exact trigger of the adaptive immune response in GCA is not known, experiments in the GCA-SCID mouse model have shown that blood-borne lipopolysaccharide serves as an effective TLR ligand in temporal arteries. Other constituents of microorganisms or some self-antigens (e.g., oxidized lipids) might also be TLR ligands that activate the arterial dendritic cells.

Once its TLRs have been engaged, dendritic cells differentiate from the resting to the active state and release cytokines such as IL-6 and IL-18, which recruit, activate, and retain $CD4^+$ T cells in the blood vessel. The crucial role

of dendritic cells in activating T cells and maintaining vasculitis has been demonstrated in the GCA-SCID mouse model. Depleting the dendritic cells markedly reduces the T cell infiltrate and suppresses the vasculitis. As noted, two separate populations of $CD4^+$ T cells become activated and expand: IFN-γ producing Th1 cells and IL-17 producing Th17 cells. IFN-γ causes macrophages to migrate, differentiate, and form granulomas.[36,44] Production of matrix metalloproteinases and lipid peroxidation agents by macrophages in the media results in the destruction of elastic laminae. The vessel attempts to counter the tissue destruction by elaborating a variety of growth factors including PDGF, vascular endothelial growth factor (VEGF), and TGF-β, which prompt smooth muscle cells in the media to revert from a contractile phenotype to a secretory one and migrate to the intima. Proliferation of the intimal smooth muscle cells results in occlusion of the lumen.[24,34]

PMR appears to result from a similar but less intense adaptive immune response in blood vessels (as evidenced by the in situ production of many inflammatory cytokines). According to this model, both PMR and GCA begin with the activation of dendritic cells at the adventitia-media border.[34,45] However, the distinguishing feature of PMR is the absence of T cells producing IFN-γ. Without IFN-γ to stimulate the recruitment and differentiation of macrophages, the level of arterial inflammation in PMR remains subclinical. Thus, the development of GCA appears to require both vascular dendritic cell activation and a disease-inducing repertoire of T cells.[24,34,45] The constitutional symptoms of PMR and GCA are attributed to the high levels of inflammatory cytokines (e.g., IL-1 and IL-6) found in the sera. Whether these serum cytokine elevations result from blood vessel inflammation alone or from some other source of inflammation is not yet clear. The attractiveness of this model is increased by its ability to explain why subsets of clinical features occur together.

## Clinical Features

The mean age at onset of GCA and PMR is approximately 79 years, with a range of approximately 50 to 90 years of age.[11,13] Younger patients with PMR have been described occasionally. Women are affected approximately twice as often as men.[21] Although the onset of the disease is usually insidious, typically evolving during weeks or months, in one-third of cases, the disease begins so abruptly that some patients recall the very day they became ill.[11,38]

### Giant Cell Arteritis

**Classic Manifestations.** The most common manifestations of GCA are constitutional symptoms, headache, visual symptoms, jaw claudication, and PMR[11] (Table 88-3). Almost all patients experience one or more constitutional symptoms including fatigue, weight loss, malaise, and fever.

Besides constitutional symptoms, headache is the most common symptom in GCA, being present in nearly three-quarters of patients.[46] The pain is typically described as boring in quality, of moderate severity, and most commonly appreciated in the temporal area. However, the description of the headache varies enormously. It can be mild to so severe that the patient seeks immediate relief by going to the emergency department. The pain may localize to any part of the skull, including the occiput (because of involvement of the occipital artery).[11,14] The most consistent characteristic is that the patient experiences the headache as something new and unusual. In untreated patients, the headache may subside over weeks, even though the disease activity continues. Often, the headache of GCA is not associated with any particular findings on physical examination. Abnormalities of the temporal artery—including enlargement, nodular swelling, tenderness, or loss of pulse—manifest in only approximately half of patients (Figure 88-4). Some patients note tenderness of the scalp, which can be aggravated by brushing or combing the hair.

Visual symptoms are common in GCA, especially loss of vision and diplopia. Vision loss can be unilateral or (less

**TABLE 88-3** Symptoms of Giant Cell Arteritis

| Symptom | Frequency (%) |
|---|---|
| Headache | 76 |
| Weight loss | 43 |
| Fever | 42 |
| Fatigue | 39 |
| Any visual symptom | 37 |
| Anorexia | 35 |
| Jaw claudication | 34 |
| Polymyalgia rheumatica | 34 |
| Arthralgia | 30 |
| Unilateral visual loss | 24 |
| Bilateral visual loss | 15 |
| Vertigo | 11 |
| Diplopia | 9 |

Modified from Smetana GW, Shmerling RH: Does this patient have temporal arteritis? *JAMA* 287:92, 2002. Data from a review of 2475 patients reported in the literature.

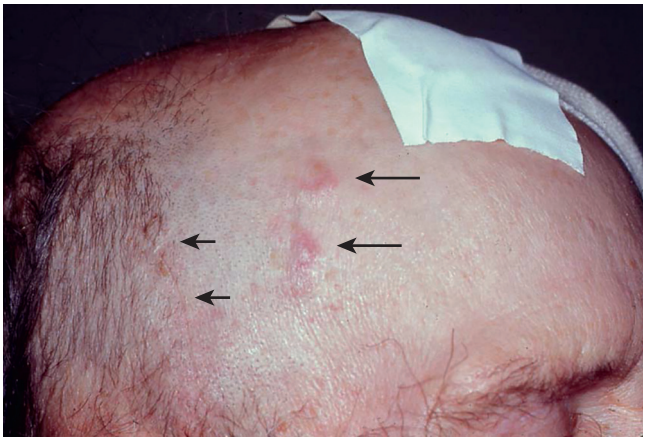


**Figure 88-4** Giant cell arteritis (GCA) involving the temporal artery. Short segments of curved artery were erythematous and tender *(long arrows)*. The bandage on the scalp covers a similar artery that was biopsied and showed GCA. A previous biopsy specimen of a proximal segment of the right temporal artery, which was normal on physical examination, was normal histologically. The faint scar from that biopsy can be seen above and anterior to the right ear *(short arrows)*.

commonly) bilateral, transient or permanent, and partial or complete.[47] Vision loss that lasts more than a few hours usually does not reverse. Loss of vision often reflects an anterior ischemic optic neuropathy caused by occlusive arteritis of the posterior ciliary artery, the chief blood supply to the head of the optic nerve. The posterior ciliary artery is a branch of the ophthalmic artery (which derives, in turn, from the internal carotid artery). Less frequently, vision loss in GCA stems from a retinal artery occlusion. Regardless of the site of the culprit lesion, vision loss in GCA is usually profound, with more than 80% of patients unable to see hand waving.[47] Vision loss is less likely to develop in GCA patients who are seen with fever or other systemic symptoms.[48,49] One possible explanation of this protective effect of fever and other systemic manifestations is that patients with prominent systemic inflammation demonstrate more extensive angiogenesis in temporal artery biopsies.[7] The angiogenesis associated with increased inflammation may result in the development of collateral circulation that reduces the chance of ischemic events.[7]

The early funduscopic appearance in the setting of blindness caused by anterior ischemic optic neuropathy is that of ischemic optic neuritis: slight pallor and edema of the optic disc, with scattered cotton-wool patches and small hemorrhages[47] (Figure 88-5). Later, optic atrophy occurs. Rarely, blindness may be the initial symptom; however, it tends to follow other symptoms by several weeks or even months. Ophthalmoscopic examination in patients without eye involvement is generally normal. In most reports, the incidence of blindness is 20% or less.[7,47,50] In a series of 245 patients from the modern era, 34 (14%) had some permanent loss of vision.[50] In 32 of these patients, the deficit developed before glucocorticoid therapy was begun; in the other 2, vision loss occurred after therapy was started. Vision loss progressed in 3 of the 32 after therapy was initiated, and it improved in 5 patients. At 5 years' follow-up, among the patients who had visual deficits caused by GCA at the time glucocorticoids were started, the risk of additional loss of vision was 13% during the follow-up period. If no loss had occurred at the beginning of glucocorticoid therapy, there was only a 1% risk of new loss of vision during the subsequent 5 years.

Another potential ocular complication of GCA is ophthalmoplegia. Diplopia usually results from ocular motor nerve palsies caused by ischemia and usually resolves after therapy is started. Oculomotor nerve involvement in GCA usually spares the pupil.[47] Rarely, arterial lesions cause infarction of the occipital cortex and vision loss.

Intermittent claudication may occur in the muscles of mastication (jaw claudication), the extremities, and, occasionally, the muscles of the tongue or those involved in swallowing.[11] In the jaw muscles, the discomfort is noted, especially when chewing meat, and may involve the muscles on one side of the mandible more than those on the other. In some instances, facial artery involvement results in spasm of the jaw muscles. More marked vascular narrowing may lead to gangrene of the scalp or tongue.

**Atypical Manifestations.** Approximately 40% of patients are seen with disease manifestations that are considered atypical[35,51-53] (Table 88-4). In these patients, headache, jaw claudication, visual symptoms, and PMR do not occur or are less prominent.

Fever occurs in as many as 40% of patients with GCA, but it is usually low grade and overshadowed by other classic symptoms. However, 15% of GCA patients may present with fever of unknown origin (FUO) in which the temperature spikes are high, dominating the clinical picture.[7,38] Although GCA causes only 2% of all cases of FUO, it is responsible for 16% of all such fevers in individuals older than 65 years.[38] Approximately two-thirds of patients experience shaking, chills, and drenching sweats, features often attributed to infection or malignancy. The median

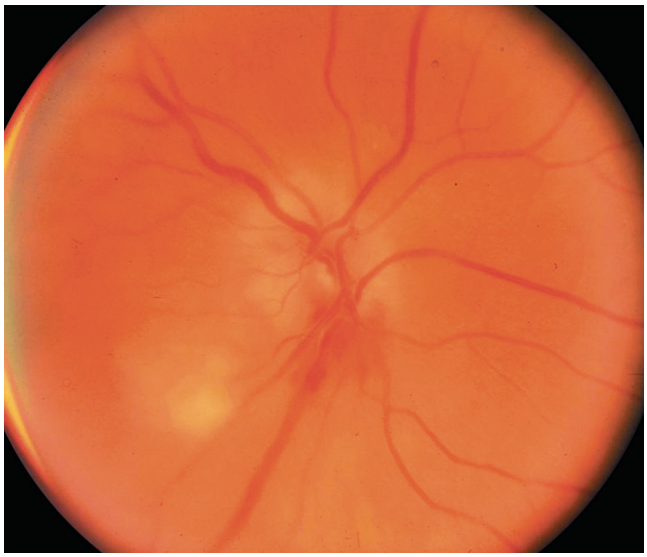


**Figure 88-5** Ophthalmoscopic appearance of the acute phase of anterior ischemic optic neuropathy seen in patients with giant cell arteritis and loss of vision. The optic disc is pale and swollen, the retinal veins are dilated, and several flame-shaped hemorrhages and a cotton-wool spot (retinal infarct) are visible. *(Courtesy Dr. Neil R. Miller.)*

**TABLE 88-4** Atypical Manifestations of Giant Cell Arteritis

| |
|---|
| Fever of unknown origin |
| Respiratory symptoms (especially cough) |
| Otolaryngeal manifestations |
| Glossitis |
| Lingual infarction |
| Throat pain |
| Hearing loss |
| Large artery disease |
| Aortic aneurysm |
| Aortic dissection |
| Limb claudication |
| Raynaud's phenomenon |
| Neurologic manifestations |
| Peripheral neuropathy |
| Transient ischemic attack, stroke |
| Dementia |
| Delirium |
| Myocardial infarction |
| Tumor-like lesions |
| Breast mass |
| Ovarian and uterine mass |
| Syndrome of inappropriate anti-diuretic hormone secretion |
| Microangiopathic hemolytic anemia |

temperature is 39.1° C, and the maximum 39.8° C. The white blood cell count in GCA-induced FUO is usually normal or nearly so (at least before the initiation of prednisone).

Neurologic problems occur in approximately 30% of patients.[1,54] These are diverse, but most common are neuropathies and transient ischemic attacks or strokes. Hemiparesis or brain stem events are to the result of narrowing or occlusion of the carotid or vertebrobasilar artery. GCA preferentially involves the posterior circulation: the 3:2 ratio of anterior to posterior strokes and transient ischemic attacks seen in the normal population reaches nearly 1:1 in patients with GCA.[55] Delirium, reversible dementia, and myelopathy have also been reported. However, the assignment of an exact cause to ischemic central nervous system events is often challenging, given the older population in which GCA occurs. The neuropathies of GCA include mononeuropathies and peripheral polyneuropathies and may affect the upper or lower extremities. Presumably, they are secondary to the involvement of nutrient arteries, but little pathologic documentation is available. Among the vasculitides, GCA has a nearly unique propensity for involving the C5 nerve root, resulting in loss of shoulder abduction.[27] Mononeuropathies affecting the hands and feet, so typical of polyarteritis and other forms of vasculitis, develop less often in patients with GCA.

Prominent respiratory tract symptoms occur in approximately 10% of patients.[56] These include cough with or without sputum, sore throat, and hoarseness. When these symptoms are severe or an initial manifestation of GCA, they may direct the attention of the examining physician away from the underlying arteritis. Vasculitis may induce these symptoms by causing ischemia or hyperirritability of the affected tissues. Otolaryngeal manifestations of GCA include throat pain, dental pain, tongue pain, glossitis, and ulceration or infarction of the tongue.[56,57]

Clinical evidence of large artery involvement occurs in 10% to 15% of patients at presentation, and in as many as 27% eventually.[3,40,58] Positron emission tomography (PET) studies with fluorodeoxyglucose (FDG) revealed that subclinical involvement of large arteries occurs in the vast majority of GCA patients. One PET study, for example, showed that 88% of 35 patients had increased FDG uptake in large arteries, with subclavian involvement in 7% and aortic involvement in 54%.[59]

Generally, clinically evident disease can be divided into early (within a year of diagnosis) and late (years after diagnosis) stages. Usually, early disease consists chiefly of large artery stenosis resulting in upper extremity claudication; bruits over the carotid, subclavian, axillary, and brachial arteries; absent or decreased pulses in the neck or arms; and Raynaud's phenomenon (Figure 88-6).[58] Angiographic features that suggest GCA are smooth-walled arterial stenoses or occlusions alternating with areas of normal or increased caliber in the absence of irregular plaques and ulcerations, located especially in the carotid, subclavian, axillary, and brachial arteries. Late disease most frequently involves thoracic aortic aneurysm.[58] The tendency for aneurysm to develop late was confirmed in one series of 41 patients in which the average time between diagnosis of GCA and recognition of this complication was 7 years.[41] Thoracic aortic aneurysm is 17 times more likely to develop in patients with GCA than in people without this disease. To place this risk in context, thoracic aortic aneurysms are twice as likely to complicate GCA as lung cancer is to result from smoking. Abdominal aortic aneurysm is also 2.4 times more common in patients with GCA.[3,41] In aggregate, nearly one out of five patients (18%) with GCA develops an aortic aneurysm or dissection.[58] Patients with large artery disease often do not have headache or other classic manifestations of GCA, and less than 50% have an abnormal temporal artery biopsy. Computed tomography (CT) angiography and magnetic resonance angiography (MRA) are the imaging modalities most commonly used to detect large artery disease in GCA.

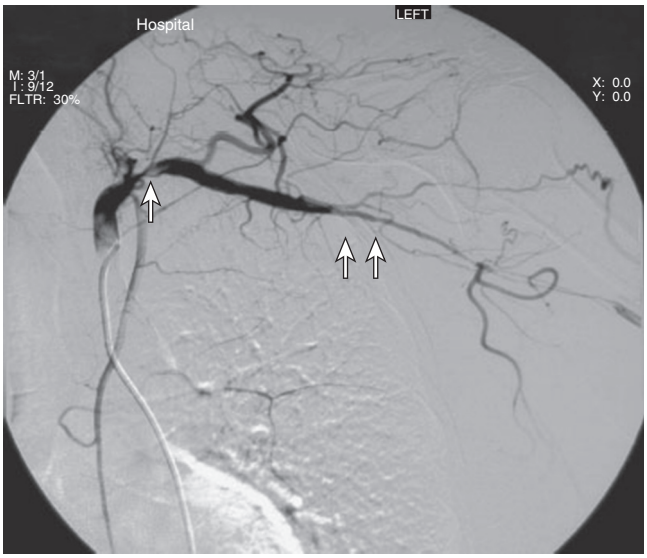



**Figure 88-6** CT angiogram showing giant cell arteritis causing abrupt narrowing of segments of the left subclavian *(arrowhead)* and axillary *(double arrowheads)* arteries. *(Courtesy Dr. Elliot Levy.)*

In women, GCA can uncommonly present as a breast or ovarian mass.[53,60] The mass lesions in these tissues result from granulomatous inflammation in and around the arteries. Angina pectoris, congestive heart failure, and myocardial infarction secondary to coronary arteritis occur rarely.

**Clinical Subsets.** Studies suggest that GCA is not just one disease but, rather, a number of clinical subsets that are explained by the differential expression of inflammatory cytokines.[61] Ischemic events including blindness, stroke, and large artery disease occur more commonly in patients who express high levels of IFN-γ and low levels of IL-6.[61] In contrast, patients who produce high amounts of IL-6 are more likely to have strong inflammatory features (such as fever and constitutional symptoms) and are less likely to develop vision loss or other ischemic events.[61-63]

## Polymyalgia Rheumatica

As in GCA, PMR patients are characteristically in good health before the onset of disease.[11] Systemic manifestations such as malaise, low-grade fever, and weight loss are present in more than half of the patients, and may be the initial symptoms of disease. High, spiking fevers are uncommon in PMR in the absence of GCA.[38] Arthralgias and myalgias may develop abruptly or evolve insidiously during weeks or

months.[2] Malaise, fatigue, and depression, along with aching and stiffness, may be present for months before the diagnosis is made. In most patients, the shoulder girdle is the first to become symptomatic; in the remainder, the hip or neck is involved at the onset. The discomfort may begin in one shoulder or hip, but usually becomes bilateral within weeks. Symptoms center on the proximal limb, axial musculature, and tendinous attachments. Morning stiffness resembling that of RA and "gelling" after inactivity are usually prominent. If the symptoms are severe, aching is more persistent. Although movement of the joints accentuates the pain, it is often felt in the proximal extremities rather than in the joints.[2] Distal joint pain and swelling occur in some cases, including diffuse distal extremity swelling with pitting edema.[64] Pain at night is common, and movement during sleep may awaken the patient. Muscle strength is generally unimpaired, although pain with movement makes the interpretation of strength-testing maneuvers difficult. Pain with movement also makes it difficult for patients to get out of bed or the bathtub. In the later stages of the syndrome, muscle atrophy may develop, and contracture of the shoulder capsule may result in limitation of passive and active motion.

As noted, the presence of bursal inflammation and synovitis in PMR has been described by many authors and is undoubtedly the cause of many of the findings in this condition.[6] A careful examination may reveal transient synovitis of the knees, wrists, and sternoclavicular joints. The shoulders and hips are covered by heavy muscles, and minimal effusions of slight synovitis are not palpable on physical examination. Synovitis has been documented by biopsies, synovial analysis, joint scintiscans, ultrasonography, and MRI.[38-40]

## Laboratory Studies

Except for the findings on arterial biopsy, laboratory results in PMR and GCA are similar (Table 88-5). A mild to moderate normochromic anemia is usually present in both diseases during their active phases. Leukocyte and differential counts are generally normal. A markedly elevated ESR and C-reactive protein (CRP) level are characteristic of both.[65,66]

**TABLE 88-5** Physical Findings and Laboratory Abnormalities in Giant Cell Arteritis

| Feature | Frequency (%) |
|---|---|
| Any temporal artery abnormality | 65 |
| Prominent or enlarged temporal artery | 47 |
| Absent temporal artery pulse | 45 |
| Scalp tenderness | 31 |
| Any funduscopic abnormality | 31 |
| Abnormal ESR | 96 |
| ESR >50 mm/hr | 83 |
| ESR >100 mm/hr | 39 |
| Anemia | 44 |

ESR, Erythrocyte sedimentation rate.

Modified from Smetana GW, Shmerling RH: Does this patient have temporal arteritis? *JAMA* 287:92, 2002.

In a study of 177 patients who had biopsy-proven GCA and measurement of both CRP and ESR, the CRP was minimally more sensitive than the ESR (86.4% vs. 84.2%).[66] The odds ratio of concordantly elevated CRP and ESR was 3.06.[66] Seven untreated patients (4%) had normal values for both ESR and CRP. The patients with normal inflammatory markers were more likely to have PMR and less likely to have constitutional symptoms or anemia.[66] An ESR higher than 100 mm/hr (Westergren method) is common, but untreated biopsy-proven cases of GCA may be associated with normal or nearly normal levels.[65,66] Of the GCA patients, 10.8% presented with an ESR of less than 50 mm/hr, and 3.6% had a rate of less than 30 mm/hr.[65] Rare individuals appear to be unable to develop an elevated ESR or CRP during any inflammatory process, including active GCA. The ESR and CRP is also liable to be relatively low or normal in patients who have been receiving corticosteroids for another condition.[65] Thus a normal ESR or CRP does not exclude the presence of GCA, especially in a patient with otherwise classic symptoms and findings. Platelet counts are often increased.

Nonspecific changes in plasma proteins are often present and include a decrease in the concentration of albumin and an increase in $\alpha_2$-globulins, fibrinogen, and other acute-phase reactant proteins. Slight increases in $\gamma$-globulins and complement may be present. Results of tests for anti-nuclear antibodies and rheumatoid factor are generally negative.

Liver function test results are mildly abnormal in approximately one-third of patients with GCA and in a slightly smaller number of those with PMR. An increased alkaline phosphatase level is the most common abnormality, but increases in aspartate transaminase and prolonged prothrombin time may also be found. Liver biopsy specimens are generally normal; granulomatous hepatitis has been observed.[38] Renal function and urinalysis are usually normal. Red blood cell casts are found in some instances, but their presence does not correlate with clinical large artery involvement.[25]

Levels of serum creatine kinase and other enzymes reflecting muscle damage are normal. Electromyograms are usually normal, and muscle biopsy shows normal histologic features or only the mild atrophy characteristic of disuse.

Synovial fluid analyses reported in GCA or PMR show evidence of mild inflammation including increased synovial fluid leukocyte counts, with a mean of 2900 cells/mm$^3$ but a range from 300 to 20,000 cells/mm$^3$; 40% to 50% are polymorphonuclear leukocytes. Synovial fluid complement levels are usually normal. In some instances, synovial biopsy has shown lymphocytic synovitis.[39,40]

Serum IL-6 levels are elevated in patients with PMR and GCA and appear to closely parallel the inflammatory activity.[67] Levels of factor VIII or von Willebrand factor are elevated in patients with GCA and PMR.

## Differential Diagnosis

Evidence that PMR and GCA are related and should be considered different manifestations of a common disease process is abundant.[11,24] The associations with age, ethnicity, geographic region, and class II HLA alleles are the same in both disorders. Moreover, both disorders involve overproduction of many of the same inflammatory cytokines.

Between 30% and 50% of patients with GCA develop PMR. Approximately 10% to 15% of patients who appear to have only PMR have positive temporal artery biopsies. In the absence of symptoms of GCA (e.g., headache, jaw claudication, visual symptoms, high fever), PMR by itself does not appear to cause vision loss and responds to low doses of glucocorticoids.[11]

The diagnosis of GCA should be considered in any patient older than 50 years who experiences loss of vision, diplopia, new form of headache, jaw claudication, PMR, FUO, unexplained constitutional symptoms, anemia, and a high ESR. GCA can cause so many forms of cranial discomfort (e.g., headache, scalp tenderness, jaw claudication, pain of the throat, gums, and tongue) that the disease should also be considered in any patient older than 50 years in whom new, unexplained "above-the-neck" pain develops. The protean manifestations of GCA mean that it should also be considered in the differential diagnosis of an older patient seen with dry cough, stroke, arm claudication, or acute C5 radiculopathy accompanied by other classic symptoms or findings of GCA.

Only a few individual symptoms or findings substantially increase or decrease the likelihood that a patient has this disease[46] (Table 88-6). For example, jaw claudication, diplopia, abnormal temporal artery signs, scalp tenderness, and ESR greater than 50 mm/hr increase the likelihood that a patient has GCA.[46] In one series of 373 patients, the presence of either jaw claudication or diplopia increased the likelihood of a positive biopsy by more than threefold; the presence of both jaw claudication and double vision had a 100% positive predictive value for a diagnostic temporal artery biopsy.[68] Conversely, the absence of headache or temporal artery abnormalities on physical examination, the presence of synovitis, and a normal ESR reduce the likelihood of GCA.

A large number of disorders can mimic GCA (Table 88-7). There are many causes of monocular vision loss besides vasculitis, including arteriosclerosis-induced thromboembolic disease.[47] Patients with nonarteritic vision loss do not have other GCA-related symptoms, signs, or findings. The funduscopic examination may help by revealing Hollenhorst plaques in cases caused by cholesterol emboli. Anterior ischemic optic neuropathy, the most common cause of vision loss in GCA, can also be caused by arteriosclerosis. Nonarteritic optic neuropathy invariably produces a small optic disc and cup-to-disc ratio, whereas GCA-related optic neuropathy results in an optic disc of variable size.[47] Thus a normal size or large cup in a patient with anterior ischemic optic neuropathy suggests GCA until proven otherwise.[47]

Constitutional symptoms with anemia and an elevated ESR in an older person may also be produced by occult infections (e.g., tuberculosis, bacterial endocarditis, HIV) or malignancy (especially lymphoma and multiple myeloma). These diagnoses highlight the value of selective serologic tests, imaging studies, and immunoelectrophoresis in appropriate patients. Systemic amyloidosis can closely mimic GCA, which is one of the few disorders other than GCA that causes jaw claudication. The amyloid deposits in the temporal artery may not be detected unless the specimen is stained with Congo red. Polyarthritis in an older patient is much more likely to be caused by RA than by GCA. In one study of 520 GCA patients, fewer than 2% developed polyarthritis before GCA was diagnosed.

**TABLE 88-6** Likelihood Ratios* for Symptoms, Signs, and Laboratory Findings in Giant Cell Arteritis

| Finding | Positive Likelihood Ratio (95% CI) | Negative Likelihood Ratio (95% CI) |
|---|---|---|
| **Symptoms** | | |
| Jaw claudication | 4.2 (2.8-6.2) | 0.72 (0.65-0.81) |
| Diplopia | 3.4 (1.3-8.6) | 0.95 (0.91-0.99) |
| Weight loss | 1.3 (1.1-1.5) | 0.89 (0.79-1.0) |
| Any headache | 1.2 (1.1-1.4) | 0.7 (0.57-0.85) |
| Fatigue | NS | NS |
| Anorexia | NS | NS |
| Arthralgia | NS | NS |
| Polymyalgia rheumatica | NS | NS |
| Fever | NS | NS |
| Visual loss | NS | NS |
| **Signs** | | |
| Beaded temporal artery | 4.6 (1.1-18.4) | 0.93 (0.88-0.99) |
| Tender temporal artery | 2.6 (1.9-3.7) | 0.82 (0.74-0.92) |
| Any temporal artery abnormality | 2.0 (1.4-3.0) | 0.53 (0.38-0.75) |
| Scalp tenderness | 1.6 (1.2-2.1) | 0.93 (0.86-1.0) |
| Synovitis | 0.41 (0.23-0.72) | 1.1 (1.0-1.2) |
| Optic atrophy | NS | NS |
| **Laboratory Results** | | |
| ESR abnormal | 1.1 (1.0-1.2) | 0.2 (0.08-0.51) |
| ESR >50 mm/hr | 1.2 (1.0-1.4) | 0.35 (0.18-0.67) |
| ESR >100 mm/hr | 1.9 (1.1-3.3) | 0.8 (0.68-0.95) |
| Anemia | NS | NS |

*Based on literature review, with the number of patients for each variable ranging from 68 to 2475.

CI, Confidence interval; ESR, erythrocyte sedimentation rate; NS, not significant.

Modified from Smetana GW, Shmerling RH: Does this patient have temporal arteritis? *JAMA* 287:92, 2002.

Criteria for the classification of GCA have been formulated and can help differentiate this arteritis from other forms of vasculitis[69] (see Table 88-1). TA, similar to GCA, can affect the aorta and the major arterial branches to the head and arms. TA, however, is a disease of young women. Anti-neutrophil cytoplasmic antibody (ANCA)–associated granulomatous vasculitis (AGV) can affect the temporal artery and, along with systemic amyloidosis, is an exception to the rule that jaw claudication is pathognomonic for GCA. AGV, however, almost always produces telltale involvement of the respiratory tract or kidneys and is associated with ANCAs. Polyarteritis nodosa can also affect the temporal artery and should be considered if the biopsy does not contain giant cells and the patient has other features atypical for GCA such as mesenteric arteritis. Fibrinoid necrosis of the vasa vasorum occurs in polyarteritis but

**TABLE 88-7** Differential Diagnosis of Giant Cell Arteritis and Polymyalgia Rheumatica

| Disease Type | Specific Entities |
|---|---|
| **Giant Cell Arteritis** | |
| Occult infections | Tuberculosis, bacterial endocarditis, human immunodeficiency virus |
| Malignancy | Lymphoma and multiple myeloma |
| Systemic amyloidosis | — |
| Other forms of vasculitis | Takayasu's arteritis, anti-neutrophil cytoplasmic antibody–associated granulomatous vasculitis, polyarteritis nodosa, primary angiitis of the central nervous system |
| | Other vascular disorders causing anterior ischemic optic neuropathy |
| **Polymyalgia Rheumatica** | |
| Early rheumatoid arthritis | — |
| Polymyositis | — |
| Chronic infections | Bacterial endocarditis |
| Fibromyalgia | — |
| Complication of medication | Statins |
| Endocrine disorders | Hypothyroidism |
| Remitting, seronegative synovitis with pitting edema | — |

rarely, if ever, in GCA. Primary angiitis of the central nervous system differs from GCA in that it affects the intracranial arteries.

The diagnosis of PMR is clinical and depends on eliciting the symptoms and findings noted earlier. Two sets of criteria for the diagnosis have been proposed[2,3] (see Table 88-2). Several disorders can mimic PMR (see Table 88-7). Distinguishing early RA from PMR can be difficult, especially in the 15% of patients who are rheumatoid factor-negative, and in those few patients with RA who have not yet developed prominent synovitis of the small joints of the hands and feet. Patients with polymyositis complain much more of weakness than of pain—the opposite symptom pattern reported by patients with PMR. In addition, in polymyositis, levels of muscle enzymes are elevated and electromyograms are abnormal. Although patients with neoplasms may have generalized musculoskeletal aching, no association exists between PMR and malignant neoplasia. Therefore, a search for an underlying tumor is not necessary unless some clinical evidence for a tumor is present or the patient has an atypically poor response to low-dose prednisone.

Some patients with chronic infections such as bacterial endocarditis may have findings that simulate PMR, and blood cultures should be obtained in patients with fever. Patients with fibromyalgia usually do not have typical morning stiffness and have laboratory test results that are normal or nearly so. Rarely, the stiffness of early Parkinson's disease can be confused with PMR if the bradykinesia and tremor of Parkinson's disease are subtle or absent. Lumbar spinal stenosis sometimes causes patients to complain of pain and stiffness in the hip girdle area. The absence of symptoms above the waist helps differentiate it from PMR. Cholesterol-lowering statin drugs can produce myalgia with or without muscle enzyme elevations but rarely mimic PMR. Hypothyroidism in the elderly can mimic many conditions, including PMR. A peculiar syndrome of remitting, seronegative synovitis with pitting edema (designated *$RS_3PE$ syndrome*) may be difficult to differentiate from PMR, and they may be related disorders. Acute symmetric polysynovitis of distal joints with pitting edema of the hands and feet develops in patients with the $RS_3PE$ syndrome. $RS_3PE$ syndrome and PMR both respond to NSAIDs and low-dose prednisone.[11,69]

## Diagnostic Evaluation in Giant Cell Arteritis

Temporal artery biopsy is the "gold standard" for diagnosing GCA.[11,70] Because GCA does not involve the artery in a continuous fashion, temporal artery biopsy should be directed to the symptomatic side, if evident. Removing a small (1 to 2 cm) section of temporal artery is usually adequate in patients who have palpable abnormalities of the vessel.[71] Otherwise, the surgeon should try to excise a 4 to 6 cm sample, and the pathologist should examine multiple sections.[11] In skilled hands, temporal artery biopsy is virtually free of morbidity or mortality. Scalp necrosis can rarely complicate active GCA but has not developed as a consequence of temporal artery biopsy.[70]

Temporal artery biopsies performed at institutions experienced in treating GCA are sensitive and have a high negative predictive value. At the Mayo Clinic, the sensitivity of temporal artery biopsy is approximately 90% to 95%, meaning that only 5% to 10% of patients with negative biopsies will subsequently be proved (by additional biopsy, angiography, or autopsy) to have GCA and require corticosteroid therapy.[70] The sensitivity figures noted previously include some patients who underwent bilateral temporal artery biopsy. Estimates of the value of bilateral biopsies vary.[69,72,73] Of 234 cases of biopsy-proven GCA, unilateral biopsy was positive in 86% and the second biopsy was positive in 14%.[69] Other studies indicate that a second temporal artery biopsy improves the diagnostic yield by only 3% to 5%.[73]

Management of a patient with a negative unilateral biopsy depends on how strongly the patient's clinical picture suggests GCA (Figure 88-7). When GCA is still strongly suspected, a second biopsy or an imaging test (see susequent discussion) should be considered. Opting for a second temporal artery biopsy probably makes most sense in patients who have jaw claudication or diplopia. Patients whose main symptom is occipital headache may be best diagnosed by biopsy of the occipital artery.[74] Patients who have signs of subclavian and axillary disease manifested by arm claudication, unequal arm blood pressures, and supraclavicular or axillary bruits may be diagnosed by angiogram, MRA, or CT scan.[61] Typically, patients with extracranial GCA have smooth, tapered stenosis or occlusion of the subclavian, axillary, and proximal brachial arteries. In one series, temporal artery biopsy was positive in only 58% of patients with larger artery involvement.[61] MRI and CT are the best-established methods for detecting aortic involvement by GCA.[11]

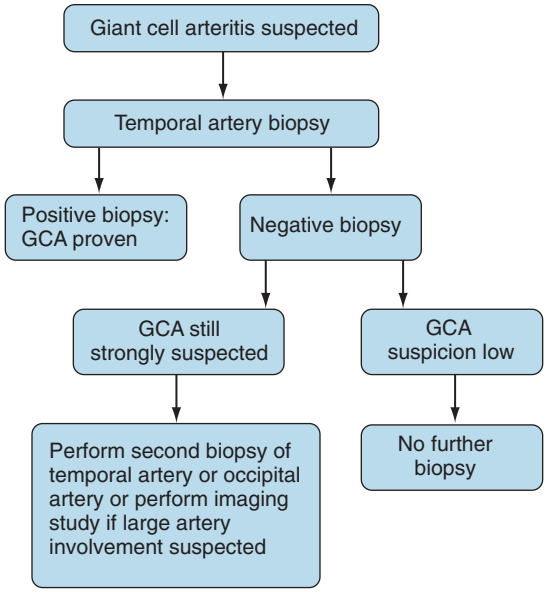



**Figure 88-7** Algorithm for diagnosing giant cell arteritis (GCA).

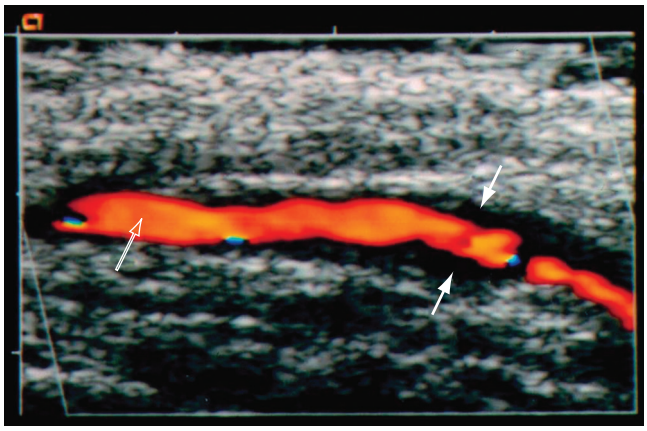


**Figure 88-8** Color duplex ultrasound examination of a swollen, tender temporal artery in a patient with giant cell arteritis. The variably thickened artery wall is visible as a clear "halo" *(solid arrows)* around the lumen in the center *(open arrow)*. *(Courtesy Dr. Gene Hunder.)*

Other imaging techniques have been proposed to assist in the diagnosis of GCA. Color duplex ultrasonography showed abnormalities of the temporal artery in 28 of 30 patients with GCA (sensitivity of 93%).[75] The most characteristic finding was a dark halo around the lumen of the temporal artery (Figure 88-8). However, the diagnostic value of ultrasonography is limited by the absence of expertise with this technique at most medical centers and conflicting estimates of its sensitivity and specificity.[76-78] One meta-analysis of 2036 patients in 23 studies estimated that the sensitivity and specificity of the halo sign were 69% and 82%, respectively. Another study found that ultrasonography did not improve the diagnostic accuracy of a carefully performed physical examination.[79] High-resolution MRI can demonstrate contrast enhancement and mural thickening of superficial cranial arteries in GCA. Small studies have found a sensitivity of 91% and a specificity of 73%, compared with temporal artery biopsy.[76-78] Best results are achieved with 3-T MRI scanners.[78] PET has shown promise in detecting occult involvement of the aorta and great vessels by GCA; however, estimates of sensitivity have varied from 65% to 100% and specificity from 77% to 99%.[76,78] PET scanning cannot image small arteries that are near highly metabolic tissues such as the brain, and thus cannot be used to study superficial extracranial arteries. Repeated PET scans do not predict the risk of relapse.[78] For the rare patient who has a compelling story for GCA but negative bilateral temporal artery biopsies, one should reconsider conditions that mimic GCA described earlier (e.g., ask the pathologist to stain the biopsied arteries with Congo red to rule out systemic amyloidosis). If no other diagnosis becomes evident, then it is reasonable to search for occult large vessel disease with an imaging test such as a PET scan or MRA to look for occult aortic disease. Rarely a patient's clinical picture strongly indicates GCA despite negative biopsies and imaging. In this case, the physician can treat the patient for GCA while watching vigilantly for the emergence of another disease.

## Treatment and Outcome

Most authorities recommend starting glucocorticoid therapy as soon as the diagnosis of GCA is strongly suspected. The main goal of treatment is to prevent loss of vision. Because vision loss is almost always permanent, it seems prudent to initiate corticosteroid therapy as early as possible, even before the biopsy is performed. Fortunately, the diagnostic yield of temporal artery biopsy is not altered by corticosteroid therapy for at least 2 weeks, and perhaps longer.[80]

### Initial Treatment for Giant Cell Arteritis

An initial dose of prednisone 40 to 60 mg/day or equivalent is adequate in nearly all cases.[11] Dividing the dose for the first 1 to 2 weeks may accelerate the rate of improvement. If the patient does not respond promptly, the dose should be increased. One double-blind, placebo-controlled, randomized trial involving 27 patients with GCA suggested that initiating treatment with intravenous methylprednisolone (15 mg/kg of ideal weight per day) for 3 days allowed more rapid tapering of oral corticosteroids and increased the likelihood of achieving a sustained remission.[81] The small size of that study and the possible overreliance on laboratory tests to define relapse raise questions about the generalizability of these results. Indeed, a randomized, multicenter, controlled trial of intravenous pulses of a single dose of 240 mg of methylprednisolone in the initial treatment of 164 GCA patients showed no benefit.[82] High-dose, intravenous-pulse methylprednisolone (1000 mg/day) for 3 days has also been tried in patients with recent loss of vision. Unfortunately, the loss remains permanent in the vast majority of patients. The occlusive nature of the vasculitis argues against any role of acute thrombolytic therapy in the treatment of blindness.

Because all patients with GCA require months of glucocorticoid therapy, measures to prevent osteoporosis should be started early, as described in Chapter 101. In addition, because traditional risk factors for atherosclerosis (e.g., smoking, hypertension, diabetes, hypercholesterolemia) might increase the risk of vision loss or stroke in GCA,[49]

reducing or eliminating these risk factors is an important part of overall management.

### Subsequent Treatment for Giant Cell Arteritis

The initial effective dose of prednisone should be continued until all reversible symptoms, signs, and laboratory abnormalities have reverted to normal.[11] This usually takes 2 to 4 weeks. After that, the dose can be gradually reduced by a maximum of 10% of the total dose each week or every 2 weeks.[11] The decision to reduce prednisone should be based on a composite assessment of the patient's symptoms, signs, and laboratory markers of inflammation. The ESR and serum concentration of CRP are generally the most convenient and helpful laboratory markers of inflammation. The ESR is reliable only if performed promptly after the blood sample is obtained. Serum levels of IL-6 appear to be the most sensitive marker of activity of GCA, but this test is not widely available.[83] CRP may be slightly more sensitive than ESR in detecting flares.[83] At some point during drug tapering, the ESR or CRP may rise above normal again, and further reductions of prednisone should be temporarily interrupted. If, over the next week or so, the patient does not develop signs or symptoms of active GCA, reductions in prednisone (at smaller decrements and at longer intervals) can usually be resumed. Doses of 10 to 20 mg/day or more are often required for several months before further reductions are possible. However, if the prednisone dose is dependent on the levels of inflammatory markers with no regard to the patient's overall clinical context, there is a risk of corticosteroid-related side effects. Gradual reductions allow the identification of the minimal suppressive dose and help avoid exacerbations that result from too-rapid tapering. Even with a gradual reduction of prednisone, more than 50% of patients experience flares of disease activity during the first year.[84] These exacerbations can usually be handled by increasing the prednisone by 10 mg above the last dose at which the disease was controlled.

Most patients with GCA have a chronic disease that requires prednisone treatment for at least 1 year and often for several years; a minority of patients run a self-limited course of only several months.[11] Glucocorticoids can eventually be reduced and discontinued in some patients. Many patients require low doses of prednisone for several years or more to control musculoskeletal symptoms.

The nearly universal experience of serious side effects associated with daily corticosteroids has prompted the search for alternative steroid-sparing treatments. Unfortunately, to date, none has been convincingly effective. Alternate-day prednisone, for example, is not effective initial therapy for GCA. Similarly, cytotoxic drugs, dapsone, anti-malarials, and cyclosporine have not been clearly shown to be effective.[11,85] The combination of weekly low-dose oral methotrexate and prednisone was steroid sparing in one placebo-controlled, double-blind treatment trial but not in another.[84] Statins do not appear to modify the course of GCA or the need for corticosteroids.[86]

Delineation of the cells and cytokines involved in the pathogenesis of GCA has invited trials of biologic agents. For example, the putative role of TNF in propagating a granulomatous vasculitic process suggested that TNF inhibitors might be effective in treating GCA. Unfortunately, neither infliximab nor etanercept is effective in GCA.[87,88] High expression of IL-6 in affected arteries and in the circulation of patients with GCA has opened the door for trying to treat with tocilizumab, a humanized monoclonal anti-IL-6 receptor antibody.[89-91] In the approximately 2 dozen patients with GCA reported in case reports and small series to have received tocilizumab, the drug appeared to be effective in reducing symptoms. Enthusiasm for this therapy must be tempered until the availability of data from large, randomized-controlled trials. In addition, most patients relapsed after the drug was stopped. The possibility that IL-6 inhibitors may suppress the systemic symptoms and signs without eliminating the vasculitis has been suggested by an autopsy in the one patient treated with tocilizumab who had appeared to respond and died of other causes, which showed persistent active arteritis.[90] A few case reports have described apparent responses in GCA patients treated with the anti-CD20 monoclonal antibody rituximab to deplete B cells or with anakinra to inhibit IL-1 receptors.[92]

No prospective, double-blind trials have tested the potential adjunctive role of aspirin or anti-coagulants in the treatment of GCA.[93] However, aspirin is theoretically appealing because, in experimental models of GCA, it inhibits IFN-γ production more effectively than prednisone.[94] In addition, three retrospective studies found that patients with GCA taking low-dose aspirin or anti-coagulant therapy had a threefold to fivefold lower risk of an ischemic event such as vision loss.[95-97] Together, these studies suggest that it is reasonable to administer low-dose aspirin to patients with GCA who do not have an excessive risk of gastrointestinal bleeding.

Arm claudication from GCA affecting the subclavian and axillary arteries usually improves or resolves with corticosteroid therapy. Rare patients with GCA who have severe upper extremity claudication unresponsive to corticosteroid therapy may benefit from balloon angioplasty. In one series of 10 patients, all improved initially after angioplasty, but symptomatic restenosis developed in 50% during 2 months.[98] In all cases, recurrent stenosis developed in vascular lesions that were greater than 3 cm long.[98]

Thoracic aortic aneurysm is greatly increased in patients with GCA. Although it can be present at the outset, aneurysms are usually noted late in the disease course, an average of 7 years after onset. Some authorities have recommended annual chest radiographs to detect thoracic aortic aneurysms.

### Treatment for Polymyalgia Rheumatica

Patients with PMR without symptoms or signs or biopsy evidence of GCA are usually treated initially with prednisone 10 to 20 mg/day or equivalent.[99,100] A systematic review of 30 studies recommended starting treatment with prednisone at 15 mg/day; lower initial doses were less effective and higher doses were more toxic.[101] Salicylates and NSAIDs have been used but are less appealing; salicylates and NSAIDs adequately control symptoms in only a minority of patients with milder symptoms and add to overall adverse drug reactions when they are used with glucocorticoids.[2,8] Prednisone therapy usually results in rapid (often overnight) and dramatic improvement of the musculoskeletal aching

and stiffness and a more gradual return of the ESR and CRP level to normal. A minority of patients with isolated PMR fail to respond to prednisone 20 mg/day after 1 week and may require as much as 30 mg/day as initial treatment. Studies suggest that these resistant cases are more likely to have ESRs greater than 50 mm/hr and high levels of IL-6. If a patient does not respond to prednisone 30 mg/day for 1 week, this should prompt a search for an alternative diagnosis (Figure 88-9). Lower doses of prednisone might not suppress an underlying arteritis if it is present. Thus, the patient must be observed carefully even though the aching improves. In patients with PMR, the dose should be reduced gradually as soon as symptoms permit. Pre-treatment ESR, CRP, and IL-6 concentrations and initial responses to therapy appear to be helpful in dividing patients into subsets with different treatment requirements.[99] If the laboratory test results become normal while the patient is receiving a smaller dose, the likelihood of an underlying active vasculitis seems to be much less, and the risk of vascular complications is smaller. However, this is not true in all instances because active arteritis has been observed even though the ESR improved.

Once the symptoms, signs, and laboratory abnormalities of PMR have resolved (usually after 2 to 3 weeks of therapy), the daily dose of prednisone can be slowly tapered. Some experts recommend tapering prednisone by 2.5 mg every week until 10 mg/day is reached, at which point the decrements should be reduced by 1 mg each month.[101] Flares are common, necessitating a dose increase to achieve remission before attempting a slower taper. The minority of patients with PMR succeed in tapering off prednisone in less than 1 year. Many require at least 2 years of low-dose prednisone.[102]

Some, but not all, studies suggest that oral methotrexate (10 mg once a week for 48 weeks) can reduce the long-term need for corticosteroids in patients with PMR.[101,103] It is not yet known whether the small but statistically significant reduction in prednisone use achieved with methotrexate results in a clinically important reduction in prednisone-related side effects.

## TAKAYASU'S ARTERITIS

TA, also known as *pulseless disease* or *occlusive thromboaortopathy*, is a form of vasculitis of unknown cause that chiefly affects the aorta and its major branches, most frequently in young women.[104,105] The disease is named for the Japanese ophthalmologist who, in 1908, described a young woman with peculiar retinal arteriovenous anastomoses caused by retinal ischemia from large vessel vasculitis.[1]

### American College of Rheumatology Classification Criteria

The ACR classification criteria for the diagnosis of TA are listed in Table 88-8.

### Epidemiology

Although TA has been described worldwide, it occurs most commonly in Japan, China, India, and Southeast Asia. The disease is also prevalent in Mexico. Whereas the incidence of TA in Japan is nearly 150 per million per year, it is only 0.2 to 2.6 per million in Western Europe and North America. TA affects women eight times more frequently than men. The median patient age at onset is 25 years; however,

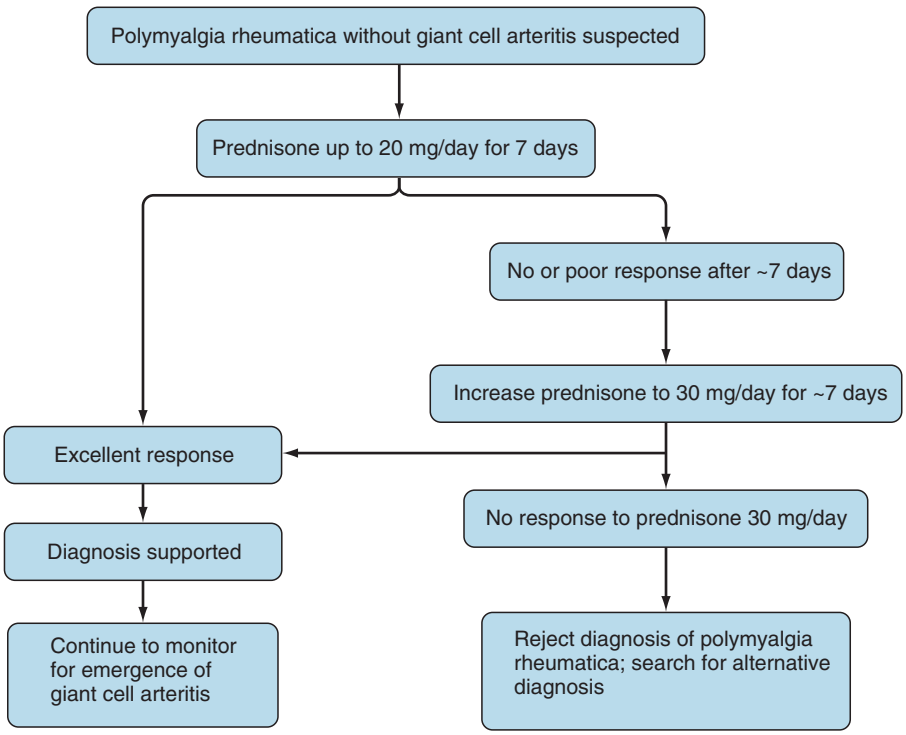



**Figure 88-9** Algorithm for diagnosing polymyalgia rheumatica without giant cell arteritis.

**TABLE 88-8** American College of Rheumatology Classification Criteria for Takayasu's Arteritis*

| |
|---|
| Onset before age 40 yr |
| Limb claudication |
| Decreased brachial artery pulse |
| Unequal arm blood pressure (>10 mm Hg) |
| Subclavian or aortic bruit |
| Angiographic evidence of narrowing or occlusion of aorta or its primary branches, or large limb arteritis |

*The presence of three or more of the six criteria is sensitive (91%) and specific (98%) for the diagnosis of Takayasu's arteritis.

American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis, *Arthritis Rheum* 33:1129, 1990; From Hellmann DB: Takayasu arteritis. In Imboden JB, Hellmann DB, Stone JH, editors: *Current rheumatology diagnosis and treatment,* New York, 2004, Lange Medical Books/McGraw-Hill, p 245.

approximately 25% of cases begin before age 20, and 10% to 20% of patients are seen after age 40 years.[104] Immunogenetic studies in Japanese patients suggest an association with several HLAs, especially HLA-Bw52, Dw12, DR2, and DQw1. Different HLA associations have been found in Koreans and Indians. No HLA association has been found in North American patients. In Mexican patients, TA has been associated with previous exposure to *Mycobacterium tuberculosis*.

## Etiology and Pathogenesis

The cause of TA is unknown. The nearly identical pathology in TA and GCA has invited speculation that the model of immunopathogenesis of GCA described earlier (see Figure 88-3) applies to TA as well.[24,44] Indeed, some have argued that TA and GCA represent a spectrum within the same disease. Similar to GCA, TA is thought to result from an autoimmune process that targets large elastic-containing arteries. Both feature panarteritis involving infiltration of dendritic cells; T cells (including αβ, γδ, and cytotoxic); natural killer cells; and macrophages. In TA, the majority of lymphocytes are perforin-secreting killer lymphocytes such as T cells and natural killer cells. The T cell receptors in TA, as in GCA, are oligoclonal, suggesting that the vasculitis is driven in both diseases by a T cell response to a specific but unknown antigen.[106] Chronic inflammation of the vessel wall leads to aneurysm formation, stenosis, or thrombosis more frequently in TA than in GCA. Dissection occurs in TA, but is rare and less frequent than in syphilitic aortitis.[107] The late phase of TA, similar to that of GCA, is characterized by intima proliferation with superimposed atherosclerosis, medial necrosis with scarring, and adventitial fibrosis. As in GCA, the inflammatory involvement in TA can be continuous or segmental, with skip areas of normal vessel interposed between involved areas.[107] It is possible that the humoral immune system may play some role in the pathogenesis of TA. Most patients with TA possess anti-endothelial cell antibodies that can damage vessels by inducing endothelial inflammatory cytokine production, adhesion molecules, and apoptosis.

The geographic clustering of cases has suggested that genetics and environmental factors participate in the pathogenesis of TA. However, immunogenetic studies (described in previous text) have not identified any other universally shared genetic risk factors. The young age of onset and the female predominance in TA and in systemic lupus erythematosus have invited speculation about the influence of female hormones in promoting an autoimmune disease process. In some countries, the apparent association of TA with high rates of exposure to tuberculosis has suggested an infectious cause. An animal model of TA has been produced in mice by using a herpesvirus that infects the smooth muscle cells of the media. In that model, the media of large elastic arteries serves as an immunoprivileged site that allows the herpesvirus to propagate a chronic inflammatory response in the aorta and its major branches.[108]

**TABLE 88-9** Clinical Features of Takayasu's Arteritis

| Feature | At Presentation (%) | Ever Present (%) |
|---|---|---|
| Vascular | 50 | 100 |
| Bruit | | 80 |
| Claudication (upper extremity) | 30 | 62 |
| Claudication (lower extremity) | 15 | 32 |
| Hypertension | 20 | 33 |
| Unequal arm blood pressures | 15 | 50 |
| Carotidynia | 15 | 32 |
| Aortic regurgitation | | 20 |
| Central nervous system | 30 | 57 |
| Lightheadedness | 20 | 35 |
| Visual abnormality | 10 | 30 |
| Stroke | 5 | 10 |
| Musculoskeletal | 20 | 53 |
| Chest wall pain | 10 | 30 |
| Joint pain | 10 | 30 |
| Myalgia | 5 | 15 |
| Constitutional | 33 | 43 |
| Malaise | 20 | 30 |
| Fever | 20 | 25 |
| Weight loss | 15 | 20 |
| Cardiac | 15 | 38 |
| Aortic regurgitation | 8 | 20 |
| Angina | 2 | 12 |
| Congestive heart failure | 2 | 10 |

Data based on a study of 60 North American patients reported by Kerr GS, Hallahan CW, Giordano A, et al: Takayasu arteritis. *Ann Intern Med* 120:919, 1994; From Hellmann DB: Takayasu arteritis. In Imboden JB, Hellmann DB, Stone JH, editors: *Current rheumatology diagnosis and treatment,* New York, 2004, Lange Medical Books/McGraw-Hill, p 243.

## Clinical Features

Although the presenting manifestations of TA are protean, the vast majority of patients are seen with symptoms and signs of vascular insufficiency (from stenosis, occlusion, or aneurysm), systemic inflammation, or both[79,104] (Table 88-9). In a North American series of 60 patients followed up at the National Institutes of Health, the most common presenting vascular symptoms were claudication (35%), reduced or absent pulse (25%), carotid bruit (20%), hypertension (20%), carotidynia (20%), lightheadedness (20%), and asymmetric arm blood pressures (15%).[104] Stroke, aortic regurgitation, and visual abnormalities were present at onset in less than 10% of patients. The extreme manifestations of

retinal ischemia noted in Takayasu's original patient are rarely seen now.[104] Permanent loss of vision, the major concern in GCA, rarely develops in TA.

Claudication affects the arms at least twice as frequently as the legs. For many young women, arm claudication first reveals itself as arm pain or fatigue experienced while trying to hold a hair dryer. Overall, bruit is the most common sign, eventually found in 80% of patients. Although bruit over the carotid artery is most frequent, it can also be found in the supraclavicular, infraclavicular, axillary, flank, chest, abdominal, and femoral areas. One-third of patients have multiple bruits.[104] Unequal arm blood pressures eventually develop in half of all patients. Headache, which is common in TA, does not correlate with carotid or vertebral disease, which develops in nearly 40% of patients.[104,109]

Constitutional, musculoskeletal, and other symptoms of systemic inflammation are also common presenting complaints.[79,104] About one in five TA patients presents with fever and malaise, which can be accompanied by night sweats and weight loss. A few patients who have minimal or no signs of vascular insufficiency may appear to have FUO for weeks or months before the diagnosis of TA becomes evident. A minority of patients present with myalgia or arthralgia (see Table 88-9). Some patients have striking midthoracic back pain, perhaps as a result of aortic inflammation irritating nociceptive nerve fibers.

Cardiac involvement occurs eventually in nearly one-third of patients (see Table 88-9).[104] Aortic regurgitation develops in 20% of patients as a result of aortic root dilation. Aortic regurgitation is important because it frequently progresses and may lead to left ventricular dilation with secondary mitral regurgitation and congestive heart failure. Aortic valve replacement is often required eventually. Angina can develop as a result of coronary artery disease. TA of the coronary arteries most often produces ostial lesions but can also produce either diffuse vasculitis of the coronary arteries or aneurysms.[110-112] Myocarditis also occurs in TA and causes potentially reversible congestive heart failure. Pericarditis is rare. TA is, along with Behçet's disease, one of the few forms of vasculitis that can affect the large pulmonary arteries. Although TA of the pulmonary arteries is rare (appearing in less than 3% of patients), affected patients can be seen with cough, chest wall pain, dyspnea, or hemoptysis.

Unlike polyarteritis or GPA, TA rarely causes peripheral neuropathies. Cutaneous manifestations develop in less than 10% of patients with TA.[104] Erythema nodosum is most common, but purpura, livedo reticularis, and ulceration may rarely occur. As in GCA, a minority of patients with TA who have active disease have a persistent, dry cough.

## Laboratory Studies

At presentation, the ESR is more frequently elevated (80%) than the CRP (~50%).[79] Mild anemia and hypergammaglobulinemia are common. The white blood cell count is usually normal or slightly elevated. The platelet count is elevated in one-third of patients, and may exceed 500,000/μL in those with active disease. The serum creatinine and urinalysis are usually normal. Any renal abnormalities are usually secondary to hypertension; unlike ANCA-associated vasculitis, TA rarely causes glomerulonephritis.

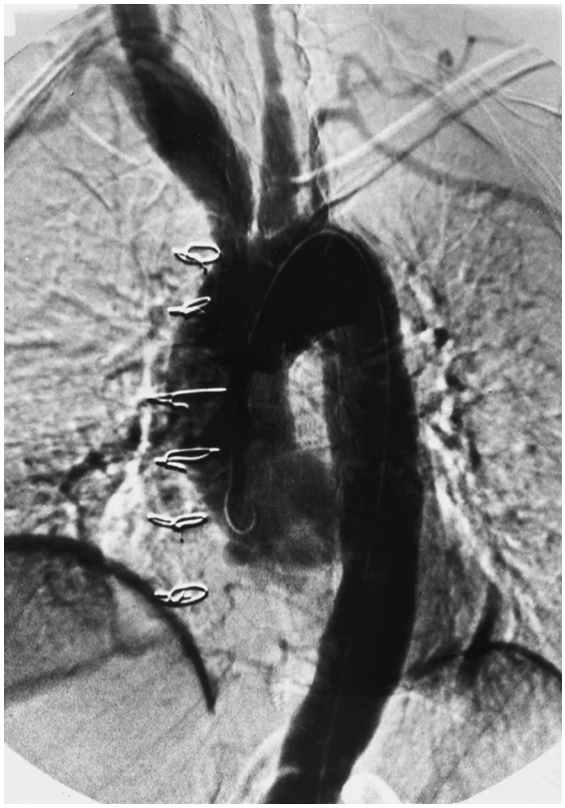


**Figure 88-10** Angiogram showing multiple changes of Takayasu's arteritis including dilation of the aortic root (with surgical wires from previous aortic valve replacement), aneurysmal dilation of the innominate and right carotid arteries, and occlusion of the distal left common carotid artery. *(From Hellmann DB, Flynn JA: Clinical presentation and natural history of Takayasu's arteritis and other inflammatory arteritides. In Perler BA, Becker GJ, editors:* Vascular intervention: a clinical approach, *New York, 1998, Thieme Medical and Scientific Publisher, pp 249–256.)*

### Imaging

Vascular abnormalities in TA can be imaged by conventional angiography, MRI, MRA, CT angiography, or ultrasonography[26,76,78,104,113] (Figure 88-10, Figure 88-11, and Figure 88-12). Each imaging technique has advantages and disadvantages (Table 88-10). The earliest detectable abnormality in TA is thickening of the vessel wall from inflammation. MRI, ultrasonography, and, to a lesser degree, CT can detect this early vessel wall thickening. Conventional angiography is invasive and provides the least sensitive method for visualizing wall thickness; however, conventional angiography is the "gold standard" for precisely delineating the stenoses, occlusions, and aneurysms that characterize the latter stages of TA. Also, only conventional angiography allows the direct measurement of central arterial blood pressure, which may be otherwise unobtainable in patients with stenotic lesions affecting all four extremities. Although MRA does not provide the same level of detail as conventional angiography, it comes close. Because MRA is not invasive and does not involve ionizing radiation, it has become the preferred imaging method for assessing the extent of vascular involvement and damage, both initially and during follow-up. The role of MRI in assessing disease activity is not clear because vessel wall edema and contrast enhancement have not correlated well with

clinical measures of disease activity.[78] Although PET scanning would be expected to be more sensitive than angiography in the early detection of vascular inflammation that precedes the development of stenoses, its sensitivity may be no greater than MRI. PET scanning has no established role in following disease activity in TA. Indeed, one study of 28 TA patients showed that PET scans did not correlate with clinical, biologic, or MRI assessment of disease activity.[114]

The most common sites of lesions in TA are the aorta (65%) and the left subclavian arteries (93%)[104] (Table 88-11). The left subclavian artery is affected slightly more frequently than the right. Carotid, renal, and vertebral arteries are also commonly affected.[104] Lesions may be stenotic (93%), occluded (57%), dilated (16%), or aneurysmal (7%). Stenotic lesions are approximately four times more common than aneurysmal lesions.[104] Stenotic segments often extend a few centimeters and may be followed by areas of dilation (see Figure 88-10). The majority of patients (53%) have vascular lesions above and below the diaphragm. However, the frequency distribution of aortic lesions varies considerably from country to country.[104]



**Figure 88-11** Magnetic resonance image (sagittal section) through the chest showing thickening of the ascending and descending thoracic aorta in a 26-year-old woman with Takayasu's arteritis. *(From Hellmann DB: Takayasu arteritis. In Imboden J, Hellmann DB, Stone JH, editors:* Current rheumatology: diagnosis & treatment, *New York, 2004, McGraw-Hill, p 244.)*

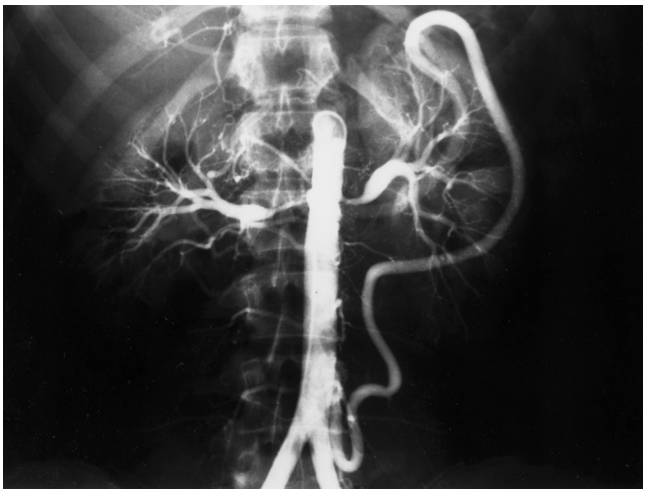


**Figure 88-12** Angiogram showing bilateral renal artery stenosis. A large left colic branch of the inferior mesenteric artery provides collateral circulation to the gut. *(From Hellmann DB, Flynn JA: Clinical presentation and natural history of Takayasu's arteritis and other inflammatory arteritides. In Perler BA, Becker GJ, editors:* Vascular intervention: a clinical approach, *New York, 1998, Thieme Medical and Scientific Publisher, pp 249–256.)*

**TABLE 88-10** Comparison of Imaging Techniques in Takayasu's Arteritis

| Technique | Advantages | Disadvantages |
|---|---|---|
| Conventional angiography | "Gold standard" image quality<br>Allows CAP measurement<br>Allows angioplasty at same time | Invasive<br>Radiation exposure<br>Does not visualize vessel wall thickness |
| Magnetic resonance angiography | Excellent image quality<br>Noninvasive<br>No ionizing radiation exposure<br>Visualizes vascular wall thickness | Image quality not "gold standard"<br>Cannot use in patients with pacemaker<br>CAP measurement not possible |
| Ultrasonography | Noninvasive<br>No ionizing radiation exposure<br>Can visualize vessel wall edema | Image quality not "gold standard"<br>Image quality affected by obesity<br>Operator dependent<br>CAP measurement not possible |
| Computed tomography angiography | Excellent image quality | Ionizing radiation exposure<br>CAP measurement not possible<br>Intravenous contrast agent required |
| Positron emission tomography | Can measure intensity of vascular inflammation | Ionizing radiation exposure<br>Vascular anatomy not well seen<br>CAP measurement not possible<br>Intravenous contrast agent required |

CAP, Central arterial blood pressure.

**TABLE 88-11** Frequency of Blood Vessel Involvement in Takayasu's Arteritis

| Blood Vessel | % Abnormal |
|---|---|
| Aorta | 65 |
| Aortic arch or root | 35 |
| Abdominal aorta | 47 |
| Thoracic aorta | 17 |
| Subclavian artery | 93 |
| Common carotid artery | 58 |
| Renal artery | 38 |
| Vertebral artery | 35 |
| Celiac axes | 18 |
| Common iliac artery | 17 |
| Pulmonary artery | 5 |

Data based on a study of 60 North America patients reported by Kerr GS, Hallahan CW, Giordano A, et al: Takayasu arteritis. *Ann Intern Med* 120:919, 1994; From Hellmann DB: Takayasu arteritis. In Imboden JB, Hellmann DB, Stone JH, editors: *Current rheumatology diagnosis and treatment,* New York, 2004, Lange Medical Books/McGraw-Hill, p 245.

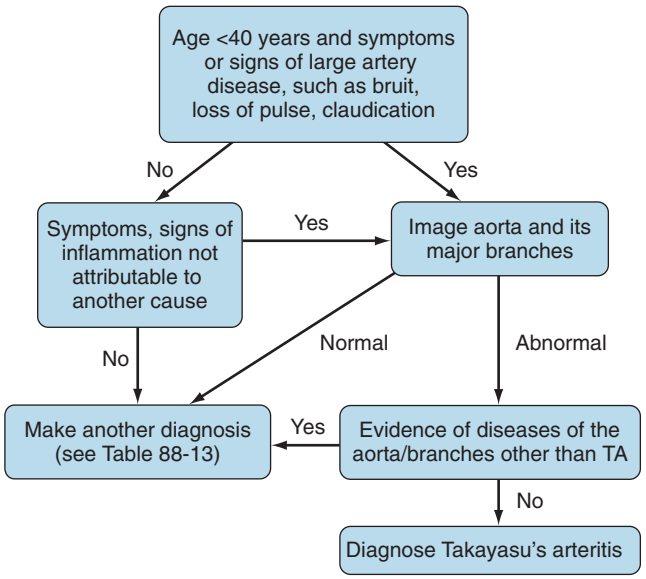



**Figure 88-13** Algorithm for the diagnosis of Takayasu's arteritis (TA).

## Diagnosis and Diagnostic Tests

The ACR has established classification criteria for the diagnosis of TA (see Table 88-8). In clinical practice, the diagnosis of TA is almost always secured by an imaging procedure (see Table 88-10) that demonstrates the characteristic abnormalities of the aorta and its major branches (Figure 88-13). Rarely, the diagnosis is first suggested when a pathologist finds granulomatous inflammation in a section of aorta or other larger artery that was removed or biopsied during a vascular surgery procedure. Unfortunately the diagnosis of TA is often delayed; the delay averaged 44 months in one large series. The most frequent impediment to a speedy diagnosis is a physician's failure to consider TA in the differential diagnosis. Although the rarity of TA helps explain its omission from diagnostic consideration, another reason is that some patients have striking features of inflammation that camouflage or overshadow the somewhat more familiar vascular abnormalities. Indeed, a few patients with TA present chiefly with FUO. Most of these patients have other, albeit subtle, manifestations of TA such as bruits, diminished pulses, unequal arm blood pressures, or aortic regurgitation. In other patients with more striking vascular abnormalities, the physician might be lured into focusing on familiar and dramatic abnormalities such as anemia or thrombocytopenia. Thus, instead of ordering an imaging test that would explain the patient's unequal and low blood pressure in the left arm, the physician mistakenly diverts the patient to a hematologist, gastroenterologist, or oncologist for additional blood tests and procedures that further delay the diagnosis.

Many of these delays can be prevented by remembering that TA should be included in the differential diagnosis of any person younger than 40 years who presents with FUO, aortic regurgitation, hypertension, or unequal or absent pulses. Delays in diagnosis can also be reduced by carefully searching for unequal or absent upper extremity pulses and by listening for bruits not only over the carotid arteries but also above and below the clavicle (for subclavian artery bruits) and over the abdomen and flanks (for renal and other mesenteric artery bruits). Recognizing that anemia and thrombocytosis can be manifestations of active inflammatory disorders such as vasculitis can also help speed the diagnosis of TA.

Once an imaging test demonstrates disease of the aorta or its major branches, the differential diagnosis narrows to a set of disorders that are usually easily differentiated (Table 88-12). Most rheumatic diseases that can affect the aorta are distinguished by their associated features. For example, Cogan's syndrome typically produces ocular inflammation (especially keratitis) and vestibuloauditory dysfunction. The one rheumatic disease that can be difficult to distinguish from TA is GCA (Table 88-13). Usually, the patient's age and the distribution of lesions allow their rapid differentiation, but it can be difficult or even impossible to distinguish TA from GCA in patients 50 years old or older. The similarity of treatment (see subsequent discussion)

**TABLE 88-12** Differential Diagnosis of Takayasu's Arteritis: Other Diseases That Can Affect the Aorta

| Disease Type | Specific Entities |
|---|---|
| Rheumatic | Giant cell arteritis, Cogan's syndrome, relapsing polychrondritis, ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus, Buerger's disease, Behçet's disease |
| Infectious | Syphilis, tuberculosis |
| Other | Atherosclerosis, ergotism, radiation-induced damage, retroperitoneal fibrosis, inflammatory bowel disease, sarcoidosis, neurofibromatosis, congenital coarctation, Marfan's syndrome, Ehlers-Danlos syndrome, $IgG_4$-related systemic disease |

Ig, Immunoglobulin.

From Hellmann DB: Takayasu arteritis. In Imboden JB, Hellmann DB, Stone JH, editors: *Current rheumatology diagnosis and treatment,* New York, 2004, Lange Medical Books/McGraw-Hill, p 243.

**TABLE 88-13** Comparison of Giant Cell Arteritis and Takayasu's Arteritis

| Feature | Giant Cell Arteritis | Takayasu's Arteritis |
|---|---|---|
| Female-male ratio | 2:1 | 8:1 |
| Age range | ≥50 yr | <40 yr |
| Average age of onset | 72 yr | 25 yr |
| Visual loss | 10%-30% | Rare |
| Involvement of aorta or its major branches | 25% | 100% |
| Histopathology | Granulomatous arteritis | Granulomatous arteritis |
| Pulmonary artery involvement | No | Occasionally |
| Renal hypertension | Rare | Common |
| Claudication | Uncommon | Common |
| Ethnic groups with highest incidence | Scandinavian | Asian |
| Corticosteroid responsive | Yes | Yes |
| Bruits present | Minority | Majority |
| Surgical intervention needed | Rare | Common |

diminishes the practical importance of solving this diagnostic dilemma.

Infections of the aorta are rare in most countries. Tertiary syphilis can be excluded by a negative fluorescent treponemal antibody test (the rapid plasma reagin test yields false negative results in approximately one-quarter of patients with late-stage syphilis). Other diseases of the aorta (see Table 88-13) are usually readily separated from TA by the history and physical examination. There has been growing appreciation for a small subset of patients who have noninfectious aortitis that is difficult to categorize. At one center, noninfectious aortitis was found in 8% of patients undergoing resection of the ascending aorta.[115] Although nearly 70% had giant cells, only a minority neatly fit a diagnosis of TA or GCA. A fraction of the cases of thoracic or abdominal aortitis are caused by $IgG_4$-related disease ($IgG_4$-RD), a systemic immune-mediated disease characterized by infiltration of $IgG_4$-bearing plasma cells in the aorta or other affected organs.[116,117]

## Treatment

### Medical Therapy

Corticosteroids are the cornerstone of treatment of active TA.[104, 118, 119] Prednisone, at a dose of 0.5 to 1 mg/kg per day, is indicated for the treatment of active disease. Criteria for active disease include new onset or worsening of two or more of the following: (1) fever or other systemic features (in the absence of other cause); (2) elevated ESR; (3) symptoms or signs of vascular ischemia or inflammation (e.g., claudication, absent pulse, and carotidynia); and (4) typical angiographic lesions.[104] Although approximately 85% of patients with TA present with active disease, approximately 15% do not.[79] The initial dose of prednisone is continued for 4 to 12 weeks before commencing a gradual taper, as is done when treating patients with GCA (see previous discussion). Although nearly two-thirds of patients achieve remission, more than half later relapse. Relapses are especially common when the prednisone dose falls below 20 mg per day.

Relapses can be treated by increasing the prednisone dose or adding an immunosuppressive agent. No agent used for TA has been evaluated in a double-blind, placebo-controlled trial. However, open trials have suggested that weekly oral methotrexate (started at 0.3 mg/kg per week, with the initial dose not to exceed 15 mg/wk) is a moderately effective corticosteroid-sparing drug.[120] Methotrexate can be gradually increased to 25 mg/wk. The emphasis is on lowering the corticosteroid dose because methotrexate seldom allows the elimination of prednisone completely; most patients continue to require at least 5 to 10 mg/day of prednisone. Small open trials have provided even more encouraging results about the effectiveness of anti-TNF inhibitors (etanercept and infliximab) in treating patients with refractory TA.[121-123] These trials emphasize that although these agents can treat TA, they rarely cure it: relapses are likely when the treatment is stopped.[121] Tocilizumab, which blocks the IL-6 receptor, has also been reported effective in individual patients.[89,90,124-126]

Case reports and small series suggest that other corticosteroid-sparing drugs may include azathioprine (2 mg/kg per day), mycophenolate mofetil (2000 mg/day), cyclosporine, tacrolimus, leflunomide, and cyclophosphamide (2 mg/kg per day).[104,119,127] The toxicity of cyclophosphamide in young women is so high that it is rarely used in TA.[104]

Efforts to prevent osteoporosis should be initiated early (as described in Chapter 101). Modifiable risk factors for atherosclerosis—especially hypertension, smoking, inactivity, diabetes, and hyperlipidemia—should be treated maximally.

### Surgical Therapy

TA is the form of vasculitis most frequently requiring revascularization procedures.[104,128,129] Unfortunately, medical therapy rarely reduces or reverses stenotic lesions. Treating stenotic or aneurysmal lesions may require bypass surgery (especially of stenotic cervicobrachial arteries, coronary arteries, or renal arteries); aortic valve replacement (for aortic regurgitation); or percutaneous transluminal angioplasty (especially for stenotic renal arteries causing hypertension).

A review of the experience with vascular interventions in TA supports several general recommendations. First, the mere presence of stenosis does not necessitate intervention. The gut, for example, has such rich collaterals that even critical stenoses of the celiac, superior, or inferior mesenteric arteries usually produce no symptoms and require no surgical intervention. Moreover, many patients with arm claudication will develop collateral circulation and improve substantially over time with medical therapy alone. For upper extremity vascular insufficiency, patiently waiting for a response to medical therapy usually pays higher dividends than undertaking rapid surgical intervention. Second,

whenever possible, surgical intervention should be deferred until TA is in remission; procedures done during active disease often produce disappointing results.[130] Third, bypass surgery yields better results than angioplasty.[118] With bypass graft procedures, autologous vessels give better results than synthetic grafts (restenosis rates of 9% vs. 36%). Patients who undergo aortic surgery are liable to develop anastomotic aneurysms; such aneurysms developed in nearly 14% of patients followed for 20 years.[128,131] Although angioplasty gives good short-term results, long-term results are often disappointing except for very short stenotic segments.[131] The experience with conventional stents has been mostly disappointing.

## Outcome

Twenty percent of TA patients have a self-limited disease. The rest have a relapsing-remitting or progressive course requiring chronic corticosteroid and/or immunosuppressive therapy. Nearly two-thirds of patients experience new angiographic lesions. In one study from the National Institutes of Health, 74% of patients experienced some form of morbidity and 47% were permanently disabled.[104] In a Japanese study of 120 patients, survival at 15 years was 83%.[131] A study of 126 TA patients in the United States reported 10 year survival of 97% and 15 year survival of 85%.[119] Patients with TA do have a higher mortality than the general population; the study estimated the standardized mortality ratio in TA to be 3.0 (95% CI, 1.0 to 8.0).[119] Age of onset older than 35 years; development of major complications (i.e., retinopathy, hypertension, aortic regurgitation, and aneurysm); or a progressive course was associated with decreased survival.[131] Congestive heart failure and renal failure are the most common causes of death.[128] Pregnancy appears to be relatively well tolerated in the presence of good medical care and in the absence of abdominal aortic involvement.[104,132,133]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 89

# Anti-neutrophil Cytoplasmic Antibody–Associated Vasculitis



Sharon Chung • Paul A. Monach

**KEY POINTS**

- The anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). They affect small- and medium-sized vessels and share clinical, pathologic, and diagnostic features.
- ANCA testing by immunofluorescence and especially enzyme-linked immunoassay is useful to diagnose ANCA-associated vasculitides in the appropriate clinical context, but its utility in assessing disease activity is unclear.
- GPA is characterized histologically by necrotizing granulomatous inflammation. Common clinical manifestations include destructive sinonasal lesions, pulmonary nodules, and pauci-immune glomerulonephritis. GPA is most commonly associated with cytoplasmic-staining ANCA and antibodies to proteinase 3.
- MPA is characterized histologically by vasculitis without granulomatous inflammation. Common clinical manifestations include rapidly progressive pauci-immune glomerulonephritis and alveolar hemorrhage. MPA is most commonly associated with perinuclear staining ANCA and antibodies to myeloperoxidase.
- EGPA is characterized histologically by eosinophilic tissue infiltration. Common clinical manifestations include asthma and peripheral eosinophilia. Most patients do not produce detectable ANCA.
- For patients with life-threatening disease, combination therapy (with steroids and either cyclophosphamide or rituximab) is needed to induce remission. Remission can be maintained with less toxic medications such as methotrexate and azathioprine. With current treatment regimens, disease remission is achieved in 75% to 93% of patients.

The anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides target small- to medium-sized vessels in multiple organ systems, with sinonasal, pulmonary, and renal involvement being particularly common. Specific diseases included in this category are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA; also known as Churg-Strauss syndrome), and renal limited vasculitis. These diseases are categorized together, given their association with antibodies directed against antigens in the cytoplasm of neutrophils—proteinase 3 (PR3) and myeloperoxidase (MPO)—and many overlapping clinical manifestations, diagnostic testing, and treatment strategies.

## CLASSIFICATION CRITERIA AND NOMENCLATURE

To help clarify and differentiate the various vasculitic syndromes, classification criteria and disease definitions have been proposed by the American College of Rheumatology (ACR) and other groups. In 1990, the ACR proposed classification criteria for both GPA and EGPA. Of note, criteria for MPA were not developed at that time. In essence, these criteria were developed to facilitate research by ensuring that the same types of patients with vasculitis were being enrolled for studies across different institutions and groups.[1]

The classification criteria for GPA were developed by comparing 85 patients who had GPA with 722 patients who had other forms of vasculitis. Four criteria were selected:

1. Abnormal urinary sediment (red blood cell casts or >5 red blood cells/high-power field)
2. Abnormal findings on a chest radiograph (e.g., nodules, cavities, or fixed infiltrates)
3. Oral ulcers or nasal discharge
4. Biopsy findings of granulomatous inflammation

The presence of two or more of these four criteria was associated with a sensitivity of 88.2% and a specificity of 92.0%.[2] ANCAs were not included in these criteria, given their relatively recent identification and the need to further determine their usefulness at the time of the publication of the criteria.

The classification criteria for EGPA were developed by comparing 20 patients with EGPA with 787 patients who had other forms of vasculitis. Six criteria were selected:

1. Asthma
2. Eosinophilia greater than 10% on white blood cell count differential
3. Mononeuropathy (including multiplex) or polyneuropathy
4. Nonfixed pulmonary infiltrates on a chest radiograph
5. Paranasal sinus abnormality
6. A biopsy specimen containing a blood vessel with extravascular eosinophils

The presence of four or more of these six criteria was associated with a sensitivity of 85% and a specificity of 99.7%.[3]

To better define the nomenclature of the systemic vasculitides, the Chapel Hill Consensus Conference (CHCC) was convened in 1994 to reach consensus on the names for the most common forms of systemic vasculitis. Vasculitides were categorized into large-, medium-, and small-vessel

groups. Of note, this conference formally defined MPA for the first time and differentiated this entity from classic polyarteritis nodosa (PAN) by defining MPA as a pauci-immune necrotizing vasculitis affecting small vessels, with or without medium-vessel involvement. PAN was defined as an arteritis of medium-sized and small vessels without involvement of the arterioles, venules, or capillaries (including glomerular capillaries).[4] The CHCC nomenclature system was reviewed in 2012 and recommended a shift from using eponyms to noneponymous replacements. Other changes include the addition of variable vessel and single-organ vasculitis, as well as vasculitis associated with systemic disease and vasculitis associated with probable etiology.[5] The names and definitions of ANCA-associated vasculitis—GPA, MPA, and EGPA, as set forth by the CHCC 2012 conference—are presented in Table 89-1. The definitions for other small-vessel vasculitides are included for comparison.

It is important to note that the ACR classification criteria and CHCC definitions are not intended to serve as diagnostic criteria. In a prospective study, the ACR classification criteria were found to perform poorly as diagnostic criteria for GPA, PAN, giant cell arteritis, and hypersensitivity vasculitis. Vasculitis was diagnosed in 51 of 198 patients (26%). The positive predictive value for the criteria for these four vasculitides ranged from 17% to 29% for all patients and 29% to 75% of patients with vasculitis.[6] In a study of GPA, EGPA, PAN, and MPA using diagnostic criteria based on the CHCC definitions, many cases fulfilled more than one set of criteria, and few patients fulfilled the MPA criteria.[7] Other groups have developed diagnostic criteria for ANCA-associated vasculitides, but these criteria are not widely accepted.[8] ACR and European League against Rheumatism (EULAR) are currently developing diagnostic criteria and updating classification criteria in their jointly sponsored Diagnostic and Classification Criteria in Vasculitis study.

## EPIDEMIOLOGY

Epidemiologic studies of the ANCA-associated vasculitides (AAV) have been hampered by the evolving classification criteria and disease definitions, along with the rarity of these diseases. Most epidemiologic investigations of AAV have studied populations of European descent. These studies suggest an overall incidence of 10 to 20 individuals per million per year, with a slight male predominance (male to female, 1.5:1). These diseases are rare in childhood and the incidence increases with age, with the peak incidence occurring in persons between 65 and 74 years of age (6 cases/100,000).[9] Compared with patients who experience the development of AAV as adults, patients with childhood-onset AAV have a higher incidence of subglottic stenosis and a lower incidence of renal involvement.[10]

**TABLE 89-1** Names and Definitions of Small Vessel Vasculitides as Presented by the 2012 Chapel Hill Consensus Conference

| Name | Definition and Comments |
|---|---|
| Small-vessel vasculitis | Vasculitis predominantly affecting small vessels, defined as small intraparenchymal arteries, arterioles, capillaries, and venules. Medium-sized arteries and veins may be affected. |
| ANCA-associated vasculitis | Necrotizing vasculitis with few or no immune deposits predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries), associated with MPO ANCA or PR3 ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity (e.g., MPO-ANCA, PR3-ANCA, ANCA-negative). |
| Granulomatosis with polyangiitis | Necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract, and necrotizing vasculitis affecting predominantly small- to medium-sized vessels (e.g., capillaries, venules, arterioles, arteries and veins). Necrotizing glomerulonephritis is common. |
| Microscopic polyangiitis | Necrotizing vasculitis with few or no immune deposits predominantly affecting small vessels (i.e., capillaries, venules, or arterioles). Necrotizing arteritis involving small- and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs. Granulomatous inflammation is absent. |
| Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) | Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, and necrotizing vasculitis predominantly affecting small- to medium-sized vessels, and associated with asthma and eosinophilia. ANCA is more frequent when glomerulonephritis is present. |
| Immune complex vasculitis | Vasculitis with moderate to marked vessel wall deposits of Ig and/or complement components predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries). Glomerulonephritis is frequent. |
| Anti-glomerular basement membrane disease | Vasculitis affecting glomerular capillaries, pulmonary capillaries, or both, with GBM deposition of anti-GBM autoantibodies. Lung involvement causes pulmonary hemorrhage, and renal involvement causes glomerulonephritis with necrosis and crescents. |
| Cryoglobulinemic vasculitis | Vasculitis with cryoglobulin immune deposits affecting small vessels (predominantly capillaries, venules, or arterioles) and associated with serum cryoglobulins. Skin, glomeruli, and peripheral nerves are often involved. |
| IgA vasculitis (Henoch-Schönlein purpura) | Vasculitis, with IgA1-dominant immune deposits, affecting small vessels (predominantly capillaries, venules, or arterioles). Often involves skin and gastrointestinal tract, and frequently causes arthritis. Glomerulonephritis indistinguishable from IgA nephropathy may occur. |
| Hypocomplementemic urticarial vasculitis (anti-C1q vasculitis) | Vasculitis accompanied by urticaria and hypocomplementemia affecting small vessels (i.e., capillaries, venules, or arterioles), and associated with anti-C1q antibodies. Glomerulonephritis, arthritis, obstructive pulmonary disease, and ocular inflammation are common. |

ANCA, Anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane; MPO, myeloperoxidase; PR3, proteinase 3.

AAVs appear to be more common in populations of European descent. In France, the overall prevalence of GPA, MPA, PAN, and EGPA was 2.0 times higher for subjects of European ancestry (104.7 cases/million) than for non-Europeans (52.5 cases/million).[11] GPA and MPA are rarely seen in persons of African descent.[12] Additional study is needed regarding the epidemiology of AAV in non-European populations.

Studies have also shown an increase in prevalence since the 1990s. Between 1994 and 2006 in Germany, the prevalence of GPA increased from 58 to 98 cases per million, MPA increased from 9 to 28 cases per million, and EGPA increased from 7 to 24 cases per million.[13] In the United Kingdom (UK), the prevalence of GPA increased from 28.8 per million in 1990 to 64.8 per million in 2005.[14] The increase in prevalence likely reflects increased awareness and diagnosis of the diseases after the development of the ACR classification criteria and CHCC definitions and ANCA testing, as well as improved survival.

In European populations, GPA is the most prevalent AAV. The incidence of GPA ranges from 4.9 to 10.5 cases per million per year, with a higher incidence in northern Europe than in southern Europe (e.g., an incidence of 10.5/million/year in Norway and 4.9/million/year in Spain). The overall prevalence ranges from 24 to 157 per million, with the highest prevalences reported in Sweden and the UK.[9,13] Variation in incidence and prevalence between countries has suggested that disease risk declines in a north-south gradient in the Northern Hemisphere.[14]

The incidence of MPA among European populations ranges from 0.5 to 11.6 cases/million/year. Unlike GPA, the highest incidence of MPA seems to occur in Southern Europe, specifically in Greece and Spain. MPA is also more common in non-European populations, with an incidence of 14.8 cases/million/year in Japan and 24.0 cases/million/year in Kuwait, the highest reported incidence thus far.[9,14] The prevalence of MPA ranges from 0 to 66 cases per million among European countries.[9]

Studies in Japan have shown the reverse in prevalence and incidence for the AAV compared with the UK. The overall prevalence and incidence of AAV appears to be similar between Japan (prevalence, 86 cases per million; incidence, 22.6 cases per million) and the UK (prevalence, 50 cases per million; incidence, 21.8 cases per million). However, more than 80% of patients in Japan are perinuclear-staining (p-ANCA) or MPO positive, whereas two thirds of patients in the UK are cytoplasmic-staining (c-ANCA) or PR3 positive. As suggested from the serologic differences, MPA is more common in Japan (occurring in 83% of patients with AAV), whereas GPA is more common in the UK (occurring in 66% of patients with AAV). Compared with patients in the UK who have GPA, renal involvement is less common in Japan.[15,16] In a study of systemic vasculitis in China, MPA was also more common than GPA (3:1), and 75% of patients were p-ANCA positive, whereas 13% were c-ANCA positive.[17] These findings suggest that granulomatosis in this family of diseases is less common in Asian populations.

EGPA is the least prevalent of the ANCA-associated vasculitides. Among European populations, the incidence of EGPA ranges from 0.5 to 6.8 cases per million individuals.[9,14,18] The incidence of EGPA among patients with asthma ranges from 34.6 to 64.4 per million.[18,19] The overall prevalence of EGPA in European populations has been estimated to range from 2 to 38 cases per million.[9,11] A gender predominance has not been clearly demonstrated. In Japan, the prevalence of EGPA has been estimated to be 17.8 cases per million, with women more frequently affected than men.[20]

### Triggering Factors

Environmental exposures, infections, and drugs have been hypothesized to be triggering factors for the development of AAV. Among environmental and occupational exposures, the most consistent associations have been observed with crystalline silica, with most studies showing an odds ratio (OR) of 2.5 to 5. Associations with other exposures such as heavy metals, farming, organic solvents, and pesticides have been studied, but results have not been consistent.[21] The influence of infection on GPA pathogenesis has been suggested by a cyclical pattern of occurrence observed in some studies.[22] However, the only clear association is seen with *Staphylococcus aureus*, where nasal carriage of *S. aureus* is associated with an increased risk of relapse in GPA.[23] The role of *S. aureus* or other infectious agents in disease initiation has not been established. Lastly, the most commonly reported drugs causing AAV include hydralazine, minocycline, propylthiouracil, and levamisole-adulterated cocaine.[24] Use of leukotriene antagonists and omalizumab has been linked to the development of EGPA. These agents may not be directly causative but instead unmask underlying EGPA by allowing corticosteroid tapering or are prescribed to patients with undiagnosed and worsening EGPA. However, a direct causal link cannot be excluded.[25-27]

## GENETICS

GPA, MPA, and EGPA are complex genetic diseases—instead of a single gene variant causing disease, multiple genetic variants, each with potentially modest effect(s), influence disease risk. Thus large cohorts of patients and control subjects are needed to identify genetic risk factors. Family-based, candidate gene, and genome-wide studies have been conducted to identify genetic susceptibility loci for the AAV. GPA is the most thoroughly studied of these three diseases.

Familial studies of GPA have suggested a genetic influence on disease susceptibility. A nationwide registry–based study in Sweden showed that the relative risk of GPA in a first-degree relative of a person with GPA was estimated to be 1.56 (95% CI, 0.35 to 6.9). This familial risk is lower than in other autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis but is of similar magnitude as the familial risk in rheumatoid arthritis.[28] First-degree relatives of patients with GPA have an increased incidence of seropositive rheumatoid arthritis (hazard ratio [HR], 1.54; 95% confidence interval [CI], 1.09 to 2.19), as well as other autoimmune diseases, including multiple sclerosis (HR, 1.92; 95% CI, 1.16 to 3.16).[29] Children of persons with GPA have an increased risk of rheumatoid arthritis (standardized incidence ratio, 1.34).[30] In an independent cohort, a genetic risk score based on rheumatoid arthritis

susceptibility genes was associated with GPA risk.[31] These findings suggest that GPA and rheumatoid arthritis share genetic risk factors.

Candidate gene studies of GPA have replicated associations with the class II human leukocyte antigen (HLA) region, *CTLA4*, *PTPN22*, and *SERPINA1*. Although genetic studies of the HLA can be difficult to interpret given the polymorphic nature of the genes and the extensive linkage disequilibrium in the area, associations between GPA and the extended haplotype containing *HLA-DPB1*0401* have been observed and replicated in ANCA-positive patients.[32,33] *Cytotoxic T-lymphocyte–associated antigen 4* (*CTLA4*) is found on T cells and inhibits T cell function. Multiple variants of *CTLA4* have been shown to confer genetic risk for GPA, with some variants affecting surface expression or messenger RNA stability.[31,34-36] *PTPN22* codes for Lyp, a tyrosine phosphatase that acts with c-src tyrosine kinase as a critical negative regulator of T cell receptor signaling. The R620W (rs2476601) variant is associated with GPA[31,37,38] and interrupts its binding with c-src tyrosine kinase, thus altering B and T cell receptor responsiveness. *SERPINA1* codes for α1 antitrypsin, a serine protease inhibitor that is a major inhibitor of PR3. The rare Z and S alleles of *SERPINA1*, which lead to a deficiency of the enzyme, have been associated with GPA.[39] Genetic variation in the HLA region, *CTLA4*, and *PTPN22* has been associated with multiple autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis.

The first genome-wide association study of GPA and MPA, published in 2012, examined a total of 2267 persons with AAV and 6858 control subjects, all of Northern European descent. The most statistically significant genetic associations in the combined (GPA and MPA) cohort were observed with *HLA-DP* (OR, 3.67; $P = 1.5 \times 10^{-71}$), *COL11A2* (OR, 1.51; $P = 7.8 \times 10^{-15}$), and *SERPINA1* (OR, 0.51; $P = 2.4 \times 10^{-9}$). However, associations based on ANCA specificity were stronger than associations with specific disease phenotypes or the overall cohort. For example, the *HLA-DP* association was strongest in anti–PR3-positive individuals (OR, 7.03; $P = 6.2 \times 10^{-89}$). Associations with *SERPINA1* were observed in anti-PR3 individuals (OR, 0.53; $P = 5.6 \times 10^{-12}$) and not in anti–MPO-positive individuals (OR, 1.1; $P = 2.2 \times 10^{-1}$). Associations with the PR3 gene were only observed in anti–PR3-positive individuals (OR, 0.73; $P = 2.6 \times 10^{-7}$). In the anti–MPO-positive subgroup, *HLA-DQ* showed stronger associations (OR, 0.65; $P = 2.1 \times 10^{-8}$).[40] These findings suggest that genetic variation may influence autoantibody specificity more strongly than clinical disease phenotype.

A second genome-wide association study focused specifically on GPA studied a total of 1020 GPA cases and 2734 control subjects who were all of European descent. This study confirmed the strong association with *HLA-DP* and found that the *HLA-DPB1*04* allele fully accounted for the association. In addition, an independent association with the *SEMA6A* (OR, 0.74; $P = 2.09 \times 10^{-8}$) was observed in the combined cohort[41] but could not be replicated by a second group.[42] A nominal association was also observed with *SERPINA1*.

A genome-wide association study of EGPA has not been published. Candidate gene studies have implicated *HLA-DRB1*0401* and the extended *IL-10.2* haplotype as genetic susceptibility loci for EGPA.[43-45]

The genetic association studies conducted thus far have primarily examined persons of European descent, and the epidemiology of GPA and MPA differs between European and Asian countries. Genetic studies of MPA in Japan have suggested associations between MPA and *IRF5*, the *HLA-DRB1*09:01-DQB1*03:03* haplotype, and *KIR2DS3*,[46,47] but these findings need to be replicated. A study of African Americans with AAV showed that the odds of having *HLA-DRB1*15* were 73.3-fold higher in PR3-ANCA–positive persons compared with healthy control subjects. In addition, a disproportionate number of persons carried the *HLA-DRB1*1501* allele of European descent compared with the *HLA-DRB1*1503* allele of African descent.[12] Further studies in non-European populations are needed to determine whether the genetic risk factors identified in European persons are broadly applicable to other ethnic groups.

## CLINICAL MANIFESTATIONS

Although the AAV can affect almost all organ systems, the frequency of organ system involvement varies greatly. Data on organ system involvement come from a small number of cohorts of 30 to 400 patients.[48-59] Some reports indicate that new organ system involvement occurs frequently over time, particularly in GPA.[48,49] The diversity of presentations within a given organ system is apparent from case series and reviews focused on that organ system and reports of particularly unusual cases.[46,49,58-61]

Not all clinical features of AAV are directly attributable to local vascular damage. This phenomenon is obvious with regard to constitutional symptoms, but in addition, the risk of venous thromboembolic events is elevated in the absence of inflammation in veins,[60,61] and the pathophysiology of arthritis is unknown. Also, the extent to which myalgia and gastrointestinal (GI) symptoms indicate vasculitis in these organ systems is not known.

As a rough approximation, the features of MPA can all be seen in GPA or EGPA, particularly in ANCA-positive patients. GPA, by definition, features perivascular necrotizing granulomatous inflammation, and EGPA features eosinophil-rich granulomatous inflammation and/or parenchymal infiltration by eosinophils, leading to greater diversity of clinical phenotypes. Unfortunately, many of the largest series and clinical trials do not allow comparison of organ system involvement in MPA, GPA, and EGPA, because either the first two or all three entities were pooled as ANCA-associated vasculitis.[56,62-65]

### Granulomatosis with Polyangiitis

Although the range of presentations and subsequent courses in GPA is extraordinarily broad, longitudinal analyses of larger cohorts generally agree on the frequency of involvement of different organ systems over time (Table 89-2).[48,49,51] Frequencies in children appear to be similar to those in adults.[50] Inflammation in the upper airway is the most common initial symptom.[48,49] Progression to systemic vasculitis, particularly with glomerulonephritis or alveolar hemorrhage, remains a common route to diagnosis, but the

**TABLE 89-2** Manifestations Over Time in the Subtypes of ANCA-Associated Vasculitis in the Largest Published Series*

| Organ System | GPA | MPA | EGPA |
|---|---|---|---|
| ENT | 83-99 | 1-20 | 48-77 |
| Joint/muscle | 59-77 | 14-54+ | 30-39+ |
| Kidney | 66-77 | 69-100 | 22-27 |
| Lung | 66-85 | 25-55 | 51-58 |
| Eye | 34-61 | 1-15 | 7 |
| Heart | 8-25 | 3-24 | 16-27 |
| Skin | 33-46 | 11-62 | 40-57 |
| Peripheral nerve | 15-40 | 13-60 | 51-76 |
| CNS | 8-11 | 5-12 | 5-14 |
| GI tract | 6-13 | 3-31 | 22-31 |
| Constitutional symptoms | 58+ | 67-84 | 49-68 |

*Numbers indicate percentages. Not all manifestations were reported in all studies. Some studies reported separate individual manifestations that were pooled into groups for this table; in such cases, the highest percentage among those separate manifestations was used, and "+" indicates that the percentage for the groups of pooled manifestations is likely to be higher.

EGPA, Eosinophilic granulomatosis with polyangiitis; ENT, ear, nose, and throat; GI, gastrointestinal; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis.

Data from Reinhold-Keller E, Beuge N, Latza U, et al: An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. *Arthritis Rheum* 43:1021–1032, 2000; Comarmond C, Pagnoux C, Khellaf M, et al: Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): clinical characteristics and long-term followup of the 383 patients enrolled in the French Vasculitis Study Group cohort. *Arthritis Rheum* 65:270–281, 2013; and Furuta S, Chaudhry AN, Hamano Y, et al: Comparison of phenotype and outcome in microscopic polyangiitis between Europe and Japan. *J Rheumatol* 41:325–333.



**Figure 89-1** Computed tomography scan of the sinuses, showing sinusitis and destruction of the nasal septum.



**Figure 89-2** Saddle nose deformity in a patient with granulomatosis with polyangiitis. *(Courtesy Dr. G. Hoffman.)*

development of ANCA testing has facilitated diagnosis at more "localized"[66,67] or "limited"[68] stages or with unusual presentations. Constitutional symptoms and inflammation in the upper and/or lower airway may develop simultaneously with systemic vasculitis or may precede it by weeks, months, or years.[67]

Frequent epistaxis and mucopurulent nasal discharge are common. The patient and subsequently the physician are often alerted to the severity of inflammation in the nasal cavity by the presence of pain and hard crusts overlying actively inflamed or eroded tissue and exacerbated by poor air flow. Inflammation of the paranasal sinuses causing pain (Figure 89-1) is also common. Ear congestion and conductive hearing loss can result from inflammation of the middle ear or the auditory tube or by obstruction of the tube at its entry into the nose. Inflammation of the subglottic trachea occurs in approximately 15%,[48,69,70] particularly in children and young adults.[71] Oral ulcers, gingivitis, and auricular chondritis are well-described but less frequent manifestations.[48,69,70,72]

Upper airway inflammation in GPA is notable for its destructive potential, but permanent damage does not always occur and is highly variable in its pace. The nasal septum is particularly at risk, and septal perforation can progress to collapse and a characteristic "saddle-nose" deformity (Figure 89-2). Bone erosion occurs elsewhere—for example, in the sinuses, hard palate, and orbit—but is much less common. Subglottic involvement often causes obstruction (Figure 89-3) as a consequence of either active inflammation or subsequent scarring and can be life-threatening.[71]

Eye and orbital involvement is also striking in GPA, both in terms of frequency (30% to 60% over time and much higher than in MPA or EGPA) and diversity.[48,51,73] Conjunctivitis and episcleritis are the most common forms and pose no danger to the patient. In contrast, scleritis can threaten vision if it is not treated aggressively. Scleritis is usually painful, whereas conjunctivitis and episcleritis are not painful, but absence of pain does not rule out scleritis, and these conditions cannot be distinguished by simple inspection. Retinal vasculitis, uveitis, oculomotor nerve

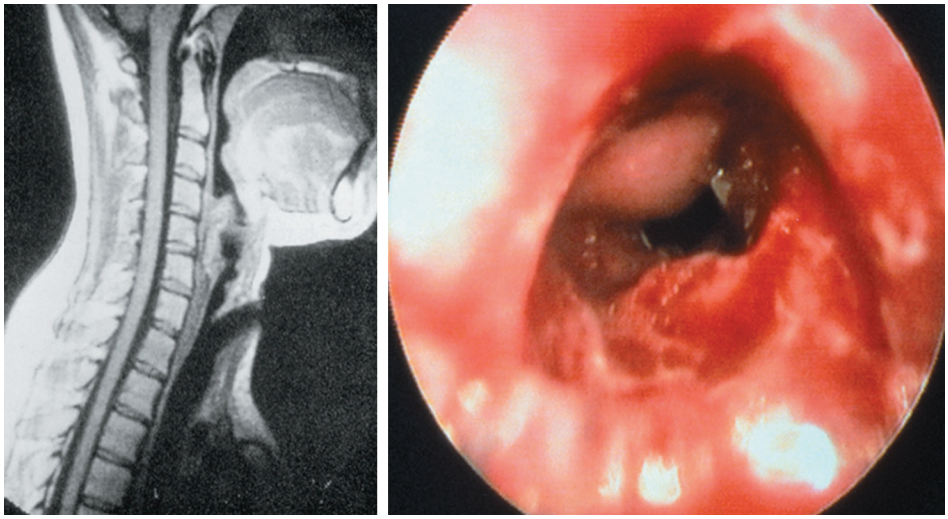


**Figure 89-3** Subglottic stenosis in a patient with granulomatosis with polyangiitis. MRI *(left)* and endoscopic view *(right)*. *(Courtesy Dr. G. Hoffman. From Hoffman GS, Kerr GS, Leavitt RY, et al: Wegener granulomatosis: an analysis of 158 patients.* Ann Intern Med *116:488–498, 1992.)*

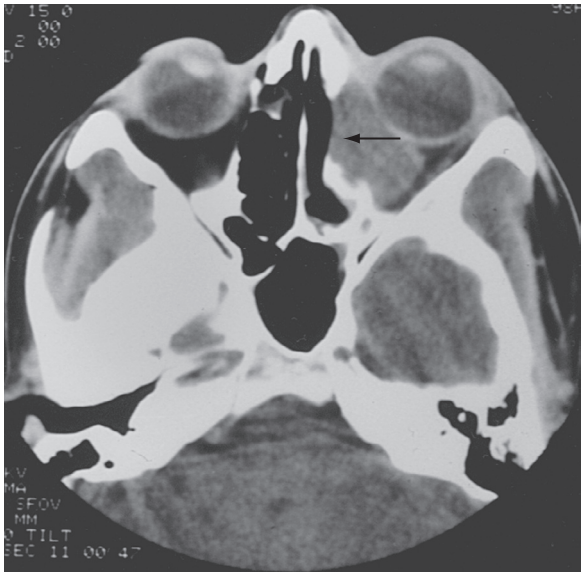


**Figure 89-4** Computed tomography scan of the orbits, showing a retro-orbital mass (orbital pseudotumor).

palsy, and optic neuropathy are less common but well described.[51,73] Inflammation of the lacrimal gland, sac, and/or duct, seen in about 10% of patients, leads to excessive tearing, and blockage of the lacrimal duct's entry into the nose can produce similar symptoms.[48,73] Orbital pseudotumor (Figure 89-4) is notable because it is both relatively common (6% to 15%) and dangerous, with risk of blindness reported at 50% despite aggressive treatment.[48,51,73]

The lung is affected in several distinct ways. Pulmonary nodules, which are necrotizing and therefore frequently cavitate (Figure 89-5), often occur prior to or in the absence of systemic vasculitis and thus are a common feature of "localized"[66,67] or "limited"[68] GPA. Nodular disease is often asymptomatic or merely produces cough. Alveolar hemorrhage, characterized by dyspnea and hemoptysis that can vary from mild to life-threatening, is also common but is distinct from nodular disease clinically and radiographically. However, dichotomizing lung disease in GPA into nodular or hemorrhagic forms understates the wide range of inflammatory disease that has been observed.[74,75] Apart from the lung parenchyma, pleuritis[48] and endobronchial lesions distal to the subglottis[76] are well described; the frequency of the latter may be underestimated because they can only be definitively diagnosed by bronchoscopy. Although clinically significant pulmonary fibrosis and bronchiectasis occur in GPA, their frequency is less than one might expect, considering how common parenchymal lung disease is; most lesions heal without scarring.

Musculoskeletal pain is common even in the early phases of disease and therefore can offer the rheumatologist the opportunity to diagnose and intervene in GPA before permanent organ damage occurs. Unfortunately, the presentations are variable and nonspecific. Monoarticular, oligoarticular, and polyarticular involvement are all seen. Arthralgia is more common than inflammatory arthritis that can be detected on physical examination.[48] Myalgia is usually not accompanied by abnormalities on examination or laboratory testing, and thus the musculoskeletal features of GPA can resemble polymyalgia rheumatica, spondyloarthropathies, rheumatoid arthritis, crystal arthropathies, the full range of connective tissue diseases, or even fibromyalgia. One distinctive articular presentation is worth emphasizing because it is relatively common in GPA but uncommon in other diseases: migratory arthralgias or arthritis of large joints, in which patients experience severe pain in a wrist, elbow, shoulder, ankle, or knee for a few days before complete resolution, to be followed soon afterward by similar symptoms in one to two other joints.

Cutaneous involvement in GPA includes the full range of presentations of cutaneous small-vessel vasculitis and, less commonly, vasculitis of small arteries. Thus purpura, papules, vesicles/bullae, ulcers, digit ischemia, subcutaneous nodules, and livedo racemosa have all been reported.[77,78] A characteristic lesion is a superficial, nonerythematous cutaneous nodule with pathologic findings showing a necrotizing, palisading granuloma, but this lesion is also seen in EGPA and other systemic diseases.[79,80]

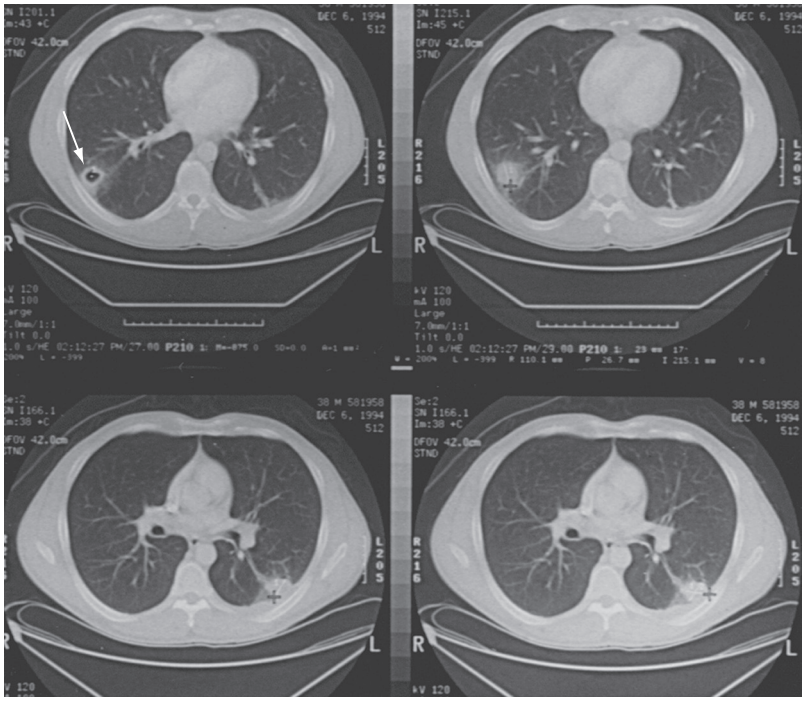


**Figure 89-5** Computed tomography of the lungs, showing pulmonary nodules. The right-sided nodule is cavitary *(arrow)*.

Peripheral neuropathy can present either as a sensory polyneuropathy or a mononeuritis multiplex with both sensory and motor deficits. Neuropathic pain is common but not universal in the setting of sensory loss. Cranial neuropathies are less frequent, with the exception of sensorineural hearing loss, the pathophysiology of which is unclear and which is an important source of morbidity.[81] Although CNS abnormalities such as strokes occur in patients with GPA, vasculitis of intracerebral arteries is thought to be rare; pachymeningitis is another well-described but uncommon manifestation.[82]

As with the CNS, patients with GPA will have cardiac problems and GI symptoms, but vasculitis of the heart muscle or the GI organs is very uncommon.[83,84] Pericarditis, in contrast, is a more common source of chest pain that is directly attributable to GPA.[48]

Glomerulonephritis (GN) is apparent in many patients at diagnosis and in more than half at some point during the course of disease.[48,49,51] On biopsy, the GN is characterized as pauci-immune and is frequently necrotizing and crescentic (Figure 89-6). The rate of progression is highly variable: renal function may deteriorate significantly within a week or slowly over months or even years. Because renal disease seldom produces gross hematuria, it is only apparent to the patient in the setting of uremia or nephrotic syndrome, and the latter occurs in a minority of patients.

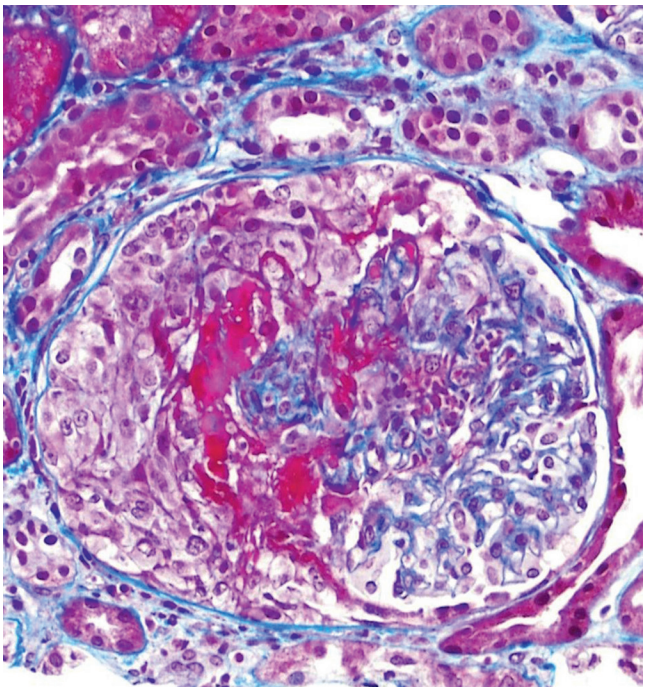


**Figure 89-6** Anti-neutrophil cytoplasmic antibody–associated crescentic glomerulonephritis. This Periodic acid–Schiff–stained section demonstrates a glomerulus with a cellular crescent partially obliterating Bowman's space. Because all types of crescentic glomerulonephritis appear similar by light microscopy, immunofluorescence is needed to distinguish among pauci-immune, immune complex, and anti–glomerular basement membrane antibody-mediated etiologies. *(Courtesy Dr. S. Bagnasco.)*

## Microscopic Polyangiitis

The clinical features of MPA consist of a limited subset of those seen in GPA (i.e., features that lack granulomatous inflammation). Large series published recently have made important contributions to the literature.[51,59] Reported frequencies of manifestations have varied much more than in GPA or EGPA (Table 89-2), likely for multiple reasons:

series from nephrology centers are expected to report very high rates of renal involvement[53]; series that include ANCA-negative patients without GN may include patients in whom the diagnosis might be debated[51,52]; confidence in defining involvement in some organ systems (e.g., cardiac, GI, and CNS) is challenging; and most interestingly, there appear to be important differences in MPA between patients in Europe and East Asia, particularly in the type of lung involvement.[59]

With those caveats, the following tentative statements can be made: GN is probably more common in MPA than in GPA, at least at diagnosis.[48,49,51,53,59,85] Cutaneous vasculitis, neuropathy,[86] constitutional and musculoskeletal symptoms, and risk of alveolar hemorrhage are probably similar. Involvement of the eye is distinctly less common. GI involvement has been reported at relatively high frequencies in some series (based on symptoms) and low frequencies in others, so it is difficult to draw conclusions.[51,59] As with GPA, it is also difficult to determine the rate of true CNS or myocardial vasculitis; subclinical myocardial abnormalities have been detected.[87] One manifestation that appears to be more common in MPA than GPA is pulmonary fibrosis, particularly but not exclusively in series from Japan and China.[59,88-90]

The duration of symptoms prior to diagnosis of MPA is wide, and the initial symptoms are often nonspecific constitutional and musculoskeletal complaints. Although symptoms commonly develop over days to months, many cases have been reported in which nonspecific symptoms had been present for years.[91] Patients who have MPA (or anti-MPO antibodies) typically present at a more advanced stage of renal failure than do patients with GPA (or anti-PR3 antibodies),[92] and histologically, renal damage at presentation is worse, on average, in patients with MPA/anti-MPO than in patients with GPA/anti-PR3.[93] However, it is not clear that GN in MPA is intrinsically worse than in GPA; alternatively, the "granulomatous" manifestations of GPA may lead to earlier diagnosis. Severe pauci-immune GN associated with anti-MPO antibodies and without any evidence of vasculitis elsewhere is well known to nephrologists and is often referred to as renal-limited vasculitis or necrotizing crescentic GN rather than MPA.[53,62]

### Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome)

The hallmarks of EGPA are asthma and peripheral eosinophilia out of proportion to what might be seen in asthma or allergic diseases, but other symptoms and findings are needed to make the diagnosis.[94] As with MPA and GPA, constitutional and musculoskeletal symptoms are common and may be the first clue to the presence of disease beyond asthma. Asthma itself is almost universal, often but not always severe, and highly variable in its onset relative to the onset of EGPA. Although EGPA is intimately linked to asthma, the conditions are distinct: patients frequently have asthma exacerbations without evidence of eosinophilic pneumonia or other manifestations of EGPA, and it is often asthma rather than EGPA-specific symptoms that requires control with chronic use of corticosteroids.[54,94]

The organ-specific manifestations of EGPA are attributable to vasculitis, parenchymal eosinophilic infiltration, or both. Reported frequencies of organ involvement are shown in Table 89-2.[54,57,58] Several comparisons to GPA and MPA are informative. Upper airway involvement is common in EGPA, but in contrast to GPA, it is usually "allergic" in character and is seldom destructive. GN and alveolar hemorrhage are uncommon and are highly associated with positive ANCA (usually anti-MPO),[54,58,95] which is detected in only 35% to 40% of patients with EGPA overall.[58,95]

Skin involvement is at least as common in EGPA as in GPA but again includes "allergic" lesions such as urticaria in addition to a wide range of presentations of cutaneous vasculitis.[77] Nodules representing necrotizing granulomas can be seen, as in GPA. The rate of GI disease is difficult to discern from series relying on symptoms, but because eosinophilic infiltration of the GI tract is well described in other settings,[96] it would not be surprising for GI disease to be more common in EGPA than in GPA or MPA.

Neuropathy, which is thought to be due to vasculitis rather than the neurotoxins of eosinophils, is more common in EGPA than in GPA or MPA,[51,54,57] and mononeuritis multiplex is frequently a useful feature in distinguishing EGPA from a hypereosinophilic syndrome (HES).[58,97] Finally, clinically important cardiac involvement is clearly a greater risk in EGPA than in MPA or GPA, with about 15% of patients having eosinophilic myocarditis severe enough to cause cardiomyopathy.[51,54] Cardiac disease resembles that seen in HES and is usually seen in ANCA-negative patients,[54,58,95] another example of the frequent separation of eosinophilic and vasculitic manifestations in EGPA.

## DIAGNOSTIC TESTING

The roles of laboratory tests, other diagnostic tests, and biopsy in diagnosing GPA, MPA, or EGPA vary with the clinical setting.

ANCAs were initially discovered and established as a clinical test using immunofluorescence staining of neutrophils. Subsequently, the target antigen for the c-ANCA pattern was identified as PR3, and the antigen for the p-ANCA pattern was identified as MPO. Although immunofluorescence staining (Figure 89-7) is often used in addition to, or as a screening test prior to, testing for anti-PR3 and anti-MPO antibodies by enzyme-linked immunosorbent assay, antigen-specific testing is much more valuable. In particular, p-ANCA immunofluorescence has poor specificity for AAV, whereas anti-MPO antibodies have high specificity for AAV.

Antibodies to PR3 or MPO are sufficiently specific that they can be considered diagnostic in cases in which vasculitis is highly likely by other criteria (Table 89-3). Examples of such clinical settings include digital ischemia (diagnosed by physical examination); noncompressive neuropathy (diagnosed by nerve conduction studies); and alveolar hemorrhage (diagnosed by either bronchoalveolar lavage or witnessed hemoptysis plus CT imaging consistent with diffuse bleeding). There is a difference of opinion in one key area. Some experts believe that a kidney biopsy should be performed whenever possible to confirm pauci-immune glomerulonephritis and give prognostic information derived from the degree of permanent damage,[93,98,99] whereas other experts might argue that red blood cell casts in the urine in the setting of anti-PR3 or anti-MPO antibodies is sufficient.

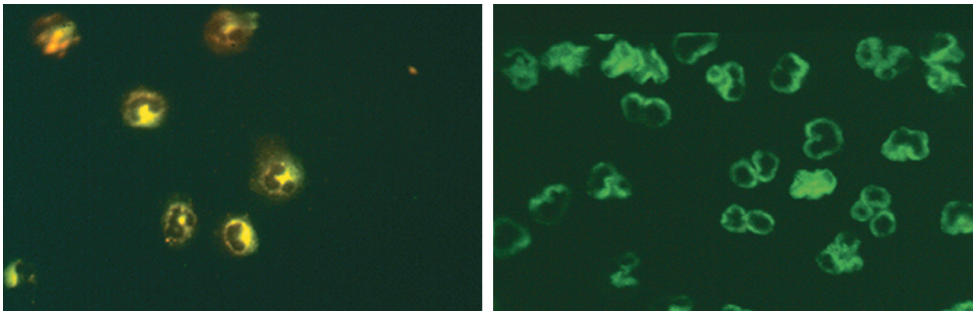


**Figure 89-7** Immunofluorescence of cytoplasmic anti-neutrophil cytoplasmic antibody (c-ANCA, *left*), which is highly correlated with anti-proteinase 3 antibodies, and perinuclear staining (p-ANCA, *right*), which is indicative of anti-myeloperoxidase antibodies. Positive immunofluorescence staining should be confirmed with antigen-specific testing to proteinase 3 and myeloperoxidase. *(Courtesy Dr. C.G.M. Kallenberg.)*

**TABLE 89-3** Clinical Settings in Which It Is Appropriate to Check ANCA and in Which Positive Testing for Anti-proteinase 3 or Anti-myeloperoxidase Antibodies Could Be Considered Diagnostic of GPA or MPA*

| Disease | Manifestation | Examination Technique | Laboratory and Other Testing | Imaging | Problems with Biopsy |
|---|---|---|---|---|---|
| GPA > MPA | Scleritis | Ophthalmologic examination | | | |
| GPA | Orbital pseudotumor | Routine inspection, or with measurement tool | | CT or MRI | |
| GPA > MPA | Sensorineural hearing loss | | Audiography | | Impossible |
| GPA | Sinonasal inflammation | Otoscope or fiberoptic examination | | CT of sinuses | All 3 characteristic features† seen in only 10% of GPA |
| GPA | Subglottic inflammation | Laryngoscopy | | CT of neck | Correlates poorly with examination or outcome |
| GPA | Pulmonary nodule(s) | | | CT of chest | 1. Trans-bronchial: all 3 diagnostic features† are seldom seen<br>2. Surgical biopsy: invasive |
| GPA/MPA | Alveolar hemorrhage | Inspection (witnessed hemoptysis) or bronchoscopy with BAL | | CT of chest | Invasive, and patient is often critically ill |
| GPA/MPA | Digit ischemia | Routine inspection | | Angiography | Impossible |
| GPA/MPA | Peripheral neuropathy | Neurologic examination: sensory and motor | Nerve conduction studies/ electromyography | | Likely to result in an area of permanent sensory deficit; small chance of chronic pain |
| GPA/MPA | Nephritis | | Urine sediment: RBC casts | | |

*If accompanied by marked peripheral eosinophilia, then EGPA would be the most probable diagnosis. Biopsy is often unnecessary to diagnose scleritis or alveolar hemorrhage, is often nonspecific or nondiagnostic for GPA in sinonasal or subglottic or orbital disease, and is impossible in digit ischemia or sensorineural hearing loss. The value of biopsy of a pulmonary nodule or peripheral nerve depends on how typical or atypical the overall presentation is for anti-neutrophil cytoplasmic antibody–associated vasculitides. Some experts recommend a kidney biopsy for all patients with suspected nephritis. Skin disease is not included in the table because the appearances of cutaneous vasculitis are nonspecific; skin biopsy is therefore particularly valuable for diagnosing vasculitis, although differentiation from other causes of cutaneous vasculitis is often impossible.

†Granulomatous inflammation, necrosis, and small-vessel vasculitis.

BAL, Bronchoalveolar lavage; CT, computed tomography; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; RBC, red blood cell.

The poor specificity of p-ANCA alone (i.e., without positive testing for antibodies to MPO) is well known. However, positive testing for anti-MPO or anti-PR3 antibodies has been well documented in three conditions other than AAV: endocarditis,[100] systemic lupus,[101] and use of cocaine adulterated with levamisole.[102] These associations are important to remember because the clinical syndromes overlap substantially with those of AAV. In all of these entities, the presence of additional autoantibodies is the rule. In the case of cocaine/levamisole, there is often a disconnect between the observed ANCA pattern by immunofluorescence and testing for the specific antigen.[102]

Other than the characteristic disease in the kidney (similar although perhaps not identical in all forms of ANCA-associated vasculitis),[98,103] the disease of MPA in other organs is difficult to distinguish from that of other

forms of small-vessel vasculitis by light microscopy. The ANCA-associated vasculitides are more likely to involve small arteries and arterioles than are vasculitides associated with immune complexes, but involvement of capillaries and venules is the most common site in all forms of small-vessel vasculitis.[103] The absence or paucity of immune complex deposition by immunofluorescence is helpful but not definitive, because immune deposits may have been consumed by phagocytes in lesions more than 48 hours old. A biopsy definitive for GPA should include necrotizing extravascular granuloma in addition to small-vessel vasculitis, but this combination of features is seen in a minority of biopsy specimens. Similarly, the combination of small-vessel vasculitis and eosinophil-rich necrotizing granuloma is seen in a minority of biopsy specimens in EGPA. Nevertheless, it is often important simply to confirm, by biopsy, the presence of vasculitis somewhere in a patient in whom MPA, GPA, or EGPA is likely, but nonvasculitic conditions are also considerations.

The presence of vasculitis, either proven by biopsy or strongly implied by documented mononeuritis multiplex,[58,97] is often essential to distinguish EGPA from the HES because only 40% of patients with EGPA test positive for ANCA, usually anti-MPO.[58,95] In turn, a high circulating eosinophil count or eosinophilic infiltration on biopsy distinguishes EGPA from MPA, GPA, and other forms of vasculitis. A high circulating eosinophil count is also often the clue that a patient has EGPA or HES rather than merely asthma and nasal polyposis.

One setting in which biopsy proof of GPA is highly desirable is in the ANCA-negative patient. Only 60% to 70% of patients with disease limited to the upper airway test positive for antibodies to PR3 or MPO, in contrast to 90% or more with systemic vasculitis.[48,49,67,68] Unfortunately, only approximately 20% of nasal biopsies will show the triad of granulomatous inflammation, necrosis, and vasculitis. Malignancies, nasal cocaine use, other chronic trauma, and a range of infections can also cause chronic inflammation, necrosis, and destruction of soft tissue and bone in the upper airway.[69,70] In the lung, GPA is one of many conditions that can cause nodules as a result of granulomatous inflammation[104]; it is presumed that the key feature of vasculitis is much more likely to be seen in an excisional biopsy than in a transbronchial or transthoracic needle biopsy.

Determination of disease activity in a patient with known AAV is challenging. Patients are susceptible to both systemic and localized infection as a result of the use of immune-suppressive drugs and to poor drainage of secretions resulting from damage to the airway, and symptoms of these infections are similar to symptoms of AAV. Fluctuation in symptoms when patients have had significant organ damage—such as the airway in GPA, congestive heart failure, particularly in EGPA, or peripheral neuropathy in all forms of AAV—may occur with or without active disease. Musculoskeletal and constitutional symptoms can occur in a wide range of acute and chronic conditions, and most patients with AAV report some degree of chronic fatigue regardless of their apparent disease status. Perhaps the greatest challenge is excluding active renal inflammation in a patient with prior kidney damage because proteinuria and hematuria can persist without evidence of additional renal damage over long periods,[105] and gradual loss of remaining kidney function may be attributable to "grumbling" disease but also may occur without any evidence of active inflammation.[53]

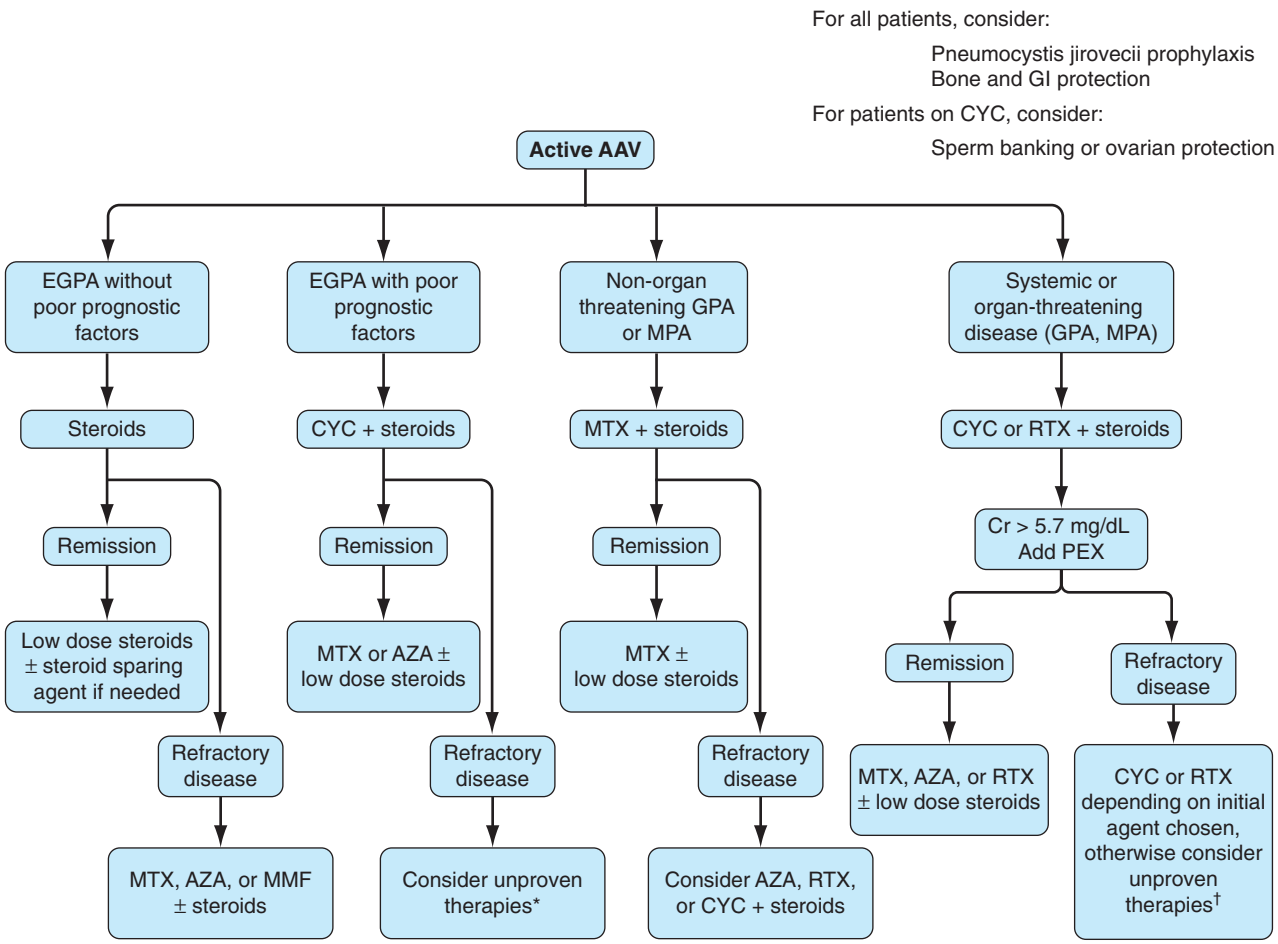
## TREATMENT

A retrospective study by Walton[106] published in 1958 showed that, without treatment, the median survival of patients with GPA was approximately 5 months, and that 81% of patients died within 1 year of diagnosis. Death was primarily due to progressive failure of multiple organ systems. The use of corticosteroids for treatment extended the median survival time to approximately 12.5 months.[107] The dismal prognosis for these diseases was not reversed until alkylating agents were used beginning in the 1960s.[107] Current treatment regimens aim to induce remission and then maintain remission using less toxic agents. Treatment is tailored to disease severity, with organ- and life-threatening manifestations warranting the most aggressive therapy (summarized in Figure 89-8). EULAR has presented guidelines for the management of primary small- and medium-vessel vasculitis.[108]

### Induction of Remission for Granulomatosis with Polyangiitis and Microscopic Polyangiitis

Since the 1960s, oral cyclophosphamide, an alkylating agent derived from nitrogen mustard, has been used at a dose of 2 mg/kg/day, along with corticosteroids, to induce remission for GPA and MPA. When oral cyclophosphamide is used, remission rates for ANCA-associated vasculitis range from 75% to 93%[48,109]—a stark contrast to the dismal median survival time prior to the use of this medication. In initial treatment regimens, cyclophosphamide was used continuously for at least 1 year. However, this prolonged use was associated with significant adverse effects. In a study of 158 patients with ANCA-associated vasculitis who were treated with cyclophosphamide for least 6 months, 46% experienced a serious infection (including pneumonias, skin infections, and bacterial and fungal sepsis), 43% experienced cyclophosphamide-induced cystitis, 2.8% experienced bladder cancer (a 33-fold increase), and 1.3% experienced lymphoma (an 11-fold increase). The risk of malignancy after cyclophosphamide use has been shown to be dose dependent. Among women between 18 and 35 years of age, 57% had cessation of menses for at least 1 year, were unable to become pregnant, or had laboratory evidence of ovarian failure.[48] In part to minimize toxicity, some studies have adjusted cyclophosphamide dosing based on renal dysfunction or advanced age. However, these adjustments have not been consistent across clinical trials. Given the toxicities observed with prolonged cyclophosphamide use, alternative cyclophosphamide regimens to minimize toxicities have been sought.

One alternative to daily oral cyclophosphamide for remission induction is to use intravenous (IV) pulse cyclophosphamide. In the CYCLOPS trial, 149 patients with either GPA or MPA received prednisolone and were randomized to receive pulse cyclophosphamide (15 mg/kg IV every 2 weeks × 3 doses, then 15 mg/kg IV every 3 weeks until remission, and then continued for 3 more months) or oral cyclophosphamide (2 mg/kg/day until remission, then




*Includes RTX, mepolizumab, hydroxurea, MTX, AZA, mycophenolate mofetil.
†Includes infliximab, mycophenolate mofetil, intravenous immunoglobulin, 15-deoxyspergualin.

**Figure 89-8** Treatment algorithm for the anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV). AZA, Azathioprine; Cr, serum creatinine; CYC, cyclophosphamide; EGPA, eosinophilic granulomatosis with polyangiitis; GI, gastrointestinal; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MTX, methotrexate; PEX, plasma exchange; RTX, rituximab.

1.5 mg/kg/day × 3 more months). After receiving cyclophosphamide, all patients received azathioprine. In the pulse cyclophosphamide arm, the mean cumulative dose was approximately half of the daily oral arm (8.2 g vs. 15.9 g), and consequently, less leukopenia was observed in the pulse arm. The median time to remission was approximately 3 months for both groups, and there was no difference in improvement in renal function between both groups.[62] Long-term follow-up (median duration 4.3 years) showed no difference in survival or renal function but a higher rate of relapse in the pulse arm compared with the daily oral arm (39.5% vs. 20.8%; HR for relapse in the daily oral arm, 0.50; 95% CI, 0.26 to 0.93; $P = 0.029$).[110]

Another alternative to daily oral cyclophosphamide for remission induction is the use of rituximab, a monoclonal antibody directed CD20, which is expressed on developing B cells from the immature B cell to the blast B cell stages. The mechanism of action for this antibody is not clearly known, but it can induce apoptosis of these developing B cells after binding and induce B cell depletion. Of note, B cell stem cells and plasma cells do not express CD20 and thus are not affected by rituximab. In the Rituximab in ANCA-Associated Vasculitis (RAVE) trial, which was double-blinded and designed to test for noninferiority, 197 patients with GPA or MPA received corticosteroid therapy and were randomized to receive either oral cyclophosphamide (2 mg/kg/day) or IV rituximab (375 mg/m$^2$ weekly × 4 doses). Patients in the oral cyclophosphamide arm were switched to azathioprine (2 mg/kg/day) after achieving remission with 3 to 6 months of cyclophosphamide therapy; patients in the rituximab arm were switched to oral placebo after the completion of four infusions. At the 6-month primary endpoint, rituximab was not inferior to cyclophosphamide at inducing remission and was potentially superior to cyclophosphamide for patients with relapsing disease. No difference was found in remission rates based on type of renal involvement, presence of alveolar hemorrhage, ANCA specificity or disease type, or whether patients became ANCA negative during the course of treatment.[65] In the 18-month follow-up study, a single course of rituximab continued to be noninferior to oral cyclophosphamide followed by azathioprine for remission maintenance. No significant difference was found between duration of complete remission, severity of relapses, or rate of adverse

events.[111] In the Rituximab versus Cyclophosphamide in ANCA-associated Vasculitis (RITUXVAS) trial, 44 patients newly diagnosed with ANCA-associated vasculitis were randomized (3 : 1) to receive rituximab along with only two infusions of cyclophosphamide versus IV cyclophosphamide for 3 to 6 months, followed by azathioprine. Rates of sustained remission and median increase in glomerular filtration rates at 12 months were similar in both groups. Rates of severe adverse events and death were also similar.[112] Both of these studies indicate that rituximab is comparable in efficacy to cyclophosphamide for remission induction.

Methotrexate has also been studied in comparison with oral cyclophosphamide for induction of remission of early systemic or localized disease. In the Nonrenal Wegener's Granulomatosis Treated Alternatively with Methotrexate (NORAM) trial, 100 patients with a new diagnosis of either MPA or GPA and without organ- or life-threatening manifestations such as severe hemoptysis, rapidly progressive neuropathy, or significant renal involvement (i.e., serum creatinine >1.7 mg/dL or 150 μmol/L, urinary red blood cell casts, or proteinuria >1 g/day) were randomized to receive either oral methotrexate up to 25 mg/week or cyclophosphamide, 2 mg/kg/day. Both treatment groups received maintenance therapy for 10 months, and then therapy was tapered and discontinued by month 12. At 6 months, the remission rate was similar in both groups (89.8% in the methotrexate arm and 93.8% in the cyclophosphamide arm, $P = 0.041$). In the methotrexate arm, remission was delayed for patients with more extensive disease or pulmonary involvement, and the relapse rate was higher at 18 months (69.5% in the methotrexate arm vs. 46.5% in the cyclophosphamide arm). Thus, although methotrexate was able to induce remission in patients without organ or life-threatening involvement, it was less effective than cyclophosphamide. Given the high relapse rate observed for both the methotrexate and cyclophosphamide treatment arms, therapy for these diseases should be continued beyond 12 months.[113]

More recently, abatacept has been studied as a remission induction agent for nonsevere, relapsing GPA. The addition of this agent to prednisone and remission maintenance therapy (e.g., methotrexate, azathioprine, or mycophenolate mofetil) was associated with disease remission and reduction in glucocorticoid doses.[114] The efficacy of this agent is being investigated in a larger trial (ClinicalTrials.gov identifier: NCT02108860).

Mycophenolate mofetil has been studied as a remission induction alternative to cyclophosphamide. In the Mycophenolate Mofetil versus Cyclophosphamide for Remission Induction of ANCA-associated Vasculitis (MYCYC) trial, 140 patients were randomized to receive either mycophenolate mofetil (2000 to 3000 mg/day) versus IV pulse cyclophosphamide. Initial results suggest that the primary remission endpoint (absence of disease activity ≥4 weeks while adherent to the glucocorticoid regimen) was reached by a similar proportion in each treatment arm (66% in the mycophenolate mofetil arm vs. 69% in the cyclophosphamide arm). However, the rate of relapse was substantially higher in the mycophenolate mofetil arm. Full publication of these results is needed to determine efficacy of mycophenolate mofetil for remission induction in the ANCA-associated vasculitides.[115,116]

## Maintenance of Remission for Granulomatosis with Polyangiitis and Microscopic Polyangiitis

Given the toxicities associated with prolonged cyclophosphamide use, a common strategy is to "induce" remission using more toxic therapies such as cyclophosphamide and then "maintain" remission using a less toxic therapy, thus decreasing overall toxicity due to cyclophosphamide. Medications studied as remission maintenance agents include azathioprine, methotrexate, mycophenolate mofetil, leflunomide, trimethoprim/sulfamethoxazole, etanercept, and rituximab.

Azathioprine was first demonstrated to be an effective remission maintenance agent in the Randomized Trial of Maintenance Therapy for Vasculitis Associated with Antineutrophil Cytoplasmic Autoantibodies (CYCAZAREM) trial, which treated 155 patients newly diagnosed with GPA or MPA with oral cyclophosphamide for at least 3 months and then randomized patients to continue cyclophosphamide or switch to azathioprine (2 mg/kg/day). The rate of relapse was similar in both groups (15.5% in the azathioprine arm vs. 13.7 in the cyclophosphamide arm, $P = 0.65$), as was the rate of adverse events. This study was the first to indicate that the duration of cyclophosphamide therapy could be reduced without significant adverse effects.[63]

Methotrexate was shown to be equally efficacious for remission maintenance when compared with azathioprine. In this study, 129 patients with GPA or MPA who achieved remission with cyclophosphamide therapy were randomized to receive either azathioprine (2 mg/kg/day) or methotrexate (up to 25 mg/week) for 12 months. The two medications were shown to have similar rates of remission maintenance, as well as adverse events, at 28 months of follow-up. Of note, 73% of patients relapsed after discontinuing the study medication at 12 months.[64] Long-term follow-up of the patients in this trial (median duration 11.8 years) showed no difference in overall 10-year survival rate, adverse event, or relapses. Anti-PR3 positivity was significantly prognostic of long-term relapses.[117]

Mycophenolate mofetil has also been investigated as an alternative remission agent in the International Mycophenolate Mofetil Protocol to Reduce Outbreaks of Vasculitides (IMPROVE) trial. After remission induction with cyclophosphamide, 156 patients were randomized to receive either azathioprine (2 mg/kg/day) or mycophenolate mofetil (2000 mg/day) and were followed up for a median of 39 months. Relapses were more common in the mycophenolate arm (HR for relapse in the mycophenolate arm, 1.65; 95% CI, 1.06 to 2.70; $P = 0.03$). Secondary outcome measures such as renal function and proteinuria and severe adverse event rates did not differ between groups.[118] Thus mycophenolate mofetil is believed to be less effective than azathioprine in maintaining remission in ANCA-associated vasculitides.

In a trial of 54 patients randomized to receive either leflunomide (30 mg/day) or methotrexate (starting at 7.5 mg/week and reaching 20 mg/week after 8 weeks), patients in the leflunomide arm had a decreased rate of relapse at 6 months (6/26 in the leflunomide vs. 13/28 in the methotrexate arm). A higher rate of major relapses in the methotrexate arm led to an early termination of the study. However, adverse event rates were higher in the

leflunomide arm.[119] Thus additional studies are needed to determine the role of leflunomide for remission maintenance therapy.

After reports of a beneficial effect of trimethoprim/sulfamethoxazole for the treatment of GPA, a double-blinded clinical trial randomized 81 patients with GPA who were in remission to receive either co-trimoxazole (800 mg of sulfamethoxazole and 160 mg of trimethoprim) or placebo for 24 months. A higher proportion of patients in the co-trimoxazole arm remained in remission compared with the placebo arm (82% vs. 60%). A subgroup analysis showed that patients with nasal or upper airway lesions benefited the most from co-trimoxazole use.[120] Thus, although co-trimoxazole is not commonly used for remission maintenance in persons with ANCA-associated vasculitis, it may be beneficial for patients with nasal or upper airway manifestations.

TNF inhibitors have had significant success as disease-modifying therapy for other autoimmune illnesses such as rheumatoid arthritis and inflammatory bowel disease. Past case series have suggested that agents such infliximab may be efficacious in controlling disease activity in GPA and MPA.[121] The most rigorously studied TNF inhibitor in GPA and MPA is etanercept for the maintenance of remission. In the Wegener's Granulomatosis Etanercept Trial (WGET), 174 patients received standard remission induction therapy (cyclophosphamide or methotrexate) and were randomized to receive concomitant etanercept or placebo, which was continued during the remission maintenance phase. There was no statistical difference in the rate of sustained remission between the etanercept and placebo arms (69.7% vs. 75.3%, $P = 0.39$) or in the time to remission. However, the incidence of solid cancers was significantly higher in the etanercept arm (six cancers vs. none, $P = 0.01$), suggesting that combining cyclophosphamide therapy with TNF therapy can substantially increase one's risk of cancer.[122] Based on these results, TNF inhibitors are generally not used for remission maintenance for ANCA-associated vasculitis.

Given the success of rituximab as a remission induction agent, it was also investigated as a remission maintenance agent. Previously published case series showed prolonged B cell depletion with rituximab to be effective in maintaining remission.[123-125] The use of rituximab as a remission maintenance agent has been more formally studied in the Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN) trial. In this study, 115 patients with either GPA, MPA, or renal limited vasculitis were randomized to receive either azathioprine (2 mg/kg/day × 12 months, followed by 1.5 mg/kg/day × 6 months, then 1 mg/kg/day × 4 months) or rituximab (500 mg IV × 2 doses at study entry and then every 6 months × 18 months), after having remission induced with cyclophosphamide. At 28 months (the primary endpoint), the major relapse rate was significantly lower in the rituximab arm (5% in the rituximab arm vs. 29% in the azathioprine arm).[126] The frequency of severe adverse events, including severe infections and malignancy, were similar in both arms. This difference in relapse rate has persisted at 39 months of follow-up (12.7% in the rituximab arm and 48.1% in the azathioprine arm; HR for relapse in the rituximab arm, 0.20; 95% CI, 0.11 to 0.45; $P < 0.0001$).[127] The international, open label, randomized controlled trial comparing rituximab with azathioprine as maintenance therapy in relapsing ANCA-associated vasculitis (RITAZAREM) trial is currently under way to further investigate the efficacy of rituximab (1000 mg IV every 4 months × 5 doses) as a remission maintenance agent.[128]

The use of low-dose glucocorticoids (prednisone or prednisolone, 5 to 10 mg/day) as a standard component of maintenance therapy is a source of considerable controversy and varying practice among experts worldwide. Unfortunately, no randomized trial investigating this question has been performed. Comparing relapse rates between trials is problematic because of different treatment regimens, different percentages of anti–MPO-positive patients or newly diagnosed patients, and different definitions of remission and relapse. With these caveats, a recent meta-analysis showed that trials that routinely continued glucocorticoids for 12 to 18 months reported markedly lower relapse rates than those in which they were discontinued earlier, usually by month 6.[129]

## Adjunctive and Alternative Agents

The role of plasma exchange in the treatment of ANCA-associated vasculitis was investigated in the Methylprednisolone or Plasma Exchange for Severe Renal Vasculitis (MEPEX) trial. In this trial, 137 patients with newly diagnosed GPA or MPA who had renal involvement confirmed by biopsy and serum creatinine greater than 5.8 mg/dL (500 μmol/L) were randomized to receive either seven plasma exchanges or 3000 mg of IV methylprednisolone, in addition to cyclophosphamide and prednisone. When compared with IV methylprednisone, plasma exchange was associated with a 24% decrease in risk to progression to end-stage renal disease at 12 months. No difference in survival was observed.[130] This therapy is being investigated further in a large ($n > 500$), multicenter, international trial (PEXIVAS[131]).

Intravenous immunoglobulin (IVIG) has been considered for patients who are unable to receive conventional immunosuppressive therapy. Case series using IVIG for remission induction have had conflicting results. In a randomized, placebo-controlled trial of 17 patients who received a single dose of IVIG (2000 mg/kg), 14 patients had significantly reduced disease activity, but this effect did not persist beyond 3 months.[132] Larger controlled clinical trials are needed to fully understand the efficacy of this agent for the treatment of ANCA-associated vasculitides.

Other alternative therapies for AAV investigated in smaller studies include anti-thymocyte globulin, which causes T cell depletion,[133] deoxyspergualin, which inhibits T cell maturation,[134] and alemtuzumab (Campath, anti-CD52), which depletes both T cells and B cells.[135]

## Treatment of EGPA

The optimal treatment of EGPA is less clearly defined when compared with GPA and MPA. In part because of the differences in clinical manifestations, patients with EGPA are frequently excluded from the randomized clinical treatment trials of GPA and MPA. Even so, the treatments found to be efficacious for GPA and MPA are used to treat EGPA,

and the same general therapeutic strategy of inducing remission with a more toxic medication and maintaining remission with a less toxic medication is also applied.

The vasculitis and asthma manifestations of EGPA are usually managed separately. Asthma treatment generally follows recommended asthma treatment guidelines for patients without EGPA. However, many patients' asthma symptoms are corticosteroid dependent, and thus additional immunosuppression can be used for steroid-sparing effects. Initial management of systemic or vasculitic manifestations of EGPA relies on corticosteroids. Patients with EGPA who do not have poor prognostic factors as defined by the original Five Factor Score (serum creatinine >1.58 mg/dL, proteinuria >1 g/day, severe GI involvement, cardiomyopathy, or CNS involvement) can be treated with corticosteroids alone, but 35% of patients relapse when corticosteroids are tapered.[136] Other immunosuppressive agents are needed in addition to corticosteroids for patients with more severe disease. As with GPA and MPA, cyclophosphamide is generally used to treat organ- or life-threatening manifestations.[137] The remission maintenance agents discussed in the previous section have not been well studied in EGPA. One randomized, open-label study showed that azathioprine was efficacious for remission maintenance when compared with cyclophosphamide,[136] but whether the efficacy of one agent such as azathioprine is equivalent or superior to the other remission-maintenance agents is unknown. For patients with and without poor prognostic factors, approximately 80% of patients require long-term use of corticosteroids to manage manifestations such as asthma.[136,137]

Current understanding of the efficacy of rituximab in EGPA is derived from case reports and case series. Most patients with EGPA in the reports were treated with rituximab because of disease refractory to usual treatments (e.g., cyclophosphamide) or medication intolerance. In one of the largest case series to date, nine patients with EGPA refractory to conventional therapy were treated with rituximab at a single center. All nine patients were either in complete or partial remission within 3 months after treatment. No flares were observed for three patients who were preemptively treated with rituximab (median follow-up for 3 years).[138] Larger studies and more experience are needed to fully determine the efficacy of rituximab in EGPA.

A promising therapy for EGPA is mepolizumab, a monoclonal antibody directed against IL-5, a cytokine critical for activation of eosinophils. Previous experience with mepolizumab in HES showed prompt normalization of peripheral eosinophil counts, as well as decreased glucocorticoid usage.[139] In two uncontrolled pilot studies of mepolizumab in EGPA, mepolizumab use led to decreased disease activity, normalization of eosinophil counts, and reduction of corticosteroid use. However, cessation of mepolizumab use was associated with disease flares.[140,141] A randomized, double-blinded, placebo-controlled trial using mepolizumab in EGPA is currently under way (ClinicalTrials.gov identifier: NCT02020889).

## OUTCOMES

Although the ANCA-associated vasculitides are still sometimes fatal (particularly as a result of alveolar hemorrhage) because of the severity of disease at onset or treatment resistance, most patients can be successfully treated, which has two important sequelae. First, the substantially increased risk of mortality in the first 6 months after diagnosis (approximately 10%) is mostly due to infection as a consequence of treatment, rather than vasculitis itself.[142] Second, the moderately increased risk of mortality long term is multifactorial, including infection, cardiovascular disease, and malignancy. The importance of permanent organ damage in increasing mortality is clear from the finding that the only factor other than advancing age that is strongly associated with long-term mortality in MPA and GPA is renal dysfunction.[142] Analogously, cardiomyopathy is by far the strongest risk factor for mortality in EGPA.[54]

Although mortality has been greatly reduced, morbidity remains high because of permanent damage (particularly renal insufficiency, sinonasal symptoms, hearing loss, neuropathy, and dyspnea) and consequences of treatment (diabetes, hypertension, osteoporosis, weakness, and certain malignancies).[92] It is also notable that patients report fatigue and reduced energy—symptoms that cannot be assessed objectively—as the most important negative consequences of having vasculitis.[143]

The incidence of cancer has been reported to be elevated in GPA. A recent study showed lower risks of bladder cancer, leukemia, and lymphoma than had been reported previously.[144,145] Because strikingly increased risk of all three of these cancers had been observed after long-term use of cyclophosphamide, it is likely that there is little increased risk with current approaches to treatment. There remains an increased risk of nonmelanoma skin cancer,[144,145] and it is difficult to assess relative risks of uncommon cancers in rare diseases.

Assuming the success of initial treatment, the different forms of AAV often relapse but often do not. Based on cohorts and follow-up of patients in clinical trials of patients with systemic vasculitis, the risk of relapse in GPA is about 60%,[48,49,111,146,147] and that of MPA is about 30%.[53,111,146-148] Whether this difference is due to the disease phenotype or ANCA specificity is uncertain.[111,146,147] Drawing a firm conclusion would require longitudinal follow-up of large numbers of MPO-positive patients with GPA separately among white and Asian cohorts, because that phenotype is more common among Asian patients.[56,90] Not surprisingly, having relapsed in the past also increases the risk of subsequent relapses.[111] Localized/limited GPA may have a higher rate of relapse than systemic/severe GPA,[67,68] and 30% to 55% of such patients may be ANCA-negative.[67,68]

Persistence of c-ANCA/PR3 and rising titers were associated with increased risk of flare in most but not all studies. The likelihood ratio is approximately 2, that is, below the level considered to be "diagnostic" for making treatment decisions with confidence.[149] Almost all studies on the value of following up on ANCA titers have focused on c-ANCA/PR3.

Relapse of vasculitis or parenchymal eosinophilic disease above and beyond sinonasal disease and asthma is about as common in EGPA as in MPA and is somewhat more common in ANCA-positive than in ANCA-negative patients.[54] Relapses of sinonasal disease and asthma are much more common (often chronic) and are the main reason that more than 80% of patients with EGPA need to

continue to take prednisone and often additional immunosuppressive drugs.[54]

The caveat to interpreting studies of relapse rates is that there may be selection bias: patients with more severe disease are more likely to enroll in clinical trials, and patients with relapsing disease are more likely to continue to be followed at referral centers. Thus the course of mild MPA (e.g., purpura, myalgia, and anti-MPO antibodies) is virtually unknown, and chronically localized/limited GPA has not been studied—in trials or outcome studies—as extensively as has systemic/severe disease.[67]

## PATHOGENESIS

MPA, GPA, and EGPA all feature leukocytoclastic vasculitis: fibrinoid necrosis of arterioles, capillaries, and venules by neutrophils, which infiltrate and die in the surrounding tissue and leave characteristic fragments of their nuclei visible under the microscope. Additional involvement of small arteries is sometimes seen, and involvement of small veins is rare.[103] Although some deposition of immunoglobulins and complement can be seen (these diseases are "pauci-immune" rather than nonimmune),[103,150] immune complex deposition is thought to play little if any role in pathogenesis. Because ANCA are highly associated with pauci-immune necrotizing vasculitis, much of the study of pathogenesis has focused on the generation and mode of action of ANCA.

Although the bulk of MPO and PR3 is intra-cellular in neutrophil granules, these proteins may be translocated to the cell surface either constitutively or by inflammatory stimuli[151] and serve as targets for ANCA to activate neutrophils.[152,153] In addition, MPO and PR3 may serve as targets extra-cellularly after degranulation, or particularly after release associated with neutrophil extra-cellular traps.[153]

The best evidence that ANCA are directly pathogenic comes from a mouse model in which anti-MPO antibodies (produced by immunizing *Mpo*–/– mice with mouse MPO) produce pauci-immune GN when transferred into normal mice.[154] Neutrophils and activation of the C5 component of complement are required to produce GN.[155-157] However, other features than GN are not seen, and GN itself is relatively mild.[154,158] More severe GN and additional features of systemic vasculitis are produced by transferring spleen cells into SCID mice,[154] but whether all of this disease is attributable to anti-MPO antibodies or even to MPO-specific T cell immunity is unclear.

Efforts to produce an analogous model using PR3 have not been successful, and other efforts to demonstrate pathogenicity of anti-PR3 autoimmunity require considerable experimental manipulation and produce limited disease. Whether this difficulty is due to the different structure and biology of PR3 in humans and rodents—or is evidence that anti-PR3 antibodies have limited pathogenicity in isolation—is not clear. Production of vasculitis using human anti-PR3 antibodies and mice with "humanized" immune systems supports the human-versus-mouse explanation and may provide the best evidence in favor of anti-PR3 antibodies causing vasculitis,[159] but as with all other rodent models of ANCA-associated vasculitis, this one has pros and cons for inferring the pathophysiology of human disease.[158] Mouse models of HES have been developed,[160] but not the combination of eosinophilic and necrotizing vasculitis that would be a more specific model for EGPA.

T cell immunity is implicated in any process that results in high-affinity antibodies, but in addition, $CD4^+$ T cells are found in the necrotizing granulomatous lesions in GPA and are likely involved in macrophage activation and neutrophil recruitment locally.[161] The target antigen(s) is/are unclear, particularly because a large percentage of patients lacking evidence for systemic vasculitis test negative for circulating ANCA. Multiple changes in the percentages of T cell subsets—Th1, Th2, Th17, and regulatory T cells—have been described in patients with different forms of AAV, in different tissue sites, and in different stages of disease activity.[161]

The number of circulating proteins related to immunity, inflammation, vascular injury, and tissue repair is so large in highly active GPA and MPA that it is difficult to obtain insight into pathophysiology from biomarker studies.[162,163] In EGPA, circulating products of eosinophils are elevated in untreated disease.[164] As expected, markers of Th2 responses are elevated,[165-167] but Th17 cells have recently been implicated as well.[166-168]

As noted in the Epidemiology and Genetics sections, known genetic risk factors and environmental exposures explain only a small portion of the risk for GPA, MPA, or EGPA. Although it is possible that rare or unique (including somatic) genetic mutations or rare environmental exposures may contribute further to risk, it is also worth emphasizing that in many autoimmune diseases, random chance is likely to play an important role in determining whether tolerance to a particular autoantigen is broken.

## CONCLUSION

In the past 30 years, significant progress has been made in diagnosing and treating ANCA-associated vasculitis. Although this progress has dramatically reduced the mortality of the AAV, patients continue to accumulate organ damage because of the chronic relapsing nature of these diseases. Thus many challenges remain. Clinicians and investigators alike would benefit from an improved understanding of the role of ANCA in disease pathogenesis, identification of clinically actionable biomarkers of disease activity, and the development of more targeted therapies to reduce treatment toxicity. With this additional knowledge, the prognosis for patients AAV may continue to improve.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 90

# Polyarteritis Nodosa and Related Disorders



Raashid Luqmani

**KEY POINTS**

- Polyarteritis nodosa (PAN), which is characterized by vasculitis of medium-sized arteries with few or no immune deposits, is relatively rare, especially in comparison with anti-neutrophil cytoplasmic antibody (ANCA)–associated small-vessel vasculitis.
- One form of PAN that is now uncommon is caused by hepatitis B virus and usually responds well to anti-viral therapy, plasma exchange, and/or glucocorticoids. Non–hepatitis B–associated PAN should be treated aggressively with cyclophosphamide and glucocorticoids, using the same approach as that used for systemic ANCA-associated vasculitis.
- The incidence and prevalence of non-hepatitis PAN is around 1 per million per annum. A significant reduction in the incidence of PAN has occurred because of a decreasing incidence of hepatitis B infection, as well as reclassification of patients (i.e., better case recognition of ANCA-associated vasculitis).
- Presenting features of PAN include an insidious onset of weight loss, purpuric skin lesions together with mononeuritis multiplex, and symptoms of mesenteric ischemia.
- Glomerular renal disease is absent in people with PAN and should prompt consideration of other diagnoses, especially ANCA-associated small-vessel vasculitis.
- Hematuria with renal impairment is rare in people with PAN but can occur as a result of renal infarction.
- "Cutaneous PAN" is a limited form of vasculitis that is unrelated to systemic PAN.
- Buerger's disease (thromboangiitis obliterans) affects both sexes and involves the upper and lower extremities; the role of tobacco use in Buerger's disease is established but not understood.
- The evidence base for treatment of other rare forms of vasculitis is limited.

## POLYARTERITIS NODOSA

### Definition and Classification

The term *periarteritis nodosa* was originally introduced in 1866 and was subsequently used to describe any form of systemic vasculitis.[1] The term has been modified to polyarteritis nodosa (PAN), and the definition has been revised to consist of "necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules."[2]

The American College of Rheumatology (ACR) criteria, which were designed to classify patients as having PAN to distinguish them from patients with other forms of primary systemic vasculitis, are of limited use because they do not differentiate between PAN and microscopic polyangiitis (MPA); both conditions are included under the umbrella of PAN. Three of 10 ACR criteria (listed in Table 90-1) are required to make a diagnosis.[3]

The sensitivity of the ACR criteria for PAN (or MPA) is 82.2%, with a specificity of 86.6% when used to classify the disease.[3] Definitions of vasculitis (including PAN) were published in 1994 by the Chapel Hill Consensus Conference (CHCC)[2] and updated in 2013.[4] MPA, granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg-Strauss syndrome) were distinguished from PAN on the basis of the pathologic findings of small-vessel involvement in the former three diseases; by contrast, small-vessel involvement is absent in PAN.

Much confusion continues to exist regarding the terms used to describe patients with different forms of vasculitis. It is worth considering this issue for a moment because, upon reviewing the cases of patients whose disease has previously been given the label of "PAN," the clinical features at presentation very often indicate another form of vasculitis, typically MPA or GPA. In an attempt to rationalize the naming of different forms of vasculitis, the European Medicines Agency (EMA) produced an algorithm to assist in the classification of patients with systemic small- and medium-vessel primary systemic vasculiti.[5] A decision tree approach was used, effectively putting PAN at the bottom of the tree.[5] In other words, patients were assigned to any other form of vasculitis when possible, and the term *PAN* was applied only as a last resort when no other diagnosis was made. This might seem like a rather negative view of the condition, but after accounting for the overuse of the term PAN in preference to other terms in the past, the true incidence and prevalence of PAN are actually low.

The EMA algorithm is particularly useful because MPA is a more common condition than PAN but is not included in the ACR classification criteria (it is considered part of PAN), whereas in the CHCC definition, PAN and MPA are treated as separate entities. Important differences exist between PAN and MPA in terms of pathogenesis, organ involvement, tendency to relapse, and prognosis.[6] In the CHCC definition, PAN is a medium-vessel disease; by comparison, MPA is predominantly a small-vessel disease that includes glomerulonephritis and pulmonary capillaritis.[2]

**TABLE 90-1** American College of Rheumatology Criteria for Classification of Polyarteritis Nodosa

| |
|---|
| Weight loss ≥4 kg |
| Livedo reticularis |
| Testicular pain or tenderness |
| Myalgias, weakness, or leg tenderness |
| Mononeuropathy or polyneuropathy |
| Diastolic blood pressure >90 mm Hg |
| Elevated blood urea nitrogen or creatinine levels |
| Hepatitis B virus |
| Arteriographic abnormality |
| Biopsy of a small- or medium-sized artery containing polymorphonuclear neutrophils |

Criteria sometimes used erroneously for microscopic polyangiitis.

From Lightfoot RW, Michel BA, Bloch DA, et al: The American College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. *Arthritis Rheum* 33:1088–1093, 1990.

**TABLE 90-2** Predictive Variables for Classification of Polyarteritis Nodosa

| Variable | Positive Prediction for PAN | Negative Prediction for PAN |
|---|---|---|
| Positive hepatitis B serology | + | |
| Arteriographic abnormalities | + | |
| Mononeuropathy or polyneuropathy | + | |
| Positive anti-neutrophil cytoplasm antibody | | + |
| Asthma | | + |
| Ear, nose, or throat signs | | + |
| Glomerulopathy | | + |
| Cryoglobulinemia | | + |

PAN, Polyarteritis nodosa.

Modified from Henegar C, Pagnoux C, Puéchal X, et al: A paradigm of diagnostic criteria for polyarteritis nodosa: analysis of a series of 949 patients with vasculitides. *Arthritis Rheum* 58:1528–1538, 2008.

Patients are assessed using the EMA algorithm if they have a clinical diagnosis of an anti-neutrophil cytoplasmic antibody (ANCA)–associated vasculitis or PAN. In the algorithm, PAN is regarded as a diagnosis of exclusion. The first part of the algorithm is to determine if the patient's disease fulfills the Lanham[7] or ACR criteria[8] for EGPA. If it does fulfill the criteria, the patient's disease is classified as EGPA. If not, the next step is to determine if the patient's disease fulfills the ACR criteria or CHCC definition for GPA,[2,9] either through histologic evidence or with appropriate surrogate markers and a positive ANCA test. If not, one proceeds down the algorithm to see if the patient's disease fulfills the definition of MPA,[2] with histology showing small-vessel vasculitis or glomerulonephritis and no surrogate markers for GPA, or surrogate markers of glomerulonephritis and a positive ANCA test. Only when EGPA, GPA, and MPA are excluded is a diagnosis of PAN possible in this schema. To fulfill the definition of PAN in this algorithm, histologic or angiographic features must be present that are consistent with the diagnosis. The disease of any remaining patients is determined to be unclassifiable. The initial validation of the algorithm made use of paper cases that were assessed by a number of experts in vasculitis. In fact, every case was assigned to one of the conditions, without the need to describe any case as unclassifiable. This outcome may not reflect clinical practice, in which some patients appear to have an overlap between different forms of vasculitis or incomplete forms of a particular vasculitis. This important issue has begun to be addressed by a task force on classification and diagnostic criteria in vasculitis.[10,11]

The French Vasculitis Study Group proposed a set of predictive items (Table 90-2) to be used as diagnostic criteria.[12] The items were derived from 949 patients with known vasculitis (including 262 patients described as having PAN) and not from undifferentiated patients, and therefore they are actually another form of classification criteria.

The combination of nonspecific constitutional symptoms and ischemic symptoms in one or more organ systems should alert the physician to the possibility of a systemic vasculitis. PAN typically presents with nonspecific symptoms, such as fever, weight loss, and myalgia, in combination with single or multiorgan manifestations resulting from ischemia or infarction. The most common organ manifestation is neurologic with mononeuritis multiplex, followed by skin lesions, abdominal pain from mesenteric ischemia, and renal infarction. Testicular pain due to ischemic orchitis is a classic feature of PAN but is rare at presentation. Some patients may present with acute abdomenal symptoms requiring surgery as a result of bowel, liver, spleen, or pancreatic infarction. Myocardial infarction, ischemic cardiomyopathy, optic ischemia, and ischemic complications of the female genital tract are possible but unusual.

## Epidemiology

The epidemiology of PAN has changed over time. Effective hepatitis B virus (HBV) immunization programs, improved blood screening for HBV, and major changes in the definition and classification of vasculitis have resulted in a substantial reduction in the true incidence of what we call PAN. Before the CHCC definition in 1994, MPA was included in the incidence and prevalence estimates of PAN, the impact of which is highlighted in a study comparing the incidence of PAN in three European regions: 4.4 to 9.7 per million by the ACR criteria versus 0 to 0.9 per million with the CHCC definition.[13]

According to ACR criteria, the incidence of PAN is 2 to 9 per million in Europe and the United States.[14] A higher incidence of 16 per million (by CHCC definition) is reported in Kuwait,[15] and 77 per million is described in an Alaskan population endemic for HBV, although it was based on only 13 actual cases of HBV PAN (in a study performed before ACR criteria or CHCC definitions were available).[16] The prevalence of PAN by ACR criteria is 31 to 33 per million in Western Europe[17-19]; it is 2 to 9 per million in Germany by CHCC definition.[20] A small study from Australia assessed the incidence and prevalence of different

forms of vasculitis between 1995 and 2005 in one area, suggesting a decrease in the incidence of PAN from 2.3 per million per annum to 1.1 per million per annum.[21]

A study from 2009 assessed the incidence and survival of patients with different forms of vasculitis and estimated the incidence of PAN to be 0.9 (between 0 and 1.7) per million per annum. This estimate compares with an almost 10-fold difference in the incidence of GPA and MPA (between 9.8 and 10.1 per million per annum).[22]

PAN can occur at any age, but the most common age range at diagnosis is 40 to 60 years. No clear gender difference exists.[19] In a multiethnic population from France, patients with European ancestry had a higher prevalence of PAN compared with patients without European ancestry.[18] Childhood-onset PAN is less common than adult-onset PAN, with a low risk of death (3.8% in a series of 52 children followed up for more than 6 years) but a significant risk of serious complications, including hypertension and cranial nerve palsy, which themselves predict worse outcome in terms of morbidity.[23]

## Etiology and Pathogenesis

PAN is often related to infection with hepatitis B.[24] The incidence of HBV-related PAN follows HBV infection rates, previously accounting for 7% to 38.5% of patients diagnosed with PAN.[18,25] The prevalence of HBV-related PAN has reduced in recent years as a result of vaccination for HBV and improved screening of blood products.[18,26] PAN develops in 1% to 5% of patients with HBV infection,[16] which confers an approximately 1000-fold increase in risk compared with the background population.[14] By contrast, in Alaska, an area endemic for HBV, a substantial increased annual incidence of HBV PAN is found (77 per million), primarily as a result of vertical transmission.[16] Documented exposure to HBV occurs from blood products, intravenous drug use, and sexual contact. Screening of blood products for HBV and mass vaccination against the virus has successfully reduced the incidence of HBV PAN, as shown in French patients, with the proportion of cases of PAN due to HBV falling from 38.5% in the 1970s to 17.4% by the period 1997-2002.[27]

In HBV-related PAN, the postulated mechanism of vasculitis includes direct vessel injury by replicating virus or deposition of immune complexes. Deposition of immune complexes leads to activation of the complement cascade, resulting in an inflammatory response and subsequent endothelial damage. The vasculitis typically occurs within the first few months after HBV infection and may be the first presenting feature of this infection. Evidence for the pathogenic nature of HBV and immune complexes is supported by the effectiveness of a treatment strategy to eradicate HBV with anti-viral therapy and removal of immune complexes by plasmapheresis without the need for long-term immunosuppression.[27,28]

Saadoun and colleagues[29] described 31 patients who had hepatitis C virus (HCV)–associated vasculitis, which they classified as PAN according to ACR criteria and CHCC definitions. This cohort, representing approximately one-fifth of their patients with HCV vasculitis, had more frequent fevers, weight loss, severe hypertension, gastrointestinal tract involvement, severe acute sensory-motor multifocal mononeuropathy, kidney and liver microaneurysms, and increased C-reactive protein compared with other patients with HCV vasculitis. In contrast, their response to therapy was more successful.

In the remainder of patients with PAN, the cause of the disease is unknown. Genetic, infectious, and environmental agents are thought to be important, but no conclusive evidence exists.[13]

A recent study of exome sequencing of six families in which multiple family members were affected has shown a link to a recessive loss of function mutation of adenosine deaminase 2.[30]

The response of idiopathic PAN to immunosuppressive therapy suggests an immunologic mechanism. In idiopathic PAN, the role of immune complexes is unclear. There is evidence of endothelial dysfunction, an increase in inflammatory cytokines, and an increase in expression of adhesion molecules. The inflammatory lesions have a propensity to occur at the sites of bifurcation in vessels, where turbulent flow is most likely. After the inciting event, focal and segmental necrotizing inflammation of medium- and small-sized arteries is present, which leads to intimal proliferation with subsequent thrombosis, resulting in ischemia or infarction of the organ or tissue supplied by these arteries.

Some case reports describe the development of PAN in patients with pre-existing hairy cell leukemia.[31,32] Most patients in these cases had undergone a splenectomy before the development of PAN. Potential mechanisms for the association between hairy cell leukemia and PAN include cross-reactivity of antibodies between tumor cells and the endothelium, direct damage of the endothelium by tumor cells, and local production of pro-inflammatory cytokines, triggering vessel wall damage.[32]

## Pathologic Features

Because of the protean manifestations of the disease, it might be appropriate to obtain biopsy specimens from several different sites when considering the diagnosis in any individual patient. Sampling should be directed by the pattern of clinical involvement suggested by the history or physical examination. Muscle, peripheral nerves, kidney, testis, and rectum, when involved, provide the best yield. However, skin involvement and positive results of a skin biopsy do not always indicate evidence of systemic involvement[33-35] (see section on cutaneous PAN).

A biopsy of a medium-sized artery will show "focal and segmental" transmural necrotizing inflammation[34,35] (Figure 90-1). If any clinical or laboratory features indicate involvement of small vessels, further assessment should be undertaken, because these features suggest an alternative form of vasculitis such as MPA.[36]

Inflammatory change at the bifurcation of vessels is reported to be common. The coexistence of different stages of inflammation and scarring, as well as normal vessel wall, is typical in patients with PAN. Areas of acute inflammation will usually have a pleomorphic cellular infiltrate of lymphocytes, neutrophils, macrophages, and eosinophils. Aneurysms can occur at the site of active lesions, and this morphologic appearance is what led to the term "nodosa" (Figure 90-2). Proliferative scarring in other areas may lead to narrowing of vessels.[33-37]



**Figure 90-1** This celiac axis angiogram from a patient with polyarteritis nodosa demonstrates an irregular vascular pattern with small aneurysms in the hepatic vessels.



**Figure 90-2** Broken livedo in a patient with cutaneous polyarteritis nodosa. *(Courtesy Professor Sunderkoetter, Department of Dermatology, University Hospital of Muenster.)*

## Clinical Features

Although PAN can present at any age, it typically occurs in the age range of 40 to 60 years. No significant gender difference exists. The diagnosis can be difficult to make because individual features can occur in a variety of other diseases. Table 90-3 summarizes the clinical findings in PAN. The presence of nonspecific constitutional symptoms such as fever, weight loss, joint and muscle pains (found in 65% to 80% of cases of PAN), and ischemic symptoms in one or more organ system should raise the possibility of a systemic vasculitis.[19,37] Organ-specific manifestations may be present at the onset of disease, or more of a low-grade course may be present, resulting in the development of specific organ involvement months to years later. Involvement of organs may be in isolation or as multisystem disease.[38] In a Swedish series, the most common manifestations occurred at disease onset: nervous system (55%), skin (44%), abdominal (33%), and renal (11%).[19] A review of 348 patients diagnosed as having PAN since the 1960s[39] suggested that their mean age at diagnosis was 51 years and the most frequent symptoms were generalized features in more than 90% of cases, neurologic features in 79%, skin involvement in 50%, abdominal involvement in 36%, hypertension in 35%, renal artery microaneurysms in 66%, and histologically proven PAN in 70%. The subset of 123 patients with hepatitis B–associated PAN was more likely to experience peripheral neuropathy, abdominal pain, cardiomyopathy, orchitis, and hypertension compared with 225 non-HBV patients with PAN. The relapse rate was 22% over a follow-up of 6 years (28% for non–HBV-related PAN compared with 11% for HBV PAN). The mortality rate was 25% overall (20% for non-HBV PAN compared with 34% in HBV-related PAN). Five-year relapse-free survival was higher in patients with HBV-related PAN (67% compared with 59%). The conclusion was that non-HBV PAN in particular had a high mortality, especially involving elderly people and was worse in patients with skin manifestations.

## Clinical Assessment of Patients

In patients in whom vasculitis is suspected, a thorough history and physical examination is necessary to identify the potential organ systems involved. Use of the Birmingham Vasculitis Activity Score as a checklist of important features of the disease is recommended[40] to guide further targeted investigation and to be used subsequently in devising a management plan for treatment. Table 90-3 lists the typical features to be assessed in people suspected of having PAN. In up to 23% of cases, we should expect new disease manifestations to appear during follow-up,[41] emphasizing the need for regular review and evaluation in all patients.

## Laboratory Testing

No specific laboratory tests are available to diagnose PAN, but some tests may be useful to support the diagnosis, identify organs that may be affected, and rule out alternative diagnoses (Table 90-4).

## Radiology

The conventional fluoroscopic contrast angiogram traditionally has been the imaging modality of choice (Figure 90-1), but increasingly, it is being replaced by less invasive, safer techniques, such as computed tomography (CT) angiography or magnetic resonance (MR) angiography. The classic findings are multiple small aneurysms, vessel ectasia, and focal occlusive lesions in medium-sized vessels, typically in the renal and mesenteric arteries. The reported

**TABLE 90-3** Organ Involvement in Polyarteritis Nodosa

| System | Comment | Frequency | Reference(s) |
|---|---|---|---|
| Constitutional | Fever and weight loss (current and previous) | >90% | 39 |
| Musculoskeletal | Arthritis, arthralgia, myalgia, or weakness; when muscle is involved, it provides a useful site for biopsy | 24%-80% | 19, 27 |
| Skin | Purpura, nodules, livedo reticularis, ulcers, bullous or vesicular eruptions, and segmental skin edema | 44%-50% | 19, 39, 112, 113 |
| Cardiovascular | Cardiac ischemia, cardiomyopathy, hypertension | 35% | 27, 34, 39 |
| Ear, nose, and throat | No involvement; nasal crusting, sinusitis, and hearing loss suggest an alternative diagnosis such as granulomatosis with polyangiitis | None | |
| Respiratory | Lung involvement not seen in PAN; abnormal respiratory findings suggest an alternative diagnosis | None | |
| Abdominal | Pain is an early feature of mesenteric artery involvement; progressive involvement may cause bowel, liver, or splenic infarction, bowel perforation, or bleeding from a ruptured arterial aneurysm; less common presentations include appendicitis, pancreatitis, or cholecystitis as a result of ischemia or infarction; the presence of abdominal tenderness or peritonitis and blood loss on rectal examination should be assessed | 33%-36% | 19, 39 |
| Renal | Vasculitis involving the renal arteries is present in many cases but does not commonly give rise to clinical features; it can present with renal impairment, renal infarcts, or rupture of renal arterial aneurysms; glomerular ischemia may result in mild proteinuria or hematuria, but red cell casts are absent because glomerular inflammation is not a feature; if evidence of glomerular inflammation exists, then an alternative diagnosis such as microscopic polyangiitis or granulomatosis with polyangiitis must be considered; hypertension is a manifestation of renal ischemia causing activation of the renin-angiotensin system | 11%-66% | 19, 39 |
| Nervous system | Mononeuritis multiplex, with sensory symptoms preceding motor deficits; central nervous system involvement is a less frequent finding and can present with encephalopathy, seizures, and stroke | 55%-79% | 19, 39 |
| Ocular | Visual impairment, retinal hemorrhage, and optic ischemia | Rare | 27, 34 |
| Other | Breast or uterine involvement is rare; testicular pain from ischemic orchitis is a characteristic feature, albeit an uncommon presentation | Rare | 114, 115 |

PAN, Polyarteritis nodosa.

sensitivity of conventional angiography is as high as 89% and specificity is 90% when performed in patients suspected of having vasculitis.[42] MR or CT angiography are less invasive alternatives to conventional angiography but are much less sensitive in demonstrating microaneurysms.[43]

MR and CT angiography have the advantage of being able to demonstrate areas of renal infarction and other potential disease. In the setting of high suspicion of PAN and normal CT or MR angiography, it is still necessary to proceed to conventional angiography. Doppler ultrasound can identify renal and hepatic aneurysms related to PAN.[44] A plain chest radiograph may be useful for excluding other diseases such as other vasculitides that may affect the lungs and also to exclude infection.

## Polyarteritis Nodosa in Children

Pediatric cases of polyarteritis have been described, but compared with adult cases, there is less certainty about the case definition. In a careful scrutiny of 110 patients initially labeled as having childhood PAN, with a mean age of 9 years and the male-to-female ratio being equal, only 57% were subsequently classified as having systemic PAN; one-third had cutaneous PAN; 5% had classic PAN associated with hepatitis B surface antigen; and 8% had ANCA$^+$ MPA.[45]

A series of 15 cases of childhood PAN from India[46] described prolonged fever, severe myalgia, skin involvement, and elevated acute phase reactants in all patients. Hypertension (93%) and peripheral neuropathy (46%) were common. Nine patients had peripheral gangrene with ulceration, and six patients had livedo reticularis. No renal, pulmonary, or testicular involvement was present. Skin biopsy specimens showed necrotizing vasculitis of medium-sized vessels.

## Microscopic Polyangiitis Versus Polyarteritis Nodosa

The French Vasculitis Study Group[12] has defined PAN as ANCA$^-$ with a clinical pattern that significantly differs from MPA. A comparison of patients with PAN versus MPA showed a difference in the skin manifestations of the 162 patients with MPA and the 248 patients with PAN.[47] Patients with MPA had a greater frequency of purpura (26%) compared with a frequency of only 19% in patients with PAN. In contrast, urticaria was more common in patients with PAN (6% vs. 1.2%). The presence or absence of HBV infection–influenced skin manifestations were less common (with a rate of only 30%) in HBV$^+$ patients compared with HBV$^-$ patients (54%). Histologic examination of the skin, however, was identical in patients with

**TABLE 90-4** Investigations for Patients with Suspected Polyarteritis Nodosa

| Test | Supports Diagnosis of PAN | Supports an Alternative Diagnosis | Comment |
|---|---|---|---|
| Elevated C-reactive protein | + | | Supports the presence of systemic inflammation, but is nonspecific |
| Elevated ESR | + | | Supports the presence of systemic inflammation, but is nonspecific |
| Elevated serum creatinine | ± | ± | A raised serum creatinine typically without hematuria or proteinuria on urinalysis, may indicate renal ischemia or infarction; significant proteinuria or hematuria (especially red cell casts) would suggest glomerular disease, which is *not* a feature of PAN |
| Abnormal liver function tests | + | | May suggest hepatitis, either from HBV or as a result of ischemic hepatitis from PAN affecting the hepatic arteries |
| Positive HBV serology | + | | Seen in HBV PAN |
| Anemia | + | | Due to chronic inflammation or gastrointestinal blood loss |
| Positive ANCA | | + | A positive ANCA would suggest an alternative type of vasculitis, such as granulomatosis with polyangiitis or MPA |
| Elevated serum creatine kinase | ± | ± | Normal or mildly elevated, despite any muscle involvement |
| Blood cultures | | + | To exclude endocarditis or other infective mimic of vasculitis |
| Positive HCV serology and cryoglobulins | | + | HCV is associated with a skin-limited manifestation of PAN, but typically it is associated with a small-vessel vasculitis related to cryoglobulinemia |
| Positive rheumatoid factor and ACPA | | + | To rule out rheumatoid arthritis, especially in the context of a patient presenting predominantly with arthritis |
| Positive ANA and anti-dsDNA | | + | In patients with clinical features consistent with SLE or other connective tissue disease |
| HIV positive | | + | |

ACPA, Anti-citrullinated protein antibody; ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; dsDNA, double-stranded DNA; ESR, erythrocyte sedimentation rate; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MPA, microscopic polyangiitis; PAN, polyarteritis nodosa; SLE, systemic lupus erythematosus.

Modified from Mukhtyar C, Guillevin L, Cid MC, et al: EULAR recommendations for management of primary small and medium vessel vasculitis. *Ann Rheum Dis* 68:310–317, 2009.

either MPA or PAN (see Chapter 89 for more details on MPA).

## Cutaneous Polyarteritis Nodosa

Cutaneous PAN is a localized chronic arteritis affecting small arteries and/or arterioles in the panniculus (medium vessels) and in the dermo-subcutaneous junction. Arteritis may or may not be present in adjacent skeletal muscle and peripheral nerves, but the condition only rarely affects small arteries or arterioles in the dermis and does not affect venules at all. Because it can also involve vessels of the muscles and nerves under the involved skin,[48] use of the term "limb-restricted vasculitis" has been recommended, but this term would not account for a few patients who have manifestations of the disease on the trunk. Unlike systemic PAN, cutaneous PAN is usually a more chronic disease. Because of the involvement of small arteries and/or arterioles,[49] the characteristic clinical features are livedo (i.e., an irregular asymmetric pattern of so-called "broken livedo" or "livedo racemosa"; Figure 90-2) and small intradermal nodules with or without ulcers (Figure 90-3). If the disease involves vessels in the local muscle and epineurium, the patients may experience neuropathy and pain. Cutaneous PAN is perhaps a misnomer because it might suggest that



**Figure 90-3** Cutaneous ulceration and livedo in a patient with cutaneous polyarteritis nodosa. *(Courtesy Professor Sunderkoetter, Department of Dermatology, University Hospital of Muenster.)*

the disease can become more widespread; however, good-quality evidence indicates that cutaneous PAN does not progress into classic or systemic PAN.[49-55]

Pathologically, the findings of a skin biopsy are indistinguishable between systemic PAN and cutaneous PAN,[35] but the absence of venule involvement in cutaneous PAN is an important distinction. In a retrospective review of patients with cutaneous PAN from a Japanese group, 22 patients with histologically proven cutaneous vasculitis underwent follow-up: 32% had a peripheral neuropathy and 27% had myalgia, suggesting a need to revise the current criteria to differentiate between the two entities of cutaneous PAN and PAN. It was suggested that the two conditions were indeed separate, but cutaneous PAN is not confined to the skin.[48] HCV infection has been associated with cutaneous PAN in one retrospective study of 16 patients, in which 5 individuals had HCV infection.[56]

## Hepatitis B Virus Polyarteritis Nodosa

Hepatitis B vasculitis occurs in people with chronic hepatitis B antigenemia, most of whom have active liver disease.[24] The manifestations vary considerably, from diffuse small-vessel vasculitis predominantly in the skin to lesions in larger vessels, as is typical of PAN. Clinical symptoms may include the entire spectrum of vasculitic manifestations, from purpura and other rashes to abdominal pain, hypertension, renal disease, and stroke. HBV-associated PAN is an increasingly rare condition as a result of better immunization programs against hepatitis B. A cohort of 115 patients with PAN was reviewed on the basis of inclusion into studies between 1972 and 2002.[27] Treatment of hepatitis B–related PAN with steroids plus an anti-viral agent plus plasmapheresis resulted in 81% of patients achieving remission and a subsequent 10% relapse rate. However, 36% subsequently died. Seroconversion of hepatitis B antibody status was associated with complete remission and no relapse. In patients with a negative ANCA test the major cause of death was gut involvement. Plasmapheresis not only achieved control of disease in combination with steroids and anti-viral therapy but facilitated seroconversion to prevent secondary long-term complications from HBV infections such as liver involvement.

## Non–Hepatitis B Virus Polyarteritis Nodosa

The French Vasculitis Study Group has combined data on PAN and MIP, focusing more on the severity of disease manifestations rather than on the type of disease in their studies of treatment or outcome. For example, Ribi and colleagues[57] described the outcome of 124 patients with a new diagnosis of either PAN or MPA in whom the outcome was likely to be good (based on a lack of poor prognostic factors as measured by the Five-Factor Score). All patients were treated with corticosteroids, followed by either azathioprine or cyclophosphamide only if they experienced a relapse. Ninety-eight of these patients achieved remission with steroids alone, but 46 of these patients had a relapse. Primary treatment with steroids failed to control the disease in 26 patients, and 49 of the original 124 patients required additional immunosuppression. Similar rates of improvement were documented by using either azathioprine or cyclophosphamide. Therefore, despite the absence of poor prognostic factors, the disease of only approximately 50% of patients could be managed with corticosteroids alone. A study of plasma exchange in 62 patients with a mixture of EGPA and what was described as PAN suggested that no clinical benefit was achieved from adding plasma exchange to standard treatment with pulse intermittent high-dose cyclophosphamide and steroid. This study from the mid-1990s describes patients with "PAN," but most of these patients probably had MPA.[58] Table 90-5 lists treatment regimens for non-HBV PAN that are based on the European League against Rheumatism guidelines on management of small- and medium-vessel vasculitis.[59]

Management of non-HBV PAN should be appropriate to the severity of the disease as defined by the prognostic Five-Factor Score and includes aggressive immunosuppression with corticosteroids and cyclophosphamide in patients with Five-Factor Score values of at least 1.[62]

## Outcome

Uncontrolled vasculitis accounts for 58% to 73% of deaths occurring within the first year of diagnosis.[60-62] Earlier diagnosis and initiation of treatment has improved outcomes. In non-HBV PAN, the 7-year survival rate is 79%, compared with a 5-year survival rate of 72.5% in HBV-related PAN. These rates are similar to the rates in patients who have vasculitis associated with ANCAs.[63] The relapse rate in patients with HBV-related PAN is low (<10%) compared with that in people with non–HBV-related PAN (relapse rates of 19.4% to 57%).[27,62] Delayed diagnosis (>3 months) increases the risk of relapse but does not affect mortality risk.[64] Seroconversion rates from hepatitis B e-antigen

**TABLE 90-5** Pulse Cyclophosphamide and Methylprednisolone for Non–Hepatitis B Virus Polyarteritis Nodosa

| Phase | Drug | Dose | Route | Frequency | Duration |
|---|---|---|---|---|---|
| Induction | Cyclophosphamide | 15 mg/kg per pulse | IV | Every 2 weeks × 3, then every 3 weeks × 3-6 | 3-6 months |
| Induction | Prednisolone | 1 mg/kg up to 60 mg/day | Oral | For 4 weeks then reduce to 15 mg/day by 3 months, then further reduce to 10 mg/day by 6 months | 6 months |
| Maintenance | Azathioprine | 2 mg/kg/day | Oral | Daily | 18-24 months, then stop |
| Maintenance | Prednisone | 7.5 mg/day | Oral | Daily | 18-24 months, then reduce |

IV, Intravenous.

positive to hepatitis B e-antibody positive (i.e., eradication of active viral infection) is achieved in almost 50% of patients treated with glucocorticoids and anti-viral agents plus limited plasmapheresis.[27]

A prognostic tool designed for use in people with PAN is effectively used to distinguish patients with higher or lower risk of poor outcome. This simple clinical score (the Five-Factor Score), assessed at diagnosis, consists of assessment of the following abnormalities: proteinuria (<1 g/day), elevated creatinine (>1.58 mg/dL), gastrointestinal involvement, central nervous system involvement, and cardiomyopathy.[65] One point per item is allocated. Survival is reduced for patients with higher scores (86% survival for 0 points, 69% survival for 1 point, and 47% survival for 2 or more points) at onset when patients are followed up for 6 years.[65] Mortality can also be predicted using the Birmingham Vasculitis Activity Score, a clinical index of disease activity.[40,62] Older age at diagnosis is an important adverse predictor of survival in the first year and after 5 years of follow-up.[60,66]

A recent study of 118 patients[67] with PAN or MPA who have a good prognosis (Five-Factor Score = 0) has shown that after more than 8 years of follow-up, the mortality rates are better than for GPA (93% at 5 years and 86% at 8 years), based on treatment with glucocorticoids alone in 88% of patients; additional immunosuppression was required in 18% of patients, rising to 47% during follow-up.

## COGAN'S SYNDROME

Cogan's syndrome[68] is a rare form of vasculitis. The median age at onset is 25 years.[69,70] Patients typically present with red painful eyes and/or hearing loss concurrently or within 4 months in 75% of patients. Features of more widespread systemic vasculitis do not develop in most patients, with the exception of aortitis and aneurysm or aortic insufficiency, which occurs in about 12% of patients.[71] With atypical Cogan's syndrome, in which the ocular manifestation is episcleritis, scleritis, iritis, uveitis, or chorioretinitis rather than interstitial keratitis, there is a worse prognosis and a higher frequency of aortic and other systemic manifestations.[72] Eye symptoms usually consist of photophobia with red and irritable eyes. The audiovestibular symptoms are rapid in onset with partial or complete hearing loss (often bilateral), vertigo, and ataxia. Although the vertigo and ataxia may improve, the hearing loss is usually permanent. In about 50% of cases, constitutional features will be present, such as weight loss, fever, lymphadenopathy, hepatosplenomegaly, and purpura. Aortic involvement is the most serious manifestation of Cogan's syndrome and accounts for most deaths. Ophthalmic and audiovestibular features may precede aortic involvement by months to several years. Rarely, widespread vasculitis develops, with purpura and gangrene.[73]

### Pathology

A mixture of acute and chronic inflammation is present in large blood vessels, especially around the internal elastic lamina. The main findings are an infiltration of neutrophils, eosinophils, mononuclear cells, and fibrosis. Occasionally, granulomas containing giant cells can be found in the lesions.

### Clinical Features

The diagnosis of Cogan's syndrome is largely based on clinical features, supported by histologic abnormalities and exclusion of other conditions. Definitive serologic markers do not exist. Most patients have leukocytosis, anemia, thrombocytosis, and an elevated sedimentation rate during active phases of their disease. Patients with aortitis show aortic root dilation and aortic insufficiency on echocardiography or MR imaging. Rare case reports demonstrate the presence of autoantibodies such as anti-endothelial cell antibodies[74] and anti-myeloperoxidase (MPO) antibodies,[75] but their role in the disease is unknown. Recent reports suggest that antibodies to heat shock protein (HSP70) are very commonly found (in more than 90% of cases) in patients with typical Cogan's syndrome, but these antibodies are much less common (17%) in atypical forms.[76]

### Treatment

No clear evidence base exists because no prospective studies of treatment have been performed in people with this rare disease. Interstitial keratitis usually responds to topical corticosteroids. Most experts manage the acute audiovestibular symptoms with high doses of glucocorticoids, with a prompt response within a month (or none at all in resistant cases). Cytotoxic drugs,[77] methotrexate,[78] and cyclosporine[79] have been used to treat the condition. The course is variable: Some patients have only a single episode and are free of active disease thereafter. The more typical course is one of waxing and waning symptoms for months or years. Most patients sustain permanent hearing loss, and almost half lose their hearing entirely; by contrast, few experience permanent vision-related effects.[71]

Cochlear implantation can successfully restore some hearing in these patients. Aortic valve replacement and surgical repair of aortic aneurysms may be required, but as in Takayasu's arteritis,[80] it would probably be best to ensure that there is no active disease at the time of surgery.

## BUERGER'S DISEASE

Buerger's disease, also known as *thromboangiitis obliterans*, is an inflammatory vaso-occlusive disease that predominantly affects the vascular supply to the lower limbs in young adult male tobacco smokers, although women and older adults[81] can also be affected. The average age at onset is 35 years. Tobacco, especially cigarette smoking, is clearly important, but the mechanism of action remains unknown. There is no clear human leukocyte antigen association.[82] Antibodies directed against collagen, elastin, and laminin have been reported in some patients.[83,84] The presence of high levels of anti-endothelial cell antibodies has been described in patients with active disease, but not in patients in remission.[85] ANCAs directed against MPO, lactoferrin, and elastase have also been associated with severe cases.[86] More recently, a link to periodontal disease and anti-cardiolipin antibody has been reported.[87]

### Pathology

In most cases Buerger's disease affects small arteries and veins in the distal extremities, but some case reports describe visceral arterial involvement, including coronary, pulmonary, and mesenteric arteries. A transmural infiltration of polymorphonuclear leukocytes and lymphocytes occurs with preservation of the internal elastic lamina.[88] Thrombosis is prominent, and microabscesses can be found in the vessel wall and surrounding tissue. The infiltrating cells are enriched in $CD3^+$ T cells, and $CD68^+$ macrophages and dendritic cells have been reported to be increased during disease activity.[89]

### Clinical Features

Buerger's disease typically begins with bilateral pain and ischemia in both lower extremities, although the upper extremities may be the site of initial symptoms. At first the symptoms can be mild, with paresthesia or pain upon exposure to cold. Most cases evolve rapidly, however, with increasing ischemic (claudicant) limb pain, digital cyanosis, splinter hemorrhages, and skin vesicles. Digital ulcers often occur, especially after minor trauma.[90] The disease typically begins distally, with symptoms worse in the tips of the toes (and fingers), but it progresses to larger, more proximal vessels over several years. Usually Buerger's disease does not cause proximal leg claudication. Superficial phlebitis occurs in about one-third of patients and may be the first symptom. Typical angiographic changes include multiple bilateral areas of narrowing or occlusion in the digital, palmar, plantar, ulnar, radial, tibial, and peroneal arteries. Small collateral vessels around the occlusion can appear to be corkscrew shaped. More proximal lesions resemble atherosclerotic occlusion. Although these findings are typical, none is pathognomonic; in the absence of pathologic confirmation, the differential diagnosis includes premature atherosclerosis; hyperviscosity syndrome; scleroderma and other rheumatic diseases; Takayasu's arteritis; embolic disease including cholesterol emboli and atrial myxomas; ergot toxicity; and thoracic outlet syndrome.

### Treatment

The most important aspect of treatment is to stop smoking. In patients who are unable to comply, nicotine substitutes may be used at least in the short term.[91]

Affected limbs must be protected from trauma and cold. Ulcers and cellulitis often require antibiotics and local wound management. Anecdotal use of calcium channel blockers and pentoxifylline reportedly has been beneficial in some, but not all, patients. Sympathectomy seems to provide little or no long-term benefit and is not usually recommended.[92] Intravenous prostacyclin is effective[93] and superior to aspirin[94] and is also superior to lumbar sympathectomy for rest pain or ischemic ulcers[92] on the basis of two large randomized controlled trials. Combining smoking cessation with intravenous prostacyclin has been effective in an open-label study of 13 cases.[95] Other approaches using bone marrow–derived mesenchymal stem cells, basic fibroblast growth factor, and granulocyte colony-stimulating factor have been effective in treatment of leg ulcers and ischemia in small open-label studies.[96-99] A series of 26 patients with Buerger's disease responded well to autologous bone marrow mononuclear cell transplantation, with a subsequent increase in the 4-year amputation-free rate from 6% in 16 untreated control subjects to 95% in treated cases.[100] Hepatocyte growth factor has been effective in a series of 22 patients with peripheral vascular disease, of whom 8 were classified as having Buerger's disease.[101]

A radical surgical approach using omental grafting is reported in large open-label surgical studies to offer more than 90% success in improving the severe ischemic complications.[102]

In patients who continue to smoke, about half require amputation, often multiple times as more proximal vessels are involved.[103] Most patients who stop smoking will stop getting worse, but a few still require amputation. However, the ischemic limb may be a source of pain and ulceration for many years.

## SUSAC'S SYNDROME

Susac's syndrome is another rare condition affecting a wide age range from children to older adults.[104-106]

The cause of this syndrome is unknown. The presentation is usually characterized by sudden onset of sensorineural hearing loss in the context of encephalopathy and branch retinal artery occlusion.[104]

The differential diagnosis includes Cogan's syndrome and GPA. The pathogenetic mechanisms suggest an endotheliopathy rather than a true vasculitis. Nevertheless, treatment with immunosuppressive agents, including glucocorticoids and cytotoxic agents, is reported to lead to improvement in some patients, but no controlled trials have been performed.[105] The diagnosis rests on the combination of clinical features and exclusion of other causes. The outcome is variable, but most patients recover some hearing.[107] Anti-endothelial cell antibodies have recently been reported in association with the disease.[108]

## VIRUS-INDUCED VASCULITIS

The most common virus-associated vasculitis today is HCV-associated cryoglobulinemia (see Chapter 91). The majority of patients with cryoglobulinemia have concomitant hepatitis C infection (see Chapter 91). Treatment with immunosuppressive drugs has been only moderately successful, and many patients die as a result of vasculitis or liver disease. Saadoun and colleagues[29] describe a cohort of 72 patients treated with combination anti-viral therapy. Patients responded well to a combination of interferon-$\alpha$ and ribavirin; however, 40% required glucocorticoids, approximately 12% had plasmapheresis, and 6% received immunosuppressive drugs for severe complications. The mortality was just over 10%. The vasculitis resulting from human immunodeficiency virus infection is discussed in Chapter 113. Although the most common rheumatic manifestation of parvovirus B19 infection is arthritis (see Chapter 114), in some children with chronic parvovirus B19 infection, a vasculitis similar to PAN has been reported to develop. Given the high incidence of the infection in children, it is not clear whether the presence of the virus is coincidental or causative in the few cases of vasculitis described.[109]

## INFECTIONS AND VASCULITIS

Much speculation still exists about the relationship between a number of other organisms and the development of vasculitis. Has the organism been directly responsible for inducing vasculitis, or did it merely act as a trigger? Is the presence of the organism or evidence of its recent or previous presence sufficient evidence to indicate that it played a key role in the onset or propagation of the disease? Did the vasculitis or its treatment change the tissue architecture, suppress immune responses, and increase the likelihood of secondary infection? A variant of a common soil organism *Burkholderia pseudomallei* (which in itself causes melioidosis) has been found in arterial biopsies from a small series of patients with giant cell arteritis.[110] Furthermore, serologic evidence of the organism's lipopolysaccharide was detected at high levels in a larger series of 61 patients with GCA, but not in any of the 102 control subjects. Therefore, with few exceptions, as discussed earlier in this chapter, the link between vasculitis and infection remains tenuous.[111]



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 91

# Immune Complex–Mediated Small-Vessel Vasculitis



John H. Stone

**KEY POINTS**

- Vasculitis mediated by immune complexes (ICs) includes a heterogeneous group of disorders linked by inefficient or dysregulated IC clearance.
- Cutaneous involvement of small blood vessels is the most prominent feature in most cases, but extracutaneous involvement occurs in some forms.
- The classic cutaneous finding in small-vessel vasculitis is palpable purpura, but a variety of other skin lesions may be found, including pustules, vesicles, urticaria, and small ulcerations.
- Direct immunofluorescence studies of involved blood vessels demonstrate characteristic types and patterns of immunoglobulin (Ig) and complement deposition.
- The most common types of IC-mediated vasculitis are hypersensitivity vasculitis, Henoch-Schönlein purpura, and mixed cryoglobulinemia. Rarer types include hypocomplementemic urticarial vasculitis and erythema elevatum diutinum.
- Hypersensitivity vasculitis most often results from a reaction to a medication or an infection.
- IgA vasculitis (Henoch-Schönlein purpura) is associated with purpura, arthritis, glomerulonephritis, and colicky abdominal pain. IgA deposition is found within blood vessel walls.
- Cryoglobulinemic vasculitis is most often associated with long-standing hepatitis C virus infection. The term *mixed cryoglobulinemia* is sometimes used for this disorder because the immunoreactants involved in the disease include both IgG and IgM.
- Other immune-mediated disorders such as systemic lupus erythematosus, Sjögren's syndrome, and rheumatoid arthritis can be associated with IC-mediated vasculitis.

The inflammation within blood vessel walls that characterizes vasculitis frequently leads to cellular destruction, damage to the vascular structures, compromised blood flow to organs, and organ dysfunction. It has been known for decades that immune complex (IC)–mediated mechanisms play critical roles in many forms of systemic vasculitis, particularly those that primarily involve small blood vessels. The use of horse serum and sulfonamides as therapeutic agents for infectious diseases in the early 1900s frequently led to small-vessel vasculitis as a result of serum sickness or hypersensitivity phenomena. *Hypersensitivity angiitis*, which is often confused with the pauci-immune form of vasculitis now called *microscopic polyangiitis* (see Chapter 87)[1,2] was one of five disorders included in the original classification of the vasculitides by Zeek in 1952.[3]

This chapter focuses on forms of small-vessel vasculitis that are mediated by IC deposition. These disorders include hypersensitivity vasculitis, immunoglobulin (Ig) A vasculitis/Henoch-Schönlein purpura (HSP), mixed cryoglobulinemia, hypocomplementemic urticarial vasculitis (UV), and erythema elevatum diutinum (EED). In addition, forms of vasculitis associated with connective tissue diseases (CTDs), particularly systemic lupus erythematosus (SLE), Sjögren's syndrome, and rheumatoid vasculitis (RV), are discussed briefly. Antiglomerular basement membrane disease and the pauci-immune forms of vasculitis such as those associated with anti-neutrophil cytoplasmic antibodies (ANCAs) are discussed in Chapter 89. Throughout this chapter, the terms *vasculitis* and *angiitis* are used interchangeably when referring to inflammation involving small blood vessels (i.e., capillaries, venules, and arterioles).

Because all forms of IC-mediated vasculitis share certain elements of pathogenesis, have many cutaneous findings in common, and have overlapping differential diagnoses, these aspects of the disorders are considered together. The epidemiology, cause, distinctive pathophysiologic mechanisms, unique clinical features, and approaches to treatment are discussed separately for each condition. Treatments are also summarized in Table 91-1.

## PATHOGENESIS

### Arthus Reaction

The Arthus reaction, described on the basis of clinical and histologic findings after the injection of horse serum into rabbits, forms the basis of our understanding of IC-mediated diseases.[4] The formation of ICs in the Arthus reaction initiates complement activation and an influx of inflammatory cells, followed by thrombus formation and hemorrhagic infarction in the areas of most intense inflammation. ICs, formed by the combination of antibody and antigen, are continuously created by the reticuloendothelial system as a means of neutralizing foreign antigens, but ICs are usually cleared swiftly and efficiently. Under some circumstances, however, ICs escape clearance and are deposited within joints, blood vessels, and other tissues, inciting inflammation and causing disease. Deposition of ICs in the blood vessel walls leads to vasculitis. Similarly, deposition of ICs within small blood vessels of the kidney—the glomeruli—causes glomerulonephritis.[5]

**TABLE 91-1** Potential Treatment Approaches for Different Forms of Immune Complex–Mediated Vasculitis

| Disease | Preferred Treatment Approach |
|---|---|
| Hypersensitivity vasculitis | Remove the offending agent<br>Brief (2- to 4-wk) course of glucocorticoids for severe cases |
| Immunoglobulin A vasculitis/Henoch-Schönlein purpura | No treatment required in the majority of cases, particularly in children (symptomatic therapy only)<br>Moderate glucocorticoid doses (prednisone, 20-40 mg/day) are of variable utility but may be used empirically in patients with disabling symptoms<br>Pulse glucocorticoids (e.g., 1 g methylprednisolone/day) have been used with anecdotal success for refractory purpura when moderate glucocorticoid doses have failed<br>Refractory glomerulonephritis may require high-dose glucocorticoids; mycophenolate mofetil is preferred as a steroid-sparing agent or when additional immunosuppression is required |
| Cryoglobulinemia | Combined anti-viral therapies and B cell depletion strategies are synergistic in treating mixed cryoglobulinemia associated with hepatitis C<br>Rituximab is possibly effective in treating idiopathic mixed cryoglobulinemia |
| Hypocomplementemic urticarial vasculitis | Low-dose prednisone (5-20 mg/day), hydroxychloroquine, dapsone<br>Higher doses of glucocorticoids in patients with serious internal organ involvement or ulcerative skin lesions<br>Anecdotal reports of success with tumor necrosis factor inhibition |
| Erythema elevatum diutinum | Dapsone or sulfapyridine |
| Connective tissue disease | Hydroxychloroquine, low-dose prednisone (5-20 mg/day), azathioprine |
| Rheumatoid vasculitis | High-dose glucocorticoids plus rituximab is an appropriate first choice for widespread necrotizing vasculitis, adding rituximab within several weeks if evidence of treatment failure is seen<br>Tumor necrosis factor inhibition may also be useful in milder cases |

## Immunogenicity

The fate of ICs formed by the reticuloendothelial system is governed by several major factors, including antigen load, antibody response, efficiency of the reticuloendothelial system, the flow dynamics of blood vessels, previous endothelial damage, and the solubility of the ICs themselves. The ratio of antibody to antigen determines the solubility of ICs. Large ICs, which are formed when antibody and antigen are present in approximately equal proportions, are identified and removed easily by the reticuloendothelial system. Smaller ICs are formed in conditions of antibody excess. These complexes remain in the serum and do not elicit an immune response within tissues. In the setting of slight antigen excess, however, ICs precipitate from the serum and become trapped within certain vascular beds. After the deposition of ICs in tissue, a cascade of pathologic events ensues: complement fixation, neutrophil recruitment, local inflammation, lysosomal release, oxygen free radical generation, and tissue injury.

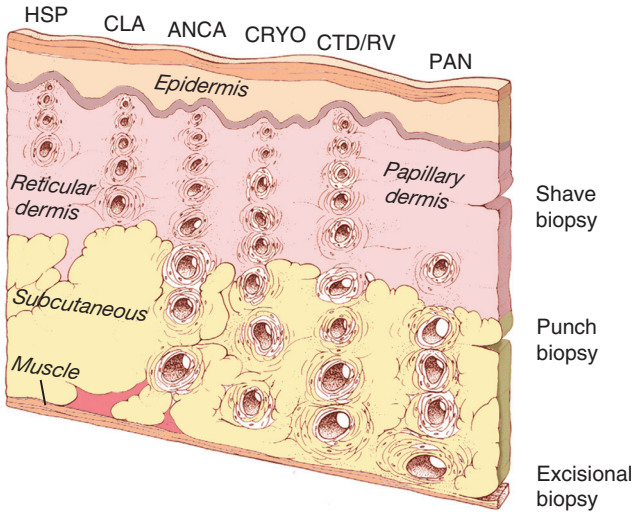



**Figure 91-1** Size of the blood vessels involved in forms of cutaneous vasculitis. The types of vasculitis with an immune complex–mediated pathogenesis include Henoch-Schönlein purpura (HSP), cutaneous leukocytoclastic angiitis (CLA), mixed cryoglobulinemia (CRYO), and connective tissue disease/rheumatoid vasculitis (CTD/RV). ANCA, Antineutrophil cytoplasmic antibody; PAN, polyarteritis nodosa.

## CUTANEOUS MANIFESTATIONS

Small blood vessels generally include capillaries, postcapillary venules, and nonmuscular arterioles—that is, vessels typically less than 50 μm in diameter. These vessels are found principally within the superficial papillary dermis (Figure 91-1). Medium-sized blood vessels—those between 50 and 150 μm in diameter—contain muscular walls and are located principally in the deep reticular dermis, near the junction of the dermis and subcutaneous tissues. Vessels larger than 150 μm in diameter are not commonly found in the skin.

Figure 91-1, which demonstrates the location and size of blood vessels involved in various types of cutaneous vasculitis, illustrates the types of blood vessels affected by several forms of IC-mediated disease. The size of a blood vessel correlates closely with its depth in the skin layers—the larger the vessel, the deeper its location. Although telltale signs of vasculitis may be evident upon inspection of the skin's surface, the epidermis is avascular. Therefore, the pathologic findings in cutaneous vasculitides lie within the dermis and subcutaneous tissues.

Palpable purpura, which is essentially pathognomonic for small-vessel vasculitis, is the most common cutaneous finding in IC-mediated vasculitis (Figure 91-2). Purpuric lesions result from the extravasation of erythrocytes through damaged blood vessel walls into tissue. Many other skin

manifestations are possible in these conditions, including vesicles, pustules, urticaria, superficial ulcerations, nonpalpable lesions (macules and patches), and splinter hemorrhages (Figure 91-3). These lesions frequently occur in combination, and careful examination usually reveals a purpuric component. Purpuric lesions *do not blanch* when pressure is applied to the skin, and they are slightly raised. After resolution, purpuric lesions may leave postinflammatory hyperpigmentation, particularly if repeated bouts occur (see Figure 91-3F).



**Figure 91-2** Palpable purpura in a patient with hypersensitivity vasculitis.

In IC-mediated vasculitis, purpuric lesions are usually distributed in a symmetric fashion over dependent regions of the body, particularly the lower legs, because of the increased hydrostatic pressure in these areas. Purpuric lesions are not always palpable, and the existence of palpable purpura does not necessarily imply an IC-mediated pathophysiology; pauci-immune forms of vasculitis such as granulomatosis with polyangiitis (GPA), microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), for example, may present with similar findings in the skin (albeit with distinctive histopathology; see Chapter 89), including either palpable or nonpalpable purpura. In contrast to pauci-immune forms of vasculitis, however, which usually lead to small, circumscribed areas of cutaneous vasculitis, cutaneous vasculitis associated with ICs often leads to diffuse, widespread lesions, usually occurring most densely on the distal lower extremities but extending up the thighs to the buttocks, abdomen, trunk, upper extremities, and occasionally even the face.

## HISTOPATHOLOGY

Full pathologic assessment of cutaneous vasculitis involves examination of a skin biopsy specimen by both light

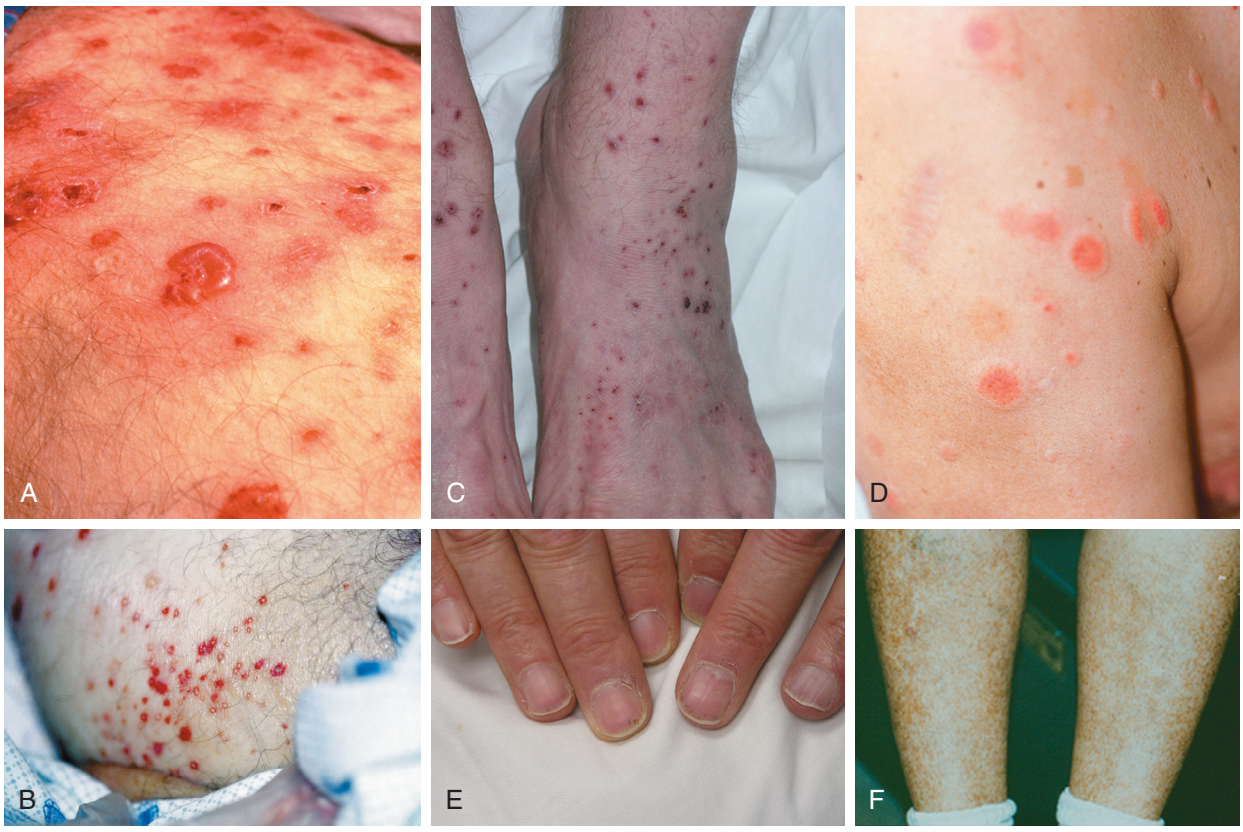



**Figure 91-3** Cutaneous findings of immune complex–mediated small-vessel vasculitis. **A,** Vesicles. **B,** Pustules. **C,** Superficial ulcerations. **D,** Urticaria. **E,** Splinter hemorrhages. **F,** Hyperpigmentation.

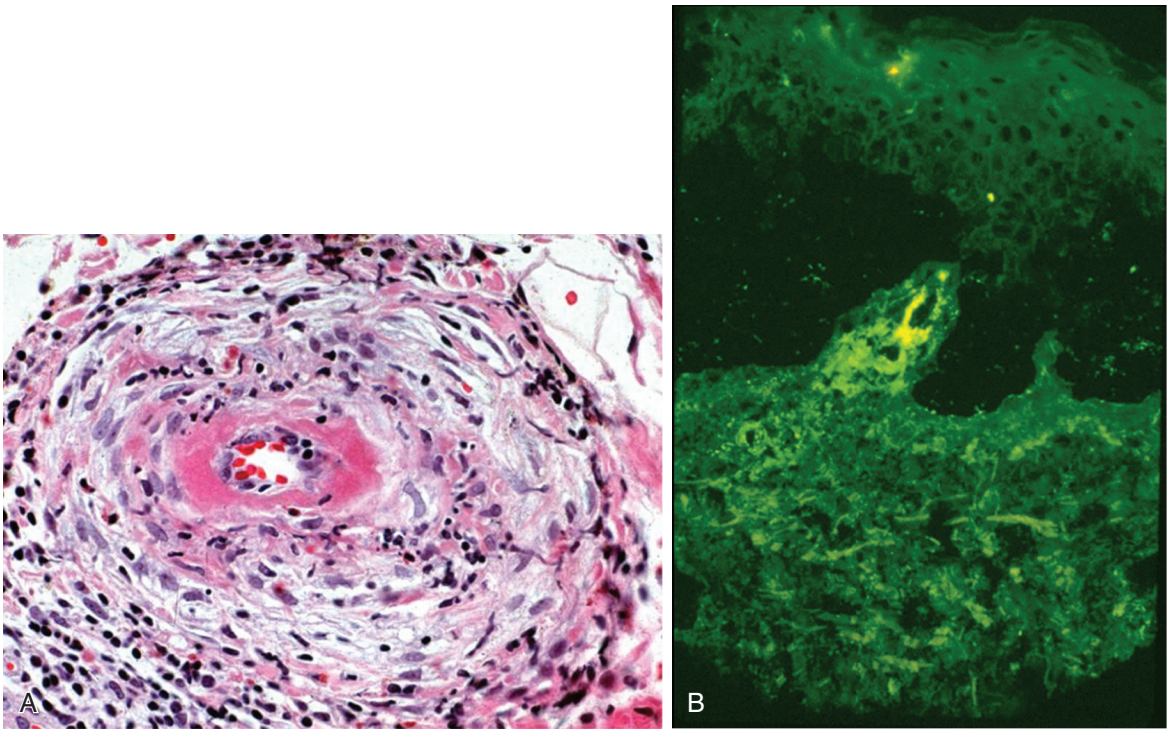



**Figure 91-4** Skin biopsy findings in immune complex–mediated small-vessel vasculitis. **A,** Light microscopy. **B,** Direct immunofluorescence demonstrating immunoglobulin A deposits. See text for description of the key features.

microscopy and direct immunofluorescence (DIF). DIF is a particularly critical procedure in the evaluation of small-vessel vasculitides. DIF studies must be planned at the time the biopsy is performed because they require a fresh skin biopsy sample.

## Light Microscopy

Figure 91-4A displays the light microscopy findings of cutaneous vasculitis. The optimal time for skin biopsy is 24 to 48 hours after the appearance of a lesion. Biopsies should be obtained from a non-ulcerated site. For ulcerated lesions—which are usually more of an issue with medium-vessel vasculitides—biopsy specimens should be taken from the edge of the ulcer. The cellular infiltrates in cutaneous vasculitis are usually composed of a combination of neutrophils and lymphocytes, but most cases demonstrate a predominance of one cell type or the other. Lymphocyte-rich infiltrates may be seen in specimens taken from either new lesions (those present <12 hours) or old lesions (those present >48 hours), regardless of the underlying type of vasculitis. Even in CTDs such as Sjögren's syndrome, the typical finding is a leukocytoclastic vasculitis rather than a lymphocytic vasculitis.[6]

The essential histologic feature in any form of cutaneous vasculitis is the disruption of blood vessel architecture by an inflammatory infiltrate within and around the vessel walls. Endothelial swelling and proliferation, leukocytoclasis (i.e., degranulation of neutrophils, leading to the production of nuclear "dust" due to apoptosis; see Figure 91-4), and extravasation of erythrocytes may be evident in the biopsy but are not essential to the diagnosis.

## Direct Immunofluorescence

Although the diagnosis of cutaneous vasculitis rests on routine histologic findings, the features revealed by hematoxylin and eosin stains do not distinguish between pauci-immune and IC-mediated disorders. DIF studies complement the histologic information, provide the only way of diagnosing HSP with certainty, and yield important clues regarding the nature of the underlying disease. The performance of *separate* biopsies for histologic and DIF analyses is recommended if sufficient numbers of lesions exist (as is usually the case with IC-mediated vasculitis).

To perform DIF studies, frozen sections are incubated with fluorescein-labeled anti-human IgG, IgM, IgA, and C3. The staining patterns of these immunoreactants may provide insight into not only the diagnosis but also the pathophysiology of certain conditions. Figure 91-4B displays the typical DIF findings in a skin lesion from a patient with IC-mediated vasculitis.

# DIFFERENTIAL DIAGNOSIS

The differential diagnosis of IC-mediated small-vessel vasculitis is shown in Table 91-2. The following three main groups of disorders are present in the differential diagnosis of IC-mediated small-vessel vasculitis:

- Other forms of IC-mediated disorders
- Forms of small-vessel vasculitis that are not mediated through ICs
- Vasculitis mimickers that involve small blood vessels

A diagnostic algorithm that includes the critical laboratory and radiographic tests is shown in Figure 91-5.

**TABLE 91-2** Differential Diagnosis of Immune Complex–Mediated Vasculitis

| |
|---|
| **Immune Complex–Mediated Vasculitides** |
| Hypersensitivity vasculitis |
| Henoch-Schönlein purpura |
| Mixed cryoglobulinemia |
| Urticarial vasculitis |
| Erythema elevatum diutinum |
| Connective tissue disease, rheumatoid vasculitis |
| **Pauci-immune Vasculitides** |
| Granulomatosis with polyangiitis |
| Eosinophilic granulomatosis with polyangiitis |
| Microscopic polyangiitis |
| **Miscellaneous Small-Vessel Vasculitides** |
| Behçet's disease |
| Malignancy associated |
| Infection |
| Inflammatory bowel disease |
| **Vasculitis Mimickers** |
| Hemorrhage |
| • Pigmented purpuric dermatoses |
| • Scurvy |
| • Immune thrombocytopenic purpura |
| Thrombosis |
| • Anti-phospholipid syndrome |
| • Thrombotic thrombocytopenic purpura |
| • Livedoid vasculopathy (atrophie blanche) |
| • Warfarin-induced skin necrosis |
| • Purpura fulminans |
| • Disseminated intravascular coagulation |
| Embolism |
| • Cholesterol emboli |
| • Atrial myxoma |
| Vascular wall pathology |
| • Calciphylaxis |
| • Amyloidosis |
| Infection |
| • Infective endocarditis |
| • Leprosy (Lucio's phenomenon) |

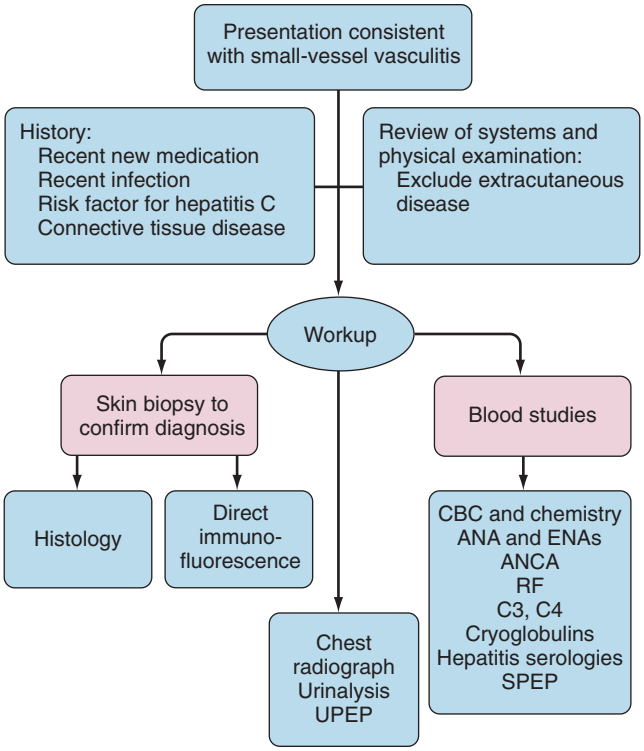



**Figure 91-5** Diagnostic algorithm for immune complex–mediated small-vessel vasculitis. The critical diagnostic test is usually a skin biopsy with hematoxylin and eosin staining and direct immunofluorescence. ANA, Anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; CBC, complete blood cell count; ENA, extractable nuclear antigen; RF, rheumatoid factor; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis.

## CLINICAL SYNDROMES

### Hypersensitivity Vasculitis

The term *hypersensitivity vasculitis* (see Chapter 87) generally refers to an IC-mediated small-vessel vasculitis of the skin that spares internal organs and usually follows drug exposures or infections. Consensus conference meetings on the nomenclature of the vasculitides have grappled with the most appropriate way to address the terminology for hypersensitivity vasculitis, a term first used by Zeek in 1952. The first Chapel Hill Consensus Conference in the early 1990s recommended eliminating the term *hypersensitivity vasculitis* in favor of *cutaneous leukocytoclastic angiitis* because of the disorder's usual confinement to the skin and its predominant cell type, the neutrophil.[1]

Twenty years later, the second Chapel Hill Consensus Conference retained the term "cutaneous leukocytoclastic angiitis" under a newly created category of *Single-Organ Vasculitis*, consistent with the idea that hypersensitivity has a predilection for the skin. That same conference also added a new nomenclature term—"drug-induced immune complex vasculitis"—under a new category, *vasculitis associated with probable etiology*. The concept of hypersensitivity vasculitis, a condition characterized pathologically by IC deposition in capillaries, postcapillary venules, and arterioles, remains firmly embedded in the medical literature and clearly encompasses both cutaneous leukocytoclastic angiitis and drug-induced immune complex vasculitis. Because pathophysiology and treatment of these hypersensitivity vasculitis subsets are similar, it makes sense to consider them together. An illness that is similar to hypersensitivity vasculitis—serum sickness—is a systemic illness that includes not only cutaneous involvement but also inflammation of joints, kidneys, and other organs; a rash occurs 1 to 2 weeks after exposure to a drug or foreign antigen.

In 1990 the American College of Rheumatology (ACR) performed a study designed to identify features that distinguish one form of vasculitis from others.[7] The resulting ACR classification criteria for hypersensitivity vasculitis are shown in Table 91-3.[8] The key historical element in evaluating a patient with possible hypersensitivity vasculitis is identifying exposures that may have triggered the reaction. However, in approximately half of all patients with presumed hypersensitivity vasculitis, no inciting agent can be identified. (According to the second Chapel Hill Consensus Conference nomenclature, most patients with such a presentation would be diagnosed most precisely as having cutaneous leukocytoclastic angiitis).

A long list of medications, infections, and other exposures can lead to the syndrome of hypersensitivity vasculitis. The typical history for a drug-induced hypersensitivity vasculitis is the occurrence of clinical symptoms approximately 7 to 14 days after starting a new medication. Although

**TABLE 91-3** American College of Rheumatology 1990 Criteria* for the Classification of Hypersensitivity Vasculitis

| |
|---|
| Age >16 years |
| Use of a possible offending medication in temporal relation to symptoms |
| Palpable purpura |
| Maculopapular rash |
| Biopsy of a skin lesion showing neutrophils around an arteriole or venule |

*The presence of three or more criteria has a sensitivity of 71% and a specificity of 84% for the diagnosis of hypersensitivity vasculitis.

From Calabrese LH, Michel BA, Bloch DA, et al: American College of Rheumatology 1990 criteria for the classification of hypersensitivity vasculitis. *Arthritis Rheum* 33:1108–1113, 1990.

**TABLE 91-4** American College of Rheumatology 1990 Criteria* for the Classification of Henoch-Schönlein Purpura

| |
|---|
| Palpable purpura |
| Age at onset <20 years |
| Bowel angina |
| Vessel wall granulocytes on biopsy |

*The presence of two criteria identified Henoch-Schönlein purpura with a sensitivity of 87% and a specificity of 88% in a group of people with forms of systemic vasculitis.

From Mills JA, Michel BA, Bloch DA, et al: The American College of Rheumatology 1990 criteria for the classification of Henoch-Schönlein purpura. *Arthritis Rheum* 33:1114–1121, 1990.

virtually any medication can be associated with the induction of a hypersensitivity vasculitis, antibiotics (particularly penicillins and cephalosporins) are the most common offenders. Other common culprits are diuretics and antihypertensive agents, but when confronted with a patient who has a potential drug-induced vasculitis, it is critical to examine and potentially discontinue any medication added within a recent time frame. Drug-induced hypersensitivity vasculitides begin to resolve within days of removal of the offending agent.

Thorough efforts are also required to exclude disease in organs other than the skin, because this finding would implicate another form of vasculitis (see Figure 91-5). For example, although hypersensitivity vasculitis can mimic the skin features of microscopic polyangiitis, it does not involve the kidneys, lungs, peripheral nerves, or other internal organs and is not associated with ANCA.

Removal of the inciting agent is the most critical therapy for hypersensitivity vasculitis when the likely agent can be identified. In patients who have been exposed to multiple medications, determining the inciting agent may be difficult and may require the withdrawal of multiple agents simultaneously until the syndrome clears, typically in 1 to 2 weeks.

The prognosis for patients with hypersensitivity vasculitis depends on the inciting cause. Treatment with glucocorticoids is reserved for patients with extensive disease and can usually be discontinued within several weeks. Patients who experience repeated disease flares may need low-dose glucocorticoids to prevent recurrences.

## IgA Vasculitis (Henoch-Schönlein Purpura)

HSP is an IC-mediated form of small-vessel vasculitis that is strongly associated with IgA deposition within blood vessel walls. Many cases of HSP occur after upper respiratory tract infections. Multiple bacterial, viral, and other infectious agents have been suggested as the cause of HSP, but the true cause remains unknown. The 1990 ACR criteria for the classification of HSP are shown in Table 91-4.[9]

A major risk factor for HSP (as well as for IgA nephropathy, which has renal findings identical to those of HSP) is aberrant glycosylation of *O*-linked glycans in the hinge region of a fraction of IgA1 molecules.[10] Rather than terminating with galactose, the aberrant galactose-deficient *O*-glycans end with *N*-acetylgalactosamine (GalNAc) or sialylated GalNAc. The terminal GalNAc moiety on the aberrantly glycosylated IgA1 may in turn be recognized by antiglycan antibodies, leading to the formation of circulating ICs that are deposited in the skin, joints, kidneys, and other organs. However, a high serum galactose-deficient–IgA1 level is not sufficient for the development of clinical symptoms. A "second hit" in the form of another environmental or inherited risk factor is required to produce HSP, which probably explains why multiple types of infections and many different drugs (e.g., antibiotics) have been linked etiologically with HSP.

The hallmarks of HSP include an upper respiratory tract infection, followed by a syndrome characterized by a purpuric rash, arthralgias, abdominal pain, and renal disease. HSP is usually viewed as a disease of childhood, and the majority of cases affect children younger than 5 years. However, adults can also be affected by HSP and have a greater tendency toward a prolonged disease course (with recurrent bouts of purpura) than do children.[11] Colicky abdominal pain, presumably secondary to gastrointestinal vasculitis, is a common characteristic of HSP and frequently occurs within a week after the onset of rash. Sometimes the gastrointestinal symptoms of HSP precede the onset of purpura, leading to a diagnostic quandary and occasionally to exploratory surgery. Endoscopy can demonstrate purpura in the upper or lower intestinal tract. Mild glomerulonephritis is common and generally self-limited, although end-stage renal disease develops in some patients.

In children with mild manifestations, the clinical history alone may be sufficient to confirm the diagnosis. In more serious cases (e.g., in the presence of renal involvement) or when there is sufficient doubt about the diagnosis, biopsy of an involved organ is essential. Unlike in other forms of IC-mediated disease, however, DIF reveals florid IgA deposition. In the proper clinical setting, this finding is diagnostic of HSP. Other forms of small-vessel vasculitis may have small quantities of IgA within blood vessels, but IgA is not the predominant immunoreactant in such cases.

In mild cases of HSP, no specific therapy is necessary. Even for patients with glomerulonephritis, it is difficult to show that treatment with glucocorticoids or immunosuppressive agents significantly alters the outcome. Nonetheless, it is often prudent to treat aggressive renal involvement with an immunosuppressive regimen including high-dose glucocorticoids. Mycophenolate mofetil is an option in patients with severe renal disease for both additional immunosuppression and steroid-sparing effects.

Recurrent skin disease, often consisting of multiple episodes occurring over many months, is common. However,

even in patients with recurrent disease, the rule is for the disorder to subside and to resolve completely over a few months to a year. In a minority of patients, some evidence of permanent renal damage persists in the form of proteinuria and hematuria. Renal failure develops as a result of HSP in fewer than 5% of patients.

## Cryoglobulinemic Vasculitis

Cryoglobulins are immunoglobulins characterized by a tendency to precipitate from serum under conditions of cold.[12] These proteins, detectable to a varying degree in a wide array of inflammatory conditions, do not always cause disease. In some patients, however, cryoglobulins bind to circulating antigen (e.g., portions of the hepatitis C virion), are deposited in the walls of small- and medium-sized blood vessels, and activate complement, leading to cryoglobulinemic vasculitis.

In contrast to most other forms of IC-mediated vasculitis, cryoglobulinemia tends to involve small- and medium-sized blood vessels. Thus the syndrome of cryoglobulinemic vasculitis can be associated with the development of large cutaneous ulcers, digital ischemia, and livedo racemosa—findings characteristic of disturbances in medium-sized vessels. The first Chapel Hill Consensus Conference provided a consensus definition for mixed cryoglobulinemia (Table 91-5).[1]

Three major types of cryoglobulinemia are recognized and are defined by the specific types of immunoglobulins with which they are associated (Table 91-6). Type I, characterized by a monoclonal gammopathy (generally IgG or IgM), differs substantially from types II and III in its clinical presentation and disease associations. Type I cryoglobulinemia, associated with Waldenström's macroglobulinemia or, less frequently, multiple myeloma, is more likely to cause syndromes related to hyperviscosity (dizziness, confusion, headache, and stroke) than necrotizing vasculitis. In contrast to the monoclonal nature of type I cryoglobulinemia, types II and III are known as *mixed cryoglobulinemias* because they are composed of both IgG and IgM. In type II cryoglobulinemia, more than 90% of cases are caused by hepatitis C infection, and the cryoproteins consist of monoclonal IgM and polyclonal IgG. Cases of type II cryoglobulinemia not associated with hepatitis C are sometimes termed *essential mixed cryoglobulinemia* because their cause is not known. Type III cryoglobulinemia, typically associated with polyclonal IgG and polyclonal IgM, is associated with many forms of chronic inflammation, including infection and autoimmune disease.

Type II and III cryoglobulinemias often present with a triad of signs and symptoms: purpura, arthralgias, and myalgias. The purpura may be extensive and confluent (Figure 91-6), sometimes involving the trunk, upper extremities, and even the face; in most cases, however, the rash is confined to the lower extremities. Other organ systems commonly involved in mixed cryoglobulinemia are the kidneys and peripheral nerves. Mixed cryoglobulinemia may cause membranoproliferative glomerulonephritis that resembles lupus nephritis histopathologically. It may also cause a vasculitic neuropathy, usually with sensory symptoms

**TABLE 91-5** Chapel Hill Consensus Conference Definitions of Immune Complex–Mediated Forms of Vasculitis

| Disease | Definition |
|---|---|
| Cutaneous leukocytoclastic angiitis | Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis |
| Immunoglobulin A vasculitis (Henoch-Schönlein purpura) | Vasculitis with immunoglobulin A–dominant immune deposits, affecting small blood vessels (capillaries, venules, and arterioles); typically involves skin, gut, and glomeruli and is associated with arthralgias or arthritis |
| Essential cryoglobulinemia | Vasculitis with cryoglobulin immune deposits, affecting small blood vessels (capillaries, venules, and arterioles) and associated with cryoglobulins in serum; skin and glomeruli are often involved |

From Jennette JC, Falk RJ, Andrassy K, et al: Nomenclature of systemic vasculitides: proposal of an international consensus conference. *Arthritis Rheum* 37:187–192, 1994.



**Figure 91-6** Confluent purpura in mixed cryoglobulinemia. Extensive purpuric lesions are often so numerous that they form confluent areas of cutaneous involvement.

**TABLE 91-6** Types of Cryoglobulins

| Cryoglobulin | RF Positivity | Monoclonality | Associated Diseases |
|---|---|---|---|
| Type I | No | Yes (IgG or IgM) | Hematopoietic malignancy (multiple myeloma, Waldenström's macroglobulinemia) |
| Type II | Yes | Yes (polyclonal IgG, monoclonal IgM) | Hepatitis C; other infection; Sjögren's syndrome; SLE |
| Type III | Yes | No (polyclonal IgG and IgM) | Hepatitis C; other infection; Sjögren's syndrome; SLE |

Ig, Immunoglobulin; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

predominating over motor symptoms. Finally, in rare cases, cryoglobulinemia is associated with alveolar hemorrhage.

Skin biopsy is the most straightforward method of confirming the diagnosis. Light microscopy of purpuric lesions demonstrates leukocytoclastic vasculitis. In addition, DIF studies reveal various types of immunoglobulin and complement deposition, depending on the type. In type II cryoglobulinemia, for example, DIF reveals IgG and IgM deposition, as well as complement components. Serologic testing may also yield clues to the presence of mixed cryoglobulinemia. To assay serum cryoglobulins, the blood is collected in a prewarmed apparatus, allowed to clot at 37° C before processing, and then refrigerated at 4° C for several days. The percentage of the serum occupied by the cryoprecipitate is referred to as the "cryocrit." The difficulties involved in performing cryoglobulin assays often lead to false-negative results. Nonspecific serologic testing may also implicate mixed cryoglobulinemia. As noted, detection of cryoglobulins is not always associated with disease.

A strong clue is the presence of an extremely low level of C4, reduced out of proportion to C3. In addition, the monoclonal component of type II cryoglobulins almost invariably has rheumatoid factor activity (i.e., it binds to the Fc portion of IgG). Thus essentially all patients with type II cryoglobulinemia have high titers of rheumatoid factor. C4 levels, rheumatoid factor titers, and cryocrits all fare poorly as markers of clinical disease activity, often remaining abnormal in the face of clinically improved disease.

In recent years, substantial progress has been made in the treatment of cryoglobulinemia. Until recently, antiviral therapies with the combination of interferon-α and ribavirin were believed to be the optimal treatment for mixed cryoglobulinemia associated with hepatitis C infection. The impact of more recent curative therapies for hepatitis C, such as sofosbuvir, on management of hepatitis-associated cryoglobulinemia still needs to be determined. Dual strategies that combine anti-viral therapies and B cell depletion approaches with rituximab might be synergistic and lead to long-term treatment responses.[13] The rationale behind rituximab use in persons with cryoglobulinemia is that peripheral B lymphocyte depletion will lead to a reduction in plasma cells that produce cryoglobulins. Studies of these treatment modalities have suggested that disease relapses are associated with the absence of virologic control and peripheral B cell recovery, implying the need to combine the two treatment strategies. The optimal timing of anti-viral and B cell depletion strategies is not clear. However, one reasonable approach is to initiate anti-viral strategies first and then to use rituximab within several weeks.

Patients who present with overwhelming illness, such as the unusual patient with alveolar hemorrhage or symptoms of hyperviscosity, are candidates for plasma exchange, with the goal of removing the pathogenic ICs as quickly as possible. One approach is to perform plasma exchange every other day with concomitant glucocorticoid therapy for a total of seven exchanges or until sufficient clinical improvement occurs, followed by B cell depletion after plasma exchange is complete (so the B cell depletion agent is not removed by plasma exchange).

The prognosis of patients with cryoglobulinemia generally depends on the underlying cause. The outcome of type I cryoglobulinemia relates closely to the success in treating the cause. Type II or III cryoglobulinemia secondary to hepatitis C can be treated effectively if the viral infection is responsive to therapy. If patients do not tolerate anti-viral therapy well or if the treatment is ineffective, low to moderate doses of prednisone may be required to control the disease.

Patients with mixed cryoglobulinemia whose disease is not associated with either hepatitis C or a lymphoproliferative malignancy often respond well to rituximab plus glucocorticoids, with glucocorticoids tapered over several weeks. Such patients often require regular treatment with rituximab (e.g., every 6 months) to prevent return of the disease.

## Hypocomplementemic Urticarial Vasculitis

Three different syndromes of UV are recognized: normocomplementemic UV, hypocomplementemic UV (HUV), and the HUV syndrome (HUVS). Normocomplementemic UV is typically a self-limited subset of hypersensitivity vasculitis. In chronic cases, normocomplementemic UV must be distinguished carefully from neutrophilic urticaria, a persistent form of urticaria not associated with vasculitis. In contrast, HUV is more likely to be a chronic disorder that has certain overlapping features with SLE: low serum complements, autoantibodies, and an interface dermatitis characterized by immunoreactant deposition (complement and immunoglobulins) at the dermal-epidermal junction in a pattern essentially identical to the lupus band test. Finally, HUVS is a severe form of the disease associated with extracutaneous disease and an array of organ system findings atypical of SLE.[14] For example, HUVS may be associated with uveitis, chronic obstructive pulmonary disease (COPD), and angioedema.

Unlike common urticaria, the UV lesions are frequently associated with moderate pain, burning, and tenderness in addition to pruritus. Whereas common urticaria typically resolves completely within 24 to 48 hours, UV lesions may take days to resolve completely, often leaving residual hyperpigmentation; they may worsen without therapy. Patients with HUV or HUVS may present with purpuric lesions that are more dominant than urticarial lesions.

The skin lesions in UV tend to be centripetal, favoring the trunk and proximal extremities more than dependent regions. The lesions are painful and associated with a burning sensation rather than the pruritus of common urticaria. Biopsy of an urticarial wheal in UV demonstrates evidence of leukocytoclastic vasculitis, including injury to the endothelial cells of the postcapillary venules, erythrocyte extravasation, leukocytoclasis, fibrin deposition, and a perivascular neutrophilic (or, less commonly, lymphocytic) infiltrate. DIF demonstrates IC deposition around blood vessels in the superficial dermis and a striking deposition of immunoglobulins and complement along the dermal-epidermal junction. In the proper setting, these findings (interface dermatitis and immunoreactant deposition within blood vessels) are diagnostic of HUV. HUVS, in contrast, is a clinical diagnosis based on the presence of UV and the occurrence of typical features in extracutaneous organ systems.

Some cases of HUV respond to therapies commonly used for the treatment of SLE, including low-dose prednisone, hydroxychloroquine, dapsone, or other immunomodulatory agents. Serious cases of HUVS, particularly those presenting with glomerulonephritis or other forms of serious organ involvement, may require high doses of glucocorticoids or biologic agents such as inhibitors of tumor necrosis factor. Both COPD and cardiac valve abnormalities are associated with HUVS and may require specific treatment as well.

The prognosis of UV is often linked to the disorder with which it is associated. SLE, COPD, angioedema, and valvular abnormalities are all known to occur in association with this disorder and may strongly influence both quality and quantity of life. Some cases of HUV and HUVS resolve completely after courses of immunosuppression.

## Erythema Elevatum Diutinum

EED is a rare, distinctive form of leukocytoclastic vasculitis limited to the skin.[15] The disorder is distinctive because of the unusual distribution of skin lesions (found symmetrically over the extensor surfaces of joints) and the prompt response to sulfone medications. The cutaneous findings are typical of any small-vessel vasculitis, with a predominance of papules, plaques, and nodules. Early lesions are often pink or yellowish and then become red or purple (Figure 91-7). The natural history of untreated lesions is for them to persist for years, becoming doughy or hard with time. The lesions have a predilection for the skin overlying the small joints of the hands and the knees, and they can also affect the buttocks. The trunk is generally spared.

The principal histopathologic findings of EED are leukocytoclastic vasculitis with fibrinoid necrosis. Although an IC basis is suspected in this disorder, DIF studies are not distinctive. EED has been associated with various CTDs, rheumatoid arthritis, other forms of vasculitis such as GPA, human immunodeficiency virus infections, and paraproteinemias (particularly IgA). EED typically responds promptly to dapsone or sulfapyridine, but long-term therapy may be required because skin lesions recur after cessation of treatment.

## Vasculitis Associated with Immune-Mediated Disease

Vasculitis rarely occurs in autoimmune diseases without overt manifestations of the underlying disorder. The diseases typically complicated by vasculitis include those related to SLE: lupus itself, mixed CTD, Sjögren's syndrome, and overlap syndromes. Although vasculitis clearly occurs in some settings, it is commonly overdiagnosed in attempts to explain perplexing disease features in patients with known rheumatic illnesses. For example, neuropsychiatric SLE is generally not caused by a true vasculitis but rather by other mechanisms that remain poorly defined. Whenever possible, the clinical hypothesis of vasculitis should be confirmed by biopsy.

Cutaneous vasculitis in these diseases almost invariably is associated with hypocomplementemia and high titers of anti-nuclear antibodies (ANAs). DIF examination of skin lesions shows granular IgG and C3 deposition in and around dermal vessels, with or without IgM, reflecting the contribution of ICs to disease pathogenesis. The phenomenon of the "in vivo ANA" is also observed in keratinocytes and dermal cells in DIF studies (Figure 91-8).

Vasculitis in patients with SLE-related disorders is more likely than other forms of vasculitis to be associated with a lymphocytic predominance. One variant of autoimmune disease–associated cutaneous vasculitis, the so-called benign hypergammaglobulinemia of Waldenström, is usually a true lymphocytic vasculitis. Patients with this disorder invariably have anti-Ro antibodies (also known as SS-A), and many have subclinical Sjögren's syndrome. Lymphocytic vasculitis typically demonstrates less disruption of blood vessel architecture than does leukocytoclastic vasculitis, perhaps because lymphocytes contain fewer of the



**Figure 91-7** Erythema elevatum diutinum. Nodules typically form over the extensor surfaces of the knuckles and other joints.

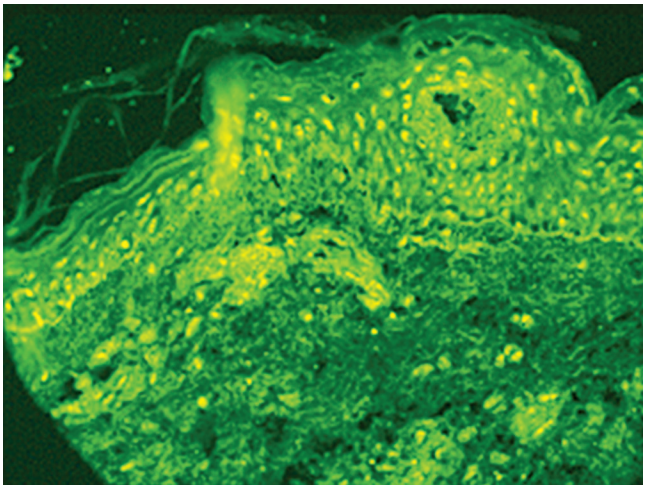


**Figure 91-8** Direct immunofluorescence study in connective tissue disease–associated vasculitis reveals tissue deposits of immunoglobulin G and an "in vivo anti-nuclear antibody" phenomenon. This phenomenon is caused by the binding of immunoreactants to targets within the nuclei of epidermal cells.

destructive enzymes found within neutrophil granules. Fibrinoid necrosis, for example, is rare in lymphocytic vasculitis. True lymphocytic vasculitis is nearly always confined to the small blood vessels of the superficial papillary dermis. Even in Sjögren's syndrome, however, the histopathology encountered in most cases is that of leukocytoclastic vasculitis.

### Rheumatoid Vasculitis

RV must be distinguished from the isolated, periungual digital vasculitis that, in the absence of severe involvement, does not require intensive, vasculitis-specific therapy. Isolated digital vasculitis in patients with rheumatoid arthritis, characterized by splinter-like lesions in the periungual region (Bywaters lesions), is not necessarily associated with a poorer prognosis than rheumatoid arthritis without digital vasculitic lesions and does not require specific therapy for vasculitis. In contrast, RV is a potentially devastating complication that may involve both medium- and small-sized blood vessels and requires the most aggressive therapeutic interventions. Many clinical manifestations of RV are indistinguishable from polyarteritis nodosa, although microaneurysms are less common in RV. RV classically occurs in patients with nodular, rheumatoid factor–positive, destructive disease who have few clinical indications of active synovitis at the time vasculitis begins. However, RV occasionally complicates early disease.

The most common presentation of RV includes purpuric lesions with or without evidence of concomitant medium-vessel vasculitis. DIF examination of the skin lesions shows granular IgM and C3 deposition in vessels, consistent with an IC-mediated pathophysiology in which rheumatoid factor, complement, and cryoglobulins may all participate. Deep cutaneous ulcers near the malleoli are a hallmark of RV and require scrupulous local care, as well as judicious immunosuppression. Mononeuritis multiplex often complicates RV. High-dose glucocorticoids plus rituximab is an appropriate first-choice regimen for many patients with RV, with the addition of cyclophosphamide if evidence of advancing disease exists. TNF inhibitors might also be reasonable options for patients with milder disease.

## CONCLUSION

IC-mediated forms of vasculitis comprise a diverse array of inflammatory disease, with a predilection for small to medium vessels (small > medium). The clinical effects often stem directly from the biochemical features of the ICs formed in response to different stimuli. The organ specificity, treatment options, and prognosis vary widely according to the specific type of vasculitis. Some forms of IC-mediated vasculitis are self-limited or require no further intervention than removal of the inciting antigen (e.g., the medication trigger in the case of drug-induced vasculitis). Glucocorticoids are the first line of treatment for the many cases in which immunosupression is required. The role of treatment with many biologic agents remains unclear, although B cell depletion fulfills an important treatment function in may patients with mixed cryoglobulinemia, rheumatoid arthritis, and other conditions.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 92

# Primary Angiitis of the Central Nervous System



Rula A. Hajj-Ali • Carol A. Langford

**KEY POINTS**

- Primary angiitis of the central nervous system (PACNS) is defined as vasculitis that is confined only to the brain, meninges, or spinal cord.
- PACNS consists of subsets including granulomatous angiitis of the central nervous system (GACNS) and atypical PACNS that are differentiated by unique histologic and imaging characteristics.
- The diagnosis of PACNS is based on compatible clinical features together with evidence from spinal fluid, brain and vascular imaging, and often a brain biopsy.
- Ruling out mimics that have a similar clinical or angiographic appearance is central to the diagnosis of PACNS.
- Reversible cerebral vasoconstriction syndrome is among the most important mimics of PACNS, with treatment and outcome implications.
- The initial treatment of PACNS includes glucocorticoids either alone or in combination with cyclophosphamide based on the subset and severity of neurologic disease.
- The outcome of PACNS is variable, with the highest rate of disability and mortality seen in people with GACNS.

Vasculitis affecting the central nervous system (CNS) most commonly occurs as a manifestation of a primary systemic vasculitis or as a secondary vasculitis in settings such as connective tissue diseases or infections. When the disease is confined only to the CNS (the brain, meninges, and spinal cord), it is referred to as *primary angiitis of the central nervous system* (PACNS).

PACNS is a rare disease that was first reported as a distinct clinical pathologic entity by Cravioto and Feigin in 1959.[1] The disease initially was described as fatal "noninfectious granulomatous angiitis with a predilection for the nervous system." Other reports of similar clinicopathologic phenotypes emerged in the literature, and "granulomatous angiitis of the central nervous system" was proposed to describe this entity.[2,3] Different terms subsequently surfaced in the literature, such as *isolated angiitis of the CNS* to encompass cases that were characterized by nongranulomatous pathologic findings.[4] Currently, PACNS is a well-accepted term for this disease that emphasizes the sole involvement of the CNS.[5,6] After the description of diagnostic criteria for PACNS proposed by Calabrese and Mallek in 1988[5] and the potential for effective treatment,[7] published cases in the literature increased tremendously, and more than 500 cases have now been described worldwide.[8,9]

## EPIDEMIOLOGY

Because of its rarity and our evolving understanding of this disease, the true incidence of PACNS is difficult to calculate.[9] In the recent era, the estimated annual incidence rate of PACNS is 2.4 cases per 1 million person-years.[9] Middle-aged men are often affected by PACNS, with a median age at onset of approximately 50 years and a male-to-female ratio of approximately 2:1.[5,6,9]

## GENETICS

The pathogenesis of PACNS is not well understood. To date, no evidence has been found to suggest a genetic predisposition, although this possibility remains under active investigation.

## CLINICAL FEATURES

**KEY POINTS**

- PACNS is divided into subsets that include granulomatous angiitis of the central nervous system (GACNS) and atypical PACNS.
- Mass-like lesions can occur in people with PACNS.
- Clinical features of GACNS typically include chronic insidious headaches along with diffuse and focal neurologic deficits.

Great progress has been made toward understanding the clinical features of PACNS despite the many challenges, which include the lack of highly specific diagnostic modalities, the sparse material for research, and the lack of controlled clinical trials. In the recent era, specific clinical and pathologic subsets of PACNS have been identified that have prognostic implications.[10-13]

### Proposed Criteria for Primary Angiitis of the Central Nervous System

In 1988, Calabrese and Mallek[5] proposed diagnostic criteria for PACNS that emphasized the importance of ruling out mimics when diagnosing PACNS. These criteria include (1) the presence of an unexplained neurologic deficit after

thorough clinical and laboratory evaluation; (2) documentation by cerebral angiography and/or tissue examination of an arteritic process within the CNS; and (3) no evidence of a systemic vasculitides or any other condition to which the angiographic or pathologic features could be secondary.

In 2009, Birnbaum and Hellmann[12] proposed changes to the criteria described by Calabrese and colleagues, incorporating levels of diagnostic certainty in their assessment. They proposed use of the term *definite diagnosis of PACNS* when tissue biopsy findings provide confirmation of vasculitis and a *probable diagnosis of PACNS* when the diagnosis is based on high-probability findings on an angiogram in the absence of tissue confirmation but with consideration of cerebrospinal fluid (CSF) profiles and neurologic symptoms to discriminate between PACNS and its mimics.

Although the original criteria by Calabrese and Mallek served as a platform of literature-based research, their interpretation has changed fundamentally as advancement of our diagnostic modalities has revealed many unexplained neurologic diseases. The importance of ruling out mimics of PACNS remains essential in the diagnostic approach of patients with suspected PACNS.

## CLINICAL SUBSETS

The original pathologic reports of PACNS as a granulomatous angiitis imposed a histologic-based nomenclature on the disease that led to the original name *granulomatous angiitis of the CNS* (GACNS). Until the 1980s, PACNS was largely considered a homogeneous entity with a uniform clinical picture and a grave prognosis. This paradigm was challenged with the introduction of direct vascular imaging as a diagnostic tool and the recognition that nongranulomatous pathologic findings occur in PACNS.

Initial attempts at subclassification of PACNS described three broad subsets: GACNS, benign angiopathy of the CNS (BACNS), and "atypical" PACNS.[14] It has since become recognized that BACNS is part of the reversible cerebral vasoconstriction syndrome (RCVS),[11] with the other PACNS subsets now being defined by pathologic or radiographic features.

### Granulomatous Angiitis of the Central Nervous System

GACNS is a subset of PACNS that is described as a clinicopathologic entity characterized by granulomatous angiitis confined to the brain. GACNS is a rare subset of PACNS, in which patients clinically present with chronic insidious headaches along with diffuse and focal neurologic deficits. Because the disease is confined to the brain, meninges, or spinal cord, signs and symptoms of systemic inflammatory diseases are usually lacking. The diagnosis of this subset is confirmed by the pathologic findings of granulomatous angiitis (Figure 92-1). Typically the CSF findings include those of an aseptic meningitis picture with negative staining for microorganisms. GACNS predominantly affects middle-aged men. The most common findings on neuroimaging include infarcts, most often bilateral, as well as high-intensity T2-weighted fluid attenuation inversion recovery (FLAIR) lesions on magnetic resonance imaging (MRI) in the subcortical white matter and deep gray matter. A cerebral angiogram is not the diagnostic modality of choice given its poor spatial resolution in the detection of small vessel vasculitis, which mainly occurs in GACNS.

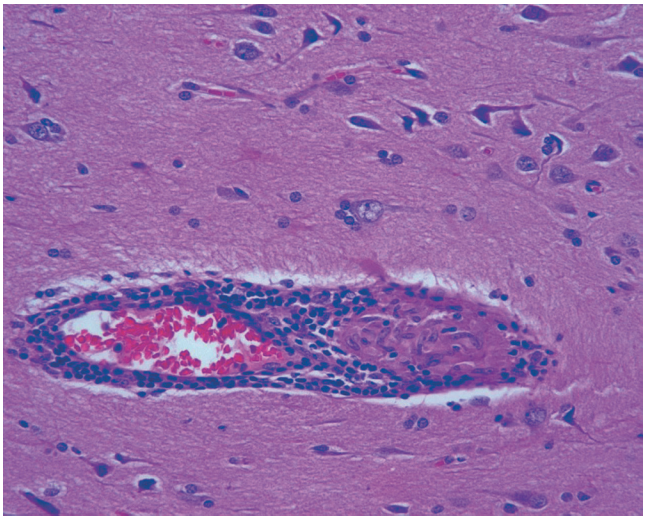


**Figure 92-1** Histopathologic findings of patients with granulomatous angiitis of the central nervous system vasculitis.

### Atypical Central Nervous System Vasculitis

Atypical CNS vasculitis, a subset of PACNS, comprises multiple manifestations of PACNS that are clinically, radiologically, or pathologically distinct from GACNS. Atypical PACNS is the most frequent and heterogeneous subset of PACNS. Included in this subset are patients with specific presentations such as lesions or patients with pathologic findings of lymphocytic infiltration rather than granulomatous angiitis.

#### Mass-like Presentation

Mass-like (ML) presentation is a rare manifestation of PACNS that occurs in less than 5% of cases. This presentation has gained attention after the recent report of a series of 38 patients with histologically confirmed PACNS who presented with a solitary cerebral mass.[15] Typically the diagnosis is unanticipated and is confirmed after pathologic examination with either biopsy samples or surgical excision of the mass. Unfortunately, no specific features on clinical assessment, neuroimaging, cerebral angiography, or CSF examination can reliably distinguish ML-PACNS from other more common causes of a solitary cerebral mass. Use of appropriate stains and cultures to rule out mycobacterial, fungal, or other infections and immunohistochemistry/gene rearrangement studies to exclude lymphoproliferative disease are essential to secure the diagnosis and exclude concomitant infectious or malignant processes.

#### Cerebral Amyloid Angiitis

Amyloid protein, in particular amyloid-β peptide, a fragment of the amyloid precursor peptide, can deposit in the brain, causing disease ranging from Alzheimer's disease to

cerebral amyloid angiopathy (CAA). CAA-related inflammation and angiocentric inflammatory reaction in CAA is referred to as amyloid-β–related angiitis (ABRA).[16] Patients with ABRA tend to be older and more prone to hallucinations and mental status changes than are other patients with PACNS. MRI cannot distinguish between ABRA and other forms of PACNS, although ABRA has a higher incidence of cerebral hemorrhage. ABRA carries a poor outcome, which could be related to older age and comorbidities.

### Angiographically Defined Central Nervous System Vasculitis

The poor specificity of the cerebral angiogram poses a major challenge in the diagnosis of PACNS. When the diagnosis of PACNS is based on angiographic findings, a thorough evaluation should be performed to rule out mimics, especially RCVS.[17,18]

### Spinal Cord Presentation

Spinal cord presentation of PACNS is a rare subset in which disease is present only in the spinal cord. The diagnosis is usually made by biopsy.[19]

### Nongranulomatous PACNS

Pathologic findings of lymphocytic infiltration rather than granulomatous findings can occur in PACNS. In this category, ruling out secondary causes of CNS vasculitis, such as infectious or lymphoproliferative diseases, should be emphasized. Adequate staining and immunophenotyping should always be carefully carried out in this category.

## DIAGNOSIS AND DIAGNOSTIC TESTS

**KEY POINTS**

- **Spinal fluid, brain and vascular imaging, and brain biopsy are central to the diagnosis of PACNS and the process of ruling out other diseases.**
- **Abnormal spinal fluid is found in 80% to 90% of patients with PACNS.**
- **Abnormalities on a cerebral angiogram are not specific for PACNS and can be seen in a wide range of other settings.**
- **Reversible cerebral vasoconstriction syndrome is an important mimic of PACNS that is characterized by thunderclap headaches, normal CSF, and abnormal findings of a cerebral angiogram in which the changes resolve within 12 weeks.**
- **Other important entities in the differential diagnosis of PACNS include infection, lymphoproliferative disease, primary systemic vasculitis, connective tissue disease, and thromboembolic disease.**

The diagnosis of PACNS is challenging because of the nonspecific clinical presentation, the lack of highly specific laboratory and radiologic tests, and the difficulty of obtaining pathologic material.

### Diagnostic Tests

#### Laboratory Findings

Elevation of acute-phase reactants, anemia, and thrombocytosis are not typical of PACNS, and if present, they should raise the possibility of a primary systemic vasculitis or another underlying disease. Laboratory testing to rule out connective tissue diseases and thromboembolic abnormalities should be performed. Infectious workup with appropriate cultures, serologies, and polymerase chain reaction testing should be directed by clinical and diagnostic findings and host risk factors.

#### Cerebrospinal Fluid Analysis

CSF is an important tool in the evaluation of PACNS. Although CSF findings are nonspecific in PACNS, their value lies in ruling out other entities. Obtaining appropriate CSF cultures, microbiologic stains, cytology, and flow cytometry is crucial in ruling out infectious and neoplastic disease. Elevated protein levels, modest lymphocytic pleocytosis, and occasionally oligoclonal bands and elevated IgG synthesis characterize the CSF in 80% to 90% of patients with pathologically documented PACNS.[5,20,21] The median CSF white blood cell count is approximately 20 cells/μL, and the median CSF protein level is approximately 120 mg/dL.[5,9]

#### Radiologic Evaluation

MRI is a sensitive modality for the diagnosis of PACNS, reaching 90% to 100%.[9,22] Abnormalities include infarcts in 50% of patients, commonly affecting the cortex and the subcortex bilaterally.[9,23] Affected areas include subcortical white matter, followed by deep gray matter, deep white matter, and the cerebral cortex.[24] Hyperintense lesions on T2-weighted sequences are common but not specific for PACNS.[25] Other abnormalities include mass lesions in 5% of patients,[15] leptomeningeal enhancement in 8% of cases,[9] and gadolinium-enhanced intracranial lesions in about one-third of patients.[9]

Cerebral vasculature imaging by catheter-directed dye angiogram or through magnetic resonance angiography (MRA) is an important modality in the diagnosis of PACNS. Alternating areas of dilatation and stenosis characterize the angiographic findings in PACNS and typically involve the vasculature on both sides but sometimes can involve single vessels.[26] Other angiographic features include smooth tapering of one or multiple vessels. Although cerebral angiograms may visualize abnormalities in medium-sized vessels, they have limited sensitivity to detect abnormalities in small vessels that are less than 500 μm in diameter. Although cerebral angiography is valuable, its specificity for the diagnosis of PACNS can be as low as 25%.[27] The reported "typical" angiographic findings for vasculitis are not specific to PACNS and can be encountered in atherosclerosis, radiation vasculopathy, or vascular spasm.[26,27] Moreover, a cerebral angiogram carries a poor positive predictive value in the diagnosis of PACNS in that the angiographic findings seen in RCVS can be consistent with those found in PACNS.[28] Vascular studies thus should be interpreted with caution and should not be considered the diagnostic

"gold standard" in PACNS. Ongoing research using high-resolution contrast-enhanced MRI to look at the vessel wall characteristics of wall thickening and wall enhancement is under way.[29] This promising technique may enable us to characterize and differentiate between CNS vasculitis, RVCS, and intracranial atherosclerosis. Recent reports indicate that vessel wall enhancement occurs at a much higher rate in PACNS compared with RCVS. Larger studies are needed to validate these interesting findings.

### Brain Biopsy

Brain biopsy is thought to carry a low morbidity and mortality in patients with PACNS[14] and is an important part of the evaluation to confirm the diagnosis and rule out mimics. When a brain biopsy is performed, an alternative diagnosis is identified in 30% to 40% of cases.[30] The interpretation of the brain biopsy results should take into consideration the potential for false-negative results because of the patchy degree of involvement and the small amount of tissue that often is able to be obtained. The finding of vasculitis on pathologic examination does not exclude the diagnosis of infection or malignancy, and appropriate stains and markers should be pursued for accurate diagnosis.

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis algorithm of PACNS is large because of the lack of highly specific clinical features or laboratory or imaging findings (Table 92-1). Excluding other entities that can have similar findings is critical in confirming a diagnosis of PACNS. Certain disease categories in particular should be considered and warrant further detailed discussion.

### Reversible Cerebral Vasoconstriction Syndromes

Distinguishing RCVS, the leading mimic of PACNS, is important because it has different implications for treatment and outcome (Table 92-2). RCVS consists of a group of disorders characterized by acute-onset headaches and reversible cerebrovasoconstriction.[11] These disorders include Call-Fleming syndrome, drug-induced angiopathy, migraine angiitis, BACNS, postpartum angiopathy, and drug-induced vasospasm. The clinical features of RCVS include acute onset of severe headaches, with or without neurologic deficit, with evidence of cerebral vasoconstriction that is reversible. The headaches are usually recurrent thunderclap headaches and can be precipitated by straining and coughing. RCVS can be associated with strokes (39%), generalized tonic-clonic seizures (17%), convexity subarachnoid hemorrhage (34%), lobar hemorrhage (20%), and brain edema (38%).[18] RCVS occurs more frequently in women than men. In contrast to PACNS, findings of CSF analysis are generally normal in people with RCVS except when a concomitant disorder or subarachnoid bleeding is present. The diagnosis of RCVS is usually considered when cerebral vascular imaging reveals multiple areas of smooth or tapered arterial narrowing followed by segments of normal-caliber or distended arteries. Typically, arterial narrowing involves multiple intracerebral arteries and their branches in both

**TABLE 92-1** Differential Diagnosis of Primary Angiitis of the Central Nervous System

| |
|---|
| **Secondary Cerebral Vasculitis** |
| Primary Systemic Vasculitides |
| Granulomatosis with polyangiitis |
| Microscopic polyangiitis |
| Eosinophilic granulomatosis with polyangiitis |
| Polyarteritis nodosa |
| Behçet's disease |
| Systemic Autoimmune Disease |
| Systemic lupus erythematosus |
| Sjögren's syndrome |
| Inflammatory myositis |
| Rheumatoid arthritis |
| Mixed connective tissue disease |
| **Other Multisystem Inflammatory Disorders** |
| Sarcoidosis |
| Susac's syndrome |
| **Infection** |
| Bacterial |
| Mycobacterial |
| Fungal |
| Viral |
| Protozoal |
| **Malignancy** |
| Central nervous system lymphoma |
| Glioma |
| Angiocentric lymphoma |
| Lymphomatoid granulomatosis |
| Metastatic malignancy |
| **Vasospastic Disorders** |
| Reversible cerebral vasoconstrictive syndrome |
| Drug exposures |
| **Other Arterial Disease** |
| Atherosclerosis |
| Fibromuscular dysplasia |
| Moyamoya disease |
| Dissection |
| **Hypercoagulable States** |
| Anti-phospholipid antibody syndrome |
| Thrombotic thrombocytopenic purpura |
| **Strokelike Syndromes** |
| CADASIL |
| Mitochondrial diseases |
| Sickle cell disease |
| Fabry's disease |
| Sneddon's syndrome |
| **Leukoencephalopathies** |
| Progressive multifocal leukoencephalopathy |
| Reversible posterior leukoencephalopathy syndrome |
| **Cerebral Hemorrhage** |
| Hypertensive |
| Aneurysmal |
| Amyloid angiopathy |
| Arteriovenous malformation |
| **Embolic Disease** |
| Thrombus |
| Cholesterol emboli |
| Myxoma |
| Endocarditis |
| Air emboli |

CADASIL, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

**TABLE 92-2** Differentiating Clinical and Radiologic Features between Reversible Cerebral Vasoconstrictive Syndrome and Primary Angiitis of the Central Nervous System

| Feature | RCVS | PACNS |
|---|---|---|
| Gender | Female predominant | Male predominant |
| Cerebrospinal fluid | Normal | Abnormal |
| Normal magnetic resonance image | 10%-15% | Very rare |
| Abnormal angiogram | 100% | 40%-50% |
| Headache pattern | Recurrent thunderclap | Insidious, chronic |
| Infarct pattern | Watershed | Small, scattered |
| Lobar hemorrhage | Common | Extremely rare |
| Convexity subarachnoid hemorrhage | Common | Very rare |

PACNS, Primary angiitis of the central nervous system; RCVS, reversible cerebral vasoconstrictive syndrome.

hemispheres, resulting in severe arterial narrowing. The diagnosis of RCVS is secured when repeat vascular imaging reveals reversibility of the cerebral vascular abnormalities, often occurring in about 6 to 12 weeks, which is in contrast to the fixed angiographic abnormalities encountered in PACNS. Controlled trials to direct treatment of RCVS are lacking. Calcium channel blockers are used for symptomatic treatment of the headaches, although no evidence exists that they alter the clinical outcome. Some experts prescribe glucocorticoid therapy, although no evidence exists that it improves the outcome.

## Primary Systemic Vasculitides

Primary systemic vasculitides such as granulomatosis with polyangiitis (GPA), microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis (EGPA; Churg-Strauss syndrome), polyarteritis nodosa, or Behçet's disease can affect the CNS, leading to inflammation and vasculitis. In GPA, CNS disease occurs in 7% to 11% of patients and presents in three ways: direct invasion of granuloma from extracranial sites, remote intracranial granuloma, and, rarely, as a result of CNS vasculitis.[31] The diagnosis of CNS vasculitis is often made on the basis of radiologic findings and by exclusion of other causes of the neurologic deficits, especially infection.[32] As in GPA, the diagnosis of CNS vasculitis in EGPA is often presumed without histologic confirmation when other mimics are excluded. Neuro-ophthalmologic manifestations including amaurosis fugax, superior oblique palsy, ischemic optic neuropathy, fourth cranial palsy, and scattered areas of retinal infarction are common presentations of CNS vasculitis in people with EGPA.[33,34] In Behçet's disease, CNS involvement occurs in 14% of cases, manifesting as meningoencephalitis involving the brain stem and rarely as a consequence of thrombosis and inflammation of the dural venous sinuses.[35-37] Pathologic evaluation in neuro-Behçet's disease typically shows a mononuclear infiltration around the small vessels of the brain, including the venous system, which is not a typical finding in PACNS.[38] True CNS vasculitis rarely occurs in Behçet's disease.[39]

## Systemic Autoimmune Diseases

The brain is a common target in connective tissue diseases. In systemic lupus erythematosus (SLE), CNS involvement occurs in 14% to 80% of adults and in 22% to 95% of children.[40] Multifocal microinfarcts, cortical atrophy, gross infarcts, hemorrhage, ischemic demyelination, and patchy multiple-sclerosis–like demyelination are typical findings in neuropsychiatric lupus. The most common microscopic brain finding in SLE is microvasculopathy, described as "healed vasculitis" consistent with hyalinization, thickening, and thrombus formation.[41,42] Rheumatoid arthritis, Sjögren's syndrome, and mixed connective tissue disease rarely affect the CNS in a vasculitic pattern.[43] CNS vasculitis is typically a late occurrence in these diseases.

## Infections

Infections are one of the most important entities that should be ruled out when considering PACNS. Vasculitis can occur in the setting of HIV, often presenting as multifocal cerebral ischemia with pathologic findings of angiocentric lymphoproliferative lesions.[44] In addition, HIV can result in granulomatous arteritis[45] or can affect the brain as a result of co-infections such as syphilis. *Treponema pallidum* infection can affect vessels in the subarachnoid space and result in thrombosis, ischemia, and infarction that can mimic PACNS.[46] Other infectious agents that have predilection to the brain include varicella-zoster virus (VZV). VZV affects the brain either by involvement of large cerebral vessels, often affecting the proximal segments of the anterior and middle cerebral arteries in immunocompetent people, or small vessel disease in immunocompromised patients.[47] A history of VZV rash is usually although not always elicited before the CNS involvement. The diagnosis of VZV angiitis is confirmed by finding VZV DNA in CSF or by the presence of reduced serum/CSF ratios of VZV IgG.[48] Tuberculosis affecting the CNS is an important cause of CNS vasculitis in endemic areas, and care must be taken to exclude it. Hepatitis C,[49] West Nile virus,[50] parvovirus B19,[51] and rarely herpes simplex virus[52] can cause CNS vasculitis. Cytomegalovirus can cause CNS vasculitis as an opportunistic infection in immunocompromised people.[53] Rarely, cysticercosis can involve middle-sized cerebral arteries.[54] A travel history and history of exposures are important features of the workup of patients with suspected PACNS.

## Lymphoproliferative Diseases

Lymphoproliferative disease with predilection to the vascular wall, such as lymphomatoid granulomatosis (LG), can cause CNS vasculitis, leading to multiple small cortical infarcts and pathologic findings of multifocal angiocentric, angiodestructive lymphoma.[55] Concomitant HIV infection is common with LG and should be ruled out.[56] Rarely, LG is associated with other systemic autoimmune diseases such as Sjögren's syndrome.[57] Other lymphoproliferative diseases such as CNS lymphoma and intravascular lymphoma can mimic PACNS.

### Miscellaneous

Atherosclerotic involvement of the intracerebral arteries is common and is often a consideration in the workup of PACNS. The poor specificity of the cerebral angiogram poses a major limitation in differentiating inflammatory causes from other vasculopathies. However, the lack of inflammatory changes of the CSF and the presence of multiple atherosclerotic risk factors should raise suspicion for atherosclerotic disease. Other entities such as antiphospholipid antibody syndrome, hypercoagulable states, and thromboembolic causes should be carefully ruled out. The workup of PACNS should include a transesophageal echocardiogram and a hypercoagulable profile to rule out thromboembolic causes, especially in patients presenting with recurrent strokes. Other rare diseases that mimic the angiographic findings of PACNS include Moyamoya disease, small vessel arterial dissection, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), radiation vasculopathy, and thromboangiitis obliterans.[58]

## TREATMENT

**KEY POINTS**

- No standardized trials relating to the treatment of PACNS have been conducted.
- Typically, GACNS is initially treated with glucocorticoids and cyclophosphamide.
- The treatment of PACNS subsets besides GACNS is based on the diagnostic subset and severity of neurologic impairment.

The treatment of PACNS is guided by expert opinion and reports of case series, as well as by extrapolation from controlled trials relating to primary systemic vasculitis. To date, no controlled trials have been conducted to investigate the treatment of PACNS. Thus it is not possible to determine a specific treatment algorithm, and therapy is based on the subtype and neurologic severity.

The report of successful treatment of GACNS with a combination of cyclophosphamide and glucocorticoids led to the regular use of this regimen.[7] Generally, patients are treated with a combination of cyclophosphamide and high-dose glucocorticoids for 3 to 6 months until remission is achieved. Cyclophosphamide is then stopped and use of a maintenance agent is initiated, following the same principles used in small-vessel vasculitis.[59] Maintenance therapy usually consists of either azathioprine or mycophenolate mofetil and rarely methotrexate given its low CNS penetration. The duration of maintenance therapy in PACNS is not well defined.

The treatment of atypical PACNS varies highly. Multiple factors affect the treatment regimen in atypical PACNS, and the treatment is usually individualized, taking into consideration the degree of the neurologic deficit and the features of the diagnosis. All atypical PACNS cases are treated with high dose of glucocorticoids initially, and the decision to add other immunosuppressive agents is based on each individual case. ABRA and ML-PACNS are two PACNS subsets in which cyclophosphamide is considered at the outset of the treatment. Use of TNF inhibitors or rituximab in patients with PACNS that is resistant to glucocorticoids and other immunosuppressive agents has thus far been confined to case reports, which provide insufficient evidence from which to draw conclusions regarding efficacy.[60-62]

Failure to respond to cyclophosphamide should prompt evaluation for an alternative diagnosis before additional treatment with an alternative immunosuppressive agent is instituted.

Assessing disease status and defining remission are crucial steps in the course of treatment of PACNS. Permanent deficits occurring after the initial event in PACNS should not erroneously be considered to be lack of remission. Disease is considered in remission when stability or improvement of the clinical and radiologic features is achieved. Serial MRI should be obtained to help in the assessment of disease activity. Adjunctive therapy for osteoporosis prevention and prophylaxis for opportunistic infections should be incorporated into the treatment plan.

## OUTCOME

**KEY POINTS**

- PACNS has an estimated mortality rate of 10% to 17%.
- Moderate to severe disability occurs in up to 20% of patients with PACNS.

Originally, PACNS was described as a fatal disease by Cravioto,[1] but an improved outlook became possible after the description of treatment with glucocorticoids and cyclophosphamide. According to recent reports, the estimated mortality rate of PACNS varies between 10% and 17%,[9,63] with one study finding that survival correlated with the initial findings of infarcts and gadolinium-enhanced lesions on MRI.[9] Salvarani and colleagues[9] and Hajj-Ali and colleagues[63] reported that 20% of patients with PACNS had moderate to severe disability as assessed by the modified Rankin disability scores and the Barthel index, respectively. Salvarani and colleagues[9] reported an improvement in disability scores over time.

## CONCLUSION

Considerable progress has been made in our understanding of PACNS. Our ability to recognize disease mimics has substantially increased our accuracy in diagnosing PACNS. The identification of RCVS in particular has clarified many of the cases that had been erroneously diagnosed as PACNS. Effective diagnosis and management of PACNS requires a multidisciplinary team to evaluate the clinical features, obtain and interpret diagnostic tests, and determine the most effective regimen that will treat the underlying disease and minimize therapeutic toxicity.

A great need exists for controlled trials to further guide treatment for patients with PACNS in their initial and maintenance phases. Prospective evaluation of long-term cohorts is necessary to better determine the rates of disability and long-term outcome of PACNS.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 93

# Behçet's Disease



William S. Kaufman • Elizabeth K. McNamara • Joseph L. Jorizzo

**KEY POINTS**

- **Behçet's disease is a complex multisystem disease characterized by oral and genital ulcers and other systemic features.**
- **The diagnosis is based on the International Criteria for Behçet's Disease and includes oral aphthae, genital aphthae, ocular lesions, cutaneous lesions, and a positive pathergy test.**
- **Cutaneous lesions display a neutrophilic vascular reaction on histopathologic examination.**
- **Treatment is based on the degree of systemic involvement. Treatments include topical corticosteroids, thalidomide, and systemic immunosuppressive and biologic agents.**
- **The prognosis is variable, and patients typically have periods of exacerbations and remissions.**

Behçet's disease is a chronic, complex multisystem disease characterized by oral and genital aphthae, cutaneous lesions, and ophthalmic, neurologic, or rheumatologic manifestations. The first description of Behçet's disease is believed to be from Hippocrates in the fifth century BC.[1] The first modern account was presented in 1937 by the Turkish dermatologist Hulusi Behçet, who reported on a patient with recurrent oral and genital aphthae and uveitis.[2]

## EPIDEMIOLOGY

Behçet's disease is seen worldwide, with the highest prevalence reported in Turkey (1 per 250 inhabitants) and Japan (11.9 patients per 100,000 inhabitants).[3,4] The prevalence, and often the severity, is increased in the Middle East and the Mediterranean (i.e., the "Silk Route").[3,4] The remainder of the Asian continent has a prevalence of 7 to 30 patients per 100,000 inhabitants.[5] It is relatively uncommon in northern Europe (0.27 to 1.18 patients per 100,000 inhabitants) and the United States (5.2 patients per 100,000 inhabitants).[5,6,7] Patients commonly manifest the diagnostic criteria in the third to fourth decades of life.[8] In the past, Behçet's disease was thought to predominantly affect males, but current epidemiologic data show a more equal male-to-female ratio.[9] Overall, the male-to-female ratio has decreased during the past 20 years, with more males diagnosed in Turkey, Greece, Italy, the Middle East, and the Far East, and more females diagnosed in Korea, China, the United States, and northern Europe.[10] Males tend to have a more severe clinical course, with increased disease-associated morbidity.[11]

## ETIOLOGY AND PATHOGENESIS

Although the pathogenesis of Behçet's disease remains enigmatic, numerous factors have been implicated. Genetics, immunologic phenomena, infectious agents, inflammatory mediators, and clotting factors are likely contributors.

### Genetics

The onset of Behçet's disease is thought to be sporadic, although familial clustering (families with multiple affected members) has been reported. Individuals with a first-degree relative with the disease are at an increased risk.[12] Additionally, children of individuals with Behçet's disease may have an earlier age of onset, suggesting genetic anticipation, characterized by a progressive increase in nucleotide repeats through consecutive generations.[13] Familial occurrence differs regionally throughout the world and is more common in Korea, Israel, Turkey, and Arab countries compared with Japan, China, and Europe.[5]

Studies have shown a significant association between the human leukocyte antigen (HLA)-B51 and Behçet's disease.[14,15,16] HLA-B51-positive patients are at an increased risk for Behçet's disease (odds ratio [OR], 5.9).[17] This association is more common in the Middle East, Mediterranean, and Japan, although not seen as often in Western nations. The disease prognosis also appears to be more severe in HLA-B51-positive patients.[5] The role of HLA-B51 in Behçet's remains unclear. It may be that HLA-B51 is not directly involved in causing the disease but is closely linked to disease-related genes.[18] Candidate genes have been localized to chromosome 6 and include the class I major histocompatibility complex (MHC) chain-related gene A (MICA) (specifically, the MICA6 allele); Perth block (*PERB*); new organization associated with HLA-B (NOB); and transporter associated with antigen processing genes (TAP).[18,19] Other hypotheses suggest that HLA-B51 may contribute to the onset of Behçet's disease by serving as a heterologous antigen, either through original antigen presentation or through viral or bacterial molecular mimicry.[18,20] A genome-wide association study confirmed the HLA-B51 relationship and also identified a second, independent association within the class I MHC region.[21]

Although Behçet's disease has many features in common with the spondyloarthropathies, especially those associated with inflammatory bowel disease (IBD), the disorder in IBD patients generally evolves in a pattern resembling reactive arthritis with an erosive axial arthritis. Erosive arthritis and *HLA-B27* are not associated with Behçet's disease.[22]

## Immune Mechanisms

Immunologic factors are thought to play a major role in Behçet's disease. Heat shock proteins, cytokines, alterations in neutrophil and macrophage activity, and autoimmune mechanisms have all been implicated.[18] Heat shock proteins are released in response to stress and may be involved in stimulating a T helper cell type I immune response through interaction with Toll-like receptors.[23] Although most of the T lymphocytes thought to be involved in this reaction are of the γδ type, the diversity of T lymphocytes seen in the disease suggests a response to multiple antigens, which may account for the various symptoms seen in Behçet's.[24] Cytokines such as IL-1, IL-8, IL-12, IL-17, IL-37, and TNF seem to be involved in the pathogenesis. Although elevated cytokine levels may serve as an indicator of disease severity, it should be appreciated that plasma TNF levels may rise and fall as an acute-phase reactant along with C-reactive protein and the erythrocyte sedimentation rate.[25,26] The production of these pro-inflammatory cytokines, which are responsible for the chronic inflammation observed, may be the result of activated macrophages.[18,27] In addition to macrophage activation, neutrophil chemotaxis and phagocytosis are increased in the lesions of Behçet's disease.[18,28] This increased activity of neutrophils leads to tissue injury in the form of the neutrophilic vascular reaction seen in lesions such as aphthae, pustular cutaneous lesions, and erythema nodosum-like lesions. Circulating immune complexes also play a role in precipitating the characteristic neutrophilic vascular reaction.[29]

The role of endothelial cell dysfunction in the pathogenesis of Behçet's disease has been suggested by decreased levels of prostacyclin in the serum of Behçet's disease patients. Increased nitric oxide concentrations in the serum, synovial fluid, and aqueous humor of individuals with Behçet's may play a role in endothelial activation, resulting in vascular inflammation and thrombosis.[30,31] Elevated homocysteine levels have been cited as the cause of the increase in nitric oxide concentrations, suggesting that hyperhomocysteinemia may represent an acquired risk factor for Behçet's, which is potentially reversible.[32-34] Anti-endothelial cell antibodies have been detected in sera from patients with Behçet's disease and have been linked to disease activity and vasculitis symptoms.[35-37] Specifically, immunoglobulin (Ig)-A type anti-endothelial cell antibodies from patients with Behçet's disease have been found to react against the target protein heterogeneous nuclear ribonucleoprotein-A2/B1 (HnRNP-A2/B1), which is associated with pre-mRNA processing, metabolism, and transport.[38]

## Infectious Agents

Several studies have suggested a role for various infectious agents in the pathogenesis of Behçet's disease. No organisms, however, have been consistently isolated. Antistreptococcal antibodies have been isolated in the serum of patients with Behçet's disease.[39] Higher concentrations of *Streptococcus sanguis* have also been found in the oral flora of patients with Behçet's disease and may contribute to the development of aphthae, which are often the initial manifestation.[40] Also, sera from patients with Behçet's disease cross-react with both human α-enolase and *S. sanguis* α-enolase, suggesting antigenic mimicry.[41] In addition to streptococcal antigens, other bacteria such as *Escherichia coli* and *Staphylococcus aureus* may trigger Behçet's disease via lymphocyte activiation.[42] Additionally, a lipoprotein of *Mycoplasma fermentans* has been found in patients with Behçet's. This lipoprotein (MALP-404) contains the specific peptide motif, which is capable of being presented by *HLA-B51*.[43] Studies have also indicated that a higher rate of *Helicobacter pylori* cytotoxin-associated gene-A antibodies appears in Behçet's patients. These antibodies may cause endothelial damage via cross-reaction with endothelial antigens. *H. pylori* eradication in these patients decreases disease severity.[44]

Herpes simplex virus (HSV) DNA has been isolated from the nuclei of peripheral blood lymphocytes by polymerase chain reaction (PCR) assay in patients with Behçet's disease.[22] HSV has also been detected by PCR in biopsy samples of genital and intestinal ulcers of Behçet's patients.[42] Other studies, however, have shown no difference in the detection of HSV in Behçet's patients with and without oral aphthae.[45]

In summary, although the etiology and exact pathogenesis of Behçet's disease remain elusive, it is likely a multifactorial process involving infectious or environmental factors that trigger an autoreactive inflammatory response in a genetically predisposed individual.

# CLINICAL FEATURES

## Aphthae

Oral aphthae (Figure 93-1) are often the initial feature of Behçet's disease and constitute a requisite diagnostic feature (although some believe that Behçet's occurs in the absence of oral aphthae). Oral ulcerations usually occur in crops of 3 to 10 or more lesions, but individual lesions may occur on the buccal mucosa, gingiva, lips, and tongue. Aphthae begin as painful papules which rapidly progress to shallow ulcerations with worsening pain. Often, a white to yellow pseudomembrane adheres to the surface of the ulcer. They usually heal spontaneously without scarring during 1 to 3



**Figure 93-1** Oral aphtha in Behçet's disease.

weeks.[46] Genital ulcers typically occur on the scrotum and penis in males and on the vulva or vaginal mucosa in females. These aphthae are similar in appearance to oral lesions, but they have a greater tendency to scar, resolve during 2 to 4 weeks, and may recur less frequently than oral ulcers.[46] Lesions in the oral mucosa are generally easy to distinguish from oral HSV, but with genital lesions, HSV should be excluded by viral culture or PCR before the lesions are accepted as a diagnostic criterion for Behçet's.

## Cutaneous Manifestations

Several cutaneous manifestations of Behçet's disease have been described: erythema nodosum-like lesions, pyoderma gangrenosum-like lesions, Sweet's syndrome-like lesions, cutaneous small vessel vasculitis, and pustular vasculitic lesions (Figure 93-2) including lesions induced by trauma—the so-called *pathergy* lesion.[46] Pathergy signifies the development of erythematous pustules or papules 24 to 48 hours following puncture of the skin with a 20- to 21-gauge sterile needle.[47] Specimens from all of these lesions demonstrate a neutrophilic vascular reaction on histopathologic analysis.[48] Acneiform or pseudofolliculitis lesions should be considered nonspecific, nondiagnostic cutaneous findings because of their common occurrence in acne vulgaris and folliculitis.

## Ophthalmic Manifestations

A variety of ocular manifestations have been reported in Behçet's patients, including anterior and posterior uveitis, retinal vasculitis, and hypopyon, with secondary glaucoma, cataract formation, decreased visual acuity, and synechiae formation. Ocular involvement occurs in 83% to 95% of men and 67% to 73% of women with Behçet's disease.[49] Men have a greater tendency for eye involvement, which may cause loss of vision.[50] Ophthalmic features most often occur in the first few years of the disease, and although ocular involvement is not commonly the presenting feature of Behçet's disease, it is a major source of serious morbidity.[51] Close ophthalmologic evaluation and follow-up are critical to prevent blindness in these patients.[52] If ocular disease does not present within a few years of diagnosis, it is less likely to be a major problem.[53]



**Figure 93-2** Pustular vasculitis lesions representing a neutrophilic vascular reaction.

## Arthritis

Arthritis in Behçet's disease is typically a nonerosive, inflammatory, symmetric, or asymmetric oligoarthritis, although polyarticular and monoarticular forms are also seen. The most commonly involved joints are the knees, wrists, ankles, and elbows, with associated enthesopathy.[54] Synovial fluid reveals formation of mucin clots and inflammatory findings of nonspecific synovitis.[55] The prevalence of arthritis among different populations ranges from 40% to 60%, and joint erosions are not observed.[52] Dilsen and colleagues[56] reported that 10% of patients with Behçet's disease had sacroiliitis. However, *HLA-B27*-positive patients were not excluded from their series, and occult IBD was not excluded as required by O'Duffy and Goldstein.[57] Other studies have shown no significant difference in the occurrence of sacroiliitis between patients with Behçet's disease and the normal population. *HLA-B27*-positive patients with erosive sacroiliitis should be included in the reactive arthritis or enteropathic arthritis disease spectrum, given the erosive, axial nature of the arthritis in the *HLA-B27* pattern. This contrasts with the classically nonerosive, nonaxial nature of arthritis in Behçet's disease.

## Other Systemic Manifestations

Central nervous system (CNS) involvement is found in 5% to 10% of patients with Behçet's disease.[58] The most common manifestations include brain stem or corticospinal tract syndromes (neuro-Behçet's syndrome), venous sinus thrombosis, increased intracranial pressure secondary to venous sinus thrombosis or aseptic meningitis, isolated behavioral symptoms, or isolated headache. In rare cases, ruptured aneurysms, peripheral neuropathy, optic neuritis, and vestibular involvement can occur.[59] Both parenchymal disease and venous thrombosis can have accompanying vasculitis.[58] Poor prognosis is associated with a progressive course, parenchymal or brain stem involvement, cerebellar symptoms, and cerebrospinal fluid abnormalities.[60] Cranial and peripheral nerve involvement may also occur.

Patients with Behçet's disease may have gastrointestinal lesions that resemble orogenital aphthae. These occur most commonly in the terminal ileum, cecum, colon, and esophagus.[33] Large aphthae may lead to perforation of the gastrointestinal mucosa. Presenting symptoms include dysphagia, abdominal pain, substernal pain, diarrhea, and melena. It is important to distinguish IBD from Behçet's disease.[61] Oral aphthae, ocular lesions, erythema nodosum-like lesions, pustular vasculitis, and pyoderma gangrenosum can occur in patients with IBD.

Pulmonary abnormalities are uncommon in Behçet's disease. Pulmonary artery aneurysms occur most frequently, followed by other complications secondary to vasculitis that affect the small pulmonary vessels. Aneurysm, thrombosis, hemorrhage, and infarction can result and cause death in patients with Behçet's disease.[34]

Renal manifestations are not common. They range from minimal changes, to proliferative glomerulonephritis, to rapidly progressive crescentic glomerulonephritis. The pathogenesis is likely to involve immune complex deposition.[62] Aphthae may also affect the bladder.

Cardiovascular complications include myocardial infarction, pericarditis, arterial and venous thromboses, and aneurysm formation. Venous involvement is more common than arterial involvement: the most frequent manifestation is venous thrombosis in the lower extremities, although thromboses that cause superior and inferior vena cava obstruction can occur.[63] Hepatic vein thrombosis is a serious potential complication of Behcet's disease, which can lead to hepatic venous outflow obstruction and associated Budd-Chiari syndrome.[64] Cardiac manifestations, either occlusive or aneurysmal, are thought to occur because of vasculitis of the vasa vasorum, which induces a thickening of the media and splitting of elastic fibers.[65] Atherosclerosis does not appear to occur at an increased rate, as is seen in many autoimmune diseases such as systemic lupus erythematosus.[66] Mortality in Behçet's disease is low and is usually related to pulmonary or CNS involvement, or to bowel perforation.[9]

Limited data are available that evaluate Behçet's in pregnancy. One case-control study reported more remissions than exacerbations during and after pregnancy, with higher rates of pregnancy complications but no changes in neonatal outcomes.[67]

## HISTOPATHOLOGY

Histopathologic analysis of specimens from the cutaneous lesions seen in Behçet's disease reveals epidermal ulceration or pustule formation and diffuse dermal infiltrate of neutrophils, lymphocytes, and histiocytes, sometimes with fully developed leukocytoclastic vasculitis. Microscopic examination of dermal capillary or venule walls shows neutrophilic infiltrates, nuclear dust, and extravasation of erythrocytes, with or without fibrinoid necrosis.[68] Immune complex-mediated vasculitis is the likely mechanism in the development of Behçet's disease.[69] A previously reported finding of lymphocytic vasculitis in patients with Behçet's disease is thought to represent an older lesion.[48]

Biopsy specimens of synovial membranes reveal a neutrophilic reaction, with occasional plasma cells and lymphocytes. Immunofluorescence microscopy may show IgG deposition along the synovial membrane.[52] Reports of synovial fluid analysis in patients with Behçet's disease show leukocyte counts that range from 300 to 36,200 cells/mm$^3$, with a predominance of neutrophils and normal glucose levels.[55]

## DIAGNOSIS

The diagnosis of Behçet's disease can be difficult, particularly in patients with a limited number of common disease features. Clinicians and investigators must rely on clinical criteria because no pathognomonic laboratory findings exist. Several sets of diagnostic criteria have been proposed, including those by O'Duffy and Goldstein,[70] Mason and Barnes,[71] and a Japanese study group.[72] In 2008, the International Team for the Revision of International Criteria for Behçet's Disease revised the established criteria on the basis of the presence of various disease manifestations, including oral aphthae, cutaneous lesions, positive pathergy, genital aphthae, and ocular involvement.[73] Each manifestation contributes 1 or 2 points, and an individual with a score of 3 or more points is diagnosed with the disease (Table 93-1). A diagnostic algorithm based on the Revised International Criteria for Behçet's disease is presented in Figure 93-3.

**TABLE 93-1** Revised International Criteria for Behçet's Disease*

| | |
|---|---|
| Oral aphthosis | 1 point |
| Skin manifestations (pseudofolliculitis, skin aphthosis) | 1 point |
| Vascular lesions (phlebitis, large vein thrombosis, aneurysm, arterial thrombosis) | 1 point |
| Positive pathergy test | 1 point |
| Genital aphthosis | 2 points |
| Ocular lesions | 2 points |

*Diagnosis of Adamantiades-Behçet's disease is made with a score of 3 points.

Modified from International Team for the Revision of International Criteria for Behçet's Disease: Clinical manifestations of Behçet's disease. The ITR-ICBD report. *Clin Exp Rheumatol* 26(Suppl 50):S1–S18, 2008.

Although not required by diagnostic criteria, IBD, systemic lupus erythematosus, reactive arthritis, and herpetic infections should first be excluded because the presenting manifestations of these conditions are often similar to those of Behçet's disease. Recurrent aphthous stomatitis and complex aphthosis, defined as recurrent oral and genital aphthae or almost constant, multiple (three or more) oral aphthae, should also be considered in the differential diagnosis of patients presenting with oral or genital aphthae.[74]

The O'Duffy-Goldstein criteria mandate the presence of recurrent oral aphthae plus at least two of the following: genital aphthae, synovitis, posterior uveitis, cutaneous pustular vasculitis, and meningoencephalitis. Patients who have only two of these findings, one being recurrent oral aphthae, are considered to have an incomplete form of Behçet's disease. One concern with regard to the International Criteria for Behçet's is the inclusion of acneiform lesions, which are a common nonspecific finding in both adolescents and adults. Therefore, our group advocates histologic confirmation of vessel-based histology to exclude acne lesions and the use of both the O'Duffy and the International Study Group criteria to exclude patients with IBD and enteropathic arthritis.[75]

The initial evaluation should include a referral for ophthalmologic consultation to identify insidious ocular involvement. Patients who have arthralgias, gastrointestinal symptoms, or neurologic abnormalities may require radiographic studies and evaluation by appropriate subspecialists. Cutaneous pustular lesions, erythema nodosum-like lesions, and pyoderma gangrenosum-like lesions should be biopsied (for both histologic evaluation and culture) to confirm the cutaneous clinical diagnosis.

## TREATMENT

Treatment of Behçet's disease is largely based on anecdotal case reports, case series, and a few randomized clinical trials. Therapeutic options should be based on the degree of disease involvement (Tables 93-2 and 93-3).[46]

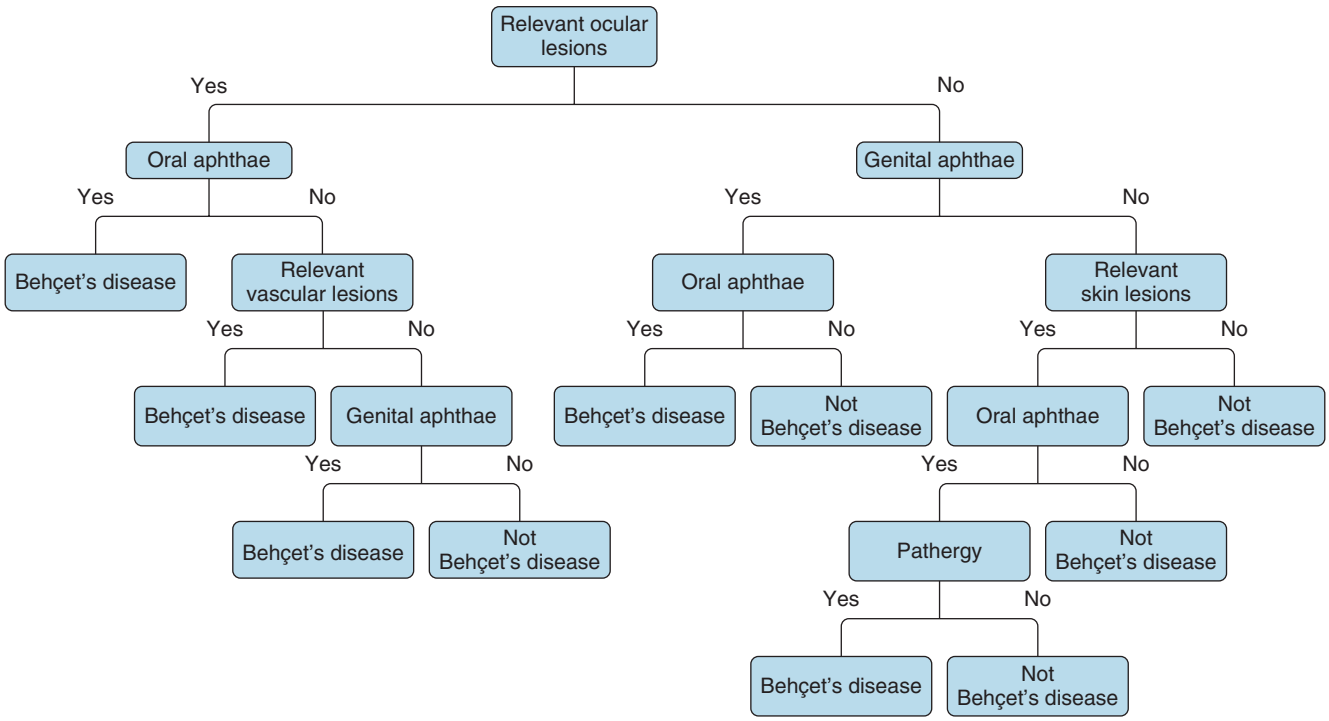



**Figure 93-3** Revised international criteria for Behçet's disease: diagnosis of Adamantiades-Behçet's disease. Classification tree format. *(Modified from International Team for the Revision of International Criteria for Behçet's Disease: clinical manifestations of Behçet's disease. The ITR-ICBD report.* Clin Exp Rheumatol *26[Suppl 50]:S1–S18, 2008.)*

**TABLE 93-2** Treatment of Behçet's Disease

| |
|---|
| **Mucocutaneous Disease Only**<br>Topical, intralesional, or aerosolized corticosteroids |
| Topical sucralfate |
| Local anesthetics |
| Topical tacrolimus |
| Colchicine (0.6-2.4 mg/day) |
| Dapsone (50-150 mg/day) |
| Combinations of agents mentioned above |
| **Severe Mucocutaneous Disease**<br>Thalidomide (50-300 mg/day) |
| Methotrexate (2.5-25 mg/wk) |
| Prednisone |
| IFN-α (3 million-9 million U/wk) |
| **Systemic Disease**<br>Prednisone |
| Azathioprine (50-200 mg twice daily) |
| Chlorambucil (4-6 mg/day) |
| Cyclophosphamide (1 g/mo, IV) |
| Cyclosporine (5-10 mg/kg/day) |
| Mycophenolate mofetil (500 mg-1.5 g, twice daily) |
| Intravenous (IV) immunoglobulin |
| Rituximab |
| TNF inhibitors |

## Mucocutaneous Disease

Patients with oral and genital aphthae can be treated with intralesional, topical, or aerosolized (not inhaled) corticosteroids. Topical tacrolimus can also be used, often in combination with superpotent topical corticosteroids. Other palliative therapies include oral tetracycline solutions, topical anesthetics, and rinses containing chlorhexidine gluconate. Oral colchicine, 0.6 to 2.4 mg daily, can decrease the size and frequency of mucocutaneous lesions.[76,77] Doses can be adjusted according to the degree of gastrointestinal upset experienced by the patient. Dapsone in a dose of 50 to 150 mg/day is often helpful alone[78] or in combination with colchicine.[74] Physicians should check glucose-6-phosphate dehydrogenase levels in all patients before beginning therapy with dapsone, and patients must be monitored for hemolytic anemia and methemoglobinemia. TNF inhibitors also improve mucosal and skin manifestations of Behçet's disease.[79,80] The oral phosphodiesterase-4 inhibitor apremilast has recently been found to decrease the number and pain of oral ulcers, in addition to improving overall disease activity.[81]

## Severe Mucocutaneous Disease

Patients in whom conservative therapies fail to produce the desired response as outlined for mucocutaneous disease may require thalidomide. Its mechanism of action is thought to be mediated by modulation of TNF and other cytokines. Previous studies have shown that thalidomide is an effective agent for the treatment of severe mucocutaneous lesions of Behçet's disease.[82,83,84] Thalidomide is known to cause severe

**TABLE 93-3** Modified EULAR 2008 Recommendations for the Management of Behçet's Disease (BD)

| No. | Recommendation |
|---|---|
| 1 | Any patient with BD and inflammatory eye disease affecting the posterior segment should be on a treatment regimen that includes azathioprine *or mycophenolate** and systemic corticosteroids. |
| 2 | If the patient has severe eye disease defined as >2 lines of drop in visual acuity on a 10/10 scale and/or retinal disease (retinal vasculitis or macular involvement), it is recommended that either cyclosporine A or infliximab be used in combination with azathioprine and corticosteroids; alternatively, IFN-α with or without corticosteroids could be used instead. |
| 3 | There is no firm evidence to guide the management of major vessel disease in BD. For the management of acute deep vein thrombosis in BD immunosuppressive agents such as corticosteroids, azathioprine, cyclophosphamide, or cyclosporine A are recommended. For the management of pulmonary and peripheral arterial aneurysms, cyclophosphamide and corticosteroids are recommended. |
| 4 | Similarly, there are no controlled data on, or evidence of benefit from, uncontrolled experience with anti-coagulants, anti-platelet, or antifibrinolytic agents in the management of deep vein thrombosis or for the use of anticoagulation for the arterial lesions of BD. |
| 5 | There is no evidence-based treatment that can be recommended for the management of gastrointestinal involvement of BD. Agents such as sulfasalazine, corticosteroids, azathioprine, TNF antagonists, and thalidomide should be tried first before surgery, except in emergencies. |
| 6 | In most patients with BD, arthritis can be managed with colchicine. |
| 7 | There are no controlled data to guide the management of CNS involvement in BD. For parenchymal involvement, agents to be tried may include corticosteroids, IFN-α, azathioprine, cyclophosphamide, methotrexate, and TNF antagonists. For dural sinus thrombosis corticosteroids are recommended. |
| 8 | Cyclosporine A should not be used in BD patients with CNS involvement unless necessary for intraocular inflammation. |
| 9 | The decision to treat skin and mucosa involvement will depend on the perceived severity by the doctor and the patient. Mucocutaneous involvement should be treated according to the dominant or codominant lesions present. Topical measures (i.e., local corticosteroids) should be the first line of treatment for isolated oral and genital ulcers. Colchicine should be preferred when the dominant lesion is erythema nodosum. *Dapsone may also be used.* Leg ulcers in BD might have different causes. Treatment should be planned accordingly. *Thalidomide*, azathioprine, IFN-α, and TNF antagonists may be considered in resistant cases. |

*The authors' modifications to the recommendations are noted in italics.
BD, Behçet's Disease; CNS, central nervous system; EULAR, European League Against Rheumatism; IFN-α, interferon-alpha; TNF, tumor necrosis factor.
From Hatemi G, Silman A, Bang D, et al: EULAR recommendations for the management of Behçet disease. *Ann Rheum Dis* 67:1656, 2008.

birth defects, and all patients and prescribing physicians must adhere to the System for Thalidomide Education and Prescribing Safety (STEPS) protocol, including monthly follow-up visits.[85] Patients receiving thalidomide can be monitored with nerve conduction studies for the development of peripheral symmetrical sensory neuropathy, if such monitoring is warranted on the basis of the clinical neurologic evaluation. Notably, the cost of treatment increased drastically when thalidomide was approved for the treatment of multiple myeloma.

Low-dose oral methotrexate (2.5 to 25 mg/wk) and low-dose prednisone are alternatives for patients with severe mucocutaneous involvement.[86] Patients receiving methotrexate should be monitored for hepatotoxicity and leukopenia. The risk of rebound after the tapering or discontinuation of systemic prednisone greatly limits its use for mucocutaneous disease alone. In addition, IFN-α is effective for severe mucocutaneous lesions and some systemic manifestations.[87,88] A review of the safety and efficacy of IFN-α by Zouboulis and Orfanos[87] recommended a 3-month, high-dose regimen of 9 million units three times per week, followed by a low, maintenance dose of 3 million units, three times per week. Patients experience the usual flulike symptoms associated with this therapy.

## Systemic Disease

Patients with systemic disease such as ocular and cardiovascular abnormalities require immunosuppressive therapy, particularly in view of the risk of morbidity and mortality resulting from untreated disease. Systemic corticosteroids may be used alone or in combination with other immunosuppressive agents such as azathioprine, IFN-α, cyclosporine, cyclophosphamide, and chlorambucil.[89] The standard of care for eye disease is prednisone plus azathioprine.[90] If this combination is not successful, one of the aforementioned immunosuppressive agents or mycophenolate mofetil can be substituted for azathioprine.[89] Rituximab has also been effective in the treatment of severe ocular manifestations of Behçet's disease.[80] Several of these immunosuppressive agents have associated hematologic toxicity, as well as the potential for the development of associated malignancies; therefore, close monitoring is essential. There have also been reports of Behçet's disease treated with anti-TNF agents.[91] A double-blind, placebo-controlled trial showed that etanercept was successful in suppressing most of the mucocutaneous manifestations of Behçet's disease.[92] Other reports support the efficacy of adalimumab and infliximab.[93,94,95] In 2008, the European League Against Rheumatism (EULAR) developed a standard of recommendations for the management of the various systemic manifestations of Behçet's disease (see Table 93-3).

# OUTCOME

Behçet's disease has a variable clinical course in most patients with a pattern of exacerbations and remissions. A delay in diagnosis after the initial manifestation of Behçet's disease is common. Most patients present initially with mucocutaneous manifestations, and evidence of ocular and neurologic involvement may appear several years after diagnosis. Patients with the finding of complex aphthosis may

represent a *forme fruste* of Behçet's disease, and they should be monitored for the development of additional abnormalities fulfilling the diagnostic criteria through regular follow-up and referral to appropriate specialists.[74] The greatest morbidity is associated with ocular manifestations (two-thirds of patients), vascular disease (one-third of patients), and CNS disease (10% to 20% of patients).[96] Ocular involvement is the most common cause of morbidity primarily manifesting as posterior uveitis and retinal vasculitis which can result in blindness. Mortality in Behçet's disease is low and is usually related to pulmonary or CNS involvement or to bowel perforation.[9]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 94

# Etiology and Pathogenesis of Hyperuricemia and Gout



Robert T. Keenan • Svetlana Krasnokutsky • Michael H. Pillinger

**KEY POINTS**

- Uric acid is the biologically active end product of human purine metabolism.
- Serum urate concentrations are determined by the balance between urate production and elimination; hyperuricemia results from urate overproduction, urate under excretion, or a combination of both.
- Specific organic anion transporters play a central role in urate excretion by the kidney.
- Hyperuricemia is defined as serum urate levels greater than 6.8 mg/dL, the limit of its solubility in serum.
- Gout pathogenesis requires the accumulation of monosodium urate at levels sufficient to drive the precipitation of crystals.
- Monosodium urate crystals drive inflammatory responses, including activation of the NLRP3 inflammasome, a multimolecular cytosolic complex that processes and generates IL-1β.
- The initiation of gouty inflammation by local leukocytes induces an influx of neutrophils into the joint; when neutrophils encounter urate crystals, they become activated and propagate further inflammation.
- Inflammation persists in chronic and tophaceous gout; macrophages continue to produce cytokines and proteases, thereby facilitating cartilage and bone destruction.

The ancient disease *gout* has a complex pathogenesis, and its modern relevance is underscored by a rise in prevalence by as much as fourfold in the past half century. Indeed, gout is now the most common inflammatory arthritis in the United States.[1,2] Gout is a disease of both metabolism and inflammation. Gout pathogenesis requires the intersection of two distinct processes: (1) the intrinsic formation of uric acid, in the form of urate, at levels sufficient to drive the precipitation of monosodium urate into crystallized forms, and (2) an inflammatory response to the crystals formed in this manner. How these processes occur and the circumstances under which they cross from adaptive to pathologic responses are the subjects of this chapter.

## EVOLUTIONARY CONSIDERATIONS

**KEY POINT**

- High baseline levels of serum urate in humans may be the result of evolutionary pressures in a prior era when hyperuricemia provided survival benefits.

The metabolic production of uric acid is ubiquitous among mammals and many other forms of animal life, and it is important to recognize that urate generation is not *a priori* pathologic. Indeed, the production of uric acid may serve one or possibly a multitude of beneficial roles, an area of interest to molecular immunologists and molecular anthropologists alike.

### Uric Acid as a Danger Signal

Uric acid is a breakdown product of purine metabolism. As such, it represents a metabolic waste molecule that might, in theory, be nothing more than a nuisance requiring excretion. However, evolution has co-opted this waste-generating process to play an important and perhaps critical role in organismal immunity. It has long been appreciated that the lysates of damaged mammalian cells can serve effectively as adjuvants—that is, they can promote immune responses to injected antigens. Recently Shi et al[3] used classic biochemical techniques to demonstrate that the major endogenous adjuvant found in damaged cells was uric acid. These investigators further demonstrated that uric acid has the capacity to promote T cell activation in response to antigen and that aggressive urate-lowering treatment could abrogate murine immune responses. Thus uric acid may serve as a *danger signal* to promote immune responses. As first proposed by Matzinger,[4] a danger signal is an intrinsically produced molecule, typically issued by an altered or damaged cell to alert the immune system to the need for an immunologic response. Viewed from this perspective, the production of uric acid in a virally infected cell, for example, might serve as an

Video available on the accompanying ExpertConsult website.

upstream "second signal" to promote antigen presentation by professional antigen-presenting cells such as dendritic cells, macrophages, or B cells. Indeed, although damaged or dying cells tend to have limited ability to manufacture proteins, their output of uric acid characteristically increases during cellular breakdown. The uric acid danger signal might also play an important role in tumor immunity, and at least one mouse model suggests that modulation of uric acid levels may directly affect immune tumor rejection.[5] Although these observations require more study, they are consistent with a paradigm in which urate production at the local cellular level may play an important role in immunity and homeostasis.

## Uric Acid and Human Evolution

Most mammals have serum urate levels in a range roughly between 0.5 and 2 mg/dL. In contrast, humans and other primates, including great apes and some New World monkeys, typically demonstrate serum urate levels between 4 and 6 mg/dL. The genetic and biochemical basis for these increases is well appreciated. During the Miocene era (10 to 25 million years ago), mutations in various primate species resulted in inactivation of the uricase gene, which codes for the enzyme that degrades uric acid to allantoic acid. Genetic studies indicate that the uricase gene underwent nonsense and other mutations not once but multiple times, across multiple hominoid lineages (Figure 94-1).[6-8] The occurrence of multiple independent loss-of-function mutations has led some biologists to hypothesize that increases in urate generation may have conferred a survival benefit for these particular species. Several compelling and not necessarily mutually exclusive hypotheses have been proposed.

These same primate species had also been subject to a unique deletion of the gene permitting organisms to produce ascorbic acid, an event that occurred in the Eocene era, 10 to 20 million years before the uricase deletions.[9] In mammals that produce it, ascorbic acid serves as the pre-eminent antioxidant in the body. Thus the loss of ascorbate production may have created an evolutionary liability, for which increases in urate provided antioxidant compensation. Other authors have suggested that the effects of hyperuricemia provided an evolutionary advantage by promoting hominoid intellectual function, either through its antioxidant effects or via activation of neurostimulatory adenosine receptors (in a manner similar to that of caffeine).[10] Although the antioxidant theory would appear compelling, critics have pointed out that (1) the production of urate itself generates oxidant molecules, diminishing any possible antioxidant benefit; (2) intra-cellular urate may have pro-oxidant effects[11]; and (3) the human/primate capacity for antioxidation may be large even in the absence of soluble antioxidants (e.g., human red blood cell membranes have extensive antioxidant capacities).[12]

Other investigators have examined the specific evolutionary pressures exerted on primate species during the Miocene era in an attempt to understand the potential advantages of urate elevations. Johnson and colleagues[13] pointed out that rapid hominoid evolution occurred during the Miocene era and that the hominoid diet at that time appears to have been mainly vegetarian and extremely low in salt. These investigators suggest that hominoids during that period may have experienced a "hypotensive crisis," particularly in the face of the transition to upright walking. They further postulate that an elevation in serum uric acid levels provided a mechanism for restoring normotension, primarily through urate-induced renovascular injury.[13,14] To model this hypothesis, these investigators exposed rats to a low-salt diet, resulting in hypotension. When treated with the uricase inhibitor oxonic acid (to mimic the primate uricase loss), the rats' urate levels rose and their blood pressures normalized. The effects of uricase inhibition on blood pressure could be reversed through the use of the urate-lowering agent allopurinol. These observations imply that what may once have been a homeostatic adaptation could now contribute to essential hypertension in today's salt-rich era. In support of the latter hypothesis, Feig and colleagues[15] identified adolescents with premature essential hypertension and hyperuricemia and treated them with the urate-lowering agent allopurinol. The result was normalization in blood pressure that reversed after allopurinol discontinuation. More recent studies have implicated elevated levels of uric acid as having a possible role in increased gluconeogenesis, with potential metabolic benefit in the Miocene era (which was also calorie poor), but perhaps contributing to the incidence of diabetes in our current calorie-rich environment.[16,17]

Although the loss of uricase promoted serum urate increases in humans and other hominoids, the levels of serum urate so achieved are generally insufficient to promote urate crystallization and gout. Rather, uricase inactivation created the circumstances under which additional increases in urate production, or impairments in urate excretion, can result in serum urate concentrations exceeding the solubility threshold. Accordingly, we next review the mechanisms of urate production and excretion, as well as the events that may tip the scales toward pathologic hyperuricemia.

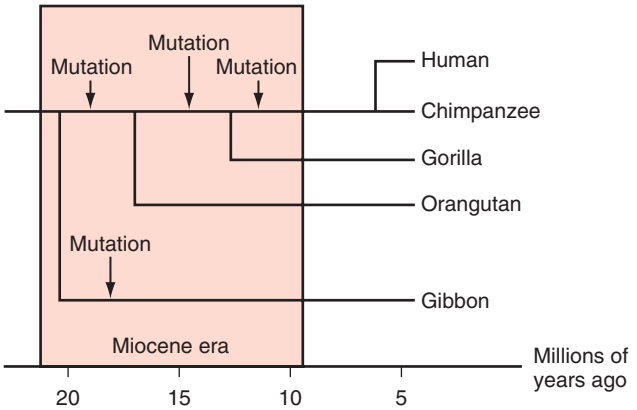



**Figure 94-1** Mutations affecting the uricase gene. During the Miocene era, hominoid species experienced not one but multiple different mutations, resulting in the loss of uricase activity. The survival of multiple species harboring different mutations suggests that loss of uricase may have conveyed a survival advantage, even as it established the necessary precondition for hyperuricemia and gout. *(Modified with permission from Wu XW, Muzny DM, Lee CC, Caskey CT: Two independent mutational events in the loss of urate oxidase during hominoid evolution,* J Mol Evol *34:78–84, 1992. Copyright Springer-Verlag GmbH.)*

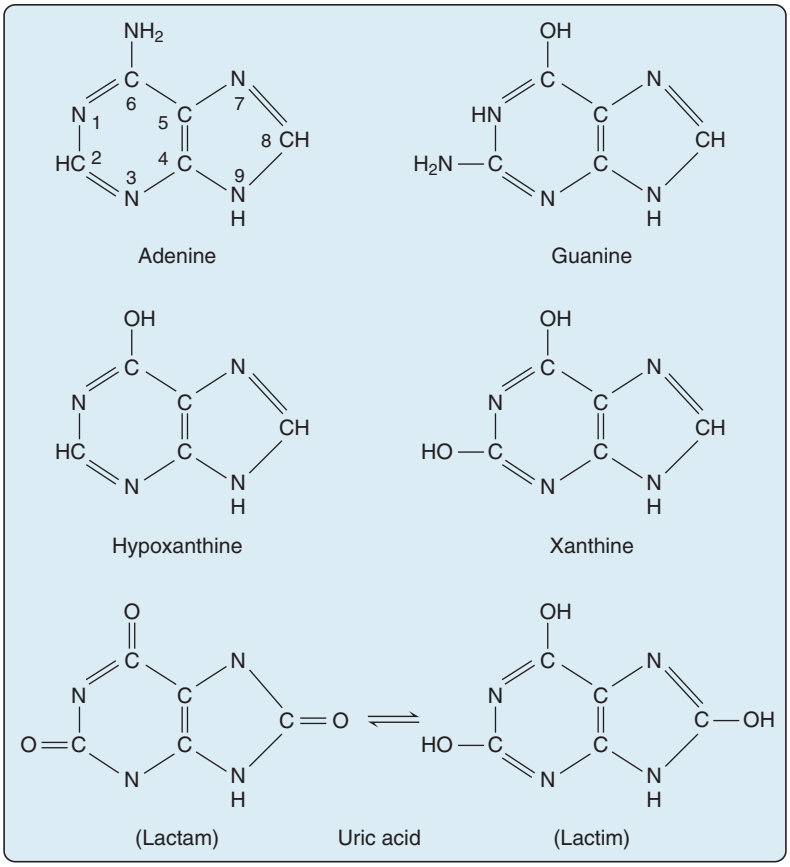



**Figure 94-2** Structure of uric acid and common purines. All purine bases may exist in the lactam form in a reversible manner as shown for uric acid.

## URIC ACID PRODUCTION AND EXCRETION: NORMAL LEVELS AND HYPERURICEMIA

Uric acid is a breakdown product of purines, and uric acid generation depends directly on both intrinsic purine production and purine intake. In humans, uric acid is an end-product metabolite; consequently, the depletion of uric acid depends directly on its excretion. The balance between uric acid production and excretion determines the serum urate level. Most people maintain a relatively stable uric acid level between 4 and 6.8 mg/dL and a total body uric acid pool of approximately 1000 mg.[18] Persons with serum uric acid levels greater than 6.8 mg/dL, even without clinical gout, may deposit uric acid crystals either occultly or in the form of appreciable masses (tophi), with the consequence that the total body urate pool may be significantly higher than in persons who are not hyperuricemic.[19,20] Such occult deposition of uric acid (the total body urate burden) may have implications for treatment because it may form a "buffering reservoir" of urate that resists initial treatment with urate-lowering agents.

### Urate Production: Purine Metabolism and Intake

**KEY POINT**

**Uric acid is the end product of human purine metabolism.**

Uric acid production depends on both purine biosynthesis and the metabolic processes that convert purines into uric acid.

#### Purine Biosynthesis

Purines are heterocyclic aromatic compounds consisting of conjoined pyrimidine and imidazole rings (Figure 94-2). In mammals, the most common expression of purines is found in the form of DNA and RNA (containing the purines adenine and guanine), as well as single-molecule nucleotides (adenosine triphosphate [ATP], adenosine diphosphate [ADP], adenosine monophosphate [AMP], cyclic AMP, and to a lesser extent, guanosine triphosphate [GTP] and cyclic guanosine monophosphate [cGMP]). Purines are also critical elements of the following energy metabolism molecules: reduced nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate (NADPH), and coenzyme Q. Purines may also serve as direct neurotransmitters; for example, adenosine may interact with receptors to modulate cardiovascular and central nervous system (CNS) function.[21]

Purine biosynthesis is initiated on a backbone of ribose-5-phosphate (Figures 94-3 and 94-4). The enzyme phosphoribosyl pyrophosphate (PRPP) synthase catalyzes the addition of a pyrophosphate moiety to form the adduct PRPP. This reaction is thought to be rate limiting. Subsequently, the enzyme glutamine-PRPP amidotransferase catalyzes the interaction of PRPP and glutamine to form

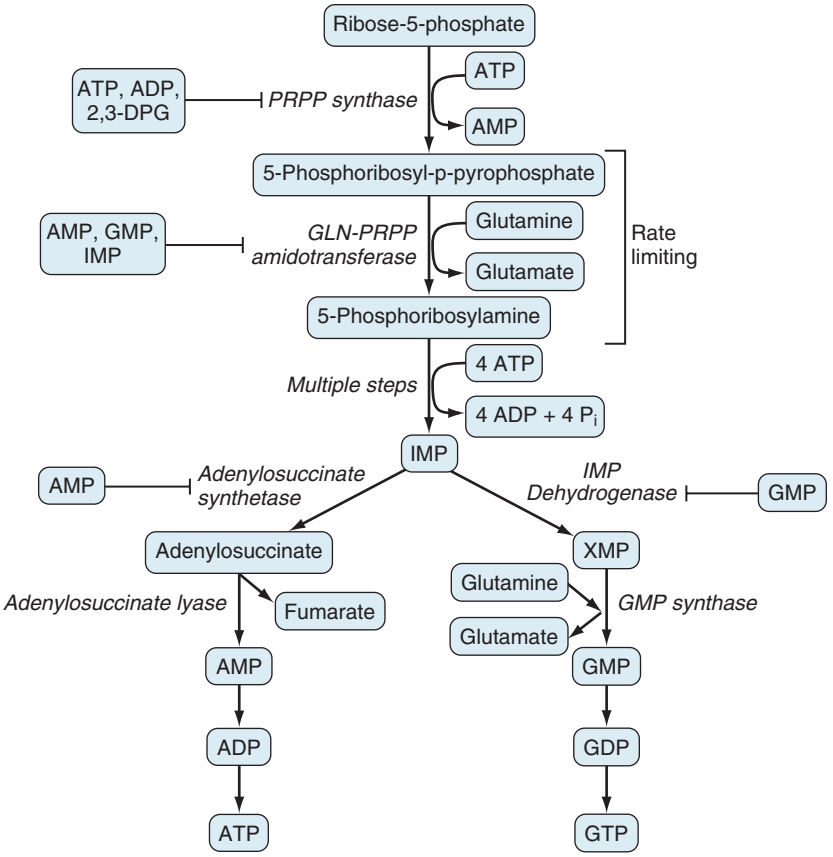



**Figure 94-3** Purine biosynthesis (see text for details). ADP, Adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate; GDP, guanosine diphosphate; GLN, glutamine; GMP, guanosine monophosphate; GTP, guanosine triphosphate; IMP, inosine monophosphate; PRPP, phosphoribosyl pyrophosphate; XMP, xanthine monophosphate.

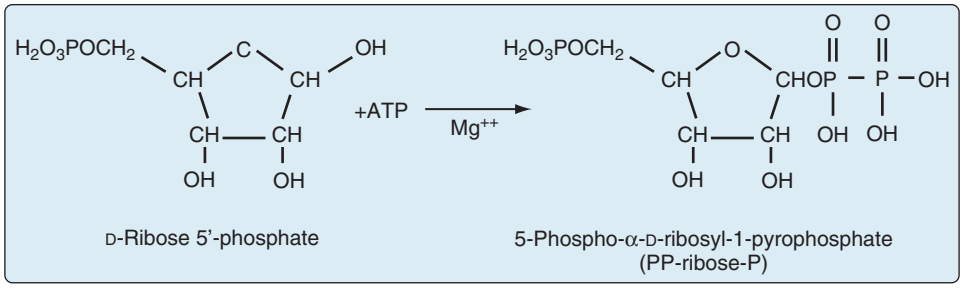



**Figure 94-4** Formation of phosphoribosyl pyrophosphate by phosphoribosyl pyrophosphate synthase. In some patients, this reaction proceeds too rapidly, promoting hyperuricemia on the basis of primary overproduction of uric acid.

5-phosphoribosyl amine, the first committed step in purine biosynthesis; 5-phosphoribosyl amine next serves as the backbone for a series of molecular additions, ending in the formation of the purine inosine monophosphate (IMP). IMP is converted into either AMP or GMP, which can then be phosphorylated into higher energy compounds (e.g., ATP and GTP). Glutamine-PRPP amidotransferase and PRPP synthase are both subject to feedback inhibition by purines themselves, providing a mechanism to slow purine biosynthesis in the setting of purine sufficiency. Collectively, the process of purine biosynthesis is highly energy dependent, requiring the consumption of multiple molecules of ATP. Thus purine biosynthesis not only directly increases the substrate load for urate generation but also increases the turnover of already formed purines that contribute to increased urate levels.[22]

## Urate Formation and Purine Salvage

Purines generated by the previously described mechanisms are susceptible to enzymatic catabolism, presumably to maintain purine homeostasis (Figure 94-5). Purines susceptible to degradation include the monophosphate nucleotides GMP and IMP. These molecules are converted by nucleotidases to their purine base forms, guanosine and inosine. In contrast to GMP and IMP, AMP is not susceptible to nucleotidase activity. However, through the activity of adenylate deaminase, AMP can undergo conversion into IMP for further degradation. Additionally, adenosine deaminase can convert adenosine to inosine for inclusion in the degradative pathway. Further catabolism of both guanosine and inosine is mediated by the common enzyme purine nucleoside phosphorylase. Guanosine is converted to

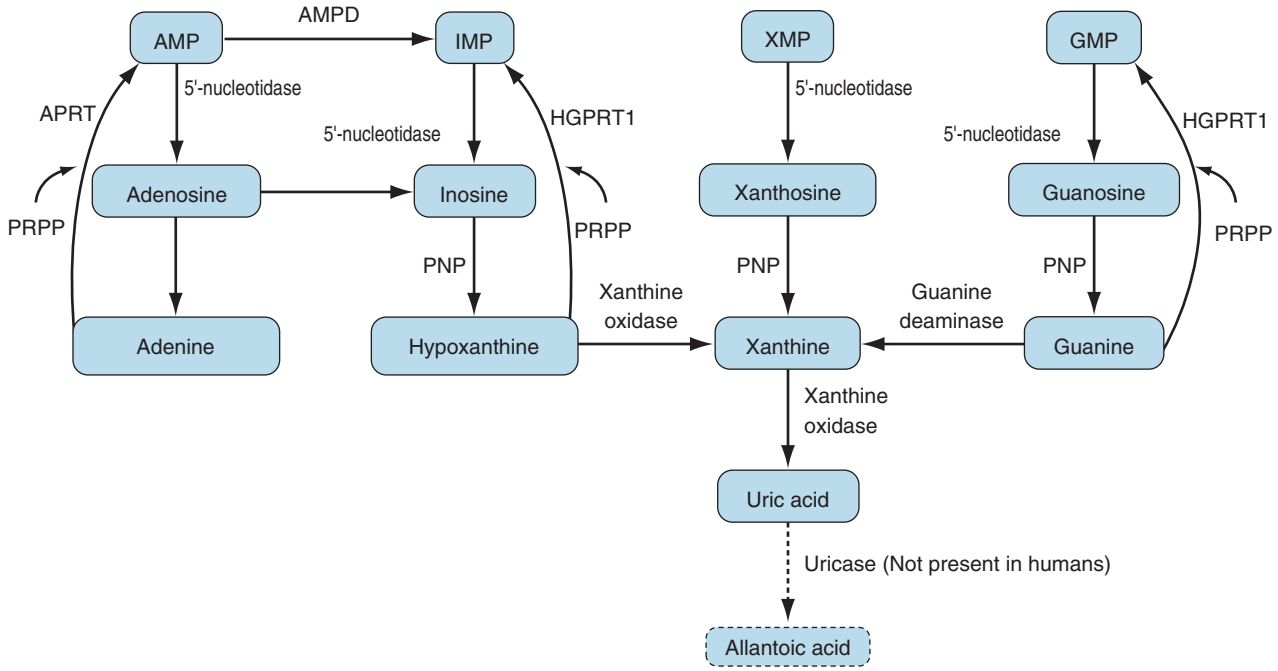



**Figure 94-5** Uric acid synthesis and purine salvage. Catabolism of purines, especially inosine monophosphate (IMP) and guanosine monophosphate (GMP), results in urate synthesis via the common substrate xanthine. Xanthine oxidase is necessary for urate synthesis from any purine and thus serves as a target for agents that inhibit uric acid synthesis (e.g., allopurinol and febuxostat). Purine salvage via hypoxanthine guanine phosphoribosyl transferase 1 (HGPRT1) returns hypoxanthine and guanine to IMP and GMP, respectively. HGPRT1 deficiencies result not only in increases in hypoxanthine and guanine and subsequent uric acid synthesis but also in the depletion of nucleotides that provide feedback inhibition on purine biosynthesis. As denoted in *gray,* mammals other than primates and some monkeys possess uricase, which converts uric acid to allantoic acid for further degradation. AMP, Adenosine monophosphate; AMPD, AMP deaminase; APRT, adenine phosphoribosyl transferase; PNP, purine nucleotide phosphorylase; PRPP, phosphoribosyl pyrophosphate; XMP, xanthine monophosphate.

guanine, whereas inosine is converted to hypoxanthine. Both guanine and hypoxanthine are subsequently converted to xanthine by the enzymes guanine deaminase and xanthine oxidase (also known as *xanthine dehydrogenase*), respectively. Xanthine from either source is then converted directly to uric acid, again by the action of xanthine oxidase. As previously noted, organisms other than humans and primates, including New World monkeys, possess an additional enzyme, uricase (urate oxidase), that converts uric acid to allantoic acid, a relatively soluble compound that can be further degraded to urea. Because of the lack of this enzyme, human and primate purine metabolism ceases with the production of uric acid.[23]

Presumably because purine synthesis is energy expensive for the cell, evolution has dictated that mechanisms exist to recover purines before they irreversibly traverse the degradative pathway. These pathways, collectively known as *purine salvage*, are intimately connected to the feedback regulation of purine synthesis. The major enzyme responsible for purine salvage, hypoxanthine/guanine phosphoribosyl transferase (HGPRT1, also known as HPRT1), catalyzes the transfer of a phosphoribose from PRPP to either hypoxanthine or guanine to form IMP or GMP, respectively (Figure 94-6). These products are then available for reinclusion in the available purine pool. A second salvage enzyme is adenine phosphoribosyl transferase, which restores adenine to AMP. However, as described earlier, most adenosine/adenine breakdown occurs via conversion to inosinic acid.

## Urate Overproduction: Primary and Secondary Causes

**KEY POINT**

**Hyperuricemia may result from urate overproduction as a result of primary or secondary causes.**

### Primary Urate Overproduction

In some patients, inborn errors of metabolism lead to urate overproduction and subsequent hyperuricemia. Several of these inborn errors of metabolism deserve mention. First, a small number of persons possess PRPP synthase enzymes that are hyperactive. The result is increased generation of PRPP. Because under normal circumstances PRPP concentrations are below the Michaelis constant of glutamine-PRPP amidotransferase for this substrate, increased PRPP levels drive amidotransferase activity and accelerate purine biosynthesis.

The second well-described class of abnormality leading to urate overproduction occurs within the purine salvage pathway. Deficiencies of HGPRT1 result in impaired purine salvage and increased substrate for uric acid generation. Additionally, because purine salvage normally results in nucleotide monophosphate generation, patients with purine salvage failure experience lower levels of nucleotide monophosphates, resulting in loss of feedback inhibition of both PRPP synthase and glutamine-PRPP amidotransferase.

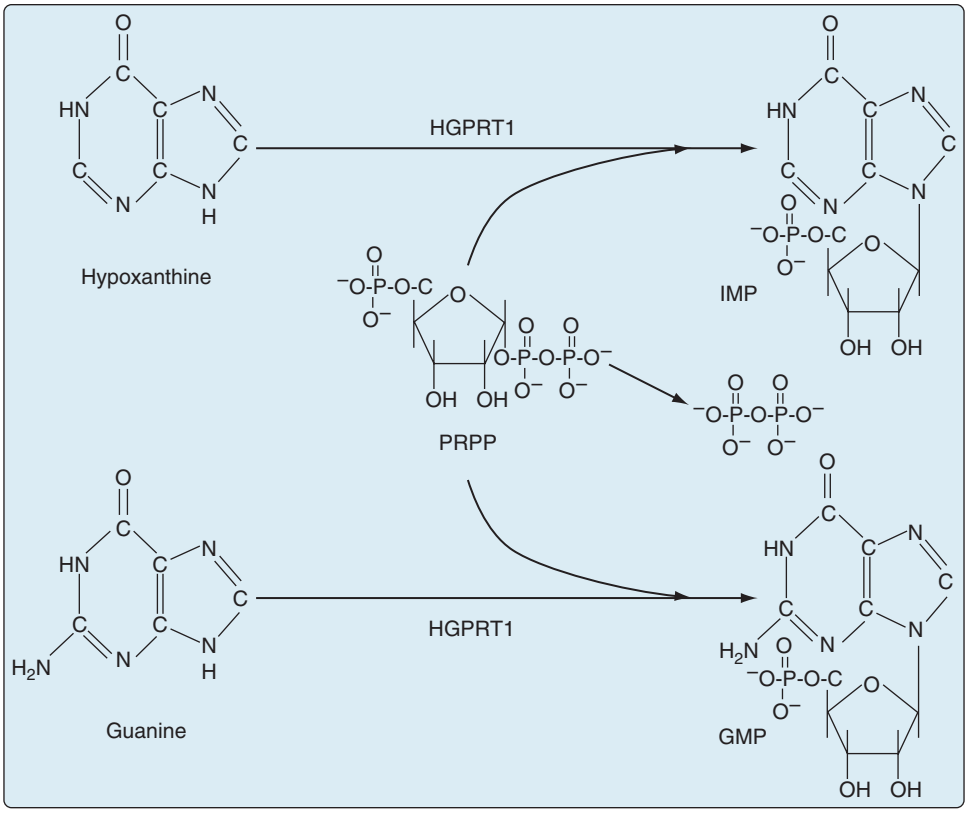



**Figure 94-6** Actions of hypoxanthine/guanine phosphoribosyl transferase 1 (HGPRT1) result in salvage of hypoxanthine and guanine to inosine monophosphate (IMP) and guanosine monophosphate (GMP). Loss of HGPRT1 activity results in hyperuricemia and, in severe cases, the neurologic deficits of Lesch-Nyhan syndrome. PRPP, Phosphoribosyl pyrophosphate.

Thus purine salvage failure resulting from HGPRT1 deficiency is compounded by purine overproduction. Recent studies have identified mutations in *ALDH16A1* (a member of the aldehyde dehydrogenase superfamily) that may promote gout by impairing the function of HGPRT1.[24]

Two major variants of HGPRT1 deficiency have been described. Complete HGPRT1 deficiency, better known as *Lesch-Nyhan syndrome*, is an X-linked recessive disorder characterized by extremely high levels of serum urate, gout flares, nephrolithiasis, mental retardation, movement disorders, and behavioral disorders, including self-mutilation. The disorder can occasionally arise by de novo mutation; female carriers are generally asymptomatic but may have elevated serum urate levels. In contrast to the gout flares and nephrolithiasis, the neurologic findings in Lesch-Nyhan syndrome are independent of hyperuricemia and unresponsive to urate-lowering drugs. Life expectancy can be greatly reduced, and these patients rarely come to the attention of adult rheumatologists.[25]

In contrast to people with Lesch-Nyhan syndrome, persons with Kelley-Seegmiller syndrome have a partial deficiency of HGPRT1[26]; they present with hyperuricemia and gout but have limited or no neurologic symptoms.[27] Several variants of Kelley-Seegmiller syndrome have been described, based on the extent of HGPRT1 inactivity and the presence or absence of neurologic findings. Kelley-Seegmiller mutations tend to occur in regions of the *HPRT1* gene other than the regions identified in persons with Lesch-Nyhan syndrome (whose mutations typically localize to the PRPP-binding region); whether such differences influence the presence or absence of neurologic symptoms has not been determined.[28]

Several hereditary defects of energy metabolism also promote hyperuricemia, mainly as a consequence of ATP consumption. Persons with glucose-6-phosphatase deficiency (type I glycogen storage, or von Gierke's disease) demonstrate a high rate of both purine and ATP turnover. The lactic acidemia that secondarily occurs in patients with glucose-6-phosphatase deficiency may also contribute to hyperuricemia by promoting decreased renal urate excretion (discussed in a subsequent section).[29] Persons with fructose-1-phosphate aldolase deficiency lack the capacity to metabolize fructose-1-phosphate. Fructose-1-phosphate accumulation causes feedback inhibition of fructokinase and fructose accumulation in the blood. As an apparent consequence of these changes, AMP accumulates and promotes hyperuricemia by the mechanisms previously described.[30] The role of fructose intake in patients without inborn errors of fructose metabolism is discussed in a subsequent section.

## Secondary Urate Overproduction and Hyperuricemia

A number of secondary causes can lead to urate overproduction and hyperuricemia. In most cases, these conditions induce increased cell turnover, with resultant purine

generation and breakdown. Chief among these must be counted diseases of erythropoietic, lymphopoietic, and myelopoietic cell turnover of both malignant and nonmalignant varieties. Among the erythropoietic diseases causing hyperuricemia, autoimmune and other hemolytic anemias (i.e., red blood cell destruction with compensatory increased red blood cell generation), sickle cell disease,[31,32] polycythemia vera,[33] and ineffective erythropoiesis (e.g., in pernicious and other forms of megaloblastic anemia, thalassemia, and other hemoglobinopathies) must be included. Patients with myeloproliferative and lymphoproliferative disorders including myelodysplastic syndrome, myeloid metaplasia, leukemias, lymphomas, and paraproteinemic diseases such as multiple myeloma and Waldenström's macroglobulinemia are also at increased risk for hyperuricemia.[34-36] In some cases, particularly in the pediatric setting, hyperuricemia and concomitant renal failure may be the first presentation of these malignancies.[37] Indeed, the level of hyperuricemia may correlate with the degree of disease and cell turnover. Patients with essential thrombocytosis may also be at increased risk for hyperuricemia.[38] An association between solid tumors and hyperuricemia has been reported[39]; given the slower turnover of solid tumor cells, solid tumor hyperuricemia tends to be less common and less severe than that seen in malignancies of bone marrow–derived cells.

Tumor lysis syndrome represents a unique form of tumor-related hyperuricemia in which cell death induced by chemotherapy not only results in hyperuricemia but also hyperphosphatemia, hyperkalemia, and hypocalcemia, often resulting in acute renal failure and arrhythmias. Although tumor lysis syndrome occurs most commonly during treatment for hematologic malignancies,[40] it also may occur during treatment of solid tumors.[41,42] Although not well documented in the literature, we have noted hyperuricemia subsequent to the use of granulocyte colony-stimulating factor (G-CSF) for myelofibrosis-associated anemia. Such use of CSFs may secondarily contribute to the new onset of gout.[43]

Although somewhat more controversial, the increased cell turnover in patients with psoriasis has also been associated with elevated levels of serum urate.[44,45] An association between sarcoidosis and hyperuricemia has also been proposed,[46] again presumably relating to increased cell turnover and/or metabolic activity. However, the epidemiologic evidence supporting sarcoidosis as a cause of hyperuricemia is less than convincing.

Conditions leading directly to the physiologic consumption/degradation of ATP also contribute to the potential for secondary purine turnover leading to hyperuricemia. Thus strenuous and prolonged exercise, particularly to levels driving anaerobic respiration, may induce transient serum urate elevations.[47,48] *Status epilepticus* is likely to mimic these events. A number of acute illnesses, including myocardial infarction and sepsis, are also accompanied by ATP catabolism and may result in transient hyperuricemia.[49] Patients with hereditary myopathies including metabolic myopathies such as glycogen storage disease types III, V, and VII (debranching enzyme deficiency, myophosphorylase deficiency, and muscle phosphofructokinase deficiency, respectively), as well as mitochondrial myopathies (including carnitine palmitoyltransferase deficiency and myoadenylate deaminase deficiency), are susceptible to increases in serum urate levels after even moderate exercise.[39,49,50] In these persons, a limited ability to synthesize ATP on demand apparently results in a rapid turnover of established ATP pools during exercise, with resultant purine and uric acid formation. Patients with medium-chain acyl-coenzyme A dehydrogenase deficiency, a defect of fatty acid metabolism, also have been shown to have elevated levels of serum urate, although the mechanism for this effect is not clear.[51]

Despite multiple potential mechanisms, abnormalities of urate overproduction account for only about 10% of patients with hyperuricemia.

## Urate Excretion: Gastrointestinal and Renal Mechanisms

**KEY POINT**

**Urate excretion occurs via the gut and the kidneys. In the kidneys, a complex series of urate transport proteins mediates a net elimination of sodium urate.**

In most patients, the serum urate level is maintained within a narrow range. Accordingly, mechanisms must exist to ensure disposal of urate, either by metabolism or excretion. As previously noted, humans possess minimal or no capacity to metabolize urate; therefore, urate excretory mechanisms play a critical role in homeostasis. The gastrointestinal tract and the kidneys each participate in urate excretion.

### Gastrointestinal Excretion of Urate

Uric acid elimination via the gastrointestinal tract has been recognized for more than 50 years but has been the subject of relatively little study. On the basis of radiolabeled urate tracer studies, Sorensen and Levinson[52-54] estimated that in healthy persons, the gastrointestinal tract is responsible for the excretion of 20% to 30% of the daily uric acid burden. Gastrointestinal uric acid excretion may become more important in settings of renal insufficiency, however, in view of animal studies suggesting that uric acid excretion via the gut may increase in a compensatory manner in the setting of renal failure and decreased renal uric acid excretion.[55] Mechanisms of uric acid transport into the gut appear to include both exocrine secretion (e.g., saliva, gastric, and pancreatic juices) and direct bowel secretory mechanisms and appear to involve a urate transporter known as ATP-binding cassette transporter G2 (ABCG2), which also plays a role in renal urate excretion (discussed in a subsequent section).[39,56] Uric acid is apparently excreted into the gut in its native form and then undergoes degradation by intestinal flora.[54]

### Renal Excretion of Uric Acid: Normal Mechanisms

In all but the most extreme circumstances of renal failure, the kidney is the primary organ for uric acid excretion. The mechanisms of renal urate excretion are complex and involve multiple steps. In the bloodstream, uric acid (in the form of urate anion) is considered to be completely or nearly completely unbound to plasma proteins. As a result, nearly 100% of the urate load entering the renal afferent arteriole

undergoes ultrafiltration by the glomerulus. As discussed further in a subsequent section, decreases in glomerular function therefore reduce urate filtration and promote increases in serum urate levels.

After ultrafiltration, urate (initially in a monovalent ion form) in the proximal tubule undergoes distinct but apparently simultaneous handling steps: (1) a *resorption step*, in which as much as 90% to 98% of the filtered urate undergoes reclamation; and (2) a *secretion step*, in which resorbed urate is transferred back into the tubule lumen. The net result is excretion of approximately 10% of the filtered load. In fully functioning nephrons, these steps are responsive to serum urate levels, such that rises in serum urate induce increased renal excretion and restoration of total body urate homeostasis. Studies have emphasized the importance of organic anion transporters (OATs) and other active and passive transport molecules in the movements of urate in both directions across the proximal tubule (Figure 94-7).[57,58]

**Urate Resorption.** Urate *resorption* in the proximal tubule depends on the action of several apical surface transporters and at least one resorption transporter at the basolateral surface (see Figure 94-7). In humans, the most important of the apical-surface transporters appears to be URAT1 (gene *SLC22A12*). URAT1 acts as a urate/anion exchanger to transfer urate from the tubule lumen to the epithelial cytosol. The major inorganic counter ion for URAT1 activity is $Cl^-$. However, organic anions such as lactate, pyrazinoate, and nicotinate can substitute for chloride, with potential clinical consequences, as discussed subsequently. The importance of URAT1 to renal urate resorption is indicated by the fact that patients with inactivating mutations of URAT1 excrete nearly 100% of their filtered urate and demonstrate low serum urate levels (along

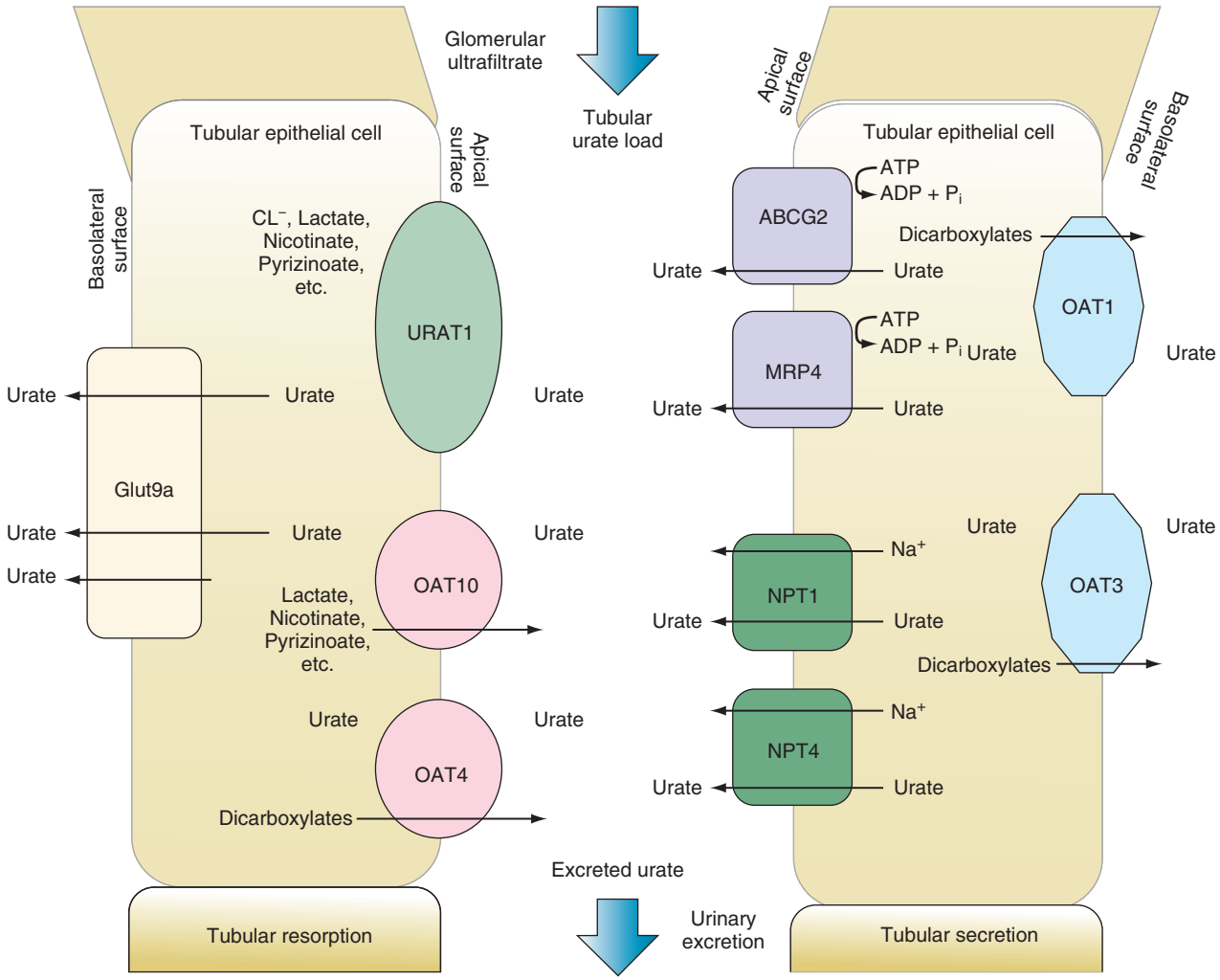



**Figure 94-7** Renal tubular handling of urate. Both urate resorption and urate secretion are handled by the epithelial cells of the proximal tubule. For simplicity, resorption is shown on the *left side* and secretion on the *right side* of the figure. **Resorption:** Multiple apical transporters (URAT1, organic anion transporter [OAT]4, and OAT10) move urate from the proximal tubule ultrafiltrate into the epithelial cytosol. Of these transporters, URAT1 appears to be most important. Both inorganic ($Cl^-$) and organic (e.g., lactate, nicotinate, and pyrazinoate) counter ions promote urate transport at this step; therefore, rises in organic acid levels can promote urate retention and hyperuricemia. Urate is subsequently transported from the cell to the interstitium by the basolateral transporter Glut9a. **Secretion:** The organic anion transporters OAT1 and OAT3 move urate from the interstitium to the epithelial cell interior, using dicarboxylates as counter ions. Urate within the epithelial cell is moved out to the tubular fluid by multiple transporters. Urate secretion by some transporters (ABCG2 and MRP4) is adenosine triphosphate (ATP) dependent, whereas other transporters (NPT1 and NPT4) move urate via cotransport of $Na^+$. Accordingly, sodium depletion can promote hyperuricemia. ADP, Adenosine diphosphate.

with increased urinary uric acid levels and risk for uric acid kidney stones).[59-63] Moreover, several well-established uricosuric drugs including probenecid, benzbromarone, losartan, and high-dose salicylates act primarily by inhibiting URAT1.[64,65] This understanding of URAT1 activity has recently led to the development and approval of lesinurad in the United States, making it the first uricosuric drug specifically designed as a URAT1 inhibitor. Conversely, other mutations in the URAT1 gene appear to convey a risk for increased urate resorption and hyperuricemia, presumably through a gain-of-function effect.

OAT4 (gene *SLC22A11*) and OAT10 (gene *SLC22A8*) are two other apical anion transporters involved in renal uric acid resorption. Like URAT1, OAT10 is an anion exchange transporter; as with URAT1, counter ions that can promote urate transport by OAT10 include lactate, pyrazinoate, and nicotinate. In contrast, although OAT4 also transports urate from the tubular lumen to the cytosol of renal epithelial cells, the counter ions it employs tend to be dicarboxylates.[57,66]

Urate transport by URAT1, OAT4, and OAT10 would lead to accumulation of urate intra-cellularly in renal tubule cells and presumably to gradients that eventually would impair further urate uptake if a mechanism did not exist to transport intra-cellular urate out of the cell at the basolateral surface. This function appears to be served by Glut9a (gene *SLC2A9*, also confusingly known as *URATv1*), which was first identified as a glucose transport family protein but has little or no glucose transport capacity. Instead, Glut9a is an effective transporter of urate from the renal epithelial cell out into the renal interstitium.[67,68] A number of different inactivating mutations of Glut9a have been identified in both humans and mice; in each case the result is impaired urate resorption, increased urate excretion, and hypouricemia.[67,69-74] Glut9a and its splice variant, Glut9b, are also expressed on other cells including chondrocytes, leukocytes, intestinal cells, hepatocytes, and vascular smooth muscle cells; the role of Glut9 proteins (and the impact of probenecid, which inhibits Glut9, as well as URAT1) on these cells is actively being explored.[75-77]

**Urate Secretion.** Other transport proteins in the renal tubule epithelium regulate the *secretion* of urate from the peritubular interstitial fluid into the tubular lumen (see Figure 94-7). At the basolateral surface, OAT1 and OAT3 (genes *SLC22A6* and *SLC22A8*, respectively) transport urate from the interstitium into the epithelial cell cytosol. These transporters act via exchange with dicarboxylate anions and transport not only urate but other organic anions and some drugs.[78] At the apical surface of proximal tubule cells, two proteins have been identified that serve as urate-extruding transporters. The multidrug resistance protein MRP4 (gene *ABCC4*) mediates ATP-dependent urate transport.[79] Whether energy failure promotes hyperuricemia by impairing MRP4 has not been established but would seem plausible. The ABCG2 transporter (gene *ABCG2*) also directly mediates renal urate excretion.[80,81] Genetic association studies have implicated two other anion transport proteins as playing a role in apical urate transport outside of the cell, namely NPT1 (gene *SLC17A1*) and NPT4 (gene *SLC17A3*).[80,82-84] Additionally, genetic studies have implicated nontransporter proteins as playing roles in urate excretion, including PDZK1, CARMIL, NHERF1, SMCT1, SLC5A8, SMCT2, and SLC2A12. It is thought that some of these proteins may contribute to a macromolecular complex regulating urate transport.[85]

## Renal Causes of Hyperuricemia

**KEY POINT**

**Decreased renal excretion of urate because of intrinsic or secondary causes results in elevated levels of serum urate.**

Most patients with hyperuricemia underexcrete urate; that is, for any given serum urate level, their degree of renal urate excretion is inadequate and less than is seen in normal control subjects (Figure 94-8). The mechanisms of urate underexcretion are various and stem from either primary or secondary renal effects.

### Primary Urate Underexcretion

In a subset of patients, hereditary defects of renal tubule urate excretion result in hyperuricemia. With the identification of the aforementioned renal urate transporters, the underlying basis for some of these defects has become apparent. For example, ABCG2 loss of function mutations promote hyperuricemia, and up to 10% of gout cases in white Europeans may be attributable to hyperuricemia induced by defects in the urate exporter ABCG2.[80,81,86] As previously noted, ABCG2 also appears to play an important role in gastrointestinal urate excretion, and ABCG2

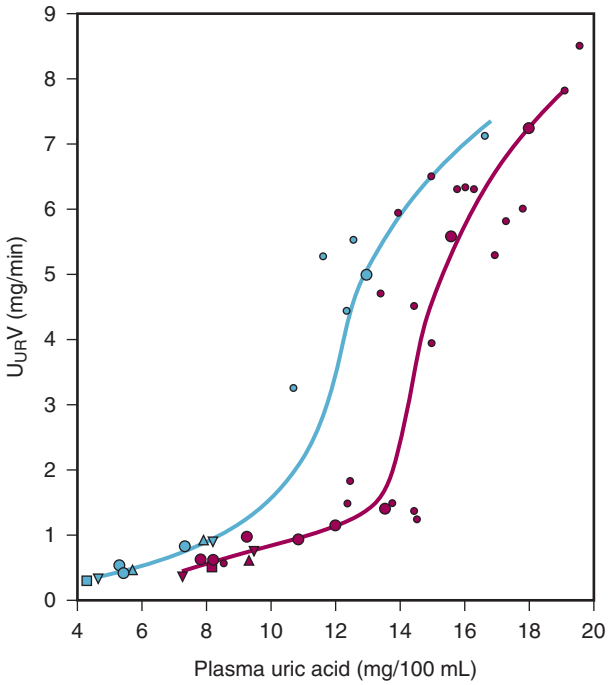



**Figure 94-8** Plasma uric acid versus renal urate excretion in underexcreters versus normal control subjects. The *red line* and *red symbols* represent urate underexcreters; the *blue line* and *blue symbols* represent normal control subjects. For any given plasma urate level, the patients who underexcrete urate demonstrate a lower degree of urinary urate; they thus require higher serum urates to produce urate-comparable urate excretion. (Large and small circles represent mean and representative individual data for the experiment, respectively.) $U_{UR}V$, Urine urate excretion rate. *(From Wyngaarden JB: Gout,* Adv Metabol Dis *2:2, 1965.)*

dysfunction may therefore also contribute to urate retention and hyperuricemia on a gastrointestinal basis.[87,88] Conversely, gain of function mutations in several transport-related proteins (e.g., PDZK1, CARMIL, and NHERF1) may promote hyperuricemia by enhancing URAT1 (i.e., renal urate resorption) activity. Patients with intrinsic tubular defects leading to net urate underexcretion typically display normal renal filtration and normal serum creatinine levels.

Familial juvenile hyperuricemic nephropathy (FJHN), also known as *medullary cystic kidney disease* (MCKD), constitutes a group of autosomal dominant disorders characterized by early hyperuricemia and progressive chronic kidney disease.[89] The hyperuricemia typically precedes the renal insufficiency and is considered to be primary. Three variants are recognized, designated MCKD 1, 2, and 3. In MCKD2, mutations in the uromodulin gene result in underproduction and/or misproduction of uromodulin (Tamm-Horsfall protein), the most prevalent protein secreted by the kidney.[90-92] The probable importance of uromodulin deficiencies to FJHN has recently been underlined by the observation that although patients with the MCDK1 and 3 subtypes of FJHN have mutations that are not in the uromodulin gene, they nevertheless display a phenotype of decreased uromodulin expression.[93] The mechanisms through which uromodulin deficiencies predispose to hyperuricemia are not yet understood, but mice transgenic for human uromodulin mutations experience renal tubular abnormalities and concentrating defects.[94]

### Secondary Causes of Renal Urate Underexcretion

A large number of underlying causes can result in nephrogenic retention of urate and subsequent hyperuricemia**.** These causes include acute or chronic renal failure, the effects of toxins and drugs, and systemic illnesses that alter renal urate handling directly or indirectly.[95]

**Age and Sex.** Classic studies by French and colleagues[96] documented that serum urate levels tend to be low in children. In males, urate levels increase precipitously at puberty, a time when females experience only a slight increase in serum urate. For women, a gradual increase over the subsequent years, followed by another rise at menopause, finally brings the serum urate level near to that of men, consistent with Hippocrates' astute aphorisms that "a young man does not take the gout until [around the time] he indulges in coition" and that "a woman does not take the gout, unless her menses be stopped."[96,97] This discrepancy between men and women, in the period between puberty and menses, suggests strongly that sex hormones play a role in urate regulation. Indeed, studies suggest that in women, both estrogen and progesterone may promote renal urate excretion.[98] Follicle-stimulating hormone has been associated with a slight increase in urate levels, though the mechanism remains unclear.[99] Conversely, an active role for androgens in promoting hyperuricemia may be inferred from Hippocrates' assertion that "Eunuchs do not take the gout" and from studies indicating that androgens and estrogens have opposing effects on renal organic anion transporters.[100] In pregnant women, increases in serum urate levels are characteristic of pre-eclampsia, a reproductive emergency consisting of hypertension and proteinuria. The hyperuricemia of pre-eclampsia is thought to result from that condition's renal dysfunction; hyperuricemia in pre-eclampsia does not lead to gout but is considered by some investigators to secondarily contribute to pre-eclamptic renal dysfunction in a positive feedback loop.[101]

**Systemic Illnesses.** Glomerular insufficiency (i.e., a reduction in the glomerular filtration rate), whether acute or chronic and for whatever reason, promotes urate underexcretion and hyperuricemia (Table 94-1). The mechanisms of hyperuricemia in patients with renal insufficiency are complex but depend first and foremost on decreased delivery of a filtered urate load to the renal tubule. At high levels of azotemia such as are rarely seen in this era of dialysis (blood urea nitrogen >100 mg/dL), hyperuricemia is practically universal. Lesser degrees of renal failure are more variably accompanied by hyperuricemia because decreases in glomerular filtration rate promote some compensatory increase in renal tubule urate secretion, and possibly because of gastrointestinal compensation as well. The effects of renal insufficiency on hyperuricemia may be more apparent in patients who also possess other risk factors for elevated urate levels. An association between congestive heart failure and hyperuricemia has also been reported. Although the direction of causality in this relationship has not been fully established, it is likely that the reduced renal perfusion seen in persons with congestive heart failure can promote urate retention.[102] Because glomerular insufficiency is a condition that accrues among older populations, it likely contributes to the higher rate of hyperuricemia and gout among older adults.[2]

**TABLE 94-1** Systemic Conditions Promoting Hyperuricemia

| |
|---|
| Overproduction |
| Hemolytic anemia |
| Sickle cell disease |
| Polycythemia vera |
| Megaloblastic anemia |
| Thalassemia |
| Myelodysplastic syndrome |
| Leukemia |
| Lymphoma |
| Multiple myeloma |
| Waldenström's macroglobulinemia |
| Essential thrombocytosis |
| Solid tumors |
| Tumor lysis syndrome |
| Psoriasis |
| Sarcoidosis |
| Metabolic myopathies |
| Mitochondrial myopathies |
| Underexcretion |
| Renal insufficiency |
| Dehydration/volume depletion |
| Lactic acidosis |
| Ketoacidosis |
| Both Overproduction and Underexcretion |
| Myocardial infarction |
| Congestive heart failure |
| Sepsis |
| Metabolic States |
| Hyperthyroidism |
| Hypothyroidism |
| Hyperparathyroidism |
| Hypoparathyroidism |
| Obesity |

Various forms of organic (metabolic) acidoses may promote renal underexcretion of urate. Thus patients with lactic acidosis (e.g., in hypoxia, sepsis, liver or kidney disease, post-surgery or myocardial infarction, during excessive anaerobic exercise, or in response to certain drugs such as metformin) may become hyperuricemic. Similarly, patients experiencing ketoacidosis (e.g., alcoholic or diabetic ketoacidosis or starvation ketosis) also may experience hyperuricemia. Lactic acidosis may also occur as a result of ketoacidosis. Although the mechanisms of these effects were previously considered to result from direct competition between the organic acids and urate for tubular excretion, they are probably better considered from the perspective of recent discoveries in renal urate transport (previously described). In particular, organic acids serve as exchange anions for the apical surface renal urate transporters URAT1 and OAT10. Such acids therefore provide a motive force for increased urate resorption in the proximal tubule.

Dehydration (volume depletion) on any basis promotes hyperuricemia.[103] Once again, the mechanisms are complex but include decreased renal perfusion and subsequently decreased urate filtration and delivery to the proximal tubule. Subsequent sodium retention will reduce tubular urate secretion, probably at the $Na^+$/urate co-transporters NPT1 and NPT4. Patients exposed to low-sodium diets may also retain urate in an effort to retain sodium.

Several metabolic and/or endocrinologic conditions have been associated with hyperuricemia, although whether these conditions represent independent associations has not been determined. These conditions include hypo- and hyperthyroidism and hypo- and hyperparathyroidism. Obesity is associated with hyperuricemia,[104] and weight loss has been shown to reduce both serum urate levels[105] and risk of gout.[106] Although it appears likely that adiposity itself may promote hyperuricemia, this relationship is complex because adiposity also may reflect diet and thyroid status. Patients who have undergone a renal transplant experience increases in the prevalence of hyperuricemia and gout (2% to 13%). It is likely that the hyperuricemia in patients who have had a renal transplant is not related to the transplant per se but to other factors such as intrinsic renal insufficiency, diuretic use, and especially the use of cyclosporine to suppress rejection (discussed further in a subsequent section).[107] Reciprocally, some studies suggest that hyperuricemia in transplant patients may contribute to worsening renal function.

The interaction between diabetes and hyperuricemia is complex and may be bidirectional (see subsequent section). An association between insulin levels and gout has been recognized, suggesting that insulin may adversely regulate urate levels, possibly through renal mechanisms. Consistent with this possibility, a recent study demonstrated that the initiation of insulin in diabetics resulted in increased serum urate levels.[108] Genetic polymorphisms in the peroxisome *proliferator-activated receptor γ (PPARγ)* gene *(PPARG)*, which are well known to be associated with obesity and metabolic abnormalities, may also be associated with increased risk of hyperuricemia. Patients with genetic polymorphism-associated metabolic abnormalities involving glucose and lipid metabolism (GCKR), cholesterol and diabetes mellitus (MYL2-CUX2), and regulation of glutamate signaling (CNIH-2) have also shown an increased susceptibility to gout.[74] Additional, wider population genome-wide association studies including fine mapping and functional analysis are needed to confirm associations with these genes.

**TABLE 94-2** Medications Promoting Hyperuricemia

| |
|---|
| Diuretics |
| Thiazide diuretics |
| Loop diuretics |
| Organic Acids |
| Salicylates (low dose) |
| Nicotinic acid |
| Pyrazinamide |
| Other |
| Cyclosporine |
| Ethambutol |
| Ethanol |
| Colony-stimulating factors |

**Medications.** Diuretics are among the most commonly used agents to treat hypertension and congestive heart failure, and it has long been appreciated that many diuretics promote hyperuricemia and subsequent gout (Table 94-2).[109,110] Despite early assessments suggesting otherwise,[111] the increase in gout risk as a result of diuretic use is substantial and may range as high as 3- to 20-fold.[112] The mechanisms through which diuretics raise serum urate are incompletely elucidated but include the induction of sodium wasting and volume depletion, with a resultant decrease in the fractional excretion of urate.[113] However, individual diuretics may also have more specific and direct effects on renal urate handling. For example, loop diuretics such as furosemide and bumetanide interact directly with the tubular urate transporter NPT4,[84] and both thiazide and loop diuretics inhibit the renal urate exporter MRP4.[114] Indeed, despite the volume-depleting effects of diuresis, not all diuretics promote hyperuricemia. For example, the potassium-sparing diuretics triamterene, amiloride, and spironolactone do not increase urate levels. Interestingly, some diuretics actually lower serum urate levels, apparently by directly promoting renal urate excretion even as they induce volume depletion. One such drug was tienilic acid, an effective diuretic and antihyperuricemic that was withdrawn from use as a result of hepatotoxicity.

Several drugs that are weak organic acids may raise serum urate by serving as counter ions to promote urate retention by URAT1 and OAT10. These drugs have also been assumed to inhibit tubular secretion, possibly by acting as urate competitors. Among these agents is the lipid-lowering drug nicotinic acid, which may not only block urate secretion but also promote urate formation.[110] Low-dose salicylates including the low doses of aspirin used for cardioprotection may also raise urate by impairing renal urate efflux.[114] At high doses, salicylates become uricosuric, apparently through the inhibition of URAT1.[115] The antituberculous agent pyrazinamide is the most potent urate-retaining agent known.[110] Pyrazinamide is metabolized to pyrazinoate and subsequently to 5-hydroxypyrazinoate[116]; it is likely that these organic anions act in a manner similar to nicotinate and salicylate. Another antituberculous agent, ethambutol, can also reduce renal tubular urate excretion

and promote hyperuricemia. However, the mechanism of action of ethambutol is not well established.[117] In contrast to calcium channel blockers that modestly promote serum urate excretion, β-blockers are associated with increased serum urate levels, possibly as a result of decreased renal perfusion.[118]

The immunosuppressant cyclosporine is well known to promote decreased renal urate excretion and hyperuricemia. Cyclosporine causes tubulointerstitial injury and arteriolar hyalinosis; reciprocally, hyperuricemia may exacerbate the nephrotoxic effects of cyclosporine.[119,120] Tacrolimus, which has a mechanism of immune action similar to that of cyclosporine and also can impair renal function, does not promote similar levels of hyperuricemia. Moreover, the effect of cyclosporine on urate retention appears to exceed its effects on renal filtration, suggesting a probable direct effect on tubular urate transport and subsequent urate excretion.[110,121,122] Most studies of the effects of cyclosporine on urate have been performed with patients who have undergone a renal transplant; the effects of cyclosporine on urate in other settings are less well established.

**Toxins.** Several toxins can affect the kidney and promote hyperuricemia. Chief among these toxins is lead.[123,124] Lead exposure is common in Western society, but but excessive exposure occurred during some periods (e.g., during the Roman Empire). A connection between lead exposure, hyperuricemia, and gout has been suspected at least since the 18th century. In the 20th century, a large cohort of patients with lead-induced hyperuricemia (saturnine gout) was recognized during and after the era of Prohibition, predominantly in the Southeastern United States and relating to the home brewing of whiskey (moonshine, or white lightning) using lead-lined vessels (typically, automobile radiators). Lead consumption results in lead distribution to a reservoir in bone and may have adverse effects on the CNS as well. In the kidney, lead poisoning leads to interstitial and perivascular fibrosis, as well as glomerular and tubular degeneration.[125,126] Although patients with lead nephropathy initially experience mild to moderate renal insufficiency, their clearance of urate is excessively limited, indicating a tubular defect in urate excretion.[127] Suggestions that lead exposure may also promote purine turnover are provocative but have been less well supported.[128] Although saturnine gout is currently a relatively rare condition, epidemiologic data suggest that it may be more prevalent than is commonly assumed.[129] In patients with moonshine nephropathy (as opposed to chronic lead poisoning from other sources), associated lifestyle factors (e.g., alcohol consumption and obesity) may play a significant role in the genesis of the hyperuricemia.[130]

Patients with chronic beryllium poisoning, usually as a result of an occupational exposure, may also experience diminished renal urate excretion and hyperuricemia.[131]

## Diet and Uric Acid

**KEY POINT**

**Diet can have significant effects on serum urate levels by serving as a source of dietary purines and by altering the metabolic production and/or renal excretion of uric acid.**

A number of dietary components can affect serum urate levels, mainly by leading to urate overproduction. Several other dietary components may have the capacity to lower serum urate levels.

### Purine-Rich Foods

Purine-rich foods are a major source of daily purine load and hence a major source of generated urate. However, not all purine-rich foods convey equivalent risk: seafood and red meat, particularly organ meats, convey an increased risk for hyperuricemia, whereas consumption of purine-rich, leafy-green vegetables apparently does not convey such a risk.[132] Earlier authors emphasized the limited effect of purine intake, with studies suggesting that alterations in purine intake may result in, at most, changes in serum urate of about 1 mg/dL.[95,133] However, such studies have rarely accounted for the role of renal urate excretion in the context of diet. Thus it is worth considering if, for a patient who is intrinsically unable to excrete serum urate, an increased dietary purine load may produce more profound increases in serum urate levels than would be seen in a patient with normal excretory capacities. In contrast to purine ingestion, protein consumption does not increase the risk of hyperuricemia and/or gout, a point of occasional confusion for practitioners, because many high-purine foods are also high in protein.[132]

### Fructose

As early as 1901, Osler[134] recognized the ability of fructose to provoke gout flares. Little attention was given to this observation until the 1960s and 1970s, when it was demonstrated that fructose loads, administered orally or intravenously, cause transient rises in serum urate levels, particularly in persons with gout.[135,136] These effects are reproduced with consumption of sucrose (which contains fructose) but not with consumption of glucose or galactose.[137] Biochemical analysis of fructose metabolism has provided insight into the mechanisms of fructose-induced hyperuricemia, which does not provide purines directly but instead alters metabolism to promote purine and hence urate synthesis (Figure 94-9).

The first step in fructose metabolism is the donation of a phosphate from ATP to form fructose-1-phosphate (enzyme phosphofructokinase), generating ADP. ADP is converted to AMP (enzyme adenylate kinase), which in turn is degraded via several steps to uric acid. In effect, fructose serves as a phosphate "sink" because the donated phosphate is no longer available for the regeneration of ATP from AMP and ADP. Because both $P_i$ and ATP inhibit the purine degradation pathway (through inhibition of AMP deaminase and 5′ nucleotidase, respectively), depletion of these compounds promotes the formation of uric acid as well.[138] Depletion of ADP/AMP also impairs feedback inhibition of PRPP synthase and promotes purine biosynthesis. Epidemiologic studies confirm a role for fructose consumption in hyperuricemia; patients who consume excessive fructose in the form of fructose-sweetened soft drinks or fruit juices demonstrate both higher serum urate levels and an increased incidence of gout.[137,139-145] The probable importance of fructose is underscored by the fact that the rise in the prevalence of gout during the past several

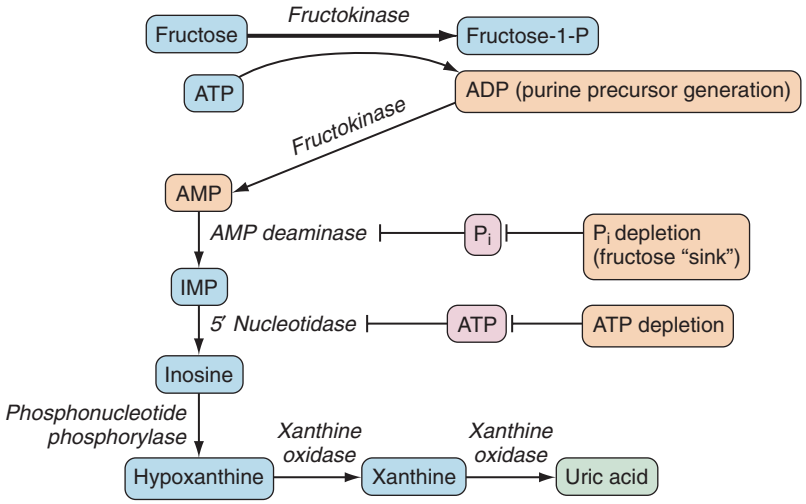



**Figure 94-9** Fructose and the generation of uric acid. Ingested fructose is converted to fructose-1-phosphate, a process that uses adenosine triphosphate (ATP), generates adenosine diphosphate (ADP), and sequesters inorganic phosphate (fructose/phosphate "sink"). The ADP generated then serves as a substrate for urate generation, whereas the depletion of ATP and $P_i$ results in decreased feedback inhibition of the enzymes that mediate uric acid synthesis. AMP, Adenosine monophosphate; IMP, inosine monophosphate.

decades has occurred in parallel with an increased industrial use of fructose, rather than dextrose (glucose), as a major additive in soft drinks and prepared foods.

### Alcoholic Beverages

Ethanol ingestion is associated with the development of gout, and ample physiologic and epidemiologic data confirm that ethanol consumption promotes the development of hyperuricemia.[146-150] Ethanol is a particularly effective agent for raising serum urate levels because it works through multiple mechanisms. Chief among these is the requirement for ATP degradation during ethanol metabolism, resulting in increased purine turnover and urate generation.[151,152] The ability of binge alcohol consumption to induce increases in lactate levels also contributes to hyperuricemia by decreasing renal urate excretion, likely through effects on URAT1, as previously discussed.[153,154] Ethanol consumption also promotes a diuresis, probably via anti-diuretic hormone suppression[155]; dehydration and volume depletion then promote renal urate retention. At acutely high levels of alcohol intake in individuals who chronically consume ethanol ketoacidosis may ensue, particularly in the setting of transient starvation and/or vomiting[156]; in such settings, uric acid secretion may be inhibited, or uric acid resorption may be promoted by 3-hydroxybutyrate and acetoacetate, in much the same manner as lactic acid.[157] As a dietary matter, some alcoholic beverages, particularly beers and ales, are high in purines, mainly in the form of guanosine.[149] Indeed, consumption of ethanol-free beer transiently raises serum urate levels, although not to the extent of beer itself.[158] The importance of purine load in alcoholic beverages may be underlined by the fact that, in contrast to beer, moderate consumption of wine (which is relatively low in purines) does not increase serum urate levels.[150,159] As previously noted, saturnine gout contracted from lead-adulterated moonshine may also contribute to hyperuricemia in the setting of alcohol consumption.[124]

### Other Dietary Components

Recent epidemiologic and physiologic data indicate that consumption of low-fat dairy products is independently associated with reduced serum urate levels and risk of gout.[132] In physiologic studies, consumption of milk or milk proteins has a direct uricosuric effect that results in lowering of serum urate.[160] Interestingly, dairy products may also have anti-inflammatory effects.[161] Regular heavy coffee consumption (4 to 6 cups daily) may have urate-lowering properties that are independent of caffeine[162-164]; these effects appear to be concordant with a reduced risk of incident gout.[165,166] In contrast, intermittent heavy coffee consumption may be transiently prohyperuricemic, perhaps as a result of caffeine-induced diuresis and volume depletion. Increased intake of vitamin C has been epidemiologically associated with decreased serum urate levels, but this effect was not confirmed in a prospective study.[167,168] A limited number of studies support the possibility that consumption of cherries, a long-standing complementary medicine treatment for gout, may produce a uricosuric effect and reduce the risk of gout flares.[169,170]

## CRYSTAL FORMATION: THE TRANSITION FROM HYPERURICEMIA TO GOUT

**KEY POINT**

**Monosodium crystal formation involves physicochemical processes but may be regulated by synovial fluid proteins and immunoglobulins.**

Soluble urate does not induce gouty flares; only crystallized urate promotes acute inflammation. Urate crystallization is therefore a critical step in the progression from hyperuricemia to frank gout.[171,172]

Uric acid is a weak organic acid (pKa1 of 5.75 [position 9] and pKa2 of 10.3 [position 3]). At physiologic pH (7.4), approximately 98% of uric acid exists in the form of monosodium urate (MSU) monohydrate. At concentrations greater than 6.8 mg/dL, MSU exceeds its limit of solubility in serum under physiologic conditions, which is the biochemical definition of hyperuricemia. Exceeding the urate solubility threshold potentiates the precipitation of needle-shaped crystals and results in inflammatory responses. However, Sir William Roberts recognized that not all patients with hyperuricemia experienced gout and drew a distinction between hyperuricemia and the crystallization of MSU.[173] Epidemiologic studies also suggest that urate crystal formation and the consequent development of acute gout may occur in a minority of subjects with hyperuricemia.[174] Therefore factors other than hyperuricemia must influence the formation of urate crystals.

In vitro models have shed light on the relationship between urate crystallization and environmental factors such as pH, temperature, salt content, vibration, and large molecules.[175-177] The fact that urate may more readily precipitate at both lower pH and lower temperature, for example, provides a possible rationale for the fact that gout flares are most commonly seen (50% as an initial presentation and eventually 90% of all gout patients) in the first metatarsophalangeal joint, which is both circumferentially exposed (i.e., relatively low in temperature) and at the farthest outpost of the systemic circulation.

Given that crystal formation and gout flares most commonly occur within the confines of the joint, other investigators have emphasized a possible role for joint biology per se in the formation of MSU crystals. For example, some investigators have suggested that soluble urate is excreted more slowly from the joint than other serum elements, providing a possible mechanism for urate concentration within the joint space.[178] Once urate crystals are formed, the fenestrated endothelium of the synovium might permit the joint space to serve as a crystal "trap," preventing crystals from being dispersed and/or dissolved in the wider circulation. Other investigators have emphasized the possible role of cartilage itself in the precipitation of urate, particularly in the setting of aging and/or osteoarthritis.[179] Potential mechanisms through which aging cartilage might facilitate MSU nucleation/crystallization include changes in the proportions and/or chemical properties of cartilage glycosaminoglycans and proteoglycans, as well as increases in the intra-cellular and extra-cellular lipid content of articular cartilage.[179-181] One recent report suggests that osteoarthritis chondrocytes may actually secrete urate into the joint space, promoting local urate excesses in the synovial fluid.[182]

Several investigators have suggested that urate crystallization may be an immune-assisted process. Kam and colleagues[183] proposed that IgG antibody binding to MSU monomers, which was previously assumed to be nonspecific, may permit the stacking of MSU crystals to occur despite dispersion forces that exist in fluid and tissue. More recently, Kanevets and colleagues[184] postulated a role for urate crystal–specific IgM antibodies in the nucleation and formation of urate crystals.

Although urate precipitation is the *sine qua non* of acute gout, not all urate precipitation may lead directly to acute gouty arthritis. Imaging studies confirm that, in patients with asymptomatic hyperuricemia, urate crystals may nonetheless deposit in both cartilage and synovium.[20,185] These deposits are of consequence not only for their potential to directly damage tissue (see the subsequent section), but also for their role as a reservoir of uncoated and potentially inflammatory urate crystals. Thus local trauma, long recognized as a possible antecedent to acute gouty flares, may physically release cartilage-deposited crystals into the joint space, where they can initiate inflammation. In addition, acute lowering of serum and synovial fluid urate levels (e.g., during initiation of urate-lowering drugs) is well documented to precipitate gouty flares[186] by a mechanism that is most likely akin to that in which glacial melting causes the sloughing off of icebergs and the exposure of previously hidden surfaces. Changes in pH and other physical parameters may have similar effects. These observations have led to a revision of the classical model of gout, wherein urate crystal deposition precedes the first acute flare and acute flares occur as a result of crystal shedding into, rather than direct spontaneous precipitation within, the affected joint(s).[187,188]

## ACUTE GOUT FLARES: THE INFLAMMATORY RESPONSE TO MONOSODIUM URATE CRYSTALS

**KEY POINT**

**Uric acid crystals activate both complement and resident synovial leukocytes, inciting neutrophil influx that itself promotes further inflammation.**

In its crystalline form, uric acid is a potent trigger of inflammation.[189] The phlogistic potential of crystalline urate in human gout was established dramatically in the 1960s by Faires and McCarty,[190] who self-injected urate crystals into their knee joints and experienced flares of acute inflammation. In the clinic, the diagnosis of gout is definitively made upon joint aspiration, with examination of synovial fluid under polarized light microscopy demonstrating the presence of urate crystals, neutrophils, and particularly intra-cellular urate crystals, confirming directed neutrophil phagocytic activity.[191] However, the inflammatory mechanisms of gout are complex and involve not only neutrophils but other cell types, numerous inflammatory mediators, and well-organized sequences of events.[192]

### Uric Acid Crystals and Complement Activation

Complement activation by the alternative pathway is a continuous process in body fluids, in which C3 component activation in the fluid phase is followed by rapid C3b deposition onto nearby surfaces.[193] On most cell surfaces C3 is routinely inactivated by regulatory proteins. In contrast, the polyanionic surfaces of uric acid crystals provide opportunities for unconstrained C3 deposition and subsequent activation of downstream complement components. Weissmann and other investigators[194,195] have demonstrated the ability of urate crystals to activate complement from C2-depleted serum, confirming activation of the alternative pathway. Interestingly, other groups have

confirmed that uric acid crystals also activate complement by the classic pathway (i.e., via C1 activation). Crystal activation of the classic pathway may occur in two ways. First, uric acid crystals may activate the classic pathway by an immunoglobulin-independent, C-reactive protein–dependent pathway.[194,196,197] In addition, it has been shown repeatedly that urate crystals possess the ability to bind antibodies. The specificity of IgG binding, as well as the question of whether antibodies bound to urate crystals can lead to additional activation of the classical pathway, is not fully resolved.[194,197,198] One result of crystal-induced complement cascades is to generate C5a, a potent vasodilator and chemoattractant for inflammatory cells such as neutrophils.[199,200] Urate crystal complement activation may also lead to generation of soluble complement membrane attack complexes, which may activate local cells to promote inflammation.[201] Other proteins of potential import to inflammation, including fibronectin and kininogen, may also adhere to urate crystals.[202]

## Cellular Response to Crystals

### Cell Recognition of Crystalline Urate

MSU crystals interact with, and potently stimulate, a range of inflammatory cells. The manner in which crystals activate cells remains an evolving subject of investigation. Several mechanisms have been proposed, including (1) crystal recognition via toll-like receptors (TLRs), (2) interactions between urate crystals and cholesterol rafts in the cell membrane, and (3) direct phagocytic mechanisms.

TLRs are critical for innate immunity and permit organisms to rapidly recognize bacteria and viruses on the basis of stereotypical features (i.e., pathogen-associated molecular patterns), rather than characteristics unique to the specific invader. Because crystalline urate might theoretically be treated as a foreign molecule, several investigators have examined whether urate crystals activate TLRs. In TLR2 and TLR4 knockout mice, decreased IL-1β and TNF production, as well as decreased neutrophil influx in the urate crystal–induced air pouch inflammation model, argue strongly for a role for TLRs in urate crystal responses.[203] Impairment of urate-driven inflammation in CD14 knockout mice also appears to support a role for TLRs, because CD14 is essential for TLR2- and TLR4-dependent signaling.[204] Interestingly, Joosten and colleagues[205] suggest that TLR2 activation by MSU crystals requires simultaneous exposure of the receptors to C18:0 free fatty acids. However, other researchers observed no effect of multiple TLR knockouts on murine models of urate-induced inflammation.[206] The role of TLRs in urate signaling therefore remains an open question.

Other authors emphasize the ability of MSU crystals to electrostatically interact with cholesterol.[207] Cholesterol-rich regions of plasma membranes (lipid rafts) are characteristically dense in signaling molecules and represent hot spots for cellular activation. Receptor-independent interactions between crystalline MSU and lipid rafts have been demonstrated in dendritic cells, resulting directly in cell activation. The mechanism behind this effect appears to relate to hydrogen bond–dependent aggregation of lipid rafts; aggregation of transmembrane receptors within the rafts then results in activation of immunoreceptor tyrosine-based activation motifs, followed by activation of the signaling molecule Syk.[208,209] Syk activation in turn can induce cell activation, including phosphatidylinositol 3 kinase (PI3K) signaling, cytoskeletal rearrangement, and crystal phagocytosis.

As previously noted, MSU crystals may become coated by immunoglobulins and other serum proteins and may serve as a substrate for complement activation. Thus protein-coated urate crystals may also activate cells via their ability to engage Ig, complement, and possibly other cell surface receptors.[210] Consistent with this model, IgG-coated but not IgM-coated urate crystals have been observed to incite greater inflammatory responses than do those that are not coated.[211]

### Intra-cellular Responses to Urate Crystal Encounters

Activation of cells by urate crystals results in the activation of a number of intra-cellular signaling molecules associated with inflammatory responses. In addition to Syk, these intra-cellular signaling molecules include PI3K; the ERK, JNK, and p38 mitogen-activated protein (MAP) kinases; phospholipases C and D; rho-family proteins; and nuclear factor κB (NF-κB). Depending on the cell type in question, activation of these molecules will result in specific phenotype alterations including cytoskeletal alterations, production of cytokines and lipid mediators, and induction of phagocytosis and superoxide generation.[212-216] Additional responses, secondary to these initial ones, include vasodilation and vascular leakiness, the upregulation of adhesion molecules on endothelial and inflammatory cell surfaces, and cellular chemotaxis, which will be discussed in a subsequent section.

It has long been appreciated that urate crystals can induce macrophages to produce IL-1β and that IL-1β produced in this manner is central to the development of gouty inflammation.[213] However, until recently, the mechanism of urate-induced IL-1β upregulation remained unknown. The NLRP3 (known as NALP3, CIAS1, or cryopyrin) inflammasome is a multimolecular, cytosolic complex with the primary purpose of generating IL-1β, as well as IL-18 and IL-33 (also see Chapter 17).[217] The pro form of IL-1β is cleaved into activated IL-1β by the inflammasome-associated enzyme caspase-1, which may also play a role in IL-1β secretion. In a seminal study, Martinon and colleagues[218] documented the ability of MSU crystals to activate the NLRP3 inflammasome and stimulate IL-1β generation (Figure 94-10). Subsequent inflammatory responses (e.g., TNF production) appear to occur as a result of autoengagement of cell surface IL-1β receptors. The importance of the inflammasome in crystal inflammation has been well documented in mouse models: macrophages from mice lacking inflammasome components generate diminished levels of IL-1β on MSU crystal exposure, and intraperitoneal injection of urate crystals into inflammasome-deficient mice, as well as mice deficient in IL-1β receptors, leads to significantly decreased neutrophil recruitment.[218] Moreover, the ability of IL-1β–directed therapies to abrogate both human gout and murine crystal-induced inflammation speaks to the centrality of IL-1β in the inflammatory response to urate crystals.[219-221]

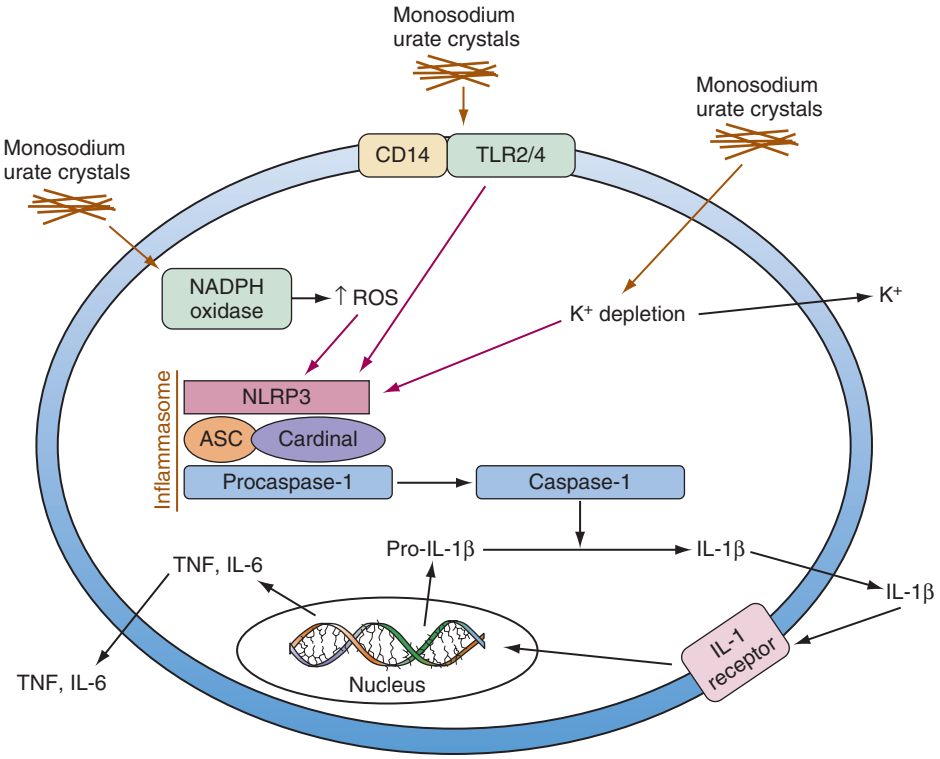



**Figure 94-10** Activation of the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome by monosodium urate crystals. Activation of the inflammasome by urate crystals results in activation of caspase-1, which cleaves and activates IL-1β and promotes IL-1β secretion. In turn, IL-1β can engage its receptors to secondarily promote the synthesis and secretion of other cytokines such as TNF and IL-6. Three possible mechanisms of inflammasome activation by urate crystals are illustrated: (1) production of reactive oxygen species (ROS), (2) activation of toll-like receptors 2 and 4 (TLR2/4), and (3) potassium depletion, which may be sensed by the inflammasome. The precise roles of these and other mechanisms of inflammasome activation by urate crystals remain a matter of investigation. ASC, Apoptosis-associated speck-like protein containing a caspase-recruitment domain; NADPH, nicotinamide adenine dinucleotide phosphate.

To date, no definitive explanation has been provided with regard to how urate crystals activate the NLRP3 inflammasome. Leading theories center on the inflammasome as an internal sensor for cellular distress resulting from either (1) oxidative stress or shifts in ion concentration or (2) disruption of phagolysosomes.[222,223] In one proposed model, crystal-induced damage to the plasma membrane promotes cellular potassium efflux, and the resultant hypokalemic state activates the inflammasome directly.[224,225] Additionally, crystal activation of the phagocyte NADPH oxidase leads to the generation of reactive oxygen species (ROS), which may then be directly or indirectly sensed by the inflammasome. The ability of large urate crystals to provoke incomplete or frustrated phagocytosis—in which a phagocyte surrounds but cannot fully engulf a target—may lead to an activated state (including oxidase activation) that also contributes to inflammasome activation.[222] The second proposed mechanism of inflammasome activation by MSU crystals is based on the ability of phagocytosed urate crystals to rupture the membranes of phagolysosomes, either by mechanical or physicochemical effects.[226] In this model, phagolysosome rupture leads to cytosolic acidification and intra-cellular release of cathepsin B, each of which has been proposed as a NLRP3 inflammasome activator.[223] These various mechanisms may not be mutually exclusive and may act synergistically (e.g., lysosomal disintegration and cathepsin-B release may themselves promote the production of ROS).

## Initiation and Propagation of the Acute Gouty Flare

The clinical picture of acute gout is one of rapid, almost explosive development of an inflammatory response. Accordingly, cellular changes during the acute flare must reflect the accelerating nature of the inflammation. Beginning with the appearance/release of urate crystals, the initial phase of gouty inflammation must depend on already available local mediators, as well as cells that are both (1) capable of urate crystal responses and (2) already in place within the joint. Clearly, Ig binding and the activation of complement on the crystal surface must be one such early response. Interaction of the crystal with local tissue cells, facilitated in part by IgG and complement opsonization, is also likely to play an early role. Best studied among the early cell type responses is that of the synovial macrophage, a resident cell in the synovial membrane. As previously noted, macrophage activation in response to crystals results in synthesis and secretion of important cytokines such as IL-1β, TNF, IL-6, and the chemokine IL-8 (CXCL8), as well as release of potentially tissue-destroying matrix metalloproteinases (MMPs) and toxic oxygen radicals.[212,213,227,228] Activation of synovial macrophages is contemporaneous with their phagocytosis of urate crystals. Human synovial fibroblasts are also capable of responding to urate crystals, leading to the generation of both inflammatory mediators and metalloproteinases.[227,229] Finally, mast cells are also resident within the synovium and appear to be important in the early phases of

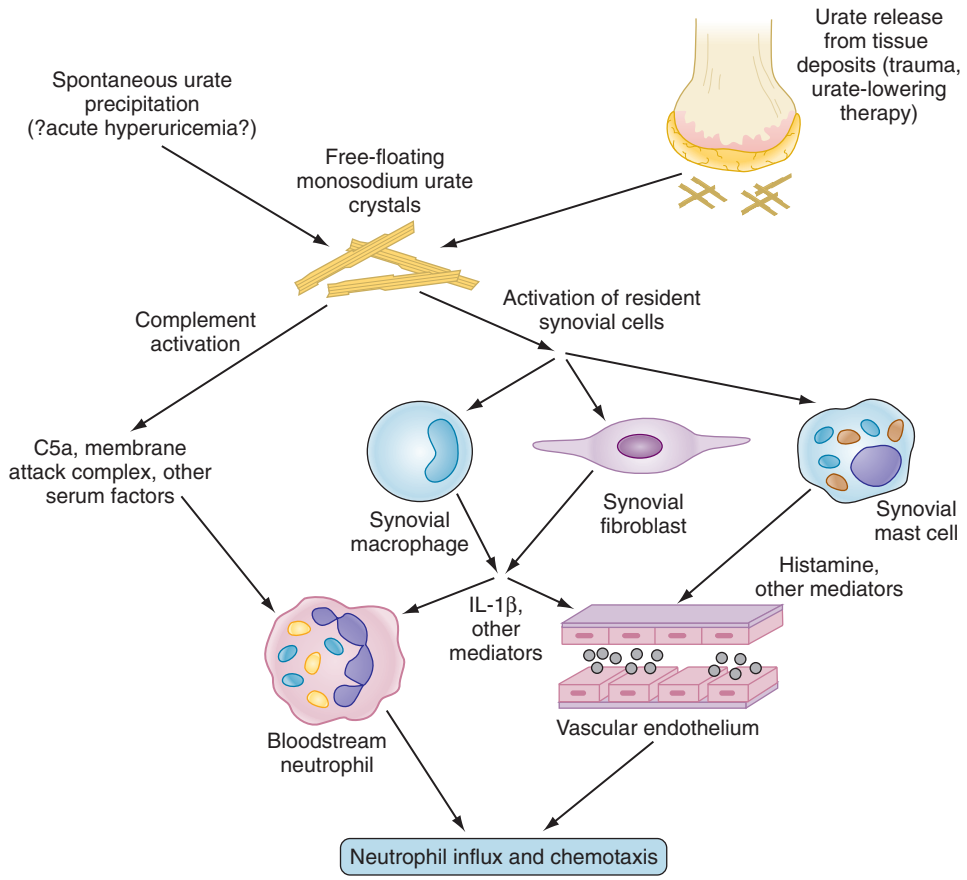



**Figure 94-11** Initial phases of urate crystal–induced activation. The presence of "fresh" urate crystals, resulting either from spontaneous precipitation or the liberation of crystals from established pools, results in direct complement activation and activation of resident cells in the synovium including macrophages, fibroblasts, and mast cells. Activated cells produce IL-1β and other cytokines, as well as multiple other mediators (not all illustrated) that in turn activate both bloodstream neutrophils and endothelial cells. These responses permit neutrophils to adhere to and traverse the endothelium, resulting in neutrophil influx and the further propagation of inflammation as neutrophils undergo direct activation by urate crystals (not illustrated). See text for additional details.

acute gouty responses, as their depletion diminishes inflammation in murine models of crystal-induced inflammation.[230] Mast cells increase in number and activity in the lining of experimental air pouches after MSU crystal injection, suggesting that additional mast cells may be secondarily attracted to the inflammatory site (Figure 94-11).[231]

These early events promote the influx of bloodstream polymorphonuclear neutrophils, the predominant cell in the inflamed gouty joint. Cytokines and other inflammatory mediators produced in the early phase of the crystal response act on the vascular endothelium to promote vasodilation and leakiness and to upregulate the expression of adhesion molecules such as selectins and intercellular adhesion molecules on the vascular surface of endothelial cell.[232] These same cytokines also promote neutrophil activation within the bloodstream, particularly the activation of the integrin adhesion molecule CD11b/CD18. A role for the crystal-generated complement component C5a in the activation of bloodstream neutrophils is also likely because C5a can potently stimulate CD11b/CD18 activation and neutrophil adhesiveness.[233] The result is that neutrophils first adhere tightly to the endothelium and then exit the vasculature, followed by chemotaxis up the C5a complement gradient, leading to encounter with, and phagocytosis of, the provoking urate crystals. The mechanisms of neutrophil activation by crystals include those previously discussed and, importantly, appear to include interactions with CD11b/CD18 (which is also a complement receptor) and the IgG receptor FcγRIIIB. Subsequent neutrophil intra-cellular signaling events include phosphorylation and/or activation of a number of tyrosine kinases including Lyn, Syk, Tec, and Src; activation of PI3K; and activation of phospholipase C.[234] Activation of MAP kinases is probably also involved because these kinases regulate neutrophil adhesion and superoxide generation.[233]

Neutrophils encountering urate crystals can rapidly promote additional inflammation and additional neutrophil influx via several mechanisms (Figure 94-12). First, neutrophils stimulated by urate crystals generate a number of inflammatory mediators and potent neutrophil chemoattractants including IL-1β, IL-8, leukotriene $B_4$ ($LTB_4$), S100A8/A9 (also known as myeloid-related proteins 8 and 14), prostaglandin $E_2$, and crystal-induced chemotactic factor.[234,235] Of these, IL-8 and $LTB_4$ are potent chemoattractants. They play a particularly important role in amplifying the chemoattractant gradient and promoting additional neutrophil influx. Second, neutrophils stimulated by crystals release a wide range of products capable of directly

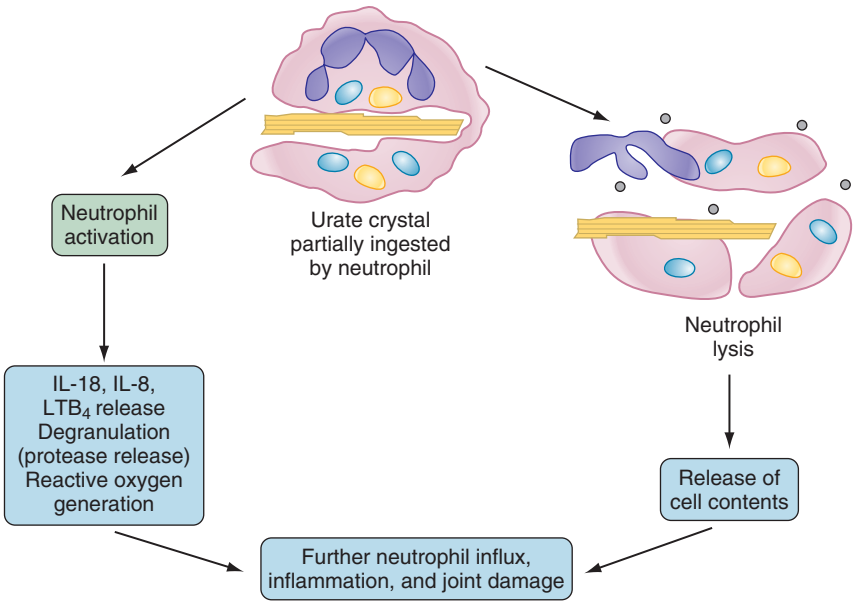



**Figure 94-12** Propagation of the acute gouty response by activated neutrophils. Neutrophils that enter the joint migrate toward and phagocytose crystals. In the case of crystals coated with Igs and complement, the resultant activation results in synthesis and/or release of inflammatory mediators such as IL-1β, IL-8, and TNF, as well as proteases and reactive oxygen species. In the case of uncoated crystals, the crystal frequently interacts with and lyses the membrane of the phagolysosome, spilling toxic contents and leading to cell lysis. In both cases, the result is local tissue damage and recruitment of additional neutrophils from the bloodstream in an explosive inflammatory cycle. $LTB_4$, Leukotriene $B_4$.

damaging local tissues, including oxygen radicals and MMPs such as MMP-8. Although these mediators are intended for digestion of foreign particles within phagolysosomes, the large size of many urate crystals results in incomplete ("frustrated") phagocytosis of the crystals, with the result that lysosomal contents are released into an unsealed phagolysosome and can escape to promote extra-cellular tissue damage ("regurgitation during feeding"). This process appears to be facilitated by crystals coated with Igs.[211] Alternatively, when uncoated crystals are phagocytosed by neutrophils, their ability to interact with the cholesterol-rich bilayers of the phagolysosome results in phagolysosome rupture, cellular necrosis, and direct release of toxic neutrophil contents.[226] In either case, the result is the potential for tissue damage and rapidly accelerating inflammation.

## Resolution of the Acute Gouty Flare

One of the more interesting features of acute gout is that, particularly in the early stages of the disease, most flares are self-limited.[236] Multiple effects have been invoked to explain this phenomenon. Urate crystals may become coated with synovial fluid proteins (e.g., apolipoproteins B and E), thus inhibiting their ability to provoke inflammation.[237] The stress of the gouty flare may promote adrenocorticotropic hormone (ACTH) secretion; in addition to its ability to induce glucocorticoid release, ACTH can bind directly to melanocyte-stimulating hormone receptors to provoke anti-inflammatory effects.[238-240]

Other investigations have emphasized the local, active events through which inflammation may be resolved. For example, in vivo studies using the urate crystal–induced mouse air pouch model of inflammation suggest that local upregulation of expression of $PGD_2$ and 15d-$PGJ_2$ (a spontaneous dehydration product of $PGD_2$) contributes to the resolution of crystal-mediated inflammation, probably through the ability of these molecules to activate the PPARγ.[241,242] Urate crystals also directly stimulate the expression of PPARγ, setting the stage for enhanced suppression of inflammation.[242] 15d-$PGJ_2$ inhibits secretion of IL-1β, IL-6, and IL-12, as well as TNF from macrophages, and downregulates the expression of inducible nitric oxide synthase. Other targets of 15d-$PJG_2$ include PGD receptors and the NF-κB pathway. 15d-$PGJ_2$ also inhibits CXC chemokine production, alters cellular adhesion molecules, and stimulates apoptosis of endothelial cells.[243] Other anti-inflammatory molecules (e.g., IL-10) are also upregulated, and the more recently identified and potent anti-inflammatory resolvins and lipoxins are almost certainly involved.[244]

With regard to the resolution of acute gouty inflammation, it is important to recognize the biologic programs that delay the production of anti-inflammatory molecules until the appropriate phase in the sequence. For example, $PGD_2$ and 15d-$PGJ_2$ production initially decline upon exposure to urate crystals, apparently facilitating the inflammatory response, but then they rebound to act during the resolution phase.[242] Similarly, the generation of anti-inflammatory lipoxins requires the accumulation of at least two active cell types (either neutrophils and activated endothelium or neutrophils and activated platelets), an evolutionary adaptation that creates an intrinsic delay before the anti-inflammatory effects are initiated, and one that may be critical to the rapid resolution of infections.[245]

Consistent with a "time-delayed" process of gout flare resolution, other studies emphasize the roles of the influxing leukocytes themselves in the resolution of the gouty flare. Several studies support the ability of influxing monocyte/macrophages to clear both urate crystals and apoptotic neutrophils that have ingested crystals, resulting in decreased crystal burden.[246,247] Moreover, these influxing macrophages

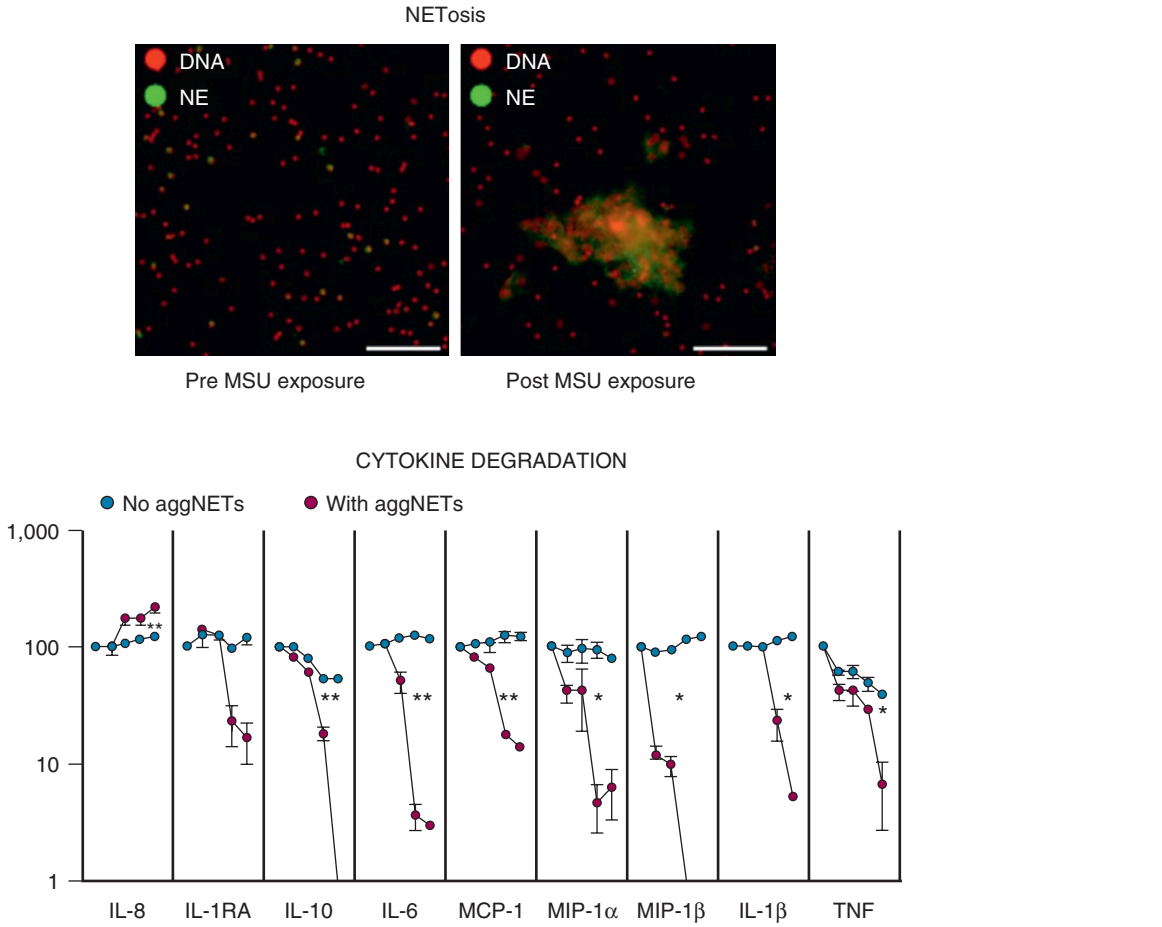



**Figure 94-13** Neutrophil extra-cellular trap (NET)osis and the resolution of acute gout. *Left,* Images of neutrophils before and after exposure to monosodium urate (MSU) crystals, showing the release of an amorphous aggregate of co-localized DNA and neutrophil elastase (NE) in response to urate. *Right,* NETosis and the stable extra-cellular expression of NE permits the rapid degradation of inflammatory cytokines, contributing to resolution of inflammation. MCP-1, Monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α. *(Modified from Schauer C, Janko C, Munoz LE, et al: Aggregated neutrophil extra-cellular traps limit inflammation by degrading cytokines and chemokines.* Nat Med *20:511–517, 2014.)*

appear to differentiate into an anti-inflammatory, M2 phenotype (in contrast to the inflammatory M1 phenotype of resident macrophages) and, upon phagocytosing crystals, to release transforming growth factor-β to provoke an anti-inflammatory effect.[248] Similarly, recent evidence suggests that neutrophils that have phagocytosed crystals may release neutrophil extra-cellular traps (NETs), a network of extracellular chromatin that may initially play a pro-inflammatory role in the gouty flare.[249] Subsequently, however, these NETs provide a stable scaffold on which extravasated neutrophil enzymes, particularly neutrophil elastase, encounter and degrade cytokines to promote inflammatory resolution (Figure 94-13).[250]

## Chronic Gouty Arthritis and Tophaceous Gout

The natural history of untreated gout may include eventual progression to a state characterized by chronic inflammation and/or the establishment of macroscopic urate deposits known as *tophi*. Even during the asymptomatic, intercritical periods of gout, low-grade chronic inflammation can persist, with continuous phagocytosis of crystals by leukocytes.[251] In patients with longstanding gout, intercritical inflammation can become frankly apparent and the cytokines, chemokines, proteases, and oxidants that participate in acute inflammation can contribute to chronic synovitis, cartilage loss, and bone erosion, signaling the progression to chronic gouty arthritis (Video 94-1).[252]

Although tophi are composed first and foremost of MSU crystals, they are complex structures in which urate (1) is embedded in a matrix of lipids, proteins, and mucopolysaccharides and (2) drives a persistent inflammatory state.[180] Alternatively, a tophus can be conceived of as a granuloma of mononucleated and multinucleated macrophages arranged in three distinguishable zones (Figure 94-14).[253] MSU crystals and debris constitute the central zone. Surrounding the central zone is the corona zone, consisting of macrophages, multinucleated macrophage-like cells, plasma cells, and mast cells. This biologically active corona is thought to account for the ultrasonographic finding of an anechoic rim that circumscribes a tophus.[254] Eventually, the corona and central zones may become encased by a connective tissue layer, the fibrovascular zone. Within this zone, macrophages express surface markers of recent migration, maturation, apoptosis, continuous recruitment, and pro-inflammatory activation.[255] T and B cells are scattered throughout both outer zones.[253,256] Although classically a late feature of gout, tophi may occur early in the disease,

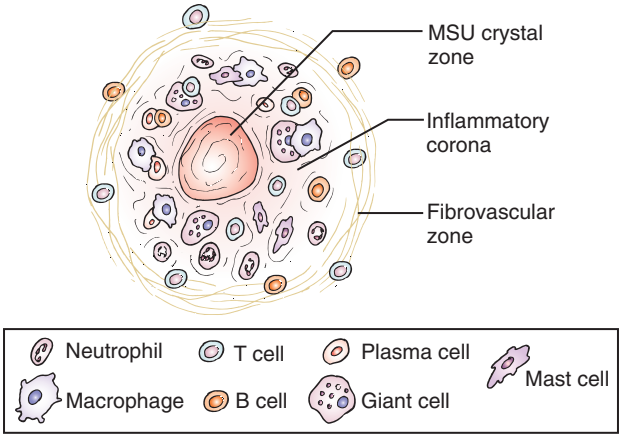



**Figure 94-14** Structure of a tophus. Note the central monosodium urate (MSU) crystal zone, the intermediate corona zone, and the outer fibrovascular zone. *(Modified from Chhana A, Dalbeth N: The gouty tophus: a review.* Curr Rheumatol Rep *17:19, 2015.)*

particularly in patients with renal insufficiency and high serum urates.[257] In some patients, tophi may develop even in the absence of any acute flares.

Tophi are important not only for their role as reservoirs of crystalline urate but also for their ability to damage the tissues in which they reside, including bone, cartilage, and tendons and other soft tissue structures[258] (see Chapter 95). Mechanical factors, induction of lytic enzymes, and synthesis of pro-inflammatory cytokines all contribute to the ability of the tophus to promote erosion and joint destruction.[179] Tophus macrophages produce IL-1β, TNF, IL-6, IL-17, MMP-2, MMP-9, and macrophage CSF (M-CSF).[255] These molecules promote further inflammation and tissue damage, as well as the maturation and activation of osteoclasts that actively resorb bone. For example, M-CSF interacts with M-CSF receptors on osteoclast progenitor cells to promote osteoclastogenesis. Tophus expression of IL-1β and other cytokines has also been shown to inhibit the viability and bone-maintaining effects of osteoblasts.[252,259] Tophus T cells promote bone erosions through the elucidation of receptor activator of NF-κB (RANK) ligand, the major promoter of osteoclastogenesis and osteoclast activation.[260] Additionally, MSU crystals on the cartilage surface drive reduced chondrocyte viability, increased chondrocyte production of degenerative enzymes, and cartilage damage.[261] Even in clinically asymptomatic patients, these inflammatory and erosive processes persist; damage may not be apparent until late in the process.

## EFFECTS OF HYPERURICEMIA IN THE ABSENCE OF GOUT

**KEY POINT**

**Even in the absence of gout, hyperuricemia and soluble serum urate are biologically active and may have previously unappreciated clinical effects.**

In addition to its roles in acute and chronic gouty arthritis, hyperuricemia may have other effects, both adverse and beneficial. Soluble urate is biologically active, with effects on renal and vascular function. Among its mechanisms of action, soluble urate inhibits synthesis of the potent vasodilator nitric oxide; induces smooth muscle cell proliferation by activating MAP kinases; and stimulates cyclooxygenase-2 and platelet-derived growth factor synthesis, all contributing to arterial vasoconstriction.[262,263] Soluble urate has also been shown to directly stimulate the renin-angiotensin system in the kidney and to induce renal interstitial and tubular inflammation.[264,265] As noted in the Evolutionary Considerations section at the beginning of this chapter, these effects may promote hypertension. Other studies suggest that hyperuricemia may also contribute to the risk for cardiovascular disease, although additional research is necessary to better assess the direction of causality.[266] Several recent clinical trials suggest that lowering serum urate levels may reduce the risk of myocardial infarction and slow the progression of renal failure.[267,268] Hyperuricemia may also promote insulin resistance in adipose cells[269-271] and drive gluconeogenesis in hepatocytes,[272] and it has been associated with reductions in $1,25(OH)_2$ vitamin D3 synthesis.[273,274] Urate may also play a role and/or serve as a biomarker in osteoarthritis.[182,275,276] In contrast to the adverse effects of serum urate, accumulating evidence suggests that hyperuricemia may protect against neurologic diseases such as dementia, multiple sclerosis, and Parkinson's and Huntington's diseases.[277-283] Recognition of the biologic complexity of uric acid will lead to a better understanding of its impact on the immune, cardiovascular, endocrine, neurologic, and musculoskeletal systems and to an improved ability to treat hyperuricemia and gout.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 95



# Clinical Features and Treatment of Gout

Christopher M. Burns • Robert L. Wortmann

**KEY POINTS**

- Hyperuricemia is defined as a serum urate level greater than 6.8 mg/dL.
- Acute gout can be treated with NSAIDs, colchicine, corticosteroids, adrenocorticotropic hormone, or IL-1 inhibitors.
- Canakinumab, an anti–IL-1β antibody, has been approved in the European Union for use in patients with acute gout under certain conditions.
- The effectiveness of treatment depends more on how quickly the therapy is initiated than on which agent is used.
- Before initiating therapy with a urate-lowering agent, the patient should be treated with low-dose colchicine or an NSAID to provide prophylaxis against further attacks.
- Regardless of whether a xanthine oxidase inhibitor (allopurinol or febuxostat) or a uricosuric agent (probenecid or benzbromarone) is used to treat hyperuricemia, the patient should receive the lowest dose that maintains the serum urate level below 6.8 mg/dL—preferably below 6 mg/dL.
- The uricase pegloticase should be taken at regular intervals, usually every 2 weeks, and therapy should be stopped when the serum urate level rises above 6 mg/dL, indicating the presence of antidrug antibodies and high risk of an infusion reaction.
- People who are hyperuricemic should be screened for commonly associated comorbid conditions, including hypertension, coronary artery disease, diabetes, obesity, and alcoholism.
- Using a specific urate-lowering agent to manage asymptomatic hyperuricemia is not recommended.
- Hyperuricemic people should try to maintain an ideal body weight, limit alcohol consumption (especially beer), and avoid sweeteners that contain fructose.
- The 2012 American College of Rheumatology Guidelines for Management of Gout has helped clarify treatment strategies.

Gout has been called the "king of diseases" and the "disease of kings." Today, the term *gout* is used to represent a heterogeneous group of diseases found exclusively in humans that include the following characteristics:

- Elevated serum urate concentration (hyperuricemia)
- Recurrent attacks of acute arthritis in which monosodium urate monohydrate crystals are demonstrable in synovial fluid leukocytes
- Aggregates of sodium urate monohydrate crystals (tophi) deposited chiefly in and around joints, which sometimes lead to deformity and crippling
- Renal disease involving glomerular, tubular, and interstitial tissues and blood vessels
- Uric acid nephrolithiasis

These manifestations can occur in various combinations.[1,2]

*Hyperuricemia* denotes an elevated level of urate in the blood. This phenomenon occurs in an absolute (or physiochemical) sense when the serum urate concentration exceeds the limit of solubility of monosodium urate in the serum, which is 6.8 mg/dL at 37°C. Thus a value greater than 6.8 mg/dL indicates supersaturation of body fluids and should be considered unhealthy. A serum urate value in excess of 7 mg/dL begins to carry an increased risk of gouty arthritis or renal stones. Unfortunately, many laboratories report normal levels of serum urate as high as 8.5 mg/dL. These values are derived from population statistics using the mean ± two standard deviations. More than 20% of the U.S. population is hyperuricemic. Therefore sampling a general population gives high "normal" values, resulting in confusion if the clinician does not consider the physiochemical (and therefore more clinically relevant) basis for defining normal.

## EPIDEMIOLOGY

**KEY POINTS**

- Hyperuricemia is common and is directly associated with serum creatinine, body mass index, age, blood pressure, and alcohol intake.
- Serum urate levels are low in childhood and increase in men at puberty and in women at menopause.
- The prevalence of gout ranges from 1% to 15% with a clear increase in incidence in recent years, perhaps related to the adoption of a Western diet and the epidemic of obesity.

Hyperuricemia is fairly common, with prevalence ranging between 2.6% and 47.2% in various populations.[3,4] A variety of factors appear to be associated with high serum urate concentrations. In adults, serum urate levels correlate strongly with serum creatinine and urea nitrogen levels, body weight, height, age, blood pressure, and alcohol intake.[5] In epidemiologic studies, body bulk (as estimated by body weight, surface area, or body mass index) has proven to be one of the most important predictors of hyperuricemia

in people of many different races and cultures, with rare exceptions.[6-8]

Serum urate concentrations vary with age and sex. Children normally have a concentration in the range of 3 to 4 mg/dL because of high renal uric acid clearance.[9] At puberty, serum urate concentrations increase by 1 to 2 mg/dL in males, and this higher level is generally sustained throughout life. In contrast, females exhibit little change in the serum urate concentration until menopause, when concentrations increase and approach those seen in adult men. The mechanism of lower serum urate levels in women is a consequence of sex hormones and is related to a higher fractional excretion of urate as a result of lower tubular urate postsecretory reabsorption.[10]

The incidence of gout varies among populations, with an overall prevalence ranging from less than 1% to 15.3%.[5] This upper limit appears to be increasing.[11,12] The prevalence increases substantially with age and with increasing serum urate concentration. The annual incidence rate of gout is 4.9% for urate levels greater than 9 mg/dL, 0.5% for values between 7 and 8.9 mg/dL, and 0.1% for values less than 7 mg/dL.[13] For serum urate values greater than 9 mg/dL, the cumulative incidence of gout reaches 22% after 5 years.

## ENVIRONMENTAL FACTORS

**KEY POINTS**

**Certain foods promote hyperuricemia and gout, including alcohol, seafood, and red meat.**

**The consumption of some foods, especially milk and yogurt, might be protective.**

An association between alcohol consumption and gout has been recognized for centuries. The risk of developing gout varies by the type of alcohol ingested.[14] Beer, which is rich in purine, carries the highest risk, which is substantially greater than that for liquor. Drinking moderate amounts of wine does not increase the risk of gout. The total quantity of alcohol consumed also strongly correlates with gout. Compared with men who did not drink any alcohol, the relative risk of gout was 1.32 for an alcohol intake of 10 to 14.9 g/day, 1.49 for 15 to 29.9 g/day, 1.96 for 30 to 49.9 g/day, and 2.53 for 50 g/day and higher, with calculations based on 12.8 g of alcohol per 12 oz serving of regular beer, 11.3 g per 12 oz serving of light beer, 11 g per 4 oz serving of wine, and 14 g per shot of liquor.

Diet also influences hyperuricemia and gout. Serum urate levels increase with meat or seafood intake and decrease with dairy intake.[15] Men in the highest quintile of seafood consumption have a 51% higher risk of developing gout, and those in the highest quintile of meat intake have a 41% higher risk. However, consumption of oatmeal and purine-rich vegetables (e.g., peas, mushrooms, lentils, spinach, and cauliflower) is not associated with an increased risk of gout. The consumption of milk one or more times a day or yogurt at least once every other day is associated with lower serum urate levels. Sweeteners containing fructose also contribute to hyperuricemia. The first step in fructose metabolism is phosphorylation by adenosine triphosphate (ATP), which triggers both an increase in purine catabolism and de novo purine synthesis.[16]

## GENETICS

**KEY POINTS**

**Rare forms of early hyperuricemia and gout have a clear genetic and metabolic basis.**

**Gout often runs in families, probably because of inherited factors affecting serum urate levels through renal urate clearance.**

**Recent genome-wide association studies have identified polymorphisms in several candidate genes that encode urate transporters in the renal proximal tubules as determinants of serum urate levels and the risk of gout.**

Since antiquity, gout has been recognized as a familial disorder. The familial incidences reported range from 11% to 80%.[17] These wide discrepancies may be attributed in part to variations in diligence and pursuit of genealogic data. In two large series, one English and one American, about 40% of subjects with gout gave a positive family history of gout. Consideration of all available data suggests that serum urate concentrations are controlled by polygenic traits. Several rare forms of hyperuricemia and gout, such as hypoxanthine phosphoribosyltransferase deficiency, phosphoribosyl-1-pyrophosphate synthetase overactivity, and familial hyperuricemia nephropathy, have a clear genetic basis, with most presenting in childhood or early adulthood.[18] However, in the vast majority of patients with gout, these discrete inborn errors of metabolism are not present and do not explain their disease. In most patients with gout, the mechanism of hyperuricemia is simply inefficient renal excretion of uric acid.[19,20] It stands to reason, then, that some of the familial predisposition to gout is related to inherited variations in renal urate handling. With the recent advances in our understanding of the transport of uric acid in the renal proximal tubule, some of this variability is being clarified. Through genome-wide association studies (GWAS), variations of the *SLC2A9*/*GLUT9* and *ABCG2* genes have been identified as important determinants of serum urate levels (see Chapter 94).[21] Curiously, the data for the role of polymorphisms in URAT1 (*SLC22A12*), a key urate transporter involved in renal proximal tubule urate reabsorption, are thus far inconsistent.[22-26] The splicing variants of glucose transporter 9 (*GLUT9*, *SLC2A9*), an electrogenic hexose transporter, mediate reabsorption of uric acid, glucose, and fructose at the renal proximal tubule epithelial cell, first at the apical membrane, then through the basolateral membrane, and on into the circulation.[27-30] Polymorphisms in *SLC2A9* are associated with a lower serum uric acid, with stronger effects seen in women; they possibly account for 0.5% to 2% of the variance in serum urate concentration in men and 3.4% to 8.8% in women.[23,28,31-33] In five of six populations studied by GWAS, *SLC2A9* polymorphisms were associated with a decreased incidence of self-reported gout, particularly in women.[28,31,32] Indeed, three patients with renal hypouricemia have been identified with

loss-of-function mutations in *SLC2A9*.[29,34] The implication is that certain polymorphisms in *SLC2A9* enhance renal uric acid excretion, lower serum urate levels, and are therefore protective against gout, particularly in women.

A gene product of *ABCG2* (human ATP-binding cassette, subfamily G2) appears to be a secretory urate transporter located in the renal proximal tubule apical border.[35] Polymorphisms in *ABCG2* are associated with a higher serum urate concentration in humans, with a more potent influence in men, potentially accounting for 1.6% to 2.1% of the variance in serum urate concentration in men and 0.5% to 0.8% in women.[24,32] One *ABCG2* polymorphism, rs2231142, has been associated with a higher incidence of gout only in males (odds ratio [OR], 2.03 for self-reported gout).[32] In a transfection model in *Xenopus* oocytes, the common mutation in human *ABCG2* encoded by rs2231142, Q141K, resulted in a 53% decrease in urate transport compared with the wild-type gene product.[35] The implication is that certain polymorphisms in *ABCG2* result in reduced proximal tubule urate secretion, increased serum urate concentrations, and a higher incidence of gout, at least in men.

Because the impact of comorbidities and environmental influences such as diet and lifestyle is so significant in gout, it remains to be seen whether risk contribution from genetic variation is clinically relevant, either directly or because of an unexpected interaction with these other factors. An example of the latter is the potential influence of the urate-fructose/glucose exchange transporter *SCL2A9* polymorphisms on the well-described association of heavy consumption of soft drinks that contain high-fructose corn syrup with elevated serum urate levels and gout.[16,36-38]

## CLINICAL FEATURES

### KEY POINTS

- The three stages of gout are asymptomatic hyperuricemia, acute and intercritical gout, and chronic gouty arthritis.
- A period of asymptomatic hyperuricemia lasts up to 20 years or more before the initial attack of gout or nephrolithiasis.
- The first gout attack generally occurs at age 40 to 60 years in men and after age 60 years in women.
- Many drugs, especially diuretics, raise serum urate levels and predispose people to gout attacks.
- Most attacks of gout, especially early in the course of the disease, are monoarticular, with a predilection for the first metatarsophalangeal joint (podagra). The attacks have a characteristic abrupt and painful onset.
- The differential diagnosis for acute gout is usually infectious arthritis or other crystal-induced synovitis, particularly pseudogout.
- Ultrasonography appears to be a useful adjunct in the diagnosis of acute and chronic gout.
- In untreated or undertreated people, chronic gout is characterized by the development of tophi and progressive joint damage.

Throughout its natural history, gout passes through three stages: (1) asymptomatic hyperuricemia, (2) episodes of acute gouty arthritis separated by asymptomatic intervals (termed *intercritical* or *interval gout*), and (3) chronic gouty arthritis, the period when tophi often become apparent.

The basic pattern of clinical gout begins with acute attacks of intensely painful arthritis. The first attack is usually monoarticular and associated with few constitutional symptoms. Later, attacks may become polyarticular and are associated with fever. Attacks vary in duration but are time limited. Over time, attacks recur at shorter intervals, last longer, and eventually resolve incompletely. This scenario leads to the development of chronic arthritis that slowly progresses to a crippling disease on which acute exacerbations are superimposed.

### Asymptomatic Hyperuricemia

Asymptomatic hyperuricemia is a condition in which the serum urate level is high, but gout—manifested by arthritis or uric acid nephrolithiasis—has not yet occurred. Most people with hyperuricemia remain asymptomatic throughout their lifetimes, although the tendency toward acute gout increases with the serum urate concentration. In the Normative Aging Study, which included only male subjects, the annual incidence rates of gout per 1000 person-years for serum urate levels of 6.0, 6.0 to 6.9, 7.0 to 7.9, 8.0 to 8.9, and greater than 9.0 mg/dL were 0.8, 0.9, 4.1, 8.4, and 49.0, respectively.[13] Based on data from the Framingham Heart Study from 1950-2002, which included 1951 men and 2476 women and had a median follow-up of 28 years, the overall incidence of gout was 4.0 in men and 1.4 in women per 1000 person-years.[39] Relative to serum urate levels, the incidence rates of gout per 1000 person-years for men with levels of less than 5.0, 5.0 to 5.9, 6.0 to 6.9, 7.0 to 7.9, and greater than 8.0 mg/dL were 0.8, 3.4, 8.0, 17.8, and 32.9, respectively ($P$ for trend <0.0001). The incidence rates of gout for women per 1000 person-years with urate levels of less than 5.0, 5.0 to 5.9, 6.0 to 6.9, 7.0 to 7.9, and greater than 8.0 mg/dL were 0.8, 2.5, 4.2, 13.1, and 27.3, respectively ($P$ for trend <0.0001). Thus, even when followed up long term, at least two-thirds of men or women with significant hyperuricemia will not experience gout attacks. The risk of nephrolithiasis increases with the serum urate level and with the magnitude of urinary uric acid excretion. The phase of asymptomatic hyperuricemia ends with the first attack of gouty arthritis or urolithiasis. In most instances, the end of the asymptomatic hyperuricemia phase occurs after at least 20 years of sustained hyperuricemia. Between 10% and 40% of gout subjects have one or more attacks of renal colic before the first articular event.[40]

### Acute Gouty Arthritis

The first attack of acute gouty arthritis usually occurs between age 40 and 60 years in men and after age 60 years in women. Onset before age 25 years should raise the possibility of an unusual form of gout, perhaps one related to a specific enzymatic defect that causes marked purine overproduction, an inherited renal disorder, or the use of certain medications, especially cyclosporine.

A single joint is involved in about 85% to 90% of first attacks, with the first metatarsophalangeal joint being the most commonly affected site.[1,17,41,42] The initial attack is oligoarticular or polyarticular in 10% to 14.5% of cases.[41,42]

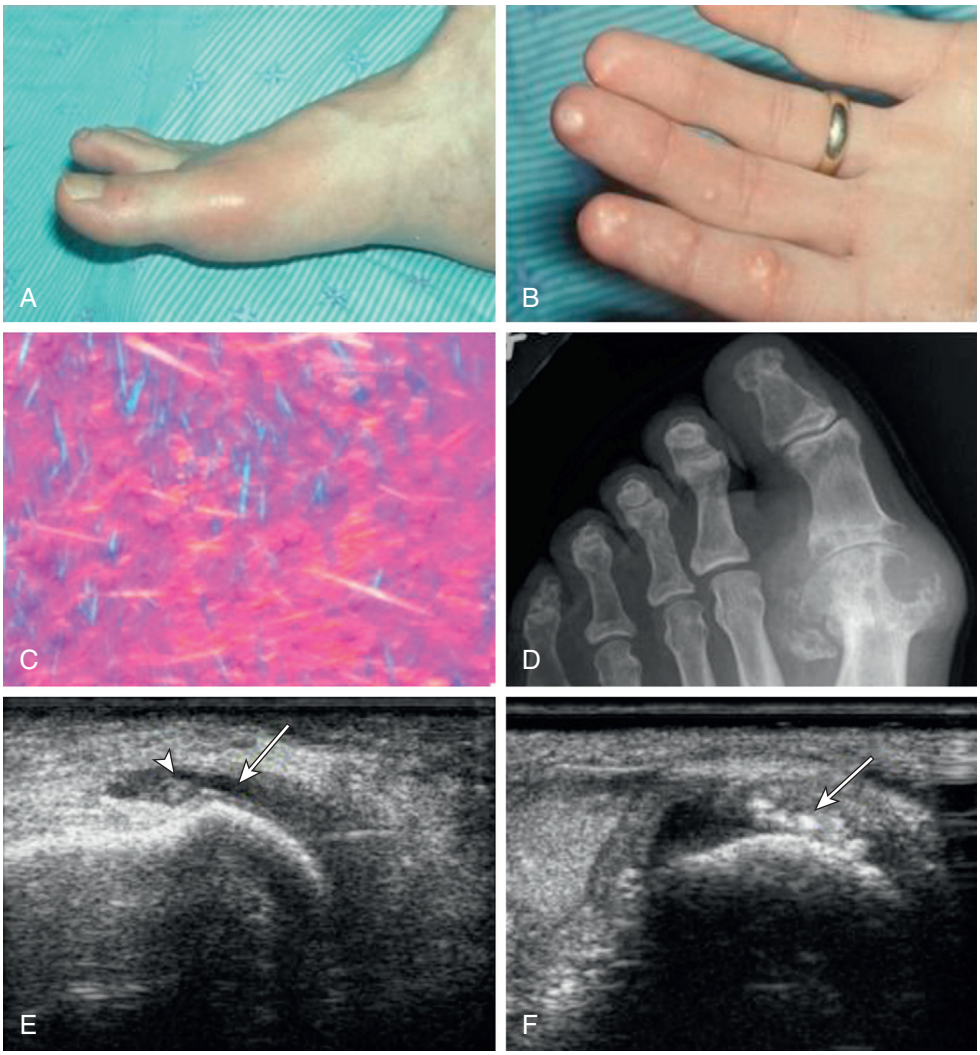



**Figure 95-1** **A** through **F,** The clinical diagnosis of gout. **A,** Podagra, or acute monoarticular inflammatory arthritis of the first metatarsophalangeal (MTP) joint. **B,** A patient with gout who has numerous subcutaneous tophi on the palmar aspect of the fingers. **C,** Monosodium urate crystals viewed under the polarizing microscope. **D,** Radiograph demonstrating typical gouty erosions at the first MTP joint, with well-demarcated erosions, away from the joint line, some with overhanging edges, and adjacent hazy tophaceous material. **E,** Ultrasound images showing the double contour sign of gout in a first MTP joint *(arrow)* with a heterogeneous, hyperechoic tophus (beneath *arrowhead*) within a hypoechoic effusion *(dark area)*. **F,** A cluster of tophi *(arrow)* in the olecranon bursa of a patient with gout. *(**D,** Courtesy Dr. Douglas Goodwin, Dartmouth Hitchcock Medical Center, Lebanon, N.H.; **F,** Courtesy Dr. John Yost, Dartmouth Hitchcock Clinic, Manchester, N.H. From Burns CM, Wortmann RL: Latest evidence on gout management: what the clinician needs to know.* Ther Adv Chronic Dis *3:271, 2012.)*

Acute gout is predominantly a disease of the lower extremities, but eventually, any joint of any extremity may be involved. Ninety percent of patients experience acute attacks in the great toe at some time during the course of their disease (Figure 95-1A). Next in order of frequency are the insteps, ankles, heels, knees, wrists, fingers, and elbows. Acute attacks rarely affect the shoulders, hips, spine, sacroiliac joints, sternoclavicular joints, acromioclavicular joints, or temporomandibular joints.[43,44] Acute gouty bursitis, tendinitis, or tenosynovitis can also occur.[45,46] Urate deposition and subsequent gout appear to have a predilection for previously damaged joints such as in Heberden's nodes of older women.[47] The differential diagnosis is usually septic arthritis or other crystal-induced arthritis, but a broader differential should be considered in confusing cases (Table 95-1).

Some patients report a history of short, trivial episodes of "ankle sprains," sore heels, or twinges of pain in the great toe before the first dramatic gouty attack. In most patients, however, the initial attack occurs with explosive suddenness and commonly begins at night after the individual has gone to sleep feeling well. Within a few hours of onset, the affected part becomes hot, dusky red, swollen, and extremely tender. Occasionally, lymphangitis may develop. Systemic signs of inflammation may include leukocytosis, fever, and elevation of the erythrocyte sedimentation rate. Radiographs usually show only soft tissue swelling during early episodes.

The course of untreated acute gout is highly variable. Mild attacks may subside in several hours or persist for only a day or two and never reach the intensity described for the classic attack. Severe attacks may last days to weeks. The skin over the joint often desquamates as the erythema subsides. With resolution, the patient becomes asymptomatic and enters the intercritical period.

Certain drugs may precipitate acute gout by either increasing or decreasing serum urate levels acutely. The

**TABLE 95-1** Differential Diagnosis of Gout

| |
|---|
| **Acute Gouty Arthritis** |
| Other crystal arthritis, especially CPPD, or pseudogout, but also basic calcium phosphate (hydroxyapatite) and others |
| Septic arthritis, including gonorrhea |
| Trauma |
| Cellulitis |
| Lyme arthritis |
| Reactive arthritis |
| Psoriatic arthritis |
| Sarcoidosis |
| Unusual presentations of other inflammatory arthritides, including rheumatoid arthritis |
| **Chronic Gouty Arthritis** |
| Rheumatoid arthritis or other chronic inflammatory arthritis |
| Calcium pyrophosphate disease |
| Osteoarthritis (especially inflammatory) |
| Lyme disease |
| Indolent infections, including mycobacteria |

**TABLE 95-2** Classification Criteria of Acute Gouty Arthritis

| |
|---|
| The presence of characteristic urate crystals in the joint fluid, or a tophus proven to contain urate crystals by chemical means or polarized light microscopy, or the presence of 6 of the following clinical, laboratory, and radiographic phenomena: |
| • More than one attack of acute arthritis |
| • Maximal inflammation developed within 1 day |
| • Attack of monoarticular arthritis |
| • Joint redness observed |
| • First metatarsophalangeal joint painful or swollen |
| • Unilateral attack involving the first metatarsophalangeal joint |
| • Unilateral attack involving the tarsal joint |
| • Suspected tophus |
| • Hyperuricemia |
| • Asymmetric swelling within a joint (radiograph) |
| • Subcortical cysts without erosions (radiograph) |
| • Negative culture of joint fluid for microorganisms during attack of joint inflammation |

Modified from Wallace SL, Robinson H, Masi AT, et al: Preliminary criteria for the classification of acute arthritis of primary gout. *Arthritis Rheum* 20:895–900, 1977.

occurrence of gout after the initiation of urate-lowering therapy is well established and particularly clinically significant. In fact, the more potent the urate-lowering effect, the more likely there is to be an acute attack.[48] Drug-induced gout as a result of increased serum urate levels occurs on occasion with diuretic therapy, administration of intravenous heparin, and use of cyclosporine.[49-51] Diuretic therapy in elderly people appears to be a particularly important precipitating factor for gouty arthritis. Other provocative factors include trauma, alcohol ingestion, surgery, dietary excess, hemorrhage, foreign protein therapy, infections, and exposure to radiographic contrast material.[52,53] Patients with gout have a 20% risk of experiencing an attack during hospitalization.[54]

The definitive diagnosis of gout is best established by aspiration of the joint and identification of intra-cellular needle-shaped crystals that have negative birefringence upon examination with compensated polarized light microscopy (Figure 95-1C). However, various alternatives have been proposed for a presumptive diagnosis. These alternatives include the triad of acute monoarticular arthritis, hyperuricemia, and a dramatic response to colchicine therapy,[55] a set of criteria proposed by the American College of Rheumatology (ACR; Table 95-2) in 1977,[56] and 10 "propositions" for diagnosis by a European League against Rheumatism (EULAR) panel of experts in 2006 (Table 95-3).[57] (The 2012 ACR Guidelines for Management of Gout assumed that the diagnosis of gout was already clearly established.[58,59]) The use of any of these schemes has limitations. First, although the diagnosis of acute gouty arthritis can be strongly suggested by the typical presentation, not all inflammation of the great toe (podagra) in hyperuricemic patients is caused by gout.[60] Second, some patients with gout are normouricemic at the time of an acute attack, a phenomenon related to alcohol use or a consequence of IL-6 generation by the acute inflammatory process.[61-63] Third, diseases other than gout occasionally can improve with colchicine therapy; these diseases include pseudogout, hydroxyapatite calcific tendinitis, sarcoid arthritis, erythema nodosum, serum sickness, rheumatoid arthritis, and familial Mediterranean fever.[17] Fourth, a recent dual-energy computed tomography (DECT) study confirmed the presence of urate in periarticular structures, especially entheses, during a suspected gout attack, perhaps accounting for the sometimes vexing problem of a joint aspiration that is negative for crystals in spite of a classic presentation.[64] Finally, the simultaneous presence of both gout and septic arthritis can be confusing clinically, with the former masking the latter.[65]

The use of ultrasonography as a means of diagnosing acute and chronic gout is gaining favor. The characteristic finding is a superficial, hyperechoic, irregular band on the surface of articular cartilage, the so-called "double contour sign" or "urate icing," which in one study was seen in 92% of gouty joints but in no joints of patients with other types of arthritis (Figure 95-1E).[66,67] Also characteristic is nonhomogeneous tophaceous material surrounded by an anechoic rim (Figure 95-1F). Further support for ultrasound comes from the demonstration of resolution of these findings in a small number of patients with gout who took urate-lowering therapy and maintained a serum urate level less than or equal to 6 mg/dL for at least 7 months.[68] Recent studies presented in abstract form have indicated excellent concordance between ultrasound readers in identifying changes, along with the presence of these findings even in people with asymptomatic hyperuricemia and the superiority of ultrasound over conventional radiography in detecting gouty erosions.[68-71] Ultrasound now appears to be a technology that will be an important ancillary approach to the diagnosis and treatment of gout. Caution is required, however, because in a recent study, the double contour sign was seen in the joints of approximately 25% of people with asymptomatic hyperuricemia.[72] As for sensitivity, the double contour sign was not present in approximately 40%

**TABLE 95-3** Propositions and Strength of Recommendation: Order According to Topic (Clinical, Urate Crystals, Biochemical, Radiographic, and Risk Factors/Comorbidities)

| | Proposition | SOR (95% CI) VAS 100 | A-B% |
|---|---|---|---|
| 1 | In acute attacks the rapid development of severe pain, swelling, and tenderness that reaches its maximum within just 6-12 hr, especially with overlying erythema, is highly suggestive of crystal inflammation, although not specific for gout | 88 (80-96) | 93 |
| 2 | For typical presentations of gout (such as recurrent podagra with hyperuricemia), a clinical diagnosis alone is reasonably accurate but not definitive without crystal confirmation | 95 (91-98) | 100 |
| 3 | Demonstration of MSU crystals in synovial fluid or tophus aspirates permits a definitive diagnosis of gout | 96 (93-100) | 100 |
| 4 | A routine search for MSU crystals is recommended in all synovial fluid samples obtained from undiagnosed inflamed joints | 90 (83-97) | 87 |
| 5 | Identification of MSU crystals from asymptomatic joints may allow definite diagnosis in intercritical periods | 84 (78-91) | 93 |
| 6 | Gout and sepsis may coexist, so when septic arthritis is suspected, Gram stain and culture of synovial fluid should still be performed even if MSU crystals are identified | 93 (87-99) | 93 |
| 7 | Although serum uric acid levels are the most important risk factor for gout, they do not confirm or exclude gout because many people with hyperuricemia do not experience gout, and during acute attacks, serum levels may be normal | 95 (92-99) | 93 |
| 8 | Renal uric acid excretion should be determined in selected patients with gout, especially those with a family history of young-onset gout, onset of gout when younger than 25 years, or renal calculi | 72 (62-81) | 60 |
| 9 | Although radiographs may be useful for differential diagnosis and may show typical features in chronic gout, they are not useful in confirming the diagnosis of early or acute gout | 86 (79-94) | 93 |
| 10 | Risk factors for gout and associated comorbidity should be assessed, including features of the metabolic syndrome (obesity, hyperglycemia, hyperlipidemia, and hypertension) | 93 (88-98) | 100 |

*A-B%: percentage of strongly to fully recommended, based on the EULAR ordinal scale (A = fully recommended, B = strongly recommended, C = moderately recommended, D = weakly recommended, E = not recommended).

CI, Confidence interval; MSU, monosodium urate; SOR, strength of recommendation; VAS, visual analogue scale (0-100 mm, 0 = not recommended at all, 100 = fully recommended).

From Zhang W, Doherty M, Pascual E, et al: EULAR evidence based recommendations for gout. Part I: diagnosis. Report of a task force of the Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). *Ann Rheum Dis* 65:1301, 2006.

of subjects despite ultrasound of multiple joints in patients with early-stage gout.[73] MRI is much more sensitive than even ultrasound at detecting gouty erosions in patients with gout whose plain radiographs are normal.[74,75] CT and DECT are also sensitive for the detection of erosions and tophi, and three-dimensional CT may have utility in the quantification of the size of tophi during clinical trials in persons with gout.[75,76]

DECT is accurate for the detection of uric acid renal stones, which have a distinctive radiographic appearance at high versus low kilovolts that allows differentiation of uric acid stones from other types of stones with computer software.[77] In several studies, DECT has been found to be useful in detecting monosodium urate crystal collections in patients with tophaceous gout.[64,78,79] A recent study evaluating DECT for the diagnosis of gout in all spectrums of disease, including early gout, found the sensitivity and specificity of DECT to be 90% and 83%, respectively (Figure 95-2).[64] A caveat is that the false-negative results were generally in patients with acute, recent-onset gout. False-positive results generally occurred in people with advanced knee osteoarthritis. The precise role of DECT and other imaging modalities for the diagnosis of acute gout in a suspicious clinical setting, either without a diagnostic tap or with a tap that reveals no crystals, remains to be seen. Whereas ultrasound has found its way into the rheumatology clinic, concerns about the cost and practicality of DECT, CT, and MRI remain.[80]

## Intercritical Gout

The terms *intercritical gout* and *interval gout* have been applied to the periods between gouty attacks. Although a few people never have a second attack, most experience a second attack within 6 months to 2 years. In the series reported by Gutman,[81] 62% had recurrences within the first year, 16% in 1 to 2 years, 11% in 2 to 5 years, and 4% in 5 to 10 years; 7% had experienced no recurrence in 10 or more years. The frequency of gout attacks usually increases over time in untreated patients. Later attacks have a less explosive onset, may be polyarticular, become more severe, and abate more slowly. Nevertheless, recovery is complete. Radiographic changes may develop during the intercritical period despite no signs of tophi on physical examination. These changes are more likely in patients with more severe hyperuricemia and more frequent acute attacks.[54,82]

The diagnosis of gout in a hyperuricemic patient with a history of acute attacks of monoarthritis may be difficult or inconclusive during the intercritical phase. Aspiration of an asymptomatic joint can be a useful adjunct in the diagnosis of gout if urate crystals are demonstrated. Joint fluids obtained from patients with gout during the intercritical phase revealed monosodium urate crystals in 12.5% to 90% of joints.[83] Such crystals in asymptomatic joints are often associated with mild synovial fluid leukocytosis, which suggests a potential to contribute to joint damage even in the intervals between attacks.

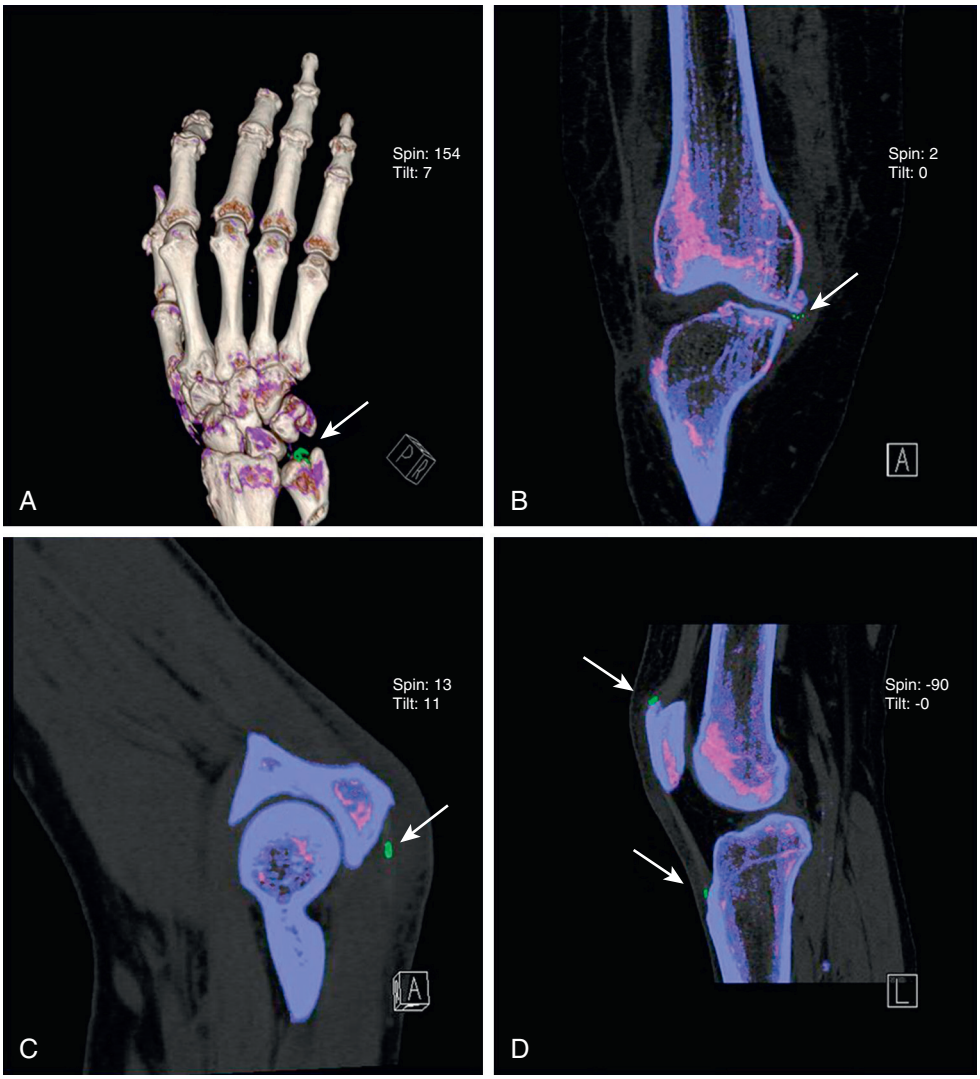



**Figure 95-2** Dual-energy computed tomography images. *Arrows* indicate monosodium urate (MSU) deposition, color coded in *green*, in patients with (**A**) acute gouty arthritis of the wrist (three-dimensional reconstruction image), (**B**) advanced osteoarthritis of the knee, (**C**) elbow pain and swelling, and (**D**) knee pain and swelling. Note that in **B,** the patient did not have active gout, and in **C** and **D**, synovial fluid analysis was initially negative, but post–dual-energy computed tomography ultrasound-guided aspiration was positive for MSU crystals. *(From Bongartz T, Glazebrook KN, Kavros SJ, et al: Dual-energy CT for the diagnosis of gout: an accuracy and diagnostic yield study.* Ann Rheum Dis *74:1072–1077, 2015.)*

## Chronic Gouty Arthritis

Eventually, the patient may enter a phase of chronic polyarticular gout with no pain-free intercritical periods. At this stage, gout is easily confused with other types of arthritis or other conditions.[84-86] The time from the initial attack to the beginning of chronic symptoms or visible tophaceous involvement is highly variable in studies of untreated patients. Hensch[87] reported intervals ranging from 3 to 42 years, with an average of 11.6 years between the first attack and the development of chronic arthritis. Ten years after the first attack, about half the individuals were still free of obvious tophi, and most of the remainder had only minimal deposits. Thereafter the proportion of people with nontophaceous involvement slowly declined, to 28% after 20 years. Two percent of the patients experienced severe crippling disease some 20 years after the initial attack.

The rate of formation of tophaceous deposits correlates with both the degree and the duration of hyperuricemia. The principal determinant is the serum urate level.[54] Gutman[88] found that the mean serum urate concentration was 9.1 mg/dL in 722 patients without tophi, 10 to 12 mg/dL in 456 patients with minimal to moderate tophi, and greater than 11 mg/dL in 11 patients with extensive tophaceous involvement. The rate of tophus formation also increases with the severity of renal disease and the use of diuretics.[89]

Tophaceous gout is the consequence of the chronic inability to eliminate urate as rapidly as it is produced. As the urate pool expands, deposits of urate crystals appear in cartilage, synovial membranes, tendons, soft tissues, and elsewhere. Tophi are rarely present at the time of an initial attack of primary gout.[90,91] They are more likely to be present in people with gout as a result of myeloproliferative diseases, in people with juvenile gout-complicating glycogen storage diseases, in people with Lesch-Nyhan syndrome, or after allograft transplantation in patients treated with cyclosporine.[17,91]

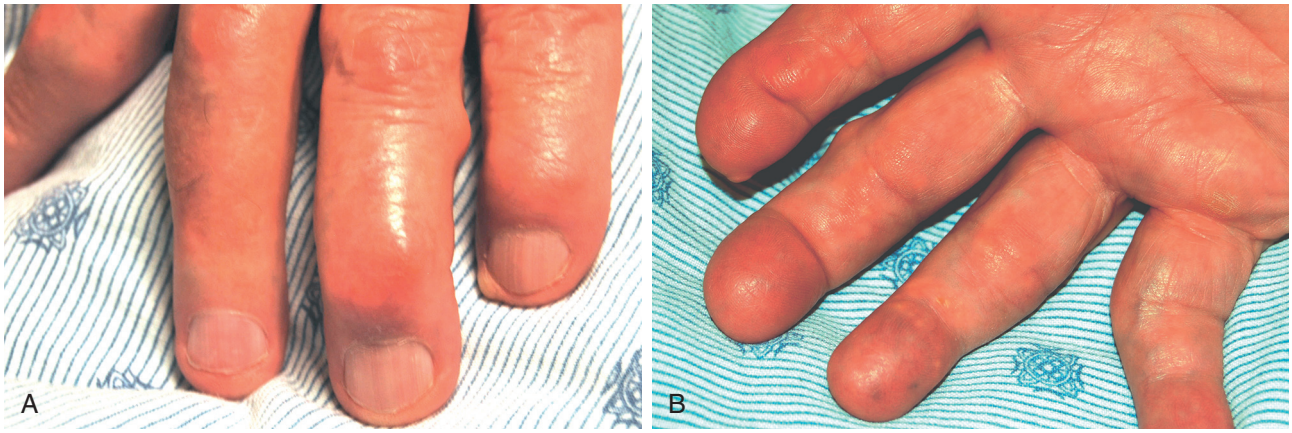



**Figure 95-3** **A,** Dorsal digits of the hand of a patient with tophaceous gout. The involvement of the third distal interphalangeal joint has an appearance similar to inflammatory osteoarthritis or psoriatic arthritis in the appropriate clinical setting. **B,** Palmar view of the same hand revealing obvious "studding" of uric acid deposits.



**Figure 95-4** A uric acid deposit in the prepatellar bursa of the right knee in a patient with tophaceous gout. The patient also has a large effusion in the suprapatellar pouch.

Tophi can occur in a variety of locations. Tophaceous deposits may produce irregular, asymmetric, moderately discrete tumescence of the fingers, hands, knees, or feet (Figures 95-1B, 95-3, 95-4, and 95-5). Tophi also form along the ulnar surfaces of the forearm as saccular distentions of the olecranon bursa (Figure 95-6), in the helix of the ear (Figure 95-7), or as fusiform enlargements of the Achilles tendon (Figure 95-8). The process of tophaceous deposition advances insidiously. Although the tophi themselves are relatively painless, acute inflammation can occur around them. Eventually, extensive destruction of the joints and large subcutaneous tophi may lead to grotesque deformities, particularly of the hands and feet, and to progressive crippling (Figure 95-9). The tense, shiny, thin skin overlying the tophus may ulcerate and extrude white, chalky, or pasty material composed of urate crystals. Secondary infections of tophi are rare.

Typical radiographic changes, particularly erosions with sclerotic margins and overhanging edges of bone, occur with the development of tophi (Figures 95-1D and 95-10).[92] These erosions may be difficult to distinguish from erosions of other causes, but the presence of a thin, overhanging calcified edge is strong evidence of gout. Calcifications can be seen in some tophi, and bony ankylosis may rarely occur. Ultrasonography, MRI, and CT, including DECT, can demonstrate tophi, with DECT providing the most specific images.[64,66-76,78-80,93]

Tophi can produce a marked limitation of joint movement by involvement of the joint structure directly or of a tendon serving the joint. Any joint can be involved, although joints of the lower extremity are primarily affected. Spinal joints do not escape urate deposition,[76,86,94] but acute gouty spondylitis is unusual. Symptoms related to nerve or spinal cord compression by tophi have rarely been observed. Tophi rarely occur in myocardium, valves, cardiac conduction system, various parts of the eye, and the larynx.[95,96]

## ASSOCIATED CONDITIONS

**KEY POINTS**

- **Gout is associated with obesity, hypertriglyceridemia, glucose intolerance and the metabolic syndrome, hypertension, atherosclerosis, and hypothyroidism.**
- **Renal insufficiency is frequently associated with hyperuricemia and gout.**
- **Hyperuricemia is a common cause of nephrolithiasis.**
- **Rarely, chronic hyperuricemia causes urate nephropathy, and acute hyperuricemia can lead to uric acid nephropathy in the tumor lysis syndrome.**
- **Alcohol use, lead intoxication, and cyclosporine treatment are associated with hyperuricemia and gout.**
- **A diagnosis of gout should prompt a search for the co-existence of associated conditions.**

The association of gout with obesity and overeating is well recognized.[97] In 6000 subjects, hyperuricemia was found in only 3.4% of persons with a relative weight at or below the 20th percentile, in 5.7% of subjects between the 21st and 79th percentiles, and in 11.4% of subjects at or above the 80th percentile.[98]




**Figure 95-5** **A,** Chronic tophaceous gout of the great toe involving both the metatarsophalangeal and interphalangeal joints. **B,** Tophaceous gout of the third toe along with extensive onychomycosis could easily be mistaken for a "sausage toe" of psoriatic arthritis.



**Figure 95-6** Uric acid deposits in the olecranon bursa and the extensor forearm in a patient with tophaceous gout.

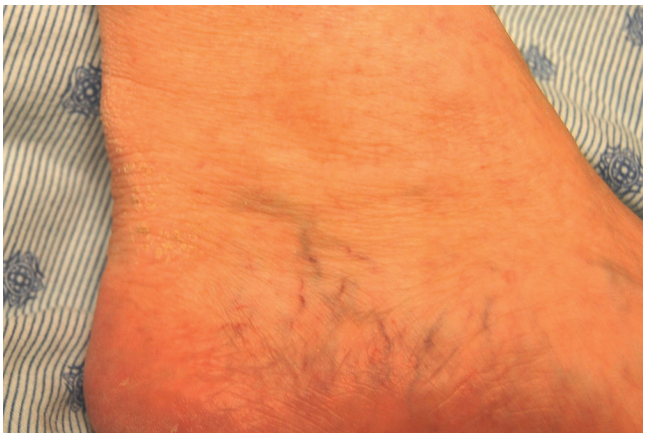


**Figure 95-8** Fusiform tophus of the Achilles tendon above the insertion on the calcaneus.



**Figure 95-7** A tophus in the helix of the ear in a patient with chronic gout.

Hypertriglyceridemia has been reported in 75% to 80% of patients with gout,[99] and hyperuricemia is found in more than 80% of patients with hypertriglyceridemia.[17] However, studies have been unable to show a correlation between serum urate and cholesterol values or a unique lipid phenotype.[100] Patients with gout who drink alcohol excessively have mean serum triglyceride levels that are higher than those of obesity-matched control subjects and of patients with gout who do not drink alcohol.[101]

Hyperuricemia has been reported in 2% to 50% of patients with diabetes mellitus, and gouty arthritis has been reported in fewer than 0.1% to 9% of these patients.[102] Abnormal glucose tolerance tests have been noted in 7% to 74% of patients with gout, depending, in part, on the criteria used.[103]

Hyperuricemia has been reported in 22% to 38% of patients with untreated hypertension. This figure increases to 67% when diuretic therapy and renal disease are present.[17] Hyperuricemia may be an indication of a potential risk for hypertension in adolescent males.[104] Hypertension is present in one-fourth to one-half of patients with classic gout, but the presence of hypertension is unrelated to the duration of gout.[97,98] Elevated serum urate concentrations are associated with increased tubular reabsorption of sodium.[105] The serum urate concentration also correlates inversely with renal blood flow and urate clearance and correlates directly with

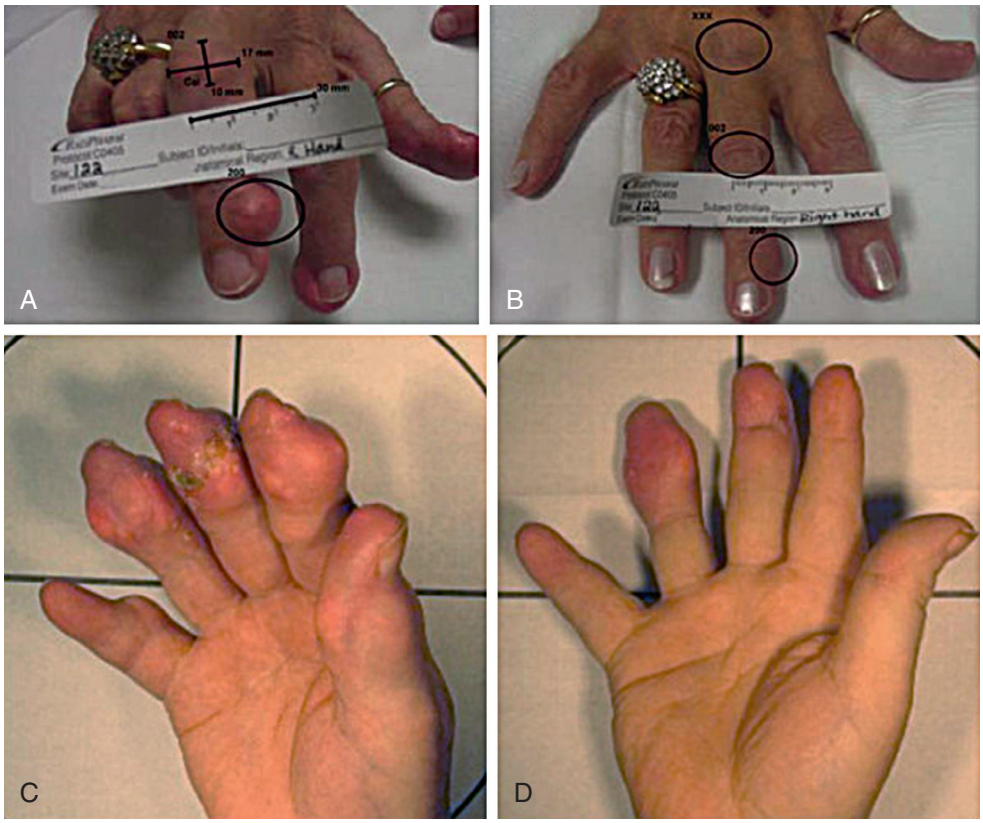



**Figure 95-9** Digital images showing complete resolution of tophi in two patients who received pegloticase biweekly. **A,** Baseline tophus on the medial aspect of the right third distal interphalangeal (DIP) joint in patient 1. **B,** Complete resolution of target tophus at week 13 in patient 1. Note also the reduction in the area of a large tophus on the third proximal interphalangeal joint. **C,** Baseline tophi on the right thumb; on the second, third, and fourth DIP joints with ulcerations; and on the fifth proximal interphalangeal joint of patient 2 after 6 months of treatment with placebo in randomized controlled trials. **D,** Complete resolution of target tophi on four digits in patient 2 after 25 weeks of treatment with twice-monthly pegloticase in an open-label extension study. *(From Baraf HS, Becker MA, Gutierrez-Urena SR, et al: Tophus burden reduction with pegloticase: results from phase 3 randomized trials and open-label extension in patients with chronic gout refractory to conventional therapy.* Arthritis Res Ther *15:R137, 2013.)*

both renovascular and total resistance. Therefore the association between hypertension and hyperuricemia may be related to the reduction of renal blood flow in persons with hypertension. In addition, uric acid causes smooth muscle proliferation in vitro and vascular disease in animal models through a mechanism that involves complex intra-cellular signaling, mitogen-activated protein kinase activation, and platelet-derived growth factor expression.[105,106]

The association between hyperuricemia and the manifestations of atherosclerosis has led to speculation that hyperuricemia is a risk factor for coronary artery disease. Some studies show no clear associations among blood pressure, blood glucose level, or serum cholesterol and serum urate concentration when adjustments are made for the effects of age, sex, and relative weight.[90,98-102,107-110] The serum urate concentrations of persons with coronary heart disease are not significantly different from the mean levels of the population.[110,111] Other studies, however, maintain that hyperuricemia is an independent risk factor for coronary artery disease[112,113] (described in a subsequent section).

The term *metabolic syndrome* has been applied to a cluster of abnormalities, including resistance to insulin-stimulated glucose uptake, hyperinsulinemia, hypertension, and dyslipoproteinemia that are characterized by high levels of plasma triglycerides and high-density lipoprotein cholesterol. Hyperuricemia closely correlates with the degree of insulin resistance[110-116] and therefore is a likely feature of metabolic syndrome. Metabolic syndrome has been associated with coronary artery disease, and hyperuricemia as a component of metabolic syndrome may explain the previously recognized association between coronary artery disease and hyperuricemia. A recent study concluded that the relationship between hyperuricemia and acute myocardial infarction is independent, but patients who experience gouty arthritis are at an increased risk for myocardial infarction. This association could not be explained by renal function, metabolic syndrome, diuretic use, or traditional cardiovascular risk factors.[117] In a prospective study, men with gout had an increased risk of all-cause mortality, and among subjects with pre-existing coronary heart disease, that gout-related increased risk was the result of an elevated risk of cardiovascular death, particularly coronary heart disease.[118] However, a very recent systematic review of the literature concluded that the data supporting an increased risk of developing cardiovascular disease as a result of hyperuricemia or gout are weak and and/or flawed, at best.[119] An up-to-date review recently explored the intricacies of this issue,[120] which will not be laid to rest until an adequately powered, randomized, controlled trial provides a definitive answer.[121]

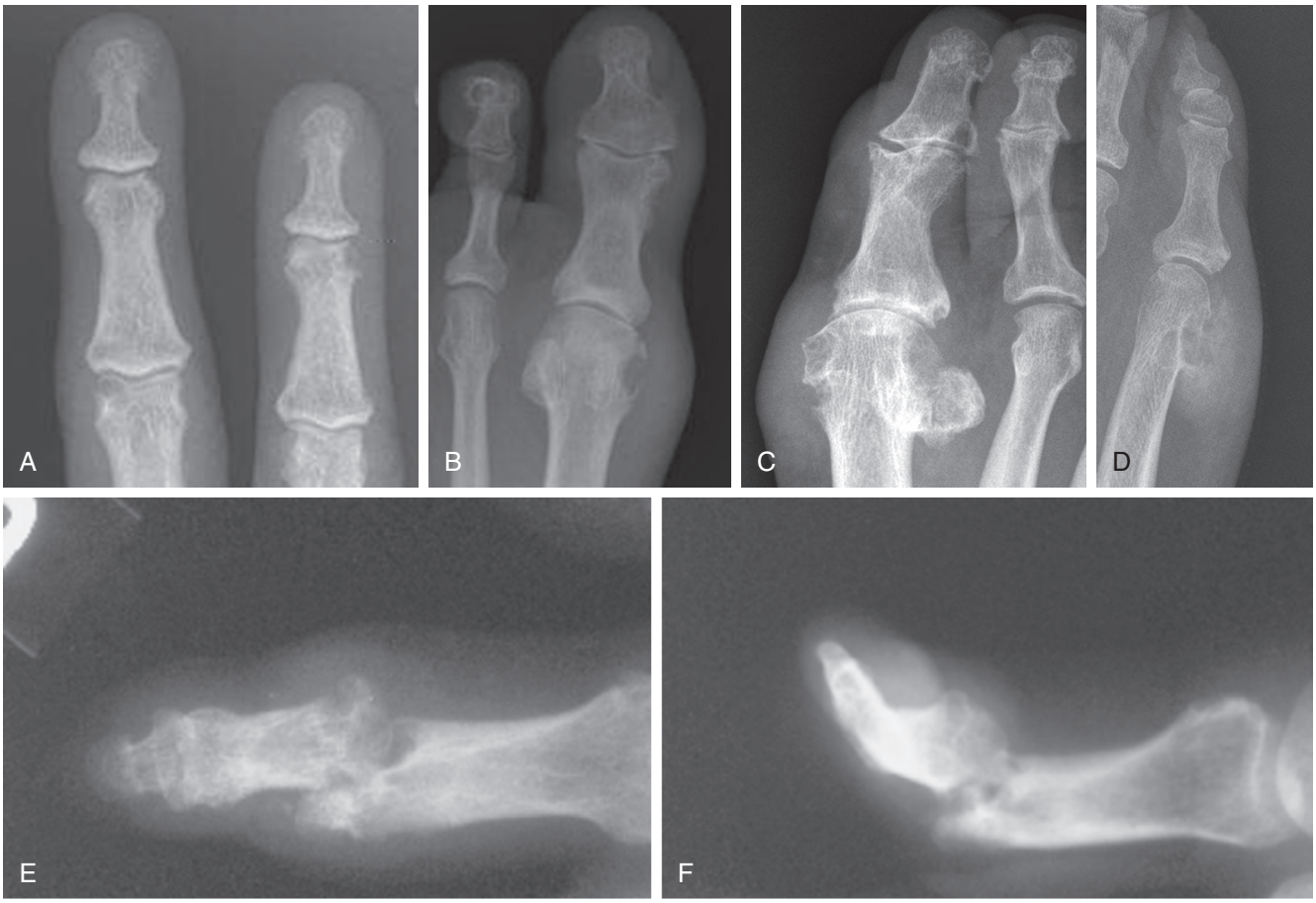



**Figure 95-10** **A** through **F,** Radiographs of damage to joints due to gout. **A,** Erosions at the third proximal interphalangeal and fourth distal interphalangeal joints of the hand. **B,** Discrete punched-out lesion at the first metatarsophalangeal (MTP) joint. **C,** More advanced disease at the first MTP joint with periarticular tophaceous material. **D,** Erosion at the fifth MTP joint. **E** and **F**, Severe destruction with malalignment.

Alcohol consumption has long been associated with hyperuricemia and gout. In susceptible persons, alcohol use can precipitate acute gouty arthritis. An epidemiologic study in Saudi Arabia, where alcohol consumption is rare, revealed an 8.4% prevalence of hyperuricemia but no cases of gout among the study group.[122] Both a decrease in the renal excretion of uric acid and an increase in uric acid production seem to be important factors in this association.[123] Ethanol increases uric acid production by accelerating the turnover of ATP. Among alcoholic beverages, beer may have more potent effects on uric acid production because of its high guanosine content.[14]

A significant increased prevalence of hypothyroidism appears to exist among both female and male patients with gouty arthritis.[124] Hyperuricemia may also be more prevalent in patients with hypothyroidism. Thyroid replacement therapy is associated with a decrease in serum urate concentration caused by an increased uric acid diuresis—a change not explained solely by a change in creatinine clearance.[125] Although the cause of hyperuricemia and gout in patients with hypothyroidism is unknown, it is speculated that urate metabolism is mediated by thyroid-stimulating hormone receptors in extrathyroidal tissues, including the kidney and that these receptors modulate urate homeostasis.

Studies of acutely ill patients in intensive care units indicate that markedly increased serum urate concentrations, in the vicinity of 20 mg/dL, are associated with hypotensive events and a poor prognosis.[126] This finding may be related to two factors. First, ischemic tissue may foster the degradation of ATP to purine end products, thereby enhancing the production of urate. The finding of increased plasma ATP degradation products associated with hyperuricemia and adult respiratory distress syndrome supports this possibility.[127] Second, the conversion of hypoxanthine to uric acid by xanthine oxidase during ischemia produces oxidant radicals, which are themselves associated with tissue injury.[128] It is possible that inhibition of xanthine oxidase with allopurinol may be a useful therapy in this setting.

Maternal serum urate concentrations normally decrease during pregnancy until the 24th week and then increase until 12 weeks after delivery.[129] An increase in the serum urate level occurs in pre-eclampsia and toxemia of pregnancy as a result of a decrease in the renal clearance of urate.[130] Perinatal mortality is markedly increased when maternal plasma urate levels are raised, usually in association with early-onset pre-eclampsia. The highest mortality rate is seen with serum urate concentrations higher

than 6 mg/dL and diastolic blood pressures greater than 110 mm Hg. Labor itself is associated with an increased serum urate level, and it remains elevated for 1 to 2 days after delivery.

Gout is rarely seen in patients with rheumatoid arthritis, systemic lupus erythematosus, or ankylosing spondylitis.[44,131-133] The basis for the decreased concurrence of these disorders is unclear, although the long-term use of NSAIDs or corticosteroids may mask the clinical features of gout in some of these patients.

## Renal Disease

After gouty arthritis, renal problems appear to be the most frequent complication of hyperuricemia. Approximately 20% to 40% of patients with gout have albuminuria, which is usually mild and often intermittent. Hyperuricemia alone may be implicated as the cause of chronic kidney disease only when the concentration of urate chronically exceeds 13 mg/dL in men or 10 mg/dL in women.[134] Before the routine treatment of asymptomatic hypertension, renal failure accounted for 10% of the deaths in patients with gout. Whether moderate hyperuricemia has a direct harmful effect on renal function is unclear. Some evidence suggests that urate damages the kidneys and leads to hypertension.[105,106]

The term *urate nephropathy* is used to describe the deposition of urate crystals in the interstitium of the medulla and pyramids, with a surrounding giant cell reaction—a distinctive histologic finding characteristic of the gouty kidney (Figure 95-11). Factors such as coexistent hypertension, chronic lead exposure, ischemic heart disease, and primary pre-existing renal insufficiency probably play important roles in the pathogenesis of this disease. Although urate nephropathy appears to exist as a distinct entity, it was not generally believed to be an important contributor to renal function in most patients with gout.[17,135] However, recent studies indicate that hyperuricemia may well play a significant role in renal function, and sustained urate-lowering therapy appears to impede renal function decline in patients with gout.[136]

In contrast, *uric acid nephropathy* is the term used to describe acute renal failure resulting from the precipitation of large quantities of uric acid crystals in the collecting ducts and ureters. This complication most commonly occurs in patients with leukemia and lymphoma as a result of rapid malignant cell turnover, often during chemotherapy.[137,138] This syndrome (also termed *acute tumor lysis syndrome*) has been more clearly defined as hyperuricemia, lactic acidosis, hyperkalemia, hyperphosphatemia, and hypocalcemia and is most commonly observed in patients with aggressive, rapidly proliferating tumors, including lymphoproliferative disorders and metastatic medulloblastoma. Uric acid nephropathy is less commonly found with other neoplasms, after epileptic seizures, after vigorous exercise with heat stress, and after angiography and coronary artery bypass surgery.[17]

In the tumor lysis syndrome, the large amount of nucleic acid in nucleotides liberated with massive cytolysis is converted rapidly to uric acid. Typically, marked hyperuricemia is present, with a mean serum urate level of 20 mg/dL (range, 12 to 80 mg/dL). The pathogenesis of acute renal failure in uric acid nephropathy is related to the precipitation of uric acid in the distal tubules and collecting ducts, the sites of maximal acidification and concentration of urine. Oliguria, or even anuria, and azotemia may occur. "Gravel" or "sand" may be noted in the urine. The ratio of urinary uric acid to creatinine in these patients typically exceeds 1; in patients with most other causes of acute renal failure, the ratio is $0.4 \pm 0.3$.[138]

Nephrolithiasis occurs in 10% to 25% of patients with primary gout, a prevalence greater than that in the general population. The likelihood of stones in a given patient with gout increases with the serum urate concentration and with amounts of urinary uric acid excretion.[41,139] It exceeds 50% with a serum urate value above 13 mg/dL or with urinary uric acid excretion rates in excess of 1100 mg every 24 hours.

Uric acid calculi account for approximately 10% of all stones in patients in the United States; elsewhere, rates range from as low as 5% up to 40% in Israel and Australia, respectively.[17] Uric acid stones can occur in patients with no history of gouty arthritis, and only 20% in this group are hyperuricemic. Other renal stone disease is associated with hyperuricemia and gout. Subjects with gout also have an increased incidence of stones that contain calcium. In addition, about 30% of patients with recurrent calcium stone disease have either an increased urinary uric acid excretion rate or hyperuricemia. A causative link between uric acid and recurrent calcium oxalate stones is provided by reports of reduced stone frequency in patients treated with allopurinol.

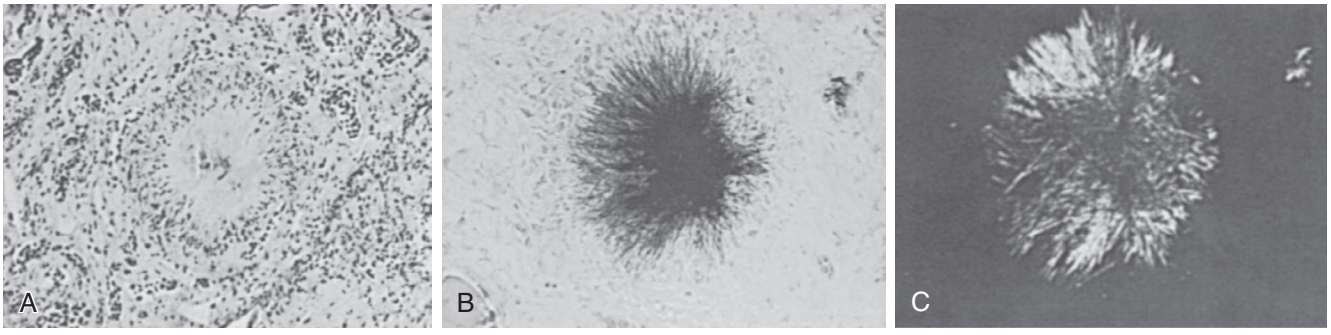



**Figure 95-11** **A,** Urate deposit in the medulla of the kidneys as seen in an alcohol-fixed section stained with hematoxylin-eosin (×250). **B,** Adjacent section of the deposit stained with methenamine silver (×250). **C,** Adjacent section of the same deposit seen with polarized light (×250).

Finally, the report of uric acid as the major constituent of a stone obtained from a patient with no apparent abnormalities of uric acid metabolism should suggest the possibility that the constituent is actually 2,8-dihydroxyadenine and that the patient has adenine phosphoribosyltransferase deficiency.[140] X-ray diffraction is required to distinguish uric acid from 2,8-dihydroxyadenine.

Familial juvenile hyperuricemic nephropathy (FJHN), sometimes called *familial juvenile gouty nephropathy*, was first described in the 1960s.[141] This disorder is inherited as an autosomal dominant trait with a high degree of penetrance and is usually associated with gout. Renal disease typically develops in the second decade of life and progresses to end-stage renal failure by midlife.[142-144] Histologic examination of kidney tissue reveals tubulointerstitial inflammation and splitting of thickened tubular basement membranes. The primary diagnostic criterion is a reduced fractional excretion of urate (defined as uric acid clearance factored by creatinine clearance × 100 equal to 5% or less; normal is 8% to 18%).[145] The dramatically low fractional excretion of urate and the early onset of disease are conspicuous characteristics of FJHN and distinguish it from other autosomal dominant hyperuricemic disorders that usually appear later in life (Table 95-4). Genotype mapping has linked the gene for FJHN to chromosome 16p12-p11.[143]

Autosomal dominant medullary cystic kidney disease (ADMCKD) is another hereditary nephropathy that usually includes gout among its constellation of symptoms. The onset of renal dysfunction occurs later than in persons with FJHN. Renal histology reveals numerous corticomedullary and intramedullary cysts in the kidneys and increased medullary connective tissue. At least two loci appear to be responsible for ADMCKD. One, termed ADMCKD1, is located on chromosome 1; the other, ADMCKD2, is a 16p locus. ADMCKD2 and FJHN loci map to approximately the same region of chromosome 16p.[146]

The chromosome 16p12 locus that harbors the candidate interval for FJHN and ADMCKD2 contains six candidate genes, including the uromodulin gene *(UMOD)*.[147-149] *UMOD* encodes the Tamm-Horsfall protein, a glycosyl phosphatidylinositol–anchored glycoprotein localized to the thick ascending limb of the loop of Henle. Amorphous deposits of uromodulin are present in the renal interstitium of patients with medullary cystic kidney disease.[150] Four different mutations in exon 4 have been identified in the *UMOD* gene. Because mutations in the same gene are responsible for both FJHN and ADMCKD, the two entities appear to be allelic variants in *UMOD* that cause decreased urinary concentrations of Tamm-Horsfall protein, with resulting hyperuricemia and progressive renal failure.[151]

**TABLE 95-4** Genetics of Renal Diseases Associated with Gout

| Condition | Inheritance | Chromosomal Location | Gene |
|---|---|---|---|
| FJHN | AD | 16p12.3<br>17cenq21.3 | Uromodulin<br>HNF-1β |
| MCKD type 1 | AD | 1q21 | ? |
| MCKD type 2 | AD, AR | 16p12.3 | Uromodulin |

AD, Autosomal dominant; AR, autosomal recessive; FJHN, familial juvenile hyperuricemic nephropathy; HNF, hepatic nuclear factor; MCKD, medullary cystic kidney disease.

## Lead Intoxication

Hyperuricemia and gout are well-recognized complications of chronic lead intoxication, with the prevalence of gout in patients with plumbism ranging between 6% and 50%.[152] Although a renal defect is recognized, it has not been well defined.[153,154] Some patients with primary gout have increased blood lead levels compared with age- and sex-matched control subjects, despite the absence of a history of overt lead exposure.[155] This finding suggests that occult chronic lead intoxication may play a causative role in some cases of primary gout (up to 36% of some gout populations).[152] In addition, patients with gout who have renal impairment seem to have an increased quantity of mobilized lead compared with gouty patients who have normal renal function.[156] These observations suggest an important role for lead in the pathogenesis of gouty nephropathy.

## Cyclosporine-Induced Hyperuricemia and Gout

Cyclosporine interferes with the renal excretion of uric acid. Hyperuricemia and gout occur with increased frequency among transplant recipients treated with cyclosporine and are even more common when diuretics are used concomitantly.[91,157] However, serum urate levels do not correlate directly with cyclosporine levels or with the degree of hypertension or renal insufficiency. The onset of gout may occur soon after transplantation, with a mean of about 17 months. Gout attacks may be typical and monoarticular, or they may affect unusual sites such as the shoulder, hip, or sacroiliac joints. Polyarticular attacks and an accelerated course, with early development of tophi, may also be observed. Nephrolithiasis develops in about 3% of patients who undergo a renal transplant. All calculi from patients treated with azathioprine are composed of calcium compounds, whereas 60% of the calculi from people treated with cyclosporine contain uric acid.[158]

# CLASSIFICATION OF HYPERURICEMIA AND GOUT

**KEY POINTS**

**Primary hyperuricemia and gout are caused by decreased renal uric acid excretion in more than 90% of affected patients and overproduction of urate in less than 10% of affected patients.**

**Secondary hyperuricemia and gout are usually related to decreased renal urate clearance as a direct or indirect consequence of the primary disease process.**

**Four known inborn errors of purine metabolism with overproduction of urate account for less than 1% of cases of secondary hyperuricemia and gout.**

The concentration of urate in body fluids is determined by the balance between production and elimination. Accordingly, hyperuricemia may be caused by an excessive rate of

**TABLE 95-5** Classification of Hyperuricemia and Gout

| Type | Metabolic Disturbance | Inheritance |
|---|---|---|
| **Primary** | | |
| **Molecular Defects Undefined** | | |
| Underexcretion (90% of primary gout) | Not established | Polygenic |
| Overproduction (10% of primary gout) | Not established | Polygenic |
| **Associated with Specific Enzyme Defects** | | |
| PRPP synthetase variants; increased activity | Overproduction of PRPP and uric acid | X-linked |
| HPRT deficiency, partial | Overproduction of uric acid; increased purine biosynthesis de novo driven by surplus PRPP; Kelley-Seegmiller syndrome | X-linked |
| **Secondary** | | |
| **Associated with Increased Purine Biosynthesis De Novo** | | |
| HPRT deficiency, "virtually complete" | Overproduction of uric acid; increased purine biosynthesis de novo driven by surplus PRPP; Lesch-Nyhan syndrome | X-linked |
| Glucose-6-phosphatase deficiency or absence | Overproduction plus underexcretion of uric acid; glycogen storage disease type I (von Gierke's disease) | Autosomal recessive |
| Fructose-1-phosphate aldolase deficiency | Overproduction plus underexcretion of uric acid | Autosomal recessive |
| **Associated with Increased ATP Degradation** | | |
| Associated with increased nucleic acid turnover | Overproduction of uric acid | Most not familial |
| Associated with decreased renal excretion of uric acid | Decreased filtration of uric acid, inhibited tubular secretion of uric acid, or enhanced tubular reabsorption of uric acid | Some autosomal dominant; some not familial; most unknown |
| **Idiopathic** | | Unknown |

ATP, Adenosine triphosphate; HPRT, hypoxanthine phosphoribosyltransferase; PRPP, phosphoribosylpyrophosphate.

urate production, a decrease in the renal excretion of uric acid, or a combination of both events.

Hyperuricemia and gout may be classified as follows (Table 95-5):

- Primary: These cases appear to be innate—that is, they are neither secondary to an acquired disorder nor the result of a subordinate manifestation of an inborn error that leads initially to a major disease, unlike gout. Some but not all cases of primary gout have a genetic basis.
- Secondary: These cases develop in the course of another disease or as a consequence of drug use.
- Idiopathic: In these cases, a more precise classification cannot be assigned.

Further subdivisions within each major category are based on the identification of overproduction, underexcretion, or both as being responsible for the hyperuricemia. Evidence of overproduction of urate is provided by determination of the 24-hour urinary uric acid excretion. For adults ingesting a purine-free diet, a total excretion of up to 600 mg/day is considered to be within the normal range.[159] For patients consuming regular diets, a value in excess of 1000 mg/day is clearly abnormal and an indication of overproduction, and values between 800 and 1000 mg/day are considered borderline. It has been suggested that overproduction of uric acid can be assessed simply by determining the ratio of uric acid to creatinine in the urine or the $C_{urate}/C_{creatinine}$ ratio. However, comparison of these two ratios with the 24-hour urinary uric acid excretion reveals a poor correlation in most patients.[160,161] Exceptions include patients with specific enzymatic deficiencies or with rapid cell lysis during chemotherapy for leukemia or lymphoma.

## Primary Gout

Renal mechanisms are responsible for hyperuricemia in most cases of gout. Genetic factors exert an important control in the renal clearance of urate.[162] A careful comparison of uric acid clearances and excretion rates over a wide but comparable range of filtered loads of urate indicates that most gouty subjects have a lower ratio of urate-to-inulin clearance ($C_{urate}/C_{inulin}$ ratio) than do nongouty subjects.[159,162,163] The excretion rates and the capacity of the excretory mechanism for uric acid are the same for people with and without gout. The excretion curve, however, is shifted. Subjects with gout require serum urate values 2 or 3 mg/dL higher than those of control subjects to achieve equivalent uric acid excretion rates. Theoretically, the shift in the excretion curve in subjects with gout may result from reduced filtration of urate, enhanced reabsorption, or decreased secretion. Patients classified as exhibiting an overproduction of uric acid represent less than 10% of the gouty population.

## Secondary Gout

Numerous secondary causes of hyperuricemia and gout can be attributed to a decrease in the renal excretion of uric acid. A reduction in the glomerular filtration rate leads to a decrease in the filtered load of urate and, consequently, to hyperuricemia. Patients with renal disease are hyperuricemic on this basis. Other factors such as decreased secretion of urate have been postulated in patients with some types of renal disease (e.g., polycystic kidney disease and lead nephropathy).

Diuretic therapy currently represents one of the most important causes of secondary hyperuricemia in humans. Diuretic-induced volume depletion leads to a decreased filtered load and enhanced tubular reabsorption of urate. A number of other drugs lead to hyperuricemia by a renal mechanism. These agents include low-dose aspirin, pyrazinamide, nicotinic acid, ethambutol, ethanol, and cyclosporine.

Decreased renal excretion of uric acid is thought to be an important mechanism for the hyperuricemia associated with several disease states. Volume depletion may be an important factor in patients with hyperuricemia that is associated with adrenal insufficiency or nephrogenic diabetes insipidus. An accumulation of organic acids leads to hyperuricemia, which is the case in starvation, alcoholic ketosis, diabetic ketoacidosis, maple syrup urine disease, and lactic acidosis of any cause (e.g., hypoxemia, respiratory insufficiency, chronic beryllium disease, and acute alcohol intoxication). The renal bases of the hyperuricemia in conditions such as chronic lead intoxication, hypoparathyroidism, pseudohypoparathyroidism, and hypothyroidism remain unclear.

Secondary gout can also result from urate overproduction. Four specific defects cause urate overproduction as a consequence of accelerated de novo purine biosynthesis: hypoxanthine phosphoribosyltransferase deficiency, phosphoribosylpyrophosphate synthetase overactivity, glucose-6-phosphatase deficiency, and fructose-1-phosphate aldolase deficiency.

## TREATMENT

### KEY POINTS

**Asymptomatic hyperuricemia generally is not treated with specific urate-lowering therapy, but identification of this condition should lead to a search for the cause and/or associated conditions.**

**Episodes of acute gouty arthritis can be treated with colchicine, NSAIDs, adrenocorticotropic hormone, and systemic or intra-articular steroids.**

**Prophylaxis against acute attacks with colchicine or NSAIDs can be effective but does not change the underlying process in the absence of concomitant urate-lowering therapy.**

**Starting urate-lowering therapy after a single attack of gout remains debatable, but recurrent attacks of gout, urate nephrolithiasis, tophaceous gout, and/or evidence of gout-induced joint damage are all accepted indications for this therapy.**

**Xanthine oxidase inhibitors and uricosuric agents are effective at lowering serum urate levels in most patients.**

**Uricases such as pegloticase should be reserved for refractory tophaceous gout.**

**A serum urate of less than 6 mg/dL is the target of therapy.**

**Prophylaxis with colchicine, NSAIDs, or, less preferably, systemic steroids should be continued for at least 6 months after initiation of urate-lowering therapy.**

**Long-term compliance with the treatment regimen remains a major issue in gout; forming a therapeutic alliance with the patient is critical.**

**Lifestyle modifications, especially reduced alcohol consumption, may help somewhat in the control of gout but are more important for the management of associated conditions such as obesity and hyperlipidemia.**

The therapeutic aims in gout are as follows (Figure 95-12):

- To terminate the acute attack as promptly as possible
- To prevent recurrences of acute gouty arthritis
- To prevent or reverse complications of the disease resulting from the deposition of sodium urate or uric acid crystals in joints, kidneys, or other sites

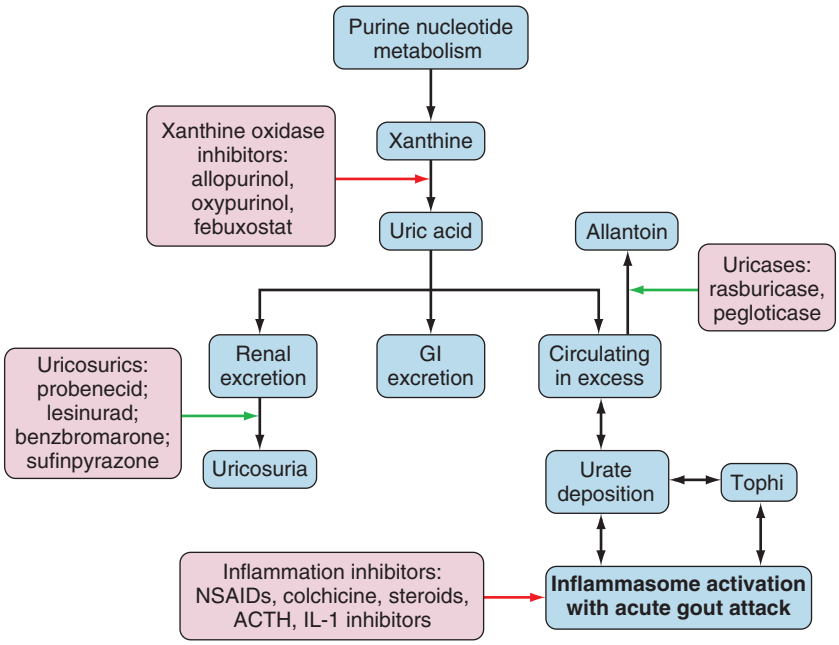



**Figure 95-12** Treatment options for gout, including inhibitors of inflammation used for acute attacks and the available approaches for lowering serum urate when appropriate. Some inflammation inhibitors may also be used for prophylaxis when initiating urate lowering therapy. See text for details. *Red arrows* indicate inhibitory effects; *green arrows* indicate promoting effects. ACTH, Adrenocorticotropic hormone; GI, gastrointestinal.

- To prevent or reverse conditions associated with gout that are deleterious, such as obesity, hypertriglyceridemia, and hypertension

## Asymptomatic Hyperuricemia

The presence of hyperuricemia is rarely an indication for specific urate-lowering therapy. Rather, upon diagnosing hyperuricemia, the following questions should be addressed:

- What is the cause of the hyperuricemia?
- Are associated findings present?
- Has damage to tissues or organs occurred as a result of the hyperuricemia?
- What, if anything, should be done?

Hyperuricemia may be the initial clue to the presence of a previously unsuspected disorder. In 70% of hyperuricemic patients, an underlying cause can be readily determined by history and physical examination. The nature of the underlying cause may be useful in predicting the potential consequences, if any, of the elevated serum urate concentration. Therefore an underlying cause should be sought in every patient with hyperuricemia.

Whether to treat hyperuricemia uncomplicated by articular gout, urolithiasis, or nephropathy is an exercise in clinical judgment, and universal agreement is lacking. When considering whether to treat asymptomatic hyperuricemia with urate-lowering agents, the following data are pertinent:

- Although there are intriguing data from animal models to the contrary,[106,107,164] no good evidence in humans has been found that renal function is adversely affected by elevated serum urate concentrations.
- The renal disease that accompanies hyperuricemia is most often related to inadequately controlled hypertension.
- Although debate exists regarding whether hyperuricemia is an independent risk factor for coronary artery disease,[110-113] thus far there is no evidence that correction of hyperuricemia has an effect on the development of heart disease (as previously discussed).

Thus it seems prudent to refrain from treating hyperuricemia with specific urate-lowering agents until symptoms develop. Rare exceptions are made for people who have a known hereditary cause of uric acid overproduction or who are at risk for acute uric acid nephropathy.

The cause of hyperuricemia should be determined and that any associated factors related to the process, such as obesity, hyperlipidemia, alcoholism, and, especially, hypertension, be addressed. Fenofibrate and losartan might be appropriate agents for the treatment of hypertriglyceridemia and hypertension, respectively, in hyperuricemic people because each has modest uricosuric effects.[165,166]

## Acute Gouty Arthritis

The acute gouty attack may be successfully terminated by any of several drugs. For practical purposes, the choice in most situations is among colchicine, an NSAID, a corticosteroid preparation, or adrenocorticotropic hormone (ACTH). In response to the recognition of the critical role that IL-1β plays in the initiation of gout attacks, trials of biologic inhibitors of IL-1β for both the treatment and prophylaxis of acute gout have been undertaken with promising results.[167-171] Although approved for the treatment of TNF receptor–associated periodic fever syndromes, these expensive agents have not been approved for gout in the United States at this writing. In the European Union, canakinumab, a humanized, monoclonal anti–IL-1β antibody, is available for the treatment of acute gout under certain circumstances. The 2012 ACR Guidelines for the Management of Gout include an algorithm for treatment of the acute gout attack[59] (Figure 95-13).

The timing of the initiation of therapy is more important than the choice of drug for acute gout.[172] With any of these agents, the sooner the drug is started, the more rapidly a complete response will be attained. Generally, colchicine is preferred for patients in whom the diagnosis of gout is not confirmed, whereas NSAIDs are preferred when the diagnosis is secure. If a patient cannot take medications by mouth or has active peptic ulcer disease, the choice is among intra-articular glucocorticoids, parenteral glucocorticoids, or ACTH. Local application of ice packs may help control the pain of an acute attack.[173] In some cases, analgesics, including narcotics, may be added as well. In almost all cases, drugs that affect serum urate concentrations, including urate-lowering agents, should not be stopped during an acute attack. Whether such drugs should be started during an acute attack is debated. Because a sudden lowering of serum urate levels is known to precipitate acute attacks, an inflammatory reaction that is already in progress may be substantially worsened by a major drop in the serum urate concentration. However, one study suggests that in selected patients this outcome does not occur, provided the patient is also receiving treatment for the acute flare.[174]

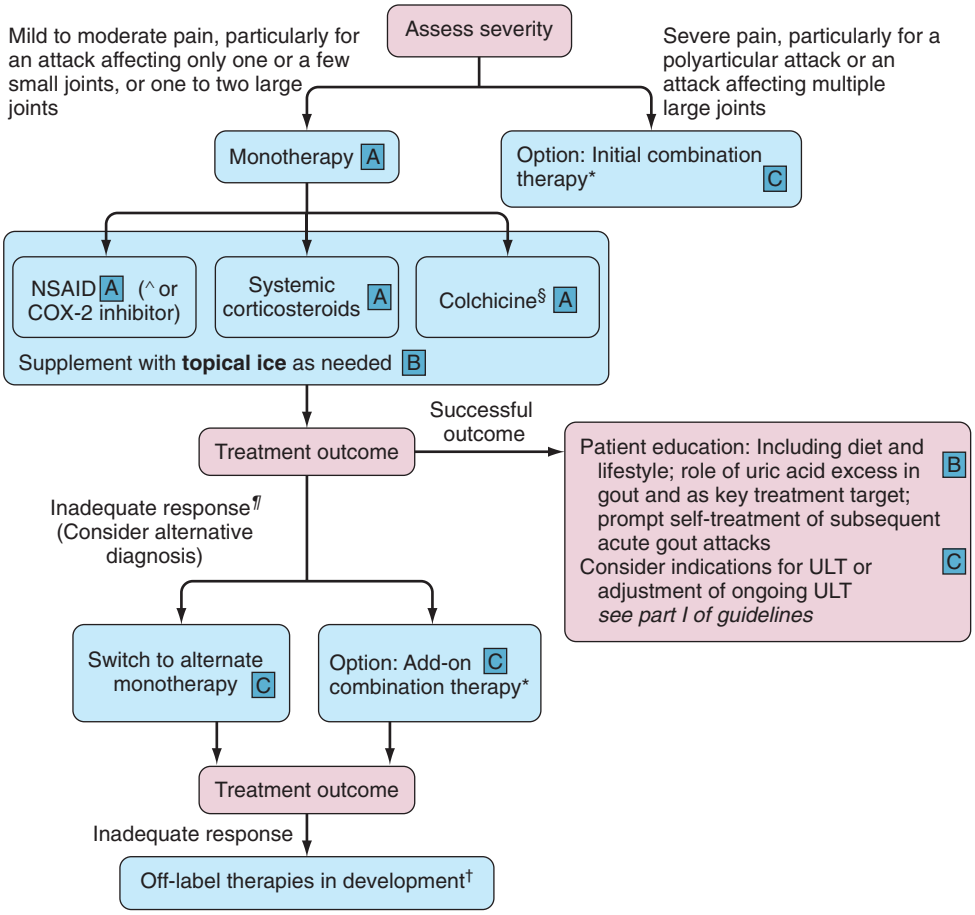
### Colchicine

Traditionally, the oral dosing schedule for colchicine was 0.5 or 0.6 mg taken hourly until one of three outcomes occurred: joint symptoms eased; nausea, vomiting, or diarrhea developed; or the patient had taken a maximum of 10 doses. If 10 doses were taken without benefit, the clinician questioned the accuracy of the diagnosis. Many clinicians have recommended for some time that doses be taken every 2 to 6 hours to reduce the incidence of adverse effects.[175] In 2009 the U.S. Food and Drug Administration (FDA) approved the first single-ingredient oral colchicine product for the treatment of acute gout flares and the prophylaxis of gout flares, as well as for familial Mediterranean fever in adults and children 4 years or older. The FDA ordered a halt to the marketing of unapproved single-ingredient colchicine preparations other than this product, which led to a dramatic increase in cost. The new recommendation for colchicine is a low-dose regimen of 1.2 mg, followed by 0.6 mg in 1 hour, for a total of 1.8 mg per attack. That protocol is based on the results of a randomized controlled trial of 184 patients with acute gout flares of less than 12 hours' duration that compared low-dose colchicine (in 74 patients) with high-dose colchicine (4.8 mg total over 6 hours in 52 patients) and with placebo (in 58 patients), with a primary endpoint of at least a 50% reduction in pain within 24 hours.[176] The low-dose approach resulted in comparable peak plasma concentrations and early gout flare

MANAGEMENT OF AN ACUTE GOUT ATTACK

General principles:

- Acute gouty arthritis attacks should be treated with pharmacologic therapy C#
- To provide optimal care, pharmacologic treatment should be initiated within 24 hours of acute gout attack onset C
- Ongoing pharmacologic ULT should not be interrupted during an acute gout attack C




#Evidence grades for recommendations:
**Level A**: Supported by multiple (i.e., more than one) randomized clinical trials or meta-analyses
**Level B**: Derived from a single randomized trial, or nonrandomized studies
**Level C**: Consensus opinion of experts, case studies, or standard of care
*§Colchicine was recommended as an appropriate option for acute gout if started within 36 hours of symptom onset.*
*^Selective COX-2 inhibition with agents available outside the United States, such as etoricoxib (Evidence A) was recommended as an option in patients with GI contraindications or intolerance to NSAIDs, but selective COX-2 inhibition shares many adverse events with NSAID therapy. COX-2 inhibition therapy with celecoxib (Evidence B) requires high doses and has unclear risk-benefit ratio at this time.*
*¶**Inadequate response** is defined as*
***<20**% improvement in pain score within **24 hours** or*
***<50**% at ≥**24 hours***
**See original referenced article for details.*
*†Off-label use of biologic IL-1 inhibitor treatment has been investigated for acute gout when nonbiologic therapeutic categories are ineffective or contraindicated, but this approach is not approved for gout by medical regulatory agencies at the time this is written.*

**Figure 95-13** Overview of management of an acute gout attack. COX-2, Cyclooxygenase-2; ULT, urate-lowering therapy. *(Modified from Khanna D, Fitzgerald JD, Khanna PP, et al: 2012 American College of Rheumatology guidelines for management of gout. Part 2: therapy and anti-inflammatory prophylaxis of acute gouty arthritis.* Arthritis Care Res *64:1447, 2012.)*

control with much fewer adverse effects, particularly diarrhea and vomiting, compared with the high-dose approach. It should be noted that these patients started treatment within 12 hours of attack onset, and there was still a greater than 30% failure to achieve the primary endpoint in both treatment arms. The appropriate dosing of colchicine for attacks of greater duration remains unclear. The low-dose recommendations are in line with those of a EULAR panel of experts.[177] The 2012 ACR Guidelines recommend starting with 1.2 mg, followed by 0.6 mg 1 hour later, and then 0.6 every 12 hours until the attack has subsided.[58] This regimen can then be followed by 0.6 mg once or twice daily for prophylaxis as needed. As always, dosages should be adjusted for renal insufficiency.

The dosing for prophylaxis of 0.6 mg once or twice a day is recommended on the basis of two randomized trials.[178,179] Patients with severe renal insufficiency should be started at 0.3 mg a day. The marketing of unapproved, injectable colchicine was prohibited by the FDA in 2008 because of an unacceptable safety profile, with the potential for serious adverse events, including death, with intravenous administration.

Peak plasma concentrations of colchicine occur within 2 hours of oral administration. Although its plasma half-life is 4 hours, levels can be detected in neutrophils 10 days after ingestion. Colchicine has a low therapeutic index, with steady-state plasma concentrations after acute treatment ranging from 0.5 to 3 ng/mL and toxic effects occurring at approximately 3 ng/mL.[180] Therefore, in most patients, the adverse effects precede or coincide with the improvement in joint symptoms. These adverse effects develop in 50% to 80% of patients using the old, high-dose regimen and include increased peristalsis, cramping abdominal pain, diarrhea, nausea, and vomiting. The drug must be stopped promptly at the first sign of gastrointestinal adverse effects.[181] The FDA prescribing guidelines recommend not using colchicine or reducing the dose in patients who take inhibitors of cytochrome P450 3A4 and of P-glycoprotein. Clarithromycin, erythromycin, cyclosporine, and disulfiram can all have serious interactions with colchicine.[182,183] In addition, the risk of myopathy is increased with the concomitant use of colchicine and other drugs with potential myotoxicity, such as statins and hydroxychloroquine.

Colchicine derives its effectiveness from its ability to interfere with acute inflammatory reactions in a variety of ways. Colchicine blocks the processing of IL-1β[184] and inhibits E-selectin–mediated adhesiveness to neutrophils.[185] Its action diminishes neutrophil L-selectin expression, random motility, chemotaxis, phospholipase $A_2$ activation, and IL-1 expression, as well as the stimulated elaboration of platelet-activating factor, crystal-induced chemotactic factor, and leukotriene $B_4$. Colchicine also inhibits endothelial cell intercellular adhesion molecule-1 (ICAM-1) expression, mast cell histamine release, and neutrophil degranulation and downregulates TNF receptors on macrophages and endothelial cells.

### Nonsteroidal Anti-inflammatory Drugs

In a patient with an established diagnosis of uncomplicated gout, the preferred agent is an NSAID, with indomethacin being the traditional choice. Although this drug may be effective in doses as low as 25 mg four times a day, an initial dose of 50 to 75 mg, followed by 50 mg every 6 to 8 hours, with a maximum dose of 200 mg in the first 24 hours, has generally been recommended. To prevent relapse, it is reasonable to continue this dose for an additional 24 hours and then taper to 50 mg every 6 to 8 hours for the next 2 days. Clinical trials have shown that oral naproxen, fenoprofen, ibuprofen, sulindac, piroxicam, and ketoprofen, as well as intramuscular ketorolac, are also effective. In fact, all members of this family of drugs can be highly effective in the treatment of acute gouty arthritis, including the cyclooxygenase-2 (COX-2) selective agents.[186]

### Corticosteroids

Intra-articular glucocorticoids are useful in the treatment of acute gout limited to a single joint or bursa.[172,187] Oral, intramuscular, or intravenous glucocorticoids can also provide relief, but these agents are usually reserved for patients who are intolerant of colchicine or NSAIDs or have medical conditions such as peptic ulcer disease or renal disease that contraindicate their use. Doses of glucocorticoids have been systematically studied, and generally, high doses (prednisone, 0.5 mg/kg, or at least 20 to 60 mg/day) are necessary. Lower doses may not be effective, as evidenced by gout flares occurring in organ transplant patients who are taking prednisone at maintenance doses of 7.5 to 15 mg a day.[188] Anecdotally, rebound attacks have been reported as steroids are withdrawn.

### Adrenocorticotropic Hormone

Single injections of intramuscular ACTH gel (25 to 80 IU) can terminate an acute gout attack.[189] More often, however, repeated administration is required every 24 to 72 hours. This treatment is effective post-operatively and may be more effective than glucocorticoids, possibly because of the mechanism of action. In addition to stimulating the adrenal cortex to produce corticosteroids, ACTH interferes with the acute inflammatory response through activation of melanocortin receptor-3.[190] Unfortunately, ACTH preparations, when they are available at all, have become prohibitively expensive. The branded ACTH gel initially approved by the FDA for a variety of indications in 1952, was reapproved in 2010 to treat infantile spasms, exacerbations of multiple sclerosis, and 17 other indications. Ironically, acute gout was not one of those indications.

### IL-1 Inhibition

In response to the recognition of the critical role that IL-1β plays in the initiation of gout attacks, trials of biologic inhibitors of IL-1β for both the treatment and prophylaxis of acute gout have been undertaken with promising results.[167-171] Although approved for the treatment of TNF receptor–associated periodic fever syndromes, these expensive agents have not yet been approved in the United States for gout. Concerns about cost and adverse effects in elderly individuals have been raised by the FDA. However, in early 2013, the European Commission approved canakinumab for patients with gout who experience frequent gout attacks (at least three attacks in the past 12 months) and in whom

NSAIDs and colchicine are contraindicated, are not tolerated, or do not provide an adequate response, and in whom corticosteroids are inappropriate. Canakinumab is a humanized monoclonal antibody directed against IL-1β and is administered as a single, subcutaneous injection of 150 mg.

### Prophylaxis

Approximately 50% of patients with gout will experience acute flares after initiation of urate-lowering therapy. The practice of giving small daily doses of colchicine as prophylaxis to prevent acute attacks is up to 85% effective in preventing those attacks.[191] Colchicine, 0.6 mg one to three times a day, is generally well tolerated, although the drug may produce a reversible axonal neuromyopathy.[192] This complication causes proximal muscle weakness with or without painful paresthesia and elevated serum levels of creatine phosphokinase, which is most often seen in patients with hypertension, renal dysfunction, or liver disease who are also taking diuretics. Rhabdomyolysis may also occur in these settings and is more common in people who are also taking a statin (hydroxymethylglutaryl–coenzyme A [HMG-CoA] reductase inhibitor) or cyclosporine.[193] Dosing of colchicine should be decreased for people with moderate to severe renal insufficiency.

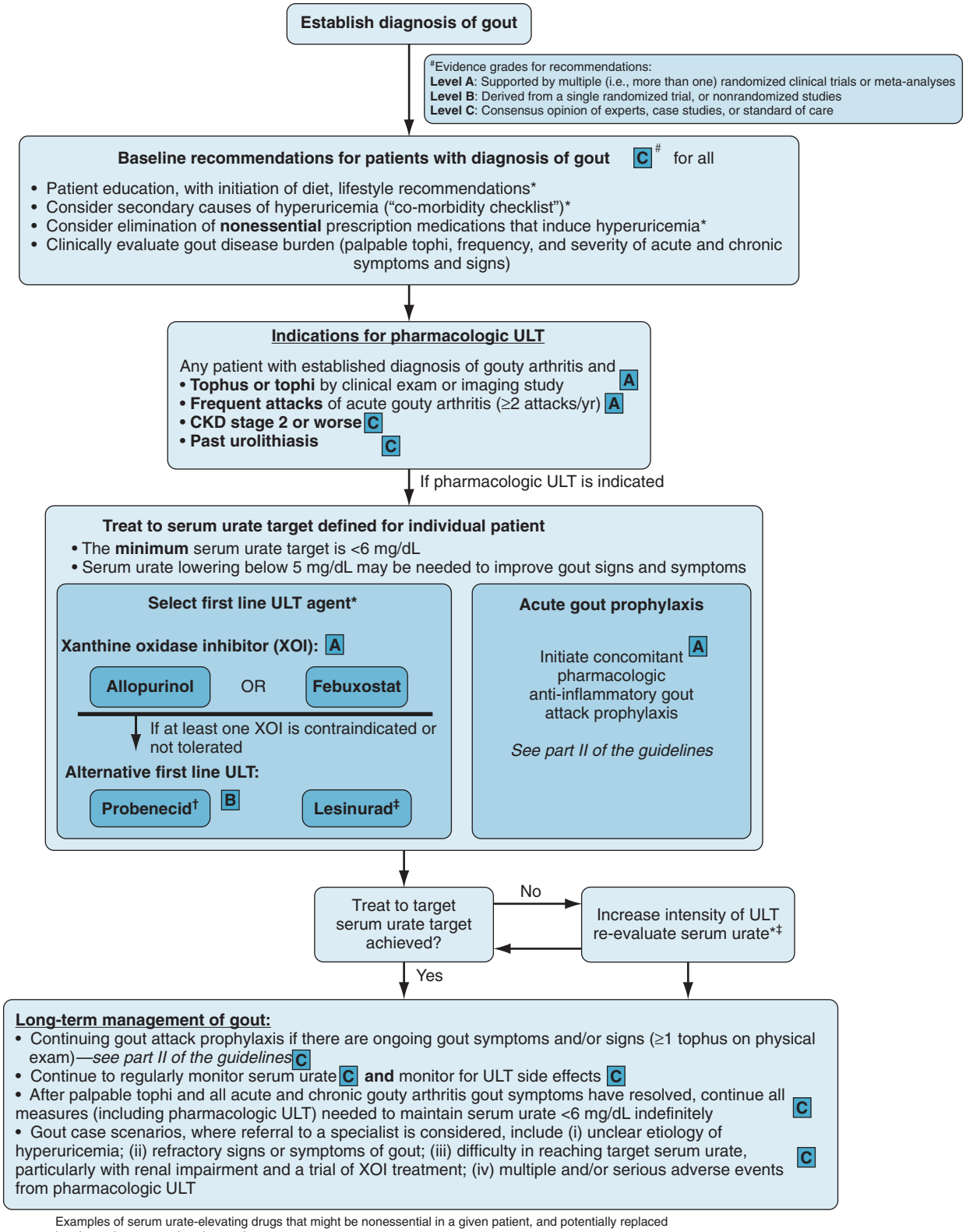
In patients who are unable to tolerate even one colchicine tablet per day, indomethacin or another NSAID has been used prophylactically at low doses (e.g., indomethacin, 25 mg twice a day, or naproxen, 250 mg a day or twice a day) with some success.[194] Some patients may require combination therapy of colchicine and NSAIDs. Peptic ulcer disease prophylaxis should be considered in at-risk patients. Maintenance doses of colchicine or an NSAID may make the difference between frequent incapacitation and uninterrupted daily activities. Prophylaxis is usually continued until the serum urate value has been maintained well within the normal range and no acute attacks have occurred for 3 to 6 months. The 2012 ACR Guidelines for Management of Gout recommend continuing prophylaxis for 6 months, or 3 months after achieving target serum urate for patients without tophi, or 6 months after achieving target serum urate with resolution of tophi.[58] It is important to warn patients that colchicine and/or NSAID discontinuation may be followed by an exacerbation of acute gouty arthritis and to advise them about what to do should an attack occur. Finally, prophylactic treatment is not recommended unless the clinician also uses urate-lowering agents. Prophylactic colchicine may block the acute inflammatory response but does not alter the deposition of crystals in tissues. When deposition continues without the warning signs of recurrent bouts of acute arthritis, tophi and destruction of cartilage and bone can occur without notice.

## Control of Hyperuricemia

Elimination of hyperuricemia with urate-lowering therapy can prevent and reverse urate deposition and perhaps improve renal function.[135,136] Today, opinion differs with regard to when in the course of gout the clinician should start urate-lowering therapy, although all persons agree that it should be initiated before the chronic inflammatory response that surrounds tophi has caused cartilage and bone erosion. Some physicians regard the first gout attack as a late event in a disorder marked by years of antecedent silent deposition of urate crystals in cartilage and other connective tissue. Other physicians believe that because tophi and symptomatic chronic gouty arthritis develop in only a minority of cases and ordinarily develop slowly after many years of recurrent acute attacks, unnecessary or premature medication can be avoided without demonstrable penalty. In practice, it is rare to have a patient who never experiences a second attack. The probability of such a benign course is greatest in patients who have only minimally elevated serum urate concentrations and normal 24-hour urinary uric acid values. Arguably, a case can be made for initiating antihyperuricemia therapy after the second attack in most patients.[194] The 2012 ACR Guidelines recommend initiation of urate-lowering therapy in patients with an established diagnosis of gouty arthritis AND tophaceous deposits by examination or imaging, frequent attacks defined as at least two per year, chronic kidney disease that is stage 2 or worse, or a history of urolithiasis (Figure 95-14).[58] Obviously, general recommendations of this type must be tempered by the clinician's best judgment. For example, a patient with gout who has experienced a single significant attack each year for the past several years should be receiving urate-lowering therapy.[195]

Urate-lowering therapy provides a definitive method for controlling hyperuricemia. Although it is important to treat and prevent acute attacks of gouty arthritis with anti-inflammatory agents, it is the long-term control of hyperuricemia that ultimately modifies the manifestations of the gouty diathesis. Once started, treatment with specific urate-lowering agents is lifelong, and the dose must be sufficient to maintain the serum urate level below 6.8 mg/dL—preferably between 5 and 6 mg/dL. Lowering the serum urate level from 11 mg/dL to 7.5 mg/dL may seem encouraging, but this change does not reverse the process; it merely slows the rate at which crystals continue to deposit. Generally, the lower the serum urate level achieved during urate-lowering therapy, the faster the reduction in tophaceous deposits.[196] The 5 to 6 mg/dL target is recommended because it is far enough below the saturation level of 6.8 mg/dL that it provides some margin for fluctuations in serum levels and avoids excessive exposure to the medication, which might increase the chance of toxicity. Particularly in cases in which extensive tophi are present on physical examination, a target serum urate of less than 4.0 mg/dL may be appropriate to achieve a timely reduction in the total body urate burden. Reduction to target levels may be achieved pharmacologically through the use of xanthine oxidase inhibitors, uricosuric agents, or uricases. Xanthine oxidase, the enzyme that catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid, is inhibited by allopurinol, oxypurinol, and febuxostat. Probenecid, lesinurad (recently approved by the FDA; see subsequent text), sulfinpyrazone, and benzbromarone are uricosuric agents that reduce serum urate concentrations by enhancing the renal excretion of uric acid. Rasburicase and pegloticase enzymatically convert urate to allantoin, which is much more soluble and readily excreted in the urine. These urate-lowering drugs do not have anti-inflammatory properties.

For patients with gout who excrete less than 800 mg of uric acid per day and have normal renal function, reduction




Examples of serum urate-elevating drugs that might be nonessential in a given patient, and potentially replaced by alternative agents that do not elevate serum urate:
- Niacin for management of hyperlipidemia
- Thiazide and loop diuretics for hypertension. However, in discussion, and without a specific vote, the TFP recognized the value of thiazide treatment in many patients with hypertension, and cautioned against imprudent cessation of thiazide treatment to lessen hyperuricemia at the cost of worsened control of blood pressure in difficult to control hypertension.
- Calcineurin inhibition with cyclosporine or tacrolimus, if it is not essential for immune suppression

*See original referenced article for details.
†Probenecid is not recommended as a first line or alternative first-line ULT agent if the CrCl is <50 (Evidence C)
‡The recently FDA-approved combination of lesinurad and an XOI is now an option, 3 years after the ACR guidelines were published

**Figure 95-14** Baseline recommendations and overall strategic plan for patients with gout. Note that this is an evolving field, and this does not include uricase or URAT1 transporter inhibitors. CKD, Chronic kidney disease; CrCl, creatinine clearance; TFP; task force panel; ULT, urate-lowering therapy; XOI, xanthine oxidase inhibitor. *(Modified from Khanna D, Fitzgerald JD, Khanna PP, et al: 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic non-pharmacologic and pharmacologic therapeutic approaches to hyperuricemia.* Arthritis Care Res *64:1431, 2012.)*

of the serum urate concentration can be achieved equally well with a xanthine oxidase inhibitor or a uricosuric drug. These agents are equally effective in preventing the deterioration of renal function in patients with primary gout.[197] In most cases, allopurinol is the drug of choice because it can be used with fewer restrictions compared with uricosuric agents.

In general, the ideal candidate for uricosuric agents is a patient with gout who is younger than 60 years and has normal renal function (creatinine clearance >80 mL/min), uric acid excretion of less than 800 mg per 24 hours on a general diet, and no history of renal calculi. When uricosuric agents are prescribed, patients should be counseled to avoid salicylate use at doses greater than 81 mg/day.[198]

In certain situations, an inhibitor of xanthine oxidase is clearly the drug of choice in a patient with gout. People with gout who excrete more than 1000 mg per 24 hours of uric acid in their urine or who have a history of renal calculi of any type should be treated with a xanthine oxidase inhibitor (Table 95-6). The incidence of renal calculi is about 35% in patients with primary gout who excrete more than 700 mg/day of uric acid.[41] The risk for uric acid stones is also greater upon initiation of uricosuric therapy. In addition, patients with tophi generally should take a xanthine oxidase inhibitor to decrease the load of urate that must be handled by the kidney. Patients with gout and mild renal insufficiency can be given either type of agent, but probenecid and sulfinpyrazone would not be expected to work when the glomerular filtration rate is less than 50 mL/min. Allopurinol is effective in the presence of renal insufficiency, but doses may need to be decreased in that situation. Febuxostat needs no adjustment in mild to moderate renal insufficiency. A final indication for a xanthine oxidase inhibitor is the failure of uricosuric agents to produce a serum urate concentration lower than 6 mg/dL or patient intolerance of the uricosuric agent. A xanthine oxidase inhibitor and a uricosuric drug may be used in combination in a patient with tophaceous gout for whom it is not possible to reduce the serum urate below 6 mg/dL with a single agent. The uricosuric agent lesinurad, a selective URAT1 inhibitor, was approved by the FDA in December of 2015 for the treatment of hyperuricemia in patients with gout who have failed to reach a target serum uric acid level of less than 6.0 mg/dL while taking allopurinol or febuxostat. In clinical trials, the combination of lesinurad 400 or 600 mg per day in combination with allopurinol 200 to 600 mg per day resulted in 74% to 79% of patients achieving serum urate levels less than 6.0 mg/dL.[198a] The combination of lesinurad 400 or 600 mg per day with feuxostat 40 or 80 mg per day resulted in 100% achieving a serum uric acid less than 5.0 mg/dL.[198b] In most settings, however, if allopurinol or febuxostat does not cause the serum urate to drop below 6 mg/dL, it is the result of insufficient dosing or poor patient compliance. The 2012 ACR Guidelines include specific recommendations regarding the use of allopurinol and uricosurics in patients with gout.[58] The FDA and EMA approved pegloticase, a mammalian uricase covalently attached to poly(ethylene glycol) with reduced immunogenicity for the treatment of difficult, tophaceous gout in 2010 and 2013, respectively.[198c] Although pegloticase offers the possibility of a rapid reduction of tophi (Figure 95-9), this drug still has a high rate of infusion reactions, including anaphylaxis, in up to 7% of patients.[198d] The incidence of treatment-induced gout flares is also very high. Therefore pegloticase is only appropriate for those patients with tophaceous gout with a large excess of total body urate and persisting gout attacks, or with damaging arthropathy, who are intolerant of or who have failed conventional treatment.[167] For a detailed discussion of the use of urate-lowering agents, see Chapter 66 of this textbook.

**TABLE 95-6** Indications for Xanthine Oxidase Inhibitor

| |
|---|
| **Hyperuricemia Associated With Increased Uric Acid Production**<br>Urinary uric acid excretion of 1000 mg or more in 24 hr |
| Hyperuricemia associated with HPRT deficiency or PRPP synthetase overactivity |
| Uric acid nephropathy |
| Nephrolithiasis |
| Prophylaxis before cytolytic therapy |
| **Intolerance or Reduced Efficacy of Uricosuric Agents**<br>Gout with renal insufficiency (GFR <60 mL/min) |
| Allergy to uricosurics |

GFR, Glomerular filtration rate; HPRT, hypoxanthine phosphoribosyltransferase; PRPP, phosphoribosylpyrophosphate.

## COMPLIANCE WITH TREATMENT

Because the disease processes involved in gout are so well understood, the diagnosis generally can be readily established. Once gout is diagnosed, the available therapies are so effective that it should be an easily managed disease. However, too many patients, including those who are accurately diagnosed, do not do well. Failure of urate-lowering therapy to attain the target urate level is usually due to improper prescribing or poor compliance.[199] The most commonly prescribed dose of allopurinol (300 mg) is effective in getting serum urate levels to less than 6.0 mg/dL in only about 40% of patients. Furthermore, a review of 16 clinical studies revealed a compliance rate for taking gout medications of only 10% to 46%.[199] Compliance is often a problem when treating chronic asymptomatic conditions, and associated alcoholism can be a factor. Perhaps more important is the fact that patients may need to take up to three different medications on three different schedules to control their symptoms and treat the disease.

It is believed that if patients understand why they are taking medications, they are more likely to be compliant. Toward this end, an analogy has been developed that helps some patients become more compliant.[200] In this analogy, urate crystals are compared with matches. The patient is told that "when the match strikes," it causes a gout attack. To "put out the fire," the patient takes an NSAID or colchicine. Although this resolves the attack, "the matches are still there." To eliminate future attacks, the patient is given prophylactic colchicine, "which makes the matches damp and harder to strike," and allopurinol (or a uricosuric agent), "which actually removes the matches from the body."

## MANAGEMENT OF GOUT AFTER ORGAN TRANSPLANTATION

The management of patients with gout after organ transplantation requires careful consideration. The use of glucocorticoids, azathioprine, or cyclosporine and the precarious status of renal function in many patients poses complex problems. Colchicine and NSAIDs may be inappropriate for the management of acute gouty arthritis in this setting because of their potential toxicities. Intra-articular glucocorticoid injections may be most helpful, and one may be forced to rely more heavily on pain medications in this setting. Prophylactic colchicine can be used in patients with normal renal function, but treatment must be monitored closely. The combination of colchicine and cyclosporine has induced rhabdomyolysis.[193]

When considering chronic therapy, it is helpful to lower the doses of cyclosporine and eliminate the use of diuretics, if possible. Uricosuric agents can be used safely, but their usefulness declines if renal function is poor. Allopurinol can be used in patients with abnormal renal function, but the dose may need to be reduced. Febuxostat may be safer in this setting but has not been studied. However, both allopurinol and febuxostat may have severe interactions with azathioprine. Azathioprine is metabolized by xanthine oxidase, and because these drugs inhibit that enzyme, the breakdown of azathioprine is slowed, increasing the effective dose. If care is not taken, significant bone marrow toxicity can result. Thiopurine *S*-methyltransferase studies should be obtained before starting azathioprine. When azathioprine and allopurinol are used together, they can be started at 25 and 50 mg/day, respectively.[201] Revised dosing for febuxostat in such a situation has not been determined. Complete blood cell counts and serum urate level concentrations are then monitored weekly when using allopurinol, and the dose is adjusted to bring the serum urate concentration down to less than 6 mg/dL. As an alternative to azathioprine, mycophenolate mofetil has been used effectively with allopurinol in some transplant patients.[202]

Uricase has been used to drastically lower serum urate levels and shrink tophi in a small number of patients with gout after cardiac transplantation.[203] This treatment has been associated with significant allergic reactions, including anaphylaxis, bronchospasm, and hemolytic anemia. The newer preparations of uricase such as pegloticase may avoid those complications and prove more effective.

## ANCILLARY FACTORS

In addition to anti-inflammatory agents, colchicine prophylaxis, and urate-lowering therapy, other factors may be decisive in determining whether the following symptoms develop: recurrent attacks, chronic gouty arthritis, kidney stones, or nephropathy. Today, dietary purine restriction solely to control serum urate levels is rarely advised. A totally purine-free diet reduces the urinary excretion of uric acid by only 200 to 400 mg/day and lowers the mean serum urate value by about 1 mg/dL. In addition, the urate-lowering agents available today are so effective that this type of dietary manipulation is rarely necessary. Nevertheless, beneficial results have been reported with a diet of moderate calorie and carbohydrate restriction and a proportionally increased intake of protein and unsaturated fat.[204] Some subjects with gout are susceptible to acute attacks after the consumption of alcoholic beverages or rich foods. Others describe idiosyncratic responses such as acute gout after eating a particular food, but such relationships are rare and questionable. A diet designed to avoid indiscretions known to precipitate acute gouty attacks in a particular individual is recommended.

In addition, diet is important with regard to other medical problems.[205] Many patients with gout are overweight, and restoration of ideal body weight through regulated calorie restriction is recommended. In addition, at least 75% of patients with primary gout have hypertriglyceridemia. The initial step in managing hypertriglyceridemia is reduction to an ideal body weight and elimination of alcohol ingestion. Fenofibrate is the only drug in its class that is uricosuric.[165] Therefore it may be a useful agent in managing lipid abnormalities.

Many patients with gout consume liberal amounts of alcohol. Acute excesses can lead to exacerbations of hyperuricemia as a result of temporary hyperlactacidemia, and chronic ingestion of alcohol may stimulate increased purine production.[123] The added purine load resulting from regular ingestion of beer may also be a contributing factor. Patients should be warned about the deleterious effects of excessive alcohol intake. Compliance with medication is also much worse among patients who consume alcohol. The 2012 ACR Guidelines include specific recommendations regarding lifestyle measures for patients with gout[58] (Figure 95-15).

About one-third of subjects with gout are hypertensive. The complications of hypertension are potentially more serious than those of hyperuricemia, and the clinician should not hesitate to use whatever drugs are necessary to control the hypertension. Many hypertensive patients with gout require a thiazide diuretic. If this medication is necessary to control hypertension, it should be used, with the recognition that the dosage of concomitant urate lowering therapy may need to be adjusted to maintain appropriate control of serum urate levels. Losartan is the only antihypertensive medication that has uricosuric properties.[166]

## CONCLUSION

Gout is an ancient malady. Although it is one of the best understood diseases, its incidence continues to rise. An epidemic of obesity and other factors have driven this increase. In addition, persistent misconceptions about the appropriate use of gout medications, poor patient compliance, and difficult comorbidities as patients live longer have led to suboptimal management of the disease. The diagnosis of gout should be substantiated by identification of intra-articular monosodium urate crystals whenever possible, guidelines for starting urate-lowering treatment should be followed, prophylaxis against flares early in such treatment should be given for at least 6 months, a serum urate of 6 mg/dL or lower should be targeted, and long-term patient compliance should be reinforced.

Major new insights into the pathogenesis of hyperuricemia and gout have occurred during the past few years, including fine details of the handling of uric acid in the

**SPECIFIC RECOMMENDATIONS:**
**GENERAL HEALTH, DIET, AND LIFESTYLE MEASURES FOR GOUT PATIENTS[#]:**

[#]Evidence grades for recommendations:
**Level A:** Supported by multiple (i.e., more than one) randomized clinical trials or meta-analyses
**Level B:** Derived from a single randomized trial, or nonrandomized studies
**Level C:** Consensus opinion of experts, case studies, or standard of care

- **Weight loss for obese patients, to achieve BMI that promotes general health**
- **Healthy overall diet***
- **Exercise (achieve physical fitness)**
- **Smoking cessation**
- **Stay well hydrated**

C

| Avoid | Limit | Encourage[†] |
|---|---|---|
| • Organ meats high in purine content (e.g., sweetbreads, liver, kidney) B | Serving sizes of:<br>• Beef, lamb, pork<br>• Seafood with high purine content (e.g., sardines, shellfish) B | • Low-fat or nonfat dairy products B |
| • High-fructose corn syrup–sweetened sodas, other beverages, or foods C | • Servings of naturally sweet fruit juices<br>• Table sugar, and sweetened beverages and desserts<br>• Table salt, including in sauces and gravies C | • Vegetables C |
| • Alcohol overuse (defined as more than two servings per day for a male and one serving per day for a female) in all gout patients B<br>• Any alcohol use in gout during periods of frequent gout attacks, or advanced gout under poor control C | • Alcohol (particularly beer, but also wine and spirits) in all gout patients B | |

*Without a specific task force panel (TFP) vote, adherence to diets for cardiac health and control of comorbidities, such as obesity, metabolic syndrome, diabetes, hyperlipidemia, and hypertension, was stressed for gout patients, as appropriate.
[†]The TFP recommendation to "encourage" intake was not intended to advocate excesses in consumption of specific dietary items. There was a lack of TFP voting consensus on cherries and cherry products, ascorbate (in supplements or foods), nuts, and legumes. The TFP did not specifically vote on the question of limits on consumption of purine-rich vegetables and legumes.

**Figure 95-15** Specific recommendations on general health, diet, lifestyle measures for patients with gout. BMI, Body mass index; TFP, task force panel. *(From Khanna D, Fitzgerald JD, Khanna PP, et al: 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic non-pharmacologic and pharmacologic therapeutic approaches to hyperuricemia.* Arthritis Care Res *64:1431, 2012.)*

renal proximal tubule and the cellular inflammatory response to monosodium urate crystals. In addition, newer therapeutic agents are available, and several others are entering the market, including IL-1 inhibitors for the treatment and prophylaxis of acute gout. The excitement generated by these changes offers an ideal opportunity to re-educate our patients and ourselves so as to improve outcomes in this highly treatable disease. Better management of gout with old and newly available drugs, as well as a concerted effort on the part of society to change negative lifestyle choices, will, it is hoped, have a positive effect on gout incidence and outcome in the years to come.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 96

# Calcium Crystal Disease:
## Calcium Pyrophosphate Dihydrate and Basic Calcium Phosphate



Robert Terkeltaub

**KEY POINTS**

- Dysregulated inorganic pyrophosphate ($PP_i$) metabolism, intimately associated with altered chondrocyte differentiation and extra-cellular matrix composition, is central in the pathogenesis of calcium pyrophosphate (CPP) dihydrate crystal deposition disease (CPPD).
- Autosomal dominant familial CPPD has been linked in multiple kindreds to certain mutations in *ANKH,* a gene encoding a $PP_i$ transporter.
- NLRP3 (cryopyrin) inflammasome activation and consequent caspase-1 activation and interleukin (IL)-1β processing and secretion drive inflammatory responses to CPP and BCP crystals.
- Degenerative arthropathy caused by CPPD often involves joints uncommonly affected by primary osteoarthritis, such as the metacarpophalangeal, wrist, and elbow joints.
- Diagnosis of CPPD in patients younger than 55 years, particularly if CPP crystal deposition is polyarticular, should prompt the differential diagnostic consideration of a primary metabolic or familial disorder. Such disorders also can first appear with clinical manifestations of CPPD in patients older than 55 years.
- High-resolution ultrasound is helpful in diagnosis of CPPD, partly because radiographic chondrocalcinosis is not detectable in all joints affected by the disease.
- CPP and basic calcium phosphate (BCP) crystal deposition in articular cartilage are very common in primary osteoarthritis (OA), particularly with advanced disease.
- CPP and BCP crystals can promote symptomatic flares of primary OA, but their net role in promoting OA progression is not well-characterized.
- BCP crystals (unlike monosodium urate and CPP crystals) are not birefringence, and specialized methods are required to identify BCP crystals in specimens from the joint.

The proposed diagnostic criteria for calcium pyrophosphate dihydrate (CPPD) are shown in Table 96-1, as adapted by the author from the European League against Rheumatism (EULAR) criteria[1] and the original criteria proposed in the past by Daniel J. McCarty and colleagues. The diagnosis is based on detection of CPP crystals by one or more methods. These include not only standard clinically applied radiography but also high-resolution ultrasound to detect hyaline articular cartilage or fibrocartilage calcifications characteristic of CPP crystal deposition (termed *chondrocalcinosis*). However, identification of CPP crystals via compensated polarized light microscopic analysis of synovial fluid in the absence of joint infection or other cause of arthritis is the gold standard for diagnosis of CPPD, particularly for acute CPP crystal-associated arthritis (classically termed *pseudogout*). Detection of typical CPP crystals in tissue sections also can be accomplished whether specimens are fixed in formaldehyde or ethanol, unlike the case for monosodium urate crystals (which dissolve in formaldehyde). Specialized crystal analytic approaches including radiography energy spectroscopy and powder diffraction analysis or atomic force microscopy can be used to distinguish CPP crystal deposition from basic calcium phosphate (BCP) crystal deposition. In assessing the form of crystals deposited in calcifications, determination of the calcium/phosphate ratios and the spacing of radiography powder diffraction lines provide the most specific information.

Consensus clinical diagnostic criteria for articular BCP crystal deposition disorders are not in place. Importantly, BCP crystals (unlike monosodium urate and CPP) do not demonstrate global birefringence, although the aggregated particles of basic calcium phosphate crystals demonstrate edge birefringence. Hence, diagnosis is predicated on detection of (1) radiographic detection of calcifications characteristic of BCP crystals, or (2) BCP crystals confirmed by transmission electron microscopy or specialized crystal analytic approaches (including those mentioned earlier for CPP crystals and discussed below). In some clinical pathology laboratories, the calcium-binding dye alizarin red S is used to stain synovial fluid samples, because BCP crystals stain strongly for alizarin red S, whereas CPP crystals stain weakly with this dye.

## EPIDEMIOLOGY

**KEY POINTS**

- True prevalence of both CPPD and pathologic articular BCP crystal deposition is not known because of limits of radiographic detection and lack of large pathology studies of joint tissues in populations at large.
- The prevalence of CPPD, including asymptomatic disease, increases with aging.
- Idiopathic/sporadic CPPD is rare in patients younger than 55 years, particularly in the absence of a history of joint trauma or knee meniscectomy.
- The epidemiology of CPPD is not uniform among populations.

**CPPD prevalence might be increasing in developed countries, mediated by both increased longevity and iatrogenic factors in such countries that promote hypomagnesemia.**

**The knee might be spared of radiographically detected chondrocalcinosis in many patients with CPPD, and should not be the sole joint screened.**

In the past, studies of the prevalence of both CPPD[2-5] and various forms of articular BCP crystal deposition disease have been predominantly based on characteristic plain radiographic features of the disease in limited numbers of joints. This is an incompletely sensitive and specific approach.[2] Other studies have been based on results of synovial fluid analyses, but definitive studies based on pathologic findings on examination of articular cartilages, or imaging approaches superior to plain radiography, have not been done. As such, the true prevalence of both CPPD and pathologic articular BCP crystal deposition is not known. However, we do know that CPPD, including asymptomatic disease, increases progressively in prevalence with aging.[2-5] Idiopathic/sporadic CPPD is rare in patients younger than 55 years, particularly in the absence of a history of joint trauma or knee meniscectomy.[6,7]

In a large U.K. community study, the age-, sex-, and knee pain–adjusted prevalence of knee CPPD was 4.5% for patients older than 40 years.[3] In some studies, women have appeared somewhat more commonly affected by CPPD than men[1]; however, in the U.K. study, there was no sex predisposition, although a strong association between primary osteoarthritis (OA) and chondrocalcinosis was confirmed.[3] This appeared to be linked more to the presence of osteophytes rather than joint space narrowing with OA. Most but not all elderly patients with CPPD of the knee also have detectable chondrocalcinosis in other joints; this may not be the case for hip CPPD, at least in association with hip OA.[8,9] Knee meniscal fibrocartilage calcification alone has been detected in approximately 15% of those aged 80 to 89 years and in approximately 30% older than 89 years.[7] Studies based on radiographs have estimated a higher prevalence of CPPD when the hands, wrists, pelvis, and knees have been surveyed.[7] Knees are spared of radiographically detected chondrocalcinosis in many with CPPD,[9] and should not be the sole joints screened for research or clinical studies.

CPPD is not uniform in epidemiology among populations. For example, in a random sample of Beijing residents older than 60 years, radiographic chondrocalcinosis was compared with whites in the American Framingham OA Study.[11] The Chinese had a much lower prevalence of knee chondrocalcinosis, and wrist chondrocalcinosis was particularly rare in Chinese elderly.[11] These findings were unexpected because there is an excess of knee OA in the Beijing population, and CPPD and OA are quite commonly associated in the knee joint. Analyses of patients of Chinese extraction in the United States would be informative.

CPPD prevalence may be increasing in developed countries, mediated both by increased longevity and iatrogenic factors in such countries that promote hypomagnesemia.[10] Such iatrogenic factors include loop diuretics,[3] proton pump inhibitors, calcineurin inhibitors (cyclosporine, tacrolimus), and post-surgical short bowel syndrome.[7,10] Other risk factors for CPPD may include low cortical bone density, for unclear reasons.[7] Knee CPPD risk appears to be increased by knee malalignment, predominantly varus, in early life.[6]

Novel reported associations of CPPD include low BMI and low cortical bone mineral density.[11a] The risk of acute episodic CPP crystal arthritis is increased in those on loop diuretics, and with hyperparathyroidism and end stage renal disease.[11a]

**Table 96-1** Proposed Diagnostic Criteria for CPPD

| | |
|---|---|
| **Criteria** | |
| I. | Demonstration of CPP crystals, obtained by biopsy or aspirated synovial fluid, by definitive means (such as characteristic radiography diffraction powder pattern) |
| II. A. | Identification of monoclinic or triclinic crystals showing a weak positive birefringence (or no birefringence) by compensated polarized light microscopy |
| B. | Presence of typical calcifications on radiographs (as discussed in text): heavy punctate and linear calcifications in fibrocartilages, articular (hyaline) cartilages, and joint capsules, especially if bilaterally symmetric |
| C. | Presence of typical findings for CPP crystal deposition in articular hyaline cartilage or fibrocartilage by high-resolution ultrasound. |
| III. A. | Acute arthritis, especially of knees, wrists, or other large joints |
| B. | Chronic arthritis, especially of knee, hip, wrist, carpus, elbow shoulder, and metacarpophalangeal joints, particularly if accompanied by acute exacerbations |
| **Diagnostic Categories** | |
| A. | Definite: criteria I or IIA must be fulfilled. |
| B. | Probable: criteria IIA or IIB or IIC must be fulfilled. |
| C. | Possible: criteria IIIA or IIIB suggests possible underlying CPPD deposition disease. |

CPPD, Calcium pyrophosphate dihydrate crystal deposition disease.
Modified from McCarty DJ: Crystals and arthritis. *Dis Month* 6:255, 1994.

## GENETICS

**KEY POINTS**

**The vast majority of CPPD is idiopathic/sporadic, but early-onset familial disease also occurs.**

**Linkage of familial CPPD to the gene *ANKH* on chromosome 5p (which encodes a transmembrane protein with functions including $PP_i$ transport) is well established.**

The vast majority of CPPD is idiopathic/sporadic, but early-onset (defined as onset before 55 years) familial disease also occurs.[12,13] Two major chromosomal linkages, 8q and 5p have been identified in studies of familial CPPD. Linkage with chromosome 8q of both early-onset OA and chondrocalcinosis appears much less common than chromosome 5p-linked chondrocalcinosis, which is linked to *ANKH* on chromosome 5p (which encodes a transmembrane protein with $PP_i$ transport and other apparent functions).[12-17] *ANKH* functions and clinical phenotypes linked to specific mutations are discussed later, for dysregulated $PP_i$ metabolism in pathologic cartilage calcification.

Familial CPPD is heterogeneous. For one example, prominent CPP and hydroxyapatite (HA) crystal deposits and cartilage and periarticular calcifications in association

with OA were described in a kindred not yet linked to a specific chromosomal locus.[18] A syndrome of spondyloepiphyseal dysplasia tarda, brachydactyly, precocious OA, and intra-articular calcifications with CPP and/or HA crystals, as well as periarticular calcifications, was linked to mutation of the procollagen type II gene in indigenous natives of the Chiloe Island region of Chile, who have high prevalence of familial CPPD. Families affected with diffuse idiopathic skeletal hyperostosis (DISH) and/or chondrocalcinosis have been identified in the Azores Islands, possibly reflecting an unidentified, shared pathogenic mechanism.[19]

## ETIOLOGY AND PATHOGENESIS

### KEY POINTS

**The loose avascular connective tissue matrices of articular hyaline cartilage, fibrocartilaginous menisci, and certain ligaments and tendons are particularly susceptible to pathologic calcification.**

**Joint cartilage pathologic calcification reflects complex interplay between organic and inorganic biochemistry of $P_i$ and $PP_i$ metabolism and transport, dysregulated chondrocyte growth factor responsiveness and differentiation, and other factors.**

**In most patients, CPPD is a systemic articular and soft-tissue metabolic disorder, likely driven by dysregulated ATP and $PP_i$ metabolism resulting from impaired mitochondrial function, linked with joint tissue aging and OA.**

**Environmental factors promoting CPPD likely include dietary mineral content and regulators of body stores of iron, calcium, phosphate, and magnesium, with downstream effects including modulation of parathyroid hormone levels and the catalytic activity of enzymes involved in $PP_i$ metabolism.**

The loose avascular connective tissue matrices of articular hyaline cartilage, fibrocartilaginous menisci, and of certain ligaments and tendons are particularly susceptible to calcification. Calcium-containing crystals deposited in the pericellular matrix of cartilage are often in the form of CPPD (chemical formula, $Ca_2P_2O_7{\cdot}H_2O$; calcium-to-phosphate ratio of 1). Crystals of BCP including partially carbonate-substituted HA ($Ca_5[PO_4]_3OH{\cdot}2H_2O$; calcium-to-phosphate ratio of 1.67) also may be deposited pathologically in articular cartilage, particularly in OA. Importantly, physiologic non-inflammatory deposition of HA is essential because HA is the principal mineral phase laid down in growth cartilage and in bone.

Inflammatory conditions also may result from deposition of HA, as well as the closely related BCP crystals, octacalcium phosphate (OCP) ($Ca_8H_2[PO_4]_6{\cdot}5H_2O$; calcium-to-phosphate ratio, 1.33) and tricalcium phosphate or "whitlockite" ($Ca_3[PO_4]_2$; calcium-to-phosphate ratio, 1.5) in periarticular structures such as the rotator cuff (calcific tendinitis) and subacromial bursa of the shoulder (see Chapter 46). CPP and BCP crystal deposition, reviewed here, are by far the most prevalent arthropathies associated with calcium-containing crystals. Articular calcium oxalate crystal deposition is less common.

Articular cartilage, unlike growth plate cartilage, is specialized to avoid the process of matrix calcification. However, the matrix of articular hyaline cartilage, like that of fibrocartilaginous menisci, lends itself well to pathologic calcification, particularly in association with certain changes in extra-cellular matrix composition and hydration in aging and OA. Joint cartilage calcification reflects a complex interplay between organic and inorganic biochemistry, ion transport, aging, genetics, inflammation, oxidative stress, and dysregulated chondrocyte growth factor responsiveness and differentiation. Pathologic cartilage calcification can reflect deficiencies of certain physiologic calcification inhibitors or upregulation of mediators that actively drive certain patterns of tissue injury, culminating in calcification within degenerating cartilage.[20]

Alteration of the concentrations of calcium, inorganic phosphate ($P_i$), $PP_i$, and the solubility products of these ions are clearly at work in promoting CPP and BCP crystal formation.[20] The levels of ambient $Mg^{2+}$ and the composition of the chondrocyte extra-cellular matrix influence the dynamics of CPP crystal formation. $Mg^{2+}$ helps to determine whether predominantly monoclinic or less inflammatory triclinic CPP crystals are predominantly formed.

Specific extra-cellular matrix effects for CPP and BCP crystals, characterized partly in experimental gel systems, include promotion of CPP crystal formation by adenosine triphosphate (ATP) and corticosteroids in conjunction with matrix type I collagen and osteopontin,[21] whereas type II collagen and intact proteoglycans appear to suppress ATP-driven CPP crystal formation in vitro. Experimental systems to analyze CPP and BCP crystal deposition have commonly used isolated matrix vesicle cell fragments from chondrocytes that are enriched in procalcifying constituents and provide a nidus for initiation of calcification, specifically with BCP crystals. Matrix vesicles are important in cartilage growth plate calcification, but it is not yet clear whether CPP and BCP crystal formation in articular cartilages is driven more by matrix vesicle–mediated effects or nucleation of crystals in association with changes in extra-cellular matrix constituents, or both pathways. CPP crystals are too large (micron size) to form within matrix vesicles, but loci of pericellular concentration of $PP_i$ may be necessary to drive CPP crystal formation at low micromolar $PP_i$ concentrations developing in cartilages with CPPD.

Besides physical effects of $Ca^{2+}$, $P_i$, and $PP_i$ on crystal nucleation and propagation, these same solutes exert a variety of mineralization-regulating effects on gene expression, differentiation, and viability in chondrocytes, mediated partly by calcium-sensing receptors and sodium-dependent inorganic phosphate cotransport in chondrocytes.[22-25] Noxious effects of excess $PP_i$ on chondrocytes, including induction of matrix metalloproteinase-13 (MMP-13) expression[26] and promotion of apoptosis[27]; noxious effects of extra-cellular ionized $Ca^{2+}$ on chondrocytes include potentiation of the ability of transforming growth factor (TGF)-β to promote $PP_i$ release.[28]

Primary idiopathic CPPD (i.e., without a precipitating cause, such as joint trauma, or a secondary metabolic disorder) is likely promoted by a systemic tendency to deposit calcium containing crystals and involves tissues beyond joint cartilages, including tendons, and possibly also the arterial wall.[11a] The systemically disordered articular and soft-tissue metabolic basis of most CPPD is reinforced by

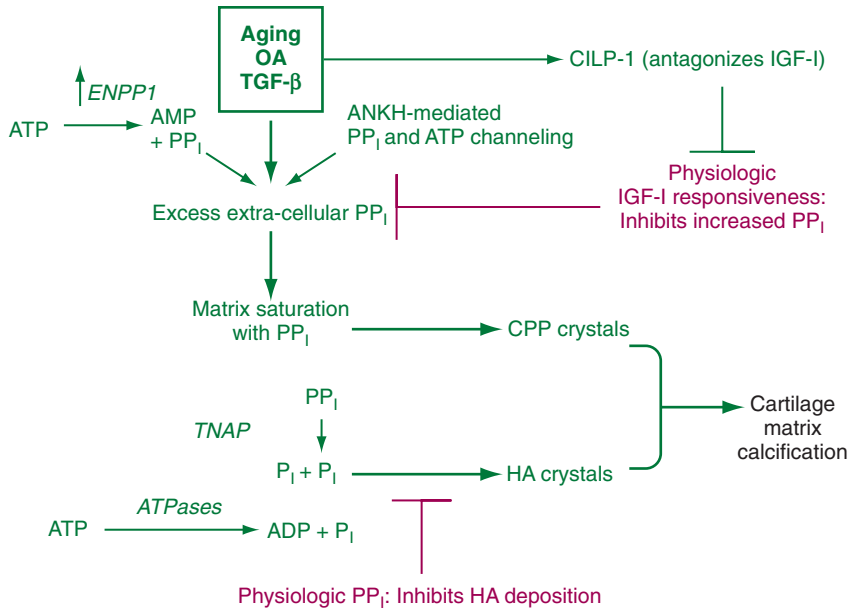



**Figure 96-1** Proposed inorganic pyrophosphate ($PP_i$)-dependent mechanisms stimulating calcium pyrophosphate (CPP) dihydrate and hydroxyapatite (HA) crystal deposition in aging and osteoarthritis (OA). Roles of adenosine triphosphate (ATP) and $PP_i$ metabolism and inorganic phosphate ($P_i$) generation in pathologic cartilage calcification. This model accounts for the association of extra-cellular $PP_i$ excess with both CPP and basic calcium phosphate (HA) crystal deposition in OA and chondrocalcinosis, as well as the paradoxical association of extra-cellular $PP_i$ deficiency (from defective *ANKH* or ectonucleotide pyrophosphatase/phosphodiesterase 1 [ENPP1] expression) with pathologic calcification of articular cartilage with HA crystals in vivo. Factors driving pathologic calcification are indicated in green and physiologic factors suppressing calcification in red. Excess $PP_i$ generation in aging cartilages in idiopathic CPPD of aging and in OA cartilages is mediated in part by increased ENPP1. In idiopathic chondrocalcinosis of aging, mean cartilage $PP_i$ and nucleotide pyrophosphatase phosphodiesterase (NPP) catalytic activity levels are double normal. ENPP1 is markedly increased at sites of meniscal cartilage calcification in vivo, and NPP1 directly induces $PP_i$ elevation and matrix calcification by chondrocytes in vitro. Depending on extra-cellular availability of substrate $PP_i$ and the activity of the pyrophosphatase tissue-nonspecific alkaline phosphatase (TNAP), the availability of substrate ATP and the activity of ATPases, as well as other factors such as substantial local $Mg^{2+}$ concentrations and HA crystal deposition, as opposed to CPP crystal deposition, may be stimulated. In this model, excess extra-cellular $PP_i$ also may result from heightened "leakiness" of intra-cellular $PP_i$ via increased *ANKH* expression in OA and abnormal *ANKH* function in familial chondrocalcinosis. Also illustrated is the role in cartilage calcification in OA and aging of increased expression of cartilage intermediate layer protein-1 (CILP-1), which inhibits the capacity of insulin-like growth factor-I (IGF-I) to suppress elevation of extra-cellular $PP_i$. AMP, Adenosine monophosphate; TGF-β, transforming growth factor-beta.

the observation that radiographic evidence of CPPD at one joint is frequently associated with CPPD at distant joints, whether OA is present or not.[8,9] However, distant joint radiographic CPPD is frequently not the case for CPPD in hip OA, as opposed to knee OA.[8,9,11a,28a]

## Dysregulated $PP_i$ Metabolism in Pathologic Articular Cartilage Calcification

Dysregulated $PP_i$ metabolism, intimately linked with altered chondrocyte differentiation, function, and extracellular matrix homeostasis, is central to crystal deposition in idiopathic and other forms of CPPD. Unlike growth plate cartilage, articular cartilage, is specialized to avoid extra-cellular matrix calcification, in large part via abundant generation and release of $PP_i$ by chondrocytes, and constitutive suppression of the procalcifying hypertrophic chondrocyte differentiation state. $PP_i$ is a potent inhibitor of the nucleation and propagation of BCP crystals.[20] Concordantly, maintenance of physiologic extra-cellular $PP_i$ levels by chondrocytes and certain other cells serves to suppress calcification with HA, as illustrated in mouse models of deficient $PP_i$ generation and transport[22,23] and a variant of human infantile arterial calcification associated with periarticular calcification.[29] The relatively unique capacity of chondrocytes to produce copious amounts of extracellular $PP_i$ is double-edged (Figure 96-1), as supersaturation of cartilage extra-cellular matrix with $PP_i$ is a major factor in promoting CPP crystal deposition.[20,31] Furthermore, excess $PP_i$ generation can promote BCP crystal deposition by providing a source for increased extra-cellular $P_i$ generation via $PP_i$ hydrolysis by the ectoenzyme tissue-nonspecific alkaline phosphatase (TNAP)[20,23] (see Figure 96-1). Depending on cartilage ATP and $PP_i$ concentrations, as well as the level of activity of $P_i$-generating ATPases and TNAP, CPP, and hyaluronic acid (HA) crystal formation may be jointly promoted in cartilage, an event that commonly occurs clinically in OA.

Heterogeneous factors increase $PP_i$ generation in the joint space in aging and OA, but many converge on increasing articular and meniscal cartilage ectonucleotide pyrophosphatase phosphodiesterase (ENPP1) and the multiple-pass transmembrane protein ANKH.[20] These factors include dysregulated chondrocyte responses to the growth factors TGF-β and insulin-like growth factor-I (IGF-I).

## Role of ENPP1 and ANKH in $PP_i$ Metabolism in Chondrocalcinosis

Sporadic aging-associated CPPD is consistently linked with excess chondrocyte $PP_i$-generating ENPP activity and

augmented $PP_i$ generation by chondrocytes.[20,31,33] In this context, ENPP1 (formerly known as *NPP1* and *plasma cell membrane glycoprotein-1 [PC-1]*) and ENPP3 (formerly known as *B10*) actively generate $PP_i$ by hydrolysis of nucleoside triphosphates including ATP.[19,33] ENPP1 plays a central role in sustaining and augmenting extra-cellular $PP_i$ in chondrocytes and certain other cells (see Figure 96-1). A substantial portion of ATP used by chondrocytes to generate extra-cellular $PP_i$ is provided by the mitochondria.[19]

ENPP family enzymes share NPP catalytic activity and modular type II transmembrane ectoenzyme structures.[19,33] ENPP1 plays by far the greatest role in augmenting extracellular $PP_i$ in chondrocytes.[31,33] Significantly, total ENPP1 deficiency states in vivo and in vitro are associated with marked depletion of plasma and extra-cellular $PP_i$.[22,29,30] In contrast, in idiopathic CPPD, cartilage NPP activity and $PP_i$ levels may average approximately double those of healthy individuals.[34]

Increased ENPP1 expression is associated with both calcification and apoptosis in degenerative human cartilages.[31] Cartilage ENPP1 mRNA and protein staining and synovial fluid ENPP enzymatic activity have most often been reported as elevated in primary knee OA, although in more advanced primary OA with associated BCP crystal deposition, either decreased or increased cartilage chondrocyte ENPP1 mRNA has been described.[35,36] Direct upregulation of ENPP1 in chondrocytic cells stimulates calcification, as well as apoptosis.[37] These effects are not shared by ENPP3, which likely has other intra-cellular "housekeeping" functions in chondrocytes.[32] ENPP2, which is also expressed in normal cartilages, functions more actively in physiology as a lysophospholipase D, and ENPP2 only modestly stimulates chondrocytes to calcify in vitro.[31,32]

*ANKH* encodes a multiple-pass transmembrane protein that functions in $PP_i$ channeling[38-41] (Figure 96-2) and possibly ATP release[43] and regulation of $P_i$ metabolism and uptake by the type III sodium-dependent $P_i$ co-transporter Pit-1.[44] ANKH promotes bidirectional movement of $PP_i$ at the plasma membrane in vitro.[41] However, the gradient for ANKH-stimulated $PP_i$ movement in chondrocytes (which generate abundant $PP_i$ both by their robust ENPP1 expression and intense matrix biosynthetic activity) is from the intra-cellular to the extra-cellular space.[19] Indeed, ANKH transport of $PP_i$ generated intra-cellularly by ENPP1[26] may be the primary means to regulate extra-cellular $PP_i$ levels.[20] Modeling of the $PP_i$ channeling function of ANKH has proposed 10 or 12 membrane-spanning domains in ANKH with an alternating inside/out orientation and with a central channel to accommodate the passage of $PP_i$[38,40] (see Figure 96-2).

ANKH is implicated in both familial and idiopathic/sporadic CPPD,[12,13,16,17] and increased ANKH expression in cartilage is seen with primary CPPD as well as with secondary CPPD in primary knee OA[26,45] (see Figure 96-1). Hypoxia, via effects of the transcription factor hypoxia-inducible factor-1α, suppresses ANKH expression.[46] It is conceivable that increased permeability to oxygen of fibrillated and fissured cartilage in OA favors increased ANKH expression. Constitutive ANKH expression is functionally implicated in maintenance of chondrocytic differentiation by articular chondrocytes.[47] However, increased ANKH, in conjunction with signaling via extra-cellular $P_i$ likely

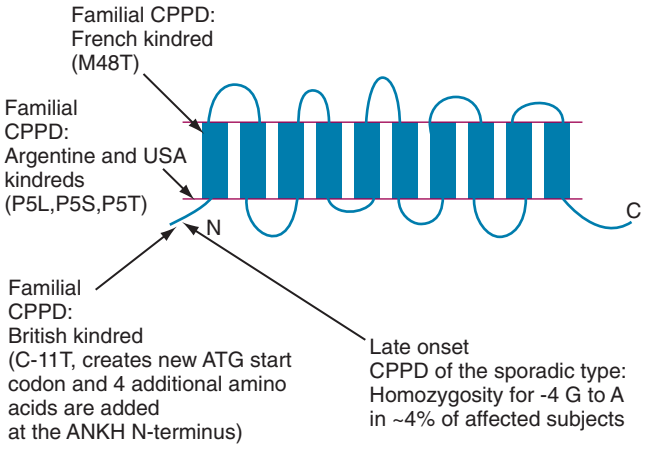



**Figure 96-2** Model for multiple-pass membrane protein structure of ANKH and for *ANKH* mutations associated with chromosome 5p-linked autosomal dominant familial calcium pyrophosphate dihydrate crystal deposition disease (CPPD) and heritable late-onset CPPD. The figure schematizes the putative multiple-pass transmembrane protein structure of ANKH, which appears to promote bidirectional proposed inorganic pyrophosphate ($PP_i$) movement between the cytosol to the extra-cellular space. The gradient for ANKH-stimulated $PP_i$ movement in chondrocytes (which generate abundant $PP_i$ both by high specific activity of ectonucleotide pyrophosphatase/phosphodiesterase 1 and robust matrix biosynthesis) is from the intra-cellular to the extra-cellular space. Distinct mutations in *ANKH* promote differences in age of onset and phenotypes in familial CPPD. The figure summarizes sites of known *ANKH* mutations clustered near the N-terminus that are associated with chromosome 5p-linked autosomal dominant familial CPPD, and all, except the French kindred M48T (which increases intra-cellular $PP_i$ and disrupts the association of ANKH with the inorganic phosphate transporter Pit-1), may act in part by increasing extra-cellular $PP_i$. A C-terminal domain *ANKH* mutation ΔE590 linked with one case of sporadic chondrocalcinosis is not depicted; *ANKH*ΔE590 appears to indirectly suppress $PP_i$ catabolism by association with impairing tissue-nonspecific alkaline phosphatase expression. The figure also depicts the –4 G to A transition in the 5′-untranslated region of *ANKH* for which homozygosity is seen in approximately 4% of late-onset sporadic CPPD, suggesting a small heritable subset. The N-terminally clustered *ANKH* mutations linked to CPPD all appear to promote chronic low-grade extra-cellular $PP_i$ excess resulting in CPP crystal formation, but there are differences in clinical and mineral metabolism phenotypes.

derived from $PP_i$, promotes chondrocyte maturation to the procalcifying hypertrophic differentiation state.[48] Figure 96-1 presents a model in which secondary alterations in chondrocyte expression of both wild-type ANKH and ENPP1 drive $PP_i$ supersaturation in cartilage in idiopathic/sporadic and OA-associated CPPD.

Mutations at different locations in *ANKH* can affect function and the skeleton in a manner including autosomal dominant CPPD[12,13,39-42] and certain other phenotypes. These include murine progressive ankylosis in the *ank/ank* mouse and human craniometaphyseal dysplasia associated with apparent decrease of the capacity to transport $PP_i$ within bone and effects on bone resorption and remodeling, putatively mediated in part by direct and indirect effects on osteoclasts.[38,41,49] In a consanguineous family with mental retardation, deafness, and ankylosis, with painful small joint soft tissue calcifications, progressive spondyloarthropathy, osteopenia, and mild hypophosphatemia, the homozygous *ANKH* missense mutation L244S was detected in all patients.[50] The mutant ANKH protein was expressed and

localized to the plasma membrane, but fibrosis and mineralization of articular soft tissues developed in homozygotes, with heterozygous carriers of the L244S mutation showing mild osteoarthritis without metabolic alterations.[50]

Clinical heterogeneity, even for CPPD associated with *ANKH* mutations,[12,13] suggests differing functional effects of ANKH mediated by specific regions of the molecule. Most of the N-terminally clustered *ANKH* coding region mutations identified to cause familial CPPD (see Figure 96-2) increase $PP_i$ transport *in vitro*.[12,13,39,40] However, some *ANKH* mutations have distinct effects on chondrocyte differentiation.[16] The M48T *ANKH* mutant in the French kindred appears functionally unique by association with increased intra-cellular $PP_i$ and interrupting interaction of ANKH with the sodium/phosphate cotransporter Pit-1.[51] This may be functionally significant because elevated $P_i$ increases both ANKH and Pit-1 expression, because ANKH and Pit-1 colocalize in chondrocytes,[51] and because $P_i$ modulates chondrocyte differentiation. An alternative mechanism of disrupting $PP_i$ metabolism may be promoted by the *ANKH* mutation ΔE590 linked with a case of sporadic CPPD[14] because *ANKH*ΔE590 appears to indirectly suppress $PP_i$ catabolism by association with impairing TNAP expression.[52] Uniquely, the familial CPPD *ANKH* mutation encoding P5S amino acid substitution was linked with secondary hyperparathyroidism and phosphaturia.[42]

Homozygosity for a single nucleotide substitution (−4 G to A) in the *ANKH* 5-untranslated region that promotes increased *ANKH* messenger RNA expression was present in approximately 4% of British subjects previously thought to have idiopathic/sporadic CPPD of aging.[16] Association with CPPD of this same noncoding region single base pair substitution was confirmed in a hospital-based study, and was independent of patient age and OA.[17]

## Imbalance of Chondrocyte Growth Factor Responses Affects $PP_i$ Metabolism in CPPD

The chondrocyte anabolic growth factor TGF-β stimulates ATP release by chondrocytes,[43] as well as ENPP1 expression and ENPP1 subcellular movement to the plasma membrane, which drive elevation of extra-cellular $PP_i$.[32] IL-1β suppresses both ENPP1 expression and extra-cellular $PP_i$ in chondrocytes and blocks the effects of TGF-β on $PP_I$.[32] The capacity of TGF-β to raise chondrocyte $PP_i$ increases with aging, as does TGF-β–stimulated NPP activity,[53] whereas growth-promoting effects of TGF-β decrease with aging in articular chondrocytes. These changes are potentially mediated by known alterations in TGF-β receptor–subunit expression in aged chondrocytes.

IGF-I, also an anabolic chondrocyte growth factor, normally suppresses extra-cellular $PP_i$ (as well as ATP release)[43] in chondrocytes (see Figure 96-1). Significantly, chondrocyte IGF-I resistance is characteristic of OA and aging cartilages[54] (see Figure 96-1). One mechanism pertinent to CPPD is that IGF-I induces cartilage intermediate-layer protein (CILP) (see Figure 96-1), the expression of which increases in aging and OA and is most abundant in the middle zone of articular cartilage where CPPD is most prevalent. CILP-1, but not the CILP-2 isoform, promotes increased extra-cellular $PP_i$ in chondrocytes indirectly by inhibiting IGF-I at the receptor level.[54]

## CPPD Deposition Disease Secondary to Primary Metabolic Disorders: Relationship to $PP_i$ Metabolism and Chondrocyte Differentiation

Hypophosphatasia, hypomagnesemic conditions (including the Gitelman's variant of Bartter's syndrome), hemochromatosis, and hyperparathyroidism are the best characterized primary metabolic disorders linked to secondary CPPD. Increased joint fluid $PP_i$ levels in each of these conditions suggests at least one common thread in the pathogenesis of chondrocalcinosis via cartilage $PP_i$ excess.[55] Magnesium is a cofactor for pyrophosphatase activity, and iron excess can suppress pyrophosphatase activity.

Hypercalcemia may promote CPP crystal deposition in hyperparathyroidism (and in familial hypocalciuric hypercalcemia)[56] by effects beyond cartilage matrix supersaturation with ionized calcium such as calcium function as a cofactor in ENPP1 catalytic activity, as well as chondrocyte-activating effects mediated by calcium transport[28] and the calcium-sensing receptor.[25] In addition, normal articular chondrocytes express parathyroid hormone/parathyroid-hormone-related protein (PTH/PTHrP) receptors, and functional responses of chondrocytes to PTH can promote proliferation, altered matrix synthesis, and mineralization.

Hypophosphatasia is the result of deficient activity of TNAP, with effects including limitation of hydrolysis $PP_i$ to generate $P_i$.[23] TNAP is a major physiologic antagonist of ENPP1-mediated elevation of extra-cellular $PP_i$.[23] Conversely, physiologic ENPP1-induced $PP_i$ generation antagonizes the essential procalcifying effects of TNAP mediated by $P_i$ generation,[23] and cartilage $PP_i$ excess presumably drives chondrocalcinosis in hypophosphatasia. *Enpp1* knockout mice and mice homozygous for the ENPP1 truncation mutant *ttw* demonstrate marked articular cartilage calcification with HA and OA, as well as ankylosing spinal ligament hyperostosis and synovial joint ossific fusion. Extra-cellular $PP_i$ levels and mineralization disturbances in soft tissues (but not long bones) of *Enpp1* knockout and TNAP-deficient mice are mutually corrected by cross-breeding.[23]

## Inflammation and Hypertrophic Chondrocyte Differentiation in Chondrocalcinosis

Regulated changes in chondrocyte differentiation and viability appear to be a mechanistically unified process that promotes joint cartilage HA and CPP crystal deposition, as well as OA. Such changes include development of foci of chondrocyte maturation to hypertrophy, with the presence of hypertrophy, as seen in histopathology of the knee cartilage in Figure 96-3, and apoptosis of chondrocytes typically found adjacent to cartilage calcifications. Articular chondrocyte hypertrophy is associated with heightened $PP_i$ generation; increased production of calcifying, membrane-limited cell fragments known as *matrix vesicles*; and certain other calcification-promoting changes in differentiation, including alteration of extra-cellular matrix composition. For example, osteopontin (which promotes CPP crystal formation) is increased and normal collagen subtype composition in the matrix is lost. TGF-β normally suppresses development of articular chondrocyte hypertrophy. Hence, altered TGF-β signal transduction in aging and OA may be involved in promoting chondrocyte hypertrophy.

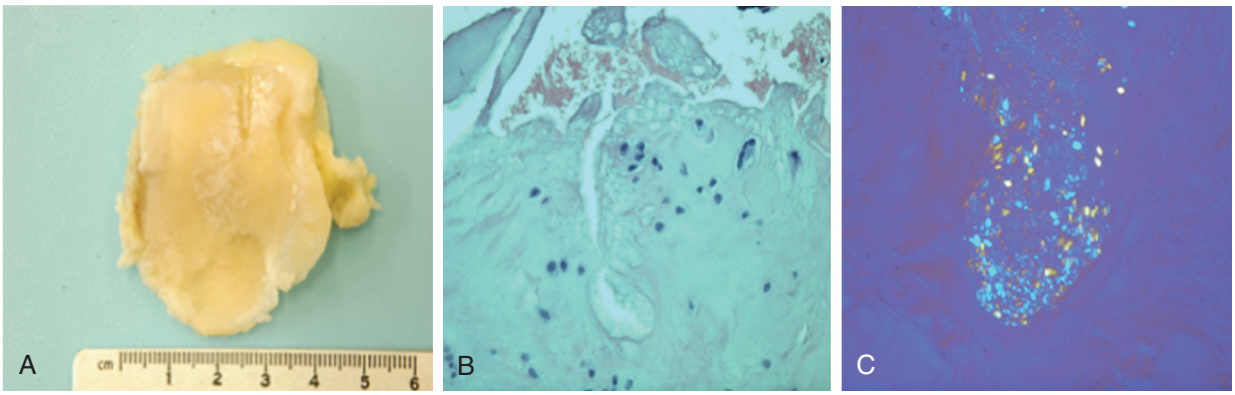



**Figure 96-3** Calcium pyrophosphate dihydrate crystal deposition disease (CPPD) arthropathy of the knee joint. **A,** Femoral condyle. There are extensive foci of chalky white particulate deposits within the articular cartilage. This is characteristic of CPPD. **B,** Histology of CPP crystal deposition within the hyaline articular cartilage. The hypertrophic chondrocytes adjacent to the crystal aggregates are within enlarged chondrons (hematoxylin and eosin, original magnification ×250). **C,** Polarized light microscopy of CPP crystal aggregates within the hyaline articular cartilage. The individual crystals have rod and rhomboid shapes and are positively birefringent (original magnification ×250). *(Courtesy Dr. Ken Pritzker, Mount Sinai Hospital Pathology Department, University of Toronto, Ontario, Canada.)*

$P_i$ taken up by Pit-1 sodium-dependent cotransport and calcium sensing can modulate chondrocyte hypertrophic differentiation and apoptosis, as well as $PP_i$-modulating responses to TGF-β.[20,31,57-59] Local upregulation of PTHrP expression also may be one of the shared features driving sequential chondrocyte proliferation and altered differentiation in growth plate chondrocytes and articular chondrocytes.[25] Chondrocyte apoptosis also promotes calcification partly through the calcifying potential of apoptotic bodies functioning as "inside-out" matrix vesicles on release from dying chondrocytes. Mitochondrial dysfunction, a central factor in tissue aging and an apparent mediator of OA progression in aging, also can stimulate oxidative stress and inflammatory changes, as well as cartilage matrix degeneration and calcification. Mitochondria are remarkably specialized to regulate calcification, and apoptosis is critically regulated by mitochondrial function. Moreover, chondrocyte ATP depletion is driven via suppression of mitochondrial oxidative phosphorylation by nitric oxide (NO) while OA evolves during aging, thereby promoting increased ATP scavenging by NPP activity and consequent augmentation of extra-cellular $PP_i$.[60]

IL-1β, which is increased in OA cartilage, stimulates articular chondrocytes to calcify the matrix.[53] NO stimulates both apoptosis and calcification in chondrocytes IL-1β and stimulates inducible nitric oxide synthase (iNOS) expression and increased NO generation, as well matrix alterations. IL-1β (as well as TNF, certain chemokines, donors of NO, and the potent oxidant peroxynitrite) also induces increased chondrocyte procalcifying transglutaminase activity.[53] TG2 acts in part by directly and indirectly promoting chondrocyte hypertrophy,[65] and also promotes activation of TGF-β.

## Special Pathogenic Aspects of Articular and Periarticular Basic Calcium Phosphate Crystal Deposition

BCP crystal deposition occurs in most hip OA, as detected by digital contact radiography, whereas plain radiography detects calcification in cartilages of less than 20% of hips of patients with OA.[61] BCP crystals also are commonly detectable within human knee cartilages with end-stage primary OA,[62,63] contribute to mineral content that can comprise 5% to 10% of knee articular cartilage weight, and typically involve multiple articular cartilage zones (including weight bearing and nonweight bearing in knee OA).[36] The process probably involves BCP crystal deposition in multiple phases of chondrocyte differentiation and functional changes, as well as viability loss. In this context, abundant cartilage NO production in OA may promote mitochondrial dysfunction, chondrocyte extra-cellular ATP depletion,[61] and lowering of extra-cellular $PP_i$, consequently favoring BCP versus CPP crystal deposition.[20] Conversely, joint fluid $PP_i$ and NPP activity are elevated in BCP-crystal–associated shoulder arthropathy (Milwaukee shoulder syndrome [MSS]), consistent with the model in Figure 96-1.

Pathologic BCP crystal deposition may occur in periarticular sites, as well as numerous organs and soft tissues. Significantly, the shoulder is the most common articular region affected by symptomatic BCP crystal deposition, in part reflecting unique shoulder structure-function (see Chapter 46). Degenerative changes promoted by biomechanical stress promote calcific tendinitis in the body of the rotator cuff. Such tendon calcifications can remain asymptomatic and also can eventually resorb, but the degenerative changes can predispose to tendon rupture. Osteopontin, a factor that normally restrains BCP crystal deposition (and is regulated by $PP_i$ and $P_i$),[22] can be detected in fibroblast-like cells and multinucleated macrophages surrounding areas of calcification in calcific tendinitis.[65] In this regard, osteopontin promotes oxidative stress, MMP activation, and macrophage recruitment and osteoclast activation.[22] The presence of multinucleated cells with cathepsin K expression and osteoclast-like functions at sites of tendon calcification[66] suggests a mechanism for both resorption of BCP crystal deposits and tendon degeneration.

## Crystal-Induced Inflammation

Some of the crystals deposited in cartilage (see supplemental Figure 96-1 on ExpertConsult.com) can subclinically

traffic to joint fluid and synovium, and the crystals can directly stimulate chondrocytes, synovial lining cells, and intra-articular leukocytes.[67-73] Inflammation triggered by CPP and BCP crystals thereby can contribute to acute flares of OA, and has the capacity to contribute to synovitis, cartilage degradation, and to potentiate worsening of OA.[67-73] Nevertheless, the net role of CPP and BCP crystals in promoting human OA progression is not well characterized.

Many pro-inflammatory mechanisms active in gout also likely mediate synovitis and cartilage degeneration associated with CPP and BCP crystal deposition.[67-73,75-78] In this regard, CPP and BCP crystals activate cells partly via non-specific activation of signal transduction pathways (e.g., mitogen-activated protein kinase activation) and induce cellular release of cyclooxygenase- and lipoxygenase-derived metabolites of arachidonic acid, cytokines including TNF, IL-1, and CXCL8[67-73] and multiple extra-cellular matrix-degrading proteases. Innate immune engagement of extracellular CPP crystals by Toll-like receptor (TLR)2[77] and CPP and BCP crystal-induced activation of the intra-cellular NLRP3 (cryopyrin) inflammasome, resulting in caspase-1 activation and IL-1β processing and release, drive cell responses to CPP and some, but not all, forms of BCP crystals in vitro and crystal-induced inflammation in vivo.[74,75,76]

In chondrocytes, BCP crystal deposits and IL-6 mutually promote each other's expression, leading to OA changes in vitro and in vivo.[76a]

The ingress of neutrophils into the joint is central in triggering acute crystal-induced synovitis, and the effects on neutrophil-endothelial interaction likely represent a major locus for prophylactic effects of low doses of colchicine for the acute arthritis of pseudogout. CXCL8 and related chemokines that bind the CXCL8 receptor CXCR2 (including CXCL1) appear to be critical in initiating and perpetuating neutrophil ingress in acute crystal-induced inflammation. However, "neutrophil extra-cellular trap" formation, an NADPH oxidase- and CXCR2 chemokine ligand-mediated process stimulated by CPP and urate crystals, is likely involved in self-limiting of acute CPP crystal-induced inflammation.[79,80]

Periarticular BCP crystals have marked inflammatory potential, such as in acute subacromial bursitis (see Chapter 46). However, BCP crystals generally trigger much less neutrophil influx into the joint space than CPP crystals. Concordantly, free intra-articular BCP crystals likely induce less pro-inflammatory cytokine expression than CPP and monosodium urate crystals, although OCP crystals could be more inflammatory than hydroxyapatite crystals.[76]

## CLINICAL FEATURES

### KEY POINTS

**In the elderly, CPPD can mimic conditions including gout, infectious arthritis, primary osteoarthritis, rheumatoid arthritis (RA), or polymyalgia rheumatica.**

**CPPD can present as fever of unknown origin, and factor in the differential diagnosis of patients with neurologic disturbances and painful cervical mass, especially in the elderly.**

**Acute CPP arthritis (also termed *pseudogout*) is a major cause of acute monoarticular or oligoarticular arthritis in the elderly. Attacks typically involve a large joint, most often the knee, less often, the wrist or ankle, and, unlike gout, rarely the first metatarsophalangeal joint.**

**Chronic degenerative arthropathy in CPPD commonly affects certain joints that are typically spared in primary OA (e.g., metacarpophalangeal joints, wrists, elbows, glenohumeral joints).**

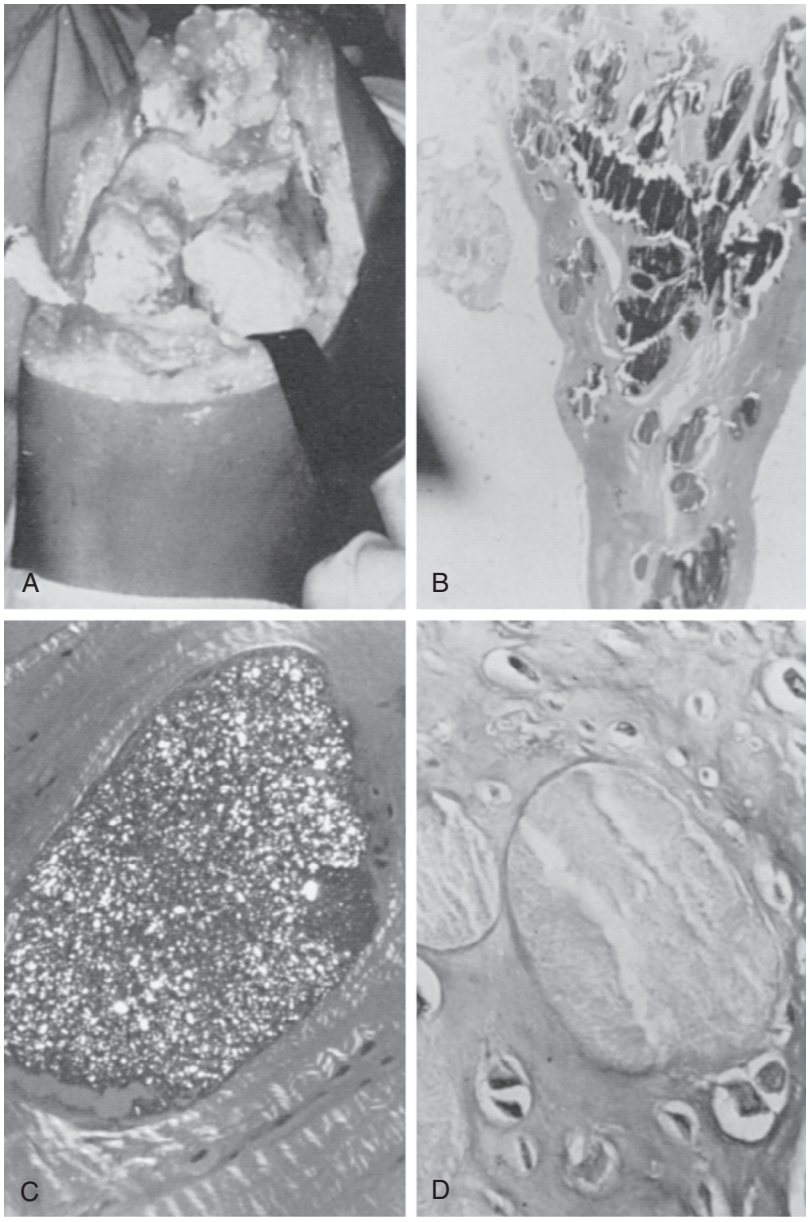
### Calcium Pyrophosphate Dihydrate Crystal Deposition Disease

The majority of elderly individuals with CPPD in the United States have primary (idiopathic/sporadic) CPPD or CPPD secondary to OA (Table 96-2). Idiopathic CPPD generally appears only after the fifth decade of life. However, patients with a history of repetitive joint trauma (and in some cases, past knee meniscectomy) may be seen with nonsystemic (monoarticular) CPPD before age 55 years. Clinical presentation of familial CPPD is often manifested in the third and fourth decades of life, but familial disease can sometimes be detected before age 20 years or first manifest clinically at an advanced age. CPPD also manifests in a variety of hereditary and metabolic conditions (including hyperparathyroidism, dialysis-dependent renal failure, and hemochromatosis) in which the CPPD-related arthropathy can be seen in patients younger than age 55 years. For unclear reasons, hemochromatosis can manifest predominantly with CPPD or as OA. The weight of evidence from

**Table 96-2** Causes of CPPD

| |
|---|
| **High Prevalence** |
| Idiopathic in association with aging (most frequent) |
| Complication of primary osteoarthritis |
| Long-term consequence of mechanical joint trauma or knee meniscectomy |
| **Moderate Prevalence** |
| Familial |
| Knee malalignment (predominantly varus) in early life |
| Associated with systemic metabolic disease (hyperparathyroidism, hemochromatosis) |
| Associated with dialysis-dependent renal failure |
| Associated with diseases, drugs, and iatrogenic factors that promote hypomagnesemia (loop diuretics, proton pump inhibitors, calcineurin inhibitors, alcohol abuse, short bowel syndrome, Gitelman's variant of Bartter's syndrome) |
| Associated with gout |
| **Low Prevalence (Largely Based on Case Reports)** |
| X-linked hypophosphatemic rickets |
| Familial hypocalciuric hypercalcemia |
| Ochronosis |
| Articular amyloidosis |
| Myxedematous hypothyroidism |
| Osteochondrodysplasias and spondyloepiphyseal dysplasias |
| Neuropathic joints |
| Wilson's disease |

CPPD, Calcium pyrophosphate dihydrate crystal deposition disease.




**Supplemental Figure 96-1** **A**, Chalky calcium pyrophosphate (CPP) crystal deposits observed on synovium, menisci, and hyaline cartilage of a 35-year-old patient with familial calcium pyrophosphate deposition disease. **B,** Menisci showing black-stained crystalline deposits under von Kossa's stain. **C,** Crystalline CPP deposits in articular cartilage. (Compensated polarized light, ×300.) **D,** Hypertrophic chondrocytes surrounding CPP deposits (hematoxylin and eosin, original magnification ×400).

controlled studies suggests that hypothyroidism (with the probable exception of myxedematous hypothyroidism) is not associated with a significantly increased prevalence of CPPD, although both disorders are clearly prevalent in aging. Initiation of thyroxine supplementation therapy has been suggested as a trigger of pseudogout.

Clinical manifestations of CPPD vary widely[81] (Table 96-3). Quite commonly, CPPD is asymptomatic. Alternatively, it can mimic OA (pseudo-osteoarthritis), gout (pseudogout) (see supplemental Figure 96-2 on ExpertConsult.com), acute-onset or insidious RA (pseudorheumatoid arthritis) (Figure 96-4; see also supplemental Figure 96-3 on ExpertConsult.com), or be seen as *pseudoneuropathic* arthropathy. Patients with CPPD also commonly are seen with episodes of hemarthrosis, often post-traumatic and in the knee. Contributions to these wide differences in clinical manifestations of monoclinic versus triclinic CPP crystals deposited, and of host factors, remain unclear. Overall, few patients with CPPD have prolonged, recurring polyarticular inflammation. Progressive degenerative arthropathy is more common. Although CPPD appears to be a common and significant public health problem in the elderly, the disease and health-related quality of life impact and the long-term course of CPPD-associated degenerative arthropathy in an unselected population have not been adequately evaluated.

**Table 96-3** Common Clinical Presentations of CPPD

| |
|---|
| Asymptomatic or incidental finding (e.g., asymptomatic knee fibrocartilage chondrocalcinosis in the elderly) |
| Recurrent acute inflammatory monoarticular arthritis (pseudogout) (e.g., wrist, knee, including provocation by trauma, concurrent medical or surgical illness, or intra-articular hyaluronan) |
| Pseudoseptic arthritis: includes inflammatory arthritis early and late after total joint arthroplasty |
| Recurrent acute hemarthrosis |
| Chronic degenerative arthritis (pseudo-osteoarthritis or pseudo-neuropathic arthritis) |
| Chronic symmetric inflammatory polyarthritis (pseudorheumatoid arthritis) |
| Systemic illness (pseudopolymyalgia rheumatica, fever of unknown origin) |
| Destructive arthritis in dialysis-dependent renal failure |
| Carpal tunnel syndrome |
| Central nervous system disease complicating ligamentum flavum or transverse ligament of atlas involvement (cervical canal stenosis, cervical myelopathy, meningismus, foramen magnum syndrome, odontoid fracture) |
| Tumoral and pseudotophaceous CPP crystal deposits |

CPPD, Calcium pyrophosphate dihydrate crystal deposition disease.

## Acute Calcium Pyrophosphate Dihydrate Crystal-Associated Synovitis (Pseudogout)

Pseudogout is a major cause of acute monoarticular or oligoarticular arthritis in the elderly. The attacks typically involve a large joint, most often the knee and less often the wrist or ankle, and, unlike gout, rarely the first metatarsophalangeal joint. Acute attacks of inflammatory pseudogout in patients with CPPD typically have a sudden onset and can be excruciatingly painful, with pronounced periarticular erythema, warmth, and swelling, comparable to gout.[1,81] In addition, arthritis in some attacks of pseudogout can be migratory or can be additive, polyarticular, and bilateral. Polyarticular pseudogout is common in familial CPPD and hyperparathyroidism.

Multiple case reports indicate that CPP inflammatory arthritis, often acute and sometimes mimicking a septic joint, can develop early or even many years after total joint arthroplasty of a joint affected by CPPD[82,83,84,85,86]; development of calcification in areas of chondroid metaplasia in the joint, including around the prosthesis, appears to be involved.[87] Acute CPP inflammatory arthritis also can be triggered by minor trauma or intercurrent medical or surgical conditions including pneumonia, myocardial infarction, cerebrovascular accident, and pregnancy. Parathyroid surgery for hyperparathyroidism frequently triggers pseudogout attacks. In addition, pseudogout of the knee can be precipitated by arthroscopy or by intra-articular administration of hyaluronan[88] that could reflect pro-inflammatory mechanisms triggered through the hyaluronan receptor CD44. Parenteral administration of granulocyte colony-stimulating factor (G-CSF) and of bisphosphonates[89] also can trigger pseudogout, the former likely by ignition of

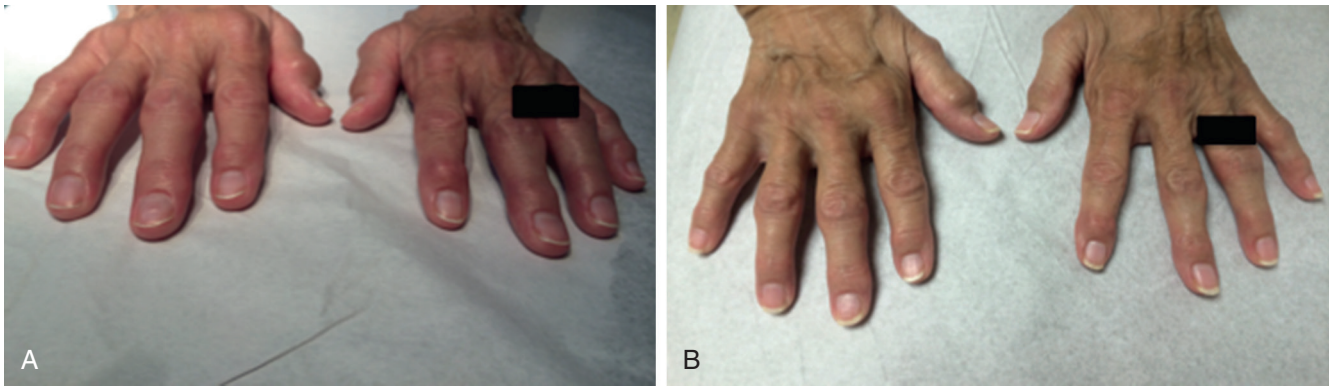
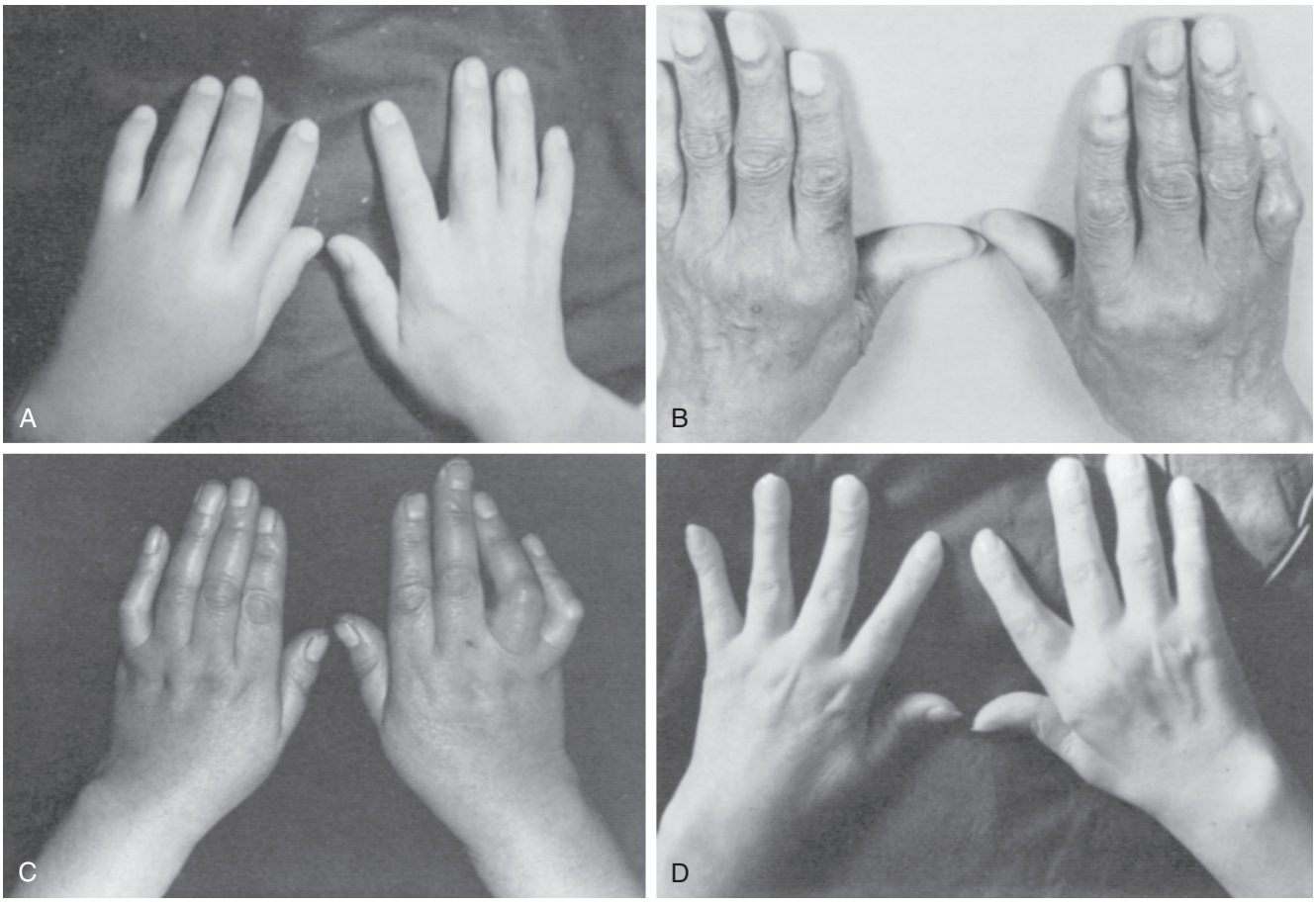



**Figure 96-4** Idiopathic symmetric pseudorheumatoid calcium pyrophosphate dihydrate crystal deposition disease (CPPD) darthropathy in an elderly female. This 66-year-old female presented with a 4- year history of hand pain, and manifested chronic symmetric proliferative synovitis diffusely involving both metacarpophalangeal (MCP) joint and proximal interphalangeal (PIP) joint rows **(A)**, and had plain radiography and ultrasound findings consistent with CPPD. After 6 months of treatment with low-dose methotrexate and hydroxychloroquine, she was symptomatically better, with decreased MCP joint row swelling and improved, although with persistent, PIP joint row swelling **(B).**




**Supplemental Figure 96-2** **A**, Acute pseudogout. The patients in **A, C,** and **D** are siblings. **B,** Pseudo-osteoarthritis. **C,** Pseudorheumatoid arthritis with boutonnière deformity. **D,** Pseudorheumatoid arthritis showing ulnar deviation, interosseous muscle atrophy, and metacarpophalangeal and wrist joint involvement.




**Supplemental Figure 96-3** Idiopathic symmetric pseudorheumatoid calcium pyrophosphate dihydrate (CPPD) deposition arthropathy in an elderly female. This 84-year-old female presented with a history of past right carpal tunnel syndrome and with chronic symmetric proliferative synovitis of both wrists and second and third metacarpophalangeal (MCP) joints, with physical findings of synovial and dorsal extensor tenosynovial swelling of the wrists and synovial swelling at the second to third MCP joints **(A).** Changes on hand and wrist plain radiographs consistent with the diagnosis of CPPD deposition disease, presented for the right wrist **(B),** included cystic changes in multiple carpal bones including the scaphoid and lunate, linear calcification on the ulnar side of the carpus *(arrow)* typical for the chondrocalcinosis of CPPD deposition, and mild narrowing of the radiocarpal joint indicative of cartilage loss.

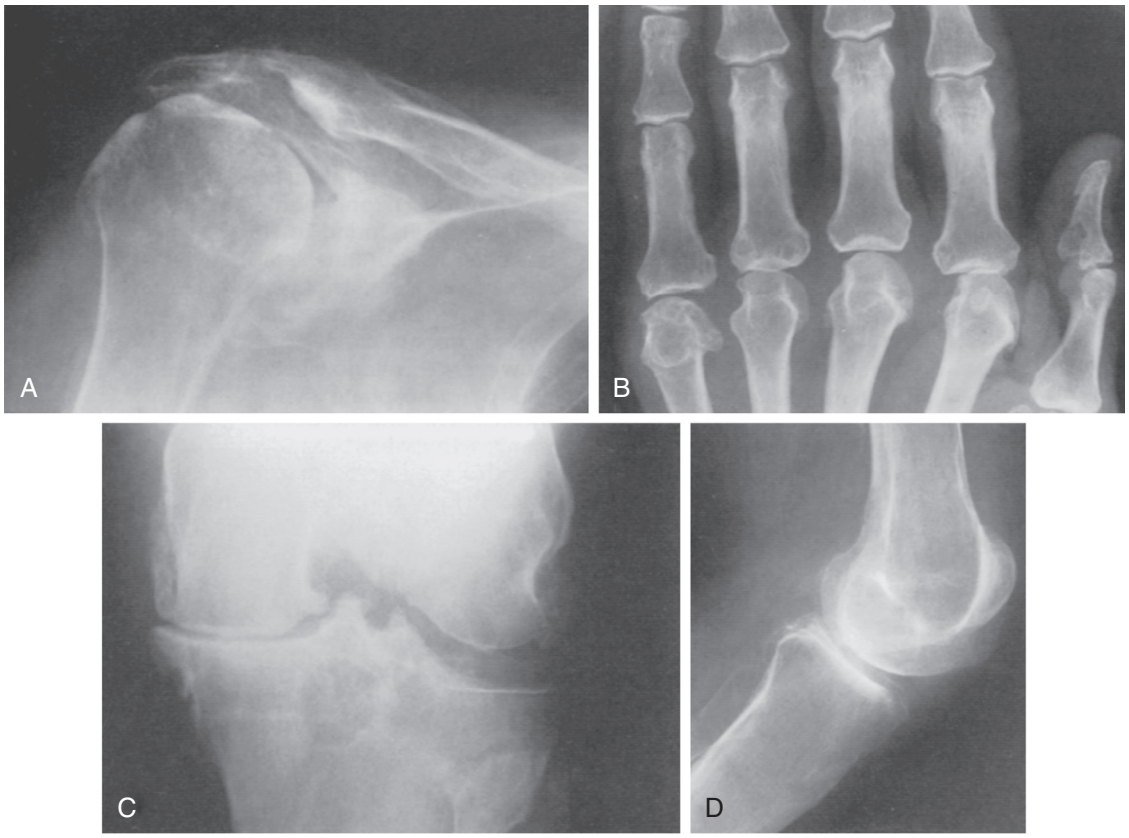



**Figure 96-5** Radiographic features of calcium pyrophosphate dihydrate crystal deposition disease (CPPD) arthropathy. **A,** Destructive shoulder arthropathy. **B,** Metacarpophalangeal joint arthropathy. **C,** Knee degenerative joint disease with large subchondral bone cyst. **D,** Wraparound patella (same patient shown in **A**).

smoldering subclinical intra-articular inflammation, the latter theoretically via pyrophosphatase inhibition, because bisphosphonates are nonhydrolyzable analogues of $PP_i$.

Acute and subacute pseudogout can be associated with fever, chills, elevated erythrocyte sedimentation rate, and systemic leukocytosis, particularly with polyarticular involvement and in the elderly.[1] Leukocyte counts in the synovial fluid are substantially elevated, and intraleukocytic CPP crystals are most often (but not universally) detectable by compensated polarized light microscopy in acute CPP arthritis (pseudogout).[1] The attacks typically last for 7 to 10 days, but also can be clustered and last for weeks to months. Occasionally, the leukocyte count in pseudogout can exceed 50,000 per $mm^3$ (pseudoseptic arthritis).

## Chronic Degenerative and Inflammatory Arthropathies

Acute pseudogout attacks may be interspersed with chronic arthropathy in CPPD, although acute flares of pseudogout may be less common in those with established chronic degenerative CPPD.[90] Chronic degenerative arthropathy in CPPD commonly affects certain joints that are typically spared in primary OA (e.g., metacarpophalangeal joints, wrists, elbows, glenohumeral joints). The development of cartilage degenerative changes in CPPD in both typical and atypical joints for primary OA suggests one or more systemic abnormalities. Significantly, the linkage of CPPD and OA is quite variable between joints.[11a]

Degenerative cartilage disease associated with sporadic CPPD may be seen as destructive arthropathy of the knees, hips, and/or shoulders, particularly in elderly females (Figures 96-5 and 96-6). The CPPD arthropathy–associated degenerative disease can be less or more destructive than that observed in primary OA. For example, patients with primary OA and CPP crystals have been reported to require knee replacement surgery more often than those with primary OA without crystals.[91] In another study, 60% of patients undergoing joint replacement had CPP or BCP crystals (and commonly both) in their knee synovial fluids, and higher mean radiographic scores correlated with the presence of calcium-containing crystals.[92] However, radiographic worsening of degenerative changes is often slow. Most patients develop changes in radiographic extent of chondrocalcinosis with time. But there is no clear correlation between the extent of calcification and progression of CPPD arthropathy. It remains unclear whether the prognosis is altered by the initial appearance of CPPD in the knee as an acute pseudogout attack alone.

Though it is not clear how CPPD modified the clinical phenotype of OA, it does appear to be associated with modifications of the radiographic phenotype.[28a] Specifically, CPPD appears to be associated with decreased knee bone stock when present in knee OA, putatively mediated in part by local PPi excess. Moreover, in a cohort study of severe symptomatic large joint OA, there was a milder imaging phenotype of hip OA linked with CPPD, with negative association seen between osteophytosis and JSN at the hip

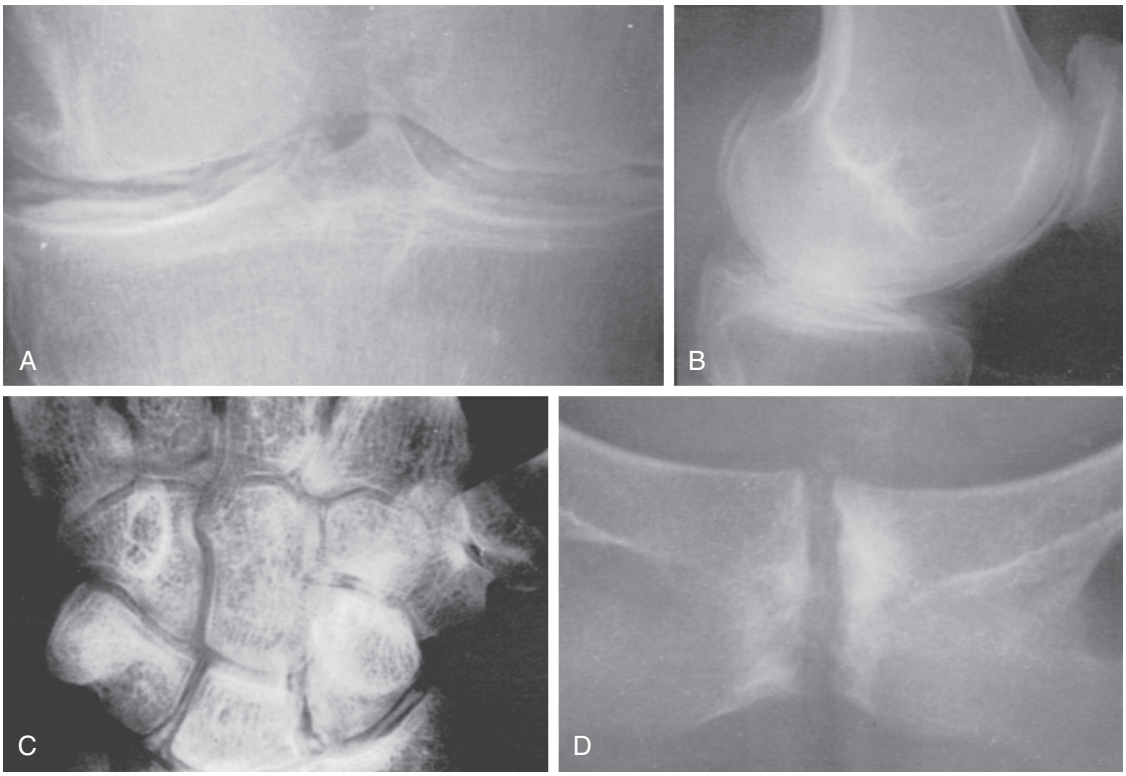



**Figure 96-6** Chondrocalcinosis of the most commonly affected joints in calcium pyrophosphate dihydrate crystal deposition disease (CPPD). **A,** Linear calcifications observed in knee menisci and fibrocartilage. **B,** Lateral view showing calcification of the articular cartilage as a line parallel to the femoral condyles. **C,** Calcification of intercarpal joints and triangular ligament. **D,** Calcification of the symphysis pubis fibrocartilage associated with subchondral bone erosions and subchondral increased bone density.

and hip CPPD, assessed by plain radiography. However, this work, in a hospital cohort, needs larger validation, since the findings may reflect difficulty in visualizing CPPD by plain radiography in more advanced hip OA, due to marked articular cartilage loss.

Pseudorheumatoid arthritis involvement in a small subset of patients with CPPD appears as a chronic, bilateral, symmetric deforming inflammatory polyarthropathy (see Figure 96-4). Many of these patients have bilateral wrist and metacarpophalangeal joint involvement. Wrist tenosynovitis and carpal tunnel syndrome, cubital tunnel syndrome, and tendon rupture may develop. Ingestion of CPP crystals by synovial lining cells and lysosomal catabolism of such ingested crystals stimulates synovial proliferation, in part via solubilization of the crystalline calcium. Such effects may contribute to regional synovial and periarticular tenosynovial proliferation promoted by CPP crystal deposition.

## Other Clinical Forms of CPPD

Concentrated (tumoral or pseudotophaceous) CPP crystal deposition can occur in periarticular structures including tendons, ligaments, bursae, and occasionally in bone.[93,94,95] Extraskeletal CPP crystal disease can occur, but appears rare.[96] CPP crystal deposits in tendons (e.g., Achilles, triceps, and obturator tendons) are usually fine and linear on radiographs. Pseudotophaceous deposits of CPP crystals have been detected in the temporal bone, around the knee and hip, and in the acromioclavicular, temporomandibular, elbow, and small hand joints. [93,94,95] Peripheral tumoral CPP crystal deposits may sometimes manifest with acute arthritic attacks. Rarely, tumoral CPP crystal deposits around the knee can mimic osteonecrosis. Tumoral CPP crystal deposition is typically associated with tissue chondroid metaplasia and behaves like a benign but locally aggressive chondroid tumor, with some of the connective tissue invasion and destruction likely mediated by CPP crystal-induced cell activation.

Axial skeletal CPPD occasionally involves the intervertebral disks, sacroiliac joints, and lumbar facet joints, and radiographic findings such as linear calcification and spinal ankylosis may appear.[97,98,99] CPPD also commonly factors in the differential diagnosis of patients with neurologic disturbances and painful cervical mass, especially in the elderly. Specifically, meningismus and clinical manifestations resembling herniated intervertebral disk, ankylosing spondylitis, and acute pseudogout of lumbar facet joints have been observed.[100] In addition, CPP crystal deposits are common in the antlanta-axial joint in aging.[101] CPPD within the ligamentum flavum or the transverse ligament of atlas can be sizeable and can progress to cause cervical canal stenosis, cervical myelopathy, and foramen magnum syndrome.[102,103] Odontoid fracture because of the calcification of the atlantoaxial joint may occur in CPPD.[103,104]

## Familial CPPD

Familial CPPD has been described in numerous countries and ethnic groups, including kindreds from Czechoslovakia, Holland, France, England, Germany, Sweden, Israel, the

United States, Canada, and Japan, and may be most prevalent in Chile and Spain. For linkages to *ANKH* on chromosome 5p, some families manifest early onset polyarthritis, which can include ankylosing intervertebral and sacroiliac joint disease, and childhood seizures have been described.[13,15,40] In others, late-onset CPPD occurs, and the disease can be oligoarticular, mild in intensity and destructiveness, and nearly indistinguishable from idiopathic CPPD.[13,15,40] Kindreds from Argentina and the Alsace region of France linked to 5p have been found to share similar phenotypic features of CPPD, including early age at onset (third decade of life), common but not universal premature OA, some cases of pseudorheumatoid arthritic peripheral joint disease, and radiographic evidence of fibrocartilage and hyaline cartilage calcifications typical of CPP crystal deposition. The most commonly affected joints in these kindreds were the knees and wrists, with pubic symphysis and intervertebral disk involvement also described.

## ARTICULAR BASIC CALCIUM PHOSPHATE CRYSTAL DISEASE

### KEY POINTS

**Unlike urate and CPP crystal deposition, acute synovitis because of HA crystal deposition is unusual. However, acute periarticular inflammatory syndromes including subacromial bursitis and a form of pseudopodagra described in young women may occur in association with HA crystal deposition in bursae, tendons, ligaments, and soft tissues.**

**Symptomatic articular and periarticular BCP crystal deposition, which may be destructive and involve the axial skeleton, may develop in patients with advanced chronic renal failure, particularly on dialysis. They may resemble or be associated with CPPD.**

Unlike urate and CPP crystal deposition, acute synovitis due to HA crystal deposition is unusual. But acute inflammatory syndromes including subacromial bursitis and "pseudopodagra" may occur in association with periarticular HA crystal deposition in bursae, tendons, ligaments, and soft tissues. Symptomatic articular and periarticular BCP crystal deposition may develop in patients with advanced chronic renal failure, particularly on dialysis (Figure 96-7), which may be destructive and involve the axial skeleton. In some cases of dialysis-dependent renal failure, destructive arthropathy associated with BCP crystal deposition may resemble or be associated with CPPD, and monosodium urate crystal deposits in the joint also may occur in the setting. Hyperparathyroidism can promote BCP-associated arthropathy and periarticular disease including calcific bursitis (see Figure 96-7). Clinically significant periarticular HA crystal deposition also may occur in certain post-traumatic conditions and the systemic autoimmune diseases scleroderma and dermatomysositis.

BCP crystal deposition has a particular predilection for the shoulder (see Chapter 46), where it may manifest as calcific tendinitis of the rotator cuff or as a destructive process associated with rotator cuff tear, which is most prevalent in the elderly and more common in females.[105] Abundant intra-articular BCP crystalline material is typically present in the distinctive non-inflammatory syndrome of rotator cuff tear and marked cartilage degeneration, an entity termed *MSS, cuff tear arthropathy*, or *apatite-associated destructive arthritis*.[105] Mechanical instability of the shoulder because of rotator cuff tear may be the driving force in many of these patients, with consequent release of BCP crystals from bone fragments into the joint space, promoting secondary synovitis and connective tissue destruction. The process may be bilateral but is generally worse on the side of the patient's dominant hand. Substantial glenohumeral joint effusions are typically seen, and synovial fluid is often blood stained but contains, at most, relatively low numbers of mononuclear leukocytes. Joints other than the shoulder, such as the knee and hip, can be affected by a condition similar to MSS, sometimes in the same individual with shoulder involvement. In contrast to primary OA, lateral tibiofemoral compartment involvement is common in BCP-associated destructive knee arthropathy. Concurrent CPPD, biomechanical abnormalities, chronic renal failure, and neuropathic factors appear to be predisposing factors. A kindred with familial OA and apparent Milwaukee shoulder-knee syndrome had an unusual type of degenerative joint disease with both intra-articular and periarticular calcifications.[106]

Aging itself is a factor in articular cartilage BCP crystal deposition.[107] Multiple studies of synovial fluids and cartilage specimens from OA, including recent work that has taken advantage of high-resolution means for BCP crystal detection,[108] have suggested that deposition of intra-articular BCP crystalline material, including HA in the pericellular matrix of chondrocytes and the capacity of joint cartilages to form such deposits, are intimately linked with OA, as well as increased chondrocyte hypertrophy and severity of OA.[92,109-111] One limitation of some of these studies is that cartilages were fixed before analysis, and calcifications can develop as a fixation artifact. However, unequivocally, cartilage and synovial fluid BCP crystals, frequently in conjunction with CPP crystals, are commonly detectable in advanced disease of the knee at the time of total joint replacement for OA.[92,111] Traffic of crystals from articular cartilage to synovium can promote calcific synovial crystal deposits at or just beneath the synovial surface, and synovium-derived rice bodies can give rise to BCP crystal deposits released into the joint space.[112]

Collectively, the abundance of HA and CPP crystals in OA joints is likely to be significant under many clinical circumstances because HA and CPP crystal-induced synovial proliferation, cytotoxic effects on chondrocytes, and synovial and chondrocyte MMP expression have the potential to promote OA progression.[111] Better surveys of joint tissues, with advanced methods for detection of BCP crystals[111,113,114] in particular, will be necessary to improve understanding of clinical impact of these calcific crystals on OA.

## DIAGNOSIS AND DIAGNOSTIC TESTS

### KEY POINTS

**The presence of radiographic evidence for chondrocalcinosis is a common finding in elderly and does not necessarily indicate that the patient's symptomatic articular problem is the result of CPPD, which is often asymptomatic.**

**The use of compensated polarized light microscopy is essential to confirm the presence of positively birefringent CPP crystals, although it should be noted that some CPP crystals are nonbirefringent.**

**High-resolution ultrasound of the affected joint is a useful and, in many cases, more sensitive approach than plain radiography in screening for CPPD.**

## Differential Diagnosis

CPPD can imitate a number of other conditions, and vice versa (see Table 96-3), which mandates attention to fulfillment of EULAR[1] and other diagnostic criteria for CPPD (see Table 96-1), with careful adherence to a diagnostic algorithm (Figure 96-8). Conversely, it is important to note that the presence of radiographic evidence for chondrocalcinosis is a common finding in the elderly and does

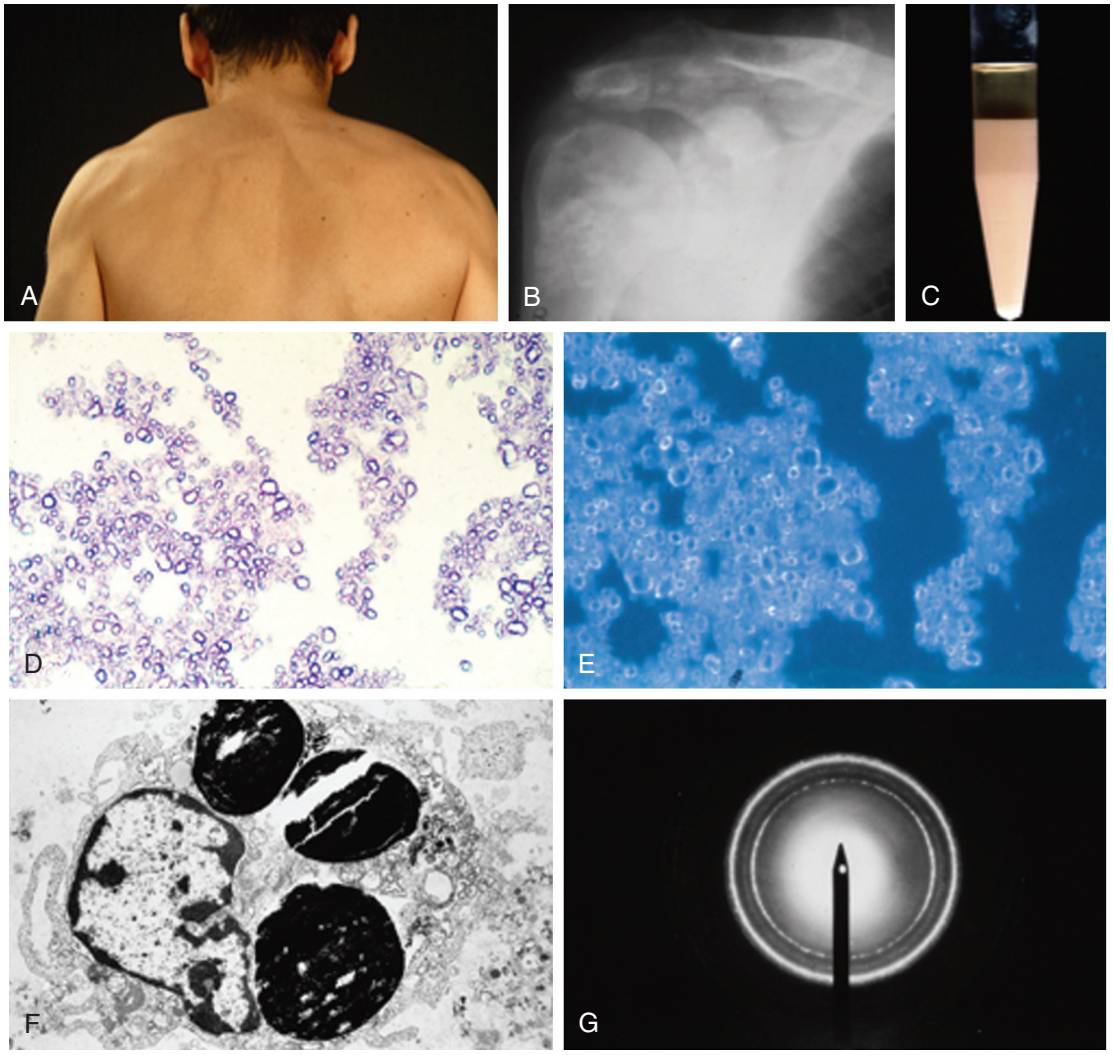



**Figure 96-7** Hydroxyapatite crystal-associated calcific bursitis of the shoulder in a patient with chronic renal failure and secondary hyperparathyroidism. **A,** Chronic soft tissue swelling involving the right shoulder caused by calcific right shoulder subacromial bursitis in a middle-aged male with a history of chronic renal failure on hemodialysis. Note the convex contour of the right shoulder compared with the left. **B,** Radiograph showing extensive calcification both within the rotator cuff and the expanded subacromial bursa surrounding the right shoulder joint. Incidental note is made of the resorption of the distal end of the clavicle consistent with the secondary hyperparathyroidism in this patient. **C,** Subacromial bursa fluid from the right shoulder. Note the milk-white appearance, with chalky sediment of the particulate material in the fluid after centrifugation consistent with crystal deposition disease. **D,** Microscopic appearance of bursa fluid aggregates of basic calcium phosphate crystals in the absence of special stains. The particles are irregular but have approximately spherical profiles. (Unstained magnification ×250.) **E,** Appearance of the bursa fluid under polarized light microscopy. Importantly, the aggregated particles of basic calcium phosphate crystals demonstrate edge birefringence but do not display intrusive birefringence, as seen in the figure. (Unstained magnification ×250.) **F,** Electron photomicrograph of a mononuclear phagocyte from this bursa fluid that contained phagocytosed electron dense *(dark black)* spherical aggregates of crystals of the basic calcium phosphate hydroxyapatite in three phagolysosomes oriented vertically to the right of the nucleus. Hundreds of tiny needle-shaped hydroxyapatite crystals are clumped in each of these dense aggregates. For perspective, the size of the mononuclear phagocyte is approximately 20 μm, and an individual (nonaggregated) hydroxyapatite crystal is approximately 0.04 × 0.01 × 0.01 μm in size. (Transmission electron microscopy, magnification ×1000.) **G,** Electron diffraction pattern of hydroxyapatite crystal aggregates. The diffraction rings are indicative of a powder pattern (i.e., small crystals). The position of the bright rings with d-spacings = 3.44 and 2.81 are characteristic of hydroxyapatite (calcium apatite). *(Courtesy Dr. Ken Pritzker, Mount Sinai Hospital Pathology Department, University of Toronto, Ontario, Canada.)*

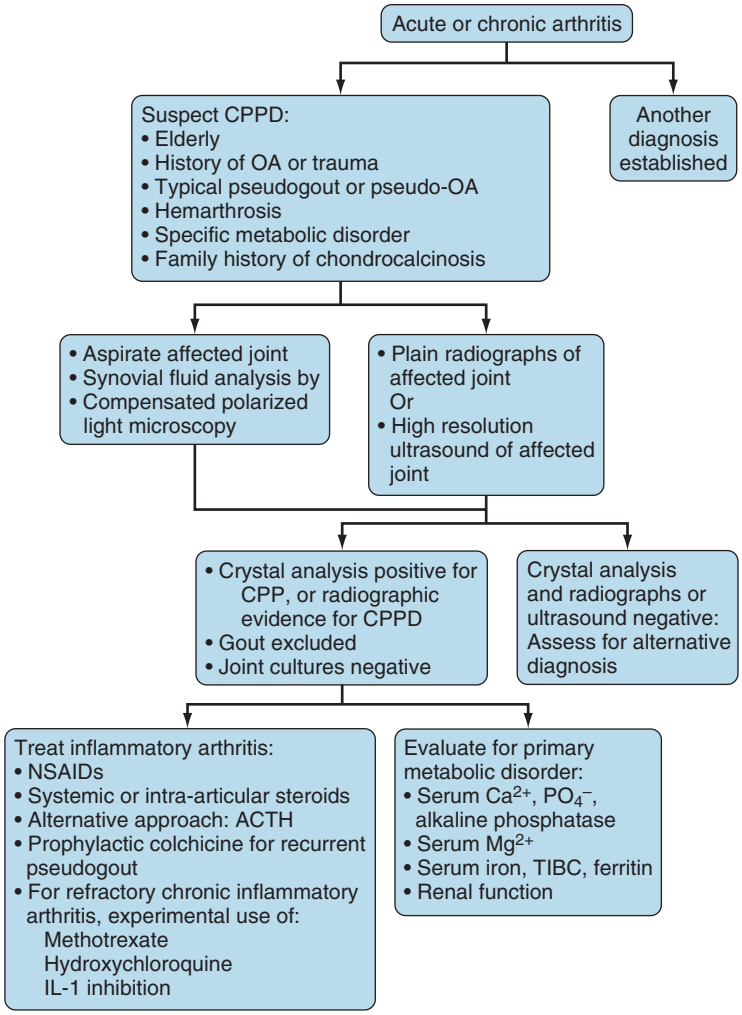



**Figure 96-8** Algorithm for diagnosis, evaluation, and treatment of CPPD. The algorithm is discussed in detail in the text. Treatment options are in line with those recently advanced by the European League Against Rheumatism. ACTH, Adrenocorticotropic hormone; IL, interleukin; NSAIDs, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; TIBC, total iron binding capacity. *(From Guerne P-A, Terkeltaub R: Calcium pyrophosphate dihydrate crystal deposition: epidemiology, clinical features, diagnosis, and treatment. In Terkeltaub R, editor:* Gout and other crystal arthropathies*, Philadelphia, 2011, Elsevier.)*

not necessarily indicate that the patient's symptomatic articular problem is the result of CPPD, which is often asymptomatic.

The demonstrable presence of CPP crystals in synovial fluid or in tissues by using compensated polarized light microscopy (as discussed earlier for gout vs. pseudogout) is definitive evidence for CPPD.[1] Although weakly birefringent relative to urate crystals and often rhomboid in shape, CPP crystals can be rod shaped and intra-cellular, thereby resembling urate crystals. Thus, the use of compensated polarized light microscopy is essential to confirm the presence of positively birefringent CPP crystals, although it should be noted that some CPP crystals are nonbirefringent.[115] The appearance and number of CPP crystals can change with storage. Therefore, clinicians should examine relatively fresh specimens collected in vials free of calcium-chelating anti-coagulants such as ethylenediaminetetraacetic acid (EDTA).

The ability of pseudogout to mimic septic arthritis (pseudoseptic arthritis) and vice versa underscores the diagnostic importance of arthrocentesis with appropriate synovial fluid crystal analysis and, in many instances, concomitant exclusion of joint infection. Significantly, crystal deposits can be "enzymatically strip-mined" by inflammation associated with joint sepsis. Hence, CPP (as well as other crystals) may be observed extra-cellularly and intra-cellularly in the synovial fluid of an infected joint.

Diagnosis of CPPD in a patient younger than 55 years, particularly if CPPD is widespread, should prompt differential diagnostic consideration of a primary metabolic or familial disorder (see Table 96-2). In the elderly, presentation of CPPD as diffuse pain and fever of unknown origin can mimic infection, polymyalgia rheumatica, and RA. A false-positive rheumatoid factor (RF) test is common in the elderly (≥30% positivity). Thus, patients with pseudorheumatoid arthritis CPPD are often RF seropositive.

## Differential Diagnostic Considerations for Basic Calcium Phosphate Crystal Deposition

BCP crystals may be detected as nonbirefringent globular clumps within leukocytes in some synovial and bursal fluids

(see Figure 96-7), and BCP crystal clumps stain with the calcium-binding dye alizarin red S under light microscopy. CPP crystals can also be detected by using alizarin red S, but they stain more weakly than BCP crystals. Relative paucity of osteophytes (so-called *atrophic degenerative arthritis*) on plain radiographs and the sizeable glenohumeral joint effusions with abundant synovial fluid BCP crystalline material associated with MSS help distinguish MSS from primary OA of the glenohumeral joint. However, destructive neuropathic shoulder arthropathy resulting from syringomyelia or alcoholism sometimes merits consideration in the differential diagnosis of MSS (see Chapter 46). Oxalate crystal deposition arthropathy can be a major differential diagnostic consideration with BCP-associated arthritis and periarticular calcifications in dialysis-dependent renal failure.

## Plain Radiographs

Chronic arthritis in CPPD has several clinical and plain radiographic features that help to differentiate it from OA. These features include involvement at sites uncommon for primary OA, such as the wrist, metacarpophalangeal joints, elbow, or shoulder, as well as radiographic heavy punctate and linear calcifications in fibrocartilages, articular (hyaline) cartilages, and joint capsules, especially if bilaterally symmetric (see Figures 96-5 and 96-6). It should be noted that faint or atypical calcifications may be caused by BCP-related vascular calcifications. Deposition of nonpathologic dicalcium phosphate dihydrate (DCPD) ($CaHPO_4{\cdot}2H_2O$, calcium-to-phosphate ratio of 1) ("brushite") crystals has been thought to cause some atypical calcifications, but brushite crystals can arise as an artifact of acid preparation of calcified tissue for pathologic analyses.

CPP crystal deposits often appear as broad linear streaks or linear "chunks" in articular hyaline and fibrocartilages on plain radiographs, whereas BCP crystal deposits in articular cartilage require high-resolution radiography for detection. Only a pattern of "atrophic" degenerative arthritis without osteophytes and variable subchondral bone thickening may be seen on plain radiographs of BCP arthritis in the shoulder and other large joints.

Patients with arthritis in whom CPPD is part of the differential diagnosis are typically screened first by plain radiography, which remains far more accessible than articular ultrasound imaging. The knee is the most common site of radiographic chondrocalcinosis, but 42% of the CPPD cases in one study had no knee involvement detectable by plain radiograph.[9] Hence, joint-specific screening is advisable for CPPD, rather than the use of knee radiographs to attempt to diagnose CPPD at sites other than the knee. The diagnosis of CPPD in one joint may warrant a search for CPPD at other sites, to assess whether the CPPD is monoarticular or part of polyarticular disease. In this circumstance, the yield is substantial for an anteroposterior (AP) view of each knee, an AP view of the pelvis (to detect symphysis pubis involvement), and posteroanterior (PA) views of both hands that include visualization of both wrists.

Calcific deposits may or may not be detectable by radiography screening of some areas in CPPD. In this circumstance, radiographic evidence other than chondrocalcinosis may point to the correct diagnosis.[116] For example, radiographic findings suggestive of CPPD, as opposed to primary OA, include radiocarpal or marked patellofemoral joint space narrowing, especially if isolated (such as the patella "wrapped around" the femur), as well as scaphoid-lunate widening and femoral cortical erosion superior to the patella. Severe progressive degeneration in the knee with subchondral bony collapse (microfractures) and fragmentation with formation of intra-articular radiodense bodies is a feature of CPPD manifesting as a "pseudoneuropathic" joint.

CPPD involving the metacarpophalangeal joints can be distinguished radiographically from RA by metacarpal squaring associated with "beaklike" osteophytes and subchondral cyst formation. Tendon calcifications (e.g., Achilles, triceps, and obturator tendons) are a valuable differential diagnostic feature of CPPD. Osteophyte formation is more variable with CPPD than with OA. Clearly, radiographic findings often fail to correlate with pathologic and clinical manifestations in CPPD.

## High-Resolution Ultrasound and Advanced Imaging

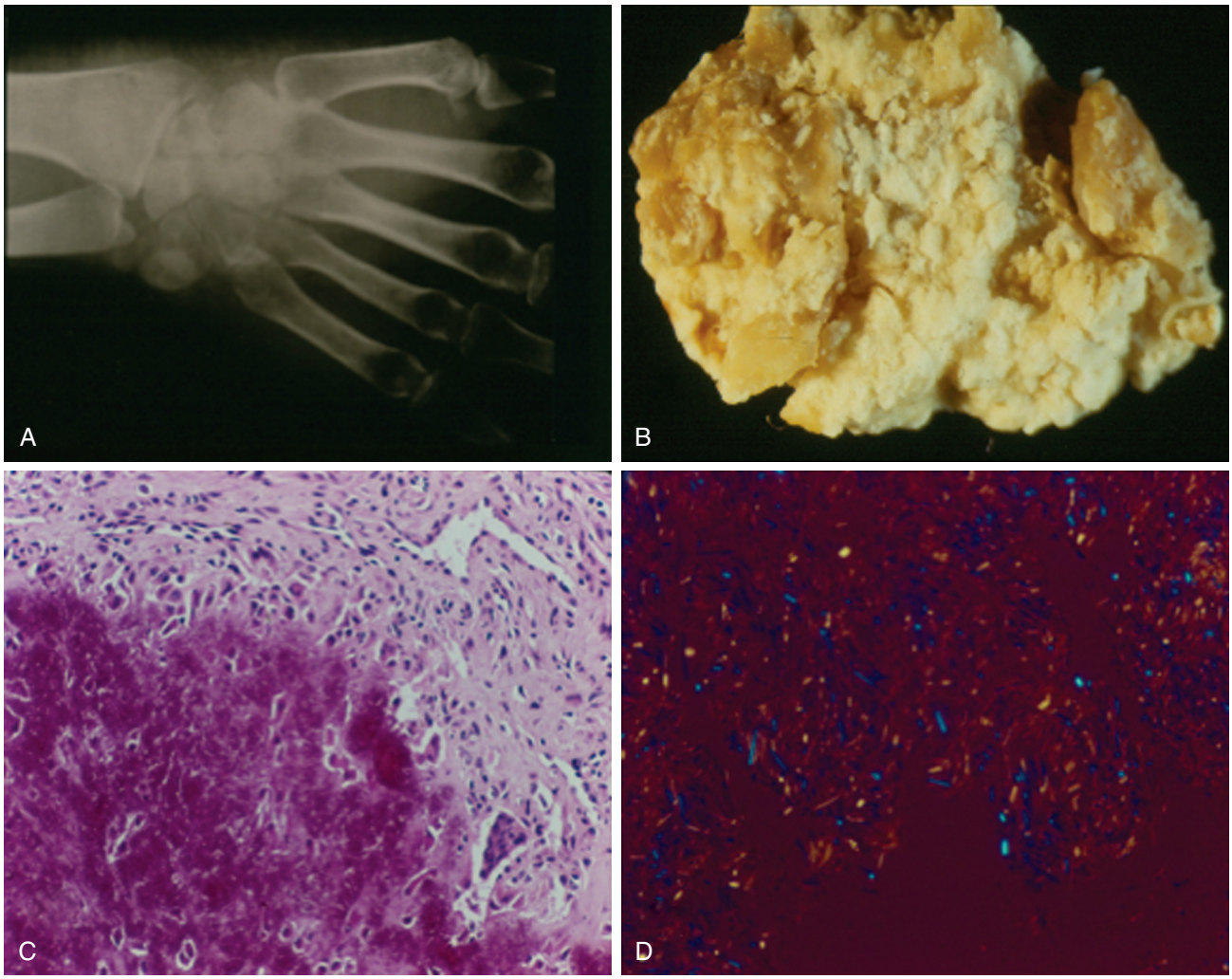
High-resolution ultrasound imaging can detect aggregated BCP-related calcifications outside of joint cartilages including in the rotator cuff of the shoulder. In addition, ultrasound detects CPP crystal deposits with high sensitivity within articular cartilage, and in plantar fascia and the Achilles and other tendons joints.[117-121] Moreover, ultrasound has higher sensitivity than plain radiography for CPPD.[119,122-124] High-resolution ultrasound for imaging CPP crystals also has high specificity, since results correlate well with positive results for synovial fluid analysis.[119] Preliminary criteria proposed for CPPD calcifications by ultrasound are summarized in Table 96-4 and Figure 96-9 (see also supplemental Figure 96-4 on ExpertConsult.com) illustrate characteristic ultrasound features of CPP crystal deposits.

Ultrasound is expedient, inexpensive, does not use irradiation, and can also assess for joint inflammation and

**Table 96-4** Preliminary Criteria for CPPD Crystal Deposition Diagnosis by High-Resolution Ultrasound

| |
|---|
| All CPPD deposits are hyperechoic and appear as one of the following patterns: |
| Thin hyperechoic bands, parallel to the surface of the hyaline cartilage (frequently observed in the knee)<br>A "punctate" pattern, composed of several thin hyperechoic spots, more common in fibrous cartilage and in tendons<br>Homogeneous hyperechoic nodular or oval deposits localized in bursae and articular recesses (frequently mobile) |
| CPPD crystal deposit calcifications always have a sparkling appearance and create posterior shadowing only when they reach dimensions of greater than 10 mm. In contrast, calcifications that present a hypoechoic appearance with posterior shadowing even at an early stage (2-3 mm in diameter) are considered as crystalline deposits of another nature, most commonly because of basic calcium phosphate crystal deposition disease. |

CPPD, Calcium pyrophosphate (CPP) dihydrate crystal deposition disease.
Modified from Frediani B, Filippou G, Falsetti P, et al: Diagnosis of calcium pyrophosphate dihydrate crystal deposition disease: ultrasonographic criteria proposed, *Ann Rheum Dis* 64:638–640, 2005.




**Supplemental Figure 96-4** Tumoral calcium pyrophosphate (CPP) dihydrate crystal deposition in wrist. **A,** Radiograph of wrist showing nodular radiodensity. **B,** CPPD pseudotumour. Nodular mass removed from wrist joint, 4 × 2 × 2 cm. **C,** Photomicroscopy demonstrating crystal deposits bounded by connective tissue cell showing cartilaginous metaplasia (hematoxylin and eosin, original magnification ×100). **D,** Compensated polarized light microscopy showing CPP crystal aggregates. Individual CPP crystals have rod and rhomboidal shapes and are positively birefringent. *(Courtesy Dr. Ken Pritzker, Mount Sinai Hospital Pathology Department, University of Toronto, Ontario, Canada.)*

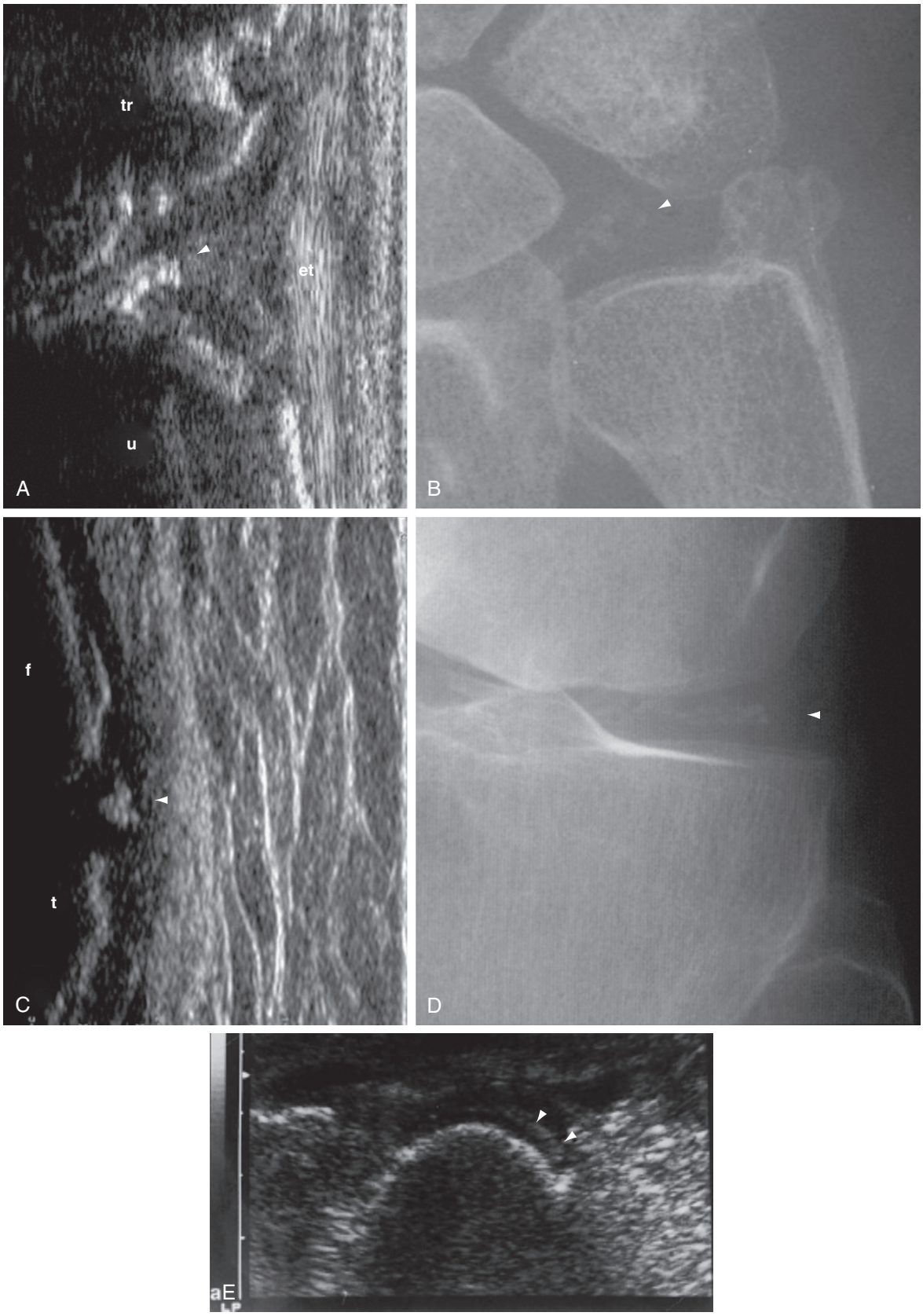



**Figure 96-9** Calcium pyrophosphate dihydrate crystal deposition disease (CPPD) detected by high-resolution ultrasound (US) versus plain radiography. **A** and **B,** Triangular fibrocartilage of the wrist. Longitudinal **(A)** lateral scan and **(B)** corresponding wrist radiograph showing hyperechoic rounded deposits within the substance of the fibrocartilage. C and D, Meniscal calcification of the knee as it appears on US **(C)** and corresponding radiograph **(D).** *Arrowhead,* fibrocartilage calcification. Et, extensor carpi ulnaris tendon; f, femur; t, tibia; tr, triquetrum bone; u, ulna. A was obtained with a Diasus (Dynamic Imaging, Livingstone, United Kindom) by using an 8- to 16-MHz linear probe. C was obtained by using a Logiq 9 (General Electric Medical Systems, Milwaukee, Wisconsin) using a 4D16L probe. **E,** Ultrasonographic manifestations of hyaline cartilage CPP crystal deposition disease as hyperechoic linear band parallel to the femoral condyle in a posterior location *(arrowheads). (**D,** From Grassi W, Meenagh G, Pascual E, Filippucci E: "Crystal clear"—sonographic assessment of gout and calcium pyrophosphate deposition disease,* Semin Arthritis Rheum *36[3]:197–202, 2006; **E,** From Foldes K: Knee chondrocalcinosis: an ultrasonographic study of the hyalin cartilage,* Clin Imaging *26[3]:194–196, 2002.)*

alternative diagnoses and guide therapeutic joint aspiration in CPPD. EULAR has endorsed ultrasound as a useful modality in CPPD diagnosis.[1] However, limitations of ultrasound include difficulty in visualizing crystal deposits in deep recesses of the joint space and the operator-dependence of the approach. Further validation studies for distinct joints and periarticular tissues are necessary before the relative priorities for roles of ultrasound and radiography in the CPPD diagnostic algorithm can be recommended. Ultrasound imaging appears particularly specific for detection of CPP crystal deposition in fibrocartilages (e.g., triangular fibrocartilage of the wrist and the mid zones of articular hyaline cartilages (see Figure 96-9). However, enthesopathies other than CPPD can also give rise to calcification of tendons and plantar fascia, and the diagnostic value for CPPD of ultrasound detection of calcification in plantar fascia and Achilles tendon[125] is not yet clear.

A recent systematic review of 27 research papers, and meta-analysis of 13 of them, on the subject of ultrasound for CPPD concluded that there were significant variations in sensitivity between joints. Sensitivity was between 0.34 (95% CI 0.02-0.65) to 0.87 (95% CI 0.76-0.99), with specificity between 0.84 (95% CI 0.52-1.00) to 1.00 (95% CI 0.99-1.00).[126]

Ultrasound, without use of synovial fluid analysis in the differential diagnosis of acute arthritis, runs the risk of missing other conditions such as infectious arthritis, which can be superimposed upon CPPD or mimic acute CPP arthritis clinically.[121] Gout can generally be differentiated, particularly because redundant hyperechoic contouring ("double contour sign") of the hyaline cartilage surface is seen more in gout, whereas CPP crystal deposition disease is typically visualized within hyaline articular cartilage.[118,120-122]

Dual-energy CT has not been studied in detail for CPPD detection, but it can specifically distinguish deposits of urate crystals from those of calcium-containing crystals.[127] MRI is not yet a reliable approach for detecting CPPD because of a lack of mobile protons in CPP crystals, and nonenhanced MRI is less sensitive in detecting knee meniscal fibrocartilage calcification than hyaline cartilage calcification.[128]

### Laboratory Diagnostic Tests

Conventional radiography or ultrasound is usually the first method to evaluate patients with suspected CPPD, but thorough laboratory evaluation of the newly diagnosed patient with CPPD routinely includes serum levels of calcium, phosphorus, magnesium, alkaline phosphatase, ferritin, iron, and total iron-binding capacity (see Figure 96-8).

Specialized techniques beyond alizarin red S staining such as radiography diffraction, Raman spectroscopy, Fourier transform infrared spectroscopy, atomic force microscopy, or transmission electron microscopy showing electron-dense clumps of needle-like crystals may be necessary to confirm BCP crystal deposition (see Figure 96-7).[108]

Raman spectroscopic identification of CPP (and urate) crystals appears to be a viable approach for rapid point of care diagnosis of acute CPP arthritis and differentiation from acute gout. In a recent study, Raman spectroscopy and compensated polarized light microscopy analyses were in agreement in 89.7% of samples, and in diagnosing acute CPP arthritis, the Kappa coefficient for agreement between the 2 methods was 0.61 (95% CI 0.42-0.81), though below the Kappa coefficient of 0.84 (95% CI 0.75-0.94) for diagnosing acute gout.[128a]

Primary symptomatic OA is commonly linked with secondary CPPD, and once positive for CPP crystals, OA synovial fluids typically remain positive for CPPD.[124] Cytocentrifugation increases the sensitivity of detection of rare CPP crystals,[129] illustrated by a study in which approximately 75% to 78% of synovial fluid samples (of patients with gout and OA) had both urate and CPP crystals.[130] Under conditions in which synovial fluid specimens are not fresh (or stored at 4° C for analysis after significant delay), Gram stain and Diff-Quik (Microptic, Barcelona, Spain) staining methods for crystal analysis in synovial fluids can provide information beyond that from compensated polarized light microscopy. Demonstration of CPP crystals in articular tissues (see Figure 96-3) can be difficult in specimens stained with hematoxylin and eosin because the acidity of hematoxylin solutions promotes decalcification. However, the decalcifying effect of hematoxylin can be diminished by limiting the staining period with Mayer's hematoxylin to 3 minutes. Studies on Raman spectroscopy for standardized laboratory diagnosis of CPP, BCP, and urate crystals in joint fluid particularly illustrate the potential for development of "point and shoot" diagnostics of crystal arthropathy,[131] which could improve on current inconsistences of crystal arthropathy diagnosis in many medical laboratories.

## TREATMENT

**KEY POINTS**

**CPPD treatment involves alleviation and prophylaxis of acute arthritic attacks, but therapy to lessen chronic and anatomically progressive sequelae of crystal deposition is not well developed for CPPD.**

**The approach to pseudogout treatment is similar to that for acute gout.**

### CPPD

A treatment algorithm is presented at the bottom of Figure 96-8 that is consonant with EULAR guidelines.[132] As in gout (see Chapter 95), therapeutic approaches to patients with CPPD involve treatment and prophylaxis of acute arthritic attacks.[132,133] Effective therapies to lessen CPP crystal deposition, and chronic and anatomically progressive sequelae of CPP crystal deposition, have not been developed for idiopathic or familial CPPD (Table 96-5). Moreover, it is not clear that appropriate treatment of hemochromatosis, hyperparathyroidism, and hypomagnesemia prevent or limit CPP crystal deposition and associated inflammation and cartilage degeneration, likely because the ability to detect chondrocalcinosis by radiography is usually indicative of advanced crystal deposition disease.

Episodes of pseudogout generally respond to NSAIDs (including cyclooxygenase-2 inhibitors) and/or intra-articular steroids,[132] although sometimes more slowly than in gout. Systemic glucocorticosteroids or adrenocorticotropic hormone [132,134] usually given as described for acute

**Table 96-5** Therapeutics for Calcium Pyrophosphate Dihydrate Crystal Deposition Disease (CPPD)

| |
|---|
| **Proven Benefits** |
| NSAIDs or COX-2 inhibitors |
| Intra-articular corticosteroids |
| Systemic corticosteroids |
| ACTH |
| Prophylactic low-dose colchicine |
| **Possible Benefits Already Observed Clinically** |
| Methotrexate for refractory chronic inflammation and recurrent pseudogout |
| Oral magnesium (for patients with hypomagnesemia) |
| **Theoretic Benefits** |
| Hydroxychloroquine for refractory chronic inflammation |
| Phosphocitrate |
| Caspase-1 or IL-1 antagonism for CPPD crystal-induced inflammation |
| TLR2 antagonism for CPPD-associated degenerative arthropathy |
| Oral calcium supplementation to suppress PTH levels |
| ANKH anion channel blockade (probenecid) |
| ENPP1 inhibition |
| TG2 inhibition |
| Polyphosphates |
| Promotion of crystal dissolution by alkaline phosphatase or polyamines |

ACTH, Adrenocorticotropic hormone; COX-2, cyclooxygenase-2; IL, interleukin; ENPP1, ecto-nucleotide pyrophosphate phosphodiesterase 1; NSAIDs, nonsteroidal anti-inflammatory drugs; PTH, parathyroid hormone; TG2, transglutaminase 2; TLR2, Toll-like receptor 2.

**Table 96-6** Therapeutics for Articular and Periarticular Basic Calcium Phosphate (BCP) Crystal Deposition

| |
|---|
| **Proven Benefits** |
| NSAIDs or selective COX-2 inhibitors |
| Local corticosteroid injection |
| Local irrigation |
| High-frequency therapeutic ultrasound to degrade BCP crystal deposits |
| **Theoretic Benefits** |
| Phosphocitrate |
| Modulators of ANKH (e.g., probenecid) or ENPP1 |

COX-2, Cyclooxygenase-2; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; NSAIDs, nonsteroidal anti-inflammatory drugs.

gout (see Chapter 95), appear effective in most cases of acute pseudogout. However, rebound arthritis after tapering systemic glucocorticosteroids in CPPD could be mediated by glucocorticosteroid induction of NLRP3 in macrophage.[135]

The response to colchicine is less consistent than that usually seen in acute gout. However, pseudogout episodes can be diminished in frequency by low-dose daily colchicine prophylaxis, as for gouty arthritis.[132,133] The self-limited nature of most acute pseudogout attacks in the knee can sometimes be accelerated by simple arthrocentesis and thorough drainage of the joint effusion, but there are currently no data for measures such as tidal irrigation in CPPD, unlike the case for MSS.[136,137]

Hydroxychloroquine[133] has been suggested to be of some benefit to patients with refractory chronic polyarticular CPPD and to reduce flares of pseudogout, and it has theoretic benefits by potentially stabilizing phagolysosomes to suppress NLRP3 inflammasome activation in response to crystal uptake.[138] Methotrexate was found promising to patients with refractory chronic polyarticular CPPD and to reduce flares of pseudogout, although in an exploratory study limited to five consecutive patients, and with patients prior to methotrexate as their own controls.[139] Though methotrexate does appear effective in some with chronic inflammatory CPPD arthritis, it clearly it has not been adequately studied to date.[139a] IL-1 antagonism (e.g., with off-label use of anakinra) has had anecdotal success,[140,141] whereas breakthrough pseudogout has been reported during TNF antagonist therapy.[142] Collectively, at this time there is insufficient evidence for hydroxychloroquine, methotrexate, and IL-1 antagonism as standard therapies for refractory inflammation in CPPD.

Only limited evidence suggests that OA patients with cartilage calcification respond to arthroscopic irrigation and daily low-dose colchicine; further substantiation is necessary. There is currently no evidence to support arthroscopic débridement as a treatment modality for CPPD deposition disease. There is insufficient evidence for beneficial effects of intra-articular hyaluronan therapy in CPPD of the knee, and risks of precipitating pseudogout appear significant with this treatment modality.

## Basic Calcium Phosphate Crystal Arthropathies

NSAIDs and local glucocorticoid injection (Table 96-6) are effective treatment options for BCP crystal-associated calcific tendinitis and subacromial bursitis (see Chapter 46). BCP crystal–associated inflammation of the rotator cuff and subacromial bursa of the shoulder can be successfully treated by using needle aspiration, irrigation, and steroid injections. Ultrasound-guided techniques, which promote resorption of rotator cuff and bursal calcifications, can enhance the success of such approaches.[133] Tidal irrigation may be of benefit for symptoms and function in MSS.[133]

## Future Directions in Treatment

Simple measures such as prevention and detection of hypomagnesemia and occult hyperparathyroidism merit long-term investigation at the population level. The potential to develop therapies for both CPPD and BCP crystal–associated arthropathies based on new molecular targets has been elevated by identification of ANKH and ENPP1 as specific molecular mediators of cartilage calcification. Intriguingly, the anion transport inhibitor probenecid suppresses *ANKH*-induced and TGF-β–induced increases in extra-cellular $PP_i$ in vitro.[38] Prevention of CPP crystal deposition by polyphosphates or promotion of CPPD dissolution by depot alkaline phosphatase and by pyrophosphatase activation-promoting polyamines could provide alternative therapeutic approaches.[143] However, incomplete CPP crystal dissolution by intra-articular lavage of patients with chondrocalcinosis of the knees with disodium EDTA and

magnesium ions in the past was a therapeutic failure, in that insignificant amounts of CPP crystals were removed, and all postlavage attacks of pseudogout mediated by crystal shedding developed in all patients.

The $PP_i$ analogue phosphocitrate, a natural compound in mammalian mitochondria and in the urinary tract, is a potent inhibitor of HA crystal formation.[144] Phosphocitrate inhibits nitric oxide–induced calcification of cartilage and also inhibits both HA and CPPD crystal–associated cell stimulation including induction of MMP-3 in fibroblasts. Systemic phosphocitrate treatment suppresses ankylosing ossification in murine progressive ankylosis of *ank/ank* mice. Moreover, an analogue of phosphocitrate (CaNaPC) decreased both the abundant meniscal cartilage HA deposition and the continuous progression of OA in the Hartley guinea pig model of spontaneous knee OA.[145] The CaNaPC treatment did not exert therapeutic effects in a rabbit knee hemimeniscectomy model of OA in which there was an absence of intra-articular calcification.[145] Such results suggest that phosphocitrate acts on calcification-mediated mechanisms of dysregulation of joint biomechanics and cartilage degeneration without exerting nonspecific chondroprotective effects. Further clinical development of phosphocitrate would be of interest but has been slowed in part by low bioavailability unless given parenterally.[144]

The use of bisphosphonates as $PP_i$ analogues can be beneficial in some cases of soft tissue calcification with HA. Lastly, the identified roles of TLR2 in chondrocyte responsiveness to CPP crystals,[77] of TLR4 in hydroxyapatite crystal–induced inflammatory responses,[146] and of NLRP3 inflammasome-mediated caspase-1 activation and IL-1β processing in CPP and BCP crystal–induced inflammation[74,75] suggest certain mediators of innate immunity including TLR2, TLR4, caspase-1, and IL-1β to be potential therapeutic targets for human forms of CPPD and hydroxyapatite crystal-driven inflammation and connective tissue destruction.

## OUTCOME

### KEY POINTS

- It is not clear whether the presence of CPP or BCP crystals in primary knee OA play a net role in promoting disease progression and more frequent knee replacement surgery, despite the fact that CPP and BCP crystals are frequently found in OA knee tissues at the time of total joint arthroplasty.
- There is no clear correlation between the extent of calcification and progression of primary CPPD arthropathy.
- The outcome of total knee arthroplasty does not appear to be significantly altered by pre-existing CPPD.

Mean radiographic scores have been found to directly correlate with the presence of calcium-containing crystals in OA in patients at the time of total joint arthroplasty.[92] However, degenerative cartilage disease associated with sporadic CPPD may be less destructive than that observed in primary OA. For example, limited prospective analysis of knee CPPD has suggested that radiographic worsening of degenerative arthritis was slow to progress. Typically, changes in radiographic extent of chondrocalcinosis are observed over time, but there is no clear correlation between the extent of calcification and progression of CPPD arthropathy.

In the Boston OA Knee Study (BOKS), and in the Health, Aging, and Body Composition (Health ABC) Study,[147] the relationship between chondrocalcinosis and the progression of knee OA was prospectively evaluated longitudinally by using MRI. In BOKS, knees with chondrocalcinosis had a decreased risk of cartilage loss compared with knees without chondrocalcinosis, and there was no difference in risk in Health ABC. Stratification by the presence of intact or damaged knee menisci produced comparable results within each cohort. In a Thai study, CPPD was identified radiographically and/or by synovial fluid analysis in 52.9% of 102 patients undergoing total knee arthroplasty.[148] Patients with and without CPPD did not differ in difficulties in performing daily activities or treatment, and those with CPPD did not undergo knee arthroplasty at an earlier age than those without CPPD. The outcome of total knee arthroplasty also does not appear to be significantly altered by pre-existing CPPD,[149] unless there is robust synovitis.[150]

In the setting of OA, processes leading to cartilage matrix calcification by CPP and BCP crystals appear to be biomarkers of both aging and secondary consequences of advanced cartilage pathology. The net role of CPP and BCP crystals in synovitis and chondrocyte pro-catabolic processes remains unclear in the progression of primary OA. Moreover, the dysregulated cartilage matrix repair that generates secondary CPPD in primary OA may be a biomarker of processes that slow cartilage tissue failure in at least some patients.

## CONCLUSION

CPPD is particularly common in aging, and is also linked with OA and cartilage matrix degeneration and abnormalities in chondrocyte differentiation and $PP_i$ metabolism, some of which are hereditary via ANKH dysregulation. Articular cartilage BCP crystal deposition is strongly linked with OA. Inflammatory responses to both CPP and BCP crystals are mediated by NLRP3 inflammasome activation and IL-1β release. CPPD can mimic multiple conditions, including gout, infectious arthritis, primary osteoarthritis, RA, and polymyalgia rheumatica. Cervical spine CPPD is common, and can be destructive. Major diagnostic approaches to CPPD include plain radiography and high-resolution ultrasound, in addition to the gold standard of synovial fluid crystal analysis with compensated polarized light microscopy. Treatment of acute CPPD arthritis is similar to that used in gout. Therapy to prevent CPP crystal deposition is not yet developed. Despite the fact that CPP and BCP crystals are frequently found in cartilage and joint fluid in advanced OA, the roles of CPP and BCP crystals in symptoms and disease progression in primary OA remain unclear.

Full references for this chapter can be found on ExpertConsult.com.

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Wellcome Trust Centre for Human Genetics: www.well.ox.ac.uk.
Teaching resource page for radiographic images of BCP and CPPD arthropathies: www.orthopaedicweblinks.com/Teaching_Resources/Radiology/more3.html.

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CHAPTER 97



# Familial Autoinflammatory Syndromes

Anna Simon • Jos W.M. Van Der Meer • Joost P.H. Drenth

**KEY POINTS**

- **Autoinflammatory disorders are characterized by recurrent or chronic inflammation without signs of infection or autoimmunity.**
- **Dysregulation of the interleukin (IL)-1β pathway is central to many autoinflammatory syndromes, especially the cryopyrin-associated periodic syndromes, deficiency of the IL-1 receptor antagonist, and familial Mediterranean fever.**
- **A severe complication of longstanding disease is type amyloid A amyloidosis, which often leads to renal failure; the risk of this complication is greatly reduced when patients receive adequate treatment.**
- **In a substantial portion of patients seen with a clear autoinflammatory phenotype, the specific diagnosis can remain elusive.**
- **Because of the central role of IL-1β in many autoinflammatory diseases, a trial of IL-1β inhibiting therapy can be warranted for diagnostic and therapeutic purposes.**

The familial autoinflammatory syndromes, often referred to as *hereditary periodic fever syndromes*, comprise rare hereditary disorders with a common phenotype of lifelong, recurrent inflammatory episodes, characterized by inflammatory symptoms such as fever, abdominal pain, diarrhea, rash, or arthralgia.[1] Between the fever episodes, patients with most of these syndromes generally feel healthy and function normally. Routine laboratory investigations during a fever attack invariably reveal a severe acute phase response with a high erythrocyte sedimentation rate, leukocytosis, and high concentrations of acute phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA). The inflammatory episodes occur without an obvious trigger, although some patients note a relationship to physical stimuli (e.g., exposure to cold), emotional stress, or the menstrual cycle. The episodes resolve spontaneously in days or weeks. Patients go undiagnosed for years, generating a high level of discouragement and frustration for patients and physicians when no diagnosis is made.[2,3] The term *autoinflammatory*, coined by McDermott and colleagues in 1999,[4] describes the phenotype of recurrent, acute inflammatory responses. It is preferable to the term *autoimmune* in these cases because typical autoimmune phenomena are not found; innate immune defects are generally responsible rather than the adaptive immune system.[5]

Several genetically distinct types of hereditary autoinflammatory syndromes are recognized. Despite the common phenotype described previously, these can often be differentiated clinically by specific characteristics—in particular, mode of inheritance, age of onset, average duration of the fever episodes and the fever-free interval, geographic region of origin of the patient, and occurrence of long-term complications such as amyloidosis or deafness (Table 97-1 and Figure 97-1). A significant number of patients with a periodic fever phenotype still do not fit into this genetically based classification, probably representing additional (genetic) defects that can lead to autoinflammatory disease. Each year, new genetic disorders are characterized. This chapter describes seven well-characterized familial autoinflammatory syndromes in detail.

## DIFFERENTIAL DIAGNOSIS

When a patient has had recurrent fever episodes for more than 2 years, an infection or a malignant disorder is unlikely. The differential diagnosis at that time might include numerous inflammatory disorders, such as juvenile rheumatoid arthritis, adult-onset Still's disease, inflammatory bowel disease, Schnitzler syndrome, and Behçet's disease, in addition to the familial autoinflammatory syndromes (Table 97-2). Because the hereditary syndromes are rare—except for familial Mediterranean fever (FMF) in individuals with a distinct ethnic background—the more common diagnoses should usually be excluded first.

The mainstay of the diagnosis of hereditary autoinflammatory syndromes is clinical assessment, with a detailed medical and family history, and preferably at least one observation of the patient during a fever episode because physical examination of the patient in a period of remission is seldom abnormal. The physician can gain another helpful clue, although not pathognomonic, from knowing the patient's ethnic origin. This clinical assessment often yields enough information to build a differential diagnosis of the specific familial autoinflammatory syndromes (see Table 97-1) to determine the direction of genetic testing (Figure 97-2). A growing number of genetic laboratories now also offer autoinflammatory syndrome gene panels, in which multiple genes are sequenced simultaneously by next-generation sequencing methods. This has great advantages; on the other hand, the finding of low-penetrant mutations or polymorphisms in these genes can lead to diagnostic problems or mistaken overdiagnosis.

When the specific diagnosis remains elusive, a trial treatment with an IL-1 inhibitor (e.g., anakinra) can give a diagnostic clue. A significant proportion of patients who suffer from an autoinflammatory disease "not otherwise specified" respond well to treatment with anakinra. The

**TABLE 97-1** Differential Diagnosis of Familial Autoinflammatory Syndromes

| | | MKD | | | CAPS | | | |
|---|---|---|---|---|---|---|---|---|
| | **FMF** | **HIDS** | **Mevalonic Aciduria** | **TRAPS** | **FCAS** | **MWS** | **CINCA** | **DIRA** |
| Mode of inheritance | Autosomal recessive | Autosomal recessive | Autosomal recessive | Autosomal dominant | Autosomal dominant | Autosomal dominant | Autosomal dominant | Autosomal recessive |
| Age at onset (yr) | <20 | <1 | <1 | <20 | <1 | <20 | <1 | Birth, <4 wk |
| Duration of attack (days)* | <2 | 4-6 | 4-5 | >14 | <2 | 1-2 | ? | Continuous |
| Cutaneous involvement | Erysipelas-like erythema | Maculopapular rash | Morbilliform rash | Migratory rash, overlying area of myalgia | Cold-induced urticaria-like lesions | Urticaria-like rash | Urticaria-like lesions | Generalized pustulosis |
| Musculoskeletal involvement | Monoarthritis common | Arthralgia, occasional oligoarthritis | Arthralgia common | Severe myalgia common, occasional frank monoarthritis | Arthralgia common, occasional mild myalgia | Lancing limb pain, arthralgia common; arthritis can occur | Epiphyseal bone formation | Sterile pustulous osteomyelitis |
| Abdominal involvement | Sterile peritonitis common | Splenomegaly, severe pain common | Splenomegaly,pain may occur | Severe pain common | None | May occur | Hepatospleno-megaly | |
| Eye involvement | Uncommon | Uncommon | Uncommon | Conjunctivitis and periorbital edema common | Conjunctivitis | Conjunctivitis, sometimes optic nerve elevation | Papilledema with possible loss of vision, uveitis | |
| Distinguishing clinical symptoms | Erysipelas-like erythema | Prominent cervical lymphadenopathy | Dysmorphic features, neurologic symptoms | Migratory nature of myalgia and rash, periorbital edema | Cold-induced urticaria-like lesions | Sensorineural hearing loss | Chronic aseptic meningitis, sensorineural hearing loss, arthropathy | |
| Gene involved | *MEFV* | *MVK* | *MVK* | *TNFRSF1A* | *CIAS1 = NLRP3* | *CIAS1 = NLRP3* | *CIAS1 = NRLP3* | *IL1RN* |
| Protein involved | Pyrin (marenostrin) | Mevalonate kinase | Mevalonate kinase | Type 1 TNF receptor | Cryopyrin | Cryopyrin | Cryopyrin | *IL1RA* |

For details on Blau syndrome, DIRA, and pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome, see text.

*Duration may vary; this is a typical duration.

CAPS, Cryopyrin-associated periodic syndrome; CINCA; chronic infantile neurologic cutaneous and articular syndrome; DIRA, deficiency of IL-1 receptor antagonist; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; MKD, mevalonate kinase deficiency; MWS, Muckle-Wells syndrome; TRAPS, TNF receptor–associated periodic syndrome.

Modified from Hull KM, Shoham N, Chae JJ, et al: The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. *Curr Opin Rheumatol* 15:61–69, 2003.

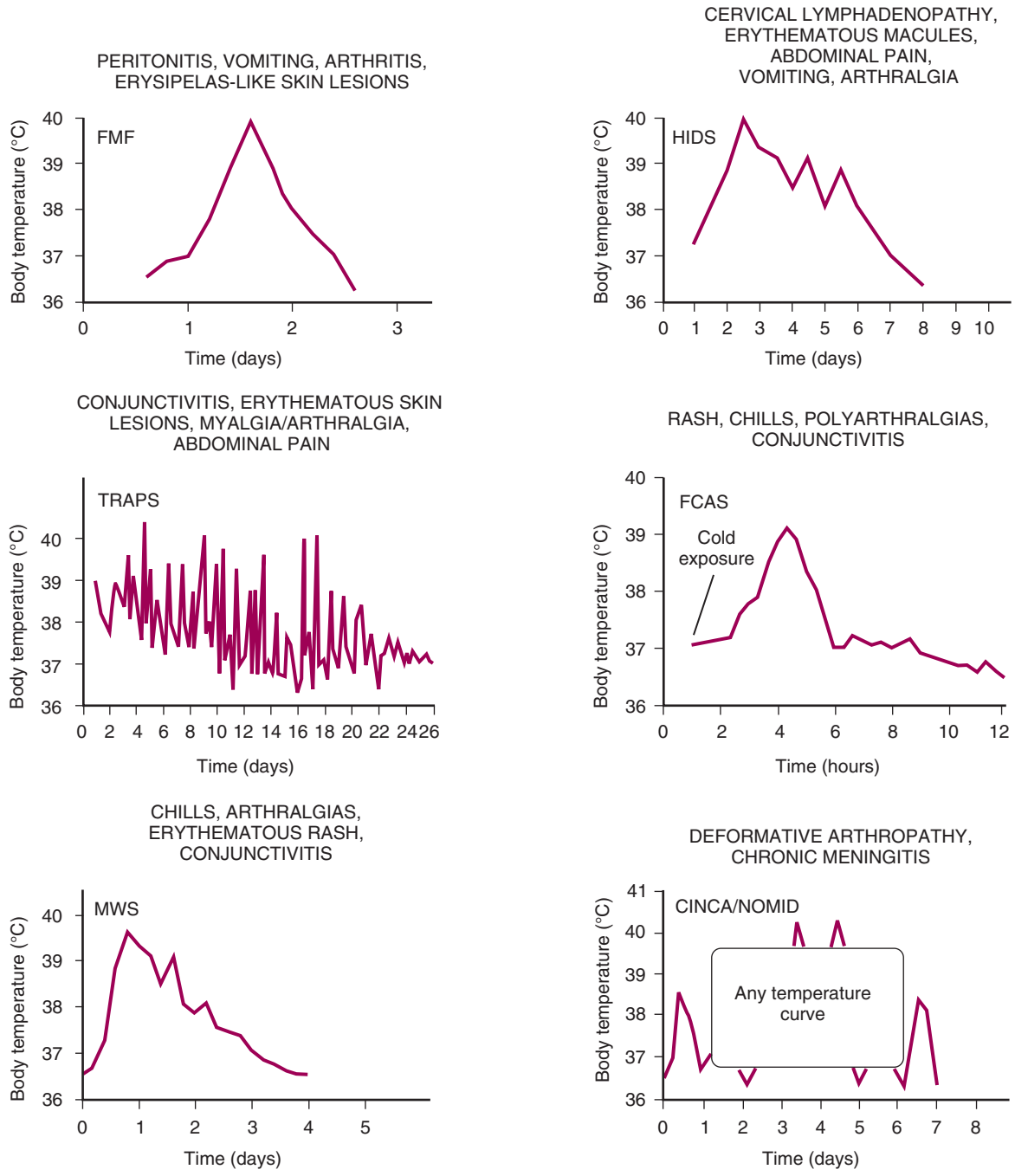



**Figure 97-1** Characteristic patterns of body temperature during inflammatory attacks in the familial autoinflammatory syndromes. Interindividual variability for each syndrome is considerable, and even for the individual patient, the fever pattern may vary greatly from episode to episode. Note the different time scales on the x-axes. CINCA/NOMID, Chronic infantile neurologic cutaneous and articular syndrome/neonatal-onset multisystemic inflammatory disease; FCAS, familial cold autoinflammatory syndrome; HIDS, hyper-IgD syndrome; MWS, Muckle-Wells syndrome; TRAPS, tumor necrosis factor receptor–associated periodic syndrome.

authors even use the (temporary) label of "anakinra-responsive disease" for this category in daily practice.

## FAMILIAL MEDITERRANEAN FEVER

### Epidemiology

FMF is the most prevalent disorder among the hereditary autoinflammatory syndromes, with more than 10,000 patients affected worldwide. It occurs primarily in people originating from the Mediterranean basin, including Armenians, Sephardic Jews, Arabs, and Turks. FMF is an autosomal recessively inherited disorder. Most families reported with an apparent autosomal dominant inheritance pattern of FMF[6] represent examples of pseudodominant inheritance because of consanguinity combined with the high carrier frequency of FMF mutations in certain populations[6-8]; however, at least three families studied do seem to show a true dominant inheritance, even after extensive genetic analysis[9,8]

**TABLE 97-2** Differential Diagnosis of Periodic Fever

1. Hereditary (see Table 97-1)
2. Nonhereditary
   a. Infectious
      i. Hidden infectious focus (e.g., aortoenteric fistula, Caroli's disease)
      ii. Recurrent reinfection (e.g., chronic meningococcemia, host defense defect)
      iii. Specific infection (e.g., Whipple's disease, malaria)
   b. Noninfectious inflammatory disorder, e.g.:
      i. Adult-onset Still's disease
      ii. Juvenile chronic rheumatoid arthritis
      iii. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis
      iv. Schnitzler syndrome
      v. Behçet's disease
      vi. Crohn's disease
      vii. Sarcoidosis
      viii. Extrinsic alveolitis
      ix. Humidifier lung, polymer fume fever
   c. Neoplastic
      i. Lymphoma (e.g., Hodgkin's disease, angioimmunoblastic lymphoma)
      ii. Solid tumor (e.g., pheochromocytoma, myxoma, colon carcinoma)
   d. Vascular (e.g., recurrent pulmonary embolism)
   e. Hypothalamic
   f. Psychogenic periodic fever
   g. Factitious or fraudulent

## Etiology

In 1997, two groups independently traced the genetic background of FMF to a hitherto unknown gene on the short arm of chromosome 16, named the Mediterranean fever (*MEFV*) gene.[10,11] At least 80 disease-linked mutations in the *MEFV* gene have been described so far, most of which are clustered in the tenth exon of this gene (for details, see the online mutation database at http://fmf.igh.cnrs.fr/infevers/). Most are missense mutations that produce a single amino acid change in the protein (Figure 97-3). There are five common mutations, accounting for the majority of FMF chromosomes: M694V (occurring in 20% to 65% of cases, depending on the population examined[12]), V726A (in 7% to 35%), M680I, M694I, and E148Q. For the first three mutations mentioned here, a founder effect has been established,[11] pointing to common ancestors at least 2500 years ago. The high frequency of the mutated *MEFV* gene in more than one Middle Eastern population has led to the hypothesis that heterozygous carriers have an as yet unknown advantage, possibly a heightened (inflammatory) resistance to an as yet unidentified endemic pathogen of the Mediterranean basin.[11] In approximately 30% of patients, only one or no mutations in the *MEFV* gene can be detected; the etiology in these patients still needs to be determined.

## Pathogenesis

The *MEFV* gene encodes for a protein of 781 amino acids, known as *pyrin* or *marenostrin*. Pyrin is expressed as a cytoplasmic protein in mature monocytes in association with microtubules[13] but is predominantly found in the nucleus in granulocytes, dendritic cells, and synovial fibroblasts.[14] Pyrin expression is induced by inflammatory mediators such as IFN-α and TNF.[15] The pyrin domain is shared by many proteins involved in apoptosis and inflammation and is a member of the death-domain superfamily that includes death domains, death-effector domains, and caspase-recruitment domains. Pyrin binds to other proteins that contain similar domains, which include the adapter protein "apoptosis-associated speck-like protein with a CARD" (ASC).

The pro-inflammatory cytokine IL-1β is central in the pathogenesis of FMF. This cytokine is expressed as an inactive precursor, which is cleaved by caspase-1 to yield the active IL-1β. Caspase-1 itself first needs to be activated through the interaction with protein complexes called *inflammasomes*. Several inflammasomes have been described. The major inflammasome complex involved in the activation of caspase-1 and IL-1β is the cryopyrin or NLRP3 inflammasome.[16,17] Two hypotheses have been proposed regarding the effect of pyrin on IL-1β processing. The "sequestration hypothesis" holds that pyrin has an inhibitory effect on caspase-1–mediated activation of IL-1β, through its prevention of the formation of the cryopyrin inflammasome by competitive binding of the adapter protein ASC and procaspase-1 and binding caspase-1.[18,19] Under this hypothesis, FMF mutations are thought to interfere with the inhibiting interactions of pyrin, resulting in decreased regulation of IL-1β activation.[19] The second hypothesis suggests that pyrin can form its own specific inflammasome for activation of IL-1β,[20] although not all the components of this proposed complex have been specified so far. The FMF mutations would increase the sensitivity of this putative pyrin inflammasome. Apart from its role in regulation of IL-1β, there is also conflicting evidence for the effect of pyrin on regulation of nuclear factor-κB (NF-κB) or apoptosis, varying from inhibition to stimulation.[1]

## Clinical Features

In approximately 90% of patients with FMF, symptoms start before age 20 years.[21] The inflammatory attacks of FMF usually last 1 to 3 days. The frequency can vary widely; 2 to 4 weeks is the most common interval (see Figure 97-1). Symptoms of serositis (i.e., peritonitis, pleuritis, synovitis) are the main feature of FMF attacks, usually accompanied by fever. Abdominal pain of 1 or 2 days' duration occurs in 95% of patients, varying in severity from severe peritonitis resembling an acute condition in the abdomen to only mild abdominal pain without overt peritonitis.[22] Arthritis (rarely destructive) is often confined to one large joint such as the knee, ankle, or wrist, and may be the only symptom. Chest pain resulting from pleuritis is usually unilateral and associated with a friction rub or transient pleural effusion. Skin involvement occurs in approximately 30% of patients, most often as erysipelas-like skin lesions on the shins or feet (Figure 97-4).[23] Other, more uncommon, symptoms are pericarditis, occurring in less than 1% of patients[24]; acute scrotal swelling and tenderness[25]; aseptic meningitis; and severe protracted myalgia, especially of the legs.

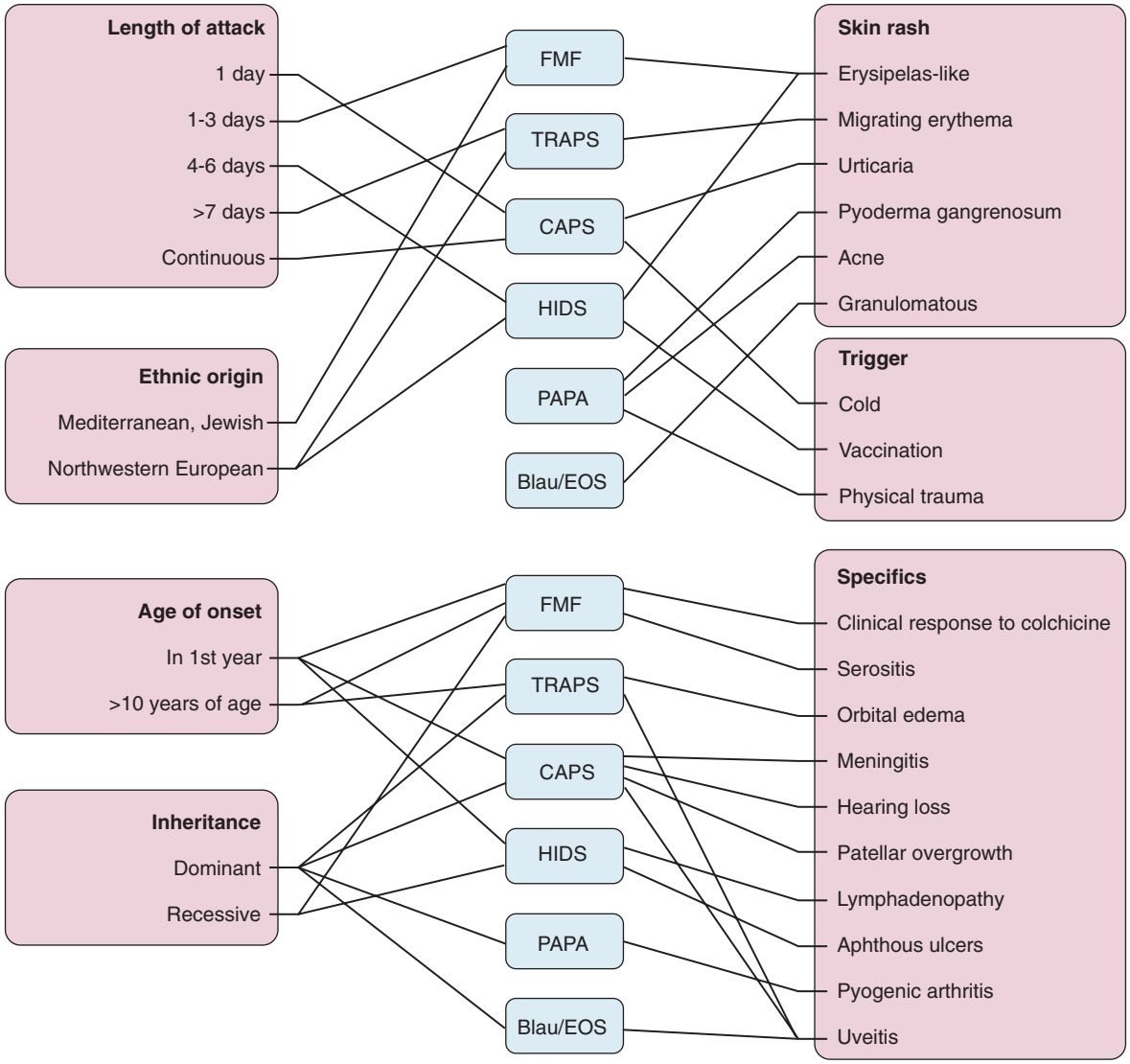



**Figure 97-2** Differential diagnosis of the familial autoinflammatory syndromes. First exclude other, more common causes of fever and inflammation in the patient. When a familial autoinflammatory syndrome seems likely, check the clinical characteristics found in the patient on the right and left of the diagram, and assign one point to each syndrome that is linked to these characteristics by a line (one characteristic could lead to or point to more than one syndrome). The final combined score assigns a rank for the likelihood of the disorders in this patient and offers help in deciding on the correct subsequent diagnostic tests. This algorithm is not evidence based but is solely derived from expert opinion. CAPS, Cryopyrin-associated periodic syndrome; EOS, early-onset sarcoidosis; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; PAPA, pyogenic sterile arthritis, pyoderma gangrenosum, and acne; TRAPS, tumor necrosis factor receptor–associated periodic syndrome.

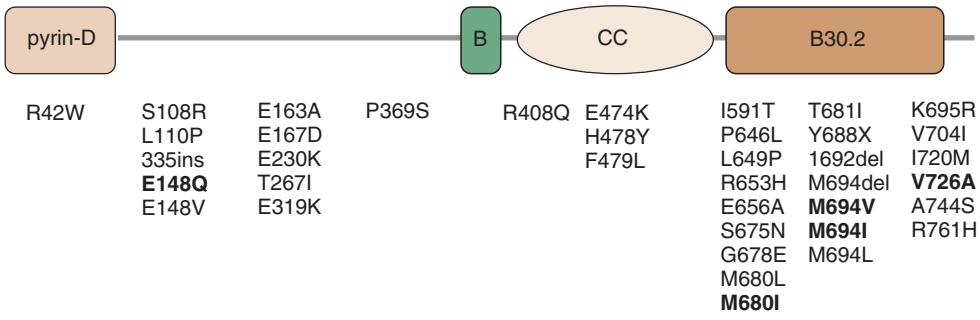



**Figure 97-3** Schematic representation of pyrin (marenostrin) protein, with four conserved domains, including a pyrin domain, a B-box (B), coiled-coil domain (CC), and a B30.2 domain. Indicated are mutations as found in familial Mediterranean fever, with the five most common missense mutations in *boldface* type. For complete listing of all currently known mutations, see INFEVERS website: http://fmf.igh.cnrs.fr/infevers/.



**Figure 97-4** Erysipelas-like eruption in a patient with a familial Mediterranean fever attack. *(Courtesy Professor A. Livneh, Heller Institute of Medical Research, Tel Hashomer, Israel.)*

**TABLE 97-3** Diagnostic Criteria for Familial Mediterranean Fever*

| |
|---|
| **Major Criteria** |
| Typical attacks† with peritonitis (generalized) |
| Typical attacks with pleuritis (unilateral) or pericarditis |
| Typical attacks with monoarthritis (hip, knee, ankle) |
| Typical attacks with fever alone |
| Incomplete abdominal attack |
| **Minor Criteria** |
| Incomplete attacks‡ involving chest pain |
| Incomplete attacks involving monoarthritis |
| Exertional leg pain |
| Favorable response to colchicine |

*Requirements for diagnosis of familial Mediterranean fever are ≥1 major criteria or ≥2 minor criteria.

†Typical attacks are defined as recurrent (≥3 of the same type), febrile (≥38° C), and short (lasting between 12 hours and 3 days).

‡Incomplete attacks are defined as painful and recurrent attacks not fulfilling the criteria for a typical attack.

From Livneh A, Langevitz P, Zemer D, et al: Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 40:1879–1885, 1997.

## Diagnosis and Diagnostic Tests

FMF is still primarily a clinical diagnosis. There is a set of validated diagnostic criteria with a reported sensitivity and specificity of 96% to 99% (Table 97-3).[26] These criteria were validated in a population with a high prevalence of FMF and a low prevalence of the other autoinflammatory disorders; however, the ethnic origin of the patient needs to be taken into account. In molecular diagnostic testing, genetic laboratories usually screen for the five most common mutations, and rare mutations are missed. *MEFV* mutations occur on both alleles in only 70% of typical cases,[27] whereas in the remaining 30%, only one or no mutation can be detected, even after sequencing. There is also evidence of reduced penetrance. Despite these limitations, molecular testing can be used as a confirmatory test. Whether or not the results are positive, treatment with colchicine is warranted in symptomatic cases of fitting ethnic origin fulfilling the diagnostic criteria.[28,29] No specific biologic marker is available to distinguish an inflammatory FMF attack from an infectious fever or appendicitis. During an inflammatory attack, there is an acute phase response, which includes elevation of SAA, CRP, and plasma fibrinogen and polymorphonuclear leukocytosis. Proteinuria in patients with FMF is highly suggestive of renal amyloidosis.

## Treatment

Colchicine is the first-line treatment for patients with FMF. It prevents inflammatory attacks completely in 60% to 75% of patients, and it significantly reduces the number of attacks in an additional 20% to 30%.[30] The average dose in adults is 1 mg daily but may be increased to 3 mg if no response is seen at the lower dose. This regimen is usually well tolerated; gastrointestinal side effects, including diarrhea and abdominal pain, generally resolve with dose reduction. More serious side effects, such as myopathy, neuropathy, and leukopenia are rare and occur primarily in patients with renal or liver impairment. During a fever attack, oral or intramuscular NSAIDs can be used for pain relief. Glucocorticoids have limited efficacy.

Compliance with colchicine use is important because colchicine prevents amyloidosis. Since the introduction of colchicine therapy, the incidence of amyloidosis in FMF has decreased dramatically, whereas in areas with a high prevalence of FMF where colchicine is not routinely available, such as Armenia, amyloidosis is still common. Colchicine's principal effect at the cellular level is to depolymerize microtubules by interacting with tubulin, inhibiting motility and exostosis of intra-cellular granules. It has a powerful anti-mitotic effect, causing metaphase arrest. It has been speculated, in cases of infertility in patients treated with colchicine, that this medication causes azoospermia. Colchicine does not have a significant adverse effect on sperm production or function, however.[31] Unfounded fear of teratogenic effects of colchicine often wrongly leads to cessation of this drug in young women who want to get pregnant, with a subsequent increased frequency and severity of attacks, which enhances problems with fertility and pregnancy. Colchicine has proven to be safe, even in early pregnancy, and treatment should not be interrupted for this reason.[32,33] It can also be used while breastfeeding.[30]

In approximately 5% to 10% of patients, FMF is refractory to colchicine use. Parenteral colchicine can be used in refractory cases,[34] but can be toxic. Currently, IL-1β inhibitors are more commonly used in colchicine-resistant FMF, as reported in several case reports and retrospective series.[35-40]

## Outcome

Recurrent attacks of peritonitis may lead to intra-abdominal or pelvic adhesions, resulting in complications such as small bowel obstruction. Another serious long-term complication of FMF is amyloid A (AA) amyloidosis. This amyloidosis is primarily found in the kidneys, resulting in renal failure, but can also occur in the gastrointestinal tract, liver, and spleen, and eventually in the heart, testes, and thyroid in late stages. The prevalence of amyloidosis varies, especially depending on the ethnic origin, but it is high in untreated patients. It is common among Sephardic Jews but rare in Ashkenazi Jews.[41]

For a variety of reasons, including peritoneal adhesions and ovulatory dysfunction, subfertility in women is

common.[32] In men, subfertility secondary to azoospermia (sometimes secondary to testicular amyloidosis) or impairment of sperm penetration has been found.[21]

## MEVALONATE KINASE DEFICIENCY (HYPER-IGD SYNDROME)

### Epidemiology

Hyper-IgD syndrome (HIDS), also known as *mevalonate kinase deficiency (MKD)*, is an autosomal recessively inherited disorder, but it is far less prevalent than FMF. Approximately 75% of patients are from Western Europe, and 50% are from the Netherlands and France.[42] Most patients with MKD are of Caucasian origin. These observations can be explained partly by a founder effect.[43] In the Netherlands, the carrier frequency of the most common mevalonate kinase mutation is 1:153.[44] Men and women are equally affected.[42]

### Etiology

MKD is caused by mutations in the gene encoding for the enzyme mevalonate kinase, located on the long arm of chromosome 12.[45-47] Patients are most often compound heterozygotes for two missense mutations (Figure 97-5). Two mutations (V377I and I268T) account for more than 85% of the patients described to date.[42]

The term *variant-type HIDS*, which was proposed for patients with an autoinflammatory disease and high IgD without MVD,[47] has been largely abandoned. Many patients with fever syndromes have a raised IgD, and this subclass is heterogeneous. It seems more useful to designate these patients with "autoinflammatory disease not otherwise specified" to indicate that a more specific diagnosis may still be found in the future.

### Pathogenesis

Mevalonate kinase is part of the isoprenoid pathway; it is the step after 3-hydroxy-3-methyl-glutaryl–coenzyme A (HMG-CoA) reductase, phosphorylating mevalonic acid. The isoprenoid pathway has many diverse end products that include cholesterol, dolichol, and ubiquinone, and it leads to isoprenylation of proteins, with a post-translational modification directing these proteins such as Rho and Ras to the cell membrane.[48]

MKD mutations lead to a constantly diminished activity of mevalonate kinase to approximately 5% to 15% of normal levels, and these levels decrease further during a fever attack.[49] Because of this reduced enzyme activity, the substrate mevalonic acid accumulates in serum and urine. Higher levels are found during the episodes of fever. There is not a dramatic shortage of any specific end product; concentrations of cholesterol, ubiquinone, and dolichol in patients are normal to slightly decreased.[1]

Another syndrome was already linked to mutations in the mevalonate kinase gene before the discovery of HIDS[50]—classic mevalonic aciduria. Patients with mevalonic aciduria carry specific mutations that cause a more severe reduction of mevalonate kinase enzyme activity, reducing it to undetectable levels. These patients constantly produce large amounts of mevalonic acid and often have more than 1000 times as much mevalonic acid in their urine than do HIDS patients.[51] Patients with mevalonic aciduria also have a more severe phenotype. Classic mevalonic aciduria and HIDS are probably two extremes of a continuous spectrum of disease related to MVD.[52]

The pathogenic link between MVD and inflammation is still unclear, but there is increasing evidence for a connection between the isoprenoid pathway and inflammation. Inhibition of the isoprenoid pathway by statins, the inhibitors of HMG-CoA reductase (the enzymatic step before that of mevalonate kinase), can have anti-inflammatory effects, ranging from increased apoptosis of inflammatory cells to reduction of expression of cytokines.[53,54] In other settings, statins seem to be pro-inflammatory, most notably in a study in which stimulation with *Mycobacterium tuberculosis* or mitogens in combination with statins increased caspase-1 activation and IL-1β secretion by monocytes, through a decrease of geranylgeraniol.[15] The ex vivo IL-1β production is increased in MKD,[55,56] whereas treatment with the IL-1 blocker anakinra is beneficial.[57,58] Current evidence points to a link between the mevalonate pathway and IL-1 through alterations of isoprenylation of the small guanosine triphosphate (GTP)ase Rac1, phosphoinositide-3-kinase (PI3K), and protein kinase B (PKB).[59-62] A defect in apoptosis may also contribute to the pathogenesis of MKD.[61] Lymphocytes from patients with MKD (who had no fever at the time of blood sampling) showed a decrease in apoptosis when stimulated with anisomycin, which was not found in patients with TNF receptor–associated periodic syndrome (TRAPS) or patients with FMF.[61] Such a decrease in apoptosis would result in increased survival of

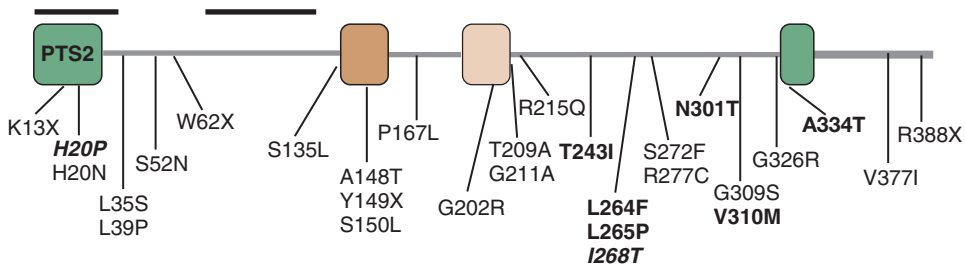



**Figure 97-5** Mevalonate kinase, with four conserved domains represented by colored boxes. Indicated are missense mutations, nonsense mutations, and two deletions *(black bars)*, which have been identified in mevalonate kinase deficiency. In *boldface* type are mutations found in mevalonic aciduria patients; in *bold and italic* are mutations found in classic hyperimmunoglobulin D syndrome and mevalonic aciduria. For complete listing of all currently known mutations, see INFEVERS website: http://fmf.igh.cnrs.fr/infevers/.

lymphocytes and may delay the resolution of the inflammatory response. An ordinarily innocuous stimulus in patients with MKD would more easily lead to a full-blown fever episode.

The cause of the characteristic high serum concentrations of IgD in this syndrome, which led to its name, is still unexplained.[62,63]

## Clinical Features

Ninety percent of patients with MKD (HIDS) experience their first fever episode in the first year of life,[42] and these episodes become most frequent in childhood and adolescence. The high fevers may lead to seizures, especially in young children. Vaccination, minor trauma, surgery, and physical or emotional stress are factors that provoke a fever episode, although often a triggering factor is not obvious.[42] The fevers often begin with cold chills and a sharp increase in body temperature. They are almost always accompanied by cervical lymphadenopathy and abdominal pain with vomiting and diarrhea. Other frequent symptoms are headache, myalgia, and arthralgia. Apart from the lymphadenopathy, physical signs frequently consist of splenomegaly and a skin rash with erythematous macules and papules (Figure 97-6) or petechiae (Figure 97-7).[42] Sometimes there are also signs of arthritis (principally large joints) and hepatomegaly. Approximately 40% of patients report painful aphthous ulcers in the mouth, vagina, or scrotum (Figure 97-8). The fever disappears spontaneously after 3 to 5 days, although it may take longer before the symptoms in joints or skin disappear completely. These inflammatory attacks occur, on average, once every 4 to 6 weeks, although this may vary from patient to patient or in an individual patient.



**Figure 97-6** Facial erythematous macules and papules in a patient with hyper-IgD syndrome during an attack.

Patients with mevalonic aciduria, the metabolic disorder that is also caused by mevalonate kinase gene mutations, experience similar but more severe inflammatory episodes versus HIDS patients. In addition, these patients suffer from psychomotor retardation, ataxia, failure to thrive, cataracts, and dysmorphic facies. Patients with classic mevalonic aciduria usually die in early childhood.[51] An intermediary clinical phenotype between classic mevalonic aciduria and HIDS has been described.[52]

## Diagnosis and Diagnostic Tests

MKD is diagnosed on the basis of a combination of characteristic clinical findings and continuously elevated IgD



**Figure 97-7** Petechiae on the leg of a patient with hyper-IgD syndrome during a febrile attack.



**Figure 97-8** Aphthous ulceration detected on the tongue of a patient with hyper-IgD syndrome. *(Courtesy Dr. K. Antila, North Carelian Central Hospital, Joensuu, Finland.)*

**TABLE 97-4** Diagnostic Indicators of Hyper-IgD Syndrome

| |
|---|
| **At Time of Attacks** |
| Elevated erythrocyte sedimentation rate and leukocytosis |
| Abrupt onset of fever (≥38.5° C) |
| Recurrent attacks |
| Lymphadenopathy (especially cervical) |
| Abdominal distress (e.g., vomiting, diarrhea, pain) |
| Skin manifestations (e.g., erythematous macules and papules) |
| Arthralgias and arthritis |
| Splenomegaly |
| **Constantly Present** |
| Elevated IgD (above upper limit of normal) measured on two occasions at least 1 mo apart* |
| Elevated IgA (≥2.6 g/L) |
| **Specific Features** |
| Mutations in mevalonate kinase gene |
| Decreased mevalonate kinase enzyme activity |

*Extremely high serum concentrations of IgD are characteristic but not obligatory.

concentrations (>100 IU/mL) (Table 97-4). There are numerous caveats concerning IgD serum concentration, however. Values may be normal in young patients (especially patients younger than 3 years).[64] Persistently normal levels have been reported in a few patients with MKD,[43] and patients with other familial autoinflammatory syndromes may also have elevated IgD concentrations, although these are usually only slightly elevated. More than 80% of MKD patients combine a high concentration of IgD with high IgA levels.[64,65] During fever attacks, a brisk acute phase response is observed, including leukocytosis, high levels of SAA and CRP, and activation of the cytokine network.[55,66]

The diagnosis can be confirmed by sequencing the mevalonate kinase gene. The best approach is to start with screening for the two most prevalent mutations, V377I and I268T. If this screening is negative, but the clinical suspicion remains high, sequencing of the entire gene can be considered. A good alternative is the measurement of urinary mevalonic acid concentrations during an attack, which are slightly elevated. Gas chromatography–mass spectroscopy is necessary to detect this slight increase, however.[67] The measurement of mevalonate kinase enzyme activity is complicated and time consuming and should be reserved for research purposes.

## Treatment

There is no established treatment regimen for MKD. IL-1β inhibition (e.g., anakinra) reduces disease severity and is currently the most promising therapy.[68,69,70] A double-blind, placebo-controlled, crossover trial of the HMG-CoA reductase inhibitor simvastatin was beneficial, with a reduction in number of days of illness in five out of six patients[71]; however, in clinical practice, the beneficial effect is not impressive. Favorable preliminary experience with the TNF antagonist etanercept has been reported,[57,72,73] as well as with the IL-6 antagonist tocilizumab.[74,75]

Some individual patients have been reported to have benefited from treatment with corticosteroids, colchicine, intravenous Ig, or cyclosporine, but these results have not been repeated in most patients.[42] Thalidomide had no effect on disease activity in a placebo-controlled trial.[76] In severe cases of classic mevalonic aciduria, stem cell transplantation has been performed with success.[77,78]

## Outcome

The long-term outcome in HIDS is relatively benign in most patients. In some patients, the fever episodes occur less frequently and become less severe later in life, starting from late adolescence.[42] Joint destruction is rare, but abdominal adhesions are seen, resulting from repeated abdominal inflammation or unnecessary diagnostic laparotomy because of suspected acute abdomen. Amyloidosis is a rare complication in HIDS, but it has been reported.[42,79-81] Regular screening for proteinuria therefore also may be advisable in HIDS patients, especially patients with frequent and severe fever episodes.

# TUMOR NECROSIS FACTOR RECEPTOR–ASSOCIATED PERIODIC SYNDROME (TRAPS)

## Epidemiology

TRAPS has an autosomal dominant inheritance pattern. It was originally described in a large family from Irish and Scottish descent as "familial Hibernian fever."[82] It is found primarily in patients from northwestern Europe but also has been described in families from Australia, Mexico, Puerto Rico, Portugal, and the Czech Republic.[83] Any ethnic group may be affected. Other abandoned nomenclature for this syndrome includes "autosomal dominant familial periodic fever"[84] and "familial perireticular amyloidosis."[85]

## Etiology

Mutations are found in the gene for the type I TNF receptor (*TNFRSF1A*), which is located on the short arm of chromosome 12.[4] These are mainly single-nucleotide missense substitutions, located in exons 2, 3, and 4, which encode for the extra-cellular domain of *TNFRSF1A*. Many of these mutations disrupt one of the highly conserved cysteine residues involved in extra-cellular disulfide bonds of the 55 kDa type I TNF receptor protein (Figure 97-9).[86]

There are some general genotype-phenotype correlations, especially when mutations are grouped in cysteine and noncysteine mutations. Noncysteine mutations have, overall, a lower penetrance than cysteine mutations, and amyloidosis is seen far more often in association with cysteine mutations.[87] Two missense mutations in *TNFRSF1A*, P46L and R92Q, have a particularly low penetrance and are found in approximately 1% to 10% of control chromosomes. These are more likely to modify inflammation than to truly cause a separate disease entity.[87-89]

## Pathogenesis

TNF is a pleiotropic molecule that induces cytokine secretion, activation of leukocytes, fever, and cachexia. Activation of the TNF receptor by TNF causes cleavage and shedding of its extra-cellular part into the circulation, where it can then act as a TNF inhibitor. However,

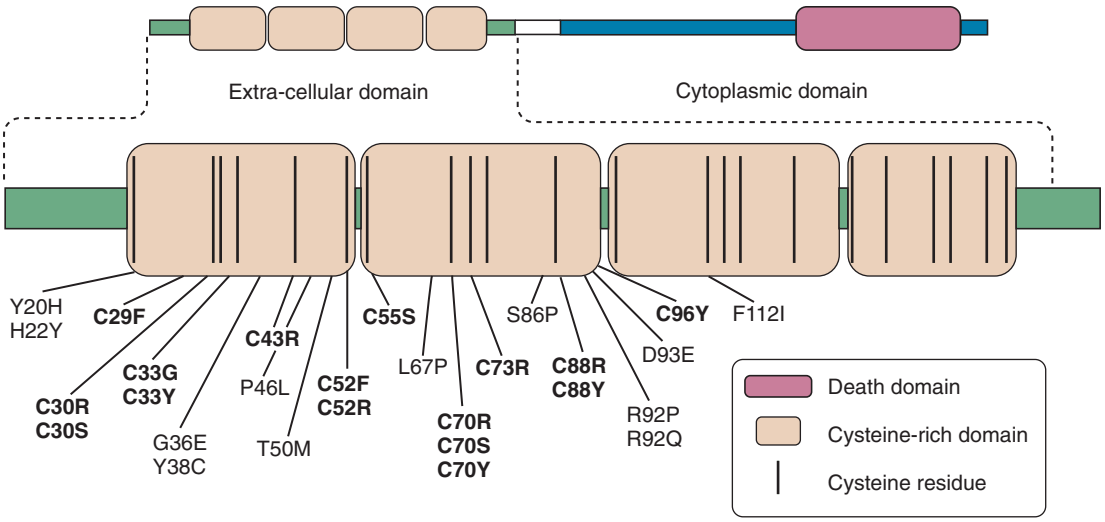



**Figure 97-9** Schematic representation of the TNF receptor type 1 protein (TNFRSF1A), depicting mutations found in TNF receptor–associated periodic syndrome up to this time (except for one intron mutation affecting a splice site). Mutations disrupting cysteine residues are in *boldface* type. For complete listing of all currently known mutations, see INFEVERS website: http://fmf.igh.cnrs.fr/infevers/.

TRAPS-associated mutations in the TNF receptor lead not to an increase but rather to a loss of TNF-signaling function, including less binding of TNF,[90,91] less cell surface expression,[91-94] and decreased TNF-induced NF-κB activation.[92,95,96] The mutated TNFRSF1A is retained intracellularly, pooled in the endoplasmic reticulum.[91,92,96,97] Mutant TNFR1 cannot associate with the wild-type version but can form aggregates by self-interaction.[91,92] This cytoplasmic receptor aggregation results in ligand-independent signaling.[96,98] Mitochondrial-derived reactive oxygen species appear to mediate this effect.[99] This new hypothesis also might offer an explanation for the observation that blocking IL-1β works better in some TRAPS patients than blocking TNF.[96,100]

An alternative hypothesis, the "shedding hypothesis," was postulated on the finding of reduced shedding, which leads to prolonged TNF signaling and uncontrolled inflammation. Not all TRAPS mutations cause decreased shedding, however, and although serum concentrations of the shedded soluble TNFRSF1A in TRAPS patients during periods without symptoms are often found to be significantly reduced compared with normal patients, this is not always the case.[1] The hypothesis of reduced shedding, although attractive by its simplicity, is not supported as the sole cause of the fever attacks in TRAPS, and additional mechanisms seem to be at work.

## Clinical Features

The clinical features can vary much more among individual TRAPS patients than is generally seen in FMF or HIDS.[83,86] The age of onset can vary, even within the same family, with a documented range of 2 weeks to 53 years old.[83,101] There is also a large variation in duration and frequency of the fever episodes in TRAPS. On average, attacks last 3 to 4 weeks and recur two to six times each year. Episodes also may be limited to a few days (see Figure 97-1). Although the index patient, through whom the diagnosis is made, often displays well-defined inflammatory attacks, affected family members may have less typical symptoms, such as episodic mild arthritis.



**Figure 97-10** Migrating erythematous rash during a TNF receptor–associated periodic syndrome attack. *(Courtesy Dr. T. Fiselier, University Medical Center St. Radboud, Nijmegen, the Netherlands.)*

During inflammatory attacks, a high, spiking fever can be accompanied by skin lesions, myalgia and arthralgia, abdominal distress, and ocular symptoms. The most common cutaneous manifestation is a centrifugal, migratory, erythematous patch, which may overlie a local area of myalgia (Figure 97-10),[102] but urticarial plaques also may be seen. Myalgia is often located primarily in the muscles of the thighs, but it may migrate during the fever episode, affecting all of the limbs and the torso, face, and neck. Arthralgia primarily affects large joints, including hips, knees, and ankles. Clinically obvious synovitis is rarer, and when it does occur it is nonerosive, asymmetric, and monoarticular.[83] Abdominal pain occurs in 92% of TRAPS patients during inflammatory attacks; other gastrointestinal symptoms often seen include vomiting and constipation. Ocular involvement is characteristic in TRAPS, and it may involve conjunctivitis, periorbital edema, or periorbital pain in one

or both eyes. Severe uveitis and iritis have been described, and any TRAPS patient with ocular pain should be examined for these complications.[83,102] Other less frequently observed symptoms during fever attacks in TRAPS are chest pain, breathlessness, pericarditis, and testicular and scrotal pain, which may be caused by inflammation of the tunica vaginalis. One case report described a patient who presented with psychosis but no fever.[103] It was initially thought that this disorder is associated with an increased incidence of indirect inguinal hernias,[104] but this was not confirmed in other case series. Lymphadenopathy is rare in TRAPS.

## Diagnosis and Diagnostic Tests

As in the other familial autoinflammatory syndromes, laboratory investigations during inflammatory attacks show a clear acute phase response, and even in between fever attacks, such an inflammatory response may be measured. The IgD level may be elevated, but the value is almost always less than 100 IU/mL.[101,104]

Hull and colleagues[83] proposed a set of clinical diagnostic criteria for TRAPS (Table 97-5). These criteria are not validated by epidemiologic measures, but they may be used as a first step in evaluation of patients. TRAPS is ultimately a genetic diagnosis, defined by a missense mutation in the gene for *TNFRSF1A*. Clinical penetrance of TRAPS mutations is not 100%, however, even for cysteine mutations, and asymptomatic carriers are common. Also, the finding of an R92Q or P46L variant in this gene would pose a difficulty. Because they have many characteristics of a polymorphism rather than a direct disease-causing mutation (see Etiology section in preceding text), it is debatable whether such a finding should lead to a diagnosis of TRAPS.

## Treatment

In mild cases of TRAPS, NSAIDs are often sufficient. NSAIDs and glucocorticoids in high doses (>20 mg/day of oral prednisone) alleviate the symptoms of fever and inflammation in most TRAPS patients, although they do not alter the frequency of attacks. They can be used beneficially at times of attack, and glucocorticoids usually can be tapered in the course of 1 or 2 weeks, as tolerated. Anti–IL-1 therapy is considered to be the most effective, from case reports and case series.[100,105-107] Use of the TNF inhibitor etanercept, a fusion product of TNFRSF1B (the receptor that is not defective) has been partially successful in reducing disease severity.[83,87,107-110,113] Infliximab, a monoclonal antibody against TNF, is less effective than etanercept in TRAPS and seems to cause increased symptoms.[108,111,112] Intravenous infusion of a synthetic TNFRSF1A fusion protein was tried in one patient by Drewe and colleagues,[108] but this seemed to provoke a severe attack.

One patient whose symptoms were resistant to administration of etanercept responded favorably to use of oral sirolimus (4 to 6 mg daily).[111] There is no response to colchicine or immunosuppressive drugs such as azathioprine, cyclosporine, thalidomide, or cyclophosphamide.[83]

**TABLE 97-5** Diagnostic Indicators of Tumor Necrosis Factor Receptor–Associated Periodic Syndrome

| |
|---|
| 1. Recurrent episodes of inflammatory symptoms spanning >6 mo duration (several symptoms generally occur simultaneously)<br>a. Fever<br>b. Abdominal pain<br>c. Myalgia (migratory)<br>d. Rash (erythematous macular rash occurs with myalgia)<br>e. Conjunctivitis or periorbital edema<br>f. Chest pain<br>g. Arthralgia or monoarticular synovitis |
| 2. Episodes last >5 days on average (although variable) |
| 3. Responsive to glucocorticosteroids but not colchicine |
| 4. Affects family members in autosomal dominant pattern (although may not always be present) |
| 5. Any ethnicity may be affected |

From Hull KM, Drewe E, Aksentijevich I, et al: The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)* 81:349–368, 2002.

## Outcome

Reactive AA amyloidosis is the principal systemic complication of TRAPS. It occurs in approximately 15% to 25% of patients if untreated[87,114] and generally leads to renal impairment. Amyloidosis in a patient with TRAPS places other affected family members at high risk for this complication. It is principally associated with *TNFRSF1A* mutations affecting cysteine residues.[87] Because proteinuria is the initial manifestation of renal amyloidosis, urine from TRAPS patients should be screened regularly by dipstick examination, especially affected family members of a TRAPS patient with amyloidosis.

# CRYOPYRIN-ASSOCIATED PERIODIC SYNDROME

The autosomal dominant cryopyrin-associated periodic syndrome (CAPS) encompasses three clinical syndromes that all have been traced to mutations in one common gene: Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and chronic infantile neurologic cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystemic inflammatory disease (NOMID). After the recognition of the genetic defect, it became clear that there is considerable overlap among these three disorders.[115] FCAS, MWS, and CINCA/NOMID represent a spectrum of disease, with FCAS the mildest and CINCA the most severe form. Given their common genotype, they are discussed under one heading here, but clinical features and outcomes are dealt with separately.

## Epidemiology

Most articles on FCAS, first described in 1940, describe large families from Europe and North America with extensive pedigrees, but sporadic cases have been described. There seems to be a founder effect in American families of Northern European extraction.[116] MWS was first described in 1962 and has since been described in large families, although it does occur in isolated cases and small nuclear families. CINCA is rare, and, to date, some 70 cases and only a few families with the syndrome have been described.

## Etiology

The first indications that MWS and FCAS are allelic stem from early linkage studies showing that FCAS and MWS were linked to the same region on the long arm of chromosome 1 (1q44).[117,118] In 2001, the gene for FCAS and MWS was identified. In a large-scale, positional cloning effort that used three families with FCAS and one family with MWS, missense mutations in a new gene were found.[119] This gene, *CIAS1*, encodes for a protein denoted *cryopyrin* at discovery, but now generally known as NLRP3 (other names that have been used are NALP3 and PYPAF). Later studies showed that NLRP3 mutations were also associated with CINCA.[120,121] Practically all mutations are missense mutations found in exon 3 of the *NLRP3* gene, which encodes for the NOD domain of NLRP3[122] (Figure 97-11). Somatic mosaicism for *NLRP3* mutations occurs in a significant number of patients who had previously been designated as "mutation-negative" CAPS patients.[123,124]

## Pathogenesis

NLRP3 or cryopyrin was a previously unknown protein at the time of the discovery of the mutations involved in these syndromes. Since that time, it has become the focus of numerous studies, which have led to a new concept—the inflammasome.[125] NLRP3 is a member of the nucleotide-binding oligomerization domain–leucine-rich repeat (NOD-LRR) protein family.[126] It consists of a pyrin domain (PYD), a NOD (also known as NACHT domain), and a LRR domain. NLRP3 is mainly expressed in monocytes and neutrophils but is also found in human chondrocytes.[119,120,127]

NLRP3 is thought to be an intra-cellular sensor of pathogens or danger signals, regulating innate immunity. On stimulation with various ligands, which include bacterial RNA, imidazoquinolone compounds, gram-positive bacterial toxins nigericin and maitotoxin, adenosine triphosphate, and uric acid crystals, NLRP3 forms interactions with adapter proteins ASC and cardinal, which results in a multiprotein complex known as the *NLRP3 inflammasome*. The NLRP3 inflammasome activates caspase-1, which subsequently cleaves pro–IL-1β to the active IL-1β.[128,129]

There is conflicting evidence for a role of NLRP3 in the regulation of transcription factor NF-κB.[1] The ultimate effect on NF-κB is likely determined by interactions of multiple proteins. Several publications by independent groups that each developed an NLRP3-deficient mouse showed no effect, however, on NF-κB activation in these mice, whereas they did show a clear deficiency in caspase-1–mediated IL-1β activation.[1]

Monocytes from patients with mutations in the NOD of NLRP3 show increased activation of caspase-1 and subsequently increased release of IL-1β.[129,130] The key role of IL-1β in CAPS is confirmed by the success of treatment with IL-1 inhibitors all three clinical syndromes.

The exact effect of the NLRP3 mutations is still unclear. An attractive hypothesis involves a possible autoinhibitory loop of NLRP3.[130] Mutations in the NOD could interfere with this autoinhibitory mechanism of NLRP3, leading to undue and excessive activation of caspase-1 and IL-1β.[1]

## Clinical Features and Outcome

### Familial Cold Autoinflammatory Syndrome

FCAS is characterized by episodes of rash, fever, and arthralgia after generalized exposure to cold (see Figure 97-1). The disease occurs in large families as an autosomal dominant inherited disorder with an almost complete penetrance.[116] The rash usually starts on the exposed extremities and, in most episodes, extends to the remainder of the body. It consists of erythematous macules and plaques (Figures 97-12 and 97-13), urticarial lesions, and sometimes petechiae[131] and can cause a burning or itchy sensation. In one case report, FCAS was associated with Raynaud's disease.[132] In some cases, localized edematous swelling of extremities is reported. Arthralgia, present in 93% of cases, most often affects the hands, knees, and ankles but can also involve feet, wrists, and elbows.[133] Frank arthritis is not seen. Most patients (84%) also report conjunctivitis during a fever episode. Other symptoms include myalgia, profuse sweating, drowsiness, headache, extreme thirst, and nausea.

A typical feature of FCAS is the requirement of cold exposure to trigger the symptoms. The delay between cold and onset of symptoms varies from 10 minutes to 8 hours.[133] When Hoffman and colleagues[134] provoked an inflammatory attack in FCAS patients by generalized cold exposure in a cold room, patients developed rash, fever, and arthralgia

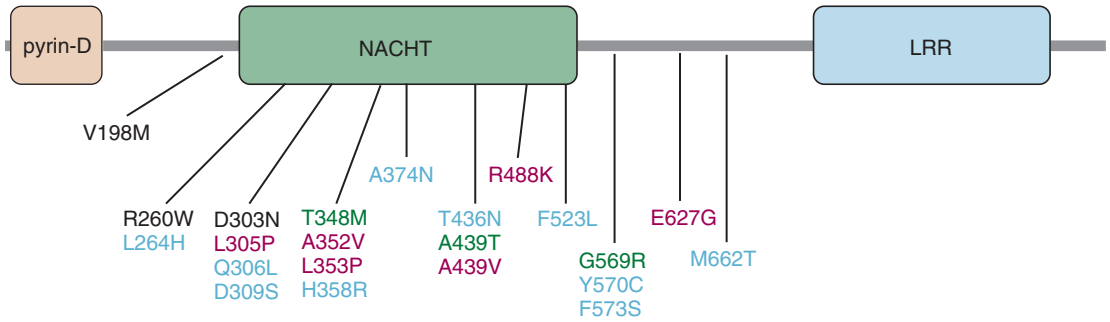



**Figure 97-11** Cryopyrin protein, containing an N-terminal pyrin domain, a nucleotide binding site (NACHT), and a leucine-rich repeat (LRR) domain. Indicated are missense mutations identified in patients with familial cold autoinflammatory syndrome *(red)*, Muckle-Wells syndrome *(green)*, or chronic infantile neurologic cutaneous and articular syndrome *(blue)*, and mutations found in common in two or all of these clinical syndromes *(black)*. For complete listing of all currently known mutations, see INFEVERS website: http://fmf.igh.cnrs.fr/infevers/.




**Figure 97-12** Fine, confluent, erythematous macules on the upper leg of a patient with familial cold autoinflammatory syndrome. *(Courtesy Dr. Johnstone, Medical College of Georgia, Augusta, Ga.)*

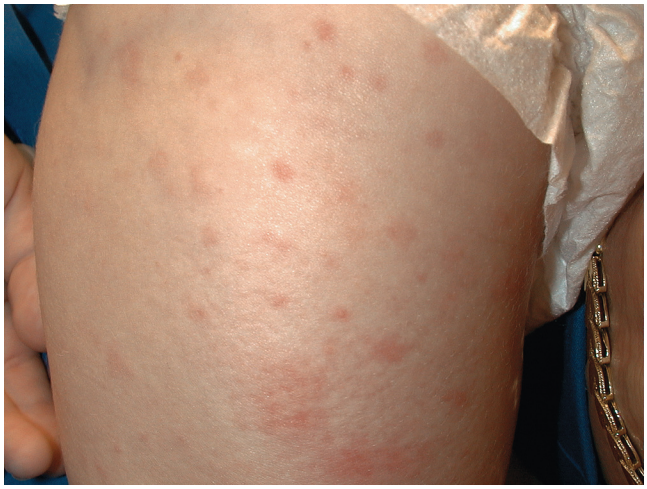


**Figure 97-13** Detail of upper leg with fine, confluent, erythematous macules in familial cold autoinflammatory syndrome. *(Courtesy Dr. Johnstone, Medical College of Georgia, Augusta, Ga.)*

within 1 to 4 hours, accompanied by increased serum and skin cytokines. The subsequent fever attack varies in length, depending on the degree of cold exposure; generally, it lasts a few hours to a maximum of 3 days. These episodes start at an early age, with 95% of patients having had their first fever episode in the first year of life—60% within the first days of life. The symptoms tend to become less severe with advancing age.[131] Type AA amyloidosis complicated by renal insufficiency has been described in at least three families with FCAS.[133]

### Muckle-Wells Syndrome

MWS is a rare autosomal dominant inflammatory disorder with incomplete penetrance. Patients have recurrent episodes of fever, abdominal pain, myalgia, urticarial rash (Figures 97-14 and 97-15), and conjunctivitis, frequently accompanied by arthralgia, arthritis with limb pain, or both.



**Figure 97-14** Urticarial skin rash in a patient with Muckle-Wells syndrome. *(Courtesy Dr. D.L. Kastner, National Institutes of Health, Bethesda, Md.)*

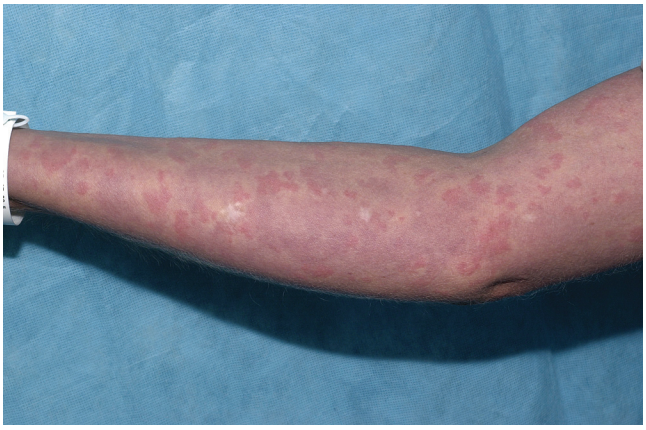


**Figure 97-15** Urticarial skin rash on the arm of a patient with Muckle-Wells syndrome. *(Courtesy Dr. D.L. Kastner, National Institutes of Health, Bethesda, Md.)*

Attacks start in adolescence and can be provoked by hunger, fatigue, and, sometimes, exposure to cold.[135] The inflammatory episodes generally last 24 to 48 hours (see Figure 97-1) and start with ill-defined malaise and transient chills and rigor, followed by aching or lancinating pains in the distal limbs and larger joints. Arthralgia is a common feature of the attacks, but synovitis of the large joints is less common.[136] The rash consists of usually aching and sometimes pruritic erythematous papules 1 to 7 cm in diameter. In a few cases, genital and buccal aphthous ulcers have been seen.[137] Ocular symptoms include uveitis and conjunctivitis. Symptoms typically start in adolescence, although they have been reported at an earlier age. Late-onset development of perceptive deafness is common in MWS. Bone involvement such as clubbing of nails and pes cavus can be seen as well. Most often, patients have a family history for the disease suggestive of autosomal dominant inheritance, but isolated cases have been reported. The most feared complication of the inflammatory attacks is type AA amyloidosis, which affects the kidneys first, leading to proteinuria and subsequent rapid progression to renal failure.

### Chronic Infantile Neurologic Cutaneous and Articular Syndrome

CINCA or NOMID is a rare congenital disorder defined by the presence of the triad of (1) neonatal-onset skin lesions, (2) chronic aseptic meningitis, and (3) recurrent fever along with joint symptoms.[138] The key clinical feature of CINCA is a skin rash accompanied by peculiar joint manifestations and central nervous system involvement. The symptoms in CINCA begin right after birth or in the first months of life with a generalized skin rash. The disease follows an unpredictable course with persistent nonpruritic and migratory rash with fever, hepatosplenomegaly, and lymphadenopathy. Central nervous system involvement is not obvious from the outset, although some patients present with seizures, spasticity, or transient episodes of hemiplegia. In most patients, there are signs of chronic persistent aseptic meningitis.[139] Cerebrospinal fluid analysis may show mild pleocytosis, and there may be increased intracranial pressure. Brain imaging shows mild ventricular dilation, prominent sulci, central atrophy, and, in long-standing cases, calcifications of fauces and dura.

In older children, headache is often a prominent feature as a sign of chronic meningitis. Mental retardation is present in many cases. Progressive sensorineural impairment leading to high-frequency hearing loss can be seen in a few cases. Ocular manifestations are prominent, with optic disc changes such as optic disc edema, pseudopapilledema, and optic atrophy, as well as anterior segment manifestations such as chronic anterior uveitis.[140] These symptoms may lead to visual impairment. Hoarseness, especially in older children, is typical. Joint and bone symptoms are a prominent feature of CINCA. Bone inflammation can give rise to major arthropathies secondary to epiphyseal and metaphyseal disorganization. Growth cartilage alterations such as enlarged epiphyses and patellar overgrowth can be an impressive feature of the disease (Figures 97-16 and 97-17). Erosive changes occur, especially in the phalanges of hands and feet. There are typical dysmorphic features such as frontal bossing and a saddle nose. These common physical features are the reason CINCA patients give the impression that totally unrelated patients are siblings. The prognosis of these patients is grave unless treated aggressively; 20% die in childhood because of infections, vasculitis, and amyloidosis.[138]



**Figure 97-16** Severe deformational arthropathy of the knees in a patient with chronic infantile neurologic cutaneous and articular syndrome. *(Courtesy Dr. A.M. Prieur, Hôpital Necker-Enfants Malades, Paris.)*

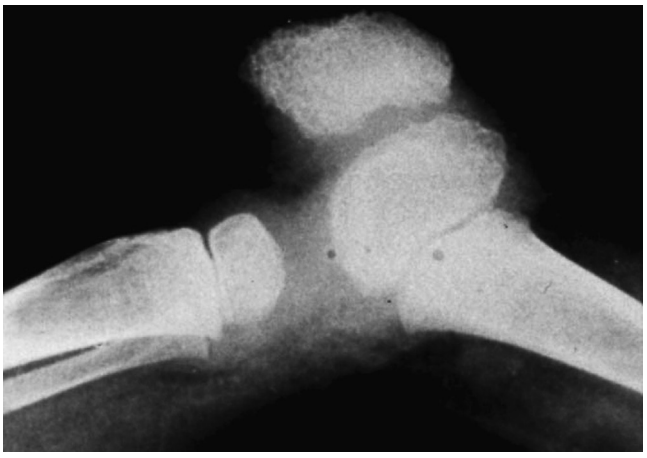


**Figure 97-17** Radiograph of the knee in a patient with chronic infantile neurologic cutaneous and articular syndrome showing greatly enlarged epiphyses and patella with punctate increased density. *(Courtesy Dr. A.M. Prieur, Hôpital Necker-Enfants Malades, Paris.)*

## Diagnosis and Diagnostic Tests

Diagnosis starts with a thorough patient and family history (see Table 97-1). Hoffman and colleagues[133] suggested a set of diagnostic criteria for FCAS after studying six large families with this syndrome (Table 97-6), but this requires validation. Laboratory examination during a fever episode in CAPS shows an acute phase response with polymorphonuclear leukocytosis and increased erythrocyte sedimentation rate, but this does not differentiate among the periodic fever disorders. Symptoms such as an urticarial rash after cold exposure highly favor a diagnosis of FCAS. The ice cube test (i.e., holding an ice cube to a patch of skin to provoke urticaria), which is diagnostic in acquired cold urticaria, is negative in FCAS. Typical facial features, such as frontal bossing and a long pediatric

**TABLE 97-6** Diagnostic Criteria for Familial Cold Autoinflammatory Syndrome

| | |
|---|---|
| 1. | Recurrent intermittent episodes of fever and rash that primarily follow generalized cold exposures |
| 2. | Autosomal dominant pattern of disease inheritance |
| 3. | Age of onset <6 mo |
| 4. | Duration of most attacks <24 hr |
| 5. | Presence of conjunctivitis associated with attacks |
| 6. | Absence of deafness, periorbital edema, lymphadenopathy, and serositis |

From Hoffman HM, Wanderer AA, Broide DH: Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. *J Allergy Clin Immunol* 108:615–620, 2001.

history including chronic aseptic meningitis, point to CINCA/NOMID.

Genetic testing of the *NLRP3* gene can subsequently help to establish the genetic diagnosis. Usually, exon 3 of this gene is screened for mutations. There seems to be genetic heterogeneity because not all patients have *NLRP3* mutations. In some patients, *NLRP3* mutation somatic mosaicism occurs, which can be missed by standard DNA diagnostic tests.[123,124]

### Treatment

Since the advent of IL-1 inhibition, it is the treatment of choice for patients with CAPS.[141] Previously, high-dose oral corticosteroids were often used and could be modestly beneficial in some patients. NSAIDs, disease-modifying anti-rheumatic drugs, and cytotoxic drugs generally do not help.

The efficacy and safety of IL-1 inhibition in CAPS has been demonstrated for several available IL-1 inhibitors in diverse trials and case series. Anakinra, the synthetic IL-1 receptor antagonist, given by daily subcutaneous injection (1 to 2 mg/kg body weight; in children higher dose may be necessary) gives rapid and sustained clinical response, with decrease of symptoms, acute phase response, and leptomeningeal lesions as seen on MRI[142,143] Mirault and colleagues[144] described improvement of sensorineural deafness in a patient with MWS on treatment with anakinra.

Placebo-controlled trials of IL-1β antibody canakinumab in patients with CAPS,[145,146] demonstrated the efficacy of an injection of 150 mg canakinumab once every 2 months, although in some patients this treatment schedule needs to be adapted, either increased or decreased according to disease severity.[147] The long-acting IL-1 TRAP (rilonacept) has also been studied in a placebo-controlled setting and is effective. A subsequent 72-week open label extension study with rilonacept demonstrated sustained clinical response and safety.[148]

## BLAU SYNDROME/EARLY-ONSET SARCOIDOSIS

### Epidemiology

Blau syndrome,[149] also known as *familial granulomatous arthritis*, and early-onset sarcoidosis (BS/EOS) are now recognized as the same disorder.[150,151] "Pediatric granulomatous arthritis" has been suggested as a new name to describe this syndrome,[152] although this might, erroneously, give the impression that the disease occurs only in children. Little is known about its epidemiology, although it is thought to occur worldwide.[150]

### Etiology

The inheritance pattern of BS/EOS is autosomal dominant. In many cases, a de novo mutation is found, which explains the relatively high incidence of sporadic cases. These sporadic cases were often classified as EOS precisely because of the absence of affected relatives, but Blau syndrome and EOS are caused by mutations in the nucleotide-binding oligomerization domain 2/caspase recruitment domain 15 gene (*NOD2/CARD15*).[150,153,154] Mutations are mostly located in exon 4 of *NOD2/CARD15*. The predominant mutations are two missense mutations at position 334 (R334Q and R334W).[152]

### Pathogenesis

The NOD2/CARD15 protein is an intra-cellular sensor for pathogenic components, analogous to the Toll-like receptors. NOD2/CARD15 activation causes a wide array of downstream effects that are still not well understood, including activation of NF-κB and mitogen-activated protein kinase pathways, turning on an innate immune response of diverse cytokines (e.g., IL-1β) and defensins.[155]

Seven of the nine different mutations of *NOD2/CARD15* linked to BS/EOS are located in the NOD domain of the protein, similar to the mutations in cryopyrin (NLRP3) in the cryopyrin-associated syndromes. The two most common mutations affect a codon at a homologous position in the NOD domain to the location of the cryopyrin R260W mutation.[156] This suggests a similar pathophysiologic effect on the function of the protein.

Polymorphisms in another part of this same *NOD2/CARD15* gene on chromosome 16 are associated with increased susceptibility to Crohn's disease[157]; the risk of Crohn's disease is increased 40-fold in individuals homozygous for these polymorphisms. Whether these polymorphisms result in a gain or loss of function of this protein is debated. There are some shared features between the two diseases. Both are characterized by granulomatous inflammation, and although bowel inflammation is not seen in Blau syndrome, Crohn's disease can manifest with uveitis, arthritis, and skin rash.

### Clinical Features and Outcome

The clinical phenotype of BS/EOS consists of recurrent granulomatous inflammations. The three typical sites affected are joints, eyes, and skin.[158] The granulomatous arthritis is most often polyarticular, with a synovitis or tenosynovitis.[150] The most frequently involved joints at presentation are wrists, ankles, knees, and proximal interphalangeal joints.[158] The uveitis associated with this disorder tends to follow a chronic, persistent course. It can be an acute anterior uveitis, but it often extends to a panuveitis.[152] Cataracts, secondary glaucoma, and significant visual impairment can result. Involvement of the skin results in a papular, erythematous skin rash with associated dermal granulomas, usually generalized and intermittent, on the trunk and extremities.[159] Other symptoms outside of the classic triad are regularly seen[158] and can include campylodactyly (contracture of multiple interphalangeal joints), generalized lymphadenopathy, cranial neuropathies, recurrent fever, interstitial lung disease, and arteritis.[153,158] In some severely affected patients, granulomatous inflammation can disseminate at an advanced stage into a systemic disease, with granulomas in liver, lung, and kidney.[152] Onset is generally before age 5 years In familial cases, genetic anticipation is often observed (i.e., the course of disease tends to be more severe in later generations). The major long-term complications are joint deformity and visual impairment.[150]

### Diagnosis

The most important aspect of diagnosis is histologic evidence of granulomas at the site of inflammation. This evidence can be obtained by biopsy of any involved site, of which skin is least invasive. One study showed that skin biopsy was diagnostic in all cases with the typical skin rash, whereas synovial biopsy was not positive in all patients, perhaps because of sampling error.[152] Genetic testing is available for *NOD2/CARD15* mutations, but in some series, not all patients with a typical clinical phenotype carried a mutation in this gene.[153]

### Treatment

There are no controlled studies of management of BS/EOS patients, and little is known about the optimal treatment.[158] There tends to be a poor response to NSAIDs. A good response to the TNF inhibitor infliximab was described in case reports,[160,161] one of which also noted that etanercept did not have the same effect. IL-1 blockade by anakinra was also reported as beneficial in case descriptions,[158,162] although in two other cases anakinra was not effective.[163] Thalidomide was effective in two children in Japan.[164] The panuveitis is usually managed by topical, subconjunctival, or systemic corticosteroids.[150]

## PYOGENIC STERILE ARTHRITIS, PYODERMA GANGRENOSUM, AND ACNE SYNDROME

### Epidemiology

Pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome is an autosomal dominant disorder first described by Lindor and colleagues.[165] Fewer than 20 families have been reported.

### Etiology/Pathogenesis

Wise and colleagues[166] identified mutations in the CD2-binding protein 1 *(CD2BP1)* gene as the cause of PAPA syndrome. *CD2BP1*, also known as *proline-serine-threonine phosphatase interacting protein 1* (PSTPIP1), is highly expressed in neutrophils.[122]

PSTPIP1 can form interactions with pyrin, the protein mutated in FMF.[167] The mutations in PAPA syndrome result in hyperphosphorylation of PSTPIP1,[167-169] which increases the strength of the interaction between PSTPIP1 and pyrin. The increased interaction between PSTPIP1 and pyrin increases IL-1β production. This activity correlated with a higher IL-1β production in response to lipopolysaccharide stimulation of peripheral blood leukocytes from a PAPA patient ex vivo compared with a healthy control.[167] It places PAPA syndrome in the same pathogenic pathway as FMF. Other studies report an increased production of TNF.[170,171] Dysregulated apoptosis may also be involved.[172]

### Clinical Features and Outcome

The episodic inflammation in this syndrome includes, as the name aptly indicates, symptoms of pyogenic sterile arthritis, pyoderma gangrenosum, and severe cystic acne. Lesions generally occur at the site of mild physical trauma, but sometimes no obvious trigger can be discerned.[165] The inflammation can be severe and eventually may lead to destruction of joints, muscle, and skin. Fever is not prominent in this syndrome. Onset is usually from age 1 to 16 years.[165,173] The acne generally starts early in puberty and persists in adulthood. Penetrance of disease manifestations can be incomplete, with varying presentation within a family.

### Diagnosis

No specific diagnostic test exists. Diagnosis is based on a finding of the typical constellation of symptoms and a positive family history. A specialized DNA diagnostics department would be able to perform the genetic test for PAPA syndrome. At this time, it is unknown whether this genetic test would detect all patients or whether other genes could be involved.

### Treatment

Only case reports or small retrospective series are available on treatment options in PAPA syndrome. High-dose steroids generally have a positive effect on the pyoderma gangrenosum but may be associated with increased acne.[165] Pyogenic arthritis is often responsive to intra-articular or oral corticosteroids.[173] Varying results have been reported with anti-cytokine treatment. The TNF inhibitor etanercept was reported to be beneficial,[170,174] as was the IL-1 inhibitor anakinra.[167,175,176] One patient with severe pyoderma gangrenosum did not respond to etanercept or anakinra reportedly responded to another TNF inhibitor, infliximab.[177]

## DEFICIENCY OF THE IL-1 RECEPTOR ANTAGONIST

Deficiency of the IL-1 receptor antagonist (DIRA) is a more recent discovery of a monogenetic disorder in the group of autoinflammatory diseases.[178,179] It was found in nine patients from six families, one from Newfoundland, Canada, three from the Netherlands, one from Puerto Rico, and one consanguineous family from Lebanon.

All patients were homozygous for mutations affecting the gene encoding the IL-1 receptor antagonist (IL-1ra), designated *IL1RN*.[178] These mutations resulted in an absence of secretion of IL-1ra. IL-1ra is an endogenous inhibitor of the inflammatory cytokines IL-1α and IL-1β. The patients showed signs of unopposed IL-1 signaling, leading to overproduction of other inflammatory cytokines and chemokines.

The clinical phenotype is present at birth or in the first month of life. Features include primarily cutaneous pustulosis and sterile pustulous osteomyelitis. Patients do not present with fever. Two children died of multiorgan failure secondary to severe inflammatory response syndrome at the ages of 2 months and 21 months, and a third child died at 9.5 years of complications of pulmonary hemosiderosis with progressive intestinal fibrosis.

The diagnosis is made by a combination of the clinical features and an impressive response to anakinra. Anakinra

**TABLE 97-7** Summary of Treatment Options

| Disorder | Treatment Options |
|---|---|
| FMF | Colchicine<br>In refractory cases or intolerance of colchicine: intravenous colchicine; IL-1 inhibition |
| HIDS | IL-1 inhibition, etanercept, (possibly simvastatin) |
| TRAPS | NSAIDs; IL-1 inhibition; etanercept |
| CAPS | IL-1 inhibition (anakinra, canakinumab, rilonacept) |
| BS/EOS | Corticosteroids; infliximab? |
| PAPA | High-dose steroids; IL-1 inhibition (anakinra); etanercept; infliximab |

BS/EOS, Blau syndrome/early-onset sarcoidosis; CAPS, cryopyrin-associated periodic syndrome; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; PAPA, pyogenic sterile arthritis, pyoderma gangrenosum, and acne; TRAPS, TNF receptor–associated periodic syndrome.

is a synthetic version of IL-1ra and thus supplements the missing protein in DIRA. Treatment with anakinra suppresses all disease symptoms, although in one patient inflammatory markers remained elevated.

## CONCLUSION

The familial autoinflammatory syndromes are characterized by recurrent episodes of fever and inflammation. This group of disorders should be considered in a patient with a history of years of such inflammatory attacks with symptom-free intervals in between (except for CINCA/NOMID, in which some symptoms and morphologic features persist). Dysregulation of the IL-1β pathway is central to many familial autoinflammatory syndromes, especially CAPS, FMF, and DIRA. Increasing availability of IL-1 inhibitors has revolutionized treatment options in many of these diseases.

The discovery of the causative genes has had an enormous impact in the field of periodic fevers. This discovery has been made possible because of the accurate phenotypic characterization of patients with periodic fever. Careful analysis and proper clustering of these patients is indispensable to allow the elucidation of the genetic background and the evaluation of possible treatment options (Table 97-7). Central periodic fever registries have afforded the opportunity to appreciate previously unrecognized symptoms, to give insight into the long-term prognosis, and to allow better evaluation of drug regimens. Despite these efforts at classification, however, many patients with periodic fever do not fall in one of the previously mentioned disease categories. Every year, new genetic disorders are recognized, and this is expected to continue.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 98

# Pathogenesis of Osteoarthritis



Paul E. Di Cesare • Dominik R. Haudenschild • Jonathan Samuels • Steven B. Abramson

## KEY POINTS

- Osteoarthritis is a degenerative joint disease that occurs primarily in older individuals, characterized by erosion of the articular cartilage, hypertrophy of bone at the margins (i.e., osteophytes), subchondral sclerosis, and a range of biochemical and morphologic alterations of the synovial membrane and joint capsule.
- Risk factors for developing osteoarthritis include age, joint location, obesity, genetic predisposition, joint malalignment, trauma, and gender.
- Morphologic changes in early osteoarthritis include articular cartilage surface irregularity, superficial clefts within the tissue, and altered proteoglycan distribution.
- Morphologic changes in late osteoarthritis include deepened clefts, increase in surface irregularities, and eventual articular cartilage ulceration, exposing the underlying bone. Chondrocytes form clusters or clones in an attempt at self-repair. In addition, marginal osteophytes can form.
- The matrix metalloproteinase family of proteinases degrades proteoglycans (aggrecanases) and collagen (collagenases).
- Suboptimal repair response of normal articular cartilage to injury typically results in secondary osteoarthritis.
- Chondrocytes sense and respond to mechanical and physicochemical stimuli via several regulatory pathways.
- Mediators classically associated with inflammation during the course of osteoarthritis include interleukin-1β and tumor necrosis factor.
- Nitric oxide, produced by the inducible isoform of nitric oxide synthase, is a major catabolic factor produced by chondrocytes in response to pro-inflammatory cytokines.
- Expression of inducible cyclooxygenase-2 is increased in osteoarthritis chondrocytes.
- Low-grade inflammation occurs in osteoarthritis synovial tissue and contributes to disease pathogenesis.
- Most current treatments aim to improve the signs and symptoms of osteoarthritis.

Osteoarthritis (OA) is a degenerative joint disease, occurring primarily in older people and characterized by erosion of the articular cartilage, hypertrophy of bone at the margins (i.e., osteophytes), subchondral sclerosis, and a range of biochemical and morphologic alterations of the synovial membrane and joint capsule. Pathologic changes in the late stages of OA include softening, ulceration, and focal disintegration of the articular cartilage; synovial inflammation may also occur. Typical clinical symptoms are pain and stiffness, particularly after prolonged activity.

In industrialized societies OA is the leading cause of physical disability, increases in health care usage, and impaired quality of life. The impact of arthritic conditions is expected to grow while the population both increases and ages in the coming decades.[1] Despite its prevalence, the precise etiology, pathogenesis, and progression of OA remain beyond our understanding, primarily because of multiple confounding factors. Without a clear-cut picture of how OA arises at the cellular or molecular level, many still consider it a result of "wear and tear," an inevitable consequence of aging. In fact, the accompanying biochemical, structural, and metabolic changes in joint cartilage have been well documented. It is now known, for example, that cytokines, mechanical trauma, and altered genetics are involved in its pathogenesis, and that these factors can initiate a degradative cascade that results in many of the characteristic alterations of articular cartilage in OA. More recently, it has become apparent that OA is a disease process that affects the entire joint structure, including cartilage, synovial membrane, subchondral bone, ligaments, and periarticular muscles. OA is thus better thought of as a group of overlapping disorders, of various etiologies and arising from a combination of systemic factors (e.g., genetics) and local factors (e.g., biomechanically or biochemically mediated events) that gradually converge to produce a condition with definable morphologic and clinical outcomes.[2] The definition of OA is being re-evaluated, and an expanded definition of the disease is likely to also include earlier markers of cell stress and degradation of the extra-cellular matrix initiated by micro injury and macroinjury.[3]

OA may be classified as primary or secondary according to its cause or major predisposing factor. What they all have in common is altered cartilage physiology. Primary OA is the most common type and has no identifiable etiology or predisposing cause. Secondary OA, although it has an identifiable underlying cause, is pathologically indistinguishable from primary OA. The most common causes of secondary OA are metabolic conditions (e.g., calcium crystal deposition, hemochromatosis, acromegaly), anatomic factors (e.g., leg length inequality, congenital hip dislocation), traumatic

events (e.g., major joint trauma, chronic joint injury, joint surgery), or the sequelae of inflammatory disorders (e.g., ankylosing spondylitis, septic arthritis). In the case of secondary OA arising from inflammatory joint disease, cartilage degeneration most likely results initially from degradative enzymes released from the synovium or leukocytes within the joint space and later from the mechanical attrition of a biomechanically altered extra-cellular matrix. Distinguishing between primary and secondary OA may be difficult because the clinical presentation and symptoms are often so similar.

## ETIOLOGY

Major factors affecting degree of risk for OA include patient age, joint location, obesity, genetic predisposition, joint malalignment, trauma, and gender.

### Age

Age is the risk factor most strongly correlated with OA.[4,5] OA is, in fact, the most common chronic disease in later life; more than 80% of people older than 75 years are affected, and OA increases progressively with age at all joint sites. Radiologic changes in OA increase while individuals age,[6] although these changes do not always correlate with clinical symptoms or disability.[7,8] Although clearly an age-related disease, OA is not, however, an inevitable consequence of aging. Age-related morphologic and structural changes in articular cartilage include fraying, softening, and thinning of the articular surface, decreased size and aggregation of matrix proteoglycans, and loss of matrix tensile strength and stiffness. These age-related tissue changes are most likely due to decrease in chondrocytes' ability to maintain and repair the tissue, as chondrocytes themselves undergo age-related decreases in mitotic and synthetic activity, exhibit decreased responsiveness to anabolic growth factors, and synthesize smaller and less uniform large aggregating proteoglycans and fewer functional link proteins.[4]

There also appears to be a direct correlation between chondrocyte apoptosis and cartilage degradation leading to OA. The combination of reduced cellularity, reduced synthetic activity of the remaining cells, and lower mechanical quality of the extra-cellular matrix produced by aged chondrocytes all contribute to increased OA risk. Age is an independent factor that predisposes articular chondrocytes to apoptosis for example the expression levels of specific proapoptotic genes (Fas, FasL, caspase-8, and p53) is higher in aged cartilage.[9,10] These changes in the cartilage matrix and chondrocyte biology may predispose the aged cartilage to more rapid OA progression in the event of minor injury.

### Joint Location

Although osteoarthritis occurs most commonly in weight-bearing joints,[11] age affects joints differentially.[12] A study comparing tensile fracture stress of cartilage in the femoral head and in the talus showed that it decreased progressively with age in the former but not in the latter.[13] Joint-specific age-related viability in articular cartilage may explain why OA is more common in hip and knee joints with increasing age but occurs rarely in the ankle.

### Obesity

Obesity is another important risk factor for OA.[14-16] Greater body mass index in both women and men has been shown to be associated with an increased risk of knee (but not hip) OA.[7,17,18] An increase in mechanical forces across weight-bearing joints is probably the primary factor leading to joint degeneration. Obesity not only increases the forces at weight-bearing joints, but may also change posture, gait, and physical activity level, any or all of which may further contribute to altered joint biomechanics.[19] The majority of obese patients exhibit varus knee deformities, which result in increased joint reactive forces in the medial compartment of the knee, thereby accelerating the degenerative process.[20]

Particularly in elderly obese individuals, heavy physical activity is an additional risk factor for the development of knee OA, whereas light to moderate activity does not appear to increase risk for knee OA and may in fact alleviate symptomatic knee OA by reducing body mass index.[7,17,18] Similarly, weight loss can reduce both radiographic knee OA progression and clinical symptoms. Recent evidence indicates that in obese patients with OA, significant weight loss dramatically improves functional status, with short-term results equivalent to those of patients who have undergone joint replacement.[21]

Yet recent work suggests that the association between obesity and osteoarthritis lies beyond the mechanical loading from a higher body mass index. Adipose tissue is now recognized as a metabolically active contributor to inflammatory cascades. Activated adipose tissue increases the synthesis of pro-inflammatory cytokines, including leptin, adiponectin, resistin, IL-1, IL-6, and TNF, whereas some regulatory cytokine levels such as IL-10 are decreased.[22,23] Specifically, the discovery of the obesity gene and its product leptin may have important implications for the onset and progression of OA and may increase our understanding of the link between obesity and OA. The fact that women have a greater proportion of total body fat and relatively higher levels of adipose-derived systemic leptin concentrations than men may partially account for the gender disparity in OA patients. Leptin, however, is produced not only by adipose cells but also by osteoblasts and chondrocytes, suggesting that local leptin production may play a role in OA. Significant levels of leptin were observed in the cartilage and osteophytes of patients with OA, yet few chondrocytes produced leptin in the cartilage of healthy patients. Leptin can induce anabolic activity in rat chondrocytes, and by acting as a pro-inflammatory agent may ultimately confer structural joint changes through various mechanisms.[24-26] The association between leptin levels and OA disease severity is supported by meta-analyses of adipokines in OA patients, especially in females.[27]

### Genetic Predisposition

Because of the prevalence of OA in the general population and its extensive clinical heterogeneity, the genetic contribution to its pathogenesis has been difficult to analyze.[28,29]

Two major cohorts (the Framingham Study and the Baltimore Longitudinal Study on Aging) support a genetic contribution to OA, with evidence for a major recessive gene and a multifactorial component, representing either polygenic or environmental factors.[30,31] Twin pair and family risk studies have indicated that the hereditable component of OA may be on the order of 50% to 65%.[28,32,33] Moreover, family, twin, and population studies have indicated differences among genetic influences that determine the site of OA (hip, spinal, knee, hand).[31,34,35] Further evidence supporting a genetic predisposition to OA is the demonstration of a significantly higher concordance for OA between monozygotic twins than between dizygotic twins. Genetic studies have identified multiple gene variations associated with an increased risk of OA.[36]

Inherited forms of OA may be caused by mutations in several genes that are expressed in cartilage, including those encoding types II, IV, V, and VI collagens, as well as cartilage oligomeric matrix protein (COMP).[37,38] Mice deficient in the type IX collagen gene and the matrilin 3 gene (human equivalent condition not yet reported) developed age-dependent OA-like changes in the knee and temporal mandibular joints.[39,40] Moreover, candidate genes in OA have been identified that are not structural proteins. The haplotype of a vitamin D receptor (VDR) that plays a vital role in controlling bone mineral density appears to be associated with a twofold risk of knee OA,[41-43] although the VDR locus is very close to the COL2A1 locus on chromosome 12q, and so the association may be to the result of linkage disequilibrium with the latter. In addition, the locus of the insulin-like growth factor (IGF)-1 gene has been associated with radiographic OA, as has an aggrecan polymorphic allele with hand OA.[29]

In population studies, genome-wide linkage scans have highlighted as many as seven chromosomal regions that may harbor OA susceptibility genes.[44] Chromosome 2q was positive in several scans, suggesting that this chromosome is likely to harbor one or more susceptibility genes. In a study of affected sibling pairs, a region of linkage stretching from 2q12 to 2q21 was reported for OA of the distal interphalangeal joint, and a previous study of affected sibling pairs in the United Kingdom demonstrated a broader region of linkage, stretching from 2q12 to 2q31.[44,45] Two IL-1 genes (*IL1α* and *IL1β*) and the gene encoding IL-1Ra *(IL1RN)* are located on chromosome 2q13 within a 430-kb genomic fragment. Given the importance of IL-1 in the perpetuation of cartilage damage in OA, it is possible that a proportion of the genetic susceptibility to OA may be encoded for by variation in the activity of ILs, and that for chromosome 2q this susceptibility could reside within the IL-1 gene clusters. Loughlin and colleagues, however, have provided evidence that the IL-1 gene cluster harbors susceptibility for knee OA, but not for hip OA.[44] These and other epidemiologic studies have highlighted potential differences in the degree of OA heritability among different joint groups and between the two sexes.[46,47] Additional OA candidate genes that have recently been identified, including nuclear receptor coactivator 3 *(NCOA3)* or sulfatase 2 *(SULF2)*[48] and aldehyde dehydrogenase family 1, subfamily A2 *(ALDH1A2)*.[49] Perhaps the most convincing genetic risk allele for OA involves growth differentiation factor-5 *(GDF-5)*, a member of the TGF-β superfamily.[50]

Genomic and postgenomic technology, in addition to defining susceptibility genotypes, will also lead to the discovery of genes and gene products that are overexpressed in OA tissues and that contribute to disease pathogenesis and progression.[51-53] Studies of differential gene expression in diseased tissue, in addition to elucidating pathogenic processes that lead to novel therapies, could also have two additional benefits: (1) identification of unique biomarkers that can be used for OA diagnosis or management, and (2) identification of candidate susceptibility genotypes, such as polymorphic variations of cytokines or growth factors, that may predispose to disease progression.[54] A realization from the many recent genome-wide studies on genetic OA risk factors is that although only a small portion of OA hereditability has been explained, the polygenic contributions to a single OA phenotype necessitates large sample sizes on the order of 50,000 to 100,000 individuals to be adequately powered.

Epigenetic regulation of gene expression is coming under increased scientific scrutiny in the context of OA pathogenesis. Epigenetics describe changes in gene expression that are heritable but without changes to the DNA sequence, typically resulting either from DNA methylation, histone modification, or noncoding RNA.[55] Joint and cartilage homeostasis requires a precise balance of many opposing anabolic and catabolic activities. Global changes in epigenetic mechanisms such as DNA hypomethylation can disturb this balance, as well as affect the expression of individual genes associated with OA.[56] Genome-wide differences in DNA methylation exist between osteoarthritic and healthy cartilage, and specific genes that show altered DNA methylation patterns include homeobox transcription factors, GDF-5, and others.[57] The mechanism by which changes in methylation affect gene expression is still under investigation, but it is clear that aberrant epigenetic regulation of gene expression is involved in OA pathogenesis.

## Joint Malalignment and Trauma

Joint malalignment or trauma may lead to rapid development of OA, or it may initiate a slow process that results in symptomatic OA years later. Probably as a result of progressive reduction in periarticular blood flow and the resultant decrease in rate of remodeling at the osteochondral junction, joints become increasingly congruent with age.[58,59] Altered joint geometry may interfere with nutrition of the cartilage, or it may alter load distribution, either of which may result in altered biochemical composition of the cartilage, irrespective of age. [60,61] Local factors, such as stresses related to joint use and joint deformity, also influence the development of OA.

Joint incongruence (e.g., malreduced intra-articular fractures, developmental dysplasia of the hip, recurrent dislocation of the patella) can lead to early-onset OA.[62] Repetitive, high-impact sports are strongly associated with joint injury and increase the risk for lower-limb OA.[63] Repetitive trauma at a subfracture level has been shown to accelerate remodeling in the zone of calcified cartilage, with reduplication of the tidemark and thinning of the noncalcified zone, resulting in stiffening of the subchondral bone, increased wear of the overlying cartilage, and, ultimately, development of OA.[64-66] Regular exercise is important in maintaining

articular cartilage structure and metabolic function. Recreational running and moderate low-impact activities have not been shown to increase the risk of OA in healthy individuals, and there is growing consensus that this is true even for individuals who are considered at risk for OA.

Articular cartilage is remarkably resistant to damage by shear forces; it is, however, highly vulnerable to repetitive impact loading.[67] When joints are subjected to in vitro cyclic loads that are easily borne by subchondral bone, cartilage degeneration still results.[68] This vulnerability accounts for the high frequency of OA in shoulders and elbows of pneumatic drill operators and baseball pitchers, ankles of ballet dancers, metacarpophalangeal joints of boxers, and knees of basketball players. The risk for knee osteoarthritis among participants in sports, however, may be more closely related to previous knee injury than to participation in sport alone.[69]

### Sex

OA is approximately twice as likely to develop in women than in men. Although women have a lower prevalence of OA than men before the age of 50 years, there is a marked increase in prevalence among women after 50 years, particularly in the knee.[70] Radiographic and interview data from the National Health and Nutrition Examination Survey (NHANES III), a representative cross-sectional health examination survey of the U.S. population, reported that the lifetime prevalence of radiographic knee osteoarthritis was 37.4% and that the prevalence of symptomatic knee osteoarthritis was 12.1% in adults age 60 years and older. Prevalence was greater among women than men (42.1% vs. 31.2%), and women had significantly more Kellgren-Lawrence grade 3-4 changes (12.9% vs. 6.5% in men).[71] Women have a greater number of joints involved and are more likely to exhibit clinical symptoms of morning stiffness, joint swelling, and nocturnal pain. The gender differences in OA incidence after age 50 years may be the result of post-menopausal estrogen deficiency. Articular chondrocytes possess functional estrogen receptors, suggesting that these cells can be regulated by estrogen. Nuclear estrogen receptors (ERs) have been detected in articular chondrocytes of humans,[72,73] rats,[72,74] monkeys,[72,75] and pigs,[76] and in human growth plate chondrocytes.[77]

Epidemiologic studies have linked estrogen replacement therapy (ERT) with a lower-than-expected risk of knee and hip OA in post-menopausal women. Clinical investigations of the association between OA and hormonal level in women have involved measurement of circulating estrogen levels in post-menopausal women, general radiographic evaluation of post-menopausal women, and examination of the effect of ERT on such variables as knee OA and cartilage volume.[78-81] In a 1996 study of more than 4000 women age 65 years or older who were assessed by pelvis radiographs for hip OA, Nevitt and colleagues[78] showed that women using oral estrogen faced a significantly reduced risk of hip OA. Estrogen users for 10 years or longer had a greater reduction in risk for hip OA than women who had used estrogen for less than 10 years.[78] In 1990 Hannan and colleagues[82], using weight-bearing radiographs in female participants in the Framingham Osteoarthritis Study ($n$ = 831; mean age, 73 years; age range, 63 to 93) to study the rate of knee OA, reported a modest but nonsignificant greater protective effect for radiographically detected OA in women who were on ERT.[82] A prospective cohort study using anteroposterior weight-bearing knee radiographs of women (mean age, 71 years; age range, 63 to 91) from the Framingham Study categorized patients into three groups according to estrogen use at biennial examination: never users ($n$ = 349), past users ($n$ = 162), and current users ($n$ = 40). When both incident and progressive radiographic knee OA cases were combined, current ERT users were found to have 60% less risk for knee OA than never users. Wluka and colleagues[80] reported on the longer-term use of ERT and its association with knee cartilage volume (measured by MRI) in post-menopausal women. Results showed that after adjusting for confounders, women using long-term ERT had more knee cartilage than controls. Beneficial effects of ERT on the severity of knee OA in ovariectomized monkeys has also been demonstrated.[83] In addition to hormone therapy, selective ER modulators appear to hold promise as gender-specific therapeutics in both pre-clinical and clinical studies.[84]

## PATHOLOGY

### Morphologic Changes

In early OA, the articular cartilage surface becomes roughened and irregular, and superficial clefts within the tissue become apparent. Histologically, the surface is fibrillated, and small cracks are apparent but limited to the upper layers of the surface zone. These changes show evidence of mechanical wear and are often accompanied by matrix swelling and chondrocyte proliferation, or, to a limited extent, apoptosis near the articular surface. Proteoglycan distribution is altered, as revealed by histochemical staining. While OA progresses, the articular cartilage surface becomes more irregular, and the superficial clefts within the tissue become enlarged and extend beyond the superficial zone and into the middle zone of cartilage. The previously isolated and focal portions of damaged cartilage become increasingly contiguous. Portions of the superficial zone are completely missing in focal lesions. While the condition worsens, the clefts deepen, surface irregularities increase, and the articular cartilage eventually ulcerates, exposing the underlying bone. While the disease continues to progress, the joint articulates on exposed bone, causing eburnation and thickening of the bone. The eburnated bone becomes denser and more metabolically active in response.

### Early Reparative, Proliferative, and Hypertrophic Changes in Cartilage

Attempts at local self-repair can be seen as an initial increase in the number of chondrocytes in the form of clusters or clones, with as many as 50 or more cells in a cluster[43] (Figure 98-1). In healthy cartilage, chondrocytes are quiescent and neither commit to proliferation nor to further hypertrophic differentiation. In contrast, in the early stages of OA, proliferating chondrocytes in clusters express higher levels of matrix proteins, including aggrecan and type II collagen,[85] as well as stem cell markers[86] and markers of hypertrophic differentiation,[85] The chondrocyte clusters with proliferative and hypertrophic markers are a hallmark of OA

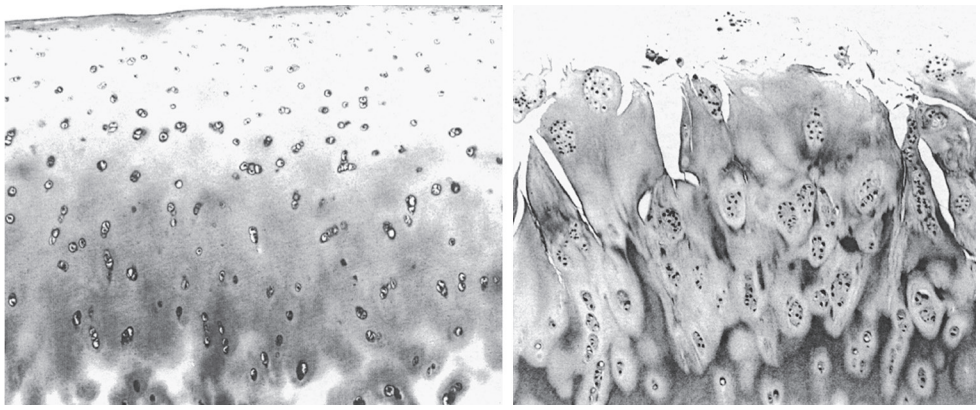



**Figure 98-1** Histologic sections of normal *(left)* and osteoarthritic *(right)* articular cartilage obtained from the femoral head. The osteoarthritic cartilage has surface irregularities, with clefts to the radial zone and cloning of chondrocytes.

and are also prominent in animal models of OA and 3D cell cultures.[87] In addition to being a repair response, chondrocyte clusters are thought to contribute to OA pathogenesis and progression through the release of matrix-degrading enzymes, growth factors, and inflammatory cytokines that affect surrounding chondrocytes and joint tissues.[87]

### Osteophyte Formation

Osteophytes consist of newly formed fibrocartilage and bone, and are most commonly formed at the peripheral margins of joints at the interface between cartilage and the periosteum. Osteophytes are thought to arise through chondrogenic differentiation of progenitor cells, most commonly from within the periosteum.[88] As such, osteophytes may be a cellular repair response to the altered growth factor environment after joint injury, and in certain cases, osteophytes can contribute to the stability of the joints.[89,90] The connection between osteophyte formation and the repair response is further strengthened by animal models of OA in which the early stages of osteophyte formation can be histologically apparent as early as 3 days after joint injury. Many growth factors that enhance in vitro chondrogenic differentiation of stem cells are also present in osteophytes, including members of the TGF-β superfamily,[91] bone morphogenetic proteins (BMPs),[92] insulin-like growth factor (IGF), and fibroblast growth factor (FGF),[88] and TGF-β, can induce osteophyte formation in animal models.[93] Although osteophytes can initially restore stability to an injured joint, while OA progresses, osteophytes can limit range of motion and become painful.

### Cartilage Hypocellularity

A reduction in cell number is observed in aging cartilage, and the reduced synthetic capability of hypocellular cartilage is a contributing factor to the initiation and progression of OA. In healthy adult femoral cartilage, the cell density varies between 24,000/mm$^3$ in the surface zone to 8000/mm$^3$ in the deep zone, with an average of 1.65% of the cartilage volume consisting of cells.[94] This number is greatly reduced in OA, through cell death via necrosis or apoptosis.[95] Necrosis can result from direct mechanical damage to cells and is not generally an active process, whereas apoptosis is an active energy-consuming process. Apoptotic chondrocyte death can be initiated by many factors that are involved in the initiation and progression of arthritis, including mechanical damage or injury, changes in cell-matrix interactions, oxidative stress resulting from nitric oxide (NO) or other reactive oxygen species, impaired mitochondrial function, and signal transduction pathways such as CD95/CD95 ligand. These pathways ultimately converge, and the execution of apoptotic cell death is mediated by the activation of caspases. Inhibition of apoptosis by interfering with caspase activation following injury is under investigation as a chondroprotective intervention to prevent secondary OA following injury. Compromised regulation of cellular senescence and autophagy can also cause cell death. The role of autophagy in inflammation and pathogenesis has been gaining attention of researchers.[96] In some circumstances cell death can be mediated by the autophagic degradation of the damaged organelles. In cartilage, autophagy can be protective, and its reduction in OA corresponds with increased apoptotic markers.[97]

## ALTERATIONS IN CARTILAGE MATRIX METABOLISM

### Biochemical Changes

These morphologic changes are accompanied by changes in the biochemical composition of the cartilage, which varies from early to later stages in the disease process. In early OA, the water content of the articular cartilage significantly increases, causing the tissue to swell and altering its biomechanical properties. This phenomenon suggests that there has been weakening of the collagen network; the type II collagen fibers have a smaller diameter than normal cartilage, and the normally tight weave in the midzone is slackened and distorted.[98-103]

In later stages of OA, type I collagen concentration within the extra-cellular matrix increases and the proteoglycan concentration falls to 50% or less than normal, with less aggregation and shorter glycosaminoglycan side chains.[99,104] Keratan sulfate concentration decreases, and the ratio of chondroitin-4-sulfate to chondroitin-6-sulfate

increases, reflecting synthesis by chondrocytes of a proteoglycan profile more typical of immature cartilage.[105] Proteoglycan concentration in the cartilage diminishes progressively until the end stages, when histologic staining detects little or none.[106]

Our understanding of the how the biochemical changes progress from the early to the later stages of OA is evolving. One of the first steps in cartilage degradation is a decrease in the density of proteoglycans. This step is thought to be at least partly reversible.[107] However, decreased proteoglycan density opens up the cartilage porosity to make it more permeable to collagenases and other proteases, and it exposes collagen fibrils. This initiates a vicious circle of positive-feedback loops that further promote cartilage degradation. For example, epitopes on collagen become accessible to the DDR2 receptor on the cell surface, which then increases matrix metalloproteinase (MMP)-13 production through activation of the Ras/Raf/MEK/ERK and p38 signal cascades.[108] The partially digested matrix components themselves have cytokine-like activity that enhances the inflammatory response and promotes matrix degradation. The destruction of the collagenous cartilage component is thought to be irreversible.

Calcium crystals (e.g., calcium pyrophosphate dihydrate [CPPD], basic calcium phosphate crystals) are commonly found in the cartilage of the elderly, and often crystal arthropathy coexists with OA.[109] The fact that calcium crystals play a role in causing or worsening OA is supported by clinical and laboratory studies, but the relationship is complex, and it is unclear whether these crystals are directly involved in OA pathogenesis.[110-112] Pyrophosphate (PP) is produced from adenosine triphosphate (ATP) by the exoenzyme nucleoside pyrophosphohydrolase.[113] High levels of PP in synovial fluid from OA patients correlate directly with the severity of joint damage.[114,115] Young or proliferating chondrocytes are a major source of PP, whereas resting chondrocytes from normal adult cartilage secrete little.[113] Thus the increased PP secretion in OA cartilage might indicate increased chondrocyte metabolic activity toward matrix repair.[116] The presence of CPPD may alter the biomechanical properties of the cartilage extra-cellular matrix and lead to cartilage breakdown. Hemochromatosis (hemosiderin), Wilson's disease (copper), ochronotic arthropathy (homogentisic acid polymers), gouty arthritis (crystals of monosodium urate), and CPPD crystal deposition disease are further examples of conditions in which the abnormal entity may alter the cartilage extra-cellular matrix, leading to either direct or indirect chondrocyte injury by increasing the stiffness of the tissue and thereby precipitating the development of OA.

## Metabolic Changes

Early OA is characterized by increased synthesis of proteoglycans, collagen, noncollagenous proteins, hyaluronate, and cell replication.[106,117] This "activation" of chondrocytes is thought to be an attempt to repair the cartilage matrix, although it is not always effective and yields a matrix of inferior quality that is more susceptible to degradation.[118] However, both anabolic and catabolic processes increase while cells attempt to repair or maintain tissue integrity,[99] and it is the imbalance between synthesis and degradation that is important in the pathogenesis of OA.[119] In later stages of OA, there is a decrease in the synthesis of matrix per cell and a decrease in cell number. Furthermore, the quality of the synthesized matrix is reduced with respect to the composition and distribution of their glycosaminoglycans, the size of the proteoglycan subunit, and their ability to aggregate with hyaluronic acid.[99,103,105,120,121] In addition to the reduced matrix production and hypocellularity, there is increased synthesis and activation of matrix degrading enzymes, and an overall decrease in the concentrations of enzyme inhibitors such as tissue inhibitors of metalloproteinases (TIMPs). Eventually OA progresses when the chondrocytic anabolic repair processes cannot keep pace with catabolic processes.[99,106] The complex interaction between matrix synthesis and degradation explains why OA typically is slowly progressive and at times remains static by morphologic criteria, but ultimately results in the overall degradation of cartilage matrix. The following sections summarize the major anabolic and catabolic factors relevant to cartilage degradation and the pathogenesis of OA.

### Anabolic Factors (TGF-β, Bone Morphogenic Proteins) and Cartilage Repair

TGF-β is essential for the formation and maintenance of cartilage (reviewed in[122]). Interference with TGF-β function in cartilage leads to OA in multiple animal models[123] and in human genetic susceptibility to OA.[124] TGF-β affects cartilage homeostasis at multiple levels: It enhances stem cell chondrogenesis to increase the pool of cells available for cartilage synthesis, and it increases matrix production in existing chondrocytes. TGF-β also increases synthesis of anti-catabolic factors such as TIMPs and plasminogen activator inhibitor (PAI)-1, proteins that inhibit the activation of latent proteinases in cartilage. TGF-β attenuates the cellular response to inflammatory cytokines, including IL-1β and TNF,[122] and TGF-β signaling through the Smad2/3 pathway can inhibit terminal differentiation and hypertrophy in chondrocytes. However, TGF-β activity can also contribute to the pathogenesis and progression of OA. For example, TGF-β signaling induces osteophyte formation in animal models. In aging cells and cells in which the ratio of the TGF-β receptors ALK1 and ALK5 is altered, TGF-β can also have opposing effects, such as activating MMP-13 and inducing terminal hypertrophic differentiation in chondrocytes.[125]

BMPs are structurally related to TGF-β but generally activate a different set of receptors and intra-cellular signaling molecules. BMPs influence all stages of embryonic chondrogenesis and can promote chondrogenic differentiation of adult mesenchymal stem cells (MSCs).[126] Recent genetic evidence suggests that impaired BMP signaling affects OA susceptibility, with the most progress reported for BMP-14 (GDF-5).[127,128] There is good evidence that a reduction of BMP-7 (OP-1) is evident in osteoarthritic cartilage,[129] with possible regulation occurring through both inhibitory microRNA[130] and promoter methylation.[131] Supplementation of BMP-7 in joints reduces experimental arthritis in animals and was proven safe in a phase I clinical trial, with no findings of ectopic bone formation in the enrolled patients during the study.[132] However, as described

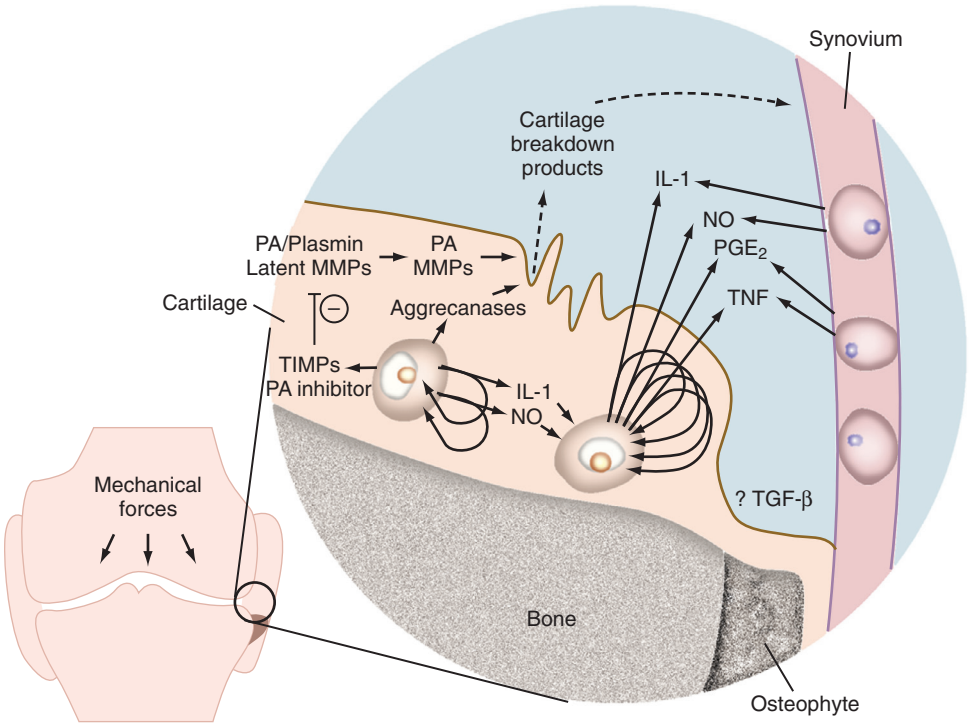



**Figure 98-2** Schematic of pathogenic mechanisms of osteoarthritis. Mechanical stress initiates altered metabolism characterized by the release of matrix metalloproteinases (MMPs), pro-inflammatory cytokines, and mediators such as nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$). Cartilage breakdown products play a role by stimulating the release of cytokines from synovial lining cells and by inducing MMP production by chondrocytes. Perpetuation of cartilage damage is amplified by the autocrine and paracrine actions of interleukin (IL)-1β and tumor necrosis factor (TNF) produced by chondrocytes. PA, Plasminogen activator; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases.

for TGF-β above, BMP signaling through certain receptors can also enhance terminal differentiation and hypertrophy in chondrocytes, processes that are hallmarks of OA progression. The effects of BMPs appear to depend on the BMP family member, with hypertrophic differentiation promoted by BMP-2 and yet suppressed by BMP-7.[133]

### Catabolic Factors and Cartilage Degradation

In addition to new matrix synthesis, cartilage remodeling also involves a degree of proteolysis. This occurs via the induction of an array of proteases, and in particular, MMPs. In OA, the cytokines IL-1 and TNF stimulate the synthesis and secretion of many proteases and MMPs (Figure 98-2).[107] IL-1 is synthesized by mononuclear cells (including synovial lining cells) in the inflamed joint and by chondrocytes as an autocrine activity.[134-136] The enzymes stimulated by IL-1 and TNF include latent collagenase, latent stromelysin, latent gelatinase, aggrecanase, and tissue plasminogen activator (TPA).[137-140] Plasminogen is either synthesized by the chondrocyte or enters the matrix by diffusion from the synovial fluid. TPA converts plasminogen to plasmin, a serine proteinase that can activate latent cartilage-degrading enzymes. A downstream mediator of IL-1– and TNF-induced cartilage degradation that is gaining increasing attention is hypoxia-inducible factor (Hif) 2α.[141,142] Hif2α is a transcription factor strongly upregulated in OA cartilage and mouse models of OA. It directly induces the expression of many cartilage-degrading enzymes, including MMP-1, -3, -9, and -12 as well as a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and, (indirectly), ADAMTS-5. In addition, a high level of Hif2α decreases the protective role of autophagy, increases the extent of cell death, and activates the RUNX2 and IHH pathways that further contribute to cartilage matrix degradation. The different classes of proteinases that are activated by these cytokines in OA are discussed in more detail below.

## CLASSES OF PROTEINASES

In OA, the synthesis and secretion of matrix-degrading enzymes by chondrocytes markedly increases.[106,117,143-145] There are four classes of proteases, which are grouped by the catalytic mechanisms of peptide bond cleavage: metalloproteinases, cysteine proteinases, serine proteinases, and aspartyl proteinases. Of these, the first three have clearly defined roles in the degradation of cartilage during the progression of OA.

### Metalloproteinases

Metalloproteinases have an enzymatic site that requires a metal ion (often zinc) for activity. Cartilage contains two families of metalloproteinases, namely ADAMTSs and MMPs. Early cartilage degeneration in OA is most likely the result of metalloproteinase enzymatic activity. Both families of metalloproteinases are upregulated in osteoarthritic cartilage, and both are highly expressed in OA cartilage at lesional sites. Because the MMPs and ADAMTSs

play major roles in the degradation of cartilage extra-cellular matrix, several MMPs and ADAMTSs are candidate targets for disease modification.[146]

The control of metalloproteinase activity in OA is complex, with regulation occurring at three different levels: synthesis and secretion, activation of latent enzyme, and inactivation by proteinase inhibitors.[147] Most metalloproteinases are not constitutively expressed, but their transcription is induced after stimulation with cytokine and growth factor signaling. Once transcribed, the transcript stability and translation of several metalloproteinases are regulated by microRNA. For example, microRNA-27b (miR-27b) regulates MMP-13 expression.[148] Also, miR140 levels are decreased in OA, which reduces its repression of ADAMTS-5.[149] Several of these miRNA are controlled by the same cytokines and growth factors that maintain cartilage homeostasis, including IL-1 and TGF-β. Although the microRNA field is still in its infancy, it is already evident that several miRNAs are involved in the pathogenesis of OA by affecting transcript stability and protein translation. Once translated into proteins, almost all MMPs are expressed as inactive proenzymes (zymogens) that require further processing for full proteolytic activity. Most MMPs contain an N-terminal prodomain that blocks or otherwise inhibits the catalytic site. The primary MMP activators are serine- and cysteine-dependent proteases (e.g., the plasminogen/plasmin cascade or furin-like proprotein convertases, and cathepsin B, respectively), as well as membrane-type MMPs.[120,150,151] Activated MMPs can then be inactivated nonspecifically by alpha-2-macroglobulin, and with more specificity by the tissue inhibitor of metalloproteinase (TIMP) family of proteins.

MMPs have historically been further divided into three groups based on their substrate specificities; *collagenases* cleave across all three chains of the native triple-helical collagens, whereas *gelatinases* preferably cleave denatured collagen, and *stromelysins* have much broader substrate specificities.[152] However, there is considerable overlap in substrates between these classifications; for example, MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1), and MMP-13 (collagenase-3) are all capable of cleaving aggrecan core protein.[153] MMPs can degrade other cartilage extra-cellular matrix molecules in addition to collagen. If combined with plasmin (that has the capability of activating many MMPs), MMPs can rapidly destroy cartilage altogether.

## Collagenases

Collagenases typically make the first cleavage in triple-helical collagen, allowing its further degradation by other proteases. Degradation of collagens is thought to be the first irreversible first step in the pathogenesis of OA because it significantly reduces the mechanical properties of cartilage. The best-studied MMPs capable of cleaving native collagens are MMP-13 and MMP-1, although MMP-8 and MMP-28 are also involved in breaking down type II collagen. Of the MMPs that degrade native collagen, MMP-13 may be the most important in OA, because it preferentially degrades type II collagen.[154] Mice deficient in MMP-13 resist developing OA, and have reduced cartilage erosion even after proteoglycan loss.[155] Expression of MMP-13 greatly increases in OA[156]; overall collagenase activity also markedly increases in human osteoarthritic cartilage cultures, suggesting that it is a major factor in OA progression and cartilage matrix degradation.[157,158] The resultant collagen fragments may then be susceptible to further cleavage by such other enzymes as MMP-2 (gelatinase A), MMP-9 (gelatinase B), MMP-3, and cathepsin B (a cysteine proteinase).

## Cathepsins

Cathepsins are a class of cysteine proteinases that can be secreted into the extra-cellular space, and can further activate MMP proenzymes. Of particular interest in OA pathogenesis is cathepsin K (known roles in bone resorption and osteoporosis), which can proteolytically cleave type II collagen and aggrecan in cartilage.[159] Cathepsin K is elevated in the synovial fluid of OA patients, cathepsin K activity is apparent in a post-traumatic model of mouse OA,[160] and treatment with cathepsin K inhibitors is protective in preclinical models of OA.[161,162]

## Aggrecanases

The aggrecanases are metalloproteases that belong to a family of extra-cellular proteases known as ADAMTS.[163] Two aggrecanases, ADAMTS-4 and ADAMTS-5, appear to be major enzymes in cartilage degradation in arthritis.[164] The roles of these enzymes appear reversed in human and mouse OA. Whereas ADAMTS-4 is predominantly associated with aggrecan degradation in human OA, in mouse models of OA, ADAMTS-5 is more important.[165-167] Recombinant ADAMTS-4 and ADAMTS-5 cleave aggrecan at five distinct sites along the core protein, and all resultant fragments have been identified in cartilage explants undergoing matrix degradation. The G1 region of aggrecan is highly resistant to proteases; however, a glutamate-alanine bond within the extended region between G1 and G2 is remarkably susceptible to proteolytic degradation.[168] Aggrecanase proteolytic activity can be modulated by altered expression, by activation by proteolytic cleavage at a furin-sensitive site, by binding to the aggrecan substrate through the C-terminal thrombospondin motif, by activation through post-translational processing of a portion of the C-terminus, and by inhibition of activity by the endogenous inhibitor tissue inhibitor of metalloproteinases 3 (TIMP-3). ADAMTS-4 and ADAMTS-5 activity is also detected in joint capsule and synovium and may be upregulated in arthritic synovium at either the message level or through post-translational processing. In addition to ADAMTS-4 and -5, several MMPs are also capable of cleaving aggrecan in vitro (MMP-1, -2, -3, -7, -8, -9, -13, and 28), as are ADAMTS-1, -8, -9, and -15.[169] It has been shown that ADAMTS-7 and -12 both bind to and degrade COMP (a prominent noncollagenous protein in cartilage) and that the latter is highly upregulated in osteoarthritic cartilage.[170,171] ADAMTS-4, -19, and -20 also have been show to degrade COMP in vitro; however, their in vivo activity in osteoarthritis has yet to be determined.[172,173]

A specific hyaluronidase has not been found in articular cartilage matrix, but one or several lysosomal enzymes that can cleave both hyaluronic acid and chondroitin-6-sulfate

have been implicated.[150] There are six or seven potential hyaluronidases in the human genome, and of these, hyaluronidase-1, -2, -3 and PH-20 are likely to be active in cartilage.[174] However, experimental evidence to date suggests that these enzymes are either exclusively lysosomal or can exist both in the lysosome and at the plasma membrane. Thus, although there is convincing evidence of extracellular degradation of hyaluronan, the precise role of the hyaluronidases in this process has not yet been clearly identified. The decrease in chondroitin sulfate chain length in osteoarthritic cartilage may be to the result of digestion by synovial fluid hyaluronidase, which may diffuse into the matrix as its permeability increases.[105] Evidence to support this theory is the finding that the hyaluronic acid concentration in OA cartilage is low, even though its rate of synthesis is considerably greater than normal.[106,117] These degradative enzymes serve to disrupt of the proteoglycan aggregate. The early result of the MMP-induced tissue degradation is thinning of the collagen fibers, loosening of the tight collagen network, and the consequent cartilage matrix swelling seen in OA.

### Enzyme Inhibitors

The balance of active and latent enzymes is controlled to some extent by at least two enzyme inhibitors: TIMP and PAI-1.[145,175,176] TIMP and PAI-1 are synthesized in increased amounts under the regulation of TGF-β.[168,177,178] If insufficient concentrations of degraded TIMP or PAI-1 are present in the matrix along with active enzymes, then increased matrix degradation occurs. Expression profiling of all known members of the MMP, ADAMTS, and TIMP gene families in normal cartilage and cartilage from patients with OA has revealed that several members are regulated in OA. Genes that showed increased expression in OA were MMP-2, -9,-13, -16, -28, ADAMTS-2, -12, -14, -16, and TIMP-3 (all at $P < 0.05$). Genes with decreased expression in OA were MMP-1, -3, -10; ADAMTS-1, -5, -9, and -15; and TIMP-1 and -4.[179] These results illustrate the complexity of the events that occur within the extra-cellular matrix regarding regulation of tissue-degrading enzymes.

## ALTERATIONS IN MATRIX SYNTHESIS

Much of what is known about changes in the extra-cellular matrix in early OA comes from animal models (e.g., the rabbit partial meniscectomy model, the canine anterior cruciate ligament–deficient model).[180,181] These animal models represent secondary OA, produced by internal derangement of the joint, and therefore may not precisely simulate the state of affairs in primary OA. Models for spontaneous osteoarthritis are reviewed and their pre-clinical utility compared in a recent review.[182] Of particular interest is an iodoacetate model, validated as the first pain model of osteoarthritis, in which intra-articular injection of iodoacetate in rats has been shown to lead to cartilage degeneration associated with pain and manifesting as time- and concentration-dependent alterations in hind limb weight bearing.[183,184] That not all animal models of osteoarthritis are equivalent becomes clear when one considers the differences in therapeutic response between young and old animals and between spontaneous and surgical models.[185]

In the dog model, the first alteration seen within days after joint destabilization is an increase in cartilage water content.[186] Initially, water content increases locally, in the tibial plateau and femoral condyle cartilage, but it soon spreads to the entire joint cartilage. Proteoglycans are more readily extractable from the matrix of experimental animals than from that of controls and reflected in the serum biomarker profile after acute injury in patients.[187] These matrix changes are also seen in spontaneously occurring dog and steer OA and in experimentally induced rabbit OA.[186,188,189] The increase in water content in osteoarthritic cartilage is to the result of loss of the collagen network's elastic restraint, enabling the hydrophilic proteoglycans to swell more than normally.[190] In early-stage OA proteoglycan concentration may increase, and the cartilage may consequently become thicker than normal and exhibit increased staining for proteoglycans.[191-193] Very shortly after the increase in cartilage water, newly synthesized proteoglycans are characterized by a higher proportion of chondroitin sulfate and a lower proportion of keratan sulfate, and proteoglycan aggregation is impaired.[181,186] These abnormal changes in extracellular matrix occur before fibrillation or any other gross morphologic changes are evident and result in a generalized decrease in stiffness that occurs in grossly normal cartilage adjacent to fibrillated areas.[194] While OA progresses, focal cartilage ulcerations develop. Proteoglycan loss is accompanied by a decrease in its ability to aggregate, persistence in abnormal glycosaminoglycan composition, and a decrease in chondroitin sulfate chain length. Once proteoglycan loss reaches a critical threshold, water content, which initially increased, falls below normal.[195]

## CHONDROCYTE SENESCENCE

The term *senescence* was originally coined to describe the replicative limit of primary cells cultured in vitro, and the concept has evolved to include premature senescence, or senescence-like changes in differentiated post-mitotic cells. Senescent cells have a unique phenotype that affects both the proteins expressed by senescent cells and their and responses to extra-cellular stimuli. Furthermore, the impact of senescence is not limited to the cells undergoing senescence; rather, the senescent cells have an impact on surrounding cells through the paracrine effects of the altered protein secretions.[196]

Premature chondrocyte senescence in OA is thought to be enhanced by oxidative injury.[197] The inflammatory cytokines IL-1β and TNF that are active in OA induce oxidative stress in chondrocytes, thereby contributing to the potential for oxidative injury that leads to senescence. Oxidative injury is also induced with excessive mechanical shear and injurious loading of explants, and this may contribute to the accumulation of senescent chondrocytes in aged cartilage.[198] TGF-β can influence both growth arrest and senescence in cultured cells, and senescent cells can overexpress TGF-β, but this effect has mostly been demonstrated in endothelial cells and not cells of mesenchymal origin. In summary, although it is clearly evident that many of the cellular mechanisms controlling senescence are also active in disease progression including OA, a causal link between senescence and OA has yet to be identified.

## BIOMECHANICS AND DISEASE MECHANISMS

### Biomechanical Changes

Two long-standing biomechanical theories of the pathogenesis of OA hold that mechanical stresses injure chondrocytes, causing them to release degradative enzymes, and that mechanical stresses initially damage the collagen network (as opposed to the cells per se).[199,200] In either case, the result is a breakdown of the matrix. Extra-cellular matrix breakdown in osteoarthritic cartilage leads to (1) loss of compressive stiffness and elasticity, resulting in greater mechanical stress on chondrocytes, and (2) an increase in hydraulic permeability, resulting in loss of interstitial fluid during compression and increased diffusion of solutes through the matrix (including the movement of degradative enzymes and their inhibitors). One important consequence is disruption of normal fluid film joint lubrication and loading dynamics due to alterations in inflammatory synovial fluid.[201-203] Joint friction, wear, lubrication, and contact mechanics are further negatively affected by the loss of cartilage proteoglycans and superficial zone protein (also called *lubricin*).[204-208]

### Muscle Weakening in Mechanical Loading

The major forces on articular cartilage, in addition to weight bearing, are caused by the contraction of the muscles that stabilize or move the joint.[70] For example, in normal walking 4 to 5 times the body weight, and in squatting as much as 10 times body weight, may be transmitted through the knee.[43] Articular cartilage is believed to be too thin to be an effective shock absorber under these high loads. What protects the joint under physiologic conditions of impact loading is joint motion, with the associated lengthening of muscles under tension and deformation of the subchondral bone.[42,70] With aging comes a weakening of these muscles, which correlates with the onset of osteoarthritis. Causative relationships have been investigated between sarcopenia and OA progression, as well as thigh muscle strength and OA. One study showed that sarcopenic obesity was more closely associated with OA than non-sarcopenic obesity.[209] However, data from the Osteoarthritis Initiative suggest that over 4 years the loss of muscle cross-sectional area is not different between painful and non-painful knees,[210] and a comparison of knees with early radiographic OA and contralateral knees with no OA did not support a correlation between loss of muscle cross-sectional area and OA.[211]

### Response of Cartilage to Mechanical Injury

The response of normal articular cartilage to injury typically results in suboptimal repair; these injuries can often result in secondary OA.[212,213] Articular cartilage produces a repair tissue with neither the original structure nor properties of normal cartilage.[214-217] Chondrocytes in areas surrounding an injured zone are unable to migrate, proliferate, repopulate, or regenerate repair tissue with similar structure, function, and biomechanical properties of normal hyaline cartilage.[195,215,218]

That articular cartilage lacks regenerative power has a long history of documentation.[219] In 1851, Redfern reported that articular cartilage wounds healed with fibrous tissue, which he believed arose from chondrocyte intercellular substance.[220] Fisher and Ito in the 1920s proposed that cartilage repair is effected by fibrous tissue resulting from proliferation of cells from bone marrow, synovial membrane, and, occasionally, surrounding articular cartilage.[221,222] It was later observed that the fibrous tissue regenerate subsequently transforms into fibrocartilage, with occasional foci of imperfect hyaline cartilage.[223-226] The common findings of these investigators was that articular cartilage lacks regenerative potential and that the regenerative fibrous tissue and fibrocartilage tissue must have originated from undifferentiated mesenchymal tissue arising from bone marrow, synovium, or the superficial layer of articular cartilage.[219]

One reason the reparative process of cartilage significantly differs from those of other tissues is that it is avascular. The healing response in vascularized tissues consists of three main phases: necrosis, inflammation, and repair.[212,217] Cartilage undergoes the initial phase of necrosis in response to injury, although typically less cell death occurs than in vascularized tissues because of chondrocytes' relative insensitivity to hypoxia.[212,217] The inflammatory phase, primarily mediated (in other tissues) by the vascular system, is largely absent in partial-thickness injuries (i.e., lesions that do not cross the tidemark), and the repair phase is severely limited given the lack of vascularity and a preceding inflammatory response. Thus no local hyperemia results, no fibrin network is produced, no subsequent clot develops to act as a scaffold for the ingrowth of repair tissue, no mediators nor cytokines are released that can stimulate cellular migration and proliferation, and no inflammatory cells, which have mitotic and reparative potential, are recruited.[195,217] In lesions that do not cross the tidemark, the burden of repair falls on the chondrocytes,[217] in a process that has been termed *intrinsic repair*.[227] Although fetal cartilage is capable of mitotic activity and replication, adult chondrocytes have little potential for replication and intrinsic repair.[225,226] Articular cartilage lesions that cross the tidemark may undergo extrinsic repair via differentiation and proliferation of MSCs from para-articular connective tissues; typically however, a fibrocartilaginous regenerate results.[227]

There are three categories of articular cartilage injury: (1) microdamage or repetitive trauma to the matrix and cells; (2) partial-thickness or superficial injuries or chondral fractures, articular surface injuries that do not penetrate the subchondral plate; and (3) osteochondral (full thickness or deep penetrating) injuries, which extend through the tidemark and into the underlying subchondral bone.[212,215,217] The host response to each type of injury differs in both timing and quality of repair.

Microdamage to the chondrocytes and/or extra-cellular matrix without gross disruption of the articular surface can be caused by a single severe impact or repetitive blunt trauma.[212,215,217] Repetitive loading of rabbit cartilage produces a surface loss of proteoglycans and an increase in chondrocyte metabolic activity.[228] Proteoglycans become more easily extractable from the articular cartilage, with a greater percentage of non-aggregated forms.[195] The cellular,

metabolic, and biochemical changes after repetitive blunt trauma resemble those in the early stages of OA: increased hydration, cellular degeneration and/or death, disruption of the collagen ultrastructure resulting in marked variation in the size and arrangements of fibers, fissuring and ulceration of the articular surface, thickening of the subchondral bone, and softening of the cartilage with loss of its compressive and tensile stiffness.[195,215,229-232] Trauma induces the release of degradative enzymes and pro-inflammatory factors (e.g., NO, TNF, IL-1) that frequently cause degradation of the surrounding matrix.[228,233,234] Eventually the material properties of the cartilage are altered—cartilage matrix thins and subchondral bone stiffens—which in turn often accelerates the degenerative process.[195] The point at which accumulated microdamage becomes irreversible is unknown, although it has been demonstrated that lost proteoglycans and matrix components may be restored if damage to chondrocytes and the collagen network is limited and the repetitive trauma is halted.[215]

Necrosis of neighboring chondrocytes follows chondral fractures and superficial lacerations, injuries that do not cross the tidemark.[212,217,235] Within 48 to 72 hours, surviving chondrocytes bordering the defect exhibit increased synthesis rates of extra-cellular matrix molecules and type II collagen, sometimes accompanied by cell proliferation and formation of clusters or clones in the periphery of the injured zone.[195,217,235,236] The increased metabolism and mitotic activity is transient, however, and is followed by a fall in metabolic rate back to normal levels, typically resulting in a suboptimal repair.[195,217] Chondrocytes proliferating on the border of the injured zone do not migrate into the defect, which remains unfilled by the newly synthesized matrix.[195,215,218] In some cases, superficial lacerations in otherwise normal joints may not progress to full-thickness loss of cartilage or OA.[195]

Lesions that cross the articular cartilage tidemark and disrupt the underlying subchondral plate elicit the three-phase repair response normally encountered in vascularized tissues. A hematoma forms in the defect that becomes organized into a fibrin clot, activating an inflammatory response. Transformation of the fibrin clot into vascular fibroblastic repair tissue[217,236] is accompanied by release of cytokines important in stimulating a repair response (e.g., TGF-β, platelet-derived growth factor, IGF, BMPs).[214] These cytokines help set in motion the recruitment, proliferation, and differentiation of undifferentiated cells into a fibrin network that serves as a scaffold for fibrocartilaginous repair tissue.[214,237,238] The origin of these MSCs has been determined to be primarily from the underlying bone marrow,[236,238,239] although there is also evidence of cartilage progenitor cells resident in other joint tissues such as the fat pad and synovium, and even within the cartilage itself.[240-242] These cells progressively differentiate into chondroblasts, chondrocytes, and osteoblasts and synthesize cartilage and bone matrices. At 6 to 8 weeks post-injury, the repair tissue contains a high proportion of chondrocyte-like cells surrounded by a matrix consisting of proteoglycans and type II collagen, with a lesser amount of type I collagen.[239,243,244] Cells in the deeper layers of the defect differentiate into osteoblasts and subsequently undergo endochondral ossification to heal the subchondral bone defect.[195]

This regenerative tissue eventually undergoes a transformation to a more fibrocartilaginous repair accompanied by a shift in the synthesis of collagen from type II to type I.[218,235,237,238,244] Typically, within 1 year from injury, the repair tissue resembles a mixture of fibrocartilage and hyaline cartilage, with a substantial component (20% to 40%) of type I collagen.[243] The size of the osteochondral defect is an important factor in the quality of repair; as a general rule, the smaller the defect the better the repair.[245] Depending on the joint, there exists a critical size defect that will not repair. Fibrocartilaginous repair is susceptible to early degenerative changes because it lacks the biomechanical properties to withstand normal physiologic joint loads.[238,243]

## MECHANOTRANSDUCTION AND GENE EXPRESSION

Chondrocytes can sense and respond to mechanical stimuli via several regulatory pathways (e.g., upstream signaling, transcription, translation, post-translational modification, vesicular transport).[246] Chondrocytes can remodel extracellular matrix in response to alterations in functional demand because physical forces influence the synthesis, assembly, and degradation of the extra-cellular cartilage matrix. High magnitude or duration loads can also cause chondrocyte death and collagen damage, and chondrocytes in the superficial zone appear to be more vulnerable to load-induced injury than those in the middle and deep zones.[247,248] Normal stimuli help chondrocytes maintain the extra-cellular matrix; abnormal stimuli can disrupt this balance.

Mechanotransduction influences the cell-mediated feedback between physical stimuli, the molecular structure of newly synthesized matrix molecules, and the resulting biomechanical tissue properties.[249] Cell-matrix interactions via integrins are believed to be the one of the important mediators in mechanotransduction in chondrocytes. In a study on the expression of COMP related to long-term cyclic compression, it was found that uniaxial unconfined dynamic compression, significantly upregulated COMP expression, which could be blocked by incubation with anti–α1-integrin antibodies.[250] Studies of bovine articular cartilage explants showed that cyclic loading increased protein synthesis by as much as 50% above free-swelling controls and had an inhibitory influence on proteoglycan synthesis, whereas static compression reduced biosynthetic activity.[251] Fibronectin and COMP were the most affected noncollagenous extracellular proteins; static compression caused a significant increase in fibronectin synthesis versus free-swelling control levels, and cyclic compression caused a significant increase in synthesis of COMP and fibronectin.

Human articular chondrocytes use the α5β1 integrin as a mechanoreceptor: mechanical stimulation signals via stretch-activated ion channels, the actin cytoskeleton and focal adhesion complex molecules.[252] The result is an anabolic response, manifested by increased aggrecan and decreased MMP-3 expression. Mechanical stimulation also activates Rho and Rho kinase pathways, which are linked to changes in the actin cytoskeleton.[253,254] Stimulation of the Rho/ROCK pathway in this context is an anabolic stimulus that leads to nuclear translocation and activation of SOX9, which is a "master regulator" of cartilage gene

expression.[255] Indian hedgehog (Ihh) protein is a key signaling molecule that controls chondrocyte proliferation and differentiation and may also be an essential mediator of mechanotransduction in cartilage: Cyclic mechanical stress was shown to induce Ihh expression by chondrocytes.[256]

Dysregulation of these anabolic pathways is thought to contribute to the progression of OA. For example, whereas the α5β1 integrin is also mechanosensitive in OA chondrocytes, the downstream signaling pathways differ, leading instead to increased cartilage breakdown.[257] Integrins and integrin-associated signaling pathways are at least partly regulated by mechanical stimulation by activation of plasma membrane apamin-sensitive $Ca^{2+}$-activated $K^+$ channels; the result is membrane hyperpolarization after cyclic mechanical stimulation.[258] Chondrocytes from normal articular cartilage exhibit membrane hyperpolarization response to cyclic pressure-induced strain, whereas chondrocytes from osteoarthritic cartilage respond by membrane depolarization and exhibit no changes in aggrecan or MMP-3 mRNA following mechanical stimulation.[259,260] The different signaling pathways responding to mechanical stimulation in healthy versus OA chondrocytes may affect the disease outcome.

In addition to cell and matrix deformation, fluid flow is also sensed by chondrocytes. A study using a tissue shear-loading model to uncouple fluid flow from cell and matrix deformation demonstrated that deformation of cells and pericellular matrix alone stimulated protein and proteoglycan synthesis.[261]

## ABNORMALITIES OF BONE

### Osteophyte Formation

Osteophytes—bony proliferations at the joint margins and in the floor of cartilage lesions—are in part responsible for the pain and restriction of joint movement in OA. Human osteoarthritic joint osteophytes synthesize cartilage with significant amounts of type I collagen and nonaggregating proteoglycans.[262] In experimentally induced OA, osteophytes may develop even though the articular cartilage appears grossly normal.[263] Osteophytes may result from penetration of blood vessels into the basal layers of degenerating cartilage, or as a result of abnormal healing of stress fractures in the subchondral trabeculae near the joint margins.[264,265] In the OA dog model, periarticular osteophyte formation begins in the marginal zone, where synovium merges with periosteum and articular cartilage, as early as 3 days after induction of knee instability.[266] Bony proliferation may result from venous congestion; in human hip OA, phlebography has demonstrated the formation of medullary varices, presumably because of changes in the medullary sinusoids, which may be compressed by subchondral cysts and thickened subchondral trabeculae.[267,268] Subchondral cysts in OA may be created by entry of synovial fluid under pressure through defects in the cartilage or may develop in necrotic areas of subchondral bone.[269] The increased venous pressure caused by the cysts and remodeled trabeculae may account for some of the pain in OA. Immobilization and glucocorticoids (but not bisphosphonates) have been shown to decrease the size and prevalence of osteophytes in experimental models of OA.[270-273]

### Subchondral Bone Sclerosis

Cancellous subchondral bone functions as a major shock absorber due to its material properties.[65] Two-thirds of subchondral bone stiffness derives from bony trabeculae, approximately one-third from intra-osseous fluid.[274] In the normal unloaded joint, the opposing surfaces are not congruous; under loading, both the cartilage and the bone deform so that a larger proportion of the opposing surfaces comes into contact, increasing joint congruity and resulting in a force distribution over the largest possible area.[275] Excessive loads may cause microfractures of subchondral trabeculae that heal via callus formation and remodeling, resulting in stiffer-than-normal bone that is less effective as a shock absorber and predisposing articular cartilage to degeneration.

Whether subchondral sclerosis precedes the onset of OA or is a change secondary to cartilage degeneration is not known. Indirect evidence supports the theory that biomechanical changes in subchondral bone may be important in OA.[274,276,277] Foss, for example, reports cases of femoral osteoporosis (which is associated with softening and greater compliance of the subchondral bone) that may have protected the hip from OA.[278] Conversely, in vitro studies have shown that stiffening of the cancellous bone with methacrylate, reducing its deformability, leads to cartilage degeneration with repetitive impact loading.[279] OA patients receiving bisphosphonates to slow bone resorption, showed an early benefit on reduced OA pain and a trend toward less joint space narrowing,[280] yet bisphosphonate treatment of patients with established hip OA did not prevent OA structural progression.[281] In pre-clinical models, preemptive treatment to slow bone resorption was effective in reducing arthritis severity.[282,283] Increased remodeling and hardening of subchondral bone is evident early in osteoarthritis, and can sometimes be detected before loss of cartilage thickness is evident radiologically.[284] An advancing tidemark of calcified cartilage is observed in OA, which changes the mechanical interface between subchondral bone and cartilage and is associated with increased subchondral vascularity. The increased calcification results in a thinning cartilage layer, which increases the mechanical stress in the adjacent cartilage. In combination with subchondral bone sclerosis, the altered mechanical environment and rapid bone remodeling may be contributing factors in cartilage degradation and OA pathogenesis.

### Bone Marrow Lesions

Bone marrow lesions (detectable by MRI) are associated with OA, and studies suggest that they contribute to the pain felt by OA patients.[285] The presence of bone marrow lesions is predictive of OA progression, the development of cartilage defects and degradation, and the need for joint replacement.[286] The relationship between bone marrow lesions and cartilage degeneration in OA is still under investigation. For example, a longitudinal study also found that the presence of cartilage defects predicts progression of bone marrow lesions, and the study authors suggest that it "remains unclear whether bone marrow lesions precede, accompany, or follow cartilage damage and volume loss in OA."[286] To address this question, a study using the

Osteoarthritis Initiative database examined patients with no radiographic OA during a period of time, and found that the presence of bone marrow lesions, and especially multiple types of bone marrow lesions, correlated well with both symptomatic and radiographic OA.[287]

Fibrosis and necrosis of the underlying bone marrow are common histologic features of the lesions visible by MRI, as are high bone turnover, altered trabecular structure, and edema. Unlike cartilage loss, bone marrow lesions are not a permanent structural change in OA. Several reports demonstrate that these lesions can resolve or at least regress, although it is more commonly observed that bone marrow lesion scores increase with time.[274] It has been postulated that edema-like lesions are less severe and reversible, whereas more advanced fibrotic and necrotic lesions are not.[288] There is a possibility that synovial fluid entering the subchondral bone marrow through intra-articular defects leads to an altered growth factor and cytokine environment that affects bone turnover. These findings suggest that there may be an altered biomechanical property of the subchondral tissues, which in turn would affect the biomechanical stresses experienced by the adjacent cartilage.

## ROLE OF INFLAMMATORY MEDIATORS IN DISEASE PROGRESSION

Whereas levels of inflammatory cytokines such as IL-1, IL-6 and TNF are elevated in the serum of patients with knee osteoarthritis,[289] these and other classic inflammatory mediators are activated within the joint tissues themselves during the course of OA. These cytokines autocatalytically stimulate their own production and induce chondrocytes to produce proteases, chemokines, NO, and eicosanoids such as prostaglandins and leukotrienes. The action of these inflammatory mediators within cartilage is predominantly to drive catabolic pathways, inhibit matrix synthesis, and promote cellular apoptosis. Thus, although osteoarthritis is not conventionally considered an inflammatory disease, "inflammatory" mediators from the affected tissues perpetuate disease progression and therefore represent potential targets for disease modification.

### Inflammatory Molecules Produced by Articular Cartilage

#### Cytokines and Chemokines

A characteristic feature of established osteoarthritis is the increased production of pro-inflammatory cytokines, such as IL-1β and TNF, by articular chondrocytes. Both IL-1β and TNF exert comparable catabolic effects on chondrocyte metabolism, decreasing proteoglycan collagen synthesis and increasing aggrecan release via the induction of degradative proteases.[143,290-295] IL-1β and TNF also induce chondrocytes and synovial cells to produce other inflammatory mediators, such as IL-8, IL-6, NO, and prostaglandin $E_2$. The actions of both cytokines are in part mediated by the activation of the transcription factor nuclear factor-κB (NF-κB), which further increases their own expression and that of other catabolic proteins such as inducible NO synthase and COX-2, thus creating an autocatalytic cascade that promotes self-destruction of articular cartilage[296,297] (Figure 98-3). Mounting evidence suggests that NF-κB mediates chondrocytes' pro-inflammatory stress responses in OA and also controls the differentiation of these cells, making NF-κB–activating factors attractive OA therapeutic targets for many reasons.[298] IL-1β and TNF are both synthesized intra-cellularly as precursors, converted through proteolytic cleavage to their mature forms by caspases—membrane-bound IL-1β–converting enzyme (ICE) and TNF-converting enzyme (TACE)—and released extra-cellularly in their active forms.[299] The expression of both ICE and TACE has been shown to be upregulated in OA cartilage.[263-265] Inhibitors of both ICE and TACE are of interest as future therapeutic small-molecule antagonists of downstream IL-1β and TNF expression, respectively. A pre-clinical model of mouse joint injury indicates that local, but not systemic, inhibition of IL-1 prevented osteoarthritis, and interestingly, that inhibition of TNF had no effect.[300] This is in contrast to a study in rabbits in which intra-articular injections of TNF inhibitor protected against experimental OA,[301] and small-scale clinical trials show some potential benefit of TNF inhibitor therapy suggesting that the activity of TNF is involved in OA pathogenesis.[302]

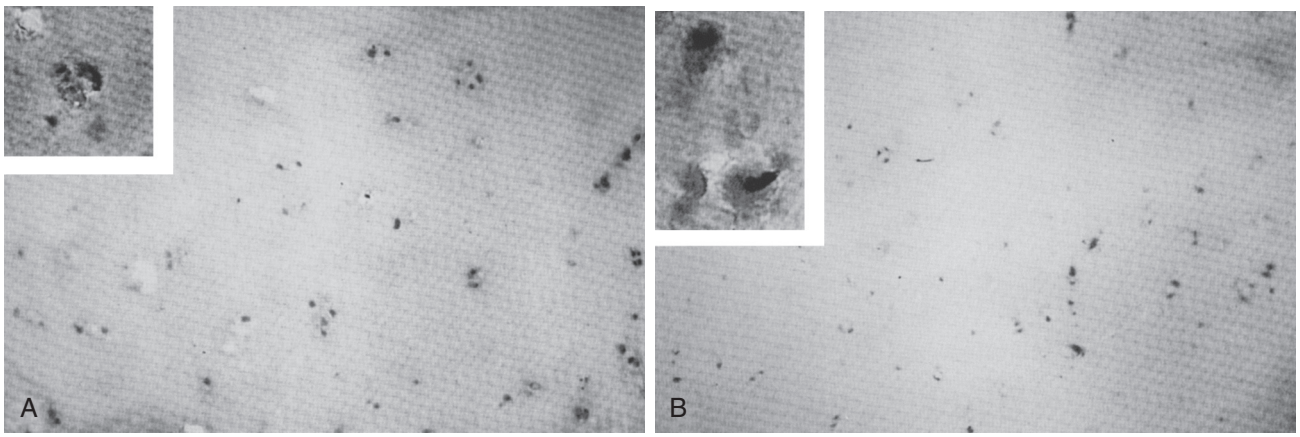



**Figure 98-3** Immunostaining of osteoarthritic cartilage specimen for inducible nitric oxide synthase **(A)** and interleukin-1β **(B)**. Note intense staining of chondrocytes in the superficial zones for both inflammatory proteins. *(From Melchiorri C, Meliconi R, Frizziero L, et al: Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis,* Arthritis Rheum *41:2165–2174, 1998.)*

The actions of IL-1 are dependent upon the engagement of two specific cell surface receptors (IL-1R), designated type I and type II. The type I receptor, which spans the plasma membrane, is responsible for signal transduction, whereas the type II receptor is a "decoy" receptor, expressed at the cell membrane but unable to signal. A relative deficit of the ratio of IL-1Ra (the competitive inhibitor to the IL-1/IL-1R complex) to IL-1 has been described in OA synovial tissue, which may permit increased IL-1 activity.[303,304] Addition of IL-1Ra, or soluble type I and II IL-1 receptors, to OA explant cultures blocks prostaglandin $E_2$ ($PGE_2$) synthesis, collagenase production, and NO production;[290,305] addition of these antagonists in culture also results in an increase in aggrecan content, likely by inhibiting degradation of newly synthesized molecules.[306] One study showed that chondrocytes with abnormal morphology, from macroscopically intact cartilage representative of early OA, demonstrated increased levels of cell-associated IL-1 and loss of type VI collagen.[307]

Encouraging results with IL-1Ra have also been reported in vivo, where gene therapy or the intra-articular administration of IL-1Ra has been shown to retard the progression of OA in experimental animal models.[308,309] Other lines of evidence also point to IL-1B as an essential link in the pathogenesis of cartilage damage, including proteoglycan loss by intra-articular injection of IL-1.[308] Clinical trials using IL-1B antagonists in OA are few and have been inconclusive. One multicenter trial with double-blinded doses of intra-articular IL-1Ra to 14 OA patients resulted in decreased pain without significant adverse events or acute injection reactions.[310] A second clinical trial found short-term improvement of OA pain outcomes with intra-articular injections of IL-1Ra, but no long-term studies of structure-modifying effects of such therapies have been reported.[311]

Osteoarthritic cartilage is also the site of increased production of both CXC and CC chemokines. These include IL-8, monocyte chemoattractant protein (MCP)-1, and normal T cell–expressed and secreted (RANTES: regulated on activation, normal T cell expressed and secreted; also known as CCL5) chemokine, as well as the receptors CCR-2 and CCR-5.[293,312,313] The expression of chemokines is low or undetectable in normal chondrocytes unless stimulated with cytokines such as IL-1 or IL-17.[314] Chemokines are detected by immunohistochemistry in the superficial and midzones of the tissue, as has been demonstrated for such other inflammatory mediators as inducible NO synthase (iNOS), IL-1β, and TNF.[312] RANTES induces expression of its own receptor, CCR-5, suggesting an autocrine/paracrine pathway of the chemokine within the cartilage. MCP-1 and RANTES promote chondrocyte catabolic activities, including induction of NO synthase, increased MMP-3 expression, inhibition of proteoglycan synthesis, and enhancement of proteoglycan release.[312,315] Consistent with these effects, treatment of normal articular cartilage with RANTES increases the release of glycosaminoglycans and profoundly reduces the intensity of safranin O staining.[312]

### Proteinases

A presumed key action of cytokines and chemokines produced in osteoarthritis is to promote cartilage proteolysis via the induction of a wide array of proteases, in particular MMPs. The two main families of MMPs are (1) the collagenases that break down type II collagen (especially MMP-1, -8, -13, and -28) and proteoglycans (MMP-3, which also cleaves pro-MMPs into their active forms), and (2) the aggrecanases, also known as the ADAMTS family, which mediate aggregen degradation in cartilage.[179,190] Both families of MMPs are expressed in OA cartilage at lesional sites, where it presumed that they play a major role in degradation of the extra-cellular matrix. A recent comprehensive analysis of specific metalloproteinases that are overexpressed by OA cartilage and synovium has revealed several MMPs and ADAMTSs that may be candidates as targets for disease modification.[146] Among the most interesting is MMP-13, which is overexpressed in murine and human OA cartilage and is the most efficient protease capable of cleaving type II collagen.[237,316] Similarly, the aggrecanase ADAMTS-5 has surfaced as the aggrecanase required for aggrecan loss in experimental OA[166] and inflammatory joint disease.[165] It has been demonstrated that mice lacking ADAMTS-5 are protected from developing OA.[166]

The expression and degradation of noncollagenous proteins and nonaggregating proteoglycans are also altered in OA cartilage and may have a direct or indirect effect on modulating the catabolic state of the chondrocyte.[317] These groups of molecules are likely to have important structural and/or biologic functions.[318,319] From their interactions with other extra-cellular matrix constituents, they can influence the supramolecular assembly of the cartilage matrix and, as a result, affect the physical properties of the tissue; by interacting directly with chondrocytes and/or neighboring cells, they can provide biologic signals on matrix properties and thereby influence cellular function.[320]

### Nitric Oxide

Nitric oxide, produced by iNOS, is a major catabolic factor produced by chondrocytes in response to pro-inflammatory cytokines such as IL-1β and TNF.[293] Considerable evidence indicates that the overproduction of NO by chondrocytes plays a role in the perpetuation of cartilage destruction in OA[321-323] (Figure 98-4). Increased concentrations of nitrites have been demonstrated in the synovial fluid of patients with OA, and iNOS has been demonstrated in OA synoviocytes and chondrocytes by in situ hybridization and immunohistochemistry.[324,325] Whereas normal cartilage does not express iNOS or produce NO without stimulation by cytokines such as IL-1, OA cartilage explants spontaneously produce large amounts of NO;[321] iNOS is also upregulated from chondrocytes by cartilage compression.[326,327]

NO exerts multiple effects on chondrocytes that promote articular cartilage degradation.[328] These include (1) inhibition of collagen and proteoglycan synthesis,[328] (2) activation of metalloproteinases,[329] (3) increased susceptibility to injury by other oxidants (e.g., $H_2O_2$),[330] and (4) apoptosis.[305] Several studies have implicated NO as an important mediator in chondrocyte apoptosis, a feature common in progressive OA.[330,331] Immunohistochemistry of joint tissue obtained from patients with OA reveals co-localization of iNOS protein and apoptosis in articular cartilage cells.[332] There is evidence that apoptosis results from the formation of peroxynitrite, a toxic free radical produced by the

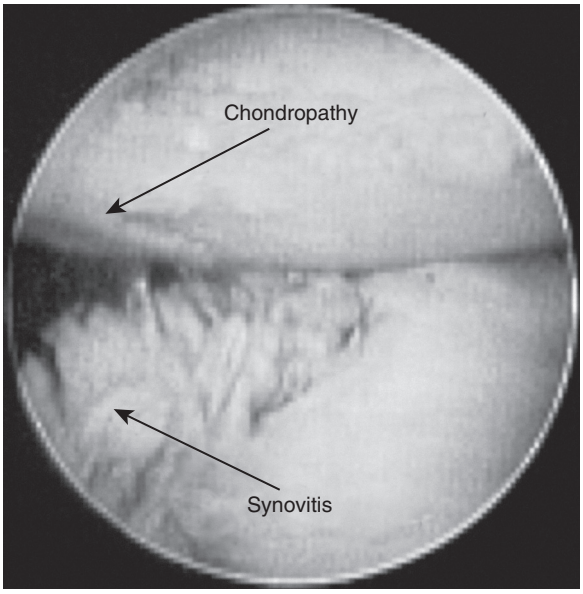



**Figure 98-4** Arthroscopic view of osteoarthritic lesion of the femoral condyle (designated chondropathy). Note that proliferative synovitis is localized to the area of the osteoarthritic lesion. *(Courtesy Maxime Dougados.)*

reaction of NO and superoxide anion. Peroxynitrite reacts with tyrosine residues on proteins, which can be detected by antibodies to nitrotyrosine. Immunostaining of OA cartilage reveals that chondrocytes that are highly positive for IL-1β also stain for nitrotyrosine, consistent with overproduction of peroxynitrite and oxidative damage.[325] The importance of NO has been corroborated in animal models of OA. In the Pond-Nuki canine model, the inhibition of NO reduced the progression of cartilage lesions.[333]

### Transforming Growth Factor-β

In most respects, TGF-β acts as a counter-regulatory molecule that opposes the effects of inflammatory mediators in cartilage. TGF-β1 has been shown to downregulate proteolytic MMP-1 and MMP-13 as well as IL-1 and TNF receptors on OA chondrocytes.[334] TGF-β2 selectively suppresses the cleavage of type II collagen by collagenases in OA cartilage in culture and limits MMP and pro-inflammatory cytokine expression.[294] Studies using the murine knee osteoarthritis model indicate that TGF-β3 protects articular cartilage, because histologic staining for this molecule revealed a lack of TGF-β3 in damaged cartilage in comparison with normals. Although premature osteophyte chondrocyte clusters express high levels of TGF-β3, other data suggest that BMP-2 is more responsible for the development of late osteophyte development.[335] Of note, TGF-β1 may also exert selected catabolic effects via the stimulation of ADAMTS-4 expression.[334]

### Hyaluronic Acid

Hyaluronic acid (HA) has been investigated as a marker of cartilage degradation that can be detected in synovial fluid and serum (see subsequent section Biomarkers of Osteoarthritis),[336] but it also appears to play a role in limiting the progression of arthritis. Karna and colleagues. found that HA in vitro counteracts the ability of IL-1β to inhibit collagen biosynthesis. At the transcriptional and post-transcriptional levels, chondrocyte cultures with IL-1β upregulated collagen synthesis markers, whereas HA negated this effect.[337] The same group found that HA similarly protects against IL-1-induced inhibition of collagen synthesis in human skin fibroblasts at the level of the IGF-I receptor.[338]

### Prostaglandins

The expression of inducible cyclooxygenase-2 (COX-2) is increased in OA chondrocytes, which spontaneously produce $PGE_2$ ex vivo.[313] The effects of prostaglandins on chondrocyte metabolism are complex, and include enhanced type II collagen synthesis, activation of metalloproteinases, and promotion of apoptosis.[339] In cartilage explants, IL-1β induces COX-2 expression and $PGE_2$ production coordinates with proteoglycan degradation. COX-2 inhibition prevents IL-1β–induced proteoglycan degradation, which can be reversed by the addition of $PGE_2$ to cultures.[340] In contrast, in vitro evidence has accumulated that selected NSAIDs) may interfere with proteoglycan synthesis.[341] Of note, another study concluded that as much as 30% of $PGE_2$ expression in OA synovial tissue stems from the COX-1 pathway.[342] Whether any differences exist between the effects of COX-1– and COX-2–derived prostaglandins on cartilage metabolism is unknown.

### F-Spondin

A novel mediator in OA cartilage, F-spondin is a neuronal extra-cellular matrix glycoprotein that appears to regulate cartilage degradation through the TGF-β and $PGE_2$ pathways. One recent article reported a sevenfold increase in expression in human OA cartilage and a significant upregulation in knee cartilage surgical specimens from rats with OA. Addition of F-spondin in vitro to OA cartilage tissue led to increased levels of activated TGF-β and production of $PGE_2$, as well as accelerated collagen degradation and reduced proteoglycan synthesis that are both dependent on these two molecules.[343]

## Inflammatory Mediators in Bone

The inflammatory mediators produced by bone in OA are less well understood than those produced by cartilage and synovium. Biomechanical and biochemical factors seem to influence the remodeling, but the underlying pathogenesis has yet to be identified. NO plays a role in bone cell function, which could have implications for OA insofar as it contributes to alterations in subchondral bone. The endothelial isoform endothelial cell NO synthase (ecNOS) is constitutively expressed in bone, where it seems to play a key role in regulating osteoblast activity and bone formation. ecNOS also mediates the effects of mechanical loading on the skeleton, where it acts along with prostaglandins to promote bone formation and suppress bone resorption.[344] In contrast, pro-inflammatory cytokines, such as IL-1 and TNF, induce iNOS in bone cells, and NO derived from this pathway potentiates bone loss.[293] Osteophyte formation and

subchondral bone remodeling likely result from local production of anabolic growth factors such as IGF-I and mostly TGF-β, which is highly expressed in osteophytes of the femoral head in OA patients.[345,346] Areas of increased radionuclide uptake ("hot spots") on bone scintigraphy have also been reported to identify OA joints more likely to progress by radiographic criteria—and/or to require surgical intervention—over a 5-year period.[260]

## Abnormalities of Synovium

Synovial lining inflammation and synovial effusions have emerged as another key feature of OA pathophysiology. OA has traditionally been classified as a non-inflammatory arthritis, largely because the synovial fluid leukocyte count in OA is typically fewer than 2000 cells/mm$^3$. Such parameters can be misleading, as low-grade inflammatory processes nevertheless occur in osteoarthritic synovial tissues and contribute to disease pathogenesis, and some degree of synovitis has been observed even in early OA. This localized synovitis may be subclinical because arthroscopic studies suggest that localized proliferative and inflammatory changes of the synovium occur in as many as 50% of OA patients, and the activated synovium may produce proteases and cytokines that accelerate damage to the adjacent cartilage.[345,346] More recently, musculoskeletal ultrasound has provided a reliable noninvasive method to detect both synovial hypertrophy and even small effusions in OA patients, using both gray-scale and power Doppler methods.[347]

Many of the clinical symptoms and signs seen in OA joints (e.g., joint swelling and effusion, stiffness, occasional redness) reflect synovial inflammation. Synovial histologic changes include synovial hypertrophy and hyperplasia with an increased number of lining cells, often accompanied by infiltration of the sublining tissue with scattered foci of lymphocytes. In contrast to rheumatoid arthritis (RA), synovial inflammation in OA is mostly confined to areas adjacent to pathologically damaged cartilage and bone. This activated synovium can release proteinases and cytokines that may accelerate destruction of nearby cartilage.[345] Whereas synovial macrophages and macrophage-produced mediators result in key inflammatory cascades and cartilage destruction in both OA and RA, it appears there may be differences between the diseases in levels of the cytokines and how they mediate destruction of the cartilage.[348]

As described earlier, the metalloproteinases that degrade cartilage are produced not only by the cartilage itself but also by the synovium. Although cartilage destruction might be directed by the chondrocytes, some degree of synovitis exists in patients even with mild OA. A comprehensive analysis by Davidson and colleagues reported several pro-inflammatory genes significantly elevated in the synovium that had not previously been reported.[146] Cartilage breakdown products, derived from the articular surface as a result of mechanical or enzymatic destruction of the cartilage, can provoke the release of collagenase and other hydrolytic enzymes from synovial cells and macrophages (Figure 98-5).[349,350] Cartilage breakdown products are also believed to result in mononuclear cell infiltration and vascular hyperplasia in the synovial membrane in OA.[351-353]

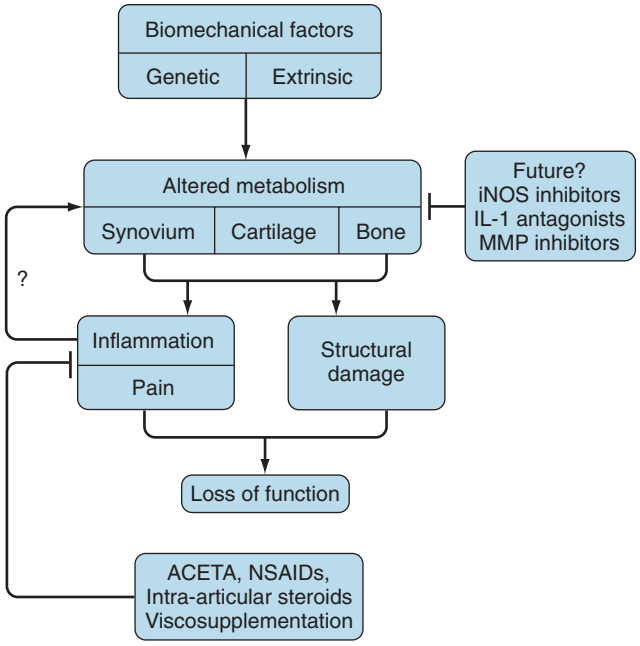



**Figure 98-5** Multiple factors that predispose to, initiate, and perpetuate osteoarthritis. In the future, structure-modifying treatments will be targeted to the biochemical processes that promote disease progression. ACETA, Acetaminophen; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase.

A consequence of the low-grade inflammatory processes is the induction of synovial IL-1β and TNF, which are likely contributors to the degradative cascade.[293] There are also reports of increased numbers of immune cells in synovial tissue, including activated B cells and T lymphocytes, as well as evidence that OA patients express cellular immunity to the cartilage proteoglycan link protein and C1 domain.[342,343] Histologic changes in OA synovium usually demonstrate mild or moderate synovitis characterized by an increase in the number of inflammatory mononuclear cells in the sublining tissue, including activated B cells and T lymphocytes.[354-359] A recent study of 10 patients with early OA (arthroscopic specimens) and 15 patients undergoing total knee arthroplasty revealed that synovial tissues from early OA had higher levels of IL-1β and TNF and increased mononuclear cell infiltration compared with late OA.[360]

Along the lines of acute episodes of OA, the inflammation may result from crystal-induced synovitis (either calcium apatite or calcium pyrophosphate dihydrate). "Milwaukee shoulder syndrome" represents a rapidly destructive form of OA with evidence of inflammation in the synovial membrane but minimal synovial fluid leukocytosis. It is typically associated with rotator cuff degeneration, severe shoulder OA, and hydroxyapatite crystal deposition in the synovial membrane.[361] The synovial fluid typically contains few cells and high levels of active collagenase. It is theorized that crystals released from the degenerating tendons trigger the release of collagenase from synovial mononuclear cells that leads to cartilage breakdown; cartilage breakdown products then further activate release of enzymes from the synovium. This inflammation is typically associated with increases in synovial IL-1 and TNF that further proteinate the degradative cascade.[362]

## BIOMARKERS

The aims of biomarker research in OA are early detection before irreversible damage occurs, predicting OA progression, and monitoring response to therapeutic intervention.[363] The National Institutes of Health–funded OA Biomarkers Network has established the "BIPED" biomarker classification, with five separate categories of surrogate markers: Burden of disease, Investigative, Prognostic, Efficacy of intervention, and Diagnostic.[345] Examples of burden of disease and for prognosis for hip and knee OA include serum COMP, serum hyaluronic acid, and urinary cross-linked C-telopeptide type II collagen (CTX-II).[364] A recent survey revealed that this classification scheme was not adopted as often as anticipated, and therefore the authors undertook a systematic review of the primary OA biomarker literature to standardize the findings according to the BIPED classification.[365]

The major structural components in cartilage are type II collagen and aggrecan, which are relatively unique to cartilage. Additional constituents of articular cartilage include the proteins COMP, cartilage intermediate layer protein (CILP), cartilage link protein, matrilin, minor collagens (types I, V, VI, IX and XI), as well as hyaluronic acid. In healthy cartilage these molecules have a relatively slow turnover rate, whereas OA is characterized by enhanced synthesis and enzymatic degradation of most of these molecules. Therefore much focus in OA biomarkers has been on detecting markers of cartilage matrix synthesis and degradation.[366] The biomarkers have been made more specific through the detection of "neoepitopes" or degraded cartilage matrix fragments generated by specific proteases enhanced in OA. The turnover of subchondral bone is also enhanced in OA, although biomarkers of bone turnover have proven less specific in identifying OA, perhaps because of the continuous bone remodeling in the skeleton as a whole. In addition to markers of matrix degradation and turnover, recent advances in the proteomics and microRNA fields have enabled detection of new OA biomarkers by using unbiased screening of serum constituents in arthritic patients.[130] The slow progression of primary OA is causing researchers to refocus the search for OA biomarkers on more rapidly progressing secondary OA after acute injury.[187]

One study of 62 patients with knee OA compared MRI findings at baseline and at 1 year, as well as levels of serum HA, osteocalcin, cartilage glycoprotein 39, COMP, and urine C-telopeptide of type II collagen, and suggested that a single measurement of serum HA or short-term increases in urine CTX-II would identify patients at greatest risk for progression of OA.[367] A comprehensive study by the Biomarkers Consortium of the Foundation for the National Institutes of Health examined OA biomarkers with commercially available kits. This study concluded that three catabolic biomarkers (CTX-II, CTX-I, and NTX-I) and one anabolic biomarker (PIIANP) were associated with OA progression, and that HA was itself not significantly associated with OA progression, but used in combination with the others it contributed to the predictive power.

Although OA is not considered an inflammatory disease in the traditional sense, it definitely involves inflammatory processes that hold promise as biomarkers for OA. For example, elevated levels of the inflammatory marker C-reactive protein (CRP) appear to be predictive of radiographic progression of long-term knee OA.[293] In a study of 1025 women, higher levels of serum CRP were associated with a statistically significant increase in both prevalent and incident knee OA and greater knee OA severity; women with bilateral knee OA had higher CRP levels than those with unilateral knee OA. Compared with women in whom knee OA did not develop, those in whom it did had a higher baseline CRP than at their index test 2.5 years earlier.[368] In another study of women (age 44 to 67 years), CRP levels were statistically greater in 105 women with knee OA than in 740 women without OA. These results must be interpreted with caution, as a recent study concluded that after adjustment of CRP levels to the patients' body mass index, serum CRP levels were no longer correlated to OA.[369] In summary, these and other studies report that inflammation markers such as CRP are modestly but significantly increased in early knee OA and can be predictive of OA that will progress over time.

Several studies have identified COMP levels as helpful assessors of the potential for, presence of, and progression of OA.[370,371] This noncollagenous extra-cellular matrix protein, synthesized by both cartilage and synovium with TGF-β1 stimulation,[363] is abundant in articular cartilage.[372-375] Both the degradation and tissue distribution of COMP exhibit marked differences in normal and OA human knee articular cartilage. Synovial fluid COMP levels are higher in individuals with knee pain or injury,[376] anterior cruciate ligament or meniscal injury[376,377] and OA[376,378] than in demographically matched healthy individuals. Similarly, serum levels of COMP are often higher in patients with more rapidly progressive joint damage[313,315,379] with some studies showing this specifically in hip OA[317,379] and knee OA.[314] Although COMP is one of the most useful serum markers for OA, the lack of specificity to OA, and relatively high natural variations in serum COMP levels currently necessitate use of additional markers. The development of specific reagents to detect degradative products of COMP may increase its utility as an OA biomarker.[371] As with other biomarkers based on anabolic and catabolic processes in cartilage, serum COMP levels indicate a specific stage of OA.

## CONCLUSION

Osteoarthritis, although classically conceived of as a degenerative consequence of aging, is a disease with an increasingly well-characterized molecular pathophysiology. Biomechanical factors, particularly in the context of genetic predisposition, obesity, and malalignment, result in chemical alterations within the joint that promote cartilage degradation. Early, anabolic changes, characterized by proliferation of chondrocytes and increased matrix production, are followed by a predominantly catabolic state, characterized by decreased matrix synthesis, increased proteolytic degradation of matrix, and chondrocyte apoptosis. Many of the features of the chondrocyte in the catabolic state are related to the production of inflammatory mediators by synovium and chondrocytes that act locally to perpetuate cartilage degradation. Although current treatments improve the signs and symptoms of disease, further characterization

of the altered metabolism in synovium, cartilage, and bone that promote disease progression should lead to future treatments that prevent structural damage in osteoarthritis.

Full references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 99

# Clinical Features of Osteoarthritis



Amanda E. Nelson • Joanne M. Jordan

**KEY POINTS**

- Osteoarthritis (OA) is the most common form of arthritis, typically affecting the hands, hips, knees, spine, and feet.
- OA can be defined radiographically, clinically, or symptomatically.
- As more sensitive measures of damage from OA become available, such as improved imaging and molecular biomarkers, definitions may change.
- Pain and functional limitations contribute substantially to disability in OA.
- Mortality is increased among individuals with OA compared with the general population.

Osteoarthritis (OA), the most common form of arthritis, is found worldwide and is strongly associated with aging. In 2005 it affected 27 million adults in the United States.[1] OA of the knee and hip has a significant functional impact because of effects on ambulation and mobility, is the cause of the majority of joint replacement procedures,[2] and is associated with considerable medical costs. In the United States in 2012, there were 671,374 hospital discharges for knee arthroplasty and 452,615 for hip arthroplasty, with estimated aggregate charges of more than US$36 billion and US$26 billion, respectively.[3] In comparison, 10 years earlier there were 386,265 discharges for knee replacement and 332,164 for hip replacement, with aggregate charges in the US$10 billion range (Figure 99-1).[3] Given the aging of society and the obesity epidemic, we can expect the burden of OA to continue to increase substantially during the next 20 years.[3]

OA affects all of the structures in and around a joint and should be considered a failure of the total joint, with the disease process affecting cartilage, subchondral bone, synovium, ligaments/tendons, meniscus, muscle, and nerve tissues. The clinically recognizable late- or end-stage joint with OA likely represents a final common pathway of many different factors, including genetics, environment, and biomechanical contributors.

## EPIDEMIOLOGY OF OSTEOARTHRITIS

OA can be defined pathologically, radiographically, or clinically. The American College of Rheumatology (ACR) criteria for the classification of OA of the hand, hip, and knee are shown in Table 99-1.[4-6] For the hand, only clinical criteria are used, with a sensitivity of 92% and a specificity of 98%. For the hip, the sensitivity and specificity of the ACR criteria are estimated to be 91% and 89%, respectively, whereas for the knee they are 91% and 86%, respectively. Because of their high specificity, these criteria are most useful for differentiating OA from inflammatory arthropathy, but they are less useful for differentiating individuals with early OA from healthy control subjects. Compared with radiographic definitions, the ACR criteria tend to underestimate the prevalence of OA.[7,8]

Radiographic demonstration of OA usually requires the presence of a definite osteophyte or joint space narrowing on plain radiographs, although MRI-based definitions are being developed.[9] Clinical OA is usually defined by abnormalities on physical examination consistent with OA, such as nodal changes in the hands, limited and painful internal rotation of the hip, or crepitus with knee movement. Symptomatic OA is usually defined as pain, aching, or stiffness in a joint with radiographic OA. Definitions can vary according to the joint site, the frequency or intensity of symptoms, and the time span over which symptoms are assessed.

### Prevalence of Radiographic Osteoarthritis

Because of the multiple definitions of OA and different sources of study participants, prevalence estimates vary. Lawrence and colleagues[1] have reviewed a few population-based studies that have estimated the prevalence of radiographic OA at the knee. Despite variations based on radiographic technique and participant age, these studies estimate that radiographic knee OA is present in 14% to 37% of U.S. adults and is more frequent in women.[10-12] For radiographic hip OA, available estimates vary more widely, from less than 1% to 27%, depending on the population being studied.[13] It is common for hand OA to be identified radiographically, especially in older individuals, but it may not be symptomatic or functionally limiting. One large study found radiographic evidence of hand OA in at least one joint in 67% of women and 55% of men aged 55 years and older, with 28% having radiographic OA in at least two of three hand joint sites (distal interphalangeal [DIP] or proximal interphalangeal [PIP] or carpometacarpal [CMC]).[14] A review of radiographic findings in foot OA reported a prevalence of 12% to 35% for first metatarsophalangeal OA,[15] whereas another review reported a prevalence from 0.1% to 61% for radiographic midfoot and

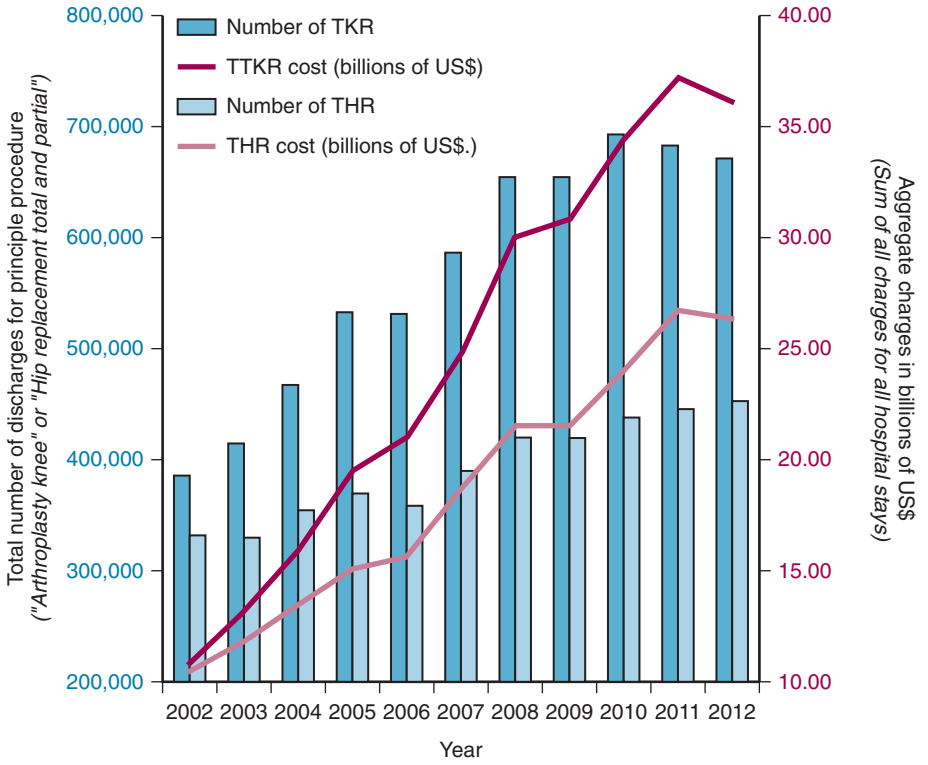



**Figure 99-1** Trends in discharge data for total knee replacement (TKR) and total/partial hip replacement (THR) in the United States from 2002-2012. The *blue bars* demonstrate the number of discharges documented in the Healthcare Cost Utilization Project National Inpatient Sample from 2002-2012 for knee *(dark blue)* and hip *(light blue)* replacement, whereas the *red lines* show the aggregate charges (sum of all charges for all hospital stays for each procedure, or the "national bill") for knee *(dark red)* and hip *(light red)* replacement. *(Generated from data on* hcupnet.ahrq.gov *on August 13, 2014.)*

forefoot OA from 30 observational and case-control studies.[16] Ankle OA, which is generally thought to be rare and usually is a result of trauma, was found to be relatively common in a cohort of patients with knee OA, with 15% having radiographic evidence of ankle OA despite a low frequency of reported injury or surgery (approximately 4%).[17] The prevalence of spinal facet joint OA is also quite high; it is reported as affecting 60% of men and 67% of women by computed tomography (CT) in the Framingham Heart Study[18] and 58% overall by radiography in the Johnston County OA Project.[19] Disk space narrowing and osteophytosis of the lumbar spine are also frequently seen on radiographs.[19]

## Prevalence of Symptomatic Osteoarthritis

A summary of prevalence estimates for symptomatic OA (i.e., symptoms in the presence of radiographic evidence of OA) of the hands, knees, and hips is presented in Table 99-2.[1] On the basis of these findings, Lawrence and colleagues[1] estimated that more than 9 million U.S. adults are affected by symptomatic knee OA and that more than 13 million have symptomatic hand OA. Nguyen and colleagues.[20] used data from the National Health and Nutrition Examination Survey (NHANES) and the Framingham study to explore trends in the frequency of symptomatic knee OA and found a dramatic increase in self-reported knee pain over approximately 20 years but no significant change in radiographic knee OA, resulting in an overall higher prevalence of symptomatic knee OA over time.

## Racial Differences in OA Prevalence

Racial differences in the prevalence of OA have been reported at several different body sites.[21] Radiographic and symptomatic knee OA were more common among African Americans compared with white people.[10,12] In the Johnston County OA Project, radiographic knee OA was present in 32% of African Americans versus 27% of whites, whereas symptomatic knee OA was present in 19% of African American and 16% of white participants.[12] Compared with white women, Chinese women had a higher prevalence of radiographic knee OA, bilateral radiographic knee OA, and symptomatic knee OA, whereas Chinese and white men had similar frequencies of these outcomes.[22] African Americans had similar[23] to slightly higher[24] frequencies of radiographic and symptomatic hip OA compared with white people, despite an apparently lower prevalence of hip OA among African populations.[25,26] Chinese people had overall lower frequencies of hip OA compared with white populations.[27] Symptomatic hand OA was present at similar frequencies in white, African American, and Mexican-American participants in NHANES III, although asymptomatic hand OA was more common in white people.[21] African Americans were much less likely to have radiographic or symptomatic hand OA phenotypes in a substudy of the Johnston County OA Project.[28,29] Compared with whites, Chinese people were also less likely to have radiographic or symptomatic hand OA.[30] Although comparisons of radiographic foot OA by race are not available in the literature, among nonobese participants in the Johnston

**TABLE 99-1** American College of Rheumatology Radiologic and Clinical Criteria for Osteoarthritis

**Hand**[5]
1. Hand pain, aching, or stiffness on most days of prior month
2. Hard tissue enlargement of ≥2 of 10 selected joints*
3. Fewer than 3 swollen MCP joints
4. Hard tissue enlargement of ≥2 DIP joints
5. Deformity of ≥2 of 10 selected joints*

Diagnosis requires items 1-3 and either 4 or 5

**Knee: Clinical**[6]
1. Knee pain for most days of prior month
2. Crepitus with active joint motion
3. Morning stiffness lasting ≤30 min
4. Bony enlargement of the knee on examination
5. Age ≥38 yr

Diagnosis requires 1 + 2 + 4, or 1 + 2 + 3 + 5, or 1 + 4 + 5

**Knee: Clinical and Radiographic**[6]
1. Knee pain for most days of prior month
2. Osteophytes at joint margins
3. Synovial fluid typical of osteoarthritis
4. Age ≥40 yr
5. Morning stiffness lasting ≤30 min
6. Crepitus with active joint motion

Diagnosis requires 1 + 2, or 1 + 3 + 5 + 6, or 1 + 4 + 5 + 6

**Hip: Clinical and Radiographic**[4]
1. Hip pain for most days of the prior month
2. ESR ≤20 mm/hr
3. Radiographic femoral and/or acetabular osteophytes
4. Radiographic hip joint space narrowing

Diagnosis requires 1 + 2 + 3, or 1 + 2 + 4, or 1 + 3 + 4

*Ten selected joints: DIP 2-3, PIP 2-3, and CMC 1 bilaterally.

CMC, Carpometacarpal; DIP, distal interphalangeal; ESR, erythrocyte sedimentation rate; MCP, metacarpophalangeal; PIP, proximal interphalangeal.

From Altman R, Asch E, Bloch D, et al: Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 29:1039–1049, 1986; Altman R, Alarcon G, Appelrouth D, et al: The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. *Arthritis Rheum* 34:505–514, 1991; and Altman R, Alarcon G, Appelrouth D, et al: The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. *Arthritis Rheum* 33:1601–1610, 1990.

**TABLE 99-2** Prevalence of Symptomatic Osteoarthritis

| Site (Age, Yr) | Source | % WITH SYMPTOMATIC OA | | |
|---|---|---|---|---|
| | | Male | Female | Total |
| Hands (≥26) | Framingham[127] | 3.8 | 9.2 | 6.8 |
| Knees | | | | |
| ≥26 | Framingham[11] | 4.6 | 4.9 | 4.9 |
| ≥45 | Framingham[11] | 5.9 | 7.2 | 6.7 |
| ≥45 | Johnston County[12] | 13.5 | 18.7 | 16.7 |
| ≥60 | NHANES III[10] | 10.0 | 13.6 | 12.1 |
| Hips (≥45) | Johnston County[24] | 8.7 | 9.3 | 9.2 |

NHANES, National Health and Nutrition Examination Survey; OA, osteoarthritis.

From Lawrence RC, Felson DT, Helmick CG, et al: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum* 58:26–35, 2008.

**TABLE 99-3** Causes of Secondary Osteoarthritis

**Metabolic**
Crystal-associated arthritis
  Calcium pyrophosphate or apatite deposition
Acromegaly
Ochronosis
Hemochromatosis
Wilson's disease
Hyperparathyroidism
Ehlers-Danlos syndrome
Gaucher's disease
Diabetes

**Mechanical/Local Factors**
Slipped capital femoral epiphysis
Epiphyseal dysplasias
Legg-Calvé-Perthes disease
Congenital dislocation
Femoroacetabular impingement
Congenital hip dysplasia
Limb-length inequality
Hypermobility syndromes
Avascular necrosis/osteonecrosis

**Traumatic**
Joint trauma (e.g., ACL tear)
Fracture through joint
Prior joint surgery (e.g., meniscectomy, ACL)
Charcot joint (neuropathic arthropathy)

**Inflammatory**
Rheumatoid arthritis or other inflammatory arthropathies
Crystalline arthropathy (gout)
History of septic arthritis

ACL, Anterior cruciate ligament.

Modified from Altman R, Asch E, Bloch D, et al: Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 29:1039–1049, 1986.

County OA Project, African Americans were more likely to have hallux valgus and hammer toes[31] and as likely as white individuals to have foot symptoms.[32] African Americans were less likely to have disk space narrowing, spinal osteophytes, or facet joint OA compared with white people.[19]

## Primary and Secondary Osteoarthritis

Historically, osteoarthritis was considered to be "primary" in the absence of an injury history or other joint disease and "secondary" if a predisposing disorder was present (Table 99-3). However, as more and more local risk factors for OA have been identified (such as femoroacetabular impingement at the hip and malalignment at the knee) and a broader range of associated factors have been discovered (genetic, biomechanical, and environmental), the division between primary and secondary OA is less clear. Many individuals who experience secondary OA are likely predisposed to the condition with or without the identified inciting event. Others who have a disorder that is linked to secondary OA may not experience clinical OA. It may be most useful to think of OA as a common pathway through which a person's genetics, history of injury or other joint damage, mechanical factors, and psychosocial milieu act on the joint, in some cases leading to an "end-stage" or "failed" joint.

## CLINICAL FEATURES

### General Symptoms and Signs

OA most commonly affects the knees, hands, feet, hips, and spine. These joints may be symptomatic, or signs of OA may be identified only on radiographs. People with OA generally describe pain in the joint(s) that is worse with activity, with limited morning stiffness (<30 minutes) and pain and stiffness with rest. This stiffness after inactivity, or "gelling" phenomenon, is often a main feature, although morning stiffness is generally less severe and of shorter duration than that seen in individuals with systemic inflammatory arthropathies. Affected joints in OA often demonstrate bony enlargement and crepitus on examination, with concomitant reductions in range of motion. Soft tissue swelling or effusion may be present, although these symptoms tend to be much less dramatic than in inflammatory arthritis. Reports of pain may be more or less than expected on the basis of structural damage.[33,34] The effects of depression, disturbed sleep, and other psychosocial factors on the pain experience in individuals with OA are increasingly being recognized.[35-37] Awareness is also increasing of the contribution of central sensitization (i.e., enhanced neural signaling leading to hypersensitivity) to chronic pain in OA, which can result in pain remote to the affected joint site and loss of pain inhibitory mechanisms. Ongoing study is needed to determine optimal methods of identification and treatment of this issue.[38]

### Joint-Specific Symptoms and Signs: Knee

Knee OA is characterized by the insidious onset of knee pain with gelling and limited range of motion. People with knee OA often describe pain and limitation with walking, transferring (as from seated to standing), and, especially, ascending or descending stairs. These reports of pain and limitation are often associated with a sensation of instability or "giving out" at the knee. A "locking" sensation at the knee can be a consequence of stiffness, loose bodies in the joint space, or meniscal lesions and may be reproducible using the McMurray test. Knees with OA often have demonstrable crepitus (although this symptom is likely more indicative of patellofemoral than tibiofemoral disease[39]) and bony enlargement. Pain may be elicited by palpation of the medial or lateral joint line, or both. Effusions, when present, are often cool and generally without erythema. They can be associated with popliteal bursa enlargement (a Baker cyst) when large. Associated pain over the anserine bursa, or even the greater trochanter, often occurs in individuals with knee OA and may be related to altered biomechanics.[40] Appreciation of such soft tissue symptoms is important because they may be amenable to corticosteroid injections with subsequent pain relief.

Malalignment, usually varus, is often seen in individuals with severe disease but can be present even in individuals with fairly mild/early-stage disease. Clinically evident varus thrust may be a risk factor for progression.[41] Other signs of severe disease include flexion deformities or joint instability. Quadriceps weakness represents an early modifiable risk factor for knee OA progression, particularly in women,[42,43] and in late stages of disease, it may be apparent as muscle atrophy.[44,45] Alterations in proprioception and vibratory sense have been demonstrated in association with knee OA, although the relation of these factors to progression and pain is still unclear.[46,47] Patellofemoral OA may be more common than tibiofemoral OA[48] and can strongly contribute to pain and disability at the knee, but it is often overlooked.[49] OA of the patellofemoral joint is characterized by crepitus and pain with ascending or descending stairs and is often located anteriorly. It can be seen in isolation or in association with tibiofemoral OA.[48] The relation between patellofemoral OA and commonly seen patellofemoral pain disorders in younger individuals remains to be elucidated.[50]

### Joint-Specific Symptoms and Signs: Hip

OA of the hip can present with groin pain, which is fairly specific,[51] but it may be described more vaguely as pain in the thigh, buttock, low back, and even in the ipsilateral knee. Therefore it is important to assess for other causes of pain in the "hip," including spinal pain (e.g., lumbar disk degeneration, spinal stenosis, facet joint OA, and sacroiliac pain); trochanteric bursitis; altered gait due to knee disease; meralgia paresthetica (i.e., lateral femoral cutaneous nerve entrapment); thigh claudication from vascular causes; or even intrapelvic causes. It is also important to consider other causes of hip and groin pain such as occult femoral neck fracture or avascular necrosis. People affected by hip OA have limitations in walking, bending, and transferring, as well as stair climbing. Internal rotation of the affected hip is often limited and can be quite painful, even in early-stage disease; it is often evident to the patient because of through difficulty in putting on socks, tying shoes, or trimming toenails. Visible deformity, hip flexion contracture, or severe limitations of range of motion are indicators of more advanced disease, which may also be associated with shortening of the affected limb as a result of superior migration of the femoral head. In young individuals presenting with groin pain that is worsened by sitting and who have pain and limitation when internally rotating and adducting the hip in the flexed position, femoroacetabular impingement is a consideration.[52]

### Joint-Specific Symptoms and Signs: Hand

The hands often provide the first clue to a diagnosis of OA. As previously mentioned, evident bony enlargement of the DIP joints (Heberden's nodes) and PIP joints (Bouchard's nodes) can be appreciated (Figure 99-2). These nodes may be acutely inflamed with warmth and tenderness or may be bland, hard enlargements and are often more marked in the dominant hand. Some patients, most commonly older women, have erosive OA characterized by episodic inflammation, pain, and swelling. It remains a matter of debate whether this type of OA is part of the continuum or is a separate disease entity.[53,54] OA involvement of the first CMC joint is particularly problematic and can lead to significant pain, limitations in functionality of the hand, and reduced grip strength.[55,56] CMC squaring, representing deformity of the joint as a result of osteophyte formation and joint space narrowing, can be seen on examination. Bilateral involvement of multiple joints is frequent, both within joint groups (multiple PIPs) and across joint groups (both DIPs and PIPs). Metacarpophalangeal joints are

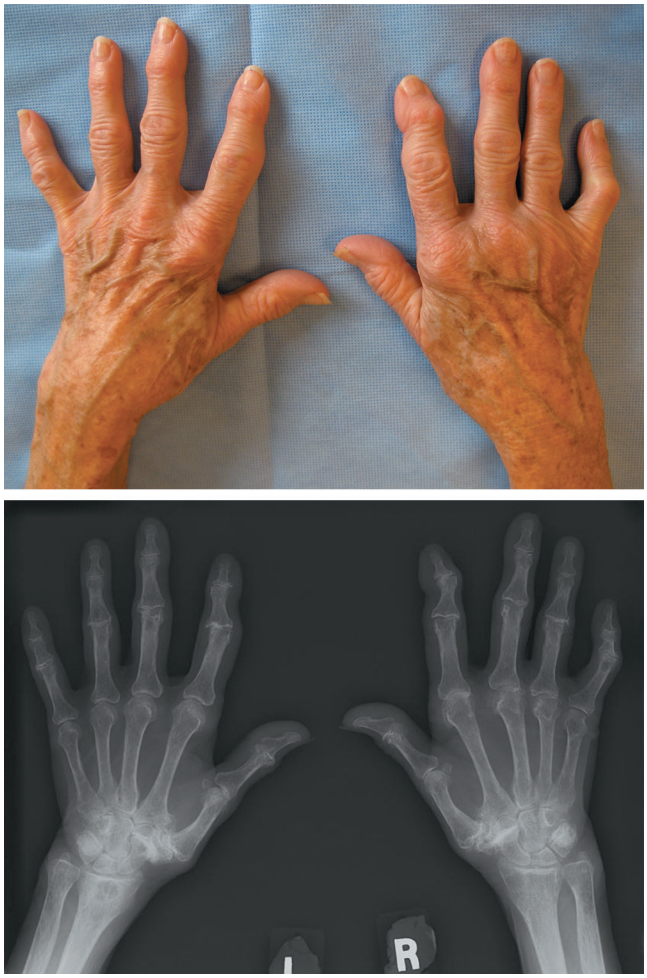



**Figure 99-2** The hands of a 79-year-old woman showing clinical *(top)* and radiographic *(bottom)* features of osteoarthritis (OA). This patient has Heberden's and Bouchard's nodes of multiple digits; radiographs show osteophytes, joint space narrowing, and cysts typical of OA, as well as "gull-wing" deformities at the third proximal interphalangeal joints suggestive of erosive OA.

affected more commonly than previously recognized,[57] although prominent involvement of these joints should prompt consideration of inflammatory/crystalline arthropathies or secondary causes of OA, such as hemochromatosis.[58] Soft tissue conditions such as De Quervain's tenosynovitis should be considered because they are associated with hand OA, can mimic or aggravate symptoms, and are amenable to conservative management.

## Joint-Specific Symptoms and Signs: Spine

Facet joint OA is prevalent among older adults. In one study, it was found that cervical spine facet joint OA and lumbar spine facet joint OA were present in 57% and 89%, respectively, of community-living adults older than 65 years.[59] The association between facet joint OA and back pain has been controversial, although a recent study using CT assessments of lumbar facet joint OA identified associations between severe facet joint OA and back pain, with an odds ratio greater than 2; individuals with multiple affected facet joints were also more likely to have back pain.[60] Facet joint OA is associated with OA of other joint sites, including the knees and hands.[19]

Osteophytosis of the spine is nearly ubiquitous in older individuals, although it is often asymptomatic.[61] Lumbar disk degeneration (LDD), characterized by disk space narrowing, end-plate sclerosis, and herniation, is often seen in association with radiographic osteophytosis,[62] although the relationship between LDD and OA of other joint sites remains controversial. [19,63]

Symptomatically, these findings can be associated with radiculopathy, a pathologic process of the nerve roots most commonly seen in older patients as a consequence of hypertrophied facet joints or uncovertebral joints, disk protrusion, osteophytosis of the vertebral body, or a combination of these features.[64,65] Cervical radiculopathy most commonly involves the seventh cervical nerve root and is caused by degenerative changes at the C6-C7 level.[64] Patients may present with pain/numbness in the shoulders, triceps area, forearm, and dorsal long finger, and triceps weakness or loss of reflex may be present.[64] Lumbosacral radiculopathy, frequently due to disk degeneration (particularly at lower lumbar levels), presents with radiating back pain that is usually exacerbated by leaning forward, sitting, or straining and is relieved by lying down.[65] Specific clinical presentation varies by level, with the lower levels more commonly affected. L4 radiculopathy can result in medial lower leg sensory changes, weak knee extension and hip adduction, and potentially reduced knee jerk.[65] At L5, foot drop and sensory symptoms of the anterolateral leg and dorsal foot may be seen, whereas S1 radiculopathy is characterized by weakness of plantar flexion, knee flexion, and hip extension with reduced ankle jerk.[65] Cauda equina syndrome, a medical emergency, occurs as a result of compression of the lower lumbar and sacral nerve roots by a central disk herniation, commonly at L4-L5. This syndrome results in bowel and bladder dysfunction, saddle anesthesia, and leg weakness.[65]

Diffuse idiopathic skeletal hyperostosis, or DISH, is characterized by exuberant osteophytosis and calcification of spinal ligaments and entheses, leading to an appearance likened to flowing candle wax.[66] People with DISH can be asymptomatic and may be identified only by radiographic appearance, or they can present in a fashion similar to that of individuals with OA or LDD. Large anterior cervical spine osteophytes, which are more often seen in DISH but are possible in LDD, can cause dysphagia as a result of compression of the esophagus.[67]

## Joint-Specific Symptoms and Signs: Shoulder

The shoulder is a common source of pain in people with OA, although the symptoms are more often due to osteophytosis and narrowing of the acromioclavicular and/or sternoclavicular joints than of the glenohumeral joint itself. Other maladies common in older adults, such as subacromial bursitis, rotator cuff disease, and adhesive capsulitis, should be assessed. Rotator cuff damage, in particular, can predispose to glenohumeral OA. Disease at the cervical spine can present as pain in the shoulder region, and thus the shoulder evaluation should include an assessment of the cervical spine. Milwaukee shoulder syndrome[68] is a rare

destructive arthropathy of the glenohumeral joint associated with large effusions, which is bilateral in 60% of cases and also affects the knees in 50% of cases.[69] Radiologically, periarticular calcifications and cuff rupture may be seen, and synovial fluid examination often reveals high red blood cell content and basic calcium crystals (particularly if Alizarin red staining is used).[69]

## Joint-Specific Symptoms and Signs: Other Joints

OA of the foot has been largely neglected in the literature to date. The foot joints most commonly affected by OA include the talocalcaneal joint, talonavicular joint, calcaneocuboid joint, metatarsocuneiform joint, and first metatarsophalangeal (MTP) joint; these joints can be affected by erosive OA as well.[70] OA of the first MTP joint is characterized by pain and hallux valgus (bunion) deformity and has radiographic features (e.g., joint space narrowing and osteophytosis) similar to OA at other joints. Loss of function at the first MTP joint as a result of ankylosis (hallux rigidus) can lead to altered gait. Ankle OA likely occurs more frequently than previously thought and may be due to biomechanical alterations from OA at other joints, in addition to accepted post-traumatic and injury-related etiologies.[17] Elbow OA is rare and may be the result of trauma, vibration damage, or other entities such as pseudogout. Temporomandibular joint OA is largely ignored in the orthopedic and rheumatologic literature but is well researched in the dental literature; it results in pain, clicking, locking, decreased range of motion, and crepitus of the joint, much like in other joints affected by OA.[71]

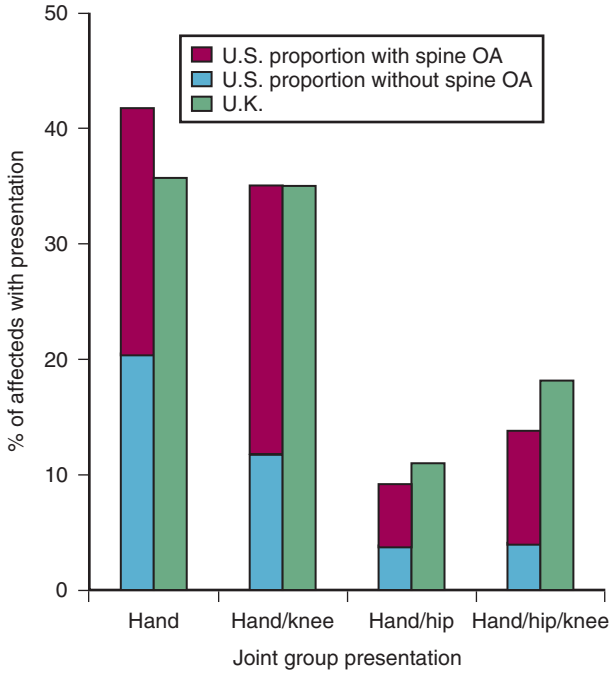



**Figure 99-3** The frequency of multiple joint involvement with radiographic osteoarthritis (OA) in the Genetics of Generalized Osteoarthritis Study. The frequencies of radiographic OA at various sites and sites in combination are shown for the United States (U.S.) in *blue*. For the United Kingdom (U.K.) they are shown in *green* for the 1963 participants meeting specified hand radiographic OA criteria. The groups represented are mutually exclusive. The frequency of spine OA (only assessed at U.S. sites) in combination with these phenotypes is indicated by the *red bars. (Modified from Kraus VB, Jordan JM, Doherty M, et al: The Genetics of Generalized Osteoarthritis [GOGO] study: study design and evaluation of osteoarthritis phenotypes.* Osteoarthritis Cartilage *15:120–127, 2007.)*

## Polyarticular Osteoarthritis

For more than 100 years, it has been recognized that OA can simultaneously occur in multiple joint sites.[72] Although the term *generalized* OA (GOA) has been used to describe this disease entity, there is no universally understood or accepted definition of what constitutes GOA (Figure 99-3).[73] In 1952, Kellgren and Moore provided the first clinical description of GOA, involving primarily Heberden's nodes and the CMC joints, with the spine, knees, hips, and feet involved in descending frequency.[74] Later studies have defined GOA as more than three or more than five joint sites affected,[75] affected joint counts,[76,77] multiple hand joint involvement,[78] nodal hand OA with other joint involvement,[57] or summed scores of OA across multiple joints.[79,80] It remains unclear which joints should be included in such definitions, and particularly whether lumbar disk degeneration and hip OA are part of the same disease entity as other joints. Clinically, it is important to recognize that a patient with OA in one joint is likely to have involvement at other sites and to assess the impact of that person's overall OA burden on their functional status, rather than focusing on a single joint.

## Nodal Osteoarthritis and Genetics

Nodal OA, which was described by Heberden in the 1800s, is a clinically apparent phenotype affecting 58% of U.S. adults older than 60 years in NHANES III.[81] Increasing numbers of nodes are associated with greater pain and functional limitation and reduced patient satisfaction.[53] The inheritance patterns of nodal OA have been studied in some detail, beginning with Stecher in the 1940s.[82] Using pedigrees and modes of mendelian inheritance, he determined that idiopathic Heberden's nodes "depend upon a single autosomal gene sex influenced to be dominant in females and recessive in males."[82] This method of inheritance was supported by an independent study 6 decades later.[83] A genetic predisposition to Heberden's nodes was also supported in a twin study in which the intraclass correlation among monozygotic twins was much higher than that among dizygotic twins (0.49 and 0.24, respectively; $P = 0.02$).[84] A microsatellite analysis identified an association between nodal OA and chromosome 2q.[85] Evidence exists of genetic anticipation in nodal OA as well, with both nodal symptom onset and associated large joint OA occurrence at significantly younger ages in offspring compared with parents (during the 40s in offspring compared with the 60s in parents).[86] More recently, in 2006, a genome-wide linkage study identified several loci associated specifically with nodal OA.[87] In another study, differences in risk factor associations were apparent in individuals with nodal OA who were undergoing total joint replacement (as a marker of severe large joint OA), compared with individuals who had non-nodal OA.[41] These investigators found that the

association between age and joint replacement was significantly stronger in nodal versus non-nodal OA; a positive association between female sex and joint replacement was apparent only for people with nodal OA; body mass index demonstrated a stronger association among people with non-nodal compared with nodal OA; and people with the nodal phenotype had a higher prevalence of OA at more than one joint.[88] Therefore nodal OA is a clinically apparent and useful phenotype that is associated with multiple joint OA involvement and likely has specific genetic risk factors.

# DIAGNOSTIC TESTING

## Diagnostic Approach

OA is diagnosed clinically, and laboratory testing is rarely required. Similarly, if the diagnosis is clear on the basis of history and clinical findings, radiographs often are not required. The purpose of additional diagnostic testing in OA is primarily to exclude potentially treatable underlying conditions. Depending on the patient's presentation, the diagnosis may be straightforward, or the differential diagnosis may include inflammatory arthropathies, such as rheumatoid arthritis, crystalline arthropathies (particularly calcium pyrophosphate crystal deposition disease), spondyloarthropathies, or a variety of other inflammatory conditions. Particularly in the case of erosive/inflammatory OA, there may be significant clinical and radiographic overlap with psoriatic arthritis, requiring further evaluation. If the presentation is of a monoarthritis, evaluation for infectious causes should be performed. Finally, trauma and other mechanical disorders (e.g., tendon or ligament injury and meniscal tears) may be seen prior to, as a part of, or subsequent to the development of OA in a given joint.

## Laboratory Testing

There is rarely an indication for testing rheumatoid factor or anti-nuclear antibodies or for performing other serologic studies in the setting of clinically suspected OA; such tests should be reserved for patients with findings suggestive of an inflammatory arthropathy. A complete blood cell count, chemistry panel, including glucose, creatinine, and liver function tests, should be obtained before initiation of pharmacologic therapy for OA, especially in older individuals with comorbid medical conditions, because of an increased risk of adverse events in this population. In cases in which there is prominent involvement of the metacarpophalangeal (MCP) joints, evaluation for hypothyroidism and hemochromatosis may be warranted.

### Synovial Fluid

The synovial fluid in people with OA is typically normal or mildly inflammatory, appearing clear and colorless to slightly yellowish. The leukocyte cell count is typically less than or equal to 2000 cells/mm$^3$ (<2 cells seen across 10 high-power fields).[89] Fluid is often obtained in the course of an intra-articular injection for a symptomatic joint, although diagnostic aspiration may also be performed in the setting of an effusion. Concomitant calcium pyrophosphate crystal disease can be identified, although other calcium crystals, or hydroxyapatite, are not seen on routine preparations.

### Molecular Biomarkers

Quantitative determination of the products of cartilage and bone metabolism has provided several putative biomarkers of OA pathophysiology. For example, one such biomarker, urinary C-telopeptide fragments of type II collagen (u-CTXII), is associated with the occurrence and progression of radiographic OA, independent of other risk factors.[80] Such biomarkers, obtained from serum, urine, or synovial fluid, eventually may provide a method for early diagnosis and monitoring of treatment effect, although currently they are used primarily in research. Interested readers are referred to a comprehensive review from 2010,[90] a 2014 overview of the 12 biomarkers with the most supportive evidence of OA associations to date,[91] and Chapter 98 in this book for additional details.

## Imaging: Conventional Radiography and General Considerations

Conventional radiography is widely available in clinical settings and is relatively inexpensive, making this modality useful to confirm the diagnosis of OA and exclude others, primarily when there is clinical uncertainty regarding the diagnosis, when the joint involved is atypical, or when evidence suggests other diagnoses such as inflammatory arthritis, fractures, Paget's disease, osteonecrosis, infection, or malignancy. Radiographs of joints affected by OA typically show osteophytes, joint space narrowing, sclerosis, and cysts of subchondral bone, which will be described qualitatively on clinical radiographs.

For research purposes, semiquantitative and quantitative assessments are used. The semiquantitative Kellgren-Lawrence (KL) grading system remains the most commonly used for research purposes.[92] KL grades range from 0 (no osteophytes or joint space narrowing) to 4 (severe joint space narrowing with subchondral sclerosis); a grade of 2 is generally considered diagnostic of OA. The KL system has several limitations, including variable definitions in the literature[93] and the categorization of all "definite" joint space narrowing as KL grade 3, thus reducing sensitivity to change, although modifications have been suggested to address this issue.[94] Additionally, identification of joint space narrowing on radiographs is not a specific finding indicative of cartilage loss but can also be reflective of meniscal disease. Other semiquantitative grading systems, such as the Osteoarthritis Research Society International (OARSI) grading system,[95] view osteophytes and joint space narrowing separately and assign grades for these specific OA features individually for various joint compartments (Figure 99-4). The choice of grading system depends on the goal of the study and the joint site of interest. Quantitative joint space width, on the other hand, is a direct measure of the space between the femur and tibia that is performed either manually or in a semi- or fully automated manner. Quantitative joint space width is commonly assessed as the minimum joint space width, but it has also been explored at fixed locations; the latter method has been shown to be comparable to MRI in the detection of OA progression.[96]

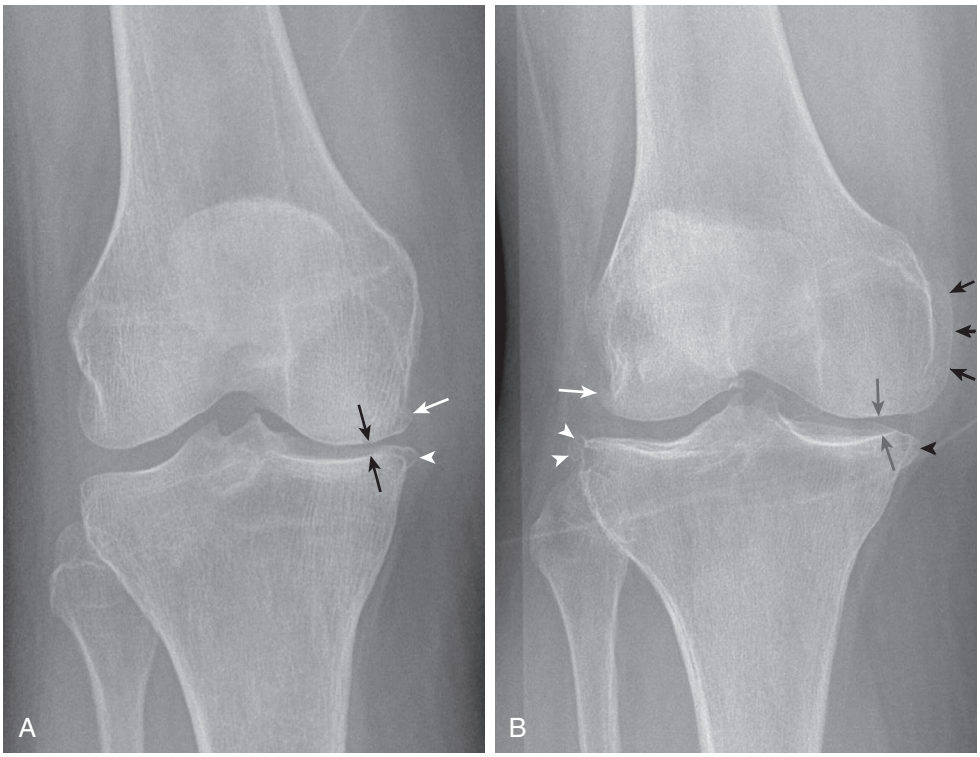



**Figure 99-4** Examples of semiquantitative radiographic assessment with use of the Kellgren-Lawrence and Osteoarthritis Research Society International (OARSI) grading schemes. **A,** Kellgren-Lawrence grade 3. No lateral femoral and tibial osteophytes are seen (OARSI grade 0). A medial femoral osteophyte: OARSI grade 1 *(white arrow);* a medial tibial osteophyte: OARSI grade 2 *(white arrowhead);* lateral tibiofemoral joint-space width: OARSI grade 0; and medial tibiofemoral joint-space narrowing: OARSI grade 2 *(black arrows)* are depicted. **B,** Kellgren-Lawrence grade 2. A lateral femoral osteophyte: OARSI grade 2 *(white arrow);* a lateral tibial osteophyte: OARSI grade 2 *(white arrowheads);* a medial femoral osteophyte: OARSI grade 3 *(short black arrows);* a medial tibial osteophyte: OARSI grade 2 *(black arrowhead);* a normal lateral tibiofemoral joint-space width: OARSI grade 0; and medial tibiofemoral joint-space width: OARSI grade 1 *(long black arrows)* are shown. *(From Guermazi A, Hunter DJ, Roemer FW: Plain radiography and magnetic resonance imaging diagnostics in osteoarthritis: validated staging and scoring.* J Bone Joint Surg Am *91[Suppl 1]:54–62, 2009.)*

## Imaging: Conventional Radiography of Specific Joints

Imaging of the knees should be bilateral while the patient is weight bearing, and for clinical purposes, it is generally anteroposterior (AP). Posteroanterior views in fixed flexion are commonly used for research, and lateral views may be necessary in some circumstances.[97] Sunrise views are needed to fully assess the patellofemoral joint. Alignment, although best determined from full-limb views, can be estimated from routine knee films.[98] An AP pelvis view should be obtained to assess hip OA and can be either supine or weight bearing. Additional views such as frog-leg, lateral, or Dunn views may be indicated for specific hip disease or to assess for femoroacetabular impingement or congenital abnormalities. An atrophic subtype of hip OA, characterized by joint space narrowing with either mild or absent osteophytosis, has been described,[99] and alternative radiographic definitions for hip OA (other than KL grading) have been proposed and used in research to address this issue.[100] For hand OA, a posteroanterior view of both hands, including the wrists, will reveal the characteristic features of OA. If there is an erosive component to hand OA, radiographs may demonstrate central erosions and "gull-wing" deformities of the interphalangeal joints (see Figure 99-2). Marked destructive changes at the DIP joints may indicate another inflammatory disorder such as psoriatic arthritis or, rarely, multicentric reticulohistiocytosis. Prominent involvement of the MCP joints may indicate a metabolic process such as hemochromatosis or calcium pyrophosphate deposition disease. Conventional radiography of the cervical and/or lumbar spine can show facet OA, disk space narrowing, and osteophytosis.

## Imaging: Advanced Modalities

Several advanced imaging modalities such as MRI, ultrasound, CT, and positron-emission tomography (PET) scans have been used in OA research, although with the exception of ultrasound, these generally specialized, expensive examinations are not indicated in routine clinical practice. A brief summary of these modalities in OA is provided in the following sections.

### Magnetic Resonance Imaging

MRI is the most well-studied advanced imaging modality in OA. Unlike radiography, it allows visualization of all joint tissues, including cartilage, menisci, ligament/tendon, synovium, effusions, and bone marrow simultaneously, although arthroscopy remains the gold standard for joint tissue assessment.[94] An MRI-based definition of tibiofemoral OA has been proposed that requires either the presence of both definite osteophytes and full-thickness cartilage loss or one of these and one of the following features: a

subchondral bone marrow lesion or cyst, meniscal disease (subluxation, maceration, or horizontal tear), partial-thickness cartilage loss, or bone attrition.[9]

Semiquantitative scoring, which can be performed using conventional acquisition techniques, is frequently used to evaluate a number of OA features simultaneously, such as the articular cartilage, meniscus, ligaments, bone marrow, synovitis, and joint effusion (Figure 99-5).[101] Several scoring systems are publicly available. Whole Organ Magnetic Resonance Imaging Score (WORMS), the oldest and most widely used semiquantitative system for MRI scoring in OA, utilizes a subregional approach to scoring of features, including cartilage, bone marrow lesions (BMLs), subchondral cysts, bone attrition, effusion, synovitis, osteophytes, meniscal tears, and cruciate and collateral ligaments.[101] The Knee Osteoarthritis Scoring System (KOSS) is similar but counts more individual lesions and includes size assessments. The Boston Leeds Osteoarthritis Knee Score (BLOKS) varies slightly in subregion definition and individually scores BMLs. The MRI Osteoarthritis Knee Score (MOAKS), which was more recently developed[102] as a response to some of the shortcomings of the other systems, refines scoring of BMLs and meniscal disease.[101] Semiquantitative scores are also available that focus on only one feature, such as cartilage or synovitis.

Overall, semiquantitative methods for knee OA are adequately responsive to change and are reliable. At the knee, semiquantitatively assessed BMLs, joint effusion, and synovitis are associated with pain, and development of knee pain is associated with increasing BML score over time.[101] Progression of cartilage loss is associated with semiquantitatively assessed meniscal damage/malposition, prevalent cartilage damage, BMLs, and effusion.[101] A recent study using MOAKS and data from the Osteoarthritis Initiative found that, among 849 individuals with no radiographic evidence of knee OA at baseline (bilateral KL grade = 0; one-third of whom had baseline frequent knee symptoms), the majority had cartilage damage (76%) and bone marrow lesions (61%) as identified by MRI, and these lesions were associated with prevalent and incident knee symptoms.[103] Semiquantitative MRI scoring has also been utilized at the hip, hand, shoulder, and spine, although to a much lesser extent than for knee OA.[101]

Quantitative assessment of MRI involves quantification of specific tissue characteristics such as thickness and volume of cartilage or size and volume of BMLs. These techniques are time and labor intensive but provide greater sensitivity to change compared with semiquantitative scoring.[101] Quantitatively assessed cartilage thickness and denuded areas of subchondral bone predict clinical outcomes such as joint replacement.[94]

Compositional imaging techniques, which allow visualization of anatomy and biochemical properties as well, have been used in the setting of very early stage disease prior to obvious structural alterations. Techniques include delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) and T1 rho, which can visualize changes in glycosaminoglycan concentrations, and T2 mapping, which reflects variation in collagen orientation and cartilage hydration status.[94] Alterations in compositional MRI have been reported in exercise/joint loading and weight change,[94] with a suggestion of possible associations with disease progression.[104]

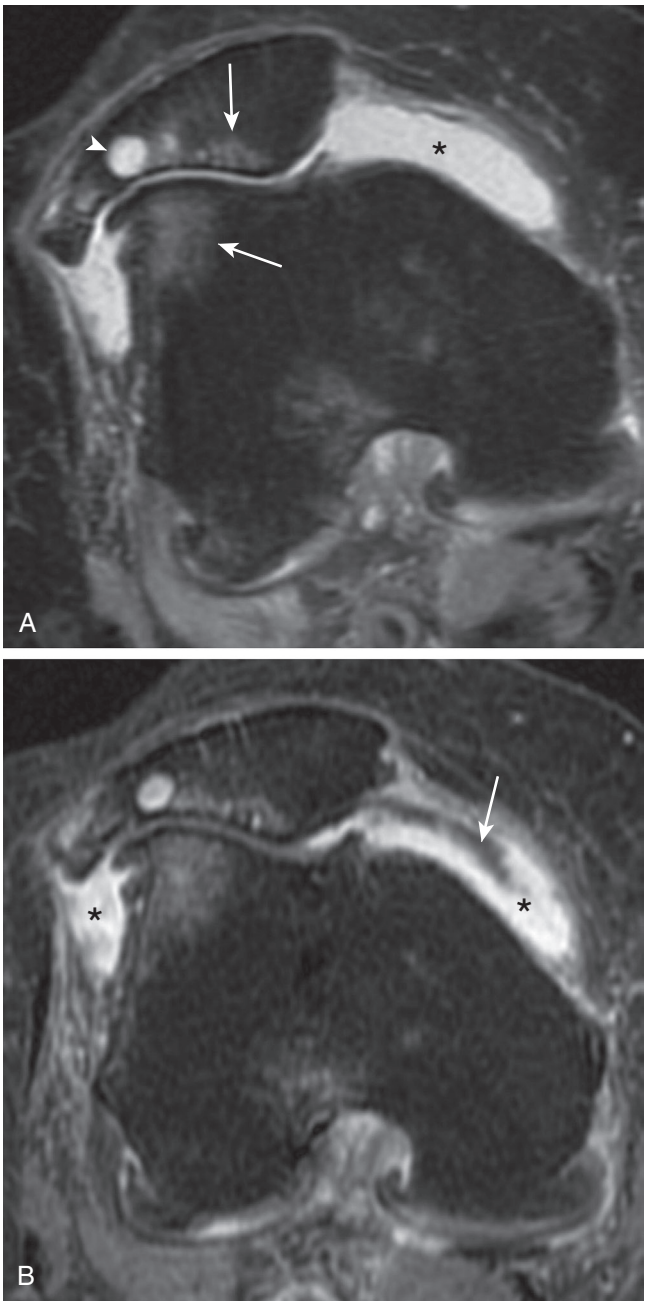



**Figure 99-5** Visualization of synovitis using nonenhanced and contrast-enhanced MRI. **A,** An axial proton-density–weighted fat-saturated image shows marked hyperintensity within the joint cavity, suggesting severe joint effusion *(asterisk)*. In addition, a large subchondral cyst is present in the lateral facet of the patella *(arrowhead)* and diffuse bone marrow edema is present in the lateral patella and trochlea *(arrows)*. **B,** An axial T1-weighted fat-saturated image after administration of contrast material clearly visualizes severe synovial thickening depicted as contrast enhancement *(asterisks)*. The *arrow* points to true amount of effusion, which is only discrete and visualized as linear hypointensity within the joint cavity. *(From Roemer FW, Eckstein F, Hayashi D, et al: The role of imaging in osteoarthritis.* Best Pract Clin Rheumatol *28:31–60, 2014.)*

## Ultrasound

Ultrasound is a point-of-care modality that can be used directly by trained providers (e.g., rheumatologists, radiologists, and orthopedists) to assess joint disease in patients. Its relatively low cost, safety (because it does not entail use of

radiation or contrast agents), short examination duration, convenience (it can be performed in the provider's office), and noninvasive nature make it an attractive OA assessment method. It has several advantages compared with conventional radiography, including lack of radiation, ability to obtain numerous views of a given joint, and ability to assess soft tissues and inflammation. Limitations include the inability of sonography to penetrate the bony cortex, which prevents assessment of BMLs or deeper aspects of the joints. A recent study using data from 311 community-dwelling people aged 61 to 63 years participating in the Newcastle Thousand Families birth cohort found a high prevalence of ultrasound osteophytosis at the DIP (70%), index PIP (23%), index MCP (10%), thumb base CMC joints (41%), knees (30%), and hips (41%), whereas knee effusion was present in 20% to 24%.[105]

For knee OA, ultrasound is more sensitive than clinical examination and is comparable with MRI in the detection of synovitis, which correlates with pain.[106] Inter-rater reliability for a variety of features of knee OA (e.g., meniscal extrusion, infrapatellar bursitis, effusion, popliteal cyst, and cartilage thickness) has been reported as moderate to good (κ values 0.5 to 0.8), although agreement on synovial hypertrophy was poor.[107] Measurement of articular cartilage by ultrasound was accurate and reproducible compared with direct anatomic measurements.[108,109] ultrasound-guided intra-articular injections also can be beneficial; a systematic review of 23 publications found that injections at the superomedial patellar, medial midpatellar, superolateral patellar, and lateral suprapatellar sites were more accurate with image guidance rather than without image guidance.[110] Ultrasound guidance also allows direct aspiration/injection of popliteal cysts for symptomatic relief.

In the hand, ultrasound is more sensitive than radiographs for the detection of erosions, osteophytes, and joint space narrowing, although estimates of ultrasound-detected cartilage thickness are correlated with radiographic determinations.[111] Generally, good agreement is found between ultrasound and MRI for structural features and inflammation.[111]

### Computed Tomography

CT is the best modality for assessment of cortical bone and soft tissue calcifications. It is also the main technique used for assessment of facet joint OA.[112] CT arthrography is the most accurate method for the evaluation of articular cartilage surface damage because of high contrast between cartilage, a contrast agent, and bone using this modality, although expense, invasiveness, and radiation exposure limit its use.[112]

### Nuclear Medicine

Bone scans (technetium 99m hydroxymethane diphosphonate scintigraphy) provide a full-body image in one test, allowing location of the site of inflammation in patients with complex symptomatology. Bone scans have been used as a measure of whole-body OA burden for evaluation of systemic biomarkers.[113] PET, most often using 18F-fluorodeoxyglucose, can detect synovitis, BMLs, and other sources of increased metabolic activity in aging and OA joints, and these findings have been associated with pain.[114,115] Limitations include cost, limited availability, poor anatomic resolution, use of ionizing radiation, and invasiveness (because of the injection of the radiopharmaceutical agent); small-parts scanners are in development and may improve feasibility for musculoskeletal imaging.[94]

## OUTCOME

### Pain and Functional Status Assessment

Numerous validated tools for assessing functional status in OA are available and widely used in research settings. As the focus on quality of care increases, these tools will likely be incorporated more frequently into clinical practice.

### Self-Reported Measures of Pain and Function

Questionnaire-based measures of pain and functional status provide rapid and repeatable patient-based assessments that can be monitored over time and for treatment response. Numerous well-studied, validated, and reliable measures are available for this purpose. For overall function, the Stanford Health Assessment Questionnaire (HAQ) disability index is often used, although it is not disease specific. For OA-specific lower extremity pain and function, the Western Ontario and McMaster Universities Arthritis Index (WOMAC; Table 99-4) is the most frequently used

**TABLE 99-4** Western Ontario and McMaster Universities Arthritis Index

| |
|---|
| **Pain Subscale** |
| How much pain do you have… |
| Walking on a flat surface? |
| Going up or down stairs? |
| At night while in bed? |
| Sitting or lying? |
| Standing upright? |
| **Stiffness Subscale** |
| How severe is your stiffness… |
| After first waking in the morning? |
| After sitting, lying down, or resting later in the day? |
| **Function Subscale** |
| What degree of difficulty do you have with… |
| Descending stairs? |
| Ascending stairs? |
| Rising from sitting? |
| Standing? |
| Bending to floor? |
| Walking on a flat surface? |
| Getting in or out of a car? |
| Going shopping? |
| Putting on socks or stockings? |
| Rising from bed? |
| Taking off socks or stockings? |
| Lying in bed? |
| Getting in and out of the bath? |
| Sitting? |
| Getting on or off the toilet? |
| Heavy domestic duties? |
| Light domestic duties? |

Modified from Bellamy N, Buchanan WW, Goldsmith CH, et al: Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to anti-rheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 15:1833–1840, 1988.

tool, although joint-specific measures such as the Hip Disability and Osteoarthritis Outcome Score (HOOS) and Knee Injury and Osteoarthritis Outcome Score (KOOS) are also validated and are often used in research. Measures are available for other joint sites as well, such as the Australian/Canadian Hand Osteoarthritis Index (AUSCAN) and Functional Index for Hand Osteoarthritis (FIHOA) for hand-specific function; Disabilities of the Arm, Shoulder, and Hand Questionnaire (DASH) for the upper extremity; and the Roland-Morris Disability Questionnaire and Oswestry Disability Index for low back disorders, among others. Various clinically important features of these measures have been established, such as responsiveness and minimal clinically important differences, all of which have been reviewed.[116]

### Performance-Based Assessment

Results on standardized performance-based tests, such as determination of walking speed and time to stand from a chair five times unassisted, have been shown to correlate with the severity and number of affected joints in OA.[117,118] The Osteoarthritis Research Society International recently provided guidelines for appropriate performance-based tests for hip or knee OA, which include a 30-second chair-stand test, 40-m fast-paced walk test, stair-climb test, timed up-and-go test, and 6-minute walk test, with the first three tests as a minimal core set of measures.[119]

## Quality of Life

OA is a chronic, progressive, and often debilitating condition, and thus assessment of quality of life is an important outcome for monitoring and treatment. Generic measures of quality of life, such as the Medical Outcomes Study Short Forms (SF-36, SF-12, and SF-6D) and Assessment of Quality of Life (AQoL), are commonly used.[116] The HOOS and KOOS previously discussed include quality-of-life domains specific to knee and hip OA.[116] The Osteoarthritis Knee and Hip Quality of Life (OAKHQOL) has been validated for this purpose.[120]

## Time to Total Joint Replacement

Total joint replacement is a "hard" endpoint in OA, but its use as an outcome measure is hampered by the prolonged time needed for assessment, the large number of patients and events required, and a variety of factors affecting an individual patient's decision to undergo this procedure, including health care system issues, procedure availability, comorbid conditions, ability to rehabilitate the joint, and patient/provider preference.[121] To date, efforts have not been successful to identify a composite index that would consider pain, functional disability, and structural degradation; that would define the "need for total joint replacement"; and that could be used as an outcome measure.[122]

## Responder Criteria

In some cases, it is more useful to categorize individuals as responders or nonresponders to treatments, given the shortcomings of outcomes based solely on imaging or self-report, particularly for clinical trials. The Outcome Measures in Rheumatology (OMERACT)-OARSI responder criteria provide a composite assessment, including threshold levels of percentage improvements in pain and function, as well as required thresholds of absolute change to define response and no response that are applicable in a variety of settings.[123]

## Mortality in Osteoarthritis

Moderate evidence indicates increased mortality in individuals with OA compared with the general population.[124] The apparent modestly increased mortality risk, determined through a review of nine studies, primarily appears to be due to cardiovascular and gastrointestinal causes. One study showed an increased mortality risk with increasing numbers of joint groups affected by OA, as well as reduced survival in individuals with hand, bilateral knee, or cervical spine involvement, but not in those with OA of the hip, foot, or lumbar spine.[125] Contributors to increased mortality risk include a combination of reduced physical activity among individuals with OA, comorbid conditions, and/or adverse medication effects such as are seen with acetaminophen and nonsteroidal anti-inflammatory drugs.[124] A recent study of Canadians aged 55 years and older with moderate to severe symptomatic hip or knee OA found that poorer WOMAC function scores and walking disability were associated with higher all-cause mortality in this population.[126]

# CONCLUSION

OA is a highly prevalent and debilitating condition with increasing prevalence as a result of the aging of our society and the ongoing obesity epidemic. OA is readily recognized on clinical grounds, although further diagnostic testing and imaging are indicated in some cases to assess for potentially treatable underlying conditions. Pain is the most common reason for a person with OA to seek medical advice, and examination often reveals loss of function. Assessment of the patient's pain symptoms and functional limitations, in the framework of that person's goals, is necessary to formulate an appropriate plan of care in the absence of disease-modifying medications. Current research in OA, in addition to ongoing efforts in both pharmacologic and nonpharmacologic therapeutic development, is focused on optimizing incident case and progression definitions and understanding the whole-body burden of OA.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 100

# Treatment of Osteoarthritis



David T. Felson

**KEY POINTS**

- Treatment approaches for painful osteoarthritis (OA) should take into account causes, frequency, and severity of pain.
- Nonpharmacologic treatments such as activity modification, education, and exercise should be a part of treatment for everyone with OA.
- Many different types of exercise can be effective for knee and hip OA. Patient adherence to exercise is the main challenge.
- Nonsteroidal anti-inflammatory drugs are moderately effective in OA, more than acetaminophen, and should be started either as topical preparations or on an as-needed basis.
- Intra-articular steroid injections are highly effective, especially within a few weeks after the injection.
- Evidence does not support the efficacy of glucosamine, chondroitin, or both to treat OA, and the evidence to support hyaluronic acid preparations is not conclusive.
- There are many unproven remedies used by patients.
- Knee and hip joint replacements are highly effective surgeries and should be offered to patients with advanced osteoarthritis who have nocturnal joint pain or pain with rest, or patients in whom pain causes work and functional impairment.

Osteoarthritis (OA) is the most common type of arthritis in the United States and throughout the world, and is the cause of more mobility disability than any other disease. At least 27 million people in the United States have symptomatic OA of the knee, hip, or hands. Symptomatic OA is defined as pain in the joint on most days of a recent month and radiographs that show osteoarthritis in the painful joint.[1] The number of affected individuals is rising with the aging of the population, higher rates of obesity (a major risk factor for disease), and frequent sports-related joint injuries that predispose to later disease, especially in the knee. More than 50% of those affected by painful knee or hip osteoarthritis will eventually undergo joint arthroplasty, although only after living many years with pain and functional loss.[2]

The primary symptom of OA is joint pain, and relieving this pain is the main goal of therapy. In OA, pain is primarily related to carrying out activities. For example, going up and down stairs or getting out of a chair often triggers knee pain in those with knee OA. Cooking or other manual activities can precipitate hand pain in people with hand OA.

The genesis of joint pain in OA is complicated. The joint is protected from damage by multiple overlapping physiologic systems. Among these are limits to excursion established by muscles, ligaments, and the joint capsule; smooth and coordinated muscle contraction instituted by both the muscles and nervous system–mediated spinal reflexes; and reflexes that provide instantaneous input on the position and tension of muscles across the joint. Activities probably induce joint pain because the load transmitted during these activities is beyond the physiologic capabilities of the joint to withstand. This can occur because the joint is already damaged and cannot withstand much loading—as often occurs in the osteoarthritic joint—or can occur with excess loads in a normal joint. In addition to factors affecting loading are those that lower the threshold for pain, including synovitis within the joint, a common feature of OA. Synovitis may itself be triggered by microscopic damage to cartilage and other structures within the joint, with shards of tissue released into the synovial fluid ingested by synovial lining cells, generating a secondary inflammatory response. In addition, the inflammatory milieu of OA can generate peripheral and even central nervous system changes that lead to chronic pain sensitization. Other factors, including psychological and sociocultural factors, contribute to the presence and severity of pain. Thus, many factors (Figure 100-1) including loading with activities, aberrant structure of the joint that increases focal loading (such as occurs with malalignment), synovitis, impaired muscle function and coordination, deficient proprioception, and nervous system sensitivity contribute to pain. Even psychological factors could theoretically be targeted in patients with painful OA. Pain causes a person to have difficulty carrying out functional activities, including work, household activities, shopping, laundry, and recreational pursuits.

Patients with painful OA often experience other symptoms that the physician should elicit during the initial examination and patient history. These symptoms, including giving way of the joint, could be the focus of treatment. Giving way or buckling tends to occur in patients who feel that their joints are unstable, and is a common feature of painful knee and, to a lesser extent, hip OA. Factors that increase the risk of giving way include muscle weakness and the co-occurrence of pain in multiple lower extremity joints. The physician should ask patients whether their knees or hips are unstable or give way because these symptoms may increase their risk of falling and, for some patients, may be more disabling than their pain.

Video available on the accompanying ExpertConsult website.

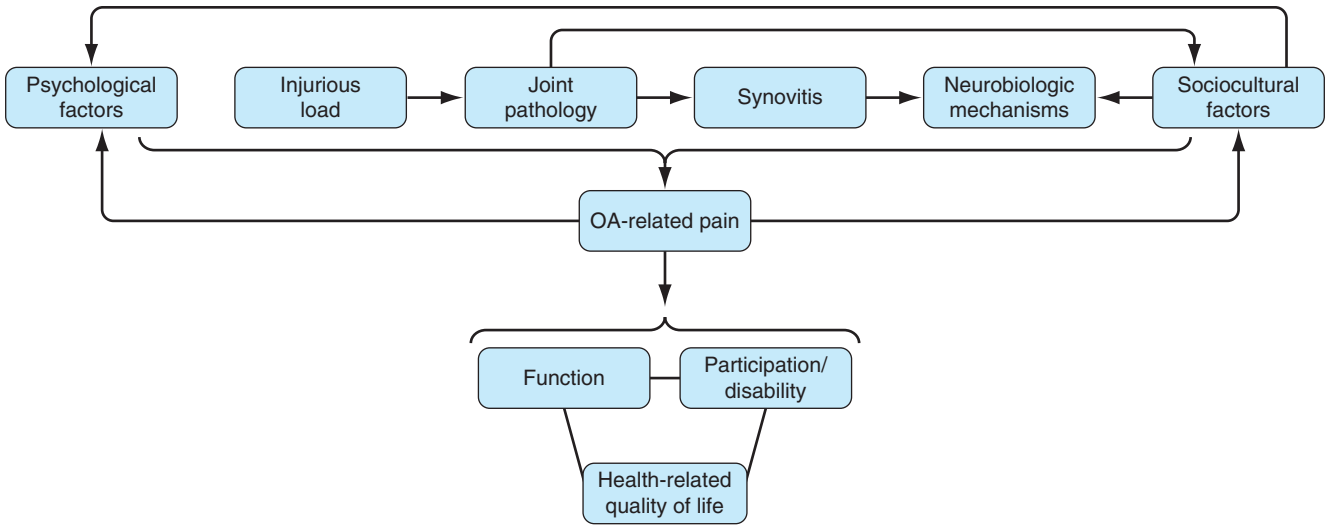



**Figure 100-1** Pathophysiology of osteoarthritis (OA)-related pain and its consequences. *(Modified from Neogi T: The epidemiology and impact of pain in osteoarthritis.* Osteoarthritis Cartilage *21[9]:1145-1153, 2013.)*

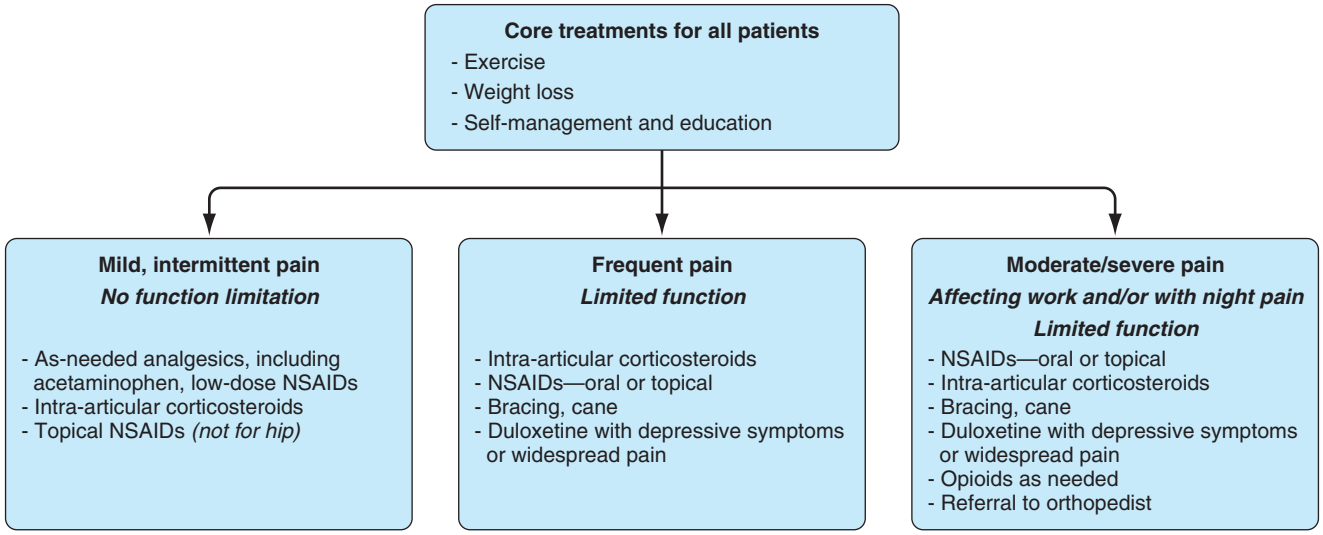



**Figure 100-2** Algorithm for management of knee and hip osteoarthritis.

The management of OA can be divided into nonpharmacologic interventions, pharmacologic interventions, and surgical options. Patients with mild or intermittent pain are likely to need only nonpharmacologic treatment, whereas those with more severe symptoms will probably require both nonpharmacologic and pharmacologic treatments (Figure 100-2).

## NONPHARMACOLOGIC TREATMENTS

### Psychosocial Interventions

As in other types of arthritis, patient education is an important first step in OA therapy. The patient should be an integral part of the decision-making team. To do this effectively, the patient should understand the nature of OA, including its natural history and treatment options. It is often reassuring for the patient to realize that OA is a common, slowly progressive ailment and is not typically as disabling or deforming as the inflammatory arthritides. A significant number of patients have already tried nonprescription medications or nutraceutical remedies before seeing a physician and will want to discuss these options. Physicians should emphasize that treatment includes nonpharmacologic and pharmacologic interventions. Organizations such as the Arthritis Foundation can be valuable sources of information geared toward patients and can provide helpful reading materials.

Some patients may develop emotional disturbances related to the pain and changes in normal daily activities that can stem from OA. These may include mood disorders such as depression or sleep disorders.

### Avoiding Painful Joint Loading

Osteoarthritic pain is activity-related, and for most affected patients, certain activities will reproducibly elicit their joint pain. The painful activities probably cause pain in part by

increasing focal loading across a damaged segment of the joint. Any of the following strategies may be successful in alleviating the pain:

- Avoiding activities that cause joint pain
- Improving the strength and conditioning of muscles that bridge the joint, to optimize their function
- Unloading the joint, either by redistributing load within the joint with a brace or a splint, or by unloading the joint during weight bearing with a cane or a crutch

The simplest effective treatment for many patients is to avoid activities that precipitate pain. For example, for the middle-aged patient whose running triggers pain in their knees, a less demanding form of weight-bearing activity may alleviate discomfort. For an older person whose daily constitutionals up and down hills cause knee pain, routing the constitutional away from hills might eliminate symptoms.

Each pound of weight increases the overall load across the knee three- to sixfold. Weight loss may have a commensurate multiplier effect, unloading both knees and hips. Obese people undergoing bariatric surgery with subsequent massive weight loss experience a marked reduction in pain.[3] Trials of modest weight loss (5 to 10 kg) suggest a moderate reduction in knee pain,[4] and the combination of exercise with weight loss appears more effective at relieving pain and reducing knee loading than weight loss alone.[5]

In hand joints affected by OA, splinting, by limiting motion, often minimizes pain for patients with involvement especially in the base of the thumb. Weight-bearing joints such as knees and hips can be unloaded by using a cane in the hand opposite to the affected joint for partial weight bearing. A physical therapist can teach the patient how to use the cane optimally, including ensuring that its height is optimal for unloading (the optimal height for a cane is with the elbow of the arm holding the cane flexed at an approximately 20-degree angle, or with the top of the cane at approximately the level of the femoral greater trochanter). Crutches or walkers can serve a similar beneficial unloading function.

## Exercise

Osteoarthritic pain in knees or hips during weight bearing results in lack of activity and poor mobility and, because OA is so common, the inactivity that results increases the risk of obesity and cardiovascular disease, thus representing a public health concern. Aerobic capacity is poor in most elders with symptomatic knee OA, and worse than others of the same age.

Weakness in muscles that bridge osteoarthritic joints is multifactorial in etiology. First, there is a decline in strength with age. Second, with limited mobility comes the muscle atrophy of disuse. Third, patients with painful knee or hip OA alter their gait to lessen loading across the affected joint, which further diminishes muscle use. Fourth, "arthrogenous inhibition" may occur, whereby contraction of muscles bridging the joint is inhibited by a nerve afferent feedback loop emanating in a swollen and stretched joint capsule; this prevents maximal attainment of voluntary maximal strength.[6] Because adequate muscle strength and conditioning are critical to joint protection, weakness in a muscle that bridges a diseased joint makes the joint more susceptible to further damage and pain. The degree of weakness correlates strongly with the severity of joint pain and the degree of physical limitation. One of the cardinal elements of the treatment of OA is to improve the functioning of muscles that surround the joint.

For knee and hip OA, trials have shown consistently that exercise lessens pain and improves physical function.[7] Most effective exercise regimens consist of aerobic and/or resistance training, the latter of which focuses on strengthening muscles across the joint. Exercises are likely to be effective, especially if they train muscles for the activities a person performs daily. Activities that increase pain in the joint should be avoided, and the exercise regimen needs to be individualized to optimize effectiveness. Range-of-motion exercises, which do not strengthen muscles and isometric exercises that strengthen muscles, but not through range of motion, are unlikely to be effective by themselves. Low-impact exercises, including water aerobics and water resistance training, are often better tolerated by patients than exercises involving impact loading, such as running or treadmill exercises. A meta-analysis of exercise trials has suggested that, although aerobic, resistance, and performance exercise were all effective for relieving pain in knee OA, single-type exercise programs were substantially more effective than programs including different types of exercise.[8] A patient should be referred to an exercise class or to a therapist who can create an individualized regimen, and then an individualized home-based regimen can be crafted. In addition to conventional exercise regimens, tai chi may be effective for knee OA. Despite the clear-cut effectiveness of exercise for knee and hip OA, there is no strong evidence that patients with hand OA benefit from therapeutic exercise.

In addition, although exercise reduces pain in knee and hip OA patients, physical therapy referral may have little efficacy in relieving pain, at least in hip OA.[9] The reason for the disappointing effect of physical therapy is unclear. Physical therapists may use a combination of treatment modalities for each patient, only some of which are effective, and also patients may not continue to carry out the prescribed exercises after their treatment sessions end.

Adherence over the long term is the major challenge to an exercise prescription. In exercise trials involving patients with knee OA, one-third to more than one-half of patients stop exercising by 6 months, and less than 50% continue regular exercise at 1 year. The strongest predictor of a patient's continued exercise is a previous personal history of successful exercise. Physicians should reinforce the exercise prescription at each clinic visit, help the patient recognize barriers to ongoing exercise, and identify convenient times for exercise to be done routinely (see Table 100-1 for strategies to improve adherence to exercise). The combination of exercise with calorie restriction and weight loss is especially effective in lessening pain.

## Strategies to Realign the Joint

For knee OA especially, misalignment across the joint increases focal load and, when the joint is loaded, such as with walking or other weight bearing, this excess load can translate into pain. Most commonly, knee OA shows varus deformities in which the medial joint is much narrower than the lateral. In normal nondiseased knees, the medial

**TABLE 100-1** Strategies to Facilitate Long-Term Adherence to Exercise and Physical Activity in People with Osteoarthritis

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| Educate patients about the disease and the benefits of exercise. |
| Develop the exercise or physical activity plan with the patient and vary activities to maintain interest and enthusiasm. |
| Use a graded progressive exercise or physical activity program, and ensure that pain and discomfort are not excessive during or after exercise. |
| Initiate exercise under expert instruction, and supervise exercise sessions if possible. |
| Supplement face-to-face instruction with other materials, such as written handouts, videos or DVDs, and online demonstrations. |
| Increase patient self-efficacy by incorporating behavioral techniques such as goal setting, positive reinforcement, use of an exercise contract, and self-monitoring by use of a diary or pedometer. |
| Include the patient's partners and family in the exercise program and garner support from family and friends. |
| Monitor the patients during the long term, with periodic reassessment by a health professional. |
| Supplement exercise instruction with an activity monitor (e.g., a wristband that tracks activity) coupled with goal setting. |

Modified from Bennell KL, Hunter DJ, Hinman RS: Management of osteoarthritis of the knee. *BMJ* 345:e4934, 2012.



**Figure 100-3** A valgus knee brace used to realign the knee.

compartment bears approximately 60% of the load during walking, but when medial knee OA and varus deformities are present, that load increases to 80% to 100% of weight-bearing load. Joint damage occurs increasingly as a consequence of that load; damage to bone occurs, as evidenced by bone marrow lesions in the medial compartment, and synovitis develops with release of shards of cartilage. Both synovitis and bone marrow lesions are sources of pain. Unloading the medial compartment in these patients by reducing the misalignment might alleviate pain and slow disease progression.

The knee is the fulcrum of a large lever arm, extending when walking from the trunk to where the foot meets the floor. When the knee has a varus (bowlegged) malalignment in the frontal or coronal plane, the knees are farther apart than the feet, and the disease in the knee is more often present in the medial than lateral compartment. A small percentage of patients (approximately 10% to 20%) with knee OA have the opposite—a valgus deformity in which the knees are close together and the feet farther apart. Generally, they have disease in the lateral compartment, which is narrower than the medial compartment. When the knee is malaligned, excessive load during weight bearing is transmitted across the narrowed compartment, often causing damage in that area. Realigning the joint and lessening that malalignment might provide therapeutic benefit. One could focus on changing the position of the foot below the knee at the bottom end of the lever arm or on the position of the trunk, above the knee at the top end of the lever arm. Examples of the former strategy include the use of various shoe orthotics, especially lateral wedge insoles, which can be used to treat medial disease. Lateral wedge insoles that are thicker on the outside than on the inside are placed into the shoe. During walking, these wedges move the knee closer to a vertical line through the center of the body, and in doing so lower the varus moment across the knee, a measure of medial load. However, despite their effect on reducing medial load during walking, trials of wedge inserts have shown[10] that they do not alleviate knee pain compared with neutral inserts, which have no effect on medial loading of the knee. The failure of this promising unloading treatment raises questions about whether unloading of the varus malaligned knee will be effective at all. Reasons for the failure of wedge inserts include the fact that they do not reduce the varus knee moment in all treated patients, the fact that their effect on medial load is small and may be insufficient to produce pain relief, and the fact that patients may not consistently place them in their shoes.

Another strategy that rectifies malalignment and unloads the knee is the use of bracing. With metal struts on the side and straps that force the knee into a more neutral alignment, valgus braces can straighten a knee that has varus malalignment (Figure 100-3). Three randomized trials of valgus bracing in patients with knee OA have been carried out, and two of these have reported that, compared either with neutral braces or with sleeves, the braces had moderate efficacy in alleviating knee pain.[11,12] Unfortunately, adherence to the use of these large bulky braces is poor, and most patients are unwilling to wear them for long periods of time. Thus, even though they are effective, valgus braces have not been a terribly popular treatment for OA. Given their availability and effectiveness, patients with obviously malaligned varus or valgus knees should be presented with the option of obtaining a knee brace. They should be shown pictures of braces so that they can anticipate what wearing them will be like. For a minority of patients, this may be an acceptable effective long-term treatment.

The patellofemoral compartment of the knee is frequently diseased in patients with OA and is a major source

of pain. Because the disease in the patella often affects one compartment preferentially, there is often malalignment of the patella in the trochlear groove; a patellar brace may push the malaligned patella back into the trochlear groove in the femur, increasing the contact area and decreasing the focal load on the diseased patellar compartment. A recent trial[6] suggests that patellar bracing may alleviate knee pain at least over the short term in patients with patellofemoral OA. Patellar braces are easier to wear than the larger bulkier tibiofemoral valgus braces and often fit easily underneath trousers. A less obtrusive option that may have similar mechanism of action is patellar taping, which has been shown[13,14] to relieve knee pain.

Another way to realign the knee is to change the position of the trunk, the top of the lever arm, during walking. In this case, the patient needs to be taught how to position his or her trunk during ambulation so as to lessen malalignment across the knee, a strategy known as "gait retraining." Although promising, this approach has not been comprehensively evaluated, and long-term patient adherence to gait retraining strategies is unclear.

## PHARMACOLOGIC TREATMENTS

### Acetaminophen (Paracetamol)

Many guidelines recommend acetaminophen either on an as-needed or continuous basis as the initial analgesic treatment for OA. Doses as high as 1 g three times daily can be used. Unfortunately acetaminophen, even in high doses, is rarely effective by itself in the treatment of OA. Meta-analyses suggest an effect size of acetaminophen versus placebo of only 0.2 standard deviations, a very small treatment effect.[15] In addition, acetaminophen is a weak cyclooxygenase (COX) inhibitor, with a greater COX-2 than COX-1 effect. There is strong evidence that the use of acetaminophen regularly causes gastrointestinal blood loss and that its overall side effect profile is therefore not necessarily benign.[16] Although its occasional use for treatment of mild OA with occasional pain is recommended, its lack of substantial efficacy suggests that it should not be a primary treatment for moderate to severe OA.[17]

### NSAIDs Including Oral and Topical Agents and COX-2 Inhibitors

NSAIDs are among the most popular treatment for OA. They have both analgesic and anti-inflammatory effects. Meta-analyses suggest that when regularly taken, oral NSAIDs are more effective than high-dose acetaminophen (effect size difference between NSAID and acetaminophen for pain is approximately 0.30, a modest but significant difference).[17] Occasionally, patients treated with NSAIDs experience dramatic and complete relief of pain, but this is not universal. NSAIDs, especially when taken orally, have many adverse effects (see Chapter 59). To lessen the risk of side effects, treatment should be started initially on an as-needed basis or by using topical agents, especially for superficial joints such as the hands and knees. If topical or occasional oral medication is insufficient to control symptoms, then daily regular treatment may be indicated.

NSAIDs cause upper gastrointestinal adverse effects in 30% to 40% of patients, including stomach discomfort, dyspepsia, nausea, and vomiting, and can cause upper gastrointestinal bleeding. These symptoms are partly the result of prostaglandin inhibition in the upper gastrointestinal tract. Prostaglandins are necessary for mucosal protection of the stomach, and inhibiting them makes the stomach mucosa vulnerable. In addition, inhibition of prostaglandins may compromise the perfusion of the superficial lining of the upper gastrointestinal tract, making it more susceptible to injury. Among the risk factors for NSAID-induced gastrointestinal symptoms and complications are higher doses of nonsteroidal drugs; also, particular nonsteroidals increase the risk of gastrointestinal symptoms and bleeding. Based on meta-analyses and a large number of observational studies,[18,19] the most dangerous NSAIDs from the perspective of upper gastrointestinal toxicity are indomethacin, ketorolac, piroxicam, and ketoprofen. The safest nonsteroidals from this perspective are nonacetylated salicylates and nabumetone. The former group of drugs, which are inexpensive and underutilized, do not inhibit gastric prostaglandins and therefore have fewer gastrointestinal side effects than conventional NSAIDs. Nabumetone is a prodrug and has no local prostaglandin-inhibiting effect when administered.

In addition to drug-related factors, there are other known risk factors for NSAID-associated gastrointestinal complications (Table 100-2). Those who need oral nonsteroidals yet who are at high risk should take gastroprotective drugs to lessen their risk. Drugs that protect against gastric and duodenal ulcers based on endoscopic studies include misoprostol, a prostaglandin analog, and proton pump inhibitors. Histamine 2 blockers are not as effective in preventing gastroduodenal complications as are these other two classes of drugs.

In addition to upper gastrointestinal side effects, oral NSAIDs also increase the risk of lower gastrointestinal tract bleeding and perforations. Lower gastrointestinal tract symptoms include diarrhea or constipation, and malabsorption or gut inflammation sometimes accompanies the diarrhea.[20] The rate of mortality from lower gastrointestinal bleeding caused by nonsteroidals may approach that of upper gastrointestinal bleeding related mortality.[21]

COX-2 inhibitors were developed in part to minimize the gastrointestinal side effects of nonsteroidals, but they work by inhibiting prostacyclin which, when inhibited, enhances platelet activation. In addition, COX-2 inhibition causes vasoconstriction. The net effect of COX-2 inhibition is to increase the risk of thrombosis, especially

**TABLE 100-2** Risk Factors for the Development of NSAID-Associated Gastroduodenal Ulcers

| | |
|---|---|
| Established Risk Factors | Advanced age (linear increase in risk)<br>History of ulcer<br>Concomitant use of corticosteroids<br>Higher doses of NSAIDs and use of more than one NSAID<br>Concomitant administration of anti-coagulants<br>Concomitant infection with *Helicobacter pylori* |
| Possible Risk Factors | Cigarette smoking<br>Alcohol |

myocardial infarction. When studies confirmed that rofecoxib increased this risk, it was removed from the market, but some NSAIDs still on the market have preferential COX-2 inhibiting effects. Large-scale observational studies and clinical trials have suggested that oral diclofenac,[22] for example, confers a substantial increased risk of myocardial infarction. Other widely used nonsteroidals such as ibuprofen modestly increase myocardial infarction risk (relative risk, ~1.2). The main exception among NSAIDs is naproxen, which does not confer an increased risk of thrombotic events. The risk of celecoxib, the most prominent remaining COX-2 inhibitor on the market, is increased only if high doses of the drug are taken, such as 400 mg per day.

In addition to effects on thrombosis, NSAIDs can also induce congestive heart failure in predisposed patients because they cause retention of fluids and they can increase blood pressure because of renal effects, although the effect on blood pressure is usually small, on the order of an increase of 1 to 2 mm Hg.

Sodium retention is common among patients taking NSAIDs and, when pronounced, can even lead to rises in serum creatinine. Other renal complications of NSAIDs are uncommon, including frank renal insufficiency, papillary necrosis, and interstitial nephritis. Oral nonsteroidals should not be used in patients with stage 4 or 5 renal disease, and should be used cautiously in those with stage 3 disease.

NSAIDs can cause confusion or dizziness, especially in elderly users. In addition, a syndrome known as "anaphylaxis" occurs among some NSAID users. This is a phenomenon mediated not by immunoglobulin (Ig)E but, rather, is a result of prostaglandin inhibition. It occurs especially in aspirin-sensitive patients who often have the syndrome of asthma and nasal or sinus polyposis. Patients in whom anaphylaxis to NSAIDs develops are sensitive to all drugs in the class, except for nonacetylated salicylates, which cause minimal cyclooxygenase inhibition.

When placed into a topical solution with a chemical moiety that enhances penetration of the skin barrier, nonsteroidals can be administered topically. Plasma concentrations of topical NSAIDs are far lower than the same amount of drug administered orally. However, if the drug is administered topically over a superficial joint such as the knees or hands, it can be detected at low concentrations in joint tissues. Randomized trials examining the efficacy of topical nonsteroidals suggest that they are more efficacious than placebo and slightly less efficacious than oral agents.[23] They are much safer than oral nonsteroidals, however, and the only major side effect associated with topical nonsteroidals is redness, burning, or itching on the skin where the medication is applied. This side effect occurs in a large percentage of patients. Strategies for the use of nonsteroidals in OA patients could include an initial trial of topical NSAIDs if the patient has painful knee or hand OA. Another option would be a trial of low-dose, oral, intermittent NSAIDs in patients who are not at high risk for the side effects. If tolerated, the drug dose can be increased.

## Corticosteroids as a Treatment for Osteoarthritis

Because synovial inflammation plays a major role in pain in OA, potent anti-inflammatories such as corticosteroids can be effective in relieving that pain. In fact, intra-articular corticosteroids decrease synovitis,[24] and may work in part by diminishing angiogenesis within the synovium, which secondarily decreases synovial inflammation. In randomized trials testing intra-articular steroids versus placebo injections in knee OA, steroids are more effective than placebo in relieving pain for 1 or 2 weeks. However, the longer-term trial data in knee OA is limited, and for some patients the effects may last much longer. In hip OA, intra-articular steroids have are more effective than placebo for at least 8 weeks.[25,26]

Evidence suggests that withdrawal of synovial fluid may enhance the efficacy of the steroid injection, and that higher doses of steroid tend to have longer-acting effects.

The effect size of intra-articular steroids as a treatment for OA in both knee and hip is large, and intra-articular corticosteroids are probably the most efficacious nonsurgical treatment, at least for pain reduction. They are especially efficacious for treating flares of disease and for cases in which disease is caused in part by the presence of intra-articular crystals, such as calcium pyrophosphate dihydrate crystals. No evidence exists that repeated corticosteroid injections into the joint are dangerous, although some case reports show that high doses of intra-articular corticosteroids may increase the risk of avascular necrosis in the treated joint.

The use of systemic steroids to treat OA, either orally or as intramuscular therapy, is unproven and is currently not recommended, although studies examining the benefits of local and systemic steroids in OA are in progress.

## Opioids

Because joint pain is the predominant symptom in OA, analgesic medications such as opioids could play a role in treatment. Opioids generally act in the central and peripheral nervous system by binding to opioid receptors. They have many side effects, including sedation, constipation, psychological effects, and dependence, which consists of unpleasant withdrawal symptoms if opioid use is discontinued. Meta-analyses[27] have suggested that opioids and tramadol, an opioid-like drug, both have efficacy in treating the pain of OA, but that compared with placebo, that the efficacy is modest at best (effect size vs. placebo of approximately 0.2 to 0.3). For example, a meta-analysis of tramadol has suggested that compared with placebo, its efficacy is somewhat less than the efficacy of oral nonsteroidals for OA. On the other hand, these same meta-analyses suggested that the adverse effect profiles of opioids in OA patients are formidable and that side effects, including major ones, are far more common among patients who take prescribed opioids than among those given placebo in trials. Because of this poor benefit to risk ratio, it is recommended that opioid use in OA be limited. Although concerns have been raised about tolerance to opioids as part of their use in other diseases, there is no evidence that the efficacy of opioids wanes with long-term use.

## Duloxetine

Selective serotonin and norepinephrine reuptake inhibitors (SNRIs) relieve pain in patients with chronic pain syndromes, including fibromyalgia. Duloxetine, an SNRI, is

approved to treat fibromyalgia, chronic musculoskeletal pain, and OA. In trials in patients with OA in which an escalating dose of as high as 120 mg a day was used, duloxetine was superior to placebo in pain reduction, with differences emerging at 2 weeks of treatment.[28,29] However, discontinuation because of side effects was considerably more common among the duloxetine-treated patients. Although these trials may demonstrate the efficacy of duloxetine (and suggest that other SNRIs may also be efficacious for the treatment of pain in OA), chronic widespread pain commonly accompanies painful knee OA. Further, nervous system adaptations occur as part of the chronic pain of OA, leading to allodynia and central sensitization. It may therefore be possible to identify patients who are more likely to respond to duloxetine, people with pain sensitization or chronic widespread pain. This has not been well studied, however.

## CONTROVERSIAL THERAPIES

### Glucosamine and Chondroitin

The signature pathology of OA is loss of hyaline articular cartilage. Both glucosamine and chondroitin are glycosaminoglycans constituents of a large macromolecule in cartilage known as *aggrecan*. These glycosaminoglycans have high negative charges, and during cartilage compression, the negatively charged molecules are forced into close proximity, increasing the electrostatic repulsion force among them. With the end of compression from weight bearing, the electrostatic force predominates and they move away from each other, allowing cartilage to reassume its usual thickness. Thus the chondroitin and glucosamine contribute to the compressive stiffness of cartilage. It is appealing to imagine that one can ingest a molecule that would then be incorporated into cartilage to improve its function. However, glycosaminoglycans are not synthesized from intact chondroitin or glucosamine molecules, and it is unlikely that ingested glucosamine or chondroitin would be incorporated intact into cartilage. While ingested as glucosamine salts (HCl, sulfate), glucosamine at least dissociates completely to glucosamine and its salt in the stomach. Thus the anion accompanying glucosamine is not relevant to the therapeutic effect of glucosamine.[30] Animal studies of glucosamine and chondroitin suggest that they may have anti-inflammatory effects.[30] Other postulated effects include an incorporation of the compounds into cartilage, effects on bone, and indirect effects on the joint via its direct effects on the liver.

Controversy exists regarding the interpretation of clinical trials testing the efficacy of glucosamine and chondroitin in humans. Although large, well-done studies have been performed that support the efficacy of each of these compounds, all nonindustry funded studies have been negative, including one large National Institute of Health (NIH)-funded multicenter study.[31,32] Other publicly funded studies from the United States, Europe, and Canada have all had null findings. One large meta-analysis has suggested that for each of glucosamine and chondroitin, the 95% confidence interval bound around their estimate of efficacy, although excluding a null effect of 0, is not large enough to signify a clinically important effect.[33] All positive trials of glucosamine have emanated from one company that produces a glucosamine sulfate compound, as opposed to the usual preparation sold in the United States: glucosamine HCl. As noted in preceding text, the glucosamine salt dissolves in the stomach so that both HCl and sulfate preparation ultimately produce the same molecule, glucosamine. Based on the results of publicly funded studies and meta-analyses summarizing all trial data on the efficacy of glucosamine and chondroitin major organizations such as the Osteoarthritis Research Society International and the American College of Rheumatology have removed glucosamine and chondroitin from lists of recommended OA treatment.[34,35] Some glucosamine and chondroitin trials have also suggested that these nutraceuticals delay joint space loss, but the NIH-funded gait study found no evidence of this.[36]

### Hyaluronic Acid Injections

When joints such as the knee move back and forth, the motion of one cartilage surface across another is almost frictionless. The lubrication provided by synovial fluid is to the result of several different molecules, among them hyaluronic acid (HA). Concentrations of hyaluronic acid in synovial fluid are diminished in individuals with OA, and that may decrease the viscosity of this fluid during disease. HA may also have direct effects on synovial fluid function. To increase synovial fluid viscosity, a number of compounds of hyaluronic acid have been developed, which are injected into the joint for therapeutic benefit. HA has previously been used in veterinary medicine; in that context it is given either intravenously or as an intra-articular injection.

Preparations of HA, when injected into the joint, are cleared usually after only a few hours, perhaps a day. Purified or synthesized HA products with large molecular weights remain a bit longer, with half-lives within the joint ranging from 17 hours to 1.5 days.[37,38] There are two fundamental types of HA molecules. The first is HA itself, which is a long nonsulfated chain of variable length with repeating disaccharide units. The second is a cross-linked hyaluronan chain that can be water insoluble as a gel. In OA, not only the concentration of HA in the synovial fluid but also the size of individual HA molecules diminishes.[39]

Because HA leaves the joint quickly, yet supposedly produces long-lasting clinical effects, other explanations for efficacy have been suggested, including the idea that synovial sites producing HA are stimulated by injections, although the evidence supporting this explanation is meager.[38] HA has also been thought to have anti-inflammatory and anti-nociceptive effects,[40] but it is difficult to imagine how HA injections would exert prolonged effects, given their transient presence in the joint.

HA injections have been approved by regulatory agencies for the treatment of OA and, depending on the preparation, the course of injections ranges from one to five, usually at weekly intervals. A large number of meta-analyses have assessed the efficacy of HA versus placebo intra-articular injection in trials. It is noteworthy that a large number of unpublished trials have been uncovered, and these have generally shown null results.[41] Among 18 large trials with blinded outcome assessment, the effect size of HA in reducing pain versus placebo was 0.11; statistically significant but clinically irrelevant. HA injections were found to increase

the risk of adverse effects, including flares of pain. Different meta-analyses have concluded that trials of HA either provide no evidence of efficacy or that the evidence suggests minimal efficacy. These meta-analyses have focused on knee OA, for which the preponderance of studies has been carried out. Two randomized trials of hip OA comparing the intra-articular HA injection with articular placebo have shown no efficacy of treatment. The recent Osteoarthritis Research Society International guidelines for OA rated hyaluronic acid as uncertain in its efficacy for knee OA and inappropriate for other types of joint OA. The American College of Rheumatology guidelines for the treatment of OA did not make recommendations regarding the use of hyaluronic acid injections for either knee or hip. The National Institute for Health and Care Excellence (NICE) guidelines recommends against offering intra-articular hyaluronate injections. Based on findings from a meta-analysis of pharmacologic treatments in OA,[42] intra-articular injections (including intra-articular placebo) are more efficacious in general than orally administered drugs, and a favorable patient response to HA may be explained, in part, by its delivery method.

### Tetracyclines

Although tetracyclines inhibit enzymes that break down cartilage, doxycycline, tested in a large trial for OA, was not found to consistently protect joints from progressive OA, or to alleviate pain.[43]

### Hydroxychloroquine

Given the potential influence of articular inflammation of joint pain and joint destruction, it is appealing to assume that a drug that abrogates inflammatory processes would be efficacious not only for rheumatoid arthritis but also for OA, especially hand OA, where inflammation is so clinically evident. However, there is an absence of evidence on the efficacy of hydroxychloroquine for OA, and it is not recommended in treatment guidelines.

## UNPROVEN REMEDIES IN OSTEOARTHRITIS

OA is a painful chronic condition that affects many millions of people throughout the world. Furthermore, approved and effective medical therapies, most of them described in this chapter, are often not very effective in alleviating all the pain and discomfort of disease. That has left a major opening for companies and entrepreneurs to market unproven remedies as effective treatments for OA. From copper bracelets to ginger to health food supplements, a multitude of treatments, many with little scientific rationale, are advertised as effective for OA pain. The market for these unproven remedies is large. Any health food store, many pharmacies, and websites with a focus on arthritis, contain ads and opportunities to purchase many of these supposedly effective treatments. Patients will ask whether there is evidence that they work, and many will be convinced that they or a close friend or relative have been helped by use of one or more of these treatments.

The threshold that distinguishes an unproven treatment from a treatment that fits within the accepted armamentarium is a fine and blurred line. To be a proven therapy, a treatment needs to have a convincing biologic rationale or mechanism of action that can be proven. Also, randomized clinical trials must demonstrate the efficacy of the treatment. Glucosamine constitutes an excellent example of this blurred line as noted above. For many unproven remedies, there is not as much evidence as there is for glucosamine. In some cases, there is one industry-sponsored trial that reports efficacy, perhaps from a developing country, where trials are often reported as positive. There may be a history of use of this treatment for OA or cultural support among particular communities for its use. There may be some suggestive evidence that a health food or vitamin contains a compound that might be therapeutic, and an industry-sponsored trial that purportedly demonstrates efficacy. Such is the case for ginger, which has some mild anti-inflammatory effects.

For many unproven remedies, the biologic rationale or mechanism of action is unclear, and there may not be a placebo-controlled randomized trial that suggests efficacy. Because the market is so large and potentially lucrative for those marketing potential treatments for OA, it is not surprising that manufacturers and producers will present rationales for treatment that may not be credible, and that randomized trials are carried out the results of which may not be trustworthy.

In evaluating whether patients should be given unproven remedies, several different considerations emerge. First, placebo treatments in OA[44] often work moderately well to relieve pain, and their effect on pain compared with no treatment at all is sometimes greater than that of effective treatments compared with placebo. Intra-articular injections are especially likely to be effective.[42] Some authors have recommended that if the unproven remedy is benign, then clinicians should provide it to patients even if it is no better than placebo. Given these considerations, when faced with a patient who insists that a specific unproven remedy has been of value for them or those close to them, the clinician should strongly consider not disabusing patients of this notion and should try to take advantage of whatever effect this treatment has, whether it is real or placebo based.

## SURGERY FOR OSTEOARTHRITIS

### Arthroscopy and Meniscal Surgery

Many knees affected by OA also have coexistent meniscal tears. These knees often are painful and sometimes have mechanical symptoms, such as catching or locking, which may be referable to their tears. Several randomized trials comparing surgery to no surgery have shown consistently that arthroscopy with meniscal surgery is not effective for these patients. In addition arthroscopy with lavage and débridement is no more effective than no surgery at all.[45,46] Even mechanical symptoms presumably related to the meniscal tears often do not respond to arthroscopic surgery.

Short of knee replacement, what surgical options are available for patients with OA? If knees have isolated compartment involvement, such as medial tibiofemoral disease, orthopedists may choose to do osteotomies, which break the

tibia and can realign the knee to correct malalignment. Alternatively, they can carry out unicondylar replacement with realignment. Both of these surgeries may delay the knee for a knee replacement or even obviate it. Of the two approaches, osteotomies are becoming less popular as there are fewer surgeons familiar with carrying them out.

## Total Joint Arthroplasty

Knee and hip replacements are efficacious and cost-effective for patients with advanced osteoarthritis who have severe pain. There is little agreement among surgeons, practitioners, or even patients as to exactly which patients ought to get knee or hip replacement.[47] Indications not only vary among surgeons, but they also vary across countries. However, a number of investigators in different countries have suggested that particular patients are good candidates for knee or hip replacements. Recommended indications are shown in Table 100-3 based on a recent survey of patients and orthopedists. Although these guidelines focus on pain and disability, surgery should be reserved for those whose symptoms have not responded to nonsurgical treatments, such as exercise and anti-inflammatory drugs. The major determinant of the need for surgery is pain, and not necessarily radiographic severity or the amount of joint destruction. Many patients with severe joint destruction have surprisingly little pain; whereas others whose radiographs do not show advanced disease are disabled by pain. Among these two contrasting groups, those with disabling pain are likely to be better candidates for knee replacement, although recent data[48] suggest that those in whom a knee replacement is most likely to fail are often those whose radiographic disease at the time of surgery is extremely mild. This may be because the knee pain experienced by these patients is not actually the result of knee OA but, rather, more diffuse pain syndromes such as fibromyalgia.

The indications for knee and hip arthroplasty are not necessarily the same. Hip arthroplasty is, on average, a more successful operation, with faster rehabilitation times than knee replacement. That suggests that the threshold for seeking a hip replacement might be lower than that for knee replacement. For patients undergoing knee replacements, 20% to 30% have persistent pain and disability, even 2 or more years after the operation, and the rehabilitation after knee replacement often takes several months, in part to regain full range of motion. Risk factors for a poor outcome after knee replacement include the presence of pain in the contralateral knee or other joints,[49] and the presence of a higher comorbidity burden. Although those with worse pain and disability have greater absolute improvements in their pain and function scores after joint replacement, they still are left with more functional limitations than those who went into joint replacement with milder functional limitations.

Although obese individuals also experience the benefits of knee and hip replacement, the failure rates of these prostheses is higher. Younger patients (those younger than 60 years) are increasingly presenting with a need for knee and hip replacement. In the United States, especially, there is a marked increase in the rates of knee and hip replacement among these people, and although prostheses last many years, it is unclear whether the durability of prostheses will be the same in patients who undergo the surgery at a younger age, in large part because they are far more active than older patients. Patients who meet clinical criteria for knee or hip replacement and are age 60 years and older are old enough to benefit from the surgery without being at high risk of loosening or other joint failure. Increasingly it is thought that patients 55 years and older have a similar benefit to risk ratio, but it is unclear whether those younger than 55 years will similarly benefit without an increased risk.

Although patients with OA that affects the thumb base (the first carpometacarpal [CMC] joint) often experience a reduction in pain and improvement in hand function after surgery, the surgical approaches are varied, and it is unclear which has better outcomes.[50] Options include trapeziectomy and the same surgery with tendon interposition and/or ligament reconstruction, CMC fusion, and even total joint replacement.

Other joints affected by OA may benefit from joint replacement. Although glenohumeral joint OA is uncommon, evidence[51] suggests that patients with this disorder who undergo shoulder joint arthroplasty experience a marked reduction in pain. Ankle joint OA, usually a consequence of trauma to the ankle, can be treated with either ankle joint replacement or ankle fusion.

**Table 100-3** Indications that Patients May Be Good Candidates for Hip or Knee Replacement

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|---|
| Patients report that arthritis symptoms are negatively impacting their overall quality of life. |
| The patient has evidence of arthritis on examination (clinical and radiographic/imaging) of the joint being considered for surgery. |
| The patient has had an adequate trial of nonsurgical arthritis treatment. |
| The patient's expectations for joint replacement surgery are achievable. |
| The patient is physically and mentally ready to have surgery. |
| The patient and surgeon agree that the potential benefits to the patient of joint replacement surgery outweigh the potential surgical risks. |

From Hawker G, Bohm ER, Conner-Spady B, et al: Perspectives of Canadian stakeholders on criteria for appropriateness for total joint arthroplasty in patients with hip and knee osteoarthritis. *Arthritis Rheumatol* 67(7):1806–1815, 2015.

## Joint Distraction

A novel and still experimental approach to treating OA is joint distraction arthroplasty, in which an external fixation frame bridging the joint is fixed with two bone pins on each side of the joint, and the frame gradually elongates, increasingly distracting the joint and separating one side of the joint from the other. The tubes with distraction are applied for approximately 2 months, after which they are removed. One effect of this distraction is to unload the osteoarthritic joint. This approach has been used most often for ankle OA, for which surgical and other treatments are limited. The joint space or bone-to-bone distance in the ankle increases in many patients after distraction arthroplasty, especially of the ankles, and more comprehensive studies have suggested that cartilage redevelops within the joint, although it is

not clear whether this is hyaline articular cartilage. Bone marrow lesions also appear to shrink or even resolve. Recent reports suggest that distraction arthroplasty might additionally be successful for knee OA,[52] at least at a follow-up of 24 months. Although promised as a treatment, distraction arthroplasty requires the patient to wear the fixation device around the affected joint for a couple of months with limited activity during that time, and the long-term effects beyond 2 years are not well documented.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 101



# Metabolic Bone Disease

Nancy E. Lane

**KEY POINTS**

- Osteoporosis is a disease defined by low bone density and deterioration of microarchitecture, which reduces bone strength and increases fracture risk.
- Major clinical risk factors for osteoporotic fractures include older age, low weight, family history of hip fracture, fracture occurring after age 50 years, glucocorticoid use, and inability to rise from a chair without assistance. More than 50% of osteoporosis in men results from secondary causes.
- Post-menopausal and age-related bone loss results from an uncoupling of bone remodeling such that bone resorption is greater than bone formation, resulting in a net loss of bone.
- Evaluation for risk of osteoporotic fractures with the Fracture Assessment Index (FRAX) for post-menopausal women and for men is critical to determine which persons have a 10-year fracture risk (for the hip and major osteoporotic fracture sites) that is high enough to warrant treatment.
- Treatment of high-turnover osteoporosis resulting from estrogen deficiency with anti-resorptive agents (e.g., estrogen, raloxifene, and bisphosphonates [alendronate, risedronate, zoledronic acid, ibandronate, and denosumab]) and an anabolic agent (recombinant human parathyroid hormone 1-34) can reduce incident vertebral fractures.
- Treatment for osteoporosis with bisphosphonates should continue for 3 to 5 years, after which, if the bone mineral density T-score is greater than –2, the subject has not sustained a fracture, and the overall risk of fracture is low, the treatment can be discontinued and the patient can be observed.
- Parathyroid hormone (PTH) increases osteoblast maturation and life span, increases trabecular bone mass and cortical thickness, improves bone strength, and decreases fractures. Anti-resorptive therapy is necessary after a full course of PTH to maintain newly formed bone mass.
- Glucocorticoid-induced bone loss results from increased osteoclast activity and reduced osteoblast activity and is most severe in the first 6 months of therapy. Bisphosphonate treatment can prevent fractures, and treatment with hPTH (1-34) can reverse glucocorticoid-induced osteoporosis and reduce incident fractures.
- Aromatase inhibitors reduce levels of serum estrogen and result in rapid bone loss in post-menopausal women undergoing adjuvant breast cancer therapy.
- Gonadotropin-releasing hormone agonists decrease testosterone and estrogen levels and cause bone loss in men being treated for prostate cancer.

Osteoporosis is characterized by low bone density and a deterioration of bone microarchitecture that reduces bone strength and increases the risk of fracture. The hallmark of osteoporosis is the loss of both bone mineral and bone matrix. Bone consists of an organic matrix (collagen and noncollagenous proteins) and an inorganic mineral component (calcium and phosphate in hydroxyapatite crystals; see Chapter 4). Normally, bone turnover is tightly coupled with osteoclast-mediated bone resorption followed by osteoblast-stimulated bone formation. This delicate balance in bone remodeling results in no net change in skeletal mass. Osteoblasts synthesize osteoid–bone matrix that subsequently undergoes mineralization and becomes mature bone matrix. The skeleton contains approximately 80% cortical bone, which is concentrated in the appendicular skeleton and femoral neck, and 20% more metabolically active trabecular bone, which is located in the spine, epiphyses, and pelvis. Osteoporosis is characterized by reduced bone strength that is usually accompanied by a reduction in bone mass. Osteomalacia encompasses disorders in which there is decreased mineralization of bone matrix. Paget's disease is a skeletal disorder characterized by increased rates of bone turnover with the development of disorganized woven bone.

## OSTEOPOROSIS

### Epidemiology and Clinical Signs

Osteoporosis, the most common metabolic bone disease, affects 200 million persons worldwide. Approximately 28 million Americans have osteoporosis or are at risk for it. *Osteoporosis*, or "porous bone," is a "disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures, especially of the hip, spine and wrist."[1] Although usually asymptomatic, osteoporosis can produce loss of height, pain, dowager's hump, and increased risk of fracture. After 50 years of age, an exponential rise in fractures occurs, with 40% of women and 13% of men experiencing one or more osteoporotic fractures in their lifetimes. In the United States alone, more than 1.5 million osteoporotic fractures occur annually, including 250,000 hip, 250,000 wrist, and 500,000 vertebral fractures. Hip fractures are associated with a 12% to 24% mortality rate in women and a nearly 25% mortality rate in men within the first year of fracture; in addition, 50% of patients are unable to ambulate independently and require long-term nursing home care.[2] These numbers will continue to grow exponentially as the elderly population of industrialized nations increases.

Bone accretion occurs during adolescence, when there is a large increment in bone mass. Peak bone density is normally achieved after puberty and into the third decade of life. However, by age 22 years, most persons have achieved nearly all of their peak bone mass. At menopause, an acceleration of bone loss usually occurs over approximately 5 to 8 years, with an annual 2% to 3% loss of trabecular bone and a 1% to 2% loss of cortical bone. Both men and women lose bone with age. Over a lifetime, women lose approximately 50% of trabecular and 30% of cortical bone; men generally lose two-thirds of these amounts.[3] Osteoporosis was previously thought to be a silent disease that was part of the normal aging process. However, the advent of bone densitometry has made it possible to accurately and reproducibly identify patients at risk for osteoporosis so that prevention and treatment strategies can be instituted to reduce the incidence of fractures. With a health care expenditure of $13.8 billion annually for osteoporosis-related fractures and a projected threefold rise in these costs during the next 40 years in the United States, the institution of effective prevention and treatment strategies to reduce fractures is of great importance.[1,4]

## Pathophysiology of Menopausal and Age-Related Bone Loss

Bone is constantly undergoing remodeling, whereby areas of bone resorption produced by osteoclastic action are replaced by bone laid down by osteoblasts. Osteoporosis results from an imbalance between bone resorption and formation. The initiation of bone remodeling is still being debated; however, the osteocytes, or terminally differentiated osteoblasts, located within the bone matrix and connected to one another and the bone surface may release chemical mediators that attract osteoclasts to the bone surface (Figure 101-1). Osteoclasts originate from hematopoietic stem cells that differentiate into colony-forming unit granulocyte-monocytes that differentiate into osteoclasts, are attracted to the bone surface, attach to the bone matrix, and resorb bone tissue. Generally, bone resorption is rapid, and a resorption pit is formed within 10 to 14 days. After bone resorption is complete, osteoblasts, which are derived from the bone marrow mesenchymal stromal cells, attach to the resorbed bone surface and produce osteoid, which is then mineralized. Bone formation can take up to 3 or 4 months, and thus a normal bone remodeling cycle in adults can last 4 to 6 months (see Figure 101-1A). A number of metabolic changes such as estrogen deficiency, immobilization, metabolic acidosis, hyperparathyroidism, and systemic and local inflammatory diseases can increase osteoclast number and activity, uncoupling bone turnover. This process results in greater bone resorption than bone formation and a net loss of bone tissue. New data show that a number of local factors in bone affect the regulation of bone formation and resorption and the coupling of these processes. These factors include insulin-like growth factors (IGFs), interleukins (IL-1, IL-6, and IL-11), TNF, receptor activator of nuclear factor-κB ligand (RANKL), and TGF-β.[5] Animal studies have shown that IL-1, IL-6, and TNF knockout mice do not lose bone with estrogen deficiency.[6] In addition, inflammatory arthritis animal models show that TNF, IL-1, and IL-6 are all strong stimulators of osteoclastic bone resorption. This link between the immune system and the maintenance of bone mass is intriguing, but additional work is required before we can understand its significance.

A number of mechanisms underlie primary osteoporosis, including a low peak bone mass as a young adult and rapid bone loss during menopause. Factors contributing to age-related bone loss include impaired calcium absorption with age, a compensatory rise in parathyroid hormone (PTH) levels, and greater resorption than formation of bone. Estrogen deficiency is associated with the release of cytokines including RANKL, IL-1, IL-6, and TNF, which leads to the recruitment and stimulation of osteoclasts in the marrow and increased production of bone-resorptive cytokines, which may contribute to menopause-related bone loss.[5] Estrogen therapy inhibits IL-1 release, and in oophorectomized rats and mice, an inhibitor of IL-1 (the IL-1 receptor antagonist) suppresses bone loss.[6] IL-6 levels also increase with age in human marrow cultures[7] and in peripheral monocytes. IL-1 and TNF induce the production of IL-6 from osteoblasts and stromal cells. Further evidence supporting a role of IL-6 in bone turnover includes data showing that oophorectomized IL-6 knockout transgenic mice do not lose bone.

Two other proteins that influence osteoclast activity have been identified: osteoprotegerin (OPG) and RANKL, which are produced by osteoblasts.[8] Estrogen deficiency increases osteoblast production of RANKL, which stimulates maturation and activity of osteoclasts by attaching to RANKL on the surface of immature and mature osteoclasts. Simultaneously, estrogen deficiency decreases osteoblast production of OPG, the decoy receptor that reduces RANKL production and activity. Adenoviral delivery of OPG ameliorates bone resorption in a mouse ovariectomy model of osteoporosis.[8] Both pre-clinical animal models and clinical trials of women with low bone mass have been completed and demonstrate that inhibition of RANKL with an anti-RANKL monoclonal antibody prevents estrogen-deficiency bone loss.[9]

In addition, a number of genetic, nutritional, and lifestyle risk factors predispose to the development of osteoporosis. Whites and Asians are at risk for low bone mass and osteoporosis, whereas African-Americans have a higher bone density and one-third to one-half the number of fractures.[1,8,9] Some studies show that African-Americans have lower vitamin D and urinary calcium levels, higher PTH levels, and skeletal resistance to the effects of PTH on bone.[10-12] Studies in twins and families show that up to 80% of the variance in bone mass is accounted for by genetic factors.[13] A maternal history of hip fracture, for example, is associated with a twofold increased risk of a hip fracture.[14] Data from Uitterlinden and colleagues[15] show that the gene encoding collagen type IA1 is associated with low bone density with increasing age and an increased risk of fracture. In the *ss* allele group, bone density was 12% lower at the femoral neck and 20% lower at the lumbar spine than that in the *SS* group, indicating an increased gene-dose effect with increasing age. However, *COLIA1* is associated with a lower baseline bone density and not an increased rate of bone loss. Further, genetically determined architectural features of bone such as a long hip axis length may contribute to increased fracture risk; conversely, a short hip axis length

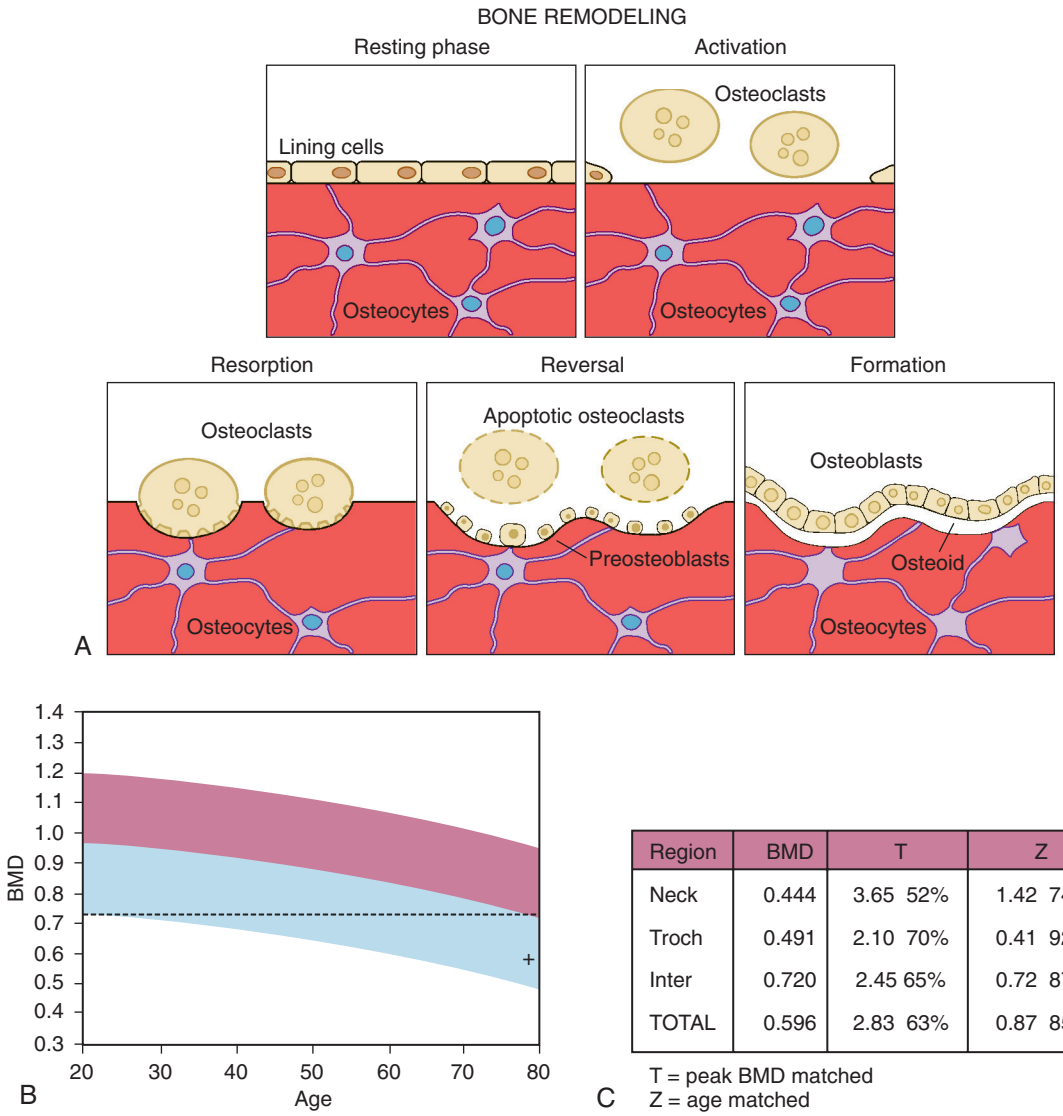



**Figure 101-1** **A,** The bone remodeling cycle. Osteocytes most likely release chemicals to the bone surface that attract osteoclasts. Osteoclasts attach to the bone matrix, create a tight ring, and release acid that lowers the pH and dissolves the mineral from the bone matrix. After the mineral is released, the demineralized matrix is broken down. The osteoclast leaves the bone surface, and an osteoblast is attracted to the area of the bone that was resorbed. The resorption phase is about 10 to 14 days. Osteoblasts produce new bone, or osteoid, that fills in the resorption pit. Also, some of the osteoblasts are left within the bone matrix as osteocytes. The osteoid mineralizes over about 3 months, and the bone remodeling cycle is complete. **B** and **C,** The bone density of a post-menopausal woman is compared with that of both young, normal control subjects and age- and gender-matched control subjects. The + shows this patient's age and bone mineral density (BMD); the blue and purple regions represent the BMD for the patient's age plotted with respect to the manufacturer's reference range (equivalent to a Z-score). The T- and Z-scores represent the number of standard deviations below young, normal control subjects and age-matched control subjects, respectively. Because the bone density provides a gradient of fracture risk, therapies can be instituted to prevent the development of osteoporosis or to treat patients at increased risk for fracture. Inter, Intertrochanteric; Troch, trochanteric.

confers some protective effect.[16] Recently, a family has been described whose members have high bone mass but are otherwise phenotypically normal. This family has a mutation (an amino acid change) in the low-density lipoprotein receptor–related protein 5 (LRP5). Using in situ hybridization to a rat tibia, expression of LRP5 was detected in areas of bone involved in remodeling. Additional studies have reported that this LRP5 mutation increases wnt signaling, which may alter bone mass through a primary defect of bone formation. Persons with this mutation demonstrate normal levels of bone resorption, but specific markers of bone formation are strikingly elevated. The observation that LRP5 is expressed at high levels in osteoblasts is consistent with LRP5 having a role in this area. Further work is now required to determine whether other mutations in the chromosome containing the LRP5 segment are associated with a variation in bone density in the general population.[17,18]

Recently, genome-wide scans have been performed in a number of cohorts and have found a number of single nucleotide polymorphisms (SNPs) that are associated with osteoporosis (fracture or bone mineral density [BMD] associations). They include VDR, ESR1, ESR2, LRP5, LRP4, SOST, GRP177, OPG, RNAK, RNAKL, COL1A, SPP1, ITGAI, SP7, and SOX6, which can be reasonably assigned as confirmed or replicated, and another 30 or so genes that are promising candidates. It is of note that confirmed and

**TABLE 101-1** Risk Factors for Osteoporosis

| |
|---|
| **Primary** |
| Previous fracture after age 30 yr |
| Family history of hip fracture |
| Cigarette smoking |
| Weight <127 lb |
| Low bone mineral density |
| **Secondary** |
| **Nonmodifiable** |
| White race |
| Advanced age |
| Frailty or poor health |
| Dementia |
| **Modifiable** |
| Low calcium intake |
| Eating disorder |
| Low testosterone levels (men) |
| Pre-menopausal estrogen deficiency (amenorrhea >1 yr or menopause at age <45 yr) |
| Excessive alcohol intake |
| Physical inactivity |
| Impaired vision |
| Neurologic disorder |
| Lack of sunlight exposure |

**TABLE 101-2** Medical Disorders and Medications Associated with Bone Loss and Osteoporosis

| |
|---|
| Primary osteoporosis |
| • Juvenile osteoporosis |
| • Post-menopausal osteoporosis |
| • Involutional osteoporosis |
| Endocrine abnormalities |
| • Glucocorticoid excess |
| • Thyroid hormone excess (supraphysiologic) |
| • Hypogonadism (including from prolactinoma or anorexia nervosa) |
| • Hyperparathyroidism |
| • Hypercalciuria |
| Processes affecting bone marrow |
| • Multiple myeloma |
| • Leukemia |
| • Gaucher's disease |
| • Systemic mastocytosis |
| Immobilization |
| • Space flight |
| Gastrointestinal diseases |
| • Gastrectomy |
| • Primary biliary cirrhosis |
| • Celiac disease |
| Renal insufficiency |
| Chronic respiratory diseases |
| Connective tissue disorders |
| • Osteogenesis imperfecta |
| • Homocystinuria |
| • Ehlers-Danlos syndrome |
| Rheumatologic disorders |
| • Ankylosing spondylitis |
| • Rheumatoid arthritis |
| • Systemic lupus erythematosus |
| Medications |
| • Anti-convulsants |
| • Heparin |
| • Methotrexate |
| • Cyclophosphamide and gonadotropin-releasing hormone agonists (hypogonadism) |
| • Lithium |
| • Cyclosporine |
| • Aluminum |
| • Excessive alcohol |
| • Pre-menopausal tamoxifen |
| • Aromatase inhibitors |

Modified from LeBoff MS: Calcium and metabolic bone disease. In *Medical knowledge self-assessment program,* Philadelphia, 1995, American College of Physicians.

promising genes are clustered in three biologic pathways: the estrogen endocrine pathway, the Wnt/beta-catenin signaling pathway, and the RANKL/RANK/OPG pathway. New biologic pathways will certainly emerge when more osteoporosis genes are identified and validated.[19] Other risk factors for osteoporosis, as enumerated in Table 101-1, include low body weight and reduced gonadal steroid levels.[13] According to some reports, lifestyle factors that may contribute to the development of osteoporosis include cigarette smoking, excessive alcohol intake, reduced physical activity, and inadequate calcium intake. Cigarette smokers have poorer health than do nonsmokers, along with impaired calcium absorption, lower estrogen levels, earlier menopause, and more fractures, and they exercise less; smoking cessation reverses this risk of osteoporosis.

In a large, prospective study of 9516 women older than 65 years, the following lifestyle factors significantly increased the risk of hip fracture: no walking for exercise, intake of more than two cups of coffee daily, current use of long-acting benzodiazepines and anticonvulsant drugs, current weight less than weight at age 25 years, height greater than 5 feet 7 inches, age older than 80 years, fracture since age 50 years, inability to stand from a chair without using arms, poor depth perception, and self-evaluation of health as fair to poor.[14] Low bone density in conjunction with a fall or trauma predisposes an individual to a fracture. Poor health and compromise of neuromuscular function increase the risk of osteoporosis and falls, which in turn increase the risk of hip fracture.[14] Importantly, elderly white women with both a low bone mass and more than two risk factors have a nearly 20-fold increased risk for fracture.

Secondary causes of bone loss that can affect women and men of all ages and races are listed in Table 101-2. Glucocorticoid therapy is the most common secondary cause of bone loss. Osteoporotic fractures develop in an estimated 30% to 50% of patients treated with glucocorticoids.[20] Glucocorticoid therapy causes bone loss through a number of different mechanisms such as producing a negative calcium balance through impaired intestinal calcium absorption, increasing urinary calcium excretion, decreasing bone formation, dose dependently increasing apoptosis of osteocytes and osteoblasts, increasing bone resorption by stimulating osteoclast activity by macrophage colony-stimulating factor, and suppressing endogenous gonadal steroid production.[21] Therapy with glucocorticoids leads to an early and, in some instances, dramatic loss of trabecular bone, with less effect on cortical bone. In hyperthyroidism (Graves' disease or toxic nodule) or supraphysiologic therapy with thyroid hormone, the ensuing accelerated bone turnover may produce a reduction in bone mass when the thyroid-stimulating hormone level is suppressed, even when thyroid hormone levels are within the normal range.[22] Athletic amenorrhea, anorexia nervosa, and other hypogonadal states, including the use of gonadotropin-releasing hormone

agonists,[23,24] may result in bone loss. In addition to estrogen deficiency, women with anorexia nervosa have low levels of IGF-I and reduced levels of adrenal androgen dehydroepiandrostenedione, which may contribute to the development of osteoporosis.[25]

### Osteoporosis in Men

Osteoporosis in men was not recognized 20 years ago but is now a major public health problem because of men's recently increased life span. The epidemiology of osteoporosis in men is just now being evaluated. Fracture risk occurs in adolescence and young adulthood and then increases after the age of 70 years. Long bone fractures occur more commonly in young men, whereas hip and spine fractures are more prevalent in men older than 70 years. The increase in fractures in older men is just as significant as it is in women, but it occurs about 10 years later in life, with an age-adjusted incidence of hip fractures in men of about one-third to one-half that of women.[26] Elderly men who sustain hip fractures have a greater risk of dying within the first year or being permanently disabled compared with women.[26] Recent data from the Study of Osteoporotic Fractures in Men (MrOS) determined that, after 15 years of follow-up, men of an older age (>75 years) who had low femoral neck BMD, a history of a previous fracture, divorced marital status, greater height, use of tricyclic anti-depressants, and a history of hyperthyroidism (Graves' disease) were independently related to an increased risk of hip fracture with low femoral neck BMD. In addition, in men with a low BMD (ranging from −1 to −2.5), the risk of hip fracture incidence increased from 4/1000 patient years to 40.2/1000 patient years).[27]

Osteoporosis in men can result from a number of secondary causes; for instance, hypogonadism causes an increase in bone turnover and rapid bone loss as gonadal function declines with age. At this time, severe hypogonadism from androgen deprivation therapy for prostate cancer is common in elderly men. The exact role of estrogen and androgens in male skeletal health is not yet known. Although estrogen is necessary for the young male skeleton, serum estrogen levels are highly correlated with bone remodeling, BMD, and the rate of BMD loss in older men; the associations are stronger than with testosterone. However, serum testosterone levels are also highly correlated with indices of bone resorption and formation. The roles of estrogen and testosterone in the male skeleton require additional investigation.[28] Some of the other common causes of osteoporosis in men that are not as frequent in women are alcoholism, gastrointestinal disorders, including hepatic disorders, and malabsorption.[26]

### Osteoporosis in Rheumatic Diseases and Other Conditions

Recently, studies have reported significant bone loss in patients with systemic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and ankylosing spondylitis. Patients with rheumatoid arthritis experience periarticular and generalized bone loss and an increased incidence of fractures compared with the general population.[29] T lymphocytes, tissue macrophages, and synovial-like fibroblasts release inflammatory cytokines (IL-1, TNF, and IL-6) and inhibitory wnt signaling proteins such as dkk-1 and RANKL, which stimulate preosteoclasts in the bone marrow and synovium to actively resorb bone; in addition, osteoblast maturation is altered.[30,31] In an animal model of inflammatory arthritis induced with collagen, animals pretreated with OPG did not have bone loss within the periarticular bone or the presence of erosions.[32]

Additional factors that might contribute to osteoporosis in patients with rheumatic diseases include decreased mobility, glucocorticoid therapy, and systemic inflammation.[33] Some data, however, show that low-dose glucocorticoid therapy in women with rheumatoid arthritis does not have adverse skeletal effects, possibly because of a decrease in disease activity in association with the suppression of inflammatory cytokines and improved physical activity and function.[34,35] Ankylosing spondylitis is also associated with fractures and reduced bone density in the spine and proximal femur, even early in the disease.[36] Patients with SLE have a high rate of osteoporotic fractures in the presence of low to normal bone mass, suggesting that systemic inflammation alters bone turnover. Increased serum levels of TNF can reduce osteoblast maturation and increase osteoclast maturation and activity; in addition, other inflammatory factors such as oxidized low-density lipoproteins and inflammatory high-density lipoproteins can direct mesenchymal stem cells to differentiate into adipocytes instead of osteoblasts and impair bone mass.[37] Infiltrative processes in the marrow such as multiple myeloma, mastocytosis, and Gaucher's disease may produce osteoporosis. Patients with Gaucher's disease show an accumulation of glucocerebrosides in macrophages in the spleen, liver, and bone marrow, which causes hepatosplenomegaly, anemia, thrombocytopenia, bone infarcts and infections, fractures, and aseptic necrosis.[38]

The immunosuppressant drug cyclophosphamide (Cytoxan) induces amenorrhea and hypogonadism, which may increase the risk of bone loss. Women who undergo premature menopause from cyclophosphamide therapy can have estrogen-deficiency bone loss in their thirties. Young women with SLE who try to preserve ovarian function while undergoing cyclophosphamide therapy by taking gonadotropin-releasing hormone agonists may also experience estrogen-deficiency bone loss. In rodent models, the immunosuppressive drug cyclosporine produces a time- and dose-dependent bone loss[39]; in contrast, azathioprine (Imuran) and rapamycin (sirolimus) do not appear to adversely affect skeletal homeostasis.[40] Therapy with both cyclosporine and prednisone in transplant recipients is associated with early accelerated bone loss after the initiation of treatment and the development of osteoporosis and fractures with continued exposure.[41]

Vitamin D deficiency may also manifest as osteopenia and fractures, but this condition is both preventable and treatable.[42] Vitamin D insufficiency is common in older patients and in persons with SLE who do not get an adequate amount of sunlight or who use potent sunscreens. Also, patients with malabsorption syndromes and liver disease can have vitamin D deficiencies. Unlike the situation in osteoporosis, very low vitamin D levels are often characterized by a mineralization defect and osteomalacia. Vitamin D deficiency is reported to be present in up to 50% of women with hip fractures.[42]

## Assessment of Bone Density and Osteoporotic Risk

Osteoporosis may first be diagnosed when a radiograph shows signs of demineralization or a spinal film shows evidence of compression fractures of vertebral bodies. Because an estimated 25% to 50% of bone mass must be lost to show osteopenia on radiographs, conventional radiography is an insensitive technique for diagnosing bone loss. Radiographs may demonstrate signs of secondary causes of osteoporosis such as the presence of subperiosteal resorption in persons with hyperparathyroidism, characteristic lytic changes or bone infarcts in persons with Gaucher's disease, local sites of lytic destruction in persons with malignancy, and pseudofractures in persons with osteomalacia. Bone densitometry makes it possible to measure the amount of bone in the relevant fracture sites of the spine, forearm, and proximal femur, as well as the total body.

Techniques for evaluating bone mass include dual-energy x-ray absorptiometry (DEXA) and quantitative computed tomography (CT) scanning of the spine.[1,2] Bone density evaluations using DEXA incorporate the attenuation of soft tissue and bone by radiographs to calculate the BMD. DEXA is both precise and safe, with a low degree of radiation exposure. With reproducibility errors of approximately 0.6% to 1.5%, this technique can detect small changes over time.[2,43,44] Further, newer DEXA techniques measure bone density rapidly, in 0.5 to 2.5 minutes. Quantitative CT scanning allows for the direct measurement of trabecular bone in the central region of the spine, but the procedure entails a comparatively high radiation exposure, and time and precision errors are usually higher than those associated with DEXA.

Figure 101-1B and C show the BMD in a postmenopausal patient compared with that of young, healthy control subjects to determine if there is reduction in BMD compared with peak bone mass (i.e., the percentage of young healthy control subjects expressed as a T-score) and with age-matched control subjects to assess whether BMD is diminished relative to an age-matched cohort (the percentage of age-matched control subjects expressed as a Z-score). An inverse relationship exists between bone density and the gradient of risk for fracture.[45] Prospective studies show that bone densitometry identifies patients with an increased gradient of risk for fracture. In 8134 women, a 1 standard deviation (SD) decrement in the bone density of the spine and the femoral neck compared with age-adjusted control subjects was associated with a 1.6- and 2.6-fold increased risk of hip fracture, respectively.[46] Measurement of bone density in the hip is more predictive of hip fracture than is measurement at another site. Studies show that in women older than 65 years, hip bone density is predictive of spine and hip fracture and that conventional spine density does not add to the diagnostic utility of a single hip bone density test in assessing the risk of fracture.

Although bone densitometry provides a quantitative measure of bone mass, in vitro studies using ultrasonography indicate that this technique also provides information about the mechanical properties of bone, including both density and elasticity. These qualities are strong predictors of bone strength. Ultrasound techniques include speed-of-sound and broadband ultrasound attenuation methods; the speed-of-sound technique reflects bone density and elasticity, and broadband ultrasound attenuation is an indicator of bone density, bone structure, and composition. Both ultrasound techniques have been approved for clinical use by the U.S. Food and Drug Administration (FDA) and have been shown to discriminate between normal and osteoporotic patients at increased fracture risk.[47] The T-score parameters used by some ultrasound machines do not correspond to T-score levels as measured by DEXA. Although ultrasonography is a radiation-free technique that may provide information about the risk of fracture and bone quality, the reproducibility of this technique and the measurement sites of mainly cortical bone or low-weight-bearing locations may make it unsuitable for monitoring small changes in bone over time. Therefore ultrasound measurements cannot reliably be used to monitor response to osteoporosis therapies. Further data are necessary to validate the clinical utility of ultrasonography.

According to the guidelines of the Scientific Advisory Board of the National Osteoporosis Foundation, bone densitometry is useful in determining which patients might benefit from therapy to protect the skeleton, including patients who have a deficiency of gonadal hormones (postmenopausal women <65 years with one or more risk factors or >65 years regardless of risk factors), post-menopausal fracture, evidence of osteopenia or a vertebral abnormality on radiographs, hyperparathyroidism, or exposure to supraphysiologic doses of glucocorticoids (Table 101-3). Bone densitometry is also used to decide when to commence therapy for osteoporosis and to assess the clinical response to therapeutic interventions.[40] Screening normal pre-menopausal women is not cost-effective.

The World Health Organization has published criteria for osteoporosis on the basis of bone density[1,48]:

- Normal bone density if the T-score is greater than −1.
- Osteopenia (low bone mass) is defined as a bone density measurement between 1 and 2.5 SD below the young-adult mean (T-score between −1 and −2.5).
- Osteoporosis is defined as a bone density measurement less than 2.5 SD below that of young, healthy control subjects (T-score <2.5).

The National Osteoporosis Foundation recommends treatment for all people who have a lumbar spine, hip, or

**TABLE 101-3** Clinical Indications for Bone Densitometry

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|---|
| All post-menopausal women <65 yr who have one or more additional risk factors for osteoporosis (besides menopause) |
| All women >65 yr regardless of additional risk factors |
| To document reduced bone density in patients with vertebral abnormalities or osteopenia on radiographs |
| Estrogen-deficient women at risk for low bone density who are considering use of estrogen or an alternative therapy, if bone density would influence the decision |
| Women who have been receiving estrogen replacement therapy for prolonged periods or to monitor the efficacy of a therapeutic intervention or interventions for osteoporosis |
| To diagnose low bone mass in people treated with glucocorticoids |
| To document low bone density in people with asymptomatic primary or secondary hyperparathyroidism |

femoral neck T-score of –2.5 or lower. However, for people who have a bone density between –1 and –2.5, the National Osteoporosis Foundation recommends performing a Fracture Risk Assessment (FRAX) using a computer program that incorporates clinical risk factors for osteoporosis with and without femoral neck BMD. FRAX also provides a 10-year risk of a hip fracture or a major osteoporotic fracture (i.e., hip, proximal humerus, and wrist). The clinical risk factors included in the FRAX program include age, weight, height, history of a fracture as an adult, parental history of a hip fracture, current glucocorticoid use, secondary causes of osteoporosis, alcohol intake of more than two drinks a day, and being a current smoker.[48,49] These risk factors are added to femoral neck T-score for the 10-year fracture risk. In the United States, a 10-year risk of hip fracture of 3% or more or a major osteoporotic fracture of 20% or more is the threshold to recommend treatment. However, the fracture risk threshold for treatment is individualized by country, so it is important to enter the country in which one is practicing medicine.

FRAX has advantages that include easily determined risk factors, global validation, application in specific regions or nations, and scores that pertain to both men and women. However, FRAX has a number of limitations; for example, it can lead to underestimates or overestimates of fracture risk in some patients because it does not include all known risk factors and some risk factors are superficial (e.g., glucocorticoid use is the question, but dose is not evaluated). Such known or suspected risk factors that contribute to fracture risk but are not included in the FRAX algorithm include immobilization, epilepsy, chronic obstructive pulmonary disease, diabetes, and depression. Lastly, the FRAX score is calibrated only for untreated patients, and thus results can be misleading for patients who are already receiving pharmacologic therapy.[48,49]

### Markers of Bone Turnover

The development of sensitive biochemical markers of bone turnover makes it possible to analyze changes in bone formation and resorption at a given point in time and obtain additional information about a patient's risk of bone loss. Only three bone formation markers are currently available. Osteocalcin, a noncollagenous matrix protein in bone, is produced exclusively by osteoblasts; it correlates with histomorphometric bone measurements. In most conditions, bone resorption and formation are tightly coupled, and osteocalcin levels reflect bone turnover. The other markers of bone formation are bone-specific alkaline phosphatase (BSAP), an enzyme that is activated as osteoblasts mature, and amino-terminal propeptide of type I procollagen (PINP), a protein whose synthesis is high in maturing osteoblasts.[50]

Sensitive indicators of bone resorption derived from the degradation of mature collagen include the urine and serum markers of type I collagen cross-links, including amino-terminal telopeptide of type I collagen (N telopeptides, or NTX) or carboxy-terminal telopeptide of type I collagen (C telopeptides, or CTX). Urinary pyridinoline cross-link, NTX, and CTX levels correlate with histomorphometric determinations of bone resorption; these biomarkers increase with estrogen deficiency at menopause and are high in patients with a variety of disorders characterized by accelerated bone turnover, including Paget's disease, osteoporosis, and rheumatoid arthritis.[51,52] Urinary excretion of N telopeptides is inversely related to total hip and spinal bone density and is more reliable than total urinary pyridinolone.[53] A few small prospective studies have found high turnover markers, and low BMD has been associated with incident fracture risk, but these results have not been replicated in larger studies.[54,55] In clinical studies, antiresorptive agents such as estrogen, bisphosphonates, and inhibitor of RANKL induce a significant decrease (30% to 80%) first in markers of resorption and then in bone formation markers, often within 3 to 6 months. Resorption markers decrease before formation markers and correlate with either maintenance of or an increase in BMD. A significant change in bone markers can be observed within months of anti-resorptive therapy, before changes in BMD occur.[55] Both bone formation and resorption marker changes over 6 to 12 months have been found to predict future fracture risk. In a study of alendronate to reduce osteoporotic fractures, patients who had more than a 30% reduction in bone alkaline phosphatase had the greatest reduction in risk for new vertebral and nonvertebral fractures. Interestingly, studies have found that reductions in markers of bone turnover, either resorption or formation markers, are associated with a reduction in fracture risk. However, long-term, prospective studies of large numbers of women are necessary to determine whether selective biochemical markers of bone turnover can predict changes in BMD or fracture risk and whether these tests should be used in standard clinical practice.

Most bone turnover marker data are derived from large studies of anti-resorptive agents. However, a bone-building anabolic agent, PTH, has been approved for the treatment of post-menopausal osteoporosis and glucocorticoid-induced bone loss. PTH stimulates osteoblast activity, and therefore PINP, osteocalcin, and other markers of bone formation increase rapidly, within a few weeks of the initiation of treatment. However, activation of the osteoblast over time results in RANKL production, which stimulates osteoclast activity. With continued PTH treatment, markers of osteoclast activity also increase, reaching levels equal to those of formation markers by 6 months of treatment. Because the overall result is an increase in bone mass, the bone turnover markers during PTH therapy reflect significant bone remodeling on both trabecular and cortical bone surfaces and within the cortex. A few small studies have found that increases in both bone formation and resorption markers predict an increase in bone mass with PTH treatment.[56-58]

### Evaluation for Secondary Bone Loss

The workup for osteoporosis is directed toward excluding secondary causes of bone loss and includes a determination of serum calcium, phosphorus, supersensitive thyroid-stimulating hormone, 25-hydroxyvitamin D (25-OHD), and intact PTH, as well as urine calcium and creatinine levels. Also, a complete blood cell count, alkaline phosphatase and liver function tests, erythrocyte sedimentation rate (in some cases), and serum and urine protein electrophoresis for patients older than 50 years may be necessary (Table 101-4). In men, additional testing for secondary causes of

**TABLE 101-4** Evaluation of Osteoporosis

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| **For All Patients** |
| Laboratory tests including SMA, CBC, and supersensitive TSH; ± PTH, alkaline phosphatase, 25-hydroxyvitamin D levels, and either measurement or estimate of 24-h urinary calcium; ± serum and urine protein electrophoresis and ESR |
| **For Selected Patients*** |
| Definitive tests for endocrine, neoplastic, and gastrointestinal disorders |
| Bone biopsy under calcified sections with double tetracycline label |
| In some patients, markers of bone turnover to identify those at risk for increased bone loss |

*Children, pre-menopausal women, men younger than 60 years, black persons, and patients with rapidly progressive disease.

CBC, Complete blood cell count; ESR, erythrocyte sedimentation rate; PTH, parathyroid hormone; SMA, sequential multiple analysis; TSH, thyroid-stimulating hormone.

Modified from *Primer on the metabolic bone diseases and disorders of mineral metabolism,* ed 6, Washington, DC, 2006, American Society of Bone and Mineral Research.

**TABLE 101-5** Calcium Requirements Recommended by the National Academy of Sciences (USA)

| Age Group | Recommended Daily Allowance (mg/day) | Recommended Daily Allowance (IU/day) |
|---|---|---|
| Children 1-8 yr | 1000 | 600 |
| Adolescents 9-18 yr | 1300 | 600 |
| Pregnant nursing females | | |
| 14-18 yr | 1300 | 600 |
| 19-50 yr | 1000 | 600 |
| Men and women | | |
| 19-50 yr | 1000 | 600 |
| Females | 1200 | |
| >51-70 yr | 1200 | 800 |
| Males | | |
| 51-70 yr | 1000 | 600 |
| >70 yr | 1200 | 800 |

Modified from the Institute of Medicine: *Brief report dietary reference intakes for calcium and vitamin D*, Washington, DC, 2010, National Academy Press, pp 1-4.

osteoporosis includes serum testosterone and luteinizing hormone.

Further tests to rule out neoplastic or endocrinologic disorders and a bone biopsy (a decalcified bone specimen is obtained after a double tetracycline label with two different fluorescent labels) should be considered in certain patients with progressive bone loss and in those in whom osteoporosis is unlikely. Identification and appropriate therapy for underlying secondary causes of osteoporosis are important. For example, treatment of vitamin D deficiency is best accomplished with vitamin D supplements. Parathyroidectomy in patients with hyperparathyroidism characterized by hypercalcemia, hypercalciuria, nephrolithiasis, age younger than 50 years, or low cortical BMD (Z-score ≤2) was associated with a large (4% to 12.8%) increase in bone density over 4 years.[59] However, bone density was stable for up to 6 years in patients with mild hyperparathyroidism.[60] In addition, treatment of hyperthyroidism, hypercortisolism, and a variety of other disorders that may cause osteoporosis can produce increments in bone mass. Reduction in the systemic inflammation associated with rheumatic diseases such as TNF-blocking agents for rheumatoid arthritis or ankylosing spondylitis or glucocorticoid-sparing agents for SLE (e.g., azathioprine and mycophenolate mofetil) can also produce increments in bone mass.[61]

# TREATMENT

## Calcium

The goals of therapy for osteoporosis are to reduce bone resorption and enhance bone formation, if possible. Bone loss occurs when the calcium intake and absorption are insufficient to balance the daily calcium losses. Prospective data show that calcium stabilizes bone mass.[62]

Table 101-5 shows the current recommendations for optimal calcium intake for women and men from the 1997 report of the Institute of Medicine to the National Academy of Sciences.[63] In the absence of kidney stones or an underlying disorder of calcium metabolism, these calcium intakes are safe. To prevent negative calcium balance, premenopausal women and men require 1000 mg and postmenopausal women require 1200 mg of total elemental calcium daily.[64] Children have increasing calcium requirements during adolescence, and data show increased bone accretion with increased calcium intake in prepubescent and pubertal children. Calcium carbonate contains 40% elemental calcium and should be taken with meals because of poor absorption in achlorhydric patients in the absence of food. Calcium citrate, which contains 24% elemental calcium, has better bioavailability and is more readily absorbed.[65] It is also absorbed well on an empty stomach in patients with achlorhydria. Recent studies underscore the fact that only recommended daily allowances of calcium and vitamin D are useful for bone health in postmenopausal women and elderly men. Additional supplementation over the recommended daily allowance has resulted in reports of increased cardiovascular disease.[66-68]

## Estrogen

Hormone replacement therapy (HRT) was once the mainstay of treatment in osteoporosis because estrogen inhibits bone resorption, produces a small rise in bone density, and reduces the risk of fracture by approximately 50% in retrospective observational studies. Cardiovascular disease is the leading cause of death in post-menopausal women. Previous data from longitudinal observational studies suggested that estrogen replacement had a beneficial effect on reducing primary and secondary cardiac events in post-menopausal women. However, in 1998, data from the 4-year Heart and Estrogen/Progestin Replacement Study were published.[69] In this study, post-menopausal women with a previous history of heart disease were randomized to receive estrogen (0.625 mg) plus progestin (2.5 mg) or placebo alone. Results showed no reduction in the overall rate of coronary heart disease or cardiac events in the treatment group; in fact, an early increase in risk for cardiac events was noted, possibly related to increased coagulability.[70]

In addition, a large, multicenter, longitudinal study by the Women's Health Initiative (WHI)—in which 162,000 women aged 50 to 79 years were randomized into a placebo group, an HRT group (if the uterus was intact), or an estrogen-only group (if the uterus was absent)—was terminated early because of an increased risk of breast cancer and cardiovascular events. The research goals for the WHI study were to determine the effects of HRT, diet modification, and calcium and vitamin D supplements on heart disease, osteoporosis, and colorectal cancer risk. After a mean follow-up of 5.3 years in an 8.5-year study, the HRT group had an increased risk of seven more cardiac events per 10,000 women taking the drug for a year, eight more invasive breast cancers, eight more strokes, and eight more pulmonary emboli, but six fewer colorectal cancers and five fewer hip fractures.[71]

At this time, the general recommendation is that HRT should be used only for vasomotor symptoms that occur at the time of menopause. When these symptoms abate, it is recommended that estrogen replacement (consisting of combined estrogen and progestin for women with an intact uterus) be stopped because the perceived cardiovascular benefits have not been substantiated and the cardiovascular disease and breast cancer risk make the benefit-to-risk ratio unacceptable for most women. It is important to acknowledge that the estrogen-only arm of the WHI study in women without a uterus did not show an increased risk of heart disease or breast cancer.

If a woman and her physician decide that she is going to take HRT or estrogen alone for vasomotor symptoms, in women with an increased risk of coagulation, transdermal estrogen replacement should be used.

### Selective Estrogen Receptor Modulators

The ideal estrogen replacement therapy would confer the beneficial effects of estrogen on bone and cardiovascular disease without increasing the risk of breast or uterine cancer. Selective estrogen receptor modulators (SERMs) are a nonsteroidal class of drugs that bind to the estrogen receptor and differ from one another in their actions on estrogen-responsive tissues, acting selectively as agonists or antagonists. Tamoxifen, the first available SERM, is an estrogen antagonist that binds to the estrogen receptor and also has estrogen-agonist effects on bone, lipids, clotting factors, and endometrium. Tamoxifen therapy in women with breast cancer produced a small increase in bone density of the spine over 2 years, with no effect on radial bone density, in association with reductions in both low-density lipoprotein and total cholesterol.[72] The Breast Cancer Prevention Trial studied 13,388 women at increased risk for breast cancer, comparing treatment with tamoxifen (20 mg daily) to placebo for 5 years.[73] Tamoxifen reduced the risk of invasive and noninvasive breast cancer by 50%, and a decreased risk of fracture was observed as well: 45% reduction at the hip and 29% at the spine. An increased incidence of low-grade endometrial cancer was noted, but no change occurred in the risk of ischemic heart disease.[73]

Raloxifene,[74] which has been approved by the FDA for the prevention and treatment of osteoporosis, is a SERM that acts as an estrogen agonist on bone, with antagonist effects on the breast and uterus.[75] Raloxifene (60 mg/day over a 2-year study period) increased BMD in the lumbar spine by 2.4%, in the total hip by 2.4%, and in the total body by 2%, with a reduction in fracture risk at 2 years similar to that seen with estrogen or alendronate (5 mg) treatment. During the 2-year study period, raloxifene reduced the incidence of vertebral fractures by nearly 40% in the treated group compared with the placebo group, and fractures recurred in 7.6% of treated women with a previous fracture, compared with 14.3% of those in the placebo group.[76] Endometrial thickness is not increased by raloxifene, but menopausal symptoms may be made worse. Raloxifene decreases low-density lipoprotein cholesterol by 12%, with a nonsignificant increase in high-density lipoprotein cholesterol, but it does not reduce C-reactive protein like estrogen does and was not found to provide cardiovascular protection.[77-80] Raloxifene also decreased the incidence of breast cancer by 76% in patients enrolled in a clinical study of osteoporosis, with breast cancer incidence studied as a secondary endpoint.[74,80] Another study compared tamoxifen and raloxifene, and yet another study evaluated raloxifene versus placebo, in the prevention of breast cancer. The first study reported that both tamoxifen and raloxifene reduced the risk of developing breast cancer, and the second study found that raloxifene reduced the risk of estrogen receptor–positive breast cancer compared with placebo in post-menopausal women.[81,82] At this time, little information is available on the use of raloxifene in men, and thus it is not recommended for male patients.

## Testosterone

Men with osteoporosis, hypogonadism, and symptoms of low libido may benefit from testosterone replacement therapy. This therapy can be administered as testosterone cypionate or enanthate (50 to 400 mg intramuscularly every 2 to 4 weeks) or as a transdermal testosterone replacement patch that is applied to the scrotal area (Testoderm, 4 to 6 mg/day) or elsewhere (Androderm, 2.5 or 5 mg/day).[83] Most studies find that bone mass increases with testosterone replacement when levels of testosterone were low at the initiation of therapy.

## Calcitonin

Calcitonin, a 32 amino acid peptide synthesized by the C cells of the thyroid gland, is a potent inhibitor of osteoclast-mediated bone resorption. Although human and salmon calcitonin are commercially available, salmon calcitonin is most commonly used because of its greater potency. On the basis of data showing an increase in total body calcium, parenteral calcitonin was approved by the FDA for the treatment of osteoporosis in 1984, and calcitonin in a nasal spray was approved for the treatment of post-menopausal osteoporosis in 1995. Parenteral calcitonin (100 IU administered subcutaneously or intramuscularly three times a week or daily) can maintain bone density or produce a small increase in bone mass in the spine and, in some instances, the forearm, particularly in patients with a high bone turnover.[84] Nasal spray calcitonin is absorbed through the nasal mucosa and is approximately 40% as potent as the parenterally administered drug (e.g., 50 to 100 IU of injectable calcitonin is comparable with 200 IU of nasal spray

calcitonin).[85] In osteoporotic women more than 5 years past menopause, nasal calcitonin (200 IU/day) increases spinal bone density by 2% to 3% compared with placebo, with no effect on proximal femur bone mass; higher doses are necessary in the early menopausal period.[85,86] Nasal spray calcitonin therapy in patients with osteoporosis is associated with a 36% reduction in vertebral fractures over 5 years.[86]

The adverse effects of parenteral calcitonin include nausea, flushing, and local irritation at the injection site. Calcitonin given intranasally is well tolerated, with the potential adverse effects of rhinitis and nasal symptoms such as dryness and crusting. Patients treated with parenteral or intranasal calcitonin may also obtain a beneficial analgesic response in the presence of osteoporotic fractures. Recently, calcitonin nasal spray was removed from use in Europe because of a concern that it may increase the risk of prostate cancer in men, but at this time it is still on formularies in the United States.

## Anti-resorptive Drugs

### Bisphosphonates

Bisphosphonates are analogues of pyrophosphate, with a P-C-P rather than a P-O-P core; they are absorbed by the hydroxyapatite of bone and suppress bone resorption. Modification of the side chains can result in the development of a variety of compounds with differing abilities to inhibit bone resorption (Table 101-6). Some bisphosphonates are administered intermittently because of a long skeletal half-life and prolonged retention in bone. These compounds must be taken on an empty stomach because gastrointestinal absorption is less than 10%.

Bisphosphonates have been used for the treatment of patients with Paget's disease of bone, hypercalcemia of malignancy, prevention and treatment of metastatic cancer to bone, and osteoporosis and for the prevention and treatment of glucocorticoid-induced osteoporosis.

Alendronate has been approved by the FDA for the prevention and treatment of osteoporosis. Data in postmenopausal women with bone density at least 2.5 SD below peak bone mass show that alendronate (10 mg/day) compared with placebo produces an 8.8% and 7.8% increase in bone density in the spine and femoral trochanter, respectively, and a 5.9% increase in the femoral neck after 3 years of therapy[87]; smaller rises (2.3% to 4.4%) occur in bone density in the spine and proximal femur in women within 0.5 to 3 years of menopause. Fosamax treatment in women with osteoporosis (T-score <2.5) yields a significant reduction in spine and hip fractures compared with the patients treated with placebo.[88] Alendronate did not reduce the incidence of clinical fractures in women who had low bone mass but not osteoporosis.[89,90] Alendronate treatment is also effective in increasing bone mass in the spine, hip, and total body and helps prevent vertebral fractures and loss of height in men with osteoporosis.[91]

Adverse effects of bisphosphonates include gastrointestinal symptoms such as stomach pain and esophagitis (caution is advised in patients with active symptoms or a history of ulcer disease), myalgias and arthralgias, and, rarely, osteonecrosis of the jaw, along with subtrochanteric fractures with long-term use.[92-96] A once-a-week preparation of alendronate (70 mg) is the most commonly used dose for the treatment of osteoporosis.[93] This preparation increased spinal and hip bone mass similarly to alendronate, 10 mg/day, over a 2-year study period.

Risedronate, another oral bisphosphonate, administered at a dose of 5 mg/day, increased bone mass and reduced the risk of new vertebral fractures 50% better than did placebo.[94-97] Another study performed to assess the effect of risedronate on hip fractures found that women with osteoporosis (defined by a femoral neck T-score of ≤–4.0) had a significant reduction in the risk of hip fracture.[90] Risedronate has been approved for the prevention and treatment of osteoporosis (35 mg once a week and now 150 mg once a month)[91] and for the treatment of Paget's disease (30 mg/day for 2 months, with retreatment if relapse occurs after 2 months).[98,99] Studies show that risedronate may be well tolerated even in patients with mild gastrointestinal symptoms. Bisphosphonates may also reduce bone pain.

Ibandronate, another aminobisphosphonate, is approved for the treatment and prevention of post-menopausal osteoporosis. In phase III studies of ibandronate (2.5 mg/day) versus placebo in post-menopausal women with osteoporosis, incident vertebral fractures were reduced about 50%. Another study compared ibandronate, 150 mg once a month, with the daily 2.5-mg dose and found similar gains in lumbar spine and hip BMD. The FDA approved ibandronate, 150 mg/month, for the treatment of osteoporosis on the basis of this bridging study.[100] Recently, intravenous (IV) ibandronate in a dose of 3 mg every 3 months was found to be similar to ibandronate, 2.5 mg/day, in terms of increasing lumbar spine and hip BMD, and the FDA has approved IV ibandronate for this indication. No data on hip fractures are available for this compound.[101]

Zoledronic acid is approved for the treatment and prevention of post-menopausal osteoporosis.[102] In phase III studies in post-menopausal women with osteoporosis treated with zoledronic acid, 5 mg once a year by IV infusion, the risk of incident vertebral fractures, hip fractures, and other major osteoporotic fractures were reduced by 68%, 40%, and 20%, respectively, compared with the placebo-treated subjects.[102] In another phase III study of patients who had sustained a hip fracture, patients were randomized to

**TABLE 101-6** Inhibition of Metaphyseal Bone Resorption in Vivo by Bisphosphonates

| Chemical Modification | Examples | Anti-resorptive Potency |
|---|---|---|
| First generation: short alkyl or halide side chain | Etidronate | 1 |
| | Clodronate | 10 |
| Second generation: $NH_2$-terminal group | Tiludronate* | 10 |
| | Pamidronate | 100 |
| | Alendronate | 100-1000 |
| Third generation: cyclic side chain | Risedronate | 1000-10,000 |
| | Ibandronate | 1000-10,000 |
| | Zoledronate | 10,0000 |

*Tiludronate has a cyclic side chain, not an $NH_2$-terminal group, but it is generally classified as a second-generation compound on the basis of its time of development and potency.

Modified from Watts NB: Treatment of osteoporosis with bisphosphonates [review], *Endocrinol Metab Clin North Am* 27:419–439, 1998.

either zoledronic acid, 5 mg once a year, or placebo, and after 3 years it was found that the incidence of osteoporotic fractures was significantly less in patients treated with zoledronic acid and that the mortality was lowered compared with the patients treated with placebo.[103] Adverse effects of zoledronic acid include arthralgias and myalgias; however, these adverse events tend to be less frequent with subsequent infusions. In patients undergoing treatment with zoledronic acid, both serum calcium and 25-OHD levels should be monitored and deficiencies should be restored to normal levels before treatment. Zoledronic acid has been approved for the treatment and prevention of post-menopausal osteoporosis (5 mg once a year), osteoporosis in men (5 mg once a year), prevention and treatment of glucocorticoid-induced osteoporosis (5 mg once a year), and Paget's disease. Zoledronic acid at a dose of 4 mg intravenously every 4 weeks is currently approved for the prevention and treatment of bone metastases in patients with breast cancer and multiple myeloma.

### RANK Ligand Inhibitor

Denosumab is a monoclonal antibody that is directed against RANKL and is approved for the treatment of post-menopausal osteoporosis. In phase III studies in post-menopausal women with osteoporosis who were treated with denosumab (60 mg subcutaneously every 6 months) for 36 months or 3 years, the incidence of vertebral fractures, hip fractures, and major osteoporotic fractures was reduced by 68%, 40%, and 20%, respectively.[104] The medication is well tolerated, but adverse events of skin infection requiring hospitalization were higher in subjects treated with denosumab than in subjects treated with placebo. Denosumab is a potent inhibitor of bone resorption with suppression of a marker of osteoclast activity.

CTX-1 is suppressed by nearly 90% a few weeks after each injection; however, the suppression of bone turnover is transient, and bone turnover and bone density return to baseline levels rapidly if the injections of denosumab are discontinued. Post-menopausal women with low bone mass who were treated with alendronate, 70 mg once a week, and then switched to denosumab, 60 mg every 6 months, had a significant gain of BMD compared with subjects who continued to take alendronate.[105] Before treatment with denosumab, serum calcium and 25-OHD should be checked and replaced if needed to achieve normal levels.

### Adverse Events Associated with Potent Anti-resorptive Agents

All potent anti-resorptive agents (bisphosphonates and RANKL inhibitor) have been associated with osteonecrosis of the jaw, a rare disorder in which a small part of the jaw bone dies; this occurrence is associated with exposure of the bone, with loss of the mucosal covering. Although osteonecrosis of the jaw has been reported as an adverse effect of all bisphosphonates and of the RANKL inhibitor, denosumab, the incidence is rare (probably between 1/10,000 to 1/100,000 in persons receiving osteoporosis medication doses); it is more common in patients with cancer who are treated with high doses and with a high frequency. Although the mechanism is still unclear, the American Dental Association recommends that the teeth be examined before the bisphosphonate or other anti-resorptive treatment is initiated and that patients taking these medications should avoid dental procedures if possible, but it is not necessary to stop the treatment if procedures are needed. In addition, the American Dental Association prefers to use the term *anti-resorptive-associated osteonecrosis of the jaw*.[92,106]

Potent anti-resorptive agents (i.e., bisphosphonates and RANKL inhibitors) are also reported to be associated with atypical subtrochanteric fractures. These fractures are somewhat unusual in that they occur below the lesser trochanter in the femur, the incidence is probably about 5 cases/10,000 patient years, they can be bilateral, and they may be related to the duration of the bisphosphonate treatment. At this time, if a patient treated with a potent anti-resorptive agent experiences mid-thigh pain, evaluation for a stress fracture should be performed because it appears that these agents first appear as painful stress fractures that eventually evolve to a displaced fracture. The risk of a subtrochanteric fracture moves to nearly zero with discontinuation of the potent anti-resorptive agents.[107,108]

The development of subtrochanteric fractures in patients taking potent anti-resorptive agents has led the FDA to make a recommendation regarding the duration of treatment of osteoporosis with these agents. The FDA rationalized that the registration trials for the bisphosphonates (alendronate, risedronate, and zoledronic acid) were 3 to 4 years in duration and that the extension trials for alendronate (FLEX), zoledronate acid (Horizon), and risedronate (VERT-MN) ranged from 3 to 10 years in length, and therefore sufficient long-term data existed from patients who were taking the drugs and had stopped taking the drugs to determine the optimal duration of bisphosphonate use for the treatment of osteoporosis. The results of the three extension studies found consistent fracture rates and maintenance of BMD in both active and placebo groups. Based on these data, the FDA recommended that the decisions regarding the discontinuation of bisphosphonates should be based on the individual patient. Younger patients with a normal BMD (T-score of the hip greater than –1.5) after 3 to 5 years of bisphosphonate therapy may be candidates to stop treatment. However, patients with an increased risk of fracture (e.g., patients of an older age, with prevalent fractures, or with BMD in the osteoporotic range [less than –2.0]) may benefit from continued bisphosphonate use.[109-112] An additional analysis by FLEX investigators that evaluated the risk of fracture in subjects treated with alendronate, 10 mg a day for about 9 years, compared with alendronate for 4.5 years and then placebo for 5 years, and concluded that subjects with a femoral neck BMD of greater than –2 were unlikely to benefit from additional treatment with a bisphosphonate after 4 years. However, subjects with a femoral neck BMD of less than –2 with fractures or less than –2.5 without fractures may benefit from continued bisphosphonate treatment.[113]

## Parathyroid Hormone

Beginning in the 1980s, small randomized studies determined that the 1-34 fragment of the PTH protein can significantly increase bone mass in the spine, with small losses or no gain at the skeletal sites rich in cortical bone.[114,115] In

2001 a recombinant human PTH (rhPTH) composed of the 34 amino acids from the amino-terminal end of the hormone, known as *Forteo*, was approved for the treatment of postmenopausal osteoporosis. In a large international, multicenter study, osteoporotic women who had sustained a fracture were randomized to receive rhPTH at a dose of 20 μg/day or 40 μg/day or placebo for an average of 21 months. Lumbar spine bone mass increased between 9% and 13% in the subjects treated with rhPTH compared with the subjects treated with placebo; hip bone mass also increased slightly. Most important, the risk of new vertebral fractures was reduced nearly 70% in both sets of subjects treated with rhPTH, and nonvertebral low-trauma fractures were reduced nearly 50% compared with the subjects treated with placebo.[116] Initially this study was supposed to continue for 3 years; however, it was stopped at approximately 21 months because of pre-clinical evidence of malignant bone tumors in animal models. Additional studies of osteoporosis in men treated with rhPTH 1-34 have reported significant gains in bone mass.[107] Forteo is given as a daily injection. Persons using this medication may experience headache, nausea, and flushing with initiation of treatment, but these adverse effects generally become less severe after a few weeks.

A number of recent studies have evaluated whether rhPTH 1-34 or rhPTH 1-84 is more effective in combination with an anti-resorptive agent (either bisphosphonates or raloxifene) than rhPTH alone in increasing BMD and reducing fractures.[117,118] Interestingly, two studies found that the combination of PTH and alendronate was less effective in stimulating bone gain at the lumbar spine in both osteopenic women and men over 1 to 1.5 years.[108,109] However, in a recent study of post-menopausal women with osteoporosis who were treated with PTH alone, denosumab alone, or a combination of PTH and denosumab for 1 year, it was found that the combination treatment increased lumbar spine BMD by 9.1% and total hip BMD by 4.9%, which was significantly greater than either treatment alone.[119]

Continued treatments for an additional 12 months with PTH and denosumab further increased lumbar spine BMD to 12% and total hip BMD to 6%, and with 1 year of additional denosumab, bone mass at both the lumbar spine and total hip remained stable.[120]

PTH stimulates new bone formation, increases bone mass, and reduces new vertebral and nonvertebral fractures, but when the medication is discontinued, the bone gained is rapidly lost. Black and colleagues[121] performed a study in which patients were given PTH for 1 year, followed by 1 year of alendronate treatment. Interestingly, the BMD gain after 1 year of PTH was about 6%; when followed by alendronate, nearly 6% BMD was gained at the spine. These data suggest that although PTH is an effective monotherapy for increasing bone mass, especially in the spine, the gain in BMD should be maintained with a potent anti-resorptive agent for a number of years. Recent data from Deal and colleagues[122] indicate that lumbar spine BMD gained after PTH therapy is maintained with raloxifene treatment.

PTH is the first bone anabolic agent approved for the treatment of osteoporosis. Patients give themselves a subcutaneous injection daily for 18 to 24 months. Other routes of administration are now being studied, including an intranasal route and a skin patch.

## New Medications in Clinical Development

### Odanacatib

Cathepsin K is an enzyme released from osteoclasts that degrades collagen I in the bone matrix. Phase II and III clinical trials in subjects with low bone mass or osteoporosis have been completed. A phase II study demonstrated that treatment with odanacatib, 50 mg weekly,[123] increased lumbar spine bone mass by 5.5% and total hip BMD by 3.2% over 2 years, and biochemical markers of bone turnover demonstrated dose-related changes. Discontinuation of the medication resulted in a rapid increase in bone turnover markers, and after 12 months, the bone mass had returned to baseline values.[124] A recent phase III study (LOFT) of odanacatib, 50 mg a day, versus placebo, showed that after 5 years there was a 54% reduction in relative risk of new or worsening vertebral fractures, a 47% relative risk reduction in clinical hip fractures, a 23% relative risk reduction of clinical nonvertebral fractures, and a 72% reduction n clinical (painful) vertebral fractures.[125] In addition, treatment with odanacatib led to a progressive increase over 5 years in BMD at the lumbar spine and total hip; compared with the placebo group, the change in BMD from baseline to 5 years was 11.2% for the lumbar spine and 9.5% for the total hip. The rates of adverse events overall in the LOFT trial were balanced between the patients treated with odanacatib and placebo. However, morphea-like skin lesions and atypical femoral fractures were observed more often in the odanocatib group than in the placebo group.[125] Odanacatib treatment for osteoporosis is currently under review by the FDA.

### Romosozumab

Romosozumab is a monoclonal antibody that inhibits the Wnt signaling antagonist sclerostin and is currently in clinical development for the treatment of osteoporosis. Sclerostin, which is produced mainly by osteocytes within the bone and then is transported through the osteocyte canalicular network to the bone surface, inhibits osteoblast maturation. Treatment with romosozumab in pre-clinical studies of estrogen-deficient bone loss found restoration of bone mass to the baseline levels.[126] A recent phase II clinical trial reported that in post-menopausal women with low bone mass, a monthly treatment with romosozumab for 12 months increased lumbar spine bone mass by 12%, femoral neck bone mass by 4%, and total hip bone mass by 4%; this increase was significant greater than with treatment with either rhPTH (1-34) or alendronate.[127] Phase III clinical studies are now ongoing to determine if treatment with romosozumab can reduce incident hip fractures in postmenopausal women with osteoporosis.

## Vitamin D

Physiologic doses of vitamin D are important to ensure normal bone mineralization. Persons 50 years and older should take at least 600 to 1000 IU of vitamin D daily as a multivitamin or combined with a calcium supplement. Hypovitaminosis D is common in the elderly population, with one study demonstrating that 57% of patients in a general medical ward were deficient in vitamin D.[128] Low

vitamin D levels increase the risk of bone loss and fracture. LeBoff and colleagues[42] found that 50% of patients admitted with acute femur fractures had vitamin D deficiency (25-OHD level <12 ng/mL), and 36.7% had secondary hyperparathyroidism. Data show seasonal variations in vitamin D levels; low 25-OHD levels during the winter and spring are associated with decreases in bone density. The importance of vitamin D to skeletal health was shown when elderly women in a nursing home who were treated with only 800 IU/day of vitamin D had a 40% reduction in incident hip fractures over 18 months compared with placebo-controlled subjects.[129] Although this dramatic effect on fractures in an elderly population may represent a correction of vitamin D insufficiency, this study underscores that adequate vitamin D replacement can effectively diminish fractures in older persons. Insufficient calcium and low 25-OHD levels are common in ambulatory patients and should be identified and treated before anti-resorptive or other therapies for osteoporosis are initiated.

Although 200 IU of vitamin D prevents bone loss in the spine, data show that a higher daily intake (800 IU) is necessary to diminish bone loss in the hip during the winter and spring. Daily treatment with 700 IU of cholecalciferol and 500 mg of calcium carbonate reduced the rate of bone loss significantly in the femoral neck, spine, and total body and decreased the incidence of nonvertebral fractures by 50%.[130] Therefore, to maintain skeletal health, patients require a vitamin D intake that results in a serum 25-OHD level of at least 30 ng/mL. To achieve this level in patients who do not receive regular sunlight, the daily intake of vitamin D needs to be higher. Replacement with 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) is not recommended because hypercalcemia and hypercalciuria are common and require regular and costly monitoring.

### Preventive Measures

Because bone loss is not completely reversible with existing therapies, prevention is essential for optimizing skeletal health. Strategies directed at increasing peak bone mass, reducing risk factors for bone loss (e.g., hypogonadism, decreased body fat, cigarette smoking, inactivity, and excessive alcohol intake), and reversing the secondary causes of osteoporosis may prevent bone loss. Patients should be advised to consume adequate vitamin D and calcium and participate in a regular weight-bearing exercise program. Weight-bearing exercise increases muscle strength and may stabilize or modestly increase bone density. Emerging data show that increased calcium intake and exercise can add to bone accretion during adolescence and that interventions such as vitamin D and calcium supplementation can reduce fractures in older patients. Thus it is highly recommended that preventive strategies or therapies be instituted at any age to diminish the risk of fractures, which rises exponentially with age.

## GLUCOCORTICOID-INDUCED OSTEOPOROSIS

Bone loss is a common sequela of therapy with glucocorticoids,[131] and glucocorticoid use increases the risk of fractures in patients with rheumatic diseases.[20] The severity of the bone loss in patients treated with glucocorticoids varies, with an approximately 3% to 20% decrease in bone density over 1 to 2 years. Glucocorticoid therapy is associated with increased fractures of the ribs and vertebrae, sites that contain predominantly trabecular bone, and it triples the risk of hip fracture in one-third of patients after 5 to 10 years of treatment.[132,133] In adults, alternate-day glucocorticoid therapy does not prevent bone loss. In patients with rheumatic diseases, concerns over the development of glucocorticoid-induced osteoporosis often limit the dose and duration of glucocorticoid therapy.

The lowest possible glucocorticoid dose should be used, along with general preventive strategies such as a regular weight-bearing exercise program, adequate calcium and vitamin D intake, and reduction of other risk factors that might contribute to the development of osteoporosis. However, data show that even patients taking 5 mg/day of prednisone have accelerated bone loss compared with control subjects. General prophylactic measures to prevent glucocorticoid-induced osteoporosis are shown in Table 101-7. Intestinal calcium absorption is impaired in patients treated with glucocorticoids, and early studies showed that this impairment could be offset with vitamin D (40 to 100 μg/day two to three times a week) or 25-OHD, which produced an increase in bone density of the forearm. However, the administration of supraphysiologic doses of vitamin D requires careful monitoring of the serum and urinary calcium concentrations in patients at high risk for bone loss (with a normal urinary calcium level and no history of nephrolithiasis). An alternative approach in patients at risk for osteoporosis and receiving long-term

**TABLE 101-7** Recommendations for the Prevention and Treatment of Glucocorticoid-Induced Osteoporosis

**Prevention**

Patients starting GC therapy at a dose equivalent to prednisone ≥5 mg/day for 3 mo or longer should:

- Modify risk factors for osteoporosis (i.e., stop smoking and decrease excessive alcohol consumption)
- Start regular weight-bearing physical exercise
- Initiate intake of calcium (total 1500 mg/day) and vitamin D (400-800 IU/day)
- Consider BMD testing to predict risk of fracture and bone loss
- Initiate bisphosphonate therapy (alendronate, 5 mg/day or 35 mg/wk, or risedronate, 5 mg/day or 35 mg/wk)

**Treatment**

Patients receiving long-term GC therapy should be tested for osteoporosis using BMD measurement. If the T-score is <–1, consider:

- Risk factor modification, including reducing risk of falls
- Regular weight-bearing physical exercises
- Calcium and vitamin D supplementation
- Replacement of gonadal steroids, if deficient
- Bisphosphonate therapy (alendronate, 10 mg/day or 70 mg/wk, or risedronate, 5 mg/day or 35 mg/wk); if bisphosphonates are contraindicated or not tolerated, consider calcitonin as a second-line agent, intravenous bisphosphonate (pamidronate or zoledronate), or parathyroid hormone 1-34
- Repeat BMD measurement annually or biannually

BMD, Bone mineral density; GC, glucocorticoid.

Modified from Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis: 2001 update, *Arthritis Rheum* 44:1496–1503, 2001.

glucocorticoid therapy is to raise the 25-OHD level into the upper-normal range (>30 ng/mL) to ensure adequate intestinal calcium absorption. This goal can usually be accomplished by administering vitamin D at 1000 to 2000 IU/day.

Because of the enhanced bone resorption in patients treated with glucocorticoids, investigators have examined the effects of inhibitors of bone resorption. The use of bisphosphonates in patients receiving chronic glucocorticoid therapy is beneficial for both the prevention and treatment of osteoporosis. The use of alendronate, 5 or 10 mg/day for 1 year in patients receiving glucocorticoid therapy, increased lumbar spine BMD by 2.1% and 2.9%, respectively, and increased femoral neck BMD by 1.2% and 1%, respectively ($P$ <0.001).[134] After 1 year of treatment, an insignificant reduction in new vertebral fractures occurred, but after 2 years of treatment, a nearly 40% reduction in new vertebral fracture risk occurred. Risedronate, 5 mg/day, was also effective in the prevention and treatment of glucocorticoid-induced bone loss.[134] Recent studies of zoledronic acid (5 mg IV/year) versus risedronate (5 mg/day) and teriparatide (20 μg/day) versus alendronate (10 mg/day) for 18 months indicated that both zoledronic acid and teriparatide improved bone mass more than the comparator, and subjects treated with teriparatide had a significant vertebral fracture risk reduction[135,136] in glucocorticoid-treated patients. Combination studies of rhPTH 1-34 with HRT were also found to be more effective than HRT alone in persons with glucocorticoid-induced osteoporosis.[137] No difference between treatments was observed with regard to fracture data; however, quantitative CT of the lumbar spine, a measure of trabecular bone only, found a nearly 35% increase in patients treated with PTH compared with the estrogen-only group after 12 months of therapy.[138]

Men taking glucocorticoids can experience a lowering of testosterone levels.[139] Men taking glucocorticoids are generally asymptomatic, but if they have evidence of low serum testosterone levels and symptoms of low libido, they can be safely treated with testosterone. Bone mass increases have been observed in men taking glucocorticoids who had low testosterone levels and were treated with testosterone.[140] However, because of the risks associated with testosterone treatment, it is more prudent to treat these patients with a bisphosphonate medication.

To prevent bone loss in patients with pulmonary diseases who require glucocorticoid therapy, treatment with inhaled glucocorticoids has been studied.[141] Inhaled glucocorticoids appear to uncouple bone turnover and increase bone loss; however, this effect is dose dependent. Less than 800 μg/day of inhaled budesonide dipropionate does not increase the risk of osteoporosis, but more than 800 μg/day does increase the risk. New inhaled steroids are more potent than older ones; for instance, Advair, 200 μg/day, is equivalent to nearly 5 mg/day of prednisone.[142] Therefore patients using steroid inhalers on a long-term basis should be screened for bone loss. Because patients who take glucocorticoids may lose a dramatic amount of bone, it is important to monitor the efficacy of a treatment intervention, assess the need for further diagnostic evaluation for other causes of bone loss, and consider alternative treatment strategies if a given therapy is ineffective in preventing bone loss or fractures. In patients at increased risk of fracture, therapy with a potent bisphosphonate or daily PTH injections are highly recommended to slow bone loss and the rate of new fractures.[143-145]

An unresolved question regarding the treatment and prevention of glucocorticoid-induced bone loss is how long to wait before initiating treatment with bone active agents after the discontinuation of the glucocorticoid treatment. It is generally recommended that a BMD measure be obtained 3 to 6 months after the discontinuation of chronic glucocorticoid use and that a decision regarding additional treatment be made on the basis of the BMD measure and any recent history of a fragility fracture.

## OSTEOMALACIA

Osteomalacia is characterized by impaired mineralization of bone matrix. Calcium, phosphate, and vitamin D are necessary for the mineralization of bone. Normally, a steep inverse relationship exists between the serum calcium and PTH concentrations. A small decrease in the serum calcium concentration leads to a rise in PTH release, which promotes distal renal calcium reabsorption, proximal tubulorenal phosphorus excretion, and resorption of calcium from bone. Vitamin D is produced in the skin in the presence of ultraviolet light or is absorbed in the intestine from dietary or supplemental sources. Activation of vitamin D to 25-OHD occurs in the liver, and activation to $1,25(OH)_2D$ occurs in the proximal tubules of the kidney. PTH, hypocalcemia, and hypophosphatemia stimulate the renal 1-hydroxylase enzyme that converts 25-OHD to $1,25(OH)_2D$, which in turn indirectly enhances intestinal calcium absorption.[146]

Osteomalacia results from reduced availability of calcium or phosphate for incorporation into the hydroxyapatite of bone or from deficient absorption or activation of vitamin D.[147] The term *rickets* applies to the defective mineralization of bone and the cartilaginous growth plate in growing children. As shown in Table 101-8, osteomalacia or rickets may result from decreased availability of vitamin D as a consequence of insufficient ultraviolet light exposure, insufficient vitamin intake, or malabsorption in patients with gastrointestinal or biliary disorders. Reduced levels of 25-OHD are caused by severe liver disease, increased renal excretion of vitamin D metabolites due to nephrotic syndrome, or accelerated metabolism of 25-OHD caused by anticonvulsant drugs. Decreased activation of $1,25(OH)_2D$ is seen in patients with renal insufficiency as a result of increased phosphate levels; the resultant lower ionized calcium levels lead to secondary or tertiary hyperparathyroidism.

A careful history is important in the diagnosis of osteomalacia or vitamin D insufficiency. For example, a history of a malabsorptive process such as gastrectomy, intestinal resection, sprue, primary biliary cirrhosis, or pancreatic deficiency may lead to the identification of vitamin D deficiency and osteomalacia.[147] Patients with osteomalacia may present with generalized pain involving the pelvis, spine, ribs, or lower extremities or with skeletal deformities such as bowing of the long bones, kyphoscoliosis, or pelvic abnormalities. Another clinical sign of osteomalacia in adults is proximal muscle weakness, which may result in an antalgic or waddling gait and difficulty ambulating.

Pain may be elicited by deep palpation of the tibia, ribs, or pubic ramus.[147] One of the radiographic signs of osteomalacia is the presence of pseudofractures, or Looser's

**TABLE 101-8** Causes of Osteomalacia and Rickets

| |
|---|
| **Vitamin D Deficiency or Dysfunction** |
| Reduced Availability |
| Nutritional deficit |
| Reduced exposure to ultraviolet light |
| Malabsorption (gastrointestinal or biliary disease or surgical resection) |
| Alteration in Metabolism |
| Reduced 25-hydroxyvitamin D from liver or gastrointestinal disease, nephrotic syndrome, or use of anticonvulsant drugs |
| Reduced 1,25-dihydroxyvitamin D from renal disease or vitamin D–dependent rickets type I |
| Alteration in Action on Target Tissues |
| Vitamin D–dependent rickets type II |
| **Phosphate Deficiency** |
| Decreased availability—dietary deficiency and phosphate-binding antacids |
| Decreased renotubular phosphate reabsorption |
| Familial—X-linked hypophosphatemic rickets and adult-onset vitamin D–resistant osteomalacia |
| Acquired—hypophosphatemic osteomalacia (phosphate diabetes) and oncogenic osteomalacia |
| Generalized renotubular disorders |
| **Acidosis** |
| Renotubular acidosis |
| Ureterosigmoidostomy |
| Carbonic anhydrase inhibitors (acetazolamide) |
| **Miscellaneous Mineralization Defects** |
| Inhibitors of mineralization—fluoride, bisphosphonates (e.g., etidronate), and chronic renal failure (aluminum) |
| Hypophosphatasia |

Modified from LeBoff MS, Brown EM: Metabolic bone disease. In Hare JW, editor: *Signs and symptoms in endocrine and metabolic disorders,* Philadelphia, 1986, JB Lippincott, pp 239–260.

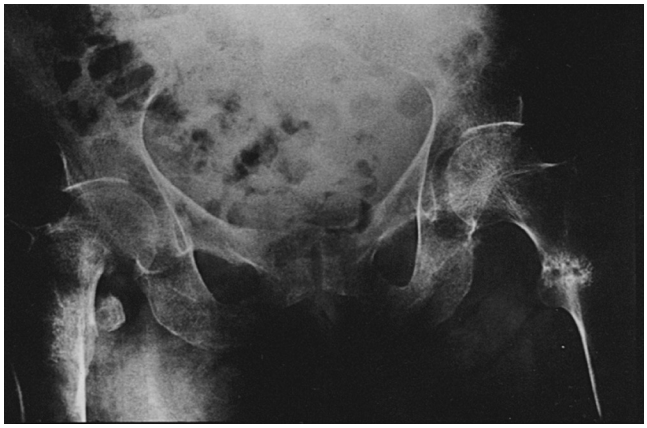


**Figure 101-2** Osteomalacia and fractures in a 65-year-old woman with malabsorption. Shown are a pseudofracture of the left lesser trochanter and an avulsion of the right lesser trochanter. This patient also had previous bilateral pubic rami fractures.

zones, which are transverse lines of rarefaction through the cortices, with incomplete healing in the ribs, scapulae, long bones (Figure 101-2), or pubic rami. Pseudofractures, however, may be indistinguishable from fractures associated with osteogenesis imperfecta or Paget's disease. Other radiographic findings in osteomalacia are vertebral fractures or protrusio acetabuli. Vitamin D deficiency may result in irreversible cortical bone loss.[147] In subtle cases of osteomalacia, a bone biopsy with a double tetracycline label may be necessary; characteristic histomorphometric findings in this disorder include increased osteoid and delayed mineralization of bone.

Rickets causes abnormalities of the epiphyseal growth plate; the clinical signs include an inability to ambulate, growth disturbances, bowing of the long bones, and short stature. Bony deformities of the skull and ribs may develop, with widened cranial sutures (craniotabes), thickened costochondral junctions (rachitic rosary), or indentation of the margins of the ribs (Harrison's grooves).

The biochemical parameters in patients with osteomalacia reflect the underlying pathophysiologic process and the compensatory biologic responses. In vitamin D deficiency states, the serum calcium levels are usually normal or slightly decreased because PTH levels rise rapidly as a compensatory response to impaired calcium absorption.[148] In renal insufficiency phosphate retention, impaired renal production of $1,25(OH)_2D$, hypocalcemia, and skeletal resistance to PTH are thought to lead to the development of hyperparathyroidism and resultant renal osteodystrophy, mixed osteomalacia, and osteitis fibrosa cystica.[149] In addition, aluminum intoxication may present with pure osteomalacia or adynamic bone disease.[148,150] Chronic vitamin D deficiency may increase the secretory demands of the parathyroid glands, thereby producing secondary or, in some instances, tertiary hyperparathyroidism. In patients with osteomalacia who do not have hepatobiliary disease, alkaline phosphatase levels are often elevated.[151]

Osteomalacia may be associated with a deficiency of phosphate, principally in patients with decreased renotubular reabsorption of phosphate. Familial hypophosphatemic vitamin D–resistant rickets in children or osteomalacia in adults usually presents with renal phosphate leak, hypophosphatemia, rachitic or osteomalacial changes, and inappropriately normal or low-normal $1,25(OH)_2D$ level for the degree of hypophosphatemia. This X-linked dominant disorder may present in young children with the inability to walk, followed by progressive bowing and skeletal deformities, without signs of proximal myopathy. The genetic locus for X-linked hypophosphatemic rickets has been mapped to Xp22.1, and the gene is named *PHEX* (phosphate-regulating gene with homology to endopeptidases on the X chromosome).[151]

Oncogenic osteomalacia or rickets is a vitamin D–resistant process associated with certain neoplasias, principally small, benign mesenchymal or endodermal tumors and, infrequently, certain malignant tumors (e.g., multiple myeloma and prostatic, oat cell, and breast carcinomas).[152,153] Such patients typically present with decreased renotubular phosphate reabsorption, hypophosphatemia, muscle weakness, diminished $1,25(OH)_2D$ levels, and normocalcemia. The benign tumors tend to be small and difficult to identify on physical examination or radiographs. Surgical removal of these tumors results in a rise in phosphate and $1,25(OH)_2D$ levels and resolution of the skeletal process. Osteomalacia may also be associated with generalized renotubular disorders and the use of certain drugs that contain inhibitors of mineralization (e.g., fluoride, etidronate, and aluminum). The evaluation of a patient suspected of having osteomalacia is outlined in Table 101-9.

Osteomalacia is often a treatable disease, but the diagnosis may be overlooked. Vitamin D deficiency can be treated with physiologic doses of vitamin D, but higher

**TABLE 101-9** Evaluation of Osteomalacia

| |
|---|
| Calcium, phosphorus, alkaline phosphatase, urinary calcium levels; 25-hydroxyvitamin D and intact parathyroid hormone levels |
| In selected patients:<br>• 1,25-Dihydroxyvitamin D levels (e.g., renal insufficiency and vitamin D–resistant osteomalacia or rickets)<br>• Vitamin D absorption test: obtain 25-hydroxyvitamin D levels at 0, 4, and 8 h (e.g., some cases of malabsorption)<br>• Tubular reabsorption of phosphate (e.g., vitamin D–resistant osteomalacia or rickets)<br>• Bone biopsy with double tetracycline labels |

doses (1000 to 2000 IU/day) may hasten the healing of bone. In the presence of intestinal malabsorption, and until the underlying malabsorptive process is corrected, large doses of vitamin D (50,000 IU once a week to three or more times a week) are often required. Careful monitoring of the serum and urinary calcium levels and 25-OHD concentrations is necessary to prevent vitamin D intoxication. Use of the active metabolite of 25-OHD (Calderol) occasionally may be necessary in resistant patients or in persons with severe liver disease who cannot achieve activation of this metabolite. The potential advantages of using 25-OHD are more stable bioavailability, a shorter half-life, and greater potency than the parent compound,[148] although the cost is greater.

In patients with hypophosphatemia and disorders of renotubular phosphate reabsorption, mineralization of bone occurs with phosphate therapy and moderately high doses of $1,25(OH)_2D$, with the latter being necessary to prevent the secondary hyperparathyroidism associated with phosphate therapy. In patients affected by renal insufficiency or failure, a phosphate binder (calcium acetate or calcium carbonate) should be used after meals to decrease intestinal phosphate absorption. Calcium citrate therapy should not be used because it augments aluminum absorption. In persons with renal failure, $1,25(OH)_2D$ therapy administered orally[148,154] (or intravenously in some patients undergoing dialysis) suppresses parathyroid cell secretion and proliferation; a threefold elevation of the PTH level is advocated by some investigators to prevent adynamic bone disease.[149] Analogues of $1,25(OH)_2D$ that do not produce hypercalcemia but decrease levels of PTH are now available and may be useful in patients with renal insufficiency.

## PAGET'S DISEASE OF BONE

Paget's disease of bone affects approximately 2% to 3% of the population older than 50 years and is uncommon in persons younger than 40 years.[155] Paget's disease is characterized by enhanced resorption of bone by giant, multinucleated osteoclasts, followed by the formation of disorganized woven bone by osteoblasts. The resultant bone is expanded, weak, and vascular, and thus affected bones may become enlarged and deformed, and the overlying skin may feel warm to the touch.[156]

### Cause

The cause of Paget's disease is uncertain, although data showing the presence of viral inclusion particles in giant pagetic osteoclasts support a viral cause, possibly associated with measles, respiratory syncytial, or canine distemper virus. Paget's disease tends to aggregate in families in an autosomal dominant pattern, and in 40% of patients, at least one other family member is affected.[157] Recently, in a family with juvenile Paget's disease, the disease was found to be associated with a polymorphism in the OPG allele.[158] Studies of additional families with this mutation may lead to the discovery of the cause of Paget's disease.

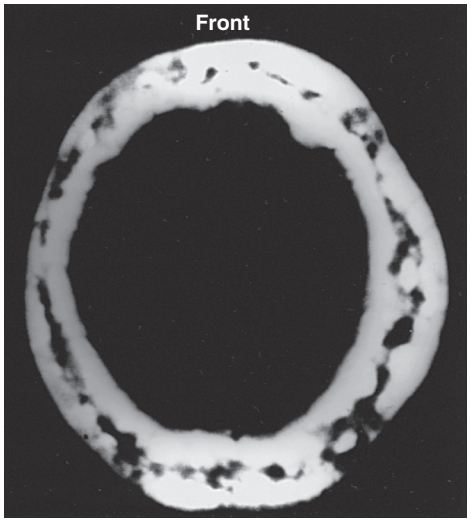



**Figure 101-3** Paget's disease of the skull in a woman with signs of increasing head size and progressive hearing loss. The alkaline phosphatase level was 2100 U/L. This computed axial tomogram shows marked thickening of the inner and outer skull tables, with osteoblastic pagetoid changes. An audiologic evaluation revealed bilateral hearing loss.

### Clinical Features

Many patients with Paget's disease are asymptomatic, and the disease is detected by the incidental finding of an elevated alkaline phosphatase level or a characteristic radiographic abnormality. Other patients present with a range of symptoms that include bone pain; skeletal deformities (e.g., bowing of long bones, an enlarged skull, and pelvic alterations); pathologic fractures; increased cardiac output (with extensive disease); and nerve compression. Paget's disease typically includes a lytic phase, a combined lytic and blastic phase, and a sclerotic or "burned out" phase occurring late in the disease process.

Radiographic signs of the three stages of Paget's disease may be present at different sites in the same patient.[157] The skeletal sites commonly involved in persons with Paget's disease include the skull (Figure 101-3), vertebrae, pelvis, sacrum, and lower extremities. Degenerative joint disease may develop adjacent to the bones and cause pain that may obscure the symptoms associated with Paget's disease.[157] Ten percent to 30% of patients with Paget's disease may experience fractures that present initially as asymptomatic or painful short fissure fractures traversing the bony cortex (Figure 101-4). Complete fractures of the bones such as the "chalk stick" fracture also occur; fractures of the long bones may be a serious complication because the increased vascularity of pagetic bone may lead to excessive blood loss.

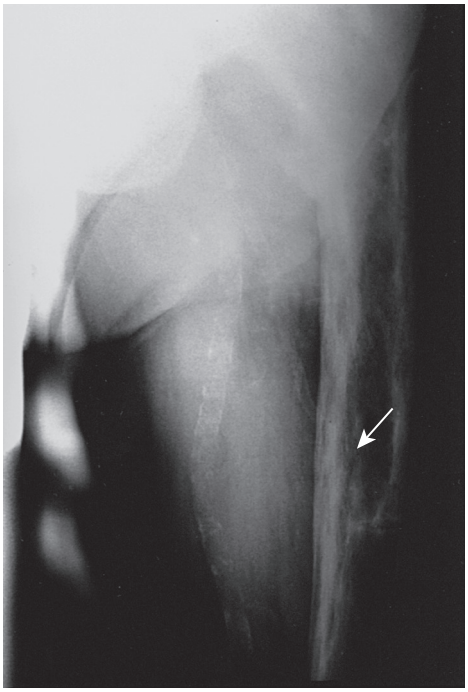


**Figure 101-4** Paget's disease of the proximal femur. Note the coarse trabeculae, thickened cortices, lateral fissure fracture, and expanding lytic region characteristic of the "blade-of-grass" lesion *(arrow)*.

Healing of fractures in pagetic bone usually occurs normally, although cases of nonunion have been reported. A rare complication of Paget's disease of bone is sarcomatous degeneration in less than 1% of patients (with osteogenic sarcomas or, less commonly, fibrosarcomas or chondrosarcomas); these patients generally have a poor prognosis. The development of a sarcoma may be heralded by the presence of a soft tissue mass, localized pain, and a rise in the alkaline phosphatase level.

Neurologic symptoms generally result from compression of the nerves by pagetic bone. Hearing loss is common and is caused by sensory loss and conduction abnormalities due to pagetic involvement of the bones of the inner ear. Paget's disease of the skull may also produce ocular and other cranial nerve palsies. Compression of the base of the skull may lead to basilar invagination, cerebellar dysfunction, or obstructive hydrocephalus, with symptoms of nausea, ataxia, incontinence, gait disturbances, and dementia. Neurologic compromise of the thoracic or lumbar spine may lead to spinal cord compression or, in the latter instance, cauda equina syndrome.

## Laboratory Findings

Biochemical indices in patients with Paget's disease usually show normal serum calcium and phosphate levels, although hypercalcemia may develop with immobilization when an uncoupling of bone resorption and formation occurs. Patients with Paget's disease may also be hypercalcemic if they coincidentally acquire primary hyperparathyroidism, which can further increase bone remodeling and worsen the disease process. Secondary hyperparathyroidism may develop in approximately 10% to 15% of patients with Paget's disease, presumably because of inadequate calcium intake to meet the skeletal demands of the heightened bone remodeling.[157]

**TABLE 101-10** Indications for Treatment of Paget's Disease of Bone

| |
|---|
| Pain |
| Hypercalcemia |
| Fractures |
| High-output cardiac failure (rare) |
| Skull involvement |
| Neurologic compromise |
| Periarticular disease |
| Prevention of progression of Paget's disease |

Alkaline phosphatase levels of bone origin (bone-specific alkaline phosphatase; BSAP) are commonly elevated in patients with significant Paget's disease because of the increased osteoblastic activity combined with bone breakdown. In the absence of liver disease, the alkaline phosphatase level typically correlates with the extent of the pagetic involvement of bone, although it may be more elevated in Paget's disease of the skull (see Figure 101-3).

Unexpectedly, circulating osteocalcin levels do not reflect disease activity in patients with Paget's disease as well as do BSAP levels. Markers of bone resorption such as urinary collagen cross-links like N telopeptides and C telopeptides are also elevated in persons with active Paget's disease. Other laboratory abnormalities in patients with Paget's disease include hypercalciuria, hyperuricuria, and hyperuricemia, possibly related to the increased turnover of osteoclasts. Serum uric acid levels should be measured periodically because of the association of Paget's disease with gouty arthritis.

## Diagnosis

Bone scans are valuable tools for assessing the extent of Paget's disease and are therefore useful as part of the initial evaluation.[155,157] As diagnostic tests, however, bone scans in general are sensitive but not specific for a number of skeletal processes. Radiographs show characteristic radiologic findings such as transverse lucent areas, osteoporosis circumscripta, enlargement of the bones, expanding lytic changes, the "blade-of-grass" lesion shown in Figure 101-4, thickened cortices, a coarse trabecular pattern, or sclerotic changes.

In patients with involvement of the skull and changes in mental status, a skull radiograph, MRI, or quantitative computed tomography may be useful to diagnose platybasia and flattening of the base of the skull, basilar invagination, or the infrequent complication of hydrocephalus. Audiologic evaluation may reveal hearing loss in patients with pagetic involvement of the skull.

## Treatment

The indications for treatment of Paget's disease (Table 101-10) include pain, hypercalcemia, fractures, high-output cardiac failure (a rare feature), and neurologic compromise.

Therapy can also be used to prevent the progression of deformity or risk of nerve compression when there is pagetic involvement of the skull, a vertebral body, or a weight-bearing bone (femur) or when disease is present adjacent to a major articular joint.

Treatment of symptomatic Paget's disease is usually directed at suppression of the enhanced bone resorption and skeletal turnover with calcitonin or bisphosphonates.[148,150] Response to therapy is monitored by the reduction of symptoms and maintenance of the alkaline phosphatase level in a mid-normal range, with retreatment once values rise 25% above normal.

### Calcitonin

Salmon calcitonin and human calcitonin inhibit the function of osteoclasts, which are active in the pagetic process; both types of calcitonin preparations come in an injectable form and are approved by the FDA for patients with Paget's disease.[159] Salmon calcitonin therapy is usually initiated at a low dose to ensure patient tolerance and then increased to a daily dose of 100 Medical Research Council units (intramuscularly or subcutaneously). After 6 months of therapy, the patient may receive a maintenance dose of 50 to 100 Medical Research Council units daily.[139] Approximately two-thirds of patients show a decrease in alkaline phosphatase levels of 50% or more in 2 to 6 months. Some patients experience resistance to the effects of calcitonin, which can be reversed in some instances by switching from salmon calcitonin to human calcitonin. Calcitonin has also been linked to an increased risk of cancer. The European Committee for Medicinal Products (CHMP) for human use found that "a higher proportion of patients treated with calcitonin for long periods of time develop cancer of various types, compared with patients taking placebo." The increase in cancer rates ranged from 0.7% for oral formulations to 2.4% for the nasal formulation. CHMP removed the nasal formulation from the market in Europe because its only indication was for osteoporosis. Because of the increased risk of cancer, the European Medicines Agency recommended that calcitonin (by injection) be used only on a short-term basis for three conditions for which it had previously been approved in the European Union: Paget's disease, acute bone loss resulting from sudden immobilization, and hypercalcemia caused by cancer. Currently the United States has put no restrictions on the duration of use of calcitonin.

### Bisphosphonates

Several bisphosphonates are currently approved by the FDA for the treatment of Paget's disease, including etidronate disodium, pamidronate, alendronate, tiludronate disodium, risedronate, and zoledronic acid.

- Etidronate disodium in tablet form is available in 200- to 400-mg doses. It is approved as a once-daily treatment for 6 months; the higher dose (400 mg) is more commonly used. No food, beverage, or medications should be consumed for 2 hours before and after taking this drug. The course should not exceed 6 months, but repeat courses can be given after rest periods, preferably of 3 to 6 months' duration.[160]
- Pamidronate disodium is available in intravenous form. The approved regimen consists of a 30-mg infusion that must be administered over 4 hours for 3 consecutive days, or 60 mg over 2 to 4 hours for 2 or more consecutive days.[161]
- Alendronate sodium is available as a 40-mg tablet that is taken once daily for 6 months; patients should wait at least 30 minutes after taking this drug before eating any food, drinking anything other than tap water, taking any medication, or lying down (the patient may sit, however).[162]
- Risedronate sodium is available as a 30-mg tablet taken once daily for 2 months; patients should wait at least 30 minutes after taking the drug before eating any food, drinking anything other than tap water, taking any medication, or lying down (the patient may sit, however).[163]
- Zoledronic acid is available as a 5-mg IV infusion. A single dose of 5 mg over 15 minutes is sufficient for 2 years. Adverse effects can include fatigue, anemia, muscle aches, swelling of the feet, and flu-like symptoms after the first infusion.[164]

In addition to anti-resorptive therapies, NSAIDs and aspirin are useful modalities to alleviate the joint pain and other symptoms that result from degenerative joint disease. Finally, surgical intervention is sometimes warranted in patients with Paget's disease and bony deformities, pathologic fractures, nerve compression, or degenerative arthritis. Orthopedic procedures such as total joint replacement and osteotomies are associated with a reduced risk of intraoperative bleeding or other complications if patients are treated medically (e.g., with calcitonin or other bisphosphonates) to reduce the disease activity and vascularity for at least 6 weeks before the procedure.

## OSTEOPOROSIS INDUCED BY OTHER MEDICATIONS

Aromatase inhibitors in women undergoing breast cancer treatment are associated with bone loss. Post-menopausal women maintain a low level of circulating estrogen because of aromatization of androgens to estrogen in tissues such as fat and muscle by the cytochrome P-450 enzyme. Inhibition of this enzyme is now used in post-menopausal women with breast cancer. There are two classes of aromatase inhibitors: nonsteroidal reversible inhibitors (anastrozole and letrozole) and steroidal reversible inhibitors (exemestane). These agents prevent the conversion of androgen to estrogen, resulting in low serum estrogen levels and increased bone remodeling. In clinical trials of aromatase inhibitors compared with either tamoxifen or placebo, fracture rates ranged from 3% to 7%.[165] In a 2-year study, significantly higher markers of bone turnover and nearly twice the lumbar spine bone loss and fracture rates were observed with anastrozole compared with tamoxifen. Although the data are just beginning to be collected regarding skeletal health in women treated for breast cancer with aromatase inhibitors, it is important to obtain a history of clinical risk factors for osteoporosis and a BMD measurement of the hip and spine. Preventive treatment should be initiated in women with normal or low bone mass and no history of fractures. Treatment of women with low bone mass (a

T-score ≤–2) should be initiated with potent anti-resorptive agents, and BMD should be monitored at least every 2 years. Recent studies with zoledronic acid and denosumab have been reported to be quite effective in this patient population.[166,167] If a woman continues to lose bone mass while taking aromatase inhibitors despite compliance with potent anti-resorptive agents, and if the patient has not been treated with radiation to the skeleton as part of the breast cancer protocol, rhPTH 1-34 treatment can be used to build up bone mass.

Gonadotropin-releasing hormone antagonists are used to treat women with endometriosis and men with prostate cancer. These compounds induce bone loss by lowering estrogen levels, resulting in accelerated bone turnover. In a study of more than 50,000 men with prostate cancer treated with androgen-deprivation therapy consisting of either gonadotropin-releasing hormone agonists or orchiectomy, an increased risk of fracture or hospitalization due to fracture was found. Although the overall risk of fracture associated with androgen-deprivation therapy was only modestly increased overall, the risk of fracture was significantly associated with the number of doses of gonadotropin-releasing hormone agonists.

Other studies have reported that androgen-deprivation therapy increases bone loss at all sites, with a 2% to 8% annual loss in the lumbar spine and a 2% to 6% loss in the hip after 1 year.[168] Given the high incidence of prostate cancer and the increasing use of this treatment, assessment of bone mass and the prevention of additional bone mass loss are probably appropriate. Oncologists currently recommend that BMD be measured at the time of initiation of androgen-deprivation therapy and that clinical risk factors for osteoporosis be reviewed, including the history of fracture after age 30 years, the family history of hip fracture, smoking history, use of glucocorticoids, low testosterone level, and rheumatoid arthritis. If the patient has a low BMD (T-score <–2.5) or a T-score between –1 and –2.5 and other risk factors, treatment with calcium and vitamin D supplementation and a bisphosphonate (zoledronic acid, alendronate, risedronate, or denosumab) should be initiated. BMD of the lumbar spine and hip should be measured at least once a year while patients receive androgen-deprivation maintenance therapy.[169-172]



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 102

# Proliferative Bone Diseases



Reuven Mader

**KEY POINTS**

- Diffuse idiopathic skeletal hyperostosis (DISH) is usually defined by the presence of large flowing osteophytes connecting at least four vertebrae, typically in the thoracic spine. However, the disease often involves the cervical and lumbar spine and peripheral joints, especially entheses.
- The cause of DISH is unclear, but it is associated with a variety of metabolic abnormalities, many of which are also seen in type 2 diabetes.
- Treatment of spinal DISH is mostly symptomatic. Patients with DISH are at increased risk for heterotopic bone formation after joint surgery.
- DISH may be associated with increased cardiovascular risk factors. Growth factors are implicated as the common cause of most, if not all, cases of hypertrophic osteoarthropathy (HOA).
- HOA usually responds dramatically to effective treatment of the primary disease such as surgical resection of a lung carcinoma.
- Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis (SAPHO) syndrome is a chronic inflammatory disorder, often relapsing, of unknown cause. It most commonly affects the anterior chest wall.
- The cause of SAPHO syndrome is unknown, but an infectious etiology has been proposed on the basis of isolation of *Propionibacterium acnes* from sternal osteosclerotic lesions.
- NSAIDs are usually the first line of treatment in SAPHO and may improve symptoms. Additional therapy with other modalities is often required.

Proliferative bone diseases encompass a variety of conditions characterized by exuberant bone and entheseal ossifications and calcifications. New bone formation is the main feature in diffuse idiopathic skeletal hyperostosis (DISH) and hypertrophic osteoarthropathy (HOA) and is a common finding in osteoarthritis. New bone formation may also accompany some spondyloarthropathies such as ankylosing spondylitis, psoriatic arthritis, and sternoclavicular syndrome, also known as SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteomyelitis). New bone formation has also been described in endocrine diseases such as thyroid disorders, acromegaly, and hypoparathyroidism (Table 102-1).[1-3] Osteoarthritis, spondyloarthropathies, and endocrine disorders are discussed elsewhere in this book.

## DIFFUSE IDIOPATHIC SKELETAL HYPEROSTOSIS

DISH is a condition characterized by calcification and ossification of soft tissues, mainly ligaments and entheses. This condition was described by Forestier and Rotes-Querol in 1950[4] and was termed *senile ankylosing hyperostosis*. It has a marked predilection for the axial skeleton, particularly the thoracic spine. Recognition that the condition is not limited to the spine and may involve peripheral joints led researchers to coin the name *DISH*, a term now widely used.[5]

DISH is characterized by the production of coarse, flowing osteophytes involving, in particular, the right side of the thoracic spine with preservation of the intervertebral disk space, as well as by ossification of the anterior longitudinal ligament. Calcification and ossification of the posterior longitudinal ligament seem to be additional skeletal manifestations of DISH. Other entheseal regions may be affected, such as the peripatellar ligaments, Achilles tendon insertion, plantar fascia, olecranon, and others.[6-8]

Diagnosis is usually based on the definition suggested by Resnick and Niwayama.[5] This radiographic approach requires the presence of flowing, coarse osteophytes on the right side of the thoracic spine, connecting at least four contiguous vertebrae, or ossification of the anterior longitudinal ligament, preserved intervertebral disk height in the involved segment, and the absence of apophyseal joint ankylosis and sacroiliac joint involvement (Table 102-2).[8] Another set of criteria, for epidemiologic purposes, was suggested by Utsinger.[7] These criteria also consider peripheral enthesopathies. With these criteria, a definite diagnosis of DISH is established by criteria similar to those suggested by Resnick and Niwayama. A probable diagnosis of DISH is possible, however, with continuous ossification or calcification, or both, of the anterolateral aspect of at least two contiguous vertebral bodies and bilateral well-corticated enthesopathies in the heel, olecranon, and patella. It has also been suggested that peripheral enthesopathies might represent early DISH, which may evolve, over time, to its full radiologic appearance.

### Epidemiology

DISH is more common in men than in women. In a series of 75 spines studied at autopsy, 28% had DISH.[9] The reported prevalence of DISH varies according to age, ethnic origin, geographic location, and clinical setting (i.e., hospital based vs. population based). In a study of a North

**TABLE 102-1** Proliferative Bone Diseases

| |
|---|
| Diffuse idiopathic skeletal hyperostosis |
| Hypertrophic osteoarthropathy |
| Thyroid disorders |
| Acromegaly |
| Hypoparathyroidism |
| Seronegative spondyloarthropathies (e.g., psoriatic arthritis and ankylosing spondylitis) |
| SAPHO |
| Osteoarthritis |

SAPHO, Synovitis, acne, pustulosis, hyperostosis, and osteomyelitis.

**TABLE 102-2** Suggested Diagnostic Criteria for Diffuse Idiopathic Skeletal Hyperostosis

| |
|---|
| Flowing calcification and ossification along the anterolateral aspect of at least four contiguous vertebral bodies |
| Preservation of intervertebral disk height in the involved vertebral segment and absence of extensive radiographic changes of degenerative disk disease |
| Absence of apophyseal joint bony ankylosis and sacroiliac joint erosion, sclerosis, or intra-articular osseous fusion |

**TABLE 102-3** Conditions Associated with Diffuse Idiopathic Skeletal Hyperostosis

| |
|---|
| Non–insulin-dependent diabetes mellitus |
| Obesity |
| High waist circumference ratio |
| Dyslipidemia |
| Hypertension |
| Hyperuricemia |
| Hyperinsulinemia |
| Elevated insulin-like growth factor-I |
| Elevated growth hormone |
| Use of retinoids |
| Genetic predisposition |

American metropolitan hospital population, the prevalence in men and women older than 50 years was reported to be 25% and 15%, respectively, and the prevalence in men and women older than 70 years was 35% and 26%, respectively.[10] Similar figures were reported for patients from Budapest.[11] Higher figures were reported for Jews living in Jerusalem, reaching a prevalence of 46% for men older than 80 years.[12] A much lower prevalence was reported from Korea, reaching only 9% in the older age group.[13] Native Africans in South Africa had a prevalence of 13.6% in patients older than 70 years with no difference between men and women.[14] In population-based, as opposed to hospital-based, studies, the reported prevalence was slightly greater than 10% in patients older than 70 years.[15] Mild DISH was found in human remains dating back 4000 years. In human remains from the sixth to eighth centuries, the prevalence of DISH was higher in men than in women, reaching 3.7%. Although these studies were performed on different and relatively young populations, the prevalence of DISH may be increasing.[16,17]

## Etiology and Pathogenesis

The cause and pathogenesis of DISH are unknown. Several genetic, metabolic, and constitutional factors, as well as other bone formation–promoting peptides, were reported to be associated with this condition. They all might contribute to bone and entheseal new bone formation.

### Genetic Factors

Genetic factors might play a role in the pathogenesis of DISH. It is known that the prevalence of canine DISH in the Boxer breed is about 40%, whereas the overall prevalence of DISH in dogs is 3.8%, suggesting a genetic basis for the risk of developing canine DISH.[18] Familial clustering of DISH or families with early presentation of DISH suggest a genetic background for this disorder in humans.[19,20] Ossification of the posterior longitudinal ligament is closely related to DISH, and the two conditions can coexist. *COL6A1*, which is the candidate gene for ossification of the posterior longitudinal ligament, was reported to be significantly associated with DISH in Japanese patients but not in Czech patients.[21,22] Although the role played by *COL6A1* has not been fully elucidated, it might be involved in ectopic bone formation in ossification of the posterior longitudinal ligament (OPLL), as well as in DISH. This finding would suggest that other factors might play an important role in the genetic predisposition for the development of DISH.

### Metabolic Factors

DISH has been linked to metabolic and constitutional factors such as obesity, a high waist circumference ratio, hypertension, type 2 diabetes mellitus (DM), hyperinsulinemia, dyslipidemia, elevated growth promoting peptides, hyperuricemia, and use of retinoids (Table 102-3).[23-31]

The association of DISH with excess body weight has been well known since the early descriptions by Forestier and other investigators.[9,32] This association was confirmed in a study in which patients with DISH were compared with healthy persons and patients with spondylosis.[33] The association of DISH with type 2 DM has been reported in several studies.[28,30,31] It has been assumed that the higher serum insulin levels in type 2 DM may contribute to the formation of DISH. No studies have been performed to address the issue of type 1 DM or the effect of exogenous insulin administration on new bone formation. However, in a recent study, the prevalence of DISH was no higher in diabetic patients than in nondiabetic subjects, suggesting re-evaluation of diabetes as a risk factor for the development of DISH.[34] More often, DISH was reported to be associated with more complex metabolic and endocrine derangements, with or without overt type 2 DM, comprising glucose intolerance, hyperinsulinemia, dyslipidemia, hyperuricemia, and elevated levels of growth hormone and insulin-like growth factor (IGF)-I.[24,27,30] Because of these metabolic abnormalities, patients with DISH have a higher likelihood of being

affected by metabolic syndrome and are subjected to a higher coronary artery disease risk.[35]

Hyperinsulinemia has a profound effect on ligaments and entheses, which is independent of age and obesity. The differentiation of mesenchymal cells in ligaments into chondrocytes and the subsequent endochondral ossification is promoted by insulin.[28,36] The enthesis provides the growth plate for tendons and ligaments in children and persists into adulthood. This particular structure is composed of collagen fibers, fibroblasts, chondrocytes, and calcified matrix, which is probably a target for the ossification process promoted by insulin.[23]

### Other Bone Formation–Promoting Pathways

Bone morphogenetic protein (BMP)-2 is a potent osteogenic factor that promotes differentiation of mesenchymal stem cells into fibroblasts and chondroblasts. It stimulates cell proliferation, alkaline phosphatase (ALP) activity, and collagen synthesis.[37,38] Its ability to promote mineralization is inhibited by matrix Gla protein, which is highly expressed in bone and cartilage. Matrix Gla protein deficiency or its altered carboxylation may cause a high level of BMP-2 activity that leads to hyperostosis.[31,39] Another possible explanation is the upregulation of BMP-2 and BMP-4 messenger RNA expression after tensile stress on ligaments from patients with OPLL.[40]

The enthesis may also be under the influence of other growth-promoting peptides. Elevated growth hormone levels have been reported in DISH. Growth hormone is capable of inducing osteoblast cell proliferation and may promote local production of IGF-I, which mediates the action of growth hormone and can stimulate ALP activity in osteoblasts.[24,31,41,42] ALP promotes the calcification process during bone formation and is considered a good indicator for the maturation stages of osteoblasts.[43] It has not yet been determined why the new bone formation is localized mainly at the ligamentous and entheseal sites. In male patients with DISH, growth hormone serum levels were not elevated compared with patients with OA, but they were much higher in the synovial fluid.[24,43] In the spine, vertebral blood supply could be a factor in the onset or progression of DISH.[44] Intraerythrocyte growth hormone levels may exceed serum growth hormone levels and could be transported to the vertebral site.[45]

The expression of various genes involved in cell division and growth is regulated by nuclear factor-κB (NF-κB), which is capable of regulating the differentiation of multipotential cells. It has been shown that activation of environmental factors such as platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β1 in ligament cells stimulates the activation of NF-κB, which influences the osteoblastic differentiation of mesenchymal cells. This event is accompanied by elevation of ALP activity in cells of patients with DISH and serves as an indicator of maturation stages of the osteoblast.[46] Inflammatory cytokines such as PDGF-BB, TGF-β1, and others may be related to the onset of non–insulin-dependent DM and may be the link between this condition and the occurrence of DISH.[47,48]

The Wnt–β-catenin pathway may induce bone formation. Wnt signaling can be inhibited by the endogenous secreted protein dickkopf-related protein 1 (DKK1; also known as dickkopf-1). Low DKK1 levels and thus reduced inhibition of the Wnt pathway might have a role in the development of extensive bony spinal bridges in a subset of patients with DISH.[49,50,51] Vitamin A and its derivatives have been implicated in the pathogenesis of DISH because of their ability to promote new bone formation. Levels of vitamin A were reported to be higher in patients with DISH compared with control subjects, and some reports showed DISH-like manifestations in young patients treated with vitamin A or its derivatives.[25,52] The role of vitamin A in the pathogenesis and clinical manifestations in DISH is still controversial.[53] Larger prospective studies are necessary to elucidate the role played by this vitamin in the pathogenesis of DISH.

Prostaglandin $I_2$ ($PGI_2$) is a potent inhibitor of bone resorption, and endothelin-1 might regulate ectopic calcification of the vasculature but may also regulate the proliferation of osteoblasts and osteoclasts. In vitro studies have suggested that endothelin-1 and $PGI_2$ might play roles in the pathogenesis of DISH by induction, through various mechanisms, of ALP activity and osteogenic differentiation in spinal ligament cells.[54-56]

There are no convincing explanations for the predilection of the hyperostotic process to affect the anterolateral aspect of the thoracic spine. The limited range of motion of the thoracic spine has been cited as a possible cause for the predilection to this site. This assumption cannot explain the involvement of the extremely mobile cervical spine or the lumbar spine, however. The less frequent involvement of the left side of the thoracic spine was ascribed to the pulsation of the aorta. This assumption was based on a few reports that described left-sided bridging osteophytes in cases with a right-sided aorta, suggesting that the aortic pulsations interfere with the production of the osteophytes.[57] Calcifications, ossification, and subsequent stiffening of ligaments and joint capsules have important pathogenetic implications. It has recently been shown that patients with DISH have a significantly higher risk of developing aortic valve sclerosis.[58] Whether this finding might share common pathogenetic mechanisms or only reflects usual risk factors has yet to be determined (Figure 102-1).

Osteoarthritis may have pathogenetic features in common with the peripheral joint manifestations of DISH. It was suggested that in the small non–weight-bearing joints in osteoarthritis, the process is caused by an increased intra-articular pressure and the subsequent development of "crash" forces.[59] This development was attributed to thickening of the collateral ligaments of these joints that enforce a constraint movement, not to primary damage to the cartilage. It seems reasonable that the joints affected by DISH may develop the same "crash" forces operating in small osteoarthritis joints as a result of this mechanism. This mechanism might explain the involvement of "atypical" joints that are not commonly affected by osteoarthritis and the hypertrophic osteoarthritic changes in the commonly affected joints.

## Clinical Manifestations

The lack of specific symptoms and signs of DISH, as well as the radiographic diagnostic criteria, have raised doubts

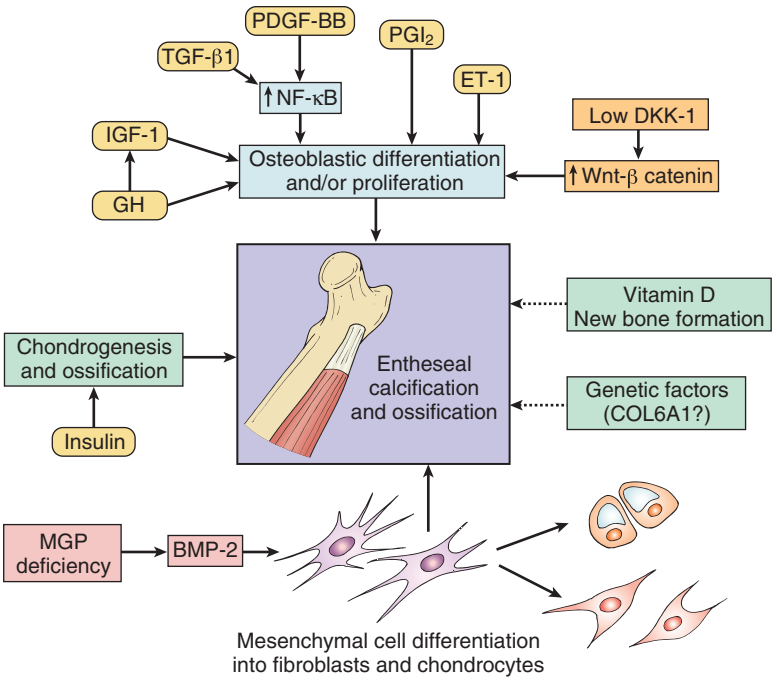



**Figure 102-1** Suggested factors that promote entheseal calcification and ossification in diffuse idiopathic skeletal hyperostosis (DISH). Growth hormone (GH) promotes osteoblastic differentiation and/or proliferation either directly or indirectly by promoting insulin-like growth factor-1 (IGF-1) production. Nuclear factor-κB (NF-κB) activity is stimulated by platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-1 (TGF-1) and promotes soft tissue ossification. Endothelin-1 (ET-1) and prostaglandin $I_2$ ($PGI_2$) may promote osteoblastic differentiation. Low Dickkopf-related protein-1 (DKK-1) levels enhance the activity of the Wnt-β catenin pathway, which induces osteoblastic differentiation and/or proliferation. Matrix Gla protein (MGP) deficiency increases bone morphogenic protein-2 (BMP-2) activity, leading to the differentiation of mesenchymal cells into chondrocytes and fibroblasts, which may calcify. Insulin induces chondrogenesis and ossification. *(From Mader R, Verlaan JJ, Buskila D: Diffuse idiopathic skeletal hyperostosis: clinical manifestations and pathogenic mechanisms.* Nat Rev Rheumatol *9:741–750, 2013.)*

about DISH as a separate entity.[60] Although the process may be asymptomatic, it has been reported to be associated with morning stiffness, dorsolumbar pain, and reduced range of motion in some patients.[7,8] Patients with DISH may have extremity pain involving peripheral large and small joints and peripheral entheses such as the heel, Achilles tendon, shoulder, patella, and olecranon.

## Spinal Involvement

Pain in the axial skeleton may involve all three segments of the spine and the costosternal and sternoclavicular joints. The level of pain and disability is significantly higher compared with healthy subjects but is not different from patients with spondylosis.[33] In some studies, up to two thirds of the patients with DISH experience morning stiffness and spinal pain, and nearly half have a reduced range of motion.[7,61] Reports of pain referable to the thoracic spine are common and are accompanied by a reduced chest expansion. However, other studies suggested that spinal DISH is not associated with increased pain, and at times patients reported less pain compared with patients who did not have DISH.[62,63]

Although similar in some aspects to osteoarthritis of the spine, DISH is a distinct clinical entity with different characteristics.[64] Classically, the portions of the spine that are involved in osteoarthritis are the lower portions of the cervical spine and the lumbar spine. Thoracic spine involvement is uncommon in osteoarthritis or occurs in late stages of the disease, as opposed to the common involvement of the thoracic spine in DISH. Thoracic spine involvement in DISH is characterized by preserved intervertebral height, whereas in spinal osteoarthritis, reduced intervertebral disk height is common. These differences in the radiologic appearance and anatomic spinal distribution probably have to do with the different pathogenetic mechanisms described earlier. It is presumed that the primary target for the osteoarthritic process is the cartilage represented in the spine by the intervertebral disks and the cartilage of the facet joints.

The wear-and-tear forces operating in the extremely mobile lower cervical and lumbar portions of the spine might explain the frequent involvement of these segments in osteoarthritis, whereas the thoracic spine is the least mobile of the spinal segments. The main targets of the disease in DISH are the spinal ligaments and entheses (Figure 102-2).[5,65] These abnormalities are not limited to the thoracic spine and may involve the lumbar spine and the cervical spine (Figure 102-3). In the lumbar spine, the large bridging osteophytes are not uniformly one-sided.[66] These sites of ossification and subsequent production of large osteophytes may result in spinal stenosis.[67] Severe complications may develop, especially when the cervical spine is affected; these complications include dysphagia, hoarseness, stridor, OPLL, myelopathy, aspiration pneumonia, sleep apnea, atlantoaxial complications, thoracic outlet syndrome, esophageal obstruction, endoscopic and intubation difficulties, and fractures (Table 102-4).[68] The high

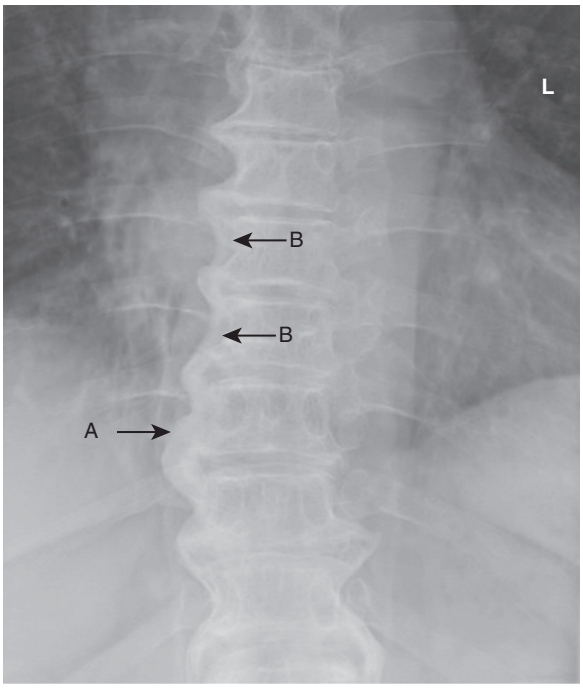



**Figure 102-2** Large, flowing, right-sided osteophytes of the thoracic spine *(A)*. Note the translucent area between the vertebral body and the ossified ligamentous tissue *(B)*.



**Figure 102-3** Severe bulky ossification of the anterior longitudinal ligament of the cervical spine.

prevalence of coexisting intervertebral disk damage in young patients with DISH suggests an important role for DISH in the pathogenesis of spondylosis in this group of patients.[69] At times, patients with DISH may assume the typical postural abnormalities of advanced ankylosing spondylitis such as accentuated kyphosis and reduced mobility

**TABLE 102-4** Clinical Manifestations of Diffuse Idiopathic Skeletal Hyperostosis in the Cervical Spine

| |
|---|
| **Spontaneous** |
| Dysphagia |
| Hoarseness |
| Stridor |
| Ossification of posterior longitudinal ligament |
| Myelopathy |
| Aspiration pneumonia |
| Sleep apnea |
| Atlantoaxial complications (e.g., pseudoarthrosis and subluxation) |
| Thoracic outlet syndrome |
| **Induced** |
| Endoscopy difficulties |
| Intubation difficulties |
| Fractures |

From Mader R: Clinical manifestations of diffuse idiopathic skeletal hyperostosis of the cervical spine. *Semin Arthritis Rheum* 32:130–135, 2002.

of the spine. These two entities, although they may coexist, can usually be distinguished by the different age of onset, clinical presentation, radiographic appearance of the spine and sacroiliac joints, and *HLA-B27* associations.[70,71]

## Extraspinal Involvement

Clinical manifestations similar or identical to those of osteoarthritis are prominent features of DISH in the peripheral joints. The peripheral joints affected by DISH have features that distinguish them from primary osteoarthritis, however. One is the more frequent involvement of joints that are not usually affected in osteoarthritis such as the metacarpophalangeal joints, elbows, and shoulders (Figure 102-4).[61,72-74] Other features are a more severe hypertrophic disease that may result in a reduced range of motion in the affected joints and calcified and/or ossified prominent enthesopathies.[74-76]

As described previously, the primary event in DISH is thickening, calcification, or ossification of ligaments and entheses. In particular, enthesopathy affecting the peripheral joints has been described.[77] The radiographic appearance of peripatellar, cruciate ligament insertion, and pericapsular osseous enthesopathies are some examples of the contribution of DISH to stiffening of the soft tissues surrounding a joint (Figure 102-5).[78] Entheseal ossification at various sites other than joints such as the heel, ribs, and pelvis is a common finding in DISH. The clinical implications of the peripheral involvement is not clear and is controversial. However, the enthesopathies may become symptomatic, exhibiting pain and swelling in the affected region. A high probability for the presence of spinal DISH was noted for ossification of the iliolumbar and sacrotuberous ligaments and with bony overgrowth of the inferior acetabular rim.[77,79] A higher level of soft tissue tenderness and lower functional status have been reported recently in patients with DISH compared with patients who do not have DISH.[80]

Patients with DISH often have higher body weight and body mass index, waist circumference, and systolic blood pressure.[33] These factors and the metabolic abnormalities described earlier put patients at an increased risk for cardiovascular diseases.[30,81,82] The diagnosis of DISH should be suspected in patients with osteoarthritis in atypical locations (e.g., the elbow), in patients with hypertrophic osteoarthritis, and in patients with large enthesopathies and entrapment neuropathies of uncertain origin. This is particularly true for patients with the associated diseases and metabolic abnormalities previously discussed. It has been shown that chest radiographs may serve as a screening tool for the diagnosis of DISH with a sensitivity of 77% and a specificity of 97%.[83]



**Figure 102-4** Severe hypertrophic osteoarthritis of the proximal and distal interphalangeal joints. Of particular interest is the involvement of the metacarpophalangeal joints with enlarged metacarpal heads, osteophytes, joint space narrowing, and subchondral sclerosis.

### Fracture Risk and Heterotropic Ossification

The sites of ossification and the subsequent production of large osteophytes may result in spinal stenosis[67] and spinal stiffening. Under these circumstances, force cannot be dissipated and the spine behaves like a long bone with an increased risk of fracture.[84] These fractures may be unrecognized, unstable, and associated with treatment delays and permanent neurologic deficits.[85]

The tendency for new bone formation puts the patient at risk for the development of heterotopic ossification after joint surgery[86] and may jeopardize the outcome. Although it is not clear what causes these ossifications, the use of irradiations or prophylactic NSAID treatment has been suggested for the patients at risk.

## Treatment

Treatment of DISH should address several issues. Treatment is expected to alleviate pain and stiffness; prevent, retard, or arrest progression; correct the associated metabolic disorders; and prevent spontaneous or induced complications (Table 102-5).

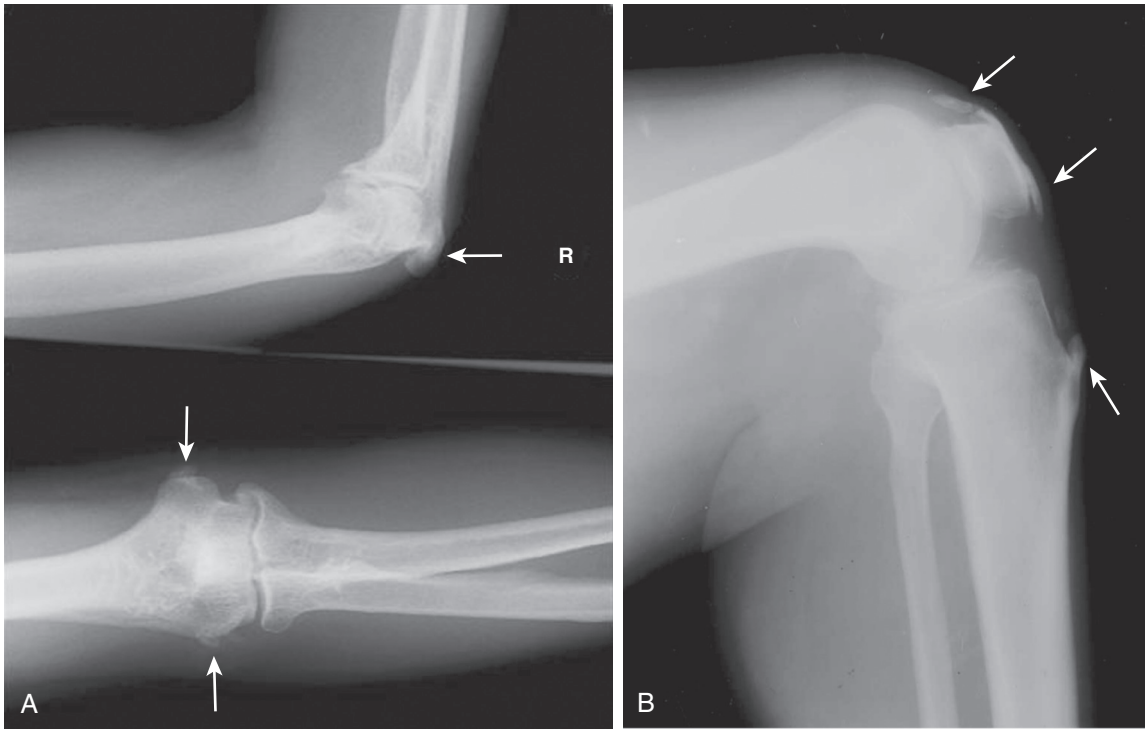



**Figure 102-5** **A** and **B,** Ossified enthesopathies in the peripatellar, olecranon, and humeral epicondyles *(arrows)*.

**TABLE 102-5** Therapeutic Targets in Diffuse Idiopathic Skeletal Hyperostosis

| |
|---|
| Symptomatic relief of pain and stiffness |
| Prevent, retard, or arrest progression |
| Treatment of associated metabolic disorder |
| Prevent spontaneous complications |
| Prevent traumatic complications |
| Prevent complications that might emerge during diagnostic or therapeutic procedures |

From Mader R: Current therapeutic options in the management of diffuse idiopathic skeletal hyperostosis. *Expert Opin Pharmacother* 6:1313–1316, 2005.

**TABLE 102-6** Future Considerations in Diffuse Idiopathic Skeletal Hyperostosis

| |
|---|
| Establish and validate diagnostic criteria that also consider the peripheral manifestations of the disease |
| Clarify the natural course and prognosis |
| Study the systemic nature and the impact on quality of life and life expectancy |
| Seek a better understanding of the pathogenetic basis for the disease |
| Offer a disease-modifying therapeutic approach |

Specific therapeutic interventions in DISH have not been systematically explored, which is probably related to the inclusion of DISH in the spectrum of osteoarthritis and the assumption that the same therapeutic interventions for osteoarthritis are suitable for DISH. More recently it was suggested that serum levels of growth hormone and IGF-I might be a useful surrogate marker for assessing DISH progression and remission.[38] It was estimated that a period of at least 10 years is necessary for the pathologic process to evolve completely.[87] This notion has been recently corroborated by a computed tomography (CT) study[88] and implies that a long observation period is necessary to show that a therapeutic intervention prevents the development of the disease, arrests its progression, or, it is hoped, reverses the pathologic changes.

Few reports have been published about remedies to alleviate the symptoms of the disease, but some investigators have reported on the beneficial effects of light exercise, heat, analgesics, and NSAIDs.[89] Exercise therapy failed to show a significant improvement in the spinal range of motion except for lumbosacral flexion.[90] More recently, the use of locally acting NSAIDs for the treatment of osteoarthritis was shown to be as effective as the same product by oral route, suggesting that locally acting NSAIDs also might be successfully used for the symptomatic relief of pain and stiffness that may be present in the peripheral joints of patients with DISH.[91] Treatment of symptomatic enthesopathies might be necessary to alleviate local pain and swelling; this treatment can be achieved through use of local soft applications such as insoles for plantar spurs or protective bandages at other sites. Infiltration of a local anesthetic with long-acting corticosteroids may offer at least temporary relief in persons with severe symptoms. When multiple sites are involved, the same therapeutic modalities mentioned for osteoarthritis may be used.

It seems appropriate to screen patients with DISH for known cardiovascular risk factors and to treat them when appropriate. General measures such as weight reduction, adequate physical activity, and a diet low in saturated fat and carbohydrates all might be important in preventing or arresting the progression of DISH. Some of these factors may have pathogenetic implications and may become therapeutic targets. On the basis of present understanding, therapeutic interventions should aim at a reduction of insulin secretion and insulin resistance. In patients with non–insulin-dependent DM, the use of biguanides, which decrease insulin resistance, may offer an advantage compared with the use of sulfonylureas, which increase insulin secretion. When coexisting hypertension is treated, medications that might improve insulin resistance such as angiotensin-converting enzyme inhibitors, calcium channel blockers, and α-blockers are preferred over medications that may worsen insulin resistance, such as thiazide diuretics and β-blockers.[92] Some growth factors that might have a role in the development of DISH such as NF-κB, PDGF-BB, TGF-β1, growth hormone, and IGF-I may become targets for specific therapeutic interventions.

In some patients, several complications can be avoided if they are taken into consideration. Aspiration pneumonia can be partially avoided if instructions in proper deglutition and preservation of an upright position after meals are carefully explained to the patient. Physicians familiar with DISH can avoid or minimize damage to the cervical spine or to soft tissues in patients who might need certain diagnostic or therapeutic interventions such as upper gastrointestinal endoscopy or endotracheal intubation. It is reasonable to adopt common measures to prevent falls and trauma, especially in elderly patients. Heterotopic ossification after orthopedic surgeries, in particular hip arthroplasty, is common in patients with DISH. Several therapeutic interventions aimed at abolishing heterotopic ossification, such as administration of NSAIDs, anti–vitamin K, and irradiation, have been reported with variable success.[93,94] Patients at high risk of developing this complication, such as patients with DISH, should be considered candidates for one of these regimens. Therapeutic options are summarized in Figure 102-6.[95] Many tasks lay ahead to better define this disorder, understand the pathogenesis, and delineate effective interventions (Table 102-6).

## HYPERTROPHIC OSTEOARTHROPATHY

HOA is a well-known entity characterized by skin and bone proliferation and large joint effusions. The hallmark and main visual manifestation is a bulbous deformity of the distal end of the digits, also known as *clubbing* or *drumsticks*. Periostosis is a progressive process with predilection for the tubular bones, principally the tibia and fibula. Periostosis is bilateral, symmetric, and spares the medullary cavity, axial skeleton, and skull. The prevalence of the condition is unknown, but it was found in skeletal human remains from thousands of years ago.[96] It may be primary or secondary.

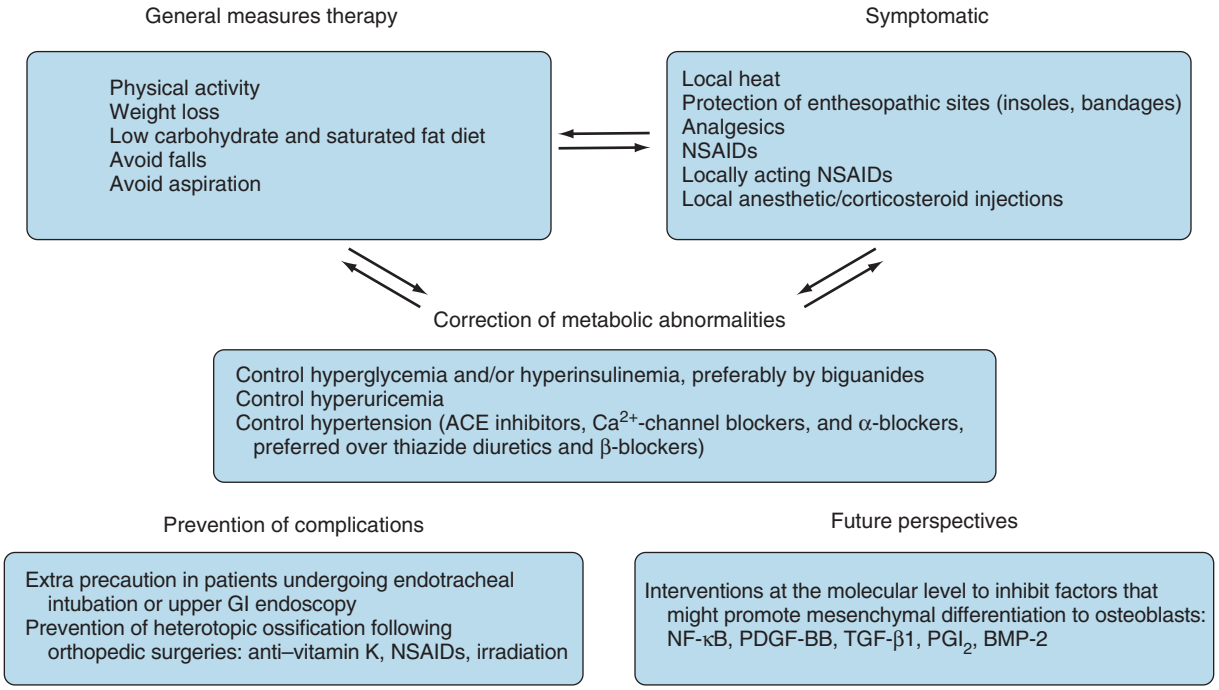



**Figure 102-6** Therapeutic options in diffuse idiopathic skeletal hyperostosis. ACE, Angiotensin-converting enzyme; BMP-2, bone morphogenetic protein-2; GI, gastrointestinal; NF-κB, nuclear factor-κB; PDGF-BB, platelet-derived growth factor–BB; $PGI_2$, prostaglandin $I_2$; TGF-β1, transforming growth factor-β1. *(From Mader R: Current therapeutic options in the management of diffuse idiopathic skeletal hyperostosis.* Expert Opin Pharmacother *6:1313–1316, 2005.)*

## Etiology

### Primary Hypertrophic Osteoarthropathy

Primary HOA is an autosomal dominant disorder characterized by periostosis, clubbing, thickening of the skin of the face and scalp, seborrhea, and hyperhidrosis. It is also termed *pachydermoperiostosis*. There is a male predominance, with a male-to-female ratio of 9 : 1; one peak of presentation occurs in the first year of life and the other in adolescence.[97] A mutation in the *HPGD* gene, which encodes the enzymes responsible for prostaglandin metabolism, results in primary HOA.[98]

### Secondary Hypertrophic Osteoarthropathy

Secondary forms of HOA manifest as isolated clubbing or with the full spectrum of the disease. Clubbing may be unilateral or bilateral and has been reported to occur in a variety of diseases, including pulmonary (most commonly cancers), cardiac, gastrointestinal, neurologic, infectious, vascular, and other diseases (Table 102-7).[99-105]

**TABLE 102-7** Etiologies of Hypertrophic Osteoarthropathy

| |
|---|
| **Unilateral** |
| Hemiplegia |
| Patent ductus arteriosus |
| Aneurysms |
| **Bilateral** |
| Pulmonary Diseases |
| Cystic fibrosis |
| Pulmonary fibrosis |
| Primary or secondary lung tumors |
| Lung and pleural infections |
| Pleural tumors |
| Heart Diseases |
| Cyanotic diseases |
| Infective endocarditis |
| Gastrointestinal Diseases |
| Cirrhosis |
| Hepatic carcinoma |
| Intestinal and esophageal malignant tumors |
| Inflammatory bowel diseases |
| Intestinal polyposis |
| Other Diseases |
| Various malignancies |
| POEMS syndrome |
| Rheumatic diseases |
| Thymoma |
| Acquired immunodeficiency syndrome |
| Thalassemia |

POEMS, Polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes.

## Pathogenesis

HOA is characterized by excessive collagen deposition, endothelial hyperplasia, edema, and new bone formation mainly involving the distal extremity and eventually progressing proximally. Various hypotheses have been generated in an attempt to explain the development of HOA. Most patients with secondary HOA have severe lung or cyanotic heart diseases. It was suggested that megakaryocytes are fragmented into platelets during their passage in

the lung capillaries. In severe lung diseases or right-to-left shunts, however, megakaryocytes or platelet aggregates bypass the pulmonary capillary bed and lodge in the peripheral vasculature of the digits.

A study showed that locally released growth factors such as vascular endothelial growth factor (VEGF) and PDGF were markedly increased in tissue samples obtained from digits of patients with HOA.[106] It is feasible that these substances might be responsible for the distal overgrowth of collagen and bone. VEGF was found to be produced by a lung tumor in a patient with HOA. In this case, serum levels of VEGF were high, and resection of the tumor reversed the digital clubbing and reduced the serum VEGF levels.[107] Activation of platelets and endothelial cells was supported by an increase in circulating von Willebrand factor antigen.[108] Other growth factors have been associated with digital clubbing, including hepatocyte growth factor, which was found to be increased in the serum of patients with lung cancer and HOA compared with patients who had lung cancer but did not have HOA.[109]

## Clinical Manifestations

HOA is often asymptomatic, and sometimes it is the patient who notes changes in the shape of his or her fingers. Symptomatic patients report a deep-seated pain in the lower extremity and over the long tubular bones, which is exacerbated by palpation. Large joint effusions are common, and the synovial fluid is thick with few white blood cells.[110] Skin hypertrophy may be confined to the nail beds or involve the face or larger areas overlying the tubular bones or joints.

The most common and apparent clinical manifestation is digital clubbing. The bulbous deformity of the fingertips is accompanied by a convex nail (watch-crystal nail). The skin around the nail bed becomes shiny and thin, with disappearance of the creases (Figure 102-7). Palpation of the base of the nail bed yields the sensation of a "floating" nail within the soft tissue. Cases of advanced clubbing can be identified easily. Several methods have been developed to diagnose early phases of the condition. Among those techniques, the digital index and the phalangeal depth ratio have been most widely used.[111,112] The digital index measures the ratio between the circumference at the level of the nail bed and the circumference at the distal interphalangeal joint of the 10 fingers. A sum of ratios greater than 10 suggests clubbing. The phalangeal depth ratio measures the ratio between the depth of the distal phalanx and the depth of the distal interphalangeal joint of the index finger. A ratio greater than 1 is considered abnormal.

No specific laboratory tests are available to diagnose HOA. Radiographs of the fingers and toes may show acroosteolysis, and periostitis manifests by cortical thickening of long bones. The process may involve few or multiple sites and can be regular or irregular in appearance. Characteristically, there is no reduction in joint space or erosions. Radioisotope bone scanning can be useful for diagnosis and for evaluating the extent of the process. Increased uptake can be seen in the cortex of long bones, sometimes in the form of splints (Figure 102-8).

## Treatment

Asymptomatic persons do not need to be treated. NSAIDs are sometimes useful in symptomatic patients. Case reports have suggested that significant pain relief was observed after treatment with octreotide or bisphosphonates.[113,114] In secondary cases of HOA, all features and symptoms promptly regress with successful treatment of the primary disease, such as correction of a heart malformation, removal of tumors, and therapy of infective endocarditis or inflammatory bowel disease. The role played by VEGF in the pathogenesis of HOA suggests that treatment with VEGF inhibitors might improve the symptoms of this condition.[115]



**Figure 102-7** Severe clubbing in a patient with advanced lung cancer.



**Figure 102-8** Increased non-nodular cortical bone uptake in a patient with bronchogenic carcinoma. *(From Vandemergel X, Blocket D, Decaux G: Periostitis and hypertrophic osteoarthropathy: etiologies and bone scan patterns in 115 cases.* Eur J Intern Med *15:375–380, 2004. Reprinted with permission from the European Federation of Internal Medicine.)*

## SAPHO SYNDROME

SAPHO syndrome is a chronic inflammatory disorder, often relapsing, of unknown cause. The term SAPHO was coined to include several associated clinical manifestations: synovitis, acne, pustulosis, hyperostosis, and osteitis.[116] The most common sites of bone involvement are in the anterior chest wall—mainly the clavicles, sternum, and sternoclavicular joint.[117] The syndrome has been associated with a variety of skin lesions such as acne conglobata, acne fulminans, palmoplantar pustulosis, and psoriasis. The condition is uncommon with an estimated prevalence of 1 in 10,000, but the exact prevalence is difficult to establish in the absence of validated diagnostic or classification criteria. Furthermore, it is presumed that the prevalence of SAPHO syndrome is underestimated, particularly in patients with mild or absent skin manifestations.[118]

### Etiology and Pathogenesis

The cause of SAPHO syndrome is unknown. An infectious cause has been proposed on the basis of the isolation of *Propionibacterium acnes*, a slowly growing anaerobic microorganism often found in acne, from sternal osteosclerotic lesions.[119] It has been postulated that *P. acnes* might act directly, inducing bony erosions through chronic indolent inflammation. It has also been suggested that *P. acnes* may trigger complement activation and promote production of IL-1, IL-8, and TNF, which contribute to both humoral and cellular pro-inflammatory responses. An imbalance between pro-inflammatory and anti-inflammatory mediators also has been suggested to contribute to the inflammatory response and the subsequent damage.[120,121]

Genetic susceptibility has not yet been found. Specifically, no associations with *HLA-B27*, other alleles often associated with psoriatic arthritis, or other candidate genes have been identified.[117,122,123]

In patients with SAPHO syndrome, the reported prevalence of common autoantibodies encountered in autoimmune diseases was 22%. The autoantibodies did not show a specific profile but suggest a possible relationship between autoimmunity and SAPHO.[124] It has been suggested that the increased association of SAPHO syndrome with inflammatory bowel disease might link SAPHO to the seronegative spondyloarthropathies, but this view has not yet been confirmed.[117,125]

### Clinical Manifestations and Imaging

SAPHO syndrome is characterized by the combination of skin and osteoarticular manifestations. The skin manifestations include palmoplantar pustulosis, severe acne, suppurative hidradenitis, and at times psoriasis. The skin manifestations can precede or follow the osteoarticular manifestations, at times by many years.[117,126,127]

The osteoarticular features of SAPHO syndrome are hyperostosis, aseptic osteitis that occasionally involves the adjacent joints, and synovitis. The early histologic findings of the bone lesions are similar to those of osteomyelitis with periosteal bone formation. Subsequently the lesions evolve into chronic inflammation with a preponderance of mononuclear infiltrate; it is only in the late phases that prominent marrow fibrosis, sclerosis, and enlarged bone trabeculae ensue.[128]

The most characteristic clinical manifestation is pain in the anterior chest wall as a result of common involvement of the clavicles, sternum, and sternoclavicular joints.[117,127] Other common sites of involvement in the axial skeleton are the vertebral bodies with vertebral sclerosis; hyperostosis; spondylodiskitis; nonmarginal syndesmophytes, at times with anterior bridging; the bone adjacent to the sacroiliac joint; and the pubic symphysis.[125,129] Extraspinal involvement is infrequent but may be observed in the form of osteitis involving the tibia, femur, or mandible and with arthritis involving the knees, hips, ankles, or small joints of the hands and feet.[117,125,130] The diagnosis is highly suspected in cases that feature usually sterile, multifocal, recurrent osteomyelitis, with or without cutaneous lesions; arthritis associated with palmoplantar pustulosis, pustular psoriasis, or severe acne; and osteitis associated with severe acne, palmoplantar pustulosis, or pustular psoriasis.[127]

Laboratory investigations may reveal a moderately elevated erythrocyte sedimentation rate or acute phase reactants such as C-reactive protein, C3, and C4. These findings may reflect the inflammatory nature of the condition but are less reliable than in other inflammatory rheumatic disorders.[117,125]

The diagnosis is usually established with the correlation between the clinical presentation and imaging findings. Bone scintigraphy has sufficient sensitivity to detect the anterior chest wall involvement, demonstrating the characteristic "bull's head" sign.[131] Plain radiographs may detect advanced lesions, but a CT scan enhances the ability to detect these lesions, in particular in flat bones. Radiographic studies of the anterior chest lesions show osteitis in the form of osteosclerosis with a homogeneous fibrillary pattern, hyperostosis in the form of periosteal reaction, and cortical thickening leading to bone hypertrophy. These lesions with eventual erosive arthritis (due to either primary arthritis or extension of the adjacent osteitis) classically involve the sternoclavicular joint and the upper costosternal and manubriosternal junctions. The spine, which is involved in about a third of the patients, presents with a similar radiographic appearance with vertebral sclerosis, hyperostosis, spondylodiscitis, nonmarginal asymmetric syndesmophytes, and at times hyperostosis in the form of osseous bridging along the anterior aspect of the spine.[126] In a recent MRI study, corner vertebral erosions were a consistent finding, frequently with involvement of the vertebral end plates and at times adjacent vertebra.[132] The meaning of these findings has not yet been fully elucidated, and further studies are necessary. Entheseal ossifications, osteitis, osteosclerosis, and periosteal new bone formation have all been reported with variable frequency in long bones.

### Treatment

NSAIDs are usually the first line of treatment and may improve symptoms. However, they often fail to control the disease, and additional therapy with corticosteroids and other modalities is required.[117,133] Several empiric therapeutic approaches have been reported in SAPHO, including NSAIDs, corticosteroids, bisphosphonates, sulfasalazine,

methotrexate, cyclosporine, leflunomide, antibiotics, and anti-tumor necrosis factor (TNF) agents,[133] but have yielded mixed results.[125,133,134] Treatment with antibiotics, usually azithromycin, has been reported to be beneficial in patients with SAPHO syndrome. However, many patients were also treated with other disease-modifying antirheumatic drugs, and the response faded after discontinuation of the antibiotic. It is not clear whether the response to the antibiotic, although transitory, is due to its antibacterial or anti-inflammatory effect.[135] In recent years it has been reported that bisphosphonates, in particular pamidronate, elicit a good or partial response in the majority of patients affected by SAPHO syndrome.[136] It seems that beyond their ability to prevent bone resorption, bisphosphonates are capable of suppressing the production of proinflammatory cytokines such as IL-1, TNF, and IL-6.[137] Treatment with TNF blockers has emerged as an effective therapy in selected cases. The results appear to be less spectacular than in other seronegative spondyloarthropathies and may be hampered by relapse of the skin manifestations.[138,139] Recently, anakinra, an IL-1 receptor antagonist, and other biologic agents have been proposed as possible therapies for this condition.[140,141]

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 103



# Osteonecrosis

Christopher Chang • Adam Greenspan • Javier Beltran • M. Eric Gershwin

## KEY POINTS

- Younger patients are affected more often by osteonecrosis than by osteoarthritis, and osteonecrosis has significantly greater long-term morbidity.
- Corticosteroids are the most common cause of nontraumatic osteonecrosis.
- The femoral head is the most common site of osteonecrosis.
- In rare instances, osteonecrosis of the jaw has been associated with bisphosphonate exposure. This phenomenon is more common with repeated intravenous infusions of bisphosphonates.
- Case reports of osteonecrosis of the jaw in association with other medications, such as denosumab, have been reported.
- The final common pathway in the pathogenesis of osteonecrosis is disruption of blood supply to a segment of bone.
- Abnormalities in lipid metabolism, bone homeostasis, regulation of apoptosis, coagulopathies, innate immunity, and oxidative stress may play a role in the pathogenesis of osteonecrosis.
- Epigenetics may alter the predisposition to develop osteonecrosis.
- MRI is currently the optimal test for early diagnosis and identification of the extent of osteonecrosis.
- Nonsurgical treatment of osteonecrosis does not change the natural history of the disease.
- Although surgical treatment of femoral head osteonecrosis has many variations, most symptomatic patients eventually require total hip arthroplasty.
- Knowledge of risk factors and early detection are crucial to the successful management of osteonecrosis.
- Because of the lack of successful treatment options, new modes of management focus on the prevention of osteonecrosis.

Osteonecrosis literally means "bone death" (*ossis* [Latin] = bone; *necrosis* = killing or causing to die). Synonyms include *avascular necrosis*, *ischemic necrosis of bone*, *aseptic necrosis*, and *subchondral avascular necrosis*. Although the term *osteonecrosis dissecans* is sometimes used synonymously with osteonecrosis, strictly speaking, it is a consequence of osteonecrosis involving fragmentation of bone that leads to fracturing or cracking of bone. The concept of bone death was first described by Hippocrates,[1] but the first clinical description of osteonecrosis was a case of sepsis-induced bone death described by Russell in 1794.[2] The first description of the occurrence of bone death in the absence of infection was published almost a century later.[3] The first report of osteonecrosis in a deep sea diver appeared in 1936.[4] The pathogenesis of osteonecrosis is complex, and immune regulation of bone homeostasis may play a significant role, but ultimately, bone death occurs as a result of complete or partial disruption of the delivery of oxygen and/or nutrients to the bone and surrounding tissues. It is likely that multiple molecular mechanisms must be in play simultaneously for osteonecrosis to occur.[5-7]

## EPIDEMIOLOGY

The prevalence of osteonecrosis is unknown, but it is estimated that 10,000 to 20,000 new patients are diagnosed every year in the United States. Osteonecrosis occurs in 15% to 80% of patients with femoral neck fractures.[6] Osteonecrosis is believed to be the cause of 10% of the 500,000 hip replacements performed in the United States each year.[8] Osteonecrosis primarily affects men, with the notable exception of osteonecrosis associated with systemic lupus erythematosus, which has a significant female predominance. Osteonecrosis primarily occurs in the third to fifth decade of life,[9] and consequently, long-term morbidity can be significant because most hip replacements have a finite period of viability.

Recently, osteonecrosis of the jaw (ONJ) has been increasingly reported as a consequence of bisphosphonate use. Other drugs and procedures, including dental implants have been associated with ONJ. In a Spanish study of a population center of 1.1 million, 70 cases of ONJ were identified, with patients in 25% and 75% of these cases receiving bisphosphonates orally and intravenously, respectively.[10]

## ETIOLOGY

Osteonecrosis has been linked to numerous conditions (Table 103-1). The strength of a causal relationship varies greatly, and in some cases only case reports have been published. The most common cause of nontraumatic osteonecrosis is corticosteroid use, which was first described in 1957.[11] Although other adverse effects of corticosteroids are perhaps better known, osteonecrosis of the femoral head is one of the more serious complications. Clearly there exist individual susceptibility differences that are not predictive, and therefore it is important that steroids be prescribed only when indicated and only for the length of time required. As will be discussed, although it is safe for some people to take

**TABLE 103-1** Conditions Associated With Osteonecrosis

| |
|---|
| Dietary, Drug, and Environmental Factors |
| Corticosteroids[190-192] |
| Bisphosphonates[159,193,194] |
| Alcoholism[22,195] |
| Cigarette smoking[22] |
| Dysbaric osteonecrosis[4,196] |
| Lead poisoning[197,198] |
| Electric shock[199,200] |
| Musculoskeletal Conditions: Compromise in Structural Integrity |
| Trauma[201] |
| Legg-Calvé-Perthes disease[32,34,35,202] |
| Congenital hip dislocation[203,204] |
| Slipped femoral capital epiphysis[205,206] |
| Metabolic Diseases: Abnormality in Fat or Other Metabolic Components |
| Fat embolism[207,208] |
| Pancreatitis[70,72,209,210] |
| Chronic liver disease[211] |
| Pregnancy[43,212] |
| Fabry's disease[213,214] |
| Gaucher's disease[44,215] |
| Gout[216] |
| Hyperparathyroidism[217] |
| Hyperlipidemia[207,208] |
| Hypercholesterolemia[216] |
| Diabetes[218] |
| Hematologic Conditions: Abnormalities in Blood Components |
| Sickle cell anema[44,219] |
| Hemophilia[47-49] |
| Hemoglobinopathies |
| Thalassemia[220] |
| Disseminated intravascular coagulation[115,221-223] |
| Thrombophilia[224] |
| Hypofibrinolysis[225,226] |
| Marrow infiltrative disorders |
| Thrombophlebitis/venous thrombosis[226] |
| Rheumatologic Conditions |
| Anti-phospholipid antibody syndrome[227] |
| Rheumatoid arthritis[228] |
| Inflammatory bowel disease[229,230] |
| Necrotizing arteritis[231] |
| Mucocutaneous lymph node syndrome[232] |
| Polymyositis[233] |
| Sarcoidosis[71] |
| Mixed connective tissue disease |
| Infectious Diseases |
| Human immunodeficiency virus infection[234,235] |
| Osteomyelitis[236] |
| Meningococcemia[237,238] |
| Severe acute respiratory syndrome (SARS)[117,118] |
| Oncologic Disorders and Their Treatment |
| Organ transplantation (with or without corticosteroid exposure)[239-244] |
| Radiation exposure[245-250] |
| Regional deep hyperthermia[251] |
| Acute lymphoblastic leukemia[252,253] |

large doses of steroids for long periods, avascular necrosis will develop in other patients who take smaller doses over shorter periods.

In a 1998 study in which the investigators reviewed associations in approximately 3000 cases of nontraumatic osteonecrosis, corticosteroid use was present in 34.7% of cases. Alcohol use was found in 21.7% of cases, and the remainder of the cases were idiopathic. Although the risk of developing osteonecrosis with corticosteroid use is small, because of the severity of the adverse event and the high morbidity associated with it, osteonecrosis is an important complication to consider when prescribing corticosteroids.

Studies have been performed to attempt to determine the duration of use and the dosages of corticosteroids necessary to precipitate osteonecrosis. Because several forms of corticosteroids exist with differing potencies and half-lives, and because dosages and duration of use vary between studies, any conclusions about a "safe" dose of corticosteroids are fraught with potential confounding variables and errors. In a study of 20 patients diagnosed with stage 1 osteonecrosis by MRI, the interval between the use of steroids and diagnosis ranged from 1 to 16 months.[12] The cumulative dose of steroids in this study ranged from 1800 to 15,505 mg (mean, 5928 mg) of prednisolone or the equivalent. In other studies, cumulative doses of steroids associated with osteonecrosis ranged from 480[13] to 4320[14] mg of dexamethasone dose equivalence. In a 2010 article, Powell and colleagues[15] collectively analyzed the available literature to derive maximum safe levels for duration, maximum daily dose, and average daily dose of corticosteroids. The study confirmed that many other confounding variables affect the development of osteonecrosis, making analysis of dose-response risk for an isolated association difficult. Nonetheless, corticosteroid-induced osteonecrosis is dependent on dosage, and the risk is higher with long-acting steroids and with parenteral usage.

Additional host-inherent risk factors also play a role in susceptibility. The incidence of osteonecrosis in a group of patients receiving glucocorticoid replacement therapy for primary or secondary adrenal insufficiency was 2.4%. In a study of patients undergoing renal transplantation, the 26 patients in whom osteonecrosis developed had taken a higher cumulative oral dose of prednisone after 1 and 3 months compared with 28 control patients undergoing renal transplantation in whom osteonecrosis did not develop.[16] A separate study estimated the incidence of osteonecrosis in patients undergoing renal transplant to be 5%.[17] No consistent evidence links the use of topical, inhaled, or nasal corticosteroids to osteonecrosis. The evidence for an association between osteonecrosis and intramuscular or intra-articular corticosteroids is limited to case reports.[18] Parenteral use poses a higher risk because of the rapid absorption and longer half-life of the drugs used. Bisphosphonate-induced osteonecrosis of the jaw is particularly interesting because the intended beneficial use of bisphosphonates with regard to bone diseases instead leads to a serious bone complication itself.[19-21]

Other associations include a link between cigarette smoking and osteonecrosis, with smokers having a threefold higher relative risk for the development of osteonecrosis, independent of all other factors.[22,23] The association between osteonecrosis and alcohol consumption was first described in 1922.[24] A study of patients with idiopathic osteonecrosis revealed that the risk of osteonecrosis increased with increasing daily consumption of alcohol.[23] The subjects were divided into three groups on the basis of their alcohol consumption of less than 400 mL/week, 400 to 1000 mL/week, and greater than 1000 mL/week, and the relative risk of osteonecrosis for these groups, independent of corticosteroid use or smoking, was 3-fold, 10-fold, and 18-fold, respectively. It was also found that liver damage is not necessary for the development of osteonecrosis in patients who

consume alcohol, although elevated liver enzymes may be present.[25] The incidence of osteonecrosis in patients who received treatment for alcoholism was 5.3%. The femoral head was again the most common site (representing 82 of 92 lesions), with the other 10 lesions in the humeral head.[26]

Musculoskeletal conditions can lead to osteonecrosis in children. Legg-Calvé-Perthes disease was first described in children between 3 and 12 years of age in 1910.[27-29] Femoral head osteonecrosis is a feature of this disease and has been linked to trauma,[30,31] congenital hip dislocation,[32] and transient synovitis.[33] Bilateral involvement is common, and associated clinical manifestations include abnormal growth and stature,[34,35] delayed skeletal maturation,[36] disproportionate skeletal growth,[35] congenital anomalies,[37] and abnormal hormone levels.[38,39] Osteonecrosis can develop in children with acute lymphoblastic leukemia as well,[40,41] but this phenomenon may be a result of steroid use. An additional risk factor for patients with acute lymphoblastic leukemia has been found to be high body mass index.[42]

Osteonecrosis has also been associated with metabolic disorders and pregnancy. Diagnosis is often delayed in mothers until months after delivery. Women who experience osteonecrosis during pregnancy tend to have a small body frame and a large weight gain.[43]

Hematologic conditions have been associated with osteonecrosis. The long-term morbidity of osteonecrosis in patients with sickle cell anemia is dismal.[44] Common issues include decreased mobility, abnormal gait, and leg-length discrepancy.[45] Osteonecrosis in patients with hemophilia has been reported, but no statistically reliable causal link has been established.[46-51]

Dysbaric osteonecrosis has been reported in construction workers exposed to high pressure environments, such as the Elbe tunnel, and may have been a feature of the first report of Caisson disease.[52] The prevalence of dysbaric osteonecrosis is 4.2% in divers and 17% in workers exposed to compressed air.[53] Patients with dysbaric osteonecrosis may have more than one lesion; in addition to the femoral head, common sites include the tibia and the humeral head and shaft. The condition is not related to decompression sickness, and although proper decompression procedures can reduce the incidence of "the bends," they do not have any effect on the development of osteonecrosis, which can occur months or years after the last exposure to high-pressure environments.

Osteonecrosis has also been associated with a number of infectious diseases, including severe acute respiratory syndrome (SARS). Many patients who contracted SARS in the early 2000s received treatment with corticosteroids, and osteonecrosis subsequently developed in some of these patients.[54] The incidence of osteonecrosis appears to be higher in this group of patients than in patients with other conditions who were treated with corticosteroids.[55] Chan and colleagues[56] reported that osteonecrosis developed in five children with SARS who were treated with corticosteroids.

## CLINICAL FEATURES

### Osteonecrosis

The primary presenting symptom in osteonecrosis is pain, although many patients may be asymptomatic in the early phases of the disease. In osteonecrosis of the femoral hip, the pain is located in the hip joint but may radiate to the groin, anterior thigh, or knee. The severity of the pain can vary, depending on the size of the infarct and whether the onset of disease is insidious or sudden. When sudden and severe disruption of blood flow occurs in trauma, and when large infarcts occur in the presence of Gaucher's disease, dysbarism, or hemoglobinopathy, pain can be intense and sudden. In other conditions with a more insidious onset, the pain can follow a gradual and slow incremental progression. The pain of osteonecrosis is usually increased with use of the joint, but in individuals with advanced disease, the pain can be persistent at rest. Limitation of range of motion is progressive and is usually a late symptom, except when it results from accompanying pain. The risk of developing osteonecrosis of the contralateral hip when one side is affected ranges from 31% to 55%.

In addition to the femoral head, osteonecrosis can affect other sites such as the humeral head,[57-60] femoral condyles[61-64] and proximal tibiae,[62,65-67] the wrists and ankles,[68] the bones of the hands and feet,[69] the vertebrae,[70-72] the jaw,[73-76] and bony structures of the face.[77] Osteonecrosis of the humeral head is the second most common location; pain is usually present in the shoulder and is associated with reduced range of motion and weakness. Pain in the ankle is the main presenting symptom in nontraumatic osteonecrosis of the talus, and in some cases, the disease has already progressed to Ficat and Arlet stage 3 by the time pain presents.[67] Kienböck's disease involves osteonecrosis of the lunate. Patients present with pain in the radiolunate joint, along with weakness and limitation of motion. Kienböck's disease appears to be related to manual labor. Soccer players have been reported to develop osteonecrosis of the foot,[78] and football players may be prone to the development of osteonecrosis of the hip.[79]

### Staging Osteonecrosis

The Ficat and Arlet method of staging osteonecrosis consists of four stages. Stages 1 and 2 are reversible, whereas stage 3 (subchondral collapse) and stage 4 (joint space narrowing and destruction of cartilage) are irreversible. The Marcus staging system consists of six stages, with the first two reversible and the subsequent four stages irreversible. The modified Steinberg staging system is based on the Marcus system and also consists of six stages. Each stage is further divided into three subclasses on the basis of the extent of femoral head involvement. In subclass A, involvement is less than 25%; in subclass B, 26% to 50% of the femoral head is involved; and in subclass C, greater than 50% of the femoral head is involved.

Table 103-2 shows the Modified Steinberg system for staging osteonecrosis. The Association of Research Circulation Osseous (ARCO) has proposed a modification to the Ficat and Arlet system, adding a stage 0 for patients with negative findings of imaging studies but who are at risk for the development of osteonecrosis. In addition, stages 1 and 3 are further stratified to take into account lesion size, location, and extent of collapse.[80] In 2001 the Japanese Ministry of Health, Labor, and Welfare proposed revising criteria for the diagnosis and staging of osteonecrosis of the femoral head.[81] Diagnostic criteria included (1) collapse of the

**TABLE 103-2** Modified Steinberg Staging Systems for Osteonecrosis

| Stage | Radiographic Appearance | Reversible |
|---|---|---|
| I | Normal radiographs, but abnormal bone scan or MRI | Yes |
| II | Lucent and sclerotic changes | Yes |
| III | Subchondral fracture without flattening | No |
| IV | Subchondral fracture with flattening or segmental depression of the femoral head | No |
| V | Joint space narrowing or acetabular changes | No |
| VI | Advanced degenerative changes | No |

femoral head without joint space narrowing or acetabular abnormality as seen on a plain radiograph, (2) demarcating sclerosis in the femoral head without joint space narrowing or acetabular abnormality, (3) "cold in hot" areas on bone scans, (4) a low-intensity band on T1-weighted MRI, and (5) histologic findings of trabecular and marrow necrosis. If a patient fulfills two of the five criteria, the diagnosis is established. The working group also proposed four types of lesions based on extensiveness and defined stages of disease according to diagnostic imaging.

## Bone Marrow Edema

Bone marrow edema is a common observation in people with osteonecrosis and is frequently accompanied by vascular congestion. Bone marrow edema is not specific for osteonecrosis and may be seen in many musculoskeletal disorders, including osteomyelitis, osteoarthritis, occult intraosseous fracture, stress fracture, osteoporosis, and sickle cell crisis.

A specific syndrome known as *bone marrow edema syndrome* was initially thought to be a precursor to osteonecrosis, but it is now believed to be a separate entity. Bone marrow edema is a transitory, self-limiting condition typically seen in middle-aged men or in women in their third trimester of pregnancy. Patients report pain, limited range of motion, and an abnormal gait. Osteopenia is detected with conventional radiographs, and MRI confirms this diagnosis with a low signal on T1-weighted images and a high signal on T2-weighted images. The three phases of bone marrow edema syndrome include an initial phase lasting about 1 month, followed by a plateau phase lasting 1 or 2 months, and finally a regression phase lasting an additional 4 to 6 months.[82] Subchondral fractures do not occur. Biopsy specimens obtained in the initial phase show diffuse interstitial edema, fragmentation of fatty marrow cells, and increased formation of new bone.[83]

A study of 24 cases of bone marrow edema syndrome of the knee showed that although migrating bone marrow edema occurred in a third of patients at 5-year follow-up, the patients were asymptomatic and MRI signal alterations had resolved. Biopsy specimens of affected bone were obtained using arthroscopic surgery and core decompression, and histologic findings revealed areas of bone marrow edema and vital trabeculae covered by osteoblasts and osteoid seams. None of the cases progressed to osteonecrosis.[84]

## Bisphosphonates, Denosumab, and Osteonecrosis of the Jaw

Bisphosphonate is a class of drug used to treat osteoporosis and diseases in which bone is not formed adequately. Two forms of bisphosphonates are available, and osteonecrosis appears to occur in association with bisphosphonates that contain nitrogen. The mechanism of action of bisphosphonate-induced ONJ appears to parallel that of glucocorticoids, with derangement in lipid metabolism, bone homeostasis, and apoptosis of bone cells all playing a role. It is interesting that the jawbone seems to be the most vulnerable bone in bisphosphonate-induced disease, as opposed to the femoral head in most other associations or causes of osteonecrosis. This phenomenon may be a result of the high bone turnover rate in the jaw or because bisphosphonates exert their action not only on bone but also on many elements of the surrounding tissue, including fibroblasts and blood vessels.

ONJ increasingly has been recognized as a significant disease, and in addition to bisphosphonate use, other causes have been reported in recent years. Many of these findings are from case reports, but epidemiologic studies are beginning to appear in the literature. For example, a case of ONJ was reported in association with the use of raloxifene. Raloxifene is a nonsteroidal benzothiophene that is used as an estrogen receptor modulator to treat osteoporosis in postmenopausal women and to reduce the risk of breast cancer.[85] Denosumab, a monoclonal antibody against receptor activator of nuclear factor-κB ligand (RANKL) that is used in the treatment of osteoporosis, has also been implicated in ONJ. In addition to case reports,[86-88] animal studies also suggest that anti-RANKL interferes with the normal bone resorptive functions of osteoclasts after dental trauma,[89,90] which may play a role in the pathogenesis of ONJ.

German investigators analyzed 1229 cases of ONJ between 2004 and 2012 for risk factors. The indication for bisphosphonate use was primarily cancer, and it was found that ONJ developed earlier in men than in women. Most of the patients (81%) were taking zoledronate at the onset of ONJ.[91]

# PATHOGENESIS

## Anatomic Considerations in Trauma-Related Osteonecrosis

The femoral head is the most common site of osteonecrosis. An understanding of the anatomy of the femoral head may help explain why that is the case.

Three arterial networks supply the femoral head and neck. The extracapsular arterial ring consists of the lateral femoral circumflex artery and the medial femoral circumflex artery, which arise from the profunda femoris. The medial femoral circumflex artery and its branches supply most of the blood to the head and neck of the femur. The lateral femoral artery winds anterolaterally, and the medial femoral artery winds posteromedially around the neck of the femur; they ultimately anastomose with each other at the superolateral aspect of the femoral head. The lateral femoral circumflex artery and the medial femoral circumflex artery further anastomose with the superior and inferior gluteal

branches of the internal iliac artery, providing collateral circulation between the femoral artery and the internal iliac artery. Small vessels known as *retinacular arteries*, which are ascending cervical branches of the extracapsular ring, form an intra-articular ring at the level of the cartilage. Epiphyseal arterial branches arise from this ring and penetrate the head and neck of the femur, including the epiphyses. The artery of the ligament of the head of the femur is a branch of the obturator artery and may be the sole supplier of blood to the proximal fragment of the head.

Some of these anatomic features may render the femoral head particularly vulnerable to ischemia. The retinacular arteries are believed to supply 80% of the femoral epiphysis. Compromise of this critical vascular system may lead to osteonecrosis originating in the anterosuperior aspect of the femoral head, as indicated by angiographic studies in early osteonecrosis in which these arteries are not visualized. A schematic of the blood supply to the femoral head is shown in Figure 103-1.

Histologically, after an infarct, a rim of bony thickening or sclerosis begins to form at the margins of the infarcted area. If the necrotic lesion is within the weight-bearing region of the femoral head, subchondral fractures occur. With repeated microfractures and continued weight bearing, the original fracture cannot heal completely and new fractures appear. The secondary fracture propagates along the junction between subchondral bone and the necrotic segment. As time goes on, the femoral head becomes flattened and eventually collapses. A nonspherical head articulating with the acetabulum produces friction, erosion, and loss of cartilage. The cycle repeats itself, and the structure of the joint deteriorates, leading to degenerative changes and eventual total joint destruction.[92-94]

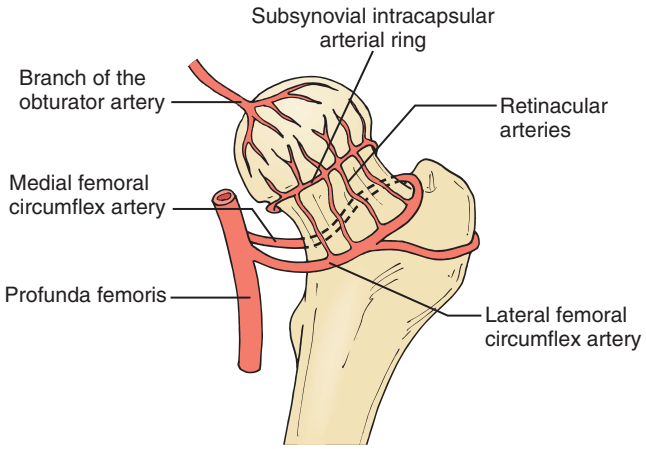



**Figure 103-1** The blood supply of the femoral head.

## Nontraumatic Osteonecrosis

Disruption of the blood supply to the femoral head can occur through a number of different mechanisms. In traumatic osteonecrosis of the femoral head, the cause of this disruption is often viewed as completely mechanical. However, as in the case of nontraumatic osteonecrosis (to be discussed in the following text), an additional component to the disruption may exist that is related to the immunologic and inflammatory changes that occur in damaged bone tissue and surrounding soft tissues.

The immunologic changes occurring in nontraumatic osteonecrosis may help explain why corticosteroids are particularly dangerous to the integrity of the blood supply of the femoral hip. Some investigators have likened osteonecrosis to "coronary disease" of the hip[95,96] and propose that the same mechanisms that cause ischemia of the myocardium may also cause ischemia of the femoral head (Table 103-3).

## Mechanical and Vascular Considerations

In Legg-Calvé-Perthes disease, obstruction to venous drainage elevates intraosseous pressure and consequently increases intra-articular pressures. In a study of patients with Legg-Calvé-Perthes disease, bone scintigraphy using technetium 99m methylene diphosphonate (Tc 99m MDP) was used to measure arterial and venous flow in the diseased hip. Although arterial flow was normal, venous drainage was significantly disrupted.[97] This disturbance was reproduced in a dog model in which injection of silicone was used to obstruct venous flow distal to the hip.[98] Ischemia resulted from the obstruction to venous drainage, leading to a cessation of endochondral ossification in the preosseous epiphyseal cartilage and the physeal plate. Widening of the joint space ensued, followed by revascularization of the epiphysis and deposition of new, immature bone. A weakened or

**TABLE 103-3** Proposed Mechanism of Disease of Common Conditions Associated with Osteonecrosis

| Associated Condition | MECHANISM OF OSTEONECROSIS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Apoptosis | Osteoblast/ Osteoclast Homeostasis | Lipid Abnormalities | Coagulation Abnormalities | Oxidative Stress | Parathyroid/ Calcium Imbalance | Vascular Plugging | Vasoactive Substances |
| Corticosteroids | X | X | X | X | X | | | X |
| Bisphosphonates | X | X | X | | | | | |
| Alcohol abuse | X | X | X | X | X | | | |
| Trauma | X | X | | | | | | X |
| Renal transplantation | X | X | | X | | X | | |
| Dialysis | | | | | | X | | |
| Sickle cell disease | | | | | | | X | |

unstable femoral epiphyseal plate resulted, and the subchondral bone became prone to segmental collapse and fracture.[99]

The pathologic mechanism of dysbaric osteonecrosis is unclear. The most intuitive explanation is that formation of gas bubbles causes arterial occlusion and ischemia. However, the true mechanism may not be quite so simple. Multiple other factors might contribute to the disease, including thromboembolic events such as platelet aggregation, erythrocyte clumping, lipid coalescence, intraosseous vessel compression as a result of extravascular gas bubbles, formation of fibrin thrombi, and narrowing of arterial lumina as a result of myointimal thickening caused by gas bubbles. The interaction between gas and blood can lead to the formation of vessel-occluding substances. All of these events can lead to redistribution of blood flow.

The increased vulnerability of bone to compression disorders has been explained by several factors, including the relative rigidity of bone and its inability to absorb increased gas pressure, inherent poor vascularization, and gas supersaturation of fatty marrow.[100] A sheep model of dysbaric osteonecrosis has been developed. Exposure to compressed air at pressures of 2.6 to 2.9 atmospheres for 24 hours results in extensive bone and marrow necrosis. The authors proposed that the initial event involving elevated intramedullary pressures leads to the formation of nitrogen gas bubbles in the fatty marrow of the long bones. Radiography shows medullary opacities and endosteal thickening. Later, neovascularization of previously ischemic fatty marrow occurs, followed by new bone formation. Osteonecrosis occurs in subchondral cortical bone with marrow fibrosis and osteocyte loss.[101]

Changes in the vasculature through injury or inflammation from other diseases may in turn lead to a compromise in blood flow. Examples include structural damage to arteriolar walls, degeneration of the tunica media, smooth muscle cell necrosis, and disruption of the internal elastic lamina. These changes can lead to eventual hemorrhagic infarction, which was observed in a study of 24 core biopsy specimens from osteonecrotic femoral heads. The changes did not occur in 11 femoral heads affected by osteoarthrosis.[102]

## Osteoimmunology

Although bone marrow is a critical component of the immune system, the bone matrix itself has often been perceived to be merely static scaffolding that functions as a support for the musculoskeletal system. In fact, it is now known that bone matrix is a dynamic tissue that is constantly replacing itself. It is estimated that about 10% of a person's bone is replaced every year. Diseases such as osteopetrosis and osteoporosis are a result of a dysfunction in the balance between bone deposition and bone resorption. The factors that regulate this homeostasis include cells of the bone matrix, immune cells, signaling molecules, cytokines and chemokines, and vitamins and hormones. Some of these regulatory factors may be present on both bone cells and immune cells, often serving different functions, thereby providing a link between the immune system and bone. Osteonecrosis, in fact, may be a result of such an imbalance in bone homeostasis. Immune factors may affect surrounding soft tissue as well, contributing to the development of osteonecrosis. The study of immune regulation of bone in osteonecrosis encompasses many of the previously proposed mechanisms of osteonecrosis, including apoptosis, oxidative stress, and genetic predisposition.

Immune factors involved in bone homeostasis include receptor activator of NF-κB (RANK) and its ligand (RANKL), IL-1, IL-6, IL-10, TGF-β, TNF, CD80, CD86, CD40, macrophage colony-stimulating factor (M-CSF), nuclear factor of activated T cell cytoplasmic (NFATc), and vitamin D (see Table 103-4 for roles and function). Many of these factors can be categorized into one of two categories—those with the overall effect of inducing osteoclastogenesis, and those that inhibit osteoclastogenesis. In addition, factors involved in cell survival and apoptosis such as Blimp-1 and Bcl-6 may also play a role. RANKL is expressed on osteoblasts and is critical for the differentiation and proliferation of osteoclasts. Because transcription of factors involved in the regulation of bone homeostasis is often influenced by glucocorticoids, this mechanism may begin to explain why steroids may be associated with osteonecrosis. The role of the innate immune system has recently come into play as well because investigators reported an upregulation in Toll-like receptor 4 signaling pathways in animal models of osteonecrosis, leading to activation of osteoclasts.[103,104]

The action of glucocorticoids is mediated by the glucocorticoid receptor, which is present on many cell types, including osteoclasts, osteoblasts, osteocytes, and cartilage. Binding of glucocorticoids to its receptor leads to the anti-inflammatory activity known to be a function of steroids. One mechanism by which this anti-inflammatory effect is mediated is through transcription of genes that inhibit the synthesis of inflammatory mediators.

## Osteoblast/Osteoclast Balance

Any disturbance in the normal homeostasis between bone deposition and bone resorption can lead to bone disease. Moreover, defective bone deposition or bone resorption in which new bone is formed in an aberrant manner can lead to disease. Alcohol can affect the ability of mesenchymal stem cells to differentiate into osteogenic lineages. During hip replacement surgery, the bone marrow in the proximal head of femurs was isolated in 33 patients with either femoral neck fractures or alcohol-induced osteonecrosis. The cells from femurs of patients with alcohol-induced osteonecrosis showed a reduced ability to differentiate into osteoblasts.[105] A subsequent study compared mesenchymal stem cells from patients with hip osteoarthritis, idiopathic osteonecrosis, and nontraumatic osteonecrosis associated with steroid or alcohol use. In idiopathic and alcohol-induced osteonecrosis, the ability of mesenchymal stem cells to differentiate into osteoblasts was decreased, but in steroid-induced osteonecrosis, it was elevated, although not to a statistically significant level. The adipogenic differentiation ability was similar in all four groups.[106]

In rats fed a diet of alcohol and glucose, lower bone mineral content and density were detected compared with control subjects. In hamsters, alcohol led to thinning of the trabeculae of the distal part of the femur. Cytologic effects included mitochondrial swelling in osteoblasts and

**TABLE 103-4** Role and Function of Immune Factors in Osteoimmunology

| Immune Factor | Ligand | Cellular Source | Function in Bone Homeostasis | OC | Immune Function |
|---|---|---|---|---|---|
| RANK | RANKL | Osteoclasts, dendritic cells | Upon binding to RANKL, signals differentiation into osteoclast | ↑ | RANKL-RANK binding leads to dendritic cell activation |
| RANKL | RANK | Osteoblasts, T helper cells | Activation of osteoclasts; overproduction can result in RA or PA | ↑ | Dendritic cell maturation |
| OPG | RANKL | | Decoy receptor for RANKL | ↓ | |
| M-CSF | CSF-1 receptor | Osteoblasts, macrophages, bone fibroblasts, stromal cells | Stimulates osteoclastogenesis | ↑ | Influences hematopoietic stem cells to differentiate into macrophages |
| TNF | TNF receptor | Macrophages, lymphocytes, mast cells, and many others | Stimulates osteoclastogenesis | ↑ | Influences multiple signaling pathways, including NF-κB, death signaling, and MAP kinase pathway |
| TGF-β | TGF-β receptor | Multiple cell lines | Induction of apoptosis | ↑ | Regulatory role, blocks activation of lymphocyte- and monocyte-derived phagocytosis |
| Blimp-1 | Bcl-6 promoter | Plasmablasts, plasma cells | Binds to Bcl-6 promoter, suppression expression | ↑ | Inhibits Tfh cell differentiation in mice[254] |
| Bcl-6 | ? | Germinal center B cells | Inhibits osteoclastogenesis | ↓ | Stimulates Tfh cell differentiation in mice |
| IL-1 | IL-1R | Macrophages, monocytes, fibroblasts, dendritic cells | Directly activates RANK signaling to promote osteoclastogenesis[255] | ↑ | Pro-inflammatory cytokine, endogenous pyrogen |
| IL-6 | IL-6R | Osteoblasts | Activation of osteoclastogenesis | ↑ | Pro-inflammatory cytokine |
| IL-10 | IL-10Rα | Monocytes, lymphocytes | Suppress bone resorption | ↓ | Anti-inflammatory cytokine, blocks NF-κB activity, regulatory cytokine |
| Vitamin D | VDR | Osteoblast, monocyte/ macrophage | Facilitate adhesion of osteoclast precursor to osteoblast[256] | ↑ | Cell proliferation and differentiation |
| Estrogens | Estrogen receptor | Ovarian follicle cells | Reduces osteoclast IL-1 responsiveness and cell survival,[257] stimulates osteoprotegerin | ↓ | Angiogenesis, endothelial healing |
| IL-17 | IL-17R | T cells | May have opposing roles of bone protection and bone loss[258] | ↑↓ | Pro-inflammatory cytokine |
| IL-18 | IL-18R | Macrophages | Inhibits TNF-mediated osteoclastogenesis in a T cell–independent manner | ↓ | Pro-inflammatory cytokine, works in synergy with IL-12 |

Examples of some of the factors involved in bone metabolism. In addition to the factors listed, others that play a role, either by themselves or in conjunction with other factors. The factors listed may have many other functions. Only select functions are listed.

Bcl-6, B cell lymphoma 6 protein; Blimp, B lymphocyte–induced maturation protein 1; CSF-1, colony-stimulating factor 1; M-CSF, macrophage colony-stimulating factor; NF-κB, nuclear factor-κB; MAP, mitogen-activated protein; OC, osteoclastogenesis; OPG, osteoprotegerin; PA, psoriatic arthritis; RA, rheumatoid arthritis; RANK, receptor activator for NF-κB; RANKL, receptor activator for NF-κB ligand; Tfh, T follicular helper cell; TGF-β, transforming growth factor-β; VDR, vitamin D receptor.

osteocytes. Partial osteonecrosis of the femoral head was detected in Merino sheep that were injected with ethanol. In humans, alcohol causes increased plasma calcium levels, decreased osteocalcin and circulating parathyroid hormone levels, reduced serum calcitriol, reduced bone volume, and an increased number of osteoclasts.

Alterations in osteoblast function may also contribute to the pathogenesis of osteonecrosis. In one study, osteoblasts were obtained from bone biopsy specimens from the intertrochanteric region of the femur and the iliac crest of 13 patients with osteonecrosis and 8 patients with hip osteoarthritis. Cell replication was measured on the basis of proliferation rate in secondary culture. Levels of alkaline phosphatase activity, collagen synthesis, and sensitivity to 1,25-dihydroxyvitamin $D_3$ were measured. The results indicated that although differentiation was not affected, the proliferation rate of osteoblastic cells was reduced in samples obtained from the patients with osteonecrosis compared with patients who had osteoarthritic hips.[107]

## Apoptosis and Osteonecrosis

Glucocorticoids can also act via their action on apoptosis of immune and bone cells. When prednisolone was administered to mice for 27 days, increased metaphyseal apoptotic activity of both osteoblasts and osteoclasts was noted.[108] The result was decreased bone turnover, density, and formation, increased formation of cancellous bone, and decreased trabecular width. The decreased bone turnover can be explained by the reduced osteoclast survival, and the reduction in trabecular width can be explained by a decrease in osteoblasts. An accumulation of apoptotic elements was also found in the region of the "fracture crescent" in the femurs of patients treated with glucocorticoids. On the

other hand, glucocorticoids may also increase osteoclast survival, leading to increased bone loss. Clearly, the effect of osteoclast survival on bone disease is more complicated than at first glance, and it involves the interaction of the osteoclast with the osteoblast. Because osteoblasts are also responsible for osteoclast differentiation under the right circumstances, a significant feedback system exists that maintains bone homeostasis.

Osteocyte death is also a feature of osteonecrosis. In a rat model, ischemia caused an induction in the expression of stress proteins, oxygen-regulated protein (ORP150), and hemoxygenase 1 (HO1). Induction of ischemia in these rates caused DNA fragmentation and the presence of apoptotic bodies in chondrocytes, bone marrow cells, and osteocytes.[109] Both alcohol and corticosteroids can induce osteocyte apoptosis, possibly via aberrations in lipid metabolism.

## Lipids and Osteonecrosis

Rabbits that were fed alcohol were found to have fatty infiltration of the liver and adipogenesis in the bone marrow. Increases in fat cell hypertrophy and proliferation, as well as a decrease in hematopoiesis in the subchondral head, were observed. Osteocytes contained triglyceride deposits, and an increase in empty osteocyte lacunae was noted. Alcohol also primarily triggered differentiation of bone marrow stromal cells into adipocytes in a dose-dependent manner. Intra-cellular lipid deposits led to the death of osteocytes.

In corticosteroid-induced osteonecrosis, the alteration in lipid metabolism parallels that of alcohol-induced osteonecrosis. In both cases, fatty infiltration of osteocytes has been postulated to occur.[110-112] Table 103-5 lists lipid-altering effects of corticosteroids and alcohol. In addition, interosseous venous stasis affects the interosseous microcirculation, which can lead to hemodynamic and structural changes in the femoral head. The resulting decrease in blood flow leads to osteonecrosis. In chickens treated with steroids, fatty infiltration of the liver and fat cell hypertrophy and proliferation in the femoral head occurred concurrently 1 week after the initiation of steroids. As in the case of alcohol-induced osteonecrosis, adipocytes contained triglyceride vesicles. In rabbits treated with steroids, it was found that interosseous pressure was increased and the size of bone marrow fat cells was larger than in control rabbits.[113] A histologic study of acetabular and proximal femoral bone in osteonecrosis of the femoral head revealed that osteonecrosis is more extensive in corticosteroid-induced compared with alcohol-induced or idiopathic osteonecrosis.[114] The reason for this phenomenon is unknown.

In ONJ, bisphosphonates inhibit protein prenylation via inhibition of the enzyme farnesyl diphosphate synthase. The normal lipid metabolism of pathways that regulate cytoskeletal integrity and osteoclastogenesis such as Rho, Rac, and Ras is disrupted, which is one of the mechanisms by which bisphosphonates exert their intended action, but their ability to disrupt normal regulation of bone metabolism may instead lead to osteonecrosis.

**TABLE 103-5** Lipid-Altering Effects of Steroids and Alcohol

| |
|---|
| Fatty liver |
| Swelling and necrosis of fat cells |
| Lipid-filled osteocytes |
| Hyperlipidemia |
| Adipogenesis of marrow stromal cells |
| Fatty infiltration of bone marrow |
| Fat emboli |

## Coagulation and Osteonecrosis

The hyperlipidemia, increased serum free fatty acids, and increased prostaglandins that are associated with alcohol-induced osteonecrosis may potentially trigger vascular inflammation and coagulation. Other triggers for intravascular coagulation include atherosclerosis and arteriolar fibroid degeneration. Jones[115] proposed that the progression of osteonecrosis from stage 1A to 1B is linked to an inability to clear procoagulants from blood or tissue. He proposed that decreased clearance of procoagulants leads to persistent levels of tissue thromboplastin and thus to arteriolar thrombosis, vascular stasis, free fatty acid–induced endothelial damage, and hypercoagulability. Studies have shown that patients with osteonecrosis had a much higher frequency of having at least one or two abnormal coagulant levels compared with normal control subjects. Of patients with osteonecrosis, 82% had at least one and 47% had at least two abnormal procoagulant levels. In normal control subjects, only 30% had one abnormal procoagulant level and only 2.5% had two or more abnormal levels. The procoagulants measured included free protein S, protein C, lipoprotein A, homocysteine, plasminogen activator inhibitor, stimulated tissue plasminogen activator, anti-cardiolipin antibodies (IgM and IgG), and resistance to activated protein C.[116]

In addition, both thrombophilia and hypofibrinolysis have been associated with osteonecrosis. Hypofibrinolysis leads to an increased likelihood of clot formation, and thrombophilia results in a decreased ability to lyse clots. Hypofibrinolysis is yet another mechanism by which corticosteroids lead to osteonecrosis—high-dose steroids lead to increased plasma plasminogen activator inhibitor, decreased tissue plasminogen activator activity, and inhibition of the fibrinolytic pathway, thus leading to a higher risk for clot formation. There is an early indication that coagulation abnormalities may play a significant role in corticosteroid-induced osteonecrosis in patients with SARS.[117,118]

## Oxidative Stress and Osteonecrosis

Alcohol consumption is associated with reduced superoxide dismutase activity. Alcohol has deleterious effects on muscle, including increased oxygen free radical–related damage, reduced myocardial contractility, defective mitochondrial function, and increased tissue enzymes.[119] When rabbits were injected with methylprednisolone, elevations in 8-hydroxy-2′deoxyguanosine, a marker of DNA oxidative injury, were observed.[120-123] These elevations coincided with the development of osteonecrosis. A polymorphism in nitric oxide synthase (NOS), which will be subsequently described, was also associated with the development of

osteonecrosis. The relationship between osteonecrosis and oxidative injury leads one to wonder if corticosteroid-induced osteonecrosis can be prevented or lessened in severity by simultaneous or prophylactic administration of antioxidants.

## Radiation and Osteonecrosis

An association between radiation exposure and osteonecrosis was reported as early as 1950.[124] The earliest reports involved primarily ONJ. Most of the evidence for radiation as a cause of osteonecrosis is derived from case reports. One of the more recent larger studies involved a case-control study to investigate risk factors for ONJ, in which 191 patients with ONJ and 573 control subjects were enrolled from 119 dental practices. The odds ratio for radiation exposure was 24.1 (4.9 to 118.4). However, when patients with cancer were excluded, the association between ONJ and radiation exposure no longer existed as an independent risk factor.[125]

Exposure to radiation may result in damage to vascularity of bone and lead to osteonecrosis in a dose-dependent manner. In particular, osteonecrosis after radiation for neoplasms is well documented and particularly affects the femoral head and the mandible. Concomitant administration of chemotherapy can accentuate the risk of osteonecrosis in patients who are receiving radiation, particularly elderly women.

Interestingly, an animal model of osteonecrosis induced by high-intensity focused ultrasound (HIFU) was developed to determine if HIFU could be used to generate osteonecrosis for animal studies. In this model, high-power acoustic energy up to 1000 W/cm$^2$ focused directly at bone was able to induce osteonecrosis of the femoral head in a canine model via the creation of a thermal injury. Typical diagnostic ultrasound transducers generally deliver intensities ranging between 0.1 to 100 W/cm$^2$, although HIFU has been studied for use in cancer and can reach 100 to 10,000 W/cm$^2$. This finding raises the possibility of ultrasound-induced osteonecrosis in humans in real-life clinical settings, although this possibility is far from substantiated.[126] The proposed mechanism by which ultrasound can induce osteonecrosis is through thermal damage induced by osteocyte damage and vascular thrombosis.

## Nitric Oxide Synthase and Osteonecrosis

Glucocorticoids can cause derangements in vascular responsiveness to vasoactive substances such as nitric oxide. Endothelial NOS (eNOS) stimulates the production of nitric oxide. Nitric oxide regulates vascular "tension" by acting as a vasodilator, inhibiting mononuclear adhesion to endothelial cells and preventing platelet aggregation. A defect in this activity can lead to increased vascular resistance and disruption to downstream blood flow, resulting in osteonecrosis.[127]

## Multihit Hypothesis

Other proposed mechanisms of osteonecrosis involve endothelial cell injury,[128] abnormal angiogenesis and repair mechanisms,[129] the effects of vasoactive substances,[130] activity of hepatic cytochrome P450 3A4,[131] and intramedullary hemorrhage.[132] Multiple mechanisms may occur simultaneously. Kenzora and Glimcher[133] were the first to introduce the concept of cumulative stress. Corticosteroid-induced osteonecrosis seems to occur with greater frequency in patients who have significant underlying illness such as systemic lupus erythematosus[134] or transplantation and less frequently or never in patients who are not chronically ill but are taking steroids for an acute event such as a head injury. Recent observations that corticosteroids induce osteonecrosis in patients with SARS further support the notion that more than one insult to the bone or surrounding tissue may be necessary to precipitate osteonecrosis. For each of the known associations of osteonecrosis, different mechanisms may predominate, such as lipid anomalies and apoptosis of osteoblasts in steroid-induced osteonecrosis, as well as elevated intraosseous pressures and coagulation abnormalities in dysbaric osteonecrosis, but additional factors may be necessary to precipitate osteonecrosis. The accumulated cell stress theory suggests that when the damaging effects of multiple events are aligned, similar to the Swiss-cheese model of risk assessment,[135,136] the involved bone is unable to recover from the chronic stress and osteonecrosis ensues.

## Microorganisms and the Microbiota in Osteonecrosis: Environmental Considerations

It has been postulated that infection is a major risk factor in the development of ONJ. Studies have attempted to define the role of microorganisms and the host response to infection as potential mechanisms for disease development and progression. Pushalkar and colleagues[137] compared microbial diversity in a small set of patients who took bisphosphonates and did or did not have osteonecrosis, as well as in normal control subjects. These investigators found less bacterial diversity in the bisphosphonate-related ONJ (BRONJ) group compared with the others, but with a predominant presence of Firmicutes. They also found lower levels of myeloperoxidase but higher levels of the proinflammatory cytokines IL-6 and TNF in the BRONJ cohort compared with control subjects. Furthermore, polymerase chain reaction analysis revealed downregulation of nucleotide-binding oligomerization domain–containing protein 2 (NOD-2) and cathepsin G and upregulation of secretory leukocyte protease inhibitor, proteinase 3, and conserved helix-loop-helix ubiquitous kinase. The respective roles of the microorganism environment itself versus the host innate immune response to this environment are still to be elucidated.[137]

In yet another study investigating the microbacterial environment in patients with BRONJ, Wei and colleagues[138] found that the predominant species in osteonecrosis lesions in patients with BRONJ included *Stretococcus* (29%), *Eubacterium* (9%), and *Pseudoramibacter* (8%) compared with the control group, in whom the most common species found were *Parvamonas* (17%), *Streptococcus* (15%), and *Fusobacterium* (15%). Wei and colleagues[138] also found that in patients with BRONJ, hematoxylin-eosin staining revealed that the bacteria were layered and packed into the scalloped edges of BRONJ bone, suggesting the role of biofilms as a potential risk factor for the development of BRONJ.[138]

Scanning electron microscope images have shown the presence of biofilms in BRONJ lesions.[139] It should also be noted that bacteria are not the only pathogens found in BRONJ lesions; fungi, especially *Candida* and *Actinomyces*, have also been found in lesions of patients with ONJ.[140]

### Genetic and Epigenetic Considerations

The degree to which genetics and the environment play a role in the pathogenesis of osteonecrosis is the subject of an ongoing investigation. Certainly, single nucleotide polymorphisms have been noted in a number of genes that may be associated with osteonecrosis. It has been argued that eNOS is an important player in the development of osteonecrosis. Nitric oxide may have beneficial effects on three systems involved in osteonecrosis—namely skeletal, vascular, and thrombotic. Each of these systems may be targets for proposed mechanisms of pathogenesis of osteonecrosis. A comparative analysis of the 26–base pair repeat polymorphism in intron 4 and the Glu298Asp polymorphism in exon 7 of the eNOS gene in patients with idiopathic, steroid-induced, and alcohol-induced osteonecrosis and normal control subjects was performed.[141] The frequency of the homozygous 4a allele was found to be higher in patients with idiopathic osteonecrosis compared with control subjects. The frequency of the 4a/b allele was found to be higher in all types of osteonecrosis when compared with control subjects. The 4a allele is known to be associated with reduced synthesis of eNOS, suggesting that nitric oxide may play a protective role with regard to the development of osteonecrosis.

Forty-one percent of patients with osteonecrosis compared with only 20% of control subjects were homozygous for the 4G/4G mutation in the plasminogen activator inhibitor-1 *(PAI-1)* gene.[142] This mutation causes increased hypofibrinolytic plasminogen activator inhibitor activity, resulting in decreased stimulated plasminogen activator activity. This observation lends support to the theory that procoagulants may play a significant role in the pathogenesis of osteonecrosis. A polymorphism in the *PAI-1* gene has also been reported to be predictive of osteonecrosis in children with acute lymphoblastic leukemia.[143]

Genetic variations in the type and levels of lipoprotein(A) have been linked to osteonecrosis. Apolipoprotein(A) (Apo[A]) is involved in lipid metabolism and the coagulation systems, and the Apo(A) low-molecular-weight phenotype is associated with an increased risk of osteonecrosis.[144-146] Polymorphisms in the promoter for vascular endothelial growth factor (VEGF) and in the receptor for IL-23 were associated with osteonecrosis in the Korean population,[147-149] reflecting the significance of the association of osteonecrosis with vascular disorders and autoimmune diseases, respectively.

Epigenetic modulation of gene expression has recently been recognized to affect more than half of all human genes. Several pathways exist for epigenetic transformation, including DNA methylation and demethylation, histone modifications such as acetylation and deacetylation, and microRNAs. MicroRNAs are short 21- to 23-nucleotide RNA molecules that bind to messenger RNA of the promoter region of genes and serve a regulatory function. Overexpression of certain microRNAs therefore may affect the expression of genes coding for various mediators of disease, such as cytokines or growth factors. In 2012, Yamasaki and colleagues[150] demonstrated that microRNA-210 is upregulated in the cells surrounding osteonecrotic bone. Furthermore, microRNA-210 is known to play a role in angiogenesis. Accompanying the upregulation of microRNA-210 in patients with osteonecrosis were also increased expression of VEGF and matrix metalloproteinase (MMP)-2 and -7 when compared with patients who had osteoarthritis.[150]

Other microRNAs such as microRNA-29a and microRNA-548d-5p have also been shown to play a role in regulation of bone resorption and osteogenesis.[151,152] MicroRNA-17-5p, which is known to play a role in cancer cell proliferation and invasion, was evaluated to test whether it also plays a role in osteoblast differentiation. The investigators found that expression of this microRNA was lower in patients who had osteonecrosis compared with patients who had osteoarthritis. This finding was accompanied by a facilitation in the differentiation of HMSC-bm cells, which is thought to be mediated by the action of microRNA-17-5p on expression of β-catenin and a subsequent increase in COL1A1.[153] MicroRNA profiles identified 27 microRNAs that were differentially expressed in patients with osteonecrosis of the femoral head. Fifteen were overexpressed, and 12 were underexpressed in patients with osteonecrosis when compared with either patients who had systemic lupus erythematosus or healthy control subjects.[154] The differentiating nature of microRNA profiles may play a future role as disease biomarkers.

Small interfering RNAs (siRNAs) have been shown to play a potential role in disease management. It is known that glucocorticoids may act in osteonecrosis by virtue of their ability to induce overexpression of the peroxisome proliferator-activated receptor γ (PPARγ) gene, leading to adipogenesis, which has already been discussed as a potential mechanism for osteonecrosis. In a rabbit model for steroid-induced osteonecrosis, siRNAs targeting PPARγ were able to reduce levels of PPARγ expression and increase levels of Runx2 and osteocalcin in rabbits treated with dexamethasone plus a recombinant adenovirus shuttle vector carrying an siRNA targeting PPARγ, compared with rabbits treated with dexamethasone alone. Treatment with dexamethasone plus the recombinant adenovirus shuttle vector led to amelioration of the marrow necrosis, adipocyte hypertrophy and proliferation, diminished hematopoiesis, thinner and sparse trabeculae, and an increased number of empty osteocyte lacunae in the femoral head seen in rabbits that only received dexamethasone or in combination with an irrelevant shuttle vector.[155] These studies, although in their early stages, offer hope for novel treatments based on pathogenesis and the physiologic and cellular derangements that are risk factors for the development of osteonecrosis.

## DIAGNOSIS

### History and Physical Examination

The diagnosis of osteonecrosis is generally made via the history, because many patients may not present until hip pain develops. By the time the patient is clinically symptomatic, the disease may be quite advanced. Therefore a

high index of suspicion is necessary for all patients taking oral or parenteral steroids. The following information should be elicited: any history of trauma; underlying disease; alcohol use; tobacco use; current medications; past medications; history of joint anomalies; presence of pain or limitation of motion; involvement in sports, especially high-impact sports; occupational history; gestational history; and the presence of liver disease or lipid abnormalities.

Physical examination includes palpating the lateral aspect of the hip for tenderness and identifying limp, leg-length discrepancy, the presence of masses, abnormal gait, muscle strength, and range of motion. The Harris hip score is frequently used to evaluate hip function and is also useful in monitoring the effectiveness of treatment (Figure 103-2).[156-158] The Harris hip score is a multidimensional observational assessment based on eight items that address pain,

**Hip Joint Evaluation System**

| **Date of assessment:** | **Name:** | | **Medical record #:** | **DOB:** |
|---|---|---|---|---|
| **Pain** | **Distance walked** | **Activities-shoes, socks** | **Public transportation** | **Limp** |
| o Totally disabled, crippled, pain in bed, bedridden<br>o Marked pain, serious limitation of activities<br>o Moderate pain, tolerable but makes concessions to pain. Some limitation of ordinary activity or work. May require occasional pain medication stronger than aspirin<br>o Mild pain, no effect on average activities, rarely moderate pain with unusual activity, may take aspirin<br>o Slight pain, occasional, no compromise in activity<br>o None, or ignores it | o Bed and chair only<br>o Two or three blocks<br>o Six blocks<br>o Unlimited | o Unable to fit or tie<br>o With difficulty<br>o With ease | o Unable to use<br>o Able to use | o Severe or unable to walk<br>o Moderate<br>o Slight<br>o None |
| **Support** | **Stairs** | **Sitting** | **Limb-length discrepancy** | |
| o Two crutches or not able to walk<br>o Two canes<br>o One crutch<br>o Cane most of the time<br>o Cane for long walks<br>o None | o Unable to do stairs in any manner<br>o Normally using a railing<br>o Normally without using a railing | o Unable to sit comfortably on any chair<br>o On a high chair for 30 minutes<br>o Comfortably, ordinary chair for one hour | _____cm | **Comments:** |
| | **Motions** | | | |
| Physician name: _____<br>Evaluator name: _____ | Hip flexion: _____<br>Hip extension: _____ | Abduction: _____<br>Adduction: _____ | Internal rotation: _____<br>External rotation: _____ | |

**Figure 103-2** Harris hip score evaluation form.

walking function, daily activity, and range of motion. Scores range from 0 (maximum disability) to 100 (no disability).

## Diagnostic Imaging

When the diagnosis of osteonecrosis is suspected clinically, it can be confirmed by imaging studies. In the early stages of osteonecrosis, plain radiographs may be completely normal. The earliest radiographic sign of osteonecrosis is the presence of a radiolucent crescent-shaped rim along the contour of the femoral head (the crescent sign) (Figure 103-3). This appearance on radiographs is the result of structural collapse of a necrotic segment of subchondral trabecular bone. At this stage, the disease is already irreversible. Later, radiographs will begin to show sclerotic changes (Figure 103-4). The appearance of radiographic "density" is secondary to compression of bone trabeculae after microfracture of the nonviable bone, calcification of detritic marrow, and repair of the necrotic area by deposition of new bone, the so-called "creeping substitution." Flattening of the articular surface of bone is a sign of further bone collapse (Figure 103-5). To show best the radiographic appearance of osteonecrosis in the femoral head and to better visualize the extent of the necrotic lesion, anteroposterior and frog-leg lateral radiographs of the hip should be obtained.

Skeletal scintigraphy (i.e., a radionuclide bone scan) using technetium-labeled diphosphonates has also been

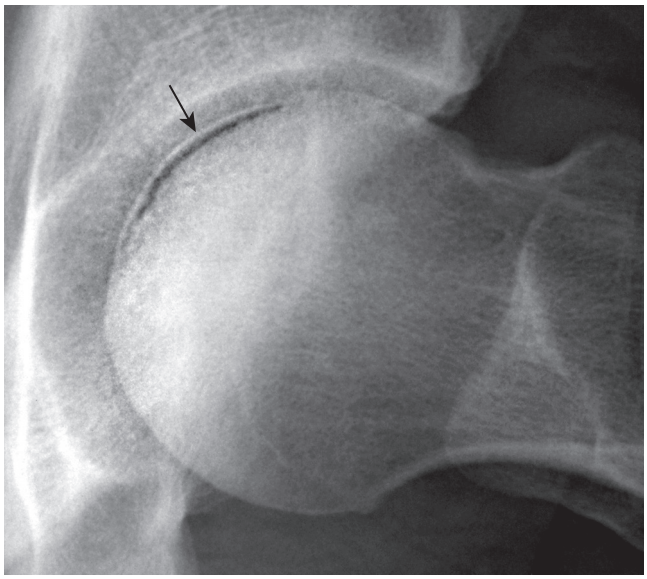


**Figure 103-3** A radiolucent crescent in the subchondral region of the left femoral head *(arrow)* is an early radiographic sign of osteonecrosis.



**Figure 103-5** Increased density of the femoral head, loss of the normal spherical shape, and flattening of the superior aspect are characteristic radiographic features of osteonecrosis.

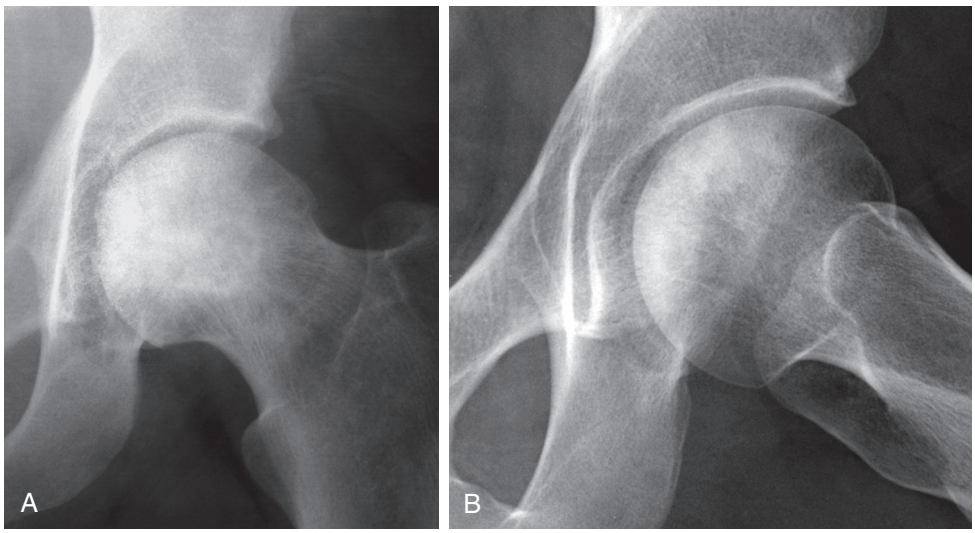



**Figure 103-4** Anteroposterior **(A)** and frog-leg lateral **(B)** views of the left hip show sclerotic changes of the femoral head typical of advanced osteonecrosis.

used to diagnose osteonecrosis. The use of this technique in the early diagnosis of this condition depends on the increase of osteoblastic activity and blood flow in the early stages of osteonecrosis. In an advanced stage of disease, the appearance may be one of increased activity in a subchondral distribution as a result of osteoblastic activity at the reactive interface around the necrotic segment; however, the center of the osteonecrotic lesion may show much less radionuclide uptake (Figure 103-6) or even a complete lack of activity, reflecting decreased metabolism in the necrotic focus as a result of interruption of the blood supply.[7]

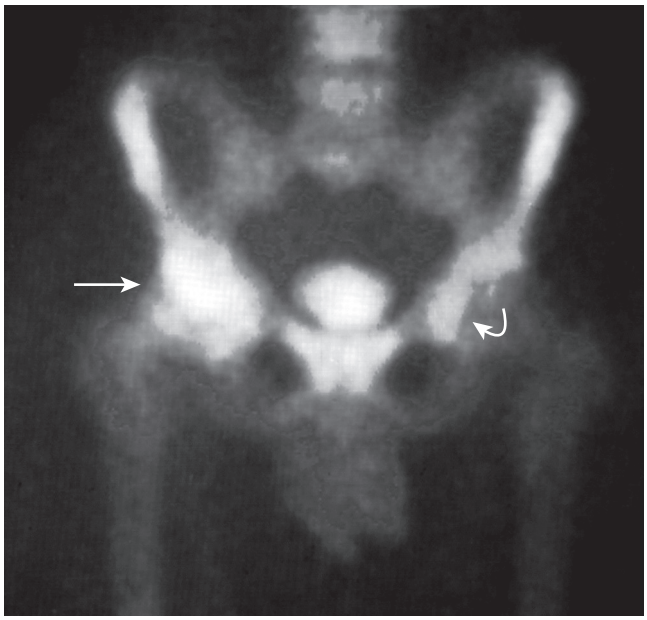


**Figure 103-6** Bone scintigraphy of osteonecrosis of both femoral heads using technetium 99m methylene diphosphonate shows moderate uptake of the radiopharmaceutical agent at the site of the osteonecrotic segment in the right femoral head and markedly increased uptake at the site of bone repair *(straight arrow)*. The left femoral head *(curved arrow)* exhibits early-stage disease.

In addition to bone scintigraphy, single-photon emission computed tomography maximizes sensitivity. A study comparing conventional radiography, MRI, computed tomography (CT), and a Tc 99m MDP three-phase bone scan in diagnosing bisphosphonate-associated osteonecrosis of the jaw showed that CT and MRI best defined the extent of the disease but that a bone scan best identified disease at an early stage. A bone scan could be an excellent screening tool for the diagnosis of osteonecrosis before further characterization of the lesions using CT or MRI.[159]

CT allows more detailed examination of the femoral head. A star-shaped structure, formed by weight-bearing bone trabeculae, gives the appearance of an asterisk on a CT scan (the asterisk sign).[160-162] This asterisk undergoes a characteristic change in ischemic bone necrosis of the femoral head, and this change was considered important for early detection of osteonecrosis. At a later stage, the collapse of necrotic bone can be well visualized (Figure 103-7).

Currently, MRI is the gold standard for imaging of osteonecrosis. Most of the staging systems for osteonecrosis are now based on MRI appearance (Table 103-6). MRI of osteonecrosis can show changes earlier than conventional radiography or CT. It can also detect bone marrow edema, a feature sometimes seen in the early phases of osteonecrosis that is not visible on conventional radiography or CT.

The typical MRI findings in osteonecrosis are intermediate or low signal intensity on T1-weighted images and high signal intensity on T2-weighted and other water-sensitive sequences (Figure 103-8). As the disease progresses, the subchondral necrotic lesion is surrounded by a low signal line on T1-weighted images and a high signal line is seen on T2-weighted images, central to the low signal line, producing the "double-line" sign (Figure 103-9). In advanced osteonecrosis, the necrotic segment exhibits low signal intensity on both T1-weighted and T2-weighted images (Figure 103-10). MRI is performed in the sagittal, coronal, and axial planes and includes T1- and T2-weighted sequences. Although excellent correlation exists between histologic findings and MRI appearance (see Table 103-6),

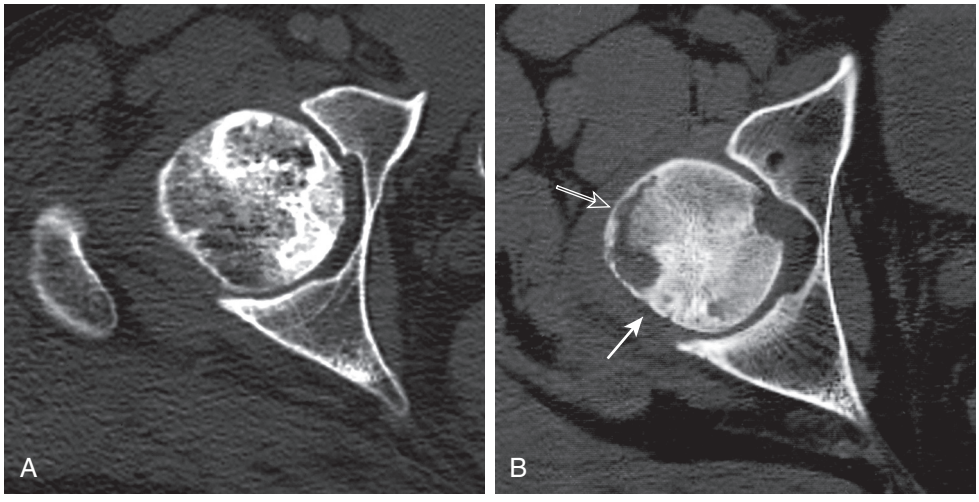



**Figure 103-7** **A,** A computed tomography scan shows osteonecrosis of the femoral head. Although several sclerotic foci are present within the trabecular bone, the integrity of the osseous structures is preserved and the femoral head exhibits a normal spherical shape. **B,** In a more advanced stage of osteonecrosis of the femoral head, note increased sclerosis in the posterior aspect *(solid arrow)* and subchondral collapse of necrotic bone anterolaterally *(open arrow)*.

**TABLE 103-6** MRI Changes and Their Correlation with Histology in Osteonecrosis

| Type of Appearance | Category of Observations | Histology | MRI Appearance |
|---|---|---|---|
| A | Fat-like | Premature fatty marrow development in the femoral neck or intertrochanteric region | Normal fat signal; sclerotic margin may be seen; circumscribing lesion |
| B | Blood-like | Bone resorption; replacement by vascular granulation tissue | High signal intensity of inner border; low signal intensity of the surrounding rim |
| C | Fluid-like | Bone marrow edema | Diffusely decreased signal on T1-weighted images; high signal on T2-weighted images |
| D | Fibrotic | Sclerosis as a result of reinforcement of existing trabeculae at the margin of live bone (repair tissue interface) | Decreased signal on T1- and T2-weighted images |

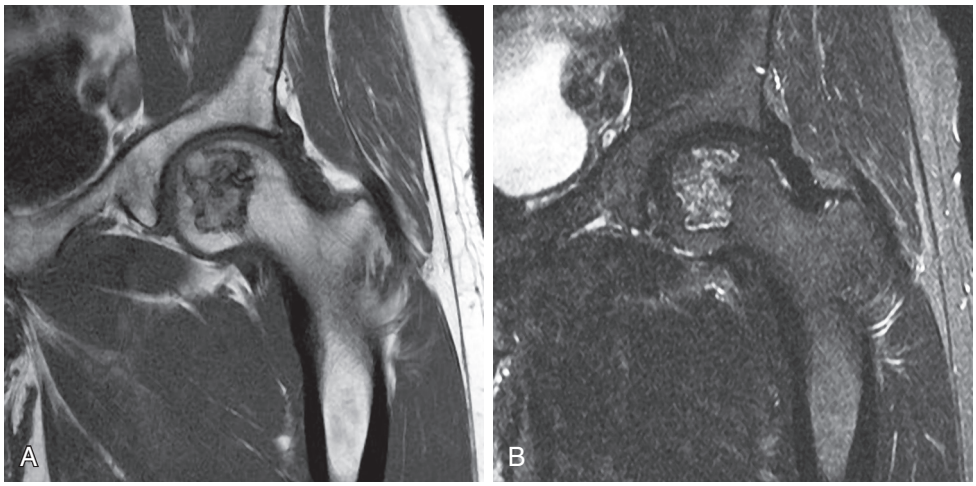



**Figure 103-8** **A,** On a T1-weighted coronal MRI of the left hip, the osteonecrotic segment in the subchondral portion of the femoral head shows low signal intensity. **B,** On a T2-weighted coronal image, the necrotic bone exhibits high signal intensity, surrounded by a sclerotic low signal rim. The low signal intensity rim surrounds a high signal intensity rim, representing the double-line sign (see also Figure 103-9). Note that the signal intensity of the necrotic area is heterogeneous in both the T1- and T2-weighted sequences.



**Figure 103-9** A coronal T2-weighted MRI scan of the right femoral head shows the double-line sign *(arrow),* which is characteristic for osteonecrosis: low signal at the periphery of the lesion and a high signal band located more centrally.

in most cases the necrotic area is composed of a variety of tissue, including areas of fibrosis and necrosis mixed with areas of blood and edema, depending on the age of the infarct. Therefore the MRI signal of the necrotic area is often very heterogeneous (see Figure 103-8), and as a result, this classification is of little clinical value. The low signal intensity band in the periphery of the osteonecrotic area correlates with a sclerotic band seen on radiographs and CT and often has a serpiginous appearance. When the osteonecrosis involves a long bone, this serpiginous band or line has been termed "smoke in a chimney."

MRI is an important tool in determining the extent of femoral head involvement in osteonecrosis. Three techniques are used to evaluate the extent of this involvement. The first is estimating head involvement. This method was first proposed by Steinberg and colleagues[163] in 1984, and it is defined by the appearance of abnormal signals on T1-weighted images. The degree of head involvement was classified into three categories: less than 15%, 15% to 30%, and greater than 30%. The second method used to evaluate the extent of involvement is the index of necrotic extent, which is determined by measuring the angle created by the extent of subchondral involvement. Lesion size was estimated using a "necrotic arc angle," defined by the angle of

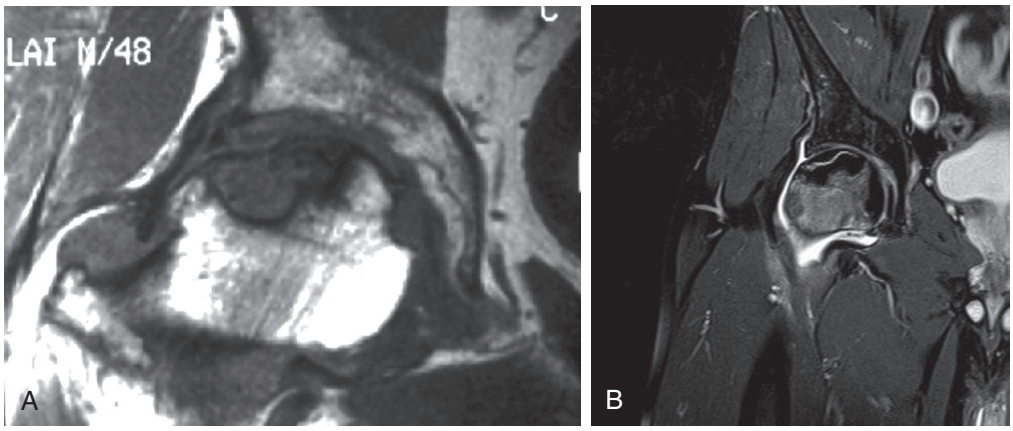



**Figure 103-10** Advanced osteonecrosis of the femoral head in two different patients. **A**, A coronal T2-weighted MRI scan demonstrates osteonecrosis of the femoral head with a subchondral fracture shown as a high signal intensity undulating subchondral line. Note the flattening of the femoral head and the presence of bone marrow edema in the femoral neck and joint effusion. **B,** A coronal T1-weighted MRI scan in another patient demonstrates low signal intensity of the femoral head with partial collapse. Note the early degenerative changes with a small marginal osteophyte of the femoral head, chondral loss, and a superior labral tear.

**TABLE 103-7** Comparative Sensitivity and Specificity of Diagnostic Radiologic Imaging Modalities in Osteonecrosis

| Radiologic Imaging | Earliest Sign Seen | Histologic Correlation | Stage | Degree of Specificity |
|---|---|---|---|---|
| Conventional radiograph | Crescent sign | Sclerotic rim of reactive bone | 2 | High |
| CT scan | Asterisk sign | Sclerotic rim surrounding a mottled area of osteolysis and sclerosis | 2 | High |
| MRI | Low signal intensity on T1-weighted images; high signal intensity on T2-weighted images | Bone marrow edema | 1 | High |
| Skeletal scintigraphy | Decreased uptake in subchondral distribution ("cold spot") | Osteonecrosis | 1 | Low |
| | Increased uptake in subchondral distribution ("hot spot") | "Creeping substitution" | 2 | Low |

the arc of the necrotic segment from the center of the femoral head. Two angles are obtained: "A," representing the necrotic arc seen on midcoronal images, and "B," representing the necrotic arc angle seen on midsagittal images. The index is a compilation of these two angles. The third method is a variation of the second, in which the angle is identified not on midcoronal or midsagittal images but on the image that shows the maximum lesion size in the sagittal and coronal planes. It is thought that this method would correct for the underestimation that may be inherent in the second method.

Table 103-7 shows a comparison of various imaging techniques used in the diagnosis and staging of osteonecrosis. Hip arthroscopy is also used in the staging of osteonecrosis. In a study comparing radiography, MRI, and arthroscopy, only moderate correlation was found among the three methods. Arthroscopy was able to detect osteochondral degeneration that was not detected by radiography or MRI in 36% of collapsed heads. Figure 103-11 is an algorithm for the diagnosis of osteonecrosis. Although the femoral head is one of the most common locations (see Figure 103-10), osteonecrosis can be seen in practically any bone. Additional examples of MRI imaging of Kienböck's disease and osteonecrosis of the knee are illustrated in Figures 103-12 and 103-13, respectively.

## Markers of Disease

The ability to find consistent and reliable markers of disease is always a welcome tool for diagnosis, determination of the extent of the disease, or even determination of the risk of acquiring the disease. The measurement of serum and urine carboxy-terminal cross-linking telopeptide of type I collagen (CTX-1), a marker of bone resorption, has been proposed as a method of evaluating the risk of ONJ as a result of bisphosphonate usage, although they are not commonly measured in the clinical setting. A serum osteocalcin level, which is easier to obtain, is another marker for bisphosphonate-related ONJ that has been suggested as a risk predictor because levels were significantly lower in the osteonecrosis group compared with a control group.[164]

# OUTCOME

The natural history of osteonecrosis depends on the size of the infarcted segment, the site of occurrence, and the clinical and radiologic staging of the disease. At the onset of the disease, range of motion may be well preserved but gradually deteriorates over time. In the early stages of the disease, when it is still reversible, patients may be asymptomatic; many patients therefore present with advanced disease.

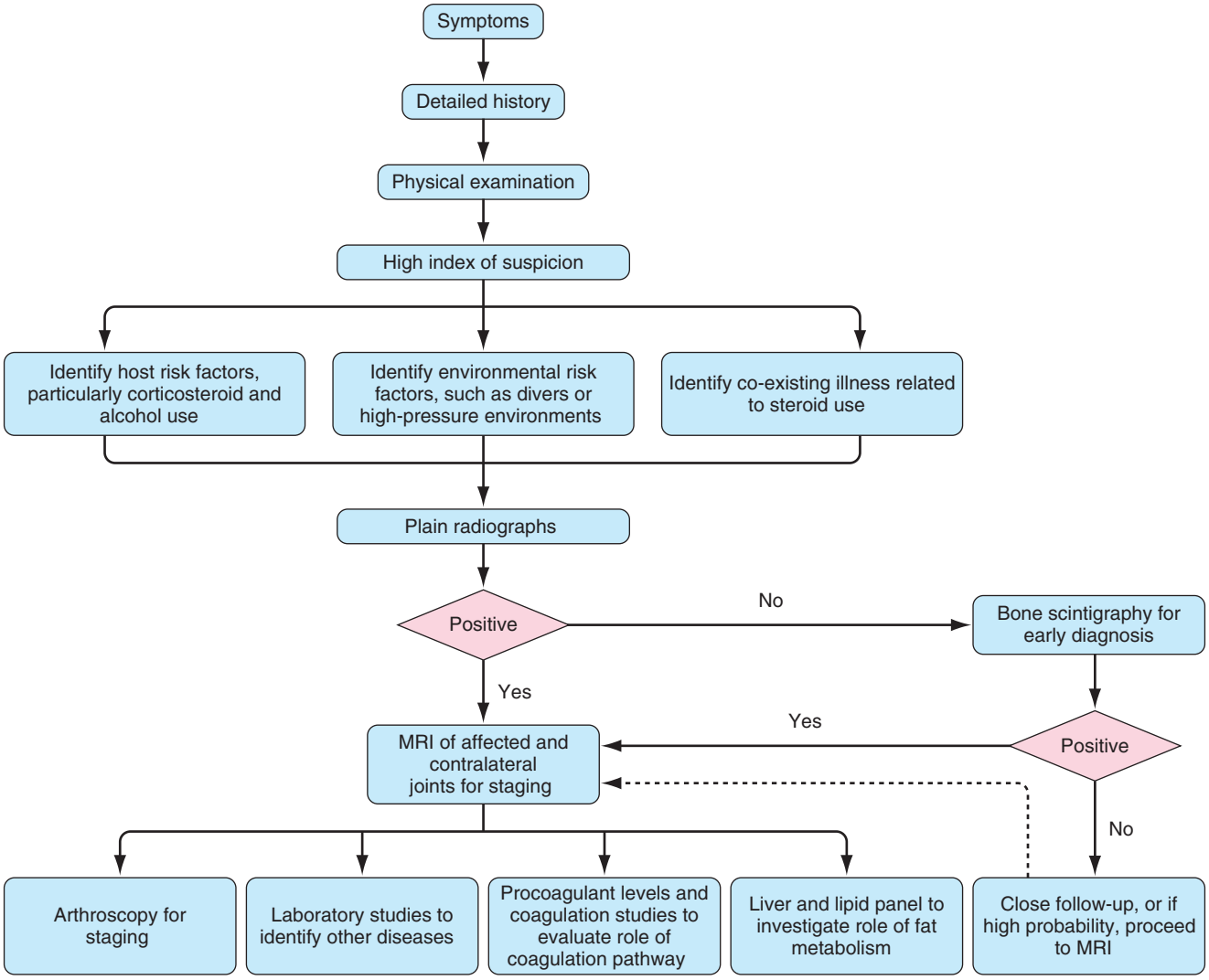



**Figure 103-11** Diagnostic algorithm for osteonecrosis.

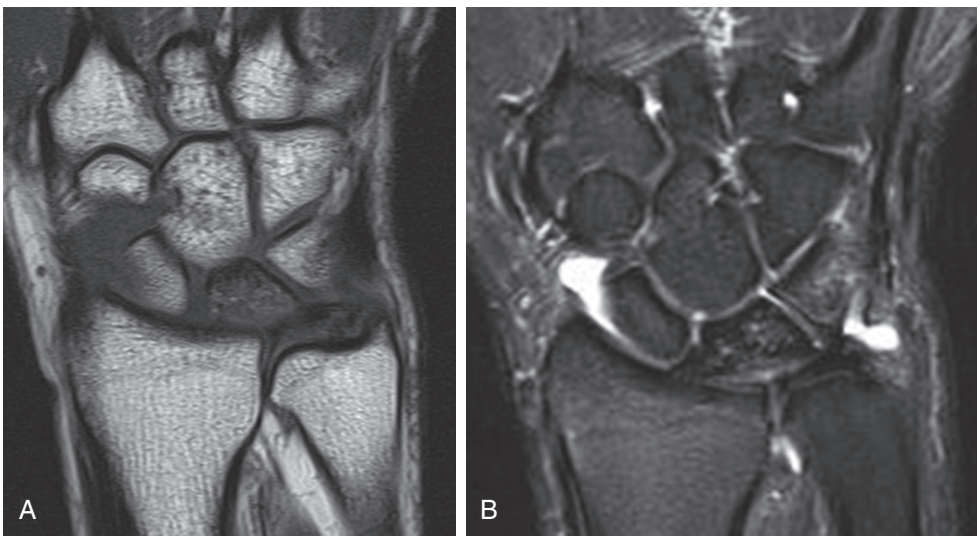



**Figure 103-12** Coronal T1-weighted **(A)** and coronal T2-weighted **(B)** MRI scans of the wrist of a patient with Kienböck's disease. Note the low signal intensity of the lunate in both pulse sequences without bone collapse, indicating the intermediate stage of osteonecrosis of the lunate.

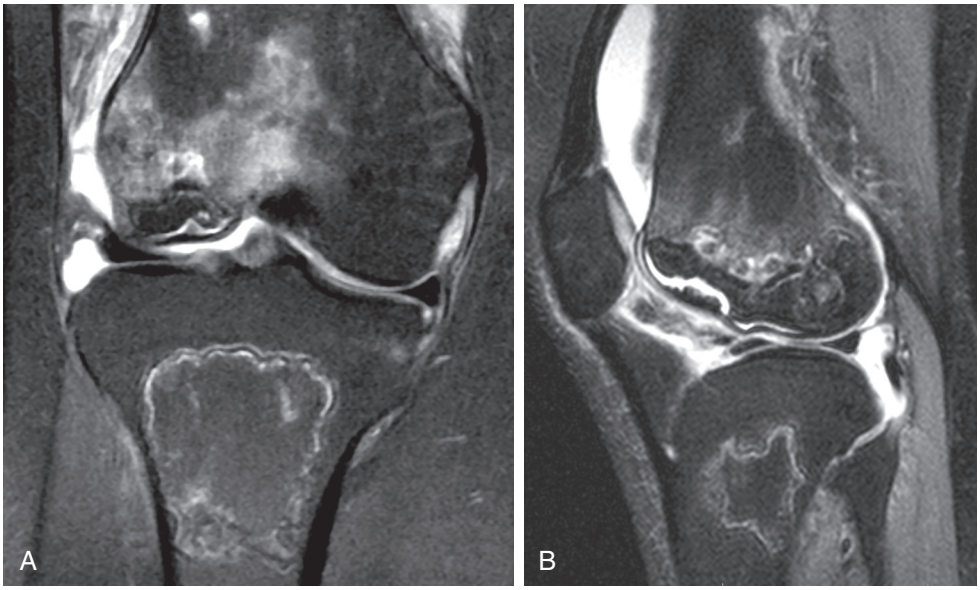



**Figure 103-13** Coronal **(A)** and sagittal **(B)** T2-weighted MRI scans of the knee in a patient with a history of prolonged corticosteroid use demonstrate multiple areas of osteonecrosis of the distal femur and proximal tibia. Note the characteristic double-line sign in the proximal tibial lesion. Note also the partial collapse of the medial femoral condyle as a result of the presence of a subchondral fracture (high signal intensity line in the subchondral bone in **A** and **B**).

Although spontaneous resolution of femoral head osteonecrosis can occur, it is rare and occurs only when the lesion size is small. A study of the prognosis of osteonecrosis of the femoral head as a function of symptoms (pain) and radiographic findings showed that in patients who were asymptomatic and had normal radiographs, progression of the disease was slow, with only 1 of 23 hips progressing to pain and radiographic changes after 5 years. When radiographic changes are already present, disease progresses to pain in 14 of 19 patients after 5 years. In a study of stage 1 osteonecrotic lesions of the hip diagnosed with MRI, 40 patients were followed up for an average of 11 years. All patients had stage 1 lesions on the contralateral hip. Overall, 35 of the 40 stage 1 hips became symptomatic, and 29 hips showed collapse. The mean interval between diagnosis and collapse was 92 months, whereas the mean interval between symptoms and diagnosis was 80 months. Most stage 1 hips eventually progress to a more advanced stage, requiring surgery, and thus these hips should be monitored closely.

## TREATMENT

### Surgery

Most cases of osteonecrosis ultimately require surgical intervention. Sometimes surgical procedures can be used in conjunction with nonsurgical approaches, as will be discussed. The more advanced the disease, the more extensive the surgery that is required.

The various surgical procedures used in the treatment of osteonecrosis include core decompression, structural bone grafting, vascularized fibula grafting, osteotomy, resurfacing arthroplasty, hemiarthroplasty, and total hip replacement. Table 103-8 shows the typical success rates for each of these procedures.

Arthroscopy is a valuable tool used in the treatment of osteonecrosis. It has been used to determine the position of the core decompression tract to the necrotic part of the femoral or humeral head,[165] and arthroscopic débridement has been used in the treatment of osteonecrosis of the capitellum of the humerus in adolescents, in individuals with Kienböck's disease, and in individuals with osteonecrosis of the scaphoid.

Core decompression, which involves the removal of a core of bone from the femoral neck and head, is indicated in less advanced stages of osteonecrosis (Figure 103-14). The core acts as a vent to reduce intraosseous pressure and intramedullary pressure, reversing ischemia and improving symptoms. Other benefits of core decompression include stimulation of angiogenesis, which leads to improved vascularization during the repair process. The effectiveness of core decompression in the treatment of nontraumatic osteonecrosis was illustrated in 34 patients with 54 affected hips. The mean age at presentation was 38 years. The patients were monitored for a mean duration of 120 months after surgery. Success was defined as absence of symptoms, no further progression of disease, and no further surgery. Clinical improvement was established in 26 hips (48%), and radiographic success was established in 20 hips (37%).

Computer-assisted core decompression has been used to provide greater precision in directing the core into the ischemic area and to minimize the duration of radiation exposure to patients.[166] Because early diagnosis improves outcome and the incidence of developing osteonecrosis in a contralateral hip is high, core decompression is frequently performed on both hips simultaneously. This approach adds little risk compared with unilateral core decompression, with the benefit of better outcomes as a result of early surgical treatment of the contralateral hip.[167]

In structural bone grafting, or bone impaction grafting, the bone graft is inserted into the necrotic segment through the core tract. The bone graft acts in similar fashion to a stent, providing support to overlying subchondral bone. The goal is to prevent collapse. This combination of

**TABLE 103-8** Surgical Treatment of Osteonecrosis

| Surgical Procedure | Rationale | Stages of Osteonecrosis | Outcome | Comments |
|---|---|---|---|---|
| Core decompression | Reduction of intraosseous and intramedullary pressure | Early stages | 37% radiographic success, 48% clinical success | Success rate depends on disease stage |
| Structural bone grafting | Provide support to overlying subchondral bone | 1 or 2 | Poor in advanced disease | 100% failure rate in stages 3 and 4 |
| Vascularized fibula grafting | Increase blood flow to graft | 2 to 4 | 96% success in stage 2, 90% in stage 3, and 57% in stage 4 | |
| Osteotomy | Shifting position of osteonecrotic segment out of weight-bearing region | 2 and 3 | Not available | |
| Resurfacing arthroplasty | Preservation of bone and joint mechanics with a metallic or ceramic shell over the femoral head | Later stages | Mean 7-year success rate is 90% | An alternative to total hip arthroscopy in later stages of disease |
| Hemiarthroplasty | Replacement of the femoral head, preservation of the anatomic acetabulum | Later stages | The failure rate for unilateral hemiarthroplasties is 50%-60% at 3 years; for bilateral hemiarthroplasties, the failure rate is 44% | Various techniques are available, some with a better outcome |
| Total hip replacement | Complete replacement of the hip joint | Late stages | 17.4% required revision after 10 years | Eventually most patients will require several hip replacements |

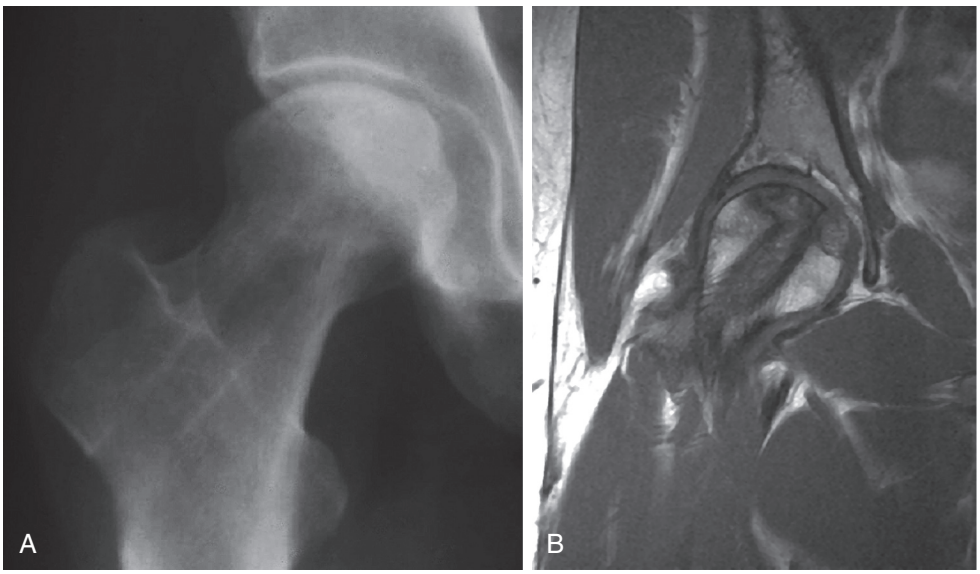



**Figure 103-14** An anteroposterior radiograph **(A)** and a T1-weighted MRI scan **(B)** in a patient who underwent core decompression demonstrate the decompression tunnel extending from the subtrochanteric area into the area of the osteonecrosis in the femoral head.

procedures is frequently used in treating stage 1 or 2 osteonecrotic femoral heads. Allogeneic and autologous bone grafts, mostly harvested from the tibia or fibula, are used. When this technique was attempted in patients with stages 3 and 4 lesions, the outcome was generally poor (100% failure after 2 to 4 years), with progression to collapse and further surgical procedures.[168] Another study investigated outcomes after structural augmentation with autogenous bone grafts in stage 1 and 2 disease in 31 patients (33 hips); all patients had significantly reduced pain, along with an improvement in Harris hip scores from 76 before surgery to 91 after surgery.[169]

Vascularized structural bone grafting also uses the core tract to insert a corticocancellous bone graft into the femoral neck and head along with its vascular pedicle. The vascular pedicle is anastomosed to a nearby vessel, adding a source of blood to the graft. The results of vascularized fibular grafting in the treatment of hips with osteonecrosis showed a survival of 61% of hips at 5-year follow-up and 42% at a median of 8 years.[170] In another study, 197 patients with 226 osteonecrotic hips were treated with a combination of autologous cancellous bone impaction and pedicled iliac bone block transfer. The anastomosis was to the ascending branch of the lateral femoral circumflex artery. Fourteen

hips required conversion to total hip arthroplasty because of collapse, severe pain, or both. Of the remaining 212 hips, 92% were considered a clinical success and 76% were considered radiographically successful. The success rate declined from stage 2 to stage 4 hips (96% for stage 2 hips, 90% for stage 3 hips, and 57% for stage 4 hips).[171] Free vascularized fibula grafting has been compared favorably with other modes of surgical treatment.[172]

Osteotomy of the femur involves shifting the position of the osteonecrotic segment by making a cut in the proximal femur so that the osteonecrotic segment is rotated or flexed out of the weight-bearing region of the acetabulum and then replacing the weight-bearing region with viable bone. Healing of the necrotic region can proceed without the stress of weight bearing. Several different osteotomy techniques have been attempted to salvage hips with stage 2 or 3 osteonecrosis.

Resurfacing arthroplasty uses a metallic or ceramic shell placed over a femoral head that has been débrided of the necrotic area. The potential advantages of resurfacing arthroplasty include preservation of joint mechanics, bone conservation,[173] more physiologic loading of the bone, a lower incidence of perioperative complications, and easier conversion to total hip arthroplasty in case of failure.[174] Complications of this procedure include femoral neck fractures, a secondary osteonecrosis when the procedure is performed for other reasons,[175] and increased metal ion levels.[176] Resurfacing arthroplasty has been recommended for patients with later-stage osteonecrosis, including those with femoral head collapse.[177] A retrospective study compared the results of limited femoral head resurfacing and total hip arthroplasty in 30 consecutive patients with Steinberg stage 3 or 4 disease. The survival rate at a 7-year mean follow-up period for the resurfacing group was 90%, whereas the survival rate at a mean 8-year follow-up for the total hip arthroplasty group was 93%.[178] A recent level 3 therapeutic study showed that hip resurfacing success rates at a 5-year follow-up were comparable with those of total hip arthroplasty in patients with osteonecrosis who were younger than 25 years.[179]

In hemiarthroplasty, only part of the hip joint is replaced. The original acetabulum is preserved, but the femoral head is replaced with a prosthesis. Two types of prostheses are used—a unipolar prosthesis and a bipolar prosthesis. In a unipolar prosthesis, the articulation is between the artificial femoral head and the acetabulum. In the bipolar prosthesis (presently the most frequently used), the articulation is within the prosthesis itself. Failure rates for hemiarthroplasties in individuals with osteonecrosis are 50% to 60% at 3 years for unipolar prostheses and 44% for bipolar prostheses. Another study evaluated the success rate of Charnley/Bicentric hemiarthroplasty in the treatment of Ficat and Arlet stage 3 osteonecrosis of the femoral head. Failures included three hips that needed to be revised to cementless total hip replacement, two hips with radiographic changes of loosening and imminent failure, and one hip with progressive loss of joint space and secondary degenerative changes. The success rate was 84.2% after a mean of 56 months.

Total hip arthroplasty is complete replacement of the hip joint with a prosthesis, including the femoral head and the acetabulum. In a study of 55 consecutive hip arthroplasty procedures, cementless total hip arthroplasty was shown to provide favorable results in advanced-stage osteonecrosis of the femoral head. Although 10 of the 48 hips available for follow-up after a minimum of 5 years required revision, all of these patients had Ficat and Arlet stage 3 or 4 disease. A study of 53 hips in 41 patients treated with cemented total hip replacement showed that at a minimum of 10 years of follow-up, 17.4% required revision. Compared with cemented total hip replacements performed for other conditions, osteonecrosis had a greater risk for loosening of acetabular and femoral components. A survivorship analysis of cemented total hip replacements in renal transplant patients with osteonecrosis of the femoral head showed excellent survival after 10 years (98.8%). After 20 years, the survival rate decreased to 63.8%.

In individuals with ONJ, the most common surgical procedure is resection of the affected bone.[180] Conservative treatment has also been used but has a higher recurrence rate. A larger extent of surgical excision and a higher number of surgical débridements were associated with a lower recurrence rate. Other modes of surgical therapy for ONJ include bone-contouring procedures, fluorescence-guided bone-contouring procedures,[181] and segmental osteotomies, but these procedures are generally reserved for more severe cases. Nonsurgical treatments, including hyperbaric oxygen therapy[182] and low-intensity laser therapy, are controversial but have been used to treat ONJ.

## Nonsurgical Approaches

The key to the successful treatment of osteonecrosis is early detection. Because many patients with osteonecrosis are relatively young, and because treatment of late-stage osteonecrosis is associated with high failure rates, earlier detection may be associated with a greater chance of preservation of the hip. The choice of conservative nonsurgical versus more aggressive surgical options depends on the clinical and pathologic staging of the disease. Figure 103-15 is an algorithm for the treatment of osteonecrosis.

Nonsurgical treatment of osteonecrosis of the femoral head includes refraining from weight bearing on the affected joint, use of analgesic and anti-inflammatory medications, and physiotherapy. Conservative medical treatment is effective only in the early stages for symptomatic relief. Nonsurgical management does not seem to alter the natural course of the disease. Electrical stimulation has been used in the treatment of osteonecrosis, in conjunction with core decompression. Electrical stimulation enhances osteogenesis and neovascularization. It also alters the balance between osteoblast and osteoclast activity, resulting in increased bone deposition and decreased bone resorption. Delivery of electrical stimulation can be performed by direct current (DC), pulsed electromagnetic field, and capacitance coupling. The success of electrical stimulation in the treatment of osteonecrosis has been rather mediocre. A study was performed of 11 hips in 8 patients with Ficat stage 2 osteonecrosis who underwent core decompression and placement of an electric stimulating coil within the core in the anterosuperior segment of the femoral head.[183] Of these 11 hips, 5 required a repeat operation, and 6 had progressive deterioration 13 months after initial placement of the coil. In addition, there was little histologic evidence that the coil did indeed

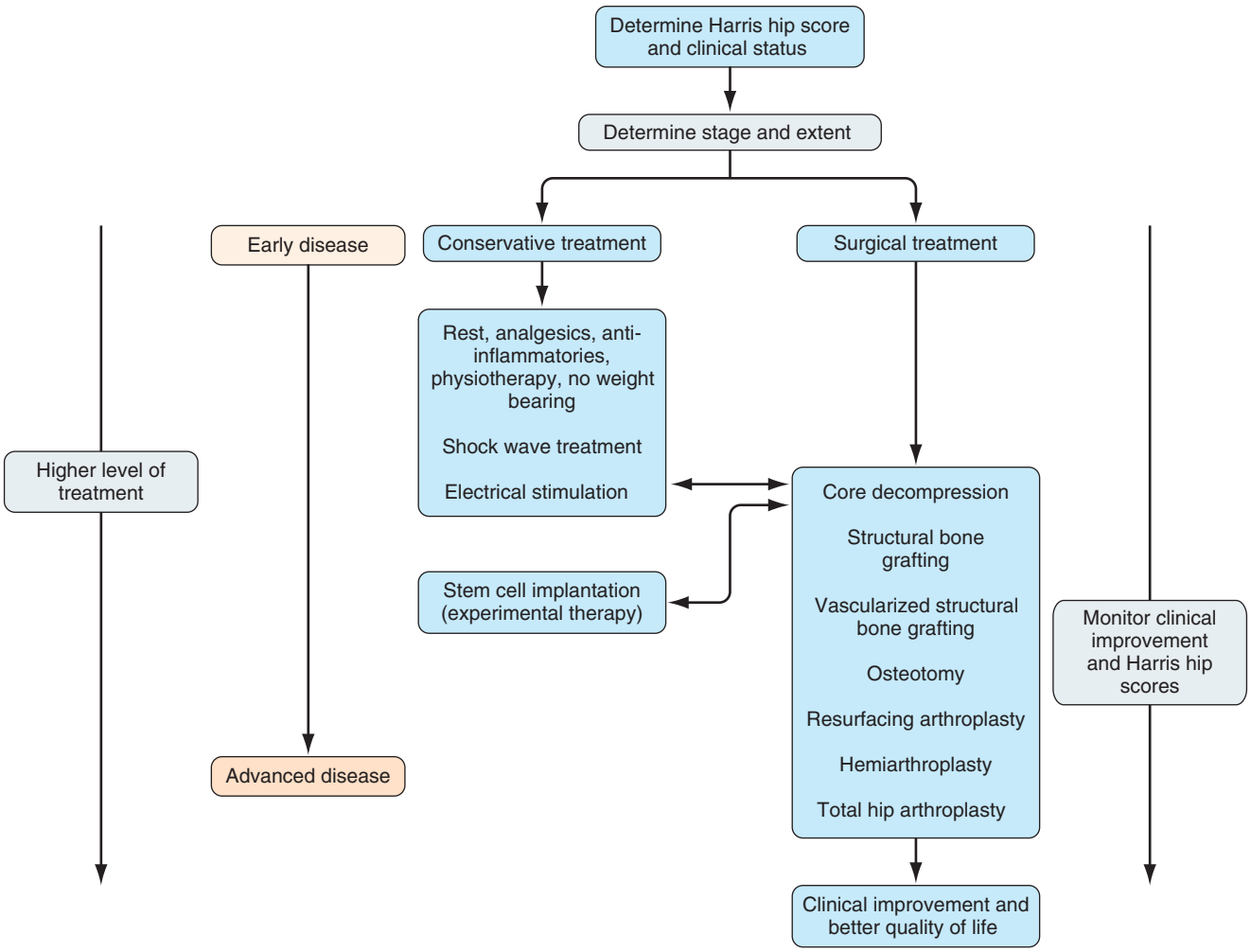



**Figure 103-15** Treatment algorithm for osteonecrosis.

generate new bone deposition around itself. The efficacy of this method of treatment is unproven.

On the other hand, another study compared the effectiveness of conservative nonsurgical treatment with core decompression with or without DC electrical stimulation.[184] The clinical symptom scores and the rate of progression to arthroplasty were best in the group with core decompression and DC electrical stimulation and worst in the nonoperative group. Capacitive coupling can be performed with or without core decompression and grafting. Forty patients with stage 1 to 3 osteonecrosis underwent core decompression and grafting; half of the patients wore active capacitive coupling units with electrodes over the femoral head for 6 months. The control group was 55 patients with osteonecrosis who were treated conservatively. Follow-ups at 2 and 4 years showed that core decompression with or without capacitive coupling provided a better clinical and radiologic outcome than did conservative treatment. Capacitive coupling did not improve the results further when used with core decompression and grafting.

Extracorporeal shock wave therapy has been used in the treatment of osteonecrosis of the femoral head. A study of 48 patients and 57 hips compared extracorporeal shock wave therapy with core decompression and bone grafting. Twenty-three patients with 29 affected hips were assigned to the shock wave group, and the remaining patients and hips were treated surgically. The patients in the shock wave group were given treatment of 6000 pulses of shock waves at 28 kV to the affected hip. The patients were evaluated radiographically and by their reports of symptoms (pain), Harris hip scores, and quality of life (daily work activity assessment). Shock wave therapy produced better results than did the nonvascularized bone grafting procedure, with comparatively less progression of disease.[185] In 35 patients with 47 osteonecrotic hips, the use of shock wave therapy led to improvements in serum nitric oxide levels, angiogenic factors such as VEGF, and osteogenic factors such as bone morphogenetic protein (BMP)-2 and osteocalcin. Levels of inflammatory markers were reduced. It is interesting to note that although these changes did not persist beyond several months, the clinical and radiographic improvement, present in 83% of hips, was present after 12 months.[186]

Conservative treatment of osteonecrosis of the talus is not promising, and the affected ankles generally continue to progress, requiring either core decompression or arthrodesis. Conservative treatment of bisphosphonate-induced ONJ includes cessation of bisphosphonate usage or surgical

débridement. Good oral hygiene, regular dental assessment, and avoidance of dental procedures during bisphosphonate usage can prevent the onset of osteonecrosis.

## RECENT DEVELOPMENTS

### Prevention Versus Treatment

A recent study evaluated the role of antioxidants in the treatment of osteonecrosis. Japanese white rabbits were divided into two groups and fed either a normal diet or a normal diet supplemented with α-tocopherol. Osteonecrosis developed in 14 of 20 rabbits in the control group but in only 5 of 21 rabbits in the experimental group. This finding suggests that oxidative stress may play a role in the pathogenesis of osteonecrosis and that a role may exist for antioxidants such as vitamin E.[187]

A group of researchers studied the use of adrenocorticotropic hormone (ACTH) in rabbits to prevent corticosteroid-induced osteonecrosis and found that if ACTH is administered along with depot methylprednisolone acetate (Depo-Medrol), osteonecrosis is reduced. The authors of this study believe that ACTH enhances osteoblast support and stimulates the production of VEGF, which stimulates the generation of new blood vessels. The result is an increase in blood flow to the vulnerable areas of bone, preventing cell death and reducing the likelihood of osteonecrosis.[188]

### Mesenchymal Stem Cells

Corticosteroids interfere with the balance of adipogenesis and osteogenesis in the differentiation of mesenchymal stem cells. Corticosteroids shunt uncommitted osteoprogenitor cells in the bone marrow into the adipocytic pathway, leading to reduced osteoblast formation. Corticosteroids have also been shown to reduce VEGF, which leads to a reduction in new blood vessel formation and potentially can lead to bone death. Alcohol has a similar effect on the differentiation of progenitor cells.

The balance between adipogenesis and osteogenesis has been targeted as a potential site for the treatment of osteonecrosis. Multipotential mesenchymal stem cells from femoral bone marrow near osteonecrosis sites are able to express messenger RNA aggrecan and type II collagen. Both are deposited into the bone matrix. These features are characteristic of chondrogenic differentiation. The mesenchymal stem cells can be differentiated into osteocytic lineage in vitro.

A pilot study evaluating the effectiveness of implantation of autologous bone marrow cells in the treatment of osteonecrosis used core decompression to implant stem cells into the necrotic lesions of the femoral head. The patients were divided into two groups—one that received core decompression alone as treatment for osteonecrosis (the control group) and one that received autologous bone marrow cell implantation along with core decompression (the treatment group). The patients were followed up for 24 months, and at that time, 5 of 8 hips in the control group, but only 1 of 10 in the treatment group, advanced to stage 3 osteonecrosis. In addition, greater improvement in pain and joint symptoms occurred in the treatment group, and the treatment seemed to be safe. Because of the small number of patients involved, further studies are necessary to confirm these results.

Twenty-eight patients with 44 necrotic hips were treated with percutaneous decompression and autologous bone marrow mononuclear cell infusion. Patients were followed up for a minimum of 2 years and evaluated for clinical and radiographic progression of the disease. Overall slowing in the progression of the disease stage appeared to occur. The mean Harris hip score improved from 58 to 86.

In 2014, Houdek and colleagues[189] reviewed the literature and published a report in which they concluded that using core decompression in conjunction with mesenchymal stem cell infusion in the form of bone marrow leads to improved pain and functionality and halts progression of osteonecrosis of the femoral head, potentially obviating the need for future invasive procedures such as total hip replacement.[189]

## CONCLUSION

Osteonecrosis is a potentially debilitating condition with significant morbidity despite medical interventions or surgery. Corticosteroid use is the most common cause of osteonecrosis, and corticosteroid-induced osteonecrosis can be reproduced in animal models. The pathogenesis of osteonecrosis is multifaceted and is still not completely understood. Why is it that corticosteroid-induced osteonecrosis is more common in patients with certain underlying diseases and not in others? Does osteonecrosis have a genetic or epigenetic basis, and is there a familial predisposition? Common pathogenic mechanisms known to be involved in osteonecrosis include osteoblast/osteoclast survival and apoptosis, lipid metabolism, and coagulation abnormalities. However, it is still unclear how these mechanisms interrelate. To better appreciate the risk factors involved in osteonecrosis, a more complete understanding of the pathogenesis is necessary. Until then, the physician should always maintain a high index of suspicion for osteonecrosis whenever known risk factors are present, especially use of corticosteroids and alcohol.


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CHAPTER 104



# Relapsing Polychondritis

Gaye Cunnane

**KEY POINTS**

- Relapsing polychondritis (RPC) is a rare, systemic autoimmune disorder characterized by episodic inflammation of cartilaginous structures.
- Classic clinical findings include "cauliflower ear" and "saddle nose" deformities.
- RPC may cause significant pathology of the upper airways, eyes, inner ears, kidneys, and blood vessels, with potentially life-threatening consequences.
- The disease may be primary or it may develop in association with other diseases, such as myelodysplasia, vasculitis, or other autoimmune/connective tissue disorders.
- Although there are no specific laboratory markers for RPC, the diagnosis is made by observing the classic clinical features and may be confirmed by the characteristic histologic findings of affected tissues.
- Because of the multisystem nature of the disease, a broad approach to diagnosis and management is essential.
- Treatment is frequently empiric because of the wide variety of disease manifestations and lack of controlled trials. However, immunosuppression is the mainstay of therapy.

Relapsing polychondritis (RPC) is a rare autoimmune disease of unknown etiology, characterized by episodic inflammation of cartilaginous structures throughout the body (e.g., the ears, nose, upper respiratory tract, chest wall, and joints). Noncartilaginous tissues may become involved, particularly those with an abundance of proteoglycans, such as the eye, inner ear, heart, blood vessels, and kidney.[1] Associated diseases such as vasculitis or myelodysplastic syndromes are present in as many as one-third of cases. The severity of the inflammatory process frequently requires the use of immunosuppressive agents. As its name suggests, RPC has an episodic pattern with periods of intense inflammation. Prognosis is variable, with a 5-year survival rate of 45% to 95%, depending on organ involvement and disease or treatment complications.[2]

This disease was first described in 1923 by Rudolf Jaksch von Wartenhorst, an internal medicine physician, when he published the case of a young male with characteristic clinical features and named the disease *polychondropathia*.[1,3] In 1960 Pearson et al. provided a more detailed description, and renamed the disease *relapsing polychondritis*.[4]

## EPIDEMIOLOGY

The worldwide incidence of RPC is unknown, but figures from Rochester, Minnesota, suggest an annual incidence of 3.5 per million in that community.[4] RPC occurs in all racial groups and with similar frequency in men and women. Peak age of onset is 40 to 50 years, but it has been described in children[5] and in people older than 80 years.[6] No familial pattern of inheritance has been shown.[7]

## PATHOLOGY

### Normal Cartilage

There are three types of cartilage tissue (hyaline, elastic, and fibrocartilage), which differ in the composition of the extra-cellular matrix. The cellular component of cartilage consists of chondrocytes, whereas the extra-cellular matrix is composed of interlinking fibrils of type II collagen, other collagens, hydrophilic proteoglycan aggregates, and a variety of matrix proteins.[8] One such protein, matrilin-1, is exclusive to the respiratory tract, ears, and xiphisternum in adults and is only found in articular cartilage before skeletal maturity.[9] Cartilage is an avascular structure that derives essential nutrients from adjacent tissue. The composition of cartilage confers resilience to external compressive forces. In healthy adults, turnover of cartilage components is slow, with incomplete repair processes.[10] Degradation of collagen fibrils occurs with age, resulting from imbalances between naturally occurring proteolytic enzymes and their inhibitors.[11]

### Relapsing Polychondritis Cartilage

There are limited data on the microscopic changes in tissue affected by RPC, partly because of the low disease incidence and also because the diagnosis is usually made on clinical grounds. In the initial stages, inflammatory cells infiltrate the perichondrium, with evidence of lymphocytes ($CD4^+$ T cells), macrophages, neutrophils, eosinophils, and plasma cells on hematoxylin-eosin staining, whereas the structure of the cartilage appears unaffected.[12,13] Immunofluorescence may reveal deposits of immunoglobulin and cartilage components at the cartilage-perichondrium junction.[14] Later, the inflammatory cells invade the cartilage, releasing proteolytic enzymes such as matrix metalloproteinases and cathepsins.[13,15] While the cartilage is destroyed, it loses its



Supplemental images available on the accompanying ExpertConsult website.

basophilic staining, while elastic and collagen fibers become disrupted.[13]

Necrosis and a reduction in cartilage components may be observed. The cartilage is subsequently replaced by fibrotic tissue, with areas of calcification. Granulation tissue and gelatinous cysts may be observed within involved areas.[13]

## PATHOGENESIS

Although the pathogenesis of RPC is unknown, evidence supports the concept of an autoimmune process. The presence of inflammatory cells and immune complexes in involved cartilage, the detection of autoantibodies to cartilage components, the association with human leukocyte antigen (HLA)-DR, the frequent concurrence with other autoimmune diseases, and the response to immunosuppressive treatment all support this hypothesis. Researchers have observed autoantibodies to types II, IX, and XI collagen in patients with this disease,[9,16-18] and autoantibodies to matrilin-1 and cartilage oligomeric protein (COMP) have been detected in patients with respiratory tract involvement.[19-22] Type II collagen neoepitope can be found in the urine of patients with active disease.[23] More recently, the serum level of soluble triggering receptor expressed on myeloid cells-1 has been cited as a potential biomarker of disease activity in RPC.[24] Pro-inflammatory chemokines involved in the recruitment of monocytes and modulation of macrophage function, such as monocyte chemoattractant protein-1, macrophage inflammatory protein 1β, and interleukin (IL)-8, are significantly elevated in active RPC compared with controls.[25] Tumor necrosis factor expression is also higher in RPC.[25] Furthermore, there is a class II HLA association with RPC. A twofold increase in HLA-DR4 has been found in RPC, although a specific subtype has not been identified.[26] There is a negative association between the extent of organ involvement in RPC and HLA-DR6.[27]

Several animal models have helped to elucidate some of the pathogenetic mechanisms underlying RPC. Chondritis can be induced in certain rat or mice species following injection of type II collagen.[28,29] In addition, mice that express HLA-DQ6ab8ab transgenes develop a spontaneous form of polychondritis with auricular, nasal, and joint involvement.[30] Experiments that used the matrilin-1–induced rodent model of RPC have demonstrated the importance of T cells, B cells, and complement components in pathogenesis of this disease.[31] Deletion of IL-10 in this model resulted in increased disease severity, suggesting a role for this endogenous cytokine in suppressing episodic inflammation.[32] The processes involved in the initiation of disease, escalation of the inflammatory response, and subsequent cartilage destruction are poorly understood. In a healthy individual, cartilage is an immunologically privileged site.[9] However, in RPC, cartilage components are exposed and vulnerable to immunologic attack, resulting in a perpetuating process of systemic inflammation and local tissue damage. In genetically susceptible individuals, cartilage microtrauma or molecular mimicry between an infectious/inciting agent and cartilage structures might instigate a cycle of inflammation similar to other autoimmune disorders.[13,33]

## CLINICAL FEATURES

RPC can be diagnosed when the characteristic clinical signs are present. The diagnostic criteria for RPC are outlined in Table 104-1. Because of the relapsing nature and rarity of the disease, the diagnosis may not be established for some time. In one study, a mean delay of 2.9 years was noted between symptom onset and confirmation of diagnosis.[34] Table 104-2 documents the frequency of clinical presentations in patients with RPC.

### Otorhinologic Disease

The most characteristic manifestation of RPC is auricular chondritis, in which the patient develops acute pain, redness, and swelling of the cartilaginous upper two-thirds of the outer ear, sparing the lobe[35] (see supplemental Figure 104-1 on ExpertConsult.com). It may be unilateral or bilateral and resolves spontaneously during a period of several

**TABLE 104-1** Diagnostic Criteria

| |
|---|
| **Major Criteria**<br>Proven inflammatory episodes of ear cartilage |
| Proven inflammatory episodes of nose cartilage |
| Proven inflammatory episodes of laryngotracheal cartilage |
| **Minor Criteria**<br>Eye inflammation |
| Hearing loss |
| Vestibular dysfunction |
| Seronegative inflammatory arthritis<br>• Diagnosis is made by two major criteria or one major plus two minor criteria.<br>• Histologic examination of affected cartilage is not required. |

Modified from Michet CJ Jr, McKenna CH, Luthra HS, et al: Relapsing polychondritis: survival and predictive role of early disease manifestations. *Ann Intern Med* 104:74–78, 1986.

**TABLE 104-2** Clinical Features of Relapsing Polychondritis

| Clinical Feature | FREQUENCY | |
|---|---|---|
| | At Presentation | In Total |
| Auricular chondritis | 39 | 85 |
| Nasal chondritis | 24 | 54 |
| Arthritis | 36 | 52 |
| Eye disease | 19 | 51 |
| Laryngotracheal bronchial disease | 26 | 48 |
| Hearing loss | 9 | 30 |
| Rash | 7 | 28 |
| Systemic vasculitis | 3 | 10 |
| Valvular dysfunction | 0 | 6 |
| Costochondritis | 2 | 2 |

Modified from Michet CJ, McKenna CH, Luthra HS, et al: Relapsing polychondritis: survival and predictive role of early disease manifestations. *Ann Intern Med* 104:74–78, 1986; and Gergely P, Poór G: Relapsing polychondritis. *Best Pract Res Clin Rheumatol* 18:723–738, 2004.

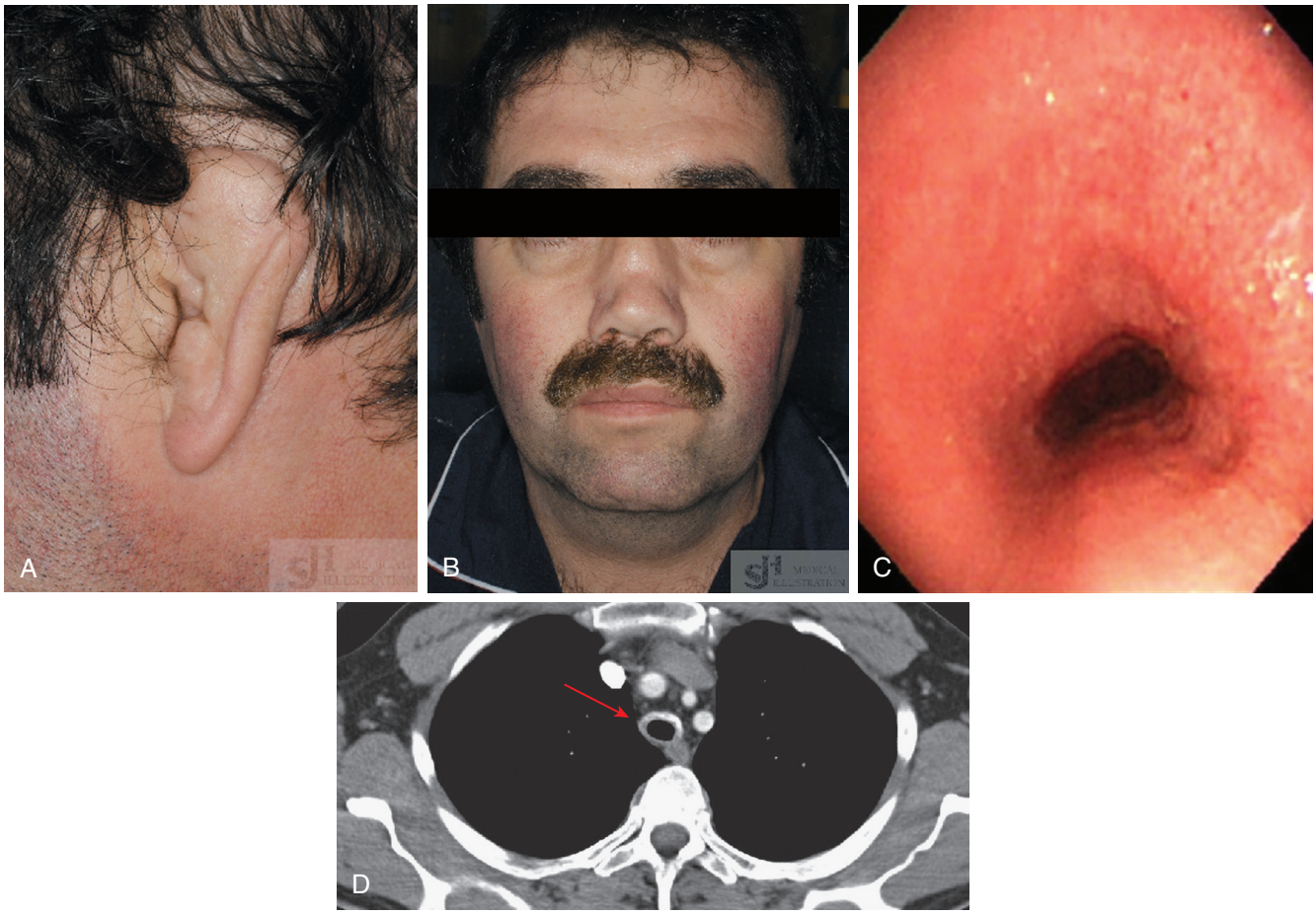



**Figure 104-1** **A,** Chronic changes of relapsing polychondritis (RPC) in the cartilaginous upper two-thirds of the ear, evident after repeated episodes of inflammation in this area. **B,** Saddle nose deformity, developed after episodic nasal chondritis. **C,** Bronchoscopy findings demonstrating mucosal hyperemia, edema with lack of normal detail of tracheal cartilaginous rings, and flattening of the trachea. **D,** Computed tomography thorax with *arrow* demonstrating tracheal flattening and calcification in a patient with RPC. *(**A** and **B,** Courtesy Mr. Anthony Edwards, Clinical Photographer, St. James's Hospital, Dublin, Ireland. **C** and **D,** Courtesy Dr. Finbarr O'Connell, Consultant Pulmonologist, St. James's Hospital, Dublin, Ireland.)*

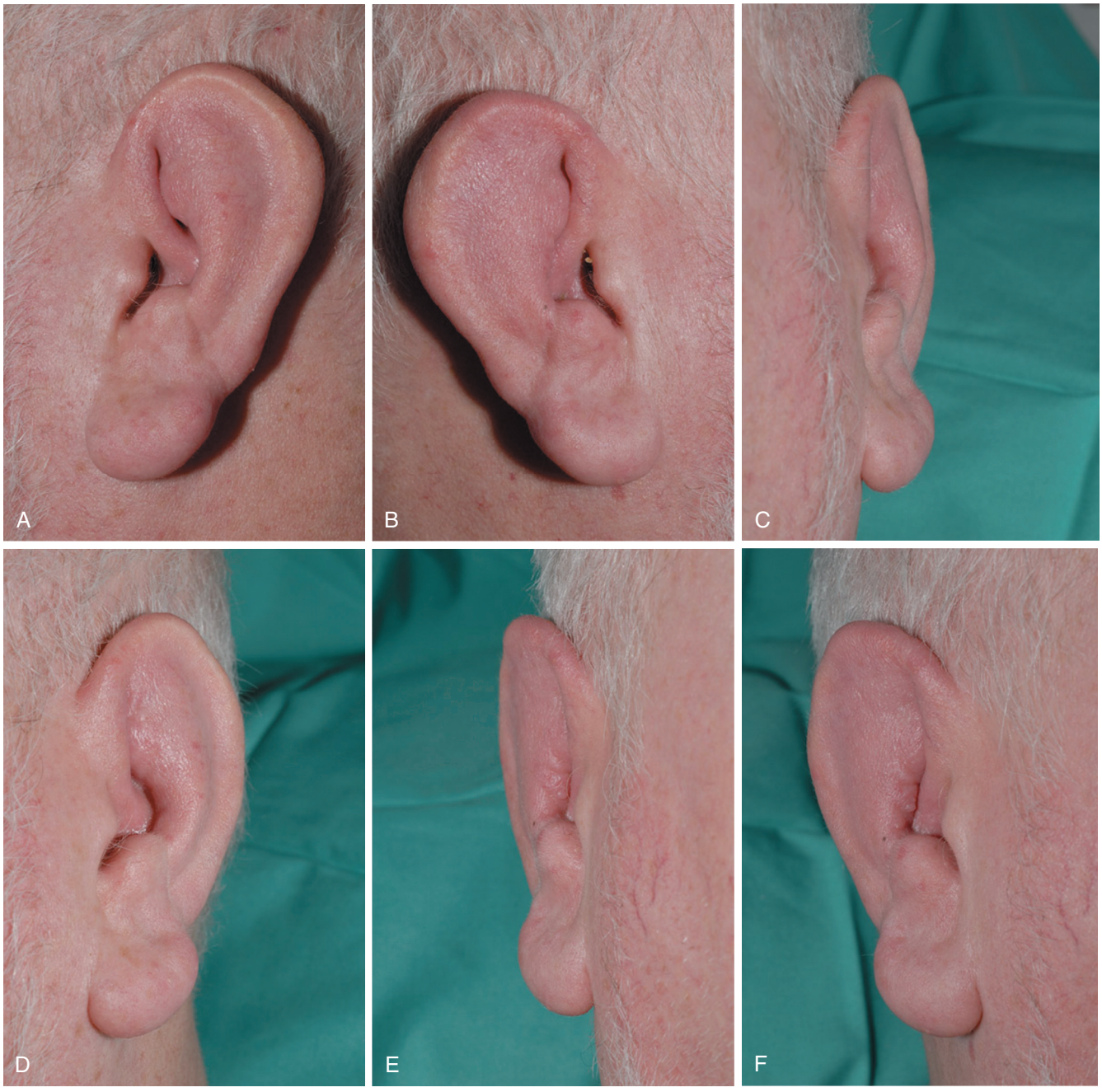
weeks. Repeated episodes result in visible damage to the ear, which takes on a deformed, flaccid appearance (Figures 104-1A and 104-2). Although classic for RPC, this is the presenting feature in only 40% of patients, although this feature eventually develops in as many as 85% of patients during the course of their disease.[6] Such swelling of the outer ear causes temporary conductive deafness. However, sensorineural deafness may result from an associated vasculitis of the internal auditory artery or its branches, leading to additional vertigo in some patients.[34,35] Nasal chondritis develops in approximately 10% of patients at presentation and as many as 70% during the course of their disease.[6,35] Repeated inflammatory episodes result in collapse of the nasal bridge and an alteration of the facial appearance—the so-called *saddle-nose* deformity (Figure 104-1B).

## Respiratory Disease

Involvement of the upper respiratory tract may be life threatening and should be investigated at an early stage in the disease course. Approximately 50% of patients with RPC develop respiratory problems, which are associated with a worse prognosis.[36] Hoarseness, dysphonia, a persistent dry cough, or anterior neck tenderness may indicate laryngeal or tracheal disease. Inflammation of the tracheobronchial tree may lead to tracheomalacia, dynamic obstruction, and acute respiratory failure, whereas repeated inflammatory episodes cause subglottic stenosis, chronic dyspnea, and increased susceptibility to infection.[37,22] Obstruction may be precipitated by attempts at intubation or bronchoscopy.[34] Computed tomography and bronchoscopy appearances of one patient with RPC are demonstrated in Figure 104-1C and D. The development of costochondritis may result in chest pain, leading to additional respiratory symptomatology.

## Cardiovascular Disease

Large and small vessel disease can occur during the course of RPC in as many as 10% of cases.[38] Such complications typically develop in patients with well-established disease, even in the setting of immunosuppression.[38-41] Inflammation of the aorta, most likely to occur at the level of the root or arch, leads to aneurysm formation and aortic incompetence, which may develop acutely with minimal prior symptoms.[34,40] Valvular problems are more common in




**Supplemental Figure 104-1** **A-F,** This series of photographs depicts the ear of a 56-year-old man with relapsing polychondritis. His disease was uncontrolled for 1 year, during which the damage shown developed in the cartilaginous structures of the ear.



**Figure 104-2** Acute on chronic changes of relapsing polychondritis in the cartilaginous upper two-thirds of the ear. *(Courtesy Professor Iain McInnes, Glasgow, United Kingdom.)*

males.[41] Aortic valve disease may be to the result of progressive dilatation of the aortic ring rather than active local inflammation.[34] Mitral regurgitation may result from valvitis or papillary muscle involvement.[34] Furthermore, myocarditis may lead to cardiac failure and conduction system abnormalities.[34,38] Small vessel vasculitis has also been reported and may cause end-organ damage to the skin, kidneys, testes, sclera, cochleovestibular system, or other areas.[34,38,42]Arterial and venous thromboses have been described in association with RPC.[34]

### Eye Disease

As many as 65% of patients with RPC will develop ocular disease, most commonly episcleritis and scleritis.[2] Recurrent inflammation can lead to thinning of the sclera, giving a bluish appearance to the eye.[1] Keratoconjunctivitis sicca may occur with associated Sjögren's syndrome. Uveitis, retinal vasculitis, and optic neuritis are rare complications. Extraocular muscle palsy, periorbital edema, and proptosis may also occur as part of the RPC symptom complex.[2]

### Renal Disease

Proteinuria and/or hematuria may be observed on urinalysis in approximately 10% of patients with RPC.[43] Renal pathology has been reported to show mesangial proliferation and segmental necrotizing crescentic glomerulonephritis.[43] Electron microscopy may demonstrate immunoglobulin and complement deposits. Renal pathology may also occur in the context of co-existing diseases such as systemic lupus erythematosus or other rheumatic diseases. Patients with RPC and renal involvement have a greater incidence of associated vasculitis and a resultant worse prognosis.[43]

### Neurologic Disease

Central and peripheral nervous system involvement is rare, affecting up to 10% of patients.[27] Cranial neuropathy is the most common manifestation, but a wide variety of problems including headache, ataxia, hemiplegia, transverse myelitis, mononeuritis multiplex, aseptic meningitis, and encephalopathy have been described. Such lesions may be vasculitic or autoimmune in nature.[35,42,44]

### Mucocutaneous

Skin and mucous membrane changes develop in approximately 50% of patients with RPC. Rashes are present in as many as 35% of patients with primary RPC, whereas 90% of patients with associated myelodysplasia have mucocutaneous involvement.[45] Oral aphthous ulcers are the most common symptom. Peripheral nodules, similar to erythema nodosum, can be noted in approximately 15% of patients.[45] Rare skin lesions include peripheral limb ulcers, livedo reticularis, panniculitis, superficial thrombophlebitis, urticaria, and angioedema. Leukocytoclastic vasculitis, dermal vessel thrombosis, or septal panniculitis may be evident on biopsy.[45]

### Joint Disease

Musculoskeletal manifestations are common and develop in 75% of patients during the course of the disease.[35] Involvement of the joints may be oligoarticular or polyarticular, most commonly affecting the ankles, wrists, hands, and feet. Recurring pain in the sternoclavicular, costochondral and manubriosternal joints has been described.[1] However, other joints, including the sacroiliac joints, may also show evidence of inflammation, although it is possible that the presence of sacroiliitis relates to the co-existence of a spondyloarthropathy.[46] The arthritis associated with RPC is typically asymmetric, episodic, and nonerosive, and it does not correlate with activity of RPC.[35]

## ASSOCIATED DISORDERS

RPC has been described in association with a variety of other disorders, including rheumatic, vasculitic, and hematologic syndromes. Its co-existence with Behçet's disease has led to the acronym *MAGIC* syndrome: mouth and genital ulcers with inflamed cartilage.[47] In older patients, RPC may be associated with myelodysplasia and a worse prognosis[48,49] (Table 104-3).

## DIFFERENTIAL DIAGNOSIS

Auricular chondritis with sparing of the earlobe is characteristic of RPC. Redness and swelling of the whole ear, including the earlobe, may be the result of trauma or exposure to temperature extremes and should be distinguished from RPC. *Pseudomonas aeruginosa* or *Staphylococcus aureus* are potential causes of unilateral otitis externa, particularly in immunocompromised patients.[50] Other

**TABLE 104-3** Diseases Associated with Relapsing Polychondritis

| |
|---|
| **Rheumatic Disorders** |
| Rheumatoid arthritis |
| Seronegative spondyloarthropathies |
| Connective tissue disorders |
| Vasculitides |
| Behçet's disease |
| **Hematologic Disorders** |
| Myelodysplasia |
| Lymphomas |
| Pernicious anemia |
| Acute lymphoblastic leukemia |
| **Endocrine Disorders** |
| Type I diabetes mellitus |
| Thyroid disorders: Hashimoto's/Graves' disease/hypothyroidism |
| **Cutaneous Disorders** |
| Leucocytoclastic vasculitis |
| Panniculitis |
| Vitiligo |
| Psoriasis |
| Lichen planus |
| **Other Disorders** |
| Inflammatory bowel disease |
| Primary biliary cirrhosis |
| Retroperitoneal fibrosis |
| Myasthenia gravis |
| Familial Mediterranean fever |

Modified from Trentham DE, Le CH: Relapsing polychondritis. *Ann Intern Med* 129:114–122, 1998; and Francès C, el Rassi R, Laporte JL, et al: Dermatologic manifestations of relapsing polychondritis. A study of 200 cases at a single center. *Medicine (Baltimore)* 80:173–179, 2001.

infections associated with chondritis include leprosy and syphilis. Damage to the nose cartilage may be to the result of trauma, local infections, or granulomatous lesions such as anti-neutrophil cytoplasmic antibody (ANCA)-associated granulomatosis vasculitis or lethal midline granuloma. Intranasal cocaine use should also be considered. A wide variety of inflammatory, rheumatic, and infectious conditions may mimic some features of RPC, particularly when multiple organs are involved. Cogan syndrome is an inflammatory autoimmune condition that results in keratitis and vestibuloauditory symptoms and may be associated with vascular inflammation, although it is not linked with chondritis.[51] Causes of systemic vasculitis (e.g., ANCA-associated vasculitis, polyarteritis nodosa, Behçet's disease, rheumatoid arthritis, or those associated with connective tissue disorders) should be taken into consideration, especially when the presentation is atypical. Valvular heart disease, particularly aortic incompetence and aortic root dilatation, may occur in conditions such as Marfan syndrome, syphilis, and idiopathic medial cystic necrosis and can also develop in relation to diseases that may coincide with RPC (e.g., ankylosing spondylitis).

## INVESTIGATIONS

### Routine Laboratory Tests

Routine laboratory tests should be performed and may demonstrate nonspecific findings such as anemia or mild thrombocytosis. The acute phase response is typically elevated during periods of inflammation. Urinalysis should always be performed to determine the presence of subclinical renal disease or infection. Autoimmune tests are nonspecific, except when associated diseases are present concurrently.

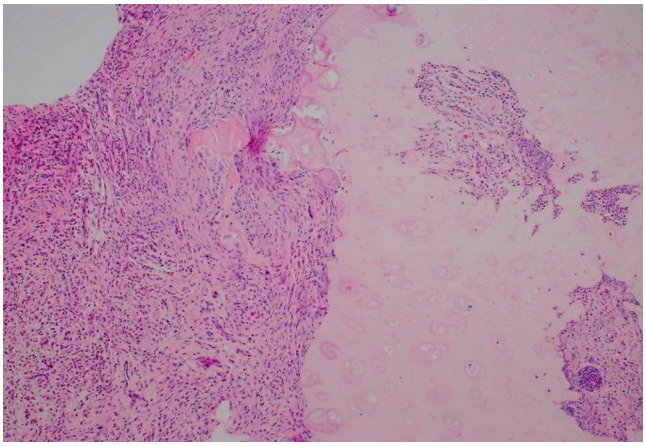


**Figure 104-3** Histopathology of tracheal biopsy of a 75-year-old man who presented with vocal cord palsy. Hematoxylin and eosin staining demonstrates a marked inflammatory response invading into cartilage. *(Courtesy Drs. Mary Toner and Mairin McMenamin, Department of Pathology, and Mr. John Kinsella, Department of Ear, Nose, and Throat, St. James's Hospital, Dublin, Ireland.)*

### Tissue Sampling/Histopathology

In typical presentations of RPC, a biopsy is not necessary for diagnosis. However, when the diagnosis is unclear, a biopsy of inflamed cartilage may show evidence of perichondritis, as described earlier (Figure 104-3).[9,34] Skin rashes may demonstrate leukocytoclastic vasculitis; other frequent findings include panniculitis, neutrophilic dermatoses, or dermal vessel occlusion.[45]

### Pulmonary Studies

Clinicians should conduct pulmonary function tests in all suspected cases of RPC, even in patients who are asymptomatic at presentation. These tests (including spirometry, lung volumes, and inspiratory/expiratory flow-volume loops) should be repeated in the event of new respiratory symptoms. If the test results are abnormal, computed tomography (CT) of the chest is recommended to detect the presence of tracheal or bronchial stenoses or dynamic airway collapse, which may be evident only during the expiratory phase of the respiratory cycle.[52-54] Extrapulmonary disease from costochondritis may cause a restrictive pattern on pulmonary function testing. Positron emission tomography/CT (PET/CT) scanning may have additional value because it can identify the presence of inflammation in areas where it is otherwise undetectable. PET/CT can also demonstrate treatment response with a reduction in inflammation after therapeutic intervention[55-57] (Figure 104-4).

Bronchoscopy is warranted only if specifically indicated because the procedure may cause inadvertent damage to the upper airways, with the risk of precipitating respiratory failure. However, indirect laryngoscopy is helpful to monitor airway involvement. Bronchoscopic ultrasound can also be used to monitor disease.[58]

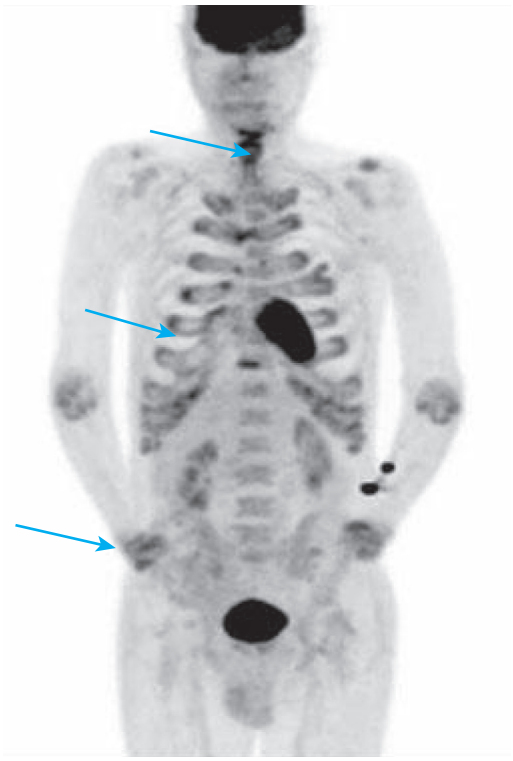


**Figure 104-4** Positron emission tomography/computed tomography scan image of a patient with relapsing polychondritis. Uptake of fluorodeoxyglucose can be seen in the trachea, costal cartilages and upper limb joints *(arrows)*. *(From Czepczyński R, Guzikowska-Ruszkowska I, Wyszomirska A: Relapsing polychondritis detected by PET/CT.* Eur J Nucl Med Mol Imaging *39:1366–1367, 2012. Courtesy of Springer.)*

### Cardiac Studies

Echocardiography is necessary to evaluate the aortic root and heart valves. Furthermore, many systemic inflammatory diseases are associated with accelerated atherosclerosis, and this may also be true for patients with RPC, although because of small numbers of cases, this has not been widely described. Nevertheless, other cardiac disease risk factors should be ascertained.

### Ophthalmologic Studies

Routine retinoscopy should be performed at diagnosis. Referral to a specialist unit is recommended if abnormalities are detected and/or if symptoms develop.

### Imaging

Plain radiographs of involved joints may show joint space narrowing or periarticular osteopenia. Erosions are not observed, unless there is an associated rheumatic disorder. Dual x-ray absorptiometry may demonstrate osteopenia as a consequence of the underlying inflammation or general debility.

### Additional Studies

At present, genetic testing offers little added benefit. Serum levels of cartilage biomarkers such as COMP or indications of an immune response to cartilage (e.g., antibodies to types II, IX, or XI collagen or matrilyin-1) are not routinely available, and their role in disease activity monitoring is unclear.[20,22,59] A recent report highlights the relevance of antiglutamate receptor GluR ε2 autoantibodies in the cerebrospinal fluid and sera of a patient with RPC-associated limbic encephalitis.[60] The potential use of other biomarkers, such as urinary type II collagen neoepitope, has been highlighted in the literature.[23] A disease activity index for RPC was recently established and may have some value in the clinical assessment of patients with this rare disease.[61]

## TREATMENT

Nonsteroidal anti-inflammatory drugs and low-dose corticosteroids may successfully control minor inflammation of the nose, ear, or chest wall. Dapsone may also be used for noncritical cartilaginous inflammation,[1,62] and colchicine is of reported use in auricular chondritis.[63] However, the presence of potentially life-threatening disease or severe chondritis requires high-dose prednisone, typically at a dose of 1 mg/kg, reducing slowly as the inflammation recedes.[1,62] Although the RPC is usually steroid responsive, it can be difficult to withdraw these drugs completely without risking a flare in disease activity. Many patients require maintenance doses of prednisone and consideration of alternative immunosuppression. Disease-modifying treatment is indicated in patients who require a steroid-sparing agent, and/or in whom life-threatening manifestations have developed.

Because of the rarity of this disease, there are no controlled trials of therapeutic interventions in RPC. However, methotrexate, cyclophosphamide, mycophenolate mofetil, azathioprine, chlorambucil, and cyclosporine have had successful results in some patients.[34,63-70] Varied outcomes have been reported with leflunomide.[71,72] Plasmapheresis has been used in resistant cases.[34,73] Furthermore, intravenous immunoglobulin has been used successfully in recalcitrant RPC.[74] There are several case reports and small case series detailing the use of biologic agents in this disease. However, there have been variable data in relation to the antitumor necrosis factor agents. In those patients who responded to treatment, a sustained reduction in the acute phase response was noted during the period of study.[75-80] There are more negative than positive case reports in relation to the use of anti–B cell drugs in RPC.[75,81,82] IL-1 receptor antagonist has demonstrated benefit in a small number of reported cases.[83,84] Tocilizumab, an anti–IL-6 agent, has had beneficial effects in this disease, including cases of refractory inflammation and vascular involvement.[85-90] However, another report found a poor therapeutic response for this drug in RPC and suggested that it should be confined to use in patients with elevated serum IL-6, which might be predictive of efficacy.[91] Abatacept, a fusion protein of cytotoxic T lymphocyte–associated protein-4 (CTLA4) and IgG1, has shown efficacy, particularly for joint and cartilage inflammation in RPC, but may have little benefit for internal organ involvement.[92,93] A successful outcome for autologous stem cell transplantation in treatment-resistant RPC has also been reported.[94] It is not clear, however, if any of these treatments overtly influence the natural history of this disease.

A holistic approach to the management of RPC is essential. Awareness of disease complications and recognition of early pathology may be lifesaving. Special consideration should be given to the management of upper airway involvement, and the anesthesia team should be made aware of disease in this region before any surgical intervention. Symptomatic airway obstruction might require tracheostomy, tracheal stenting, or night-time positive pressure ventilation to prevent airway collapse while the patient is asleep. There is limited experience with laryngotracheal reconstruction in patients with this disease, and such interventions should only take place during disease quiescence.[95] Heart surgery to treat aortic root dilatation, aneurysm formation, or valvular disease has a good outcome in most cases, although post-operative dehiscence is a complication in 12%.[40,96,97] Most cases require post-operative immunosuppression. For patients with sensorineural deafness, cochlear implantation is usually beneficial with significant restoration of hearing.[98] The successful use of bone grafting to correct saddle nose deformity has been reported.[99,100] Neither hormonal activity nor pregnancy is thought to influence disease activity in RPC.[101] However, several case reports have described the treatment of disease flares during pregnancy, typically with corticosteroids, with successful maternal and fetal outcomes.[102-103]

## OUTCOME

RPC is an episodic, progressive disease that results in tissue destruction of target organs. Some patients have a mild course, complicated only by recurrent chondritis, whereas others develop potentially life-threatening problems. The most common cause of death is pulmonary infection, arising as a result of the disease or its immunosuppressive treatment.[34] In young patients, adverse prognostic factors at presentation include anemia, hematuria, upper airway disease, arthritis, and saddle nose deformity, whereas in older patients (older than 51 years), only anemia at presentation predicts increased mortality.[6] The presence of vasculitis worsens prognosis, with a 5-year survival rate of 45%.[36] In 1986, Michet and colleagues reported the 10-year survival rate to be 55%, whereas in 1998, perhaps because of improvements in disease monitoring and treatment, Trentham and colleagues described a 94% survival rate.[6,34]

## CONCLUSION

RPC is a systemic autoimmune disorder, characterized by episodic inflammation of cartilaginous structures (e.g., the upper aspect of the ears, proximal airways, eyes, kidneys, heart valves, and blood vessels). It may occur in isolation or in association with other inflammatory diseases. Involvement of the trachea or cardiovascular system may have life-threatening consequences. Immunosuppressive treatment, including the use of biologic agents, has been shown to abrogate disease features. A holistic approach is recommended to improve the morbidity and mortality of this rare condition.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 105

# Heritable Diseases of Connective Tissue



Deborah Krakow

**KEY POINTS**

- Heritable disorders of connective tissues are a genetically diverse group of disorders and are associated with extreme variation in height, from very short individuals (dwarves) to those with tall stature.
- The osteochondrodysplasias or skeletal dysplasias are a group of more than 450 distinct disorders frequently associated with profound short stature and orthopedic complications.
- These disorders are diagnosed based on radiographic, clinical, and molecular criteria.
- There has been significant progress in elucidating the underlying molecular mechanisms in most of these disorders, providing for improved clinical diagnosis and reproductive choices for affected individuals and their families.
- Treatment options have been used in osteogenesis imperfecta, metabolic storage disorders, and Marfan syndrome, which may improve the quality of life and life span in affected individuals.

Heritable disorders of connective tissues are a heterogenous group of disorders characterized by abnormalities in skeletal tissues, which include cartilage, bone, tendon ligament, and muscle. Disorders of connective tissues have been classified based on clinical findings and molecular criteria. They are subclassified into those that primarily affect cartilage and bone, which are the skeletal dysplasias, and those that have a more profound effect on connective tissue, which include the Ehlers-Danlos syndrome, Marfan syndrome, and other disorders caused by abnormal extra-cellular matrix molecules, such as cutis laxa syndrome resulting from mutations in the gene that encodes fibulin 5.[1] The skeletal dysplasias are associated with abnormalities in the size and shape of the appendicular and axial skeleton and frequently result in disproportionate short stature. Until the early 1960s, most individuals with short stature were considered to have pituitary dwarfism, achondroplasia (short-limbed dwarfism), or Morquio disease (short-trunked dwarfism). Presently, there are more than 450 well-characterized disorders that are classified primarily on the basis of clinical, radiographic, and molecular criteria. Disorders of connective tissues are genetic in origin and result from mutations in genes that encode extra-cellular matrix proteins, transcription factors, tumor suppressors, signal transducers, enzymes, chaperones, intra-cellular binding proteins, RNA-processing molecules, ciliary proteins, and genes of unknown function.[2]

## SKELETAL DYSPLASIAS

The skeletal dysplasias, or osteochondrodysplasias, are defined as disorders associated with a generalized abnormality in the skeletal. Although each skeletal dysplasia is relatively rare, collectively the birth incidence of these disorders is almost 1 in 5000.[3] These disorders range in severity from precious arthropathy to perinatal lethality caused by pulmonary insufficiency. Individuals with these disorders can have significant complications, including orthopedic, neurologic, and psychological. Many of these individuals seek medical attention for orthopedic complaints from ongoing pain, arthritic complaints in large joints, and back pain primarily caused by ongoing abnormalities in bone, cartilage, tendons, and ligaments.

### Embryology

The human skeleton (from the Greek *skeletos*, dried up) is a complex organ consisting of 206 bones (126 appendicular, 74 axial, 6 ossicles). The skeleton, including tendon, ligaments, and muscles in addition to cartilage and bone, has multiple embryonic origins and serves many key functions throughout life, including linear growth, mechanical support for movement, a blood and mineral reservoir, and protection of vital organs.

The patterning and architecture of the skeleton occurs during fetal development. During that period, the number, size, and the shape of the future skeletal elements are determined, and this process is under complex genetic control.[4] Uncondensed mesenchyme undergoes cellular condensations (cartilage anlagen) at sites of future bones, and this occurs by two mechanisms.[5] In the process of endochondral ossification, mesenchyme first differentiates into a cartilage model (anlagen), and then the center of the anlagen degrades, mineralizes, and is removed by osteoclast-like cells. This process spreads up and down the bones and allows for vascular invasion and influx of osteoprogenitor cells. The periosteum in the midshaft region of the bone produces osteoblasts, which then synthesis the cortex. This is known as the *primary ossification center*. At the ends of the cartilage anlagen, a similar process leading to the removal of cartilage occurs (secondary center of ossification), leaving a portion of cartilage model "trapped" between the expanding primary and secondary ossification center. This area is referred to as a *cartilage growth plate* or *epiphyses*. There are four chondrocyte cell types in the growth plate: reserve, resting, proliferative, and hypertrophic. These growth plate chondrocytes

undergo a tightly regulated program of proliferation, hypertrophy, degradation, and then replacement by bone, leading to the primary spongiosa. This is the major mechanism underlying skeletogenesis and is the means by which bones increase in length and the articular surfaces increase in diameter. In contrast, the flat bones of the cranial vault and part of the clavicles and pubis form by intramembraneous ossification, in which fibrous tissue, derived from mesenchymal cells, differentiate directly into osteoblasts, which then directly lay down bone. These processes are under specific temporal and genetic control, and abnormalities in the genes that encode these pathways frequently lead to skeletal dysplasias.[5]

## Cartilage Structure

Collagen accounts for two-thirds of the adult weight of adult articular cartilage and provide significant strength and structure to the tissue. Collagens are a family of proteins that consist of single molecules (monomers) that associate into three polypeptide chains to form a triple helix structure. In the triple helix, every third amino acid is a glycine residue, and the general chain structure is denoted as Gly-X-Y, where the X and Y amino acids are commonly proline and hydroxyproline. The collagen helix can be composed of identical chains (homotrimeric), as in type II collagen, or can consist of different collagen chains (heterotrimeric), as seen in type I collagen.[6]

Collagens are widely distributed throughout the body, and 33 collagen gene products are expressed in a tissue-specific manner, leading to 19 triple-helical collagens. Collagens are further classified by the structures they form in the extra-cellular matrix. The most abundant collagens are the fibrillar types (I, II, III, V, and XI), and their extensive cross-linking provides mechanical strength that is necessary for high-stress tissue, such as cartilage, bone, and skin.[7] Another collagen species is the fibril-associated collagens with interrupted triple helices (FACIT) and include types IX, XII, XIV, and XVI collagens. These collagens interact with fibrillar collagens as well as other extra-cellular molecules, including aggrecan, cartilage oligomatrix protein (COMP), and other sulfated proteoglycans.[8] Types VIII and X collagen are nonfibrillar, short-chain collagens, and type X collagen is the most abundant extra-cellular matrix molecule expressed by hypertrophic chondrocytes during endochondral ossification.[8] The major collagens of articular cartilage are fibrillar collagen, including types II, IX, XI, and X. In developing cartilage, the core fibrillar network is a cross-linked copolymer of types II, IX, and XI collagen,[9] and form the backbone complex to which other structural proteins bind. Mutations in genes that encode these collagens result in varying skeletal dysplasias (Table 105-1) and

**TABLE 105-1** Classification of the Chondrodysplasias

| Dysplasia | Mode of Inheritance | Gene |
|---|---|---|
| **Achondroplasia Group** | | |
| Achondroplasia | AD | *FGFR3* |
| Thanatophoric dysplasia, type I | AD | *FGFR3* |
| Thanatophoric dysplasia, type II | AD | *FGFR3* |
| Achondroplasia | AD | *FGFR3* |
| Hypochondroplasia | AD | *FGFR3* |
| SADDAN | AD | *FGFR3* |
| Osteoglophonic dysplasia | AD | *FGFR1* |
| **Severe Spondylodyplastic Dysplasias** | | |
| Achondrogenesis IA | AR | *TRIP11* |
| Opsismodysplasia | AR | *INPPL1* |
| **Metatropic Dysplasia Group** | | |
| Fibrochondrogenesis | AR | *COL11A1, COL11A2* |
| Schneckenbecken dysplasia | AR | *SLC35D1, INNPI1* |
| Metatropic dysplasia | AD | *TRPV4* |
| **Short Rib Dysplasia (Polydactyly) Group** | | |
| Short rib polydactyly syndromes, including asphyxiating thoracic dysplasia | AR | *DYNC2H1, NEK1, WDR60, WDR35, IFT80, WDR34, IFT172, TCTEXD1, DYNC2LI1* |
| Chondroectodermal dysplasia | AR | *EVC1, EVC2* |
| Thoracolaryngopelvic dysplasia | AD | Unknown |
| **Omodysplasia Group** | | |
| Omodysplasia I | AD | Unknown |
| Omodysplasia II | AR | GPC6 |
| **Filamin-Related Disorders** | | |
| Atelosteogenesis I | AD | *FLNB* |
| Atelosteogenesis III | AD | *FLNB* |
| Larsen syndrome | AD | *FLNB* |
| Otopalatodigital syndrome type II | XLR | *FLNA* |
| Osteodysplasty, Melnick-Needles | XLD | *FLNA* |
| **Diastrophic Dysplasia Group** | | |
| Achondrogenesis IB | AR | *DTDST* |
| Achondrogenesis II | AR | *DTDST* |
| Diastrophic dysplasia | AR | *DTDST* |
| Recessive multiple epiphyseal dysplasia | AR | *DTDST* |

**TABLE 105-1** Classification of the Chondrodysplasias—cont'd

| Dysplasia | Mode of Inheritance | Gene |
|---|---|---|
| **Dys-segmental Dysplasia Group** | | |
| Dys-segmental dsyplasia | AR | *HSPG2* |
| Silverman-Handmaker type | | |
| Dyssegmental dsyplasia | AR | *HSPG2* |
| Rolland-Desbuquois | | |
| **Type II Collagenopathies** | | |
| Achondrogenesis II | AD | *COL2A1* |
| Kniest dysplasia | AD | *COL2A1* |
| Spondyloepiphyseal dysplasia congenital | AD | *COL2A1* |
| Spondyloepiphyseal dysplasia, Strudwick type | AD | *COL2A1* |
| Spondyloperipheral dysplasia | AD | *COL2A1* |
| Arthro-opthamalopathy, (Stickler syndrome) | AD | *COL2A1* |
| **Type XI Collagenopathies** | | |
| Stickler dysplasia | AD | *COL11A1* |
| Otospondylomegaepiphyseal dysplasia (OSMED) | AR | *COL11A2* |
| Weissenbacher-Zweymuller syndrome | AD | *COL11A2* |
| **Other Spondyloepi-(meta)-physial dysplasias [SE(M)Ds]** | | |
| Spondyloepimetaphyseal dysplasia, Pakistani type | AR | *ATPSK2* |
| Spondyloepiphyseal dysplasia tarda | XLR | *SEDL* |
| Progressive pseudorheumatoid dysplasia | AR | *WISP3* |
| Dyggve-Melchior-Clausen dysplasia | AR | *Dymeclin* |
| Wolcott-Rallison dysplasia | AR | *EIF2AK3* |
| Acrocapitofemoral dysplasia | AR | *IHH* |
| Schimke immunoosseous dysplasia | AR | *SMARCAL1* |
| Sponastrime | AR | Unknown |
| Spondyloepimetaphyseal dysplasia, with joint laxity | AR | *B3GALT6* |
| | AD | *KIF22* |
| **Multiple Epiphyseal Dysplasia and Pseudoachondroplasia** | | |
| Multiple epiphyseal dysplasia, types Fairbanks and Ribbing | AD | *COL9A1, COL9A2, COL9A3, MATN3, COMP* |
| Pseudoachondroplasia | AD | *COMP* |
| **Chondrodysplasia Punctata** | | |
| Chondrodysplasia punctata, rhizomelic type | AR | *PEX7, DHAPAT, AGPS* |
| Chondrodysplasia punctata, Conradi-Hunermann type | XLD | *EBP* |
| Hydrops-ectopic-calcifications-moth-eaten bones | AR | *LBR* |
| Chondrodysplasia punctata, brachytelephalangic type | XLR | *ARSE* |
| **Metaphyseal Dysplasias** | | |
| Metaphyseal chondrodysplasia, type Jansen | AD | *PTHrP* |
| Eiken dysplasia | AR | *PTHrP* |
| Bloomstrand dysplasia | AR | *PTHrP* |
| Metaphyseal chondrodysplasia, type Schmidt | AD | *COL10A1* |
| Metaphyseal chondrodysplasia, McKusick type | AR | *RMRP* |
| Metaphyseal chondrodysplasia, with pancreatic insufficiency, and cyclin neutropenia | AR | *SBDS* |
| Adenosine deaminase deficiency | AR | *ADA* |
| **Spondylometaphyseal Dysplasias** | | |
| Spondylometaphyseal dysplasia, type Koslowski | AD | *TRPV4* |
| Spondylometaphyseal Dysplasia, type corner fracture | AD | Unknown |
| **Brachyolmia Spondylodysplasias** | | |
| Brachyolmia (Hobek type) (Toledo type) | AR | Unknown |
| Brachyolmia (Maroteaux type) | AR | *TRPV4* |
| Brachyolmia (AD) | AD | *TRPV4* |
| **Mesomelic Dysplasias** | | |
| Dyschondroseosis | XLD | *SHOX* |
| Mesomelic dysplasia, type Langer | XLR | *SHOX* |
| Mesomelic dysplasia, type Robinow | AD | |
| | AR | *ROR2* |
| **Acromelic and Acromesomelic Dysplasias** | | |
| Acromicric dysplasia | AD | *FBN1* |
| Geleophysic dysplasia | AR | *ADAMTSL2* |
| | AD | *FBN1* |
| Trichorhinophalangeal dysplasia, type I | AD | *TRPS1* |
| Trichorhinophalangeal dysplasia, type II | AD | *TRPS2* |
| Acrodysostosis | AD | *PDE4D, PRKAR1A* |
| Grebe dysplasia | AR | *CDMP1* |
| Acromesomelic dysplasia, Hunter-Thompson | AR | *CDMP1* |
| Acromesomelic dysplasia, type Maroteaux | AR | *NPRB* |
| **Dysplasia with Prominent Membranous** | | |

*Continued*

**TABLE 105-1** Classification of the Chondrodysplasias—cont'd

| Dysplasia | Mode of Inheritance | Gene |
|---|---|---|
| **Bone Involvement** | | |
| Cleidocranial dysplasia | AD | *CBFA1* |
| **Bent Bone Dysplasias** | | |
| Campomelic dysplasia | AD | *SOX9* |
| Stuve-Wiedemann dysplasia | AR | *LIFR* |
| **Multiple Dislocations with Dysplasias** | | |
| Desbuquois syndrome | AR | *CANT1, XYLT1* |
| Pseudodiastrophic dysplasia | AR | Unresolved |
| Spondyloepimetaphyseal dysplasia with joint laxity | AR | *B3GALT6* |

AD, Autosomal dominant; AR, autosomal recessive; SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans; SP, sporadic; XLR, X-linked recessive; XLD, X-linked dominant.

highlight the importance of these molecules in skeletal development.

## Classification and Nomenclature

As mentioned above, in the 1970s there was recognition of the genetic and clinical heterogeneity of these disorders and a new awareness of the complexity of these disorders. As a result, there have been multiple attempts to classify these disorders in a manner that clinicians and scientists could effectively use to diagnose and determine their pathogenicity (International Nomenclature of Constitutional Diseases of Bone, 1970, 1977, 1983, 1992, 2001, 2005, and 2010).[2] The initial categories were purely descriptive and clinically based. With the recent explosion in determining the genetic basis of these diseases, the classification has evolved into one that combines the older clinical one (including the eponyms and Greek terms) and also blends these disorders into families that share a molecular basis or pathway. Some of the chondrodysplasia families are listed in Table 105-1. The most widely used method for differentiating the skeletal disorders has been through the detection of skeletal radiographic abnormalities. Radiographic classifications are based on the different parts of the long bones that are abnormal (epiphyses, metaphyses, diaphyses) (Figure 105-1). These epiphyseal, metaphyseal, and diaphyseal disorders can be further differentiated depending on whether or not the spine is involved (spondyloepiphyseal, spondylometaphyseal dysplasias, or spondyloepimetaphyseal dysplasias). Moreover, each of these classes of these disorders can be further differentiated into distinct disorders based on other clinical and radiographic findings.

## Clinical Evaluation and Features

The skeletal dysplasias are generalized disorders of the skeleton and usually result in disproportionate short stature. Affected individuals usually present with the complaint of disproportionate short stature, and this finding needs to be documented on the appropriate growth curves for sex and ethnicity if possible. As a general comment, most individuals with disproportionate short stature have skeletal dysplasias, and those with proportionate short stature have endocrine, nutritional, prenatal-onset growth deficiency, or other nonskeletal dysplasia genetic disorders. However, there are exceptions to the rule, such as congenital hypothyroidism, which is associated with disproportionate short stature, and disorders such as mild osteogenesis imperfecta and hypophosphatasia can be associated with relatively normal body proportions.

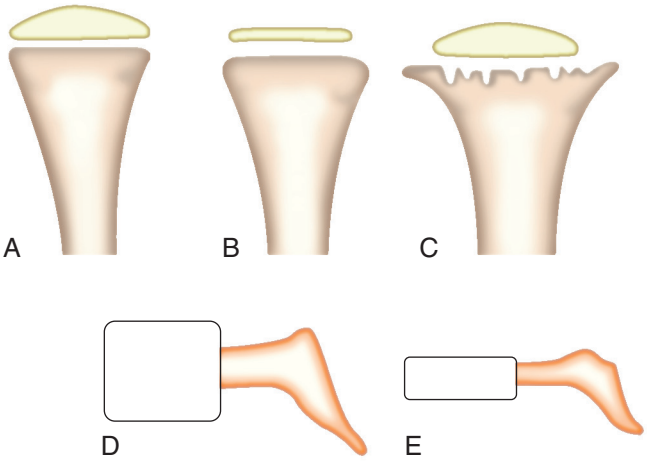



**Figure 105-1** Classification of chondrodysplasias based on radiographic involvement of the long bones **(A-C)** and vertebrae (**D** and **E**). **A** and **D** are normal. **B,** epiphyseal abnormality. **C,** metaphyseal abnormality. **E,** spondylo-abnormality.

A disproportionate body habitus may not be immediately visible on physical examination. Therefore the physician must take anthropodemetric measurements, such as upper/lower segment (U/L) ratio, sitting height, and arm span, when considering the possibility of a skeletal dysplasia, and measurements should be taken in centimeters. Sitting height is an accurate measure of head and trunk length, but it requires special equipment for precise measurements. Upper/lower ratios are easy to obtain and provide an accurate measurement of proportion. The lower segment is measured from the symphysis pubis to the floor at the inside of heel. The upper segment is measured by subtracting the lower segment measurement from the total height. For example, a Caucasian child between the ages of 8 to 10 years has a U/L segment ratio of approximately 1.0 and an U/L segment ratio of 0.95 as an adult. Individuals seen with disproportionate short stature will have altered ratios depending on whether they have short limbs, short trunk, or both. For example, an individual with short limbs and relatively normal trunk will have an increased U/L segment ratio, and an individual with relatively normal limbs but a short trunk will have a diminished U/L segment ratio (Figure 105-2). Another means of determining whether

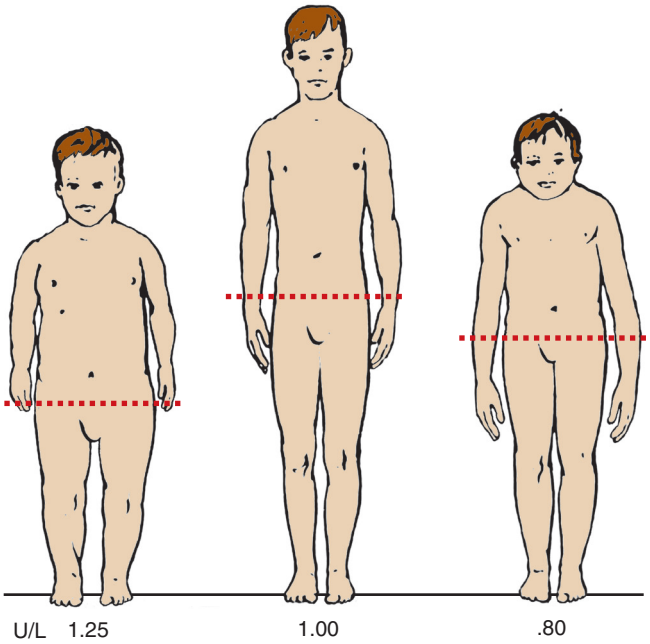



**Figure 105-2** Upper segment length/lower segment length in 8- to 10-year-old individuals with short-limb and short-trunk dwarfism. The child on the left has short limbs and an increased upper/lower ratio; the child on the right has a short trunk and reduced upper/lower ratio.

there is disproportion is based on arm span measurements, which are measured from tip to tip of the third finger. Arm span length is typically very close to total height (1:1) in an average-proportioned individual. A short-limbed individual will have an arm span considerably shorter than their height.

As in any disorder that has a genetic basis, it is critical for the physician to obtain an accurate family history, and this should include any history of previously affected children or parental consanguinity. The skeletal dysplasias are genetically heterogenous and can be inherited as autosomal dominant, autosomal recessive, X-linked recessive, and X-dominant disorders, and rarer genetic mechanisms of disease, including germline mosaicism, somatic mosaicism, uniparental disomy, and microdeletion syndromes, have been seen. For many patients and families, accurate diagnosis and recurrence risk can have significant impact on their reproductive decisions. Another consideration for patients with short stature is that there is increased nonassertive mating, which leads to reproductive outcomes that have been previously unknown.[10] For example, homozygous achondroplasia is lethal, and many newborns who inherit two dominant mutations (compound heterozygotes) do quite poorly because of increased lethality and severe effects on the skeleton.[11] The physician should also obtain accurate history relative to the onset of short stature and whether it developed immediately in the postnatal period or was noticed at age 2 or 3 years. Of the 450 skeletal dysplasias, approximately 100 of them have onset in the prenatal period, but many do not manifest disproportionate short stature and joint discomfort until childhood.[2]

A detailed physical examination may reveal a diagnosis or help differentiate the most likely group of possible diagnoses. It is critical that once disproportion and short stature has been established and the limbs are involved, to determine which segment is involved: upper segment (rhizomelic; humeri and femora), middle segment (mesomelic; radii, ulnae, tibiae, and fibulae), and distal segment (acromelic; hands and feet). Numerous head and facial dysmorphisms are seen in the skeletal disorders. Affected individuals frequently have disproportionately large heads or relative macrocephaly. Frontal bossing of the metopic bossing and flattened nasal bridge is very characteristic of achondroplasia, one of the most common skeletal dysplasias.[12] Cleft palate and micrognathia are commonly found in the type II collagen abnormalities, abnormally flattened of the midface with a turned-up nose is frequently found in the chondrodysplasia punctata disorders,[13] and abnormal swollen pinnae are seen in diastrophic dysplasia.[14] Individuals with skeletal dysplasias should be screened for ophthalmologic and hearing abnormalities because some of these disorders are associated with eye abnormalities and hearing loss.

Further evaluation of the hands and feet can lead to further differentiation of these disorders. Postaxial polydactyly is characteristically found in chondroectodermal dysplasia and the short-rib polydactyly syndrome disorders (see Table 105-1). Short, hypermobile, radially displaced thumbs and medially deviated halluxes are seen in diastrophic dysplasia. Nails can be abnormally hypoplastic in chondroectodermal dysplasia and short and broad in cartilage hair hypoplasia. Club feet or equinovarus may be seen in many disorders, including Kniest dysplasia, spondyloepiphyseal dysplasia congenita, Larsen syndrome, more severe forms of osteogenesis imperfecta, and diastrophic dysplasia. Bone fractures occur most commonly in two types of disorders: those that result from undermineralized bone (osteogenesis imperfecta, hypophosphatasia, achondrogenesis IA), or those that result from overmineralized bone (osteopetrosis syndromes and dysosteosclerosis).

Other organ systems, beyond the skeleton, can be involved though not commonly. Congenital cardiac defects are seen in chondroectodermal dysplasia (atrial septal defect), the short-rib polydactyly syndrome disorders (complex outlet defects, including isolated ventricular septal defects) and in Larsen syndrome (ventricular septal defect). Gastrointestinal anomalies are rare among the skeletal disorders, but congenital megacolon can be seen in cartilage hair hypoplasia, malabsorption syndrome in Shwachman-Diamond syndrome, and omphaloceles in otopalatodigital syndrome and atelosteogenesis I.

## Diagnostic Tests and Diagnosis

After obtaining a thorough family history and physical examination, the next step is to obtain a full set of skeletal radiographs or genetic skeletal survey. A full series of skeletal views, including anterior, lateral, and Towne views of the skull, anterior and lateral views of the entire spine, and anteroposterior pelvis and extremities, with separate views of the hands and feet, is optimal, especially after the newborn period. Most of the important clues to diagnosis are in skeletal radiographs that are obtained before puberty. Once the epiphyses have fused to the metaphyses, determining the precise diagnosis can be exceedingly challenging. If an adult is evaluated, all attempts should be made to obtain any available childhood radiographs. There are many subtle

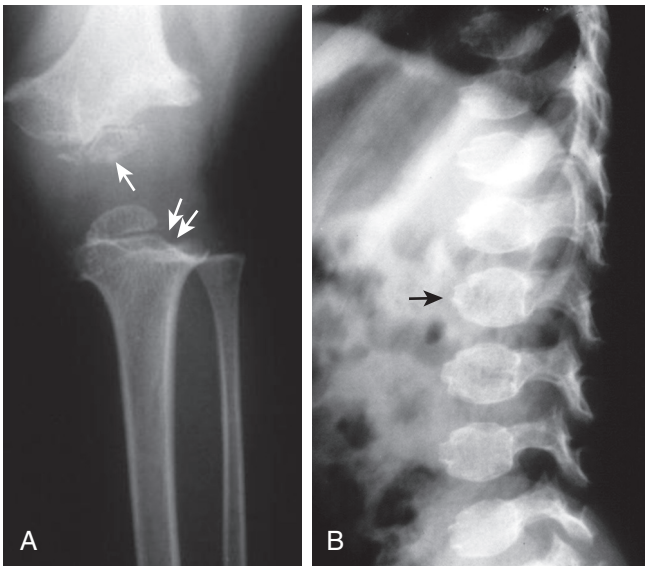



**Figure 105-3** Radiographs showing abnormalities. **A,** Distal femur and proximal tibia and fibula showing small irregular epiphyses *(single arrow)* and irregular metaphyses *(double arrows)*. **B,** Lateral view of the spine showing abnormally rounded vertebral bodies with anterior central beaking *(arrow)*.

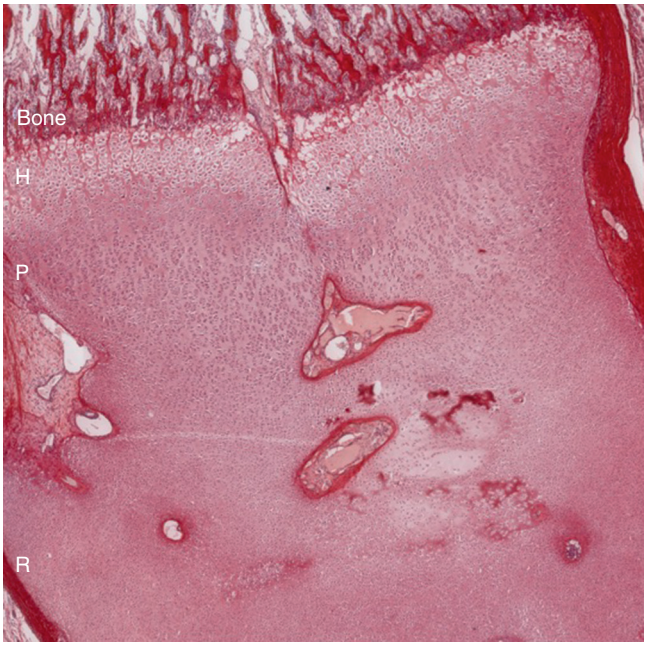



**Figure 105-4** Histologic analysis of a control 17-week human fetal growth plate stained with picrosirius red, showing the morphology of the growth plate. The defined zones of the growth plate are labeled, *H*, hypertrophic, *P*, proliferative, and *R*, resting.

clues in these skeletal radiographs that can lead to a precise diagnosis. For example, punctuate calcifications in the areas of the epiphyses in the chondrodysplasia punctata disorders, which are not seen in adulthood; multiple ossification centers of the calcaneus in more than 20 disorders[15]; and the pattern of hand shortening can aid in differentiating many disorders.

After obtaining radiographs, the physician should pay close attention to the specific parts of the skeleton (spine, limbs, pelvis, skull) involved and to the location of the lesions (epiphyses, metaphyses, and spondylcomponents) (Figure 105-3). As mentioned in preceding text, these radiographic abnormalities can change with age. If possible to obtain, radiographs of patients across a few ages will aid in diagnosis. Fractures can be seen in osteogenesis imperfecta (all types; see Table 105-1) and severe hypophosphatasia. In older individuals, fractures may be seen in disorders associated with increased mineralization, such as the osteopetrosis syndromes.

Morphologic studies of chondroosseus tissue have revealed specific abnormalities in many of the skeletal dysplasias.[16] In these disorders, histologic evaluation of chondroosseous morphology can aid in making an accurate diagnosis, and absence of histopathologic alterations can rule out diagnoses (Figure 105-4). These studies need to be performed on cartilage growth plate, and although commonly performed on perinatal lethal skeletal disorders at time of autopsy, obtaining growth plate histology on individuals with nonlethal disorders is difficult. If affected individuals (children) are undergoing surgery, an iliac crest biopsy can be evaluated, although admittedly this is performed with markedly less frequency than in the past, particularly while our molecular understanding has markedly increased. However, histomorphology studies done on these disorders have led to important insights on the pathogenesis of these disorders. On morphologic grounds, the chondrodysplasias can be broadly classified into those disorders that (1) have a qualitative abnormality in endochondral ossification, (2) have abnormalities in cellular morphology, (3) have abnormalities in matrix morphology, and (4) in which the abnormality is primarily localized to the area of chondroosseous transformation. For example, in thanatophoric dysplasia, there is a defect in endochondral ossification with a very short, if almost hypertrophic, zone, a shortened proliferative zone, and overgrowth of the periosteum. In pseudoachondroplasia, there is a distinct lamellar pattern (alternating electron-dense and electron-lucent lamellae) in the rough endoplasmic reticulum of chondrocytes and a grossly abnormal matrix in diastrophic dysplasia, which leads to a characteristic ring around the chondrocytes. All of these findings are characteristic and diagnostic for these disorders and illustrates how morphology studies can play in integral part in the investigation of these disorders. Further, disorders across a phenotypic spectrum have been placed in bone families based on similar cartilage growth plate morphologic abnormalities.

There has been significant progress in gene identification in these disorders, which has impact for affected individuals. As illustrated in Table 105-1, for those disorders in which the gene is identified, molecular diagnostic testing is potentially available. Molecular diagnosis can be used to confirm a clinical and radiographic diagnosis, predict carrier status in those families at risk for a recessive disorder, and, for some individuals, allow prenatal diagnosis of at-risk fetuses. With commercial availability of whole-exome sequence analysis, many high and low bone mass parallel sequencing panels are available, as well as whole-exome sequence analysis for rare disorders not available on panels. If there is no question regarding definitive diagnosis, then disease genes can be directly interrogated. GeneTests (www.genetests.org) is a

publically funded medical genetics website developed for physicians that provides information on specific diseases and available genetic testing.

## Treatment

The optimal management of this diverse set of disorders requires an understanding of their medical, skeletal, and psychosocial consequences. This is often best accomplished by centers that have a multidisciplinary approach, which include adult and pediatric physicians, as well as a group of physicians, geneticists, orthopedists, rheumatologists, otolaryngologists, neurologists, neurosurgeons, and ophthalmologists who are committed to the care of these patients.

Most medical complications in these disorders result from orthopedic complications, and they vary depending on the specific disorder. For example, in disorders associated with significant odontoid hypoplasia, such as Morquio disease, type II collagenopathies, metatropic dysplasia, and Larsen syndrome, flexion-extension films should be monitored at regular intervals to assess C1-C2 subluxation. If there is evidence for subluxation, surgery for C1-C2 fixation is indicated. Many geneticists support flexion and extension radiographic evaluation, including MRI of the neck in all cases of skeletal dysplasias, because so many of these disorders have cervical vertebral abnormalities.[17] Genu varum, lateral curvature of the lower extremity, is common in many skeletal disorders because of overgrowth of the fibula. This causes knee or ankle pain in many individuals, especially children, and consideration should be made for correction by osteotomies. Children and adults with skeletal dysplasias should have regular eye and hearing examinations because they are at increased risk for myopia, retinal degeneration, glaucoma, and hearing loss, depending on the disorder.

Frequently, patients with these disorders have significant joint pain and, in some cases, joint limitations. Because most of these disorders result from mutations in genes critical to cartilage function, the cartilage at the joint surfaces may not provide adequate support and cushioning function. Many of these patients seek attention for joint pain. Evaluation should include radiographic views and MRI, when appropriate, to determine the etiology of the pain. In some disorders (e.g., the type II collagenopathies, pseudoachondroplasia, multiple epiphyseal dysplasia, and cartilage hair hypoplasia), by adulthood so little cartilage remains at the knee or hips that joint replacement is indicated for pain relief. Last, weight control in adults with short stature is an ongoing issue and contributes to inactivity, loss of function, adult-onset diabetes, hypertension, and coronary disease.[18]

Achondroplasia is the most common of the nonlethal skeletal dysplasias (approximately 1/20,000) and serves as an example on how to approach these disorders, particularly because the incidence and type of medical complications change during the life span of the individual. The majority of these individuals are of normal intelligence, have a normal life span, and lead independent and productive lives. The mean final height in achondroplasia is 130 cm for men and 125 cm for women, and specific growth charts have been developed to document and track linear growth, head circumference, and weight in these individuals.[19,20]

In early infancy, there is potentially serious compression of the cervicomedullary spinal cord secondary to a narrow foramen magnum, cervical canal, or both. Clinically, these infants show central apnea, sleep apnea, profound hypotonia, motor delay, or excessive sweating. MRI with flow studies is necessary to document the obstruction and, if present, requires decompression surgery. Other complications include upper airway obstruction, thoracolumbar kyphosis, and hydrocephalous in a small number of individuals.[21] From early childhood and while the child begins to walk, several orthopedic manifestations occur, including progressive bowing of the legs because of fibular overgrowth, lumbar lordosis, and hip flexion contractures. Recurrent ear infections lead to chronic serous otitis media and deafness. Tympanic membrane tube placement is indicated in many of these patients. Craniofacial abnormalities lead to dental malocclusion, and appropriate treatment is necessary. As adults, the main potential medical complication is impingement of the spinal root canals, and this can be manifested by lower limb paresthesias, claudication, clonus, bladder, or bowel dysfunction. It is critical that these complaints are addressed because without appropriate decompression surgery, paralysis of the spinal cord can result.[21]

Growth hormone has not been effective in increasing height in this disorder.[22] Surgical limb lengthening has been used successfully to increase limb length by as much as 12 inches,[23] but patients need to recognize that this technique needs to be done during the teen years, is performed during a 2-year period, and is associated with complications. Because of the discovery of the disease gene in 1994,[24] the molecular consequences of the activating mutation in fibroblast growth factor receptor 3 gene *(FGFR3)* have been elucidated. Although the mutation has numerous effects on receptor kinase signaling pathways, increased activation of ERK (mitogen-activated protein kinase) is responsible for much of the proliferation defect seen in achondroplasia cartilage growth plates.[25] Inhibitors of the pathway have been identified and potential treatments are being proposed.[26]

Throughout their lives, individuals with achondroplasia, other skeletal dysplasias, and their families experience various psychosocial challenges.[27] This can be addressed by specialized medical and social support systems. Interactions with advocacy groups such as *Little People of America* (*LPA*; http://www.lpaonline.org) can provide both emotional support and medical information.

## Biochemical and Molecular Abnormalities

Based on clinical, radiographic, and histomorphologic similarities, skeletal dysplasias have been placed into bone dysplasia families.[28] These families share a common pathophysiologic or pathway mechanisms. In recent years, there has been an explosion in our understanding of the basic biology of these disorders. This has resulted from advances in the human genome project that improved various methodology, including candidate gene approach, linkage analysis, positional cloning, human/mouse synteny, whole-exome and whole-genome analysis, allowing identification of the responsible disease genes (see Table 105-1). With gene discovery in more than 350 of these osteochondrodysplasias, these genes can be placed into several categories

designed to understand their pathogenesis: (1) defects in extra-cellular proteins, (2) defects in metabolic pathways (enzymes, ion channels, and transporters), (3) defects in folding and degradation of macromolecules, (4) defects in hormones and signal transduction, (5) defects in nuclear proteins, (6) defects in oncogenes and tumor suppressor genes, (7) defects in RNA and DNA processing molecules, (8) defects in intra-cellular structural proteins, (9) defects in chaperones, (10) defects in ciliary components, and (11) genes of unknown function. There are still skeletal dysplasias for which the disease-producing genes are unknown. Below are just some examples of the molecular mechanisms involved in the skeletal dysplasias.

## DEFECTS IN EXTRA-CELLULAR STRUCTURAL PROTEINS

### Type II Collagen and Type XI Collagen

Because type II collagen was found primarily in cartilage, the nucleus pulposus, and the vitreous of the eye, it was hypothesized that skeletal disorders with significant spine and eye abnormalities would result from defects in type II collagen. Indeed, type II collagen defects have been identified in a spectrum of disorders ranging from lethal to mild arthropathy, which include achondrogenesis II, hypochondrochogenesis, spondyloepiphyseal dysplasia congenital, spondyloperipheral, spondyloepimetaphyseal dysplasia, Strudwick type, Kniest dysplasia, Stickler syndrome, and "precocious" familial arthropathy. These disorders are referred to as *type II collagenopathies*, and they all result from heterozygosity for mutations in the gene *COL2A1*, which encodes type II collagen.[29,30] Biochemical analysis of cartilage derived from these individuals shows electrophoretically detectable abnormal type II collagen. Further, type I collagen is not normally present in cartilage; however, in the presence of abnormal type II collagen, there is increased type I collagen in the growth plate.

Mutations that result in a substitution for a triple-helical glycine residue appear to be the most common type of mutation.[31-33] There are some correlations between the location of the mutation and the disease phenotype. In spondyloepiphyseal dysplasia congenita (SEDC), the glycine substitutions are scattered throughout the molecule; however, in Kniest dysplasia, the mutations are in the more amino-terminal end of the molecule.[34-36] Stickler syndrome is genetically heterogenous and results from mutations in *COL2A1* and *COL11A1*, and non-ocular forms in *COL11A2*.[37,38] In Stickler syndrome, the *COL2A1* and *COL11A1* mutations tend to be nonsense mutations resulting in premature translation stop codons; however, patients with *COL11A1* mutations tend to have a more severe eye phenotype and hearing loss than patients with *COL2A1* mutations.

Individuals heterozygous for various *COL11A2* mutations have a non-ocular form of Stickler syndrome, which is consistent with the absent expression of *COL11A2* in the vitreous humor. Otospondylomegaepiphyseal dysplasia (OSMED) is a rare autosomal recessive disorder caused by loss of function mutations in *COL11A2*.[39] This disorder has radiographic similarities to Kniest dysplasia but is associated with profound sensorineural hearing loss and lack of ocular involvement. To add to the spectrum of type XI mutations, both dominantly and recessively inherited mutations in *COL11A1* and *COL11A2*, respectively, can produce the very severe skeletal dysplasia, fibrochondrogenesis.[40,41]

### Cartilage Oligomeric Matrix Protein

Heterozygosity for mutations in COMP lead to both pseudoachondroplasia and multiple epiphyseal dysplasia.[42] COMP is a member of the thrombospondin family of protein and consists of an epidermal growth factor domain and a calcium-binding calmodulin domain.[43] In both pseudoachondroplasia and MED, disease-producing mutations occur in the calmodulin domain, with a minority in the globular carboxyterminal domain. Both these disorders are associated with early-onset osteoarthritis, particularly of the hips and the knee, leading to joint replacements in many individuals in early adulthood.[44]

### Conclusion

Although these osteochondrodysplasias are relatively rare disorders, affected individuals have significant skeletal complications throughout their lives, first because of patterning defects, then effects on linear growth, and, finally, loss of normal structural cartilage as a cushion later in life. The explosion in delineating molecular defects has shown the complexity of cartilage as a tissue and the large number of cellular processes necessary for a normal skeleton.

## OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta (OI) is a heritable disorder of bone and was one of the first genetic disorders hypothesized to be a defect in collagen.[45] Although an osteochondrodysplasia, it will be discussed separately from the chondrodysplasias delineated above. OI is actually a generalized disorder of connective tissue that predominantly affects the skeletal system and affects a significant numbers of individuals (estimates at approximately 1/20,000 individuals)

Initially, there were four types of recognized OI in the clinical classification of Sillence.[46] However, there are now numerous types of recognized OI on the basis of expanding genetic heterogeneity, yet most of the forms of OI can still be classified into mild, moderate, severe, and lethal. Because there is enormous clinical variability in these types, the subtypes will be discussed separately (Table 105-2). However, these disorders all share the same phenotypic finding of hypomineralization of the skeleton, although interesting bone quality is associated with overbrittleness.[47]

### Mild Osteogenesis Imperfecta (Type I OI)

Affected individuals with type I OI disease have relatively mild severity in terms of their clinical course, the extent of skeletal deformity, and the radiologic appearance of the skeleton (see Table 105-2). They also account for the vast majority of individuals with OI. Individuals usually have mild short stature for their age or their unaffected family members, but are not as severe as seen in the other forms. Many of these individuals experience numerous fractures, especially in childhood, and it is common for

**TABLE 105-2** Classification and Molecular Basis of Osteogenesis Imperfecta (OI)

| Sillence Classification | OI Phenotypic Grouping | Mode of Inheritance | Biochemical Findings | Gene(s) | Mechanisms |
|---|---|---|---|---|---|
| I | Mild | AD | 50% reduction in type I collagen synthesis | *COL1A1*<br>*COL1A2* | Structural defects<br>Haploinsufficiency |
| IV | Moderate | AD<br>AR | Structural alterations of type I collagen chains; overmodification | *COL1A1*<br>*COL1A2*<br>*TMEM38B*<br>*SP7* | Structural defects |
| III | Severe progressive deforming | AD<br>AR | Structural alterations of type I collagen chains; overmodification<br>Structural alterations of type I collagen chains; overmodification<br>Delayed processing of type I procollagen<br>Normal type I collagen biochemical analysis<br>Distinct bone histology | *COL1A1*<br>*COL1A2*<br>*CRTAP*<br>*P3H1*<br>*PPIB*<br>*BMP1*<br>*FKBP10*<br>*SERPINH1*<br>*WNT1*<br>*SERPINF1*<br>*PEDF* | Structural defects<br>3-Hydroxylation defects<br>Molecular chaperones<br>Osteoblast maturation |
| II | Perinatal lethal | AD<br>AR | Structural alterations of type I collagen chains; overmodification | *COL1A1*<br>*COL1A2*<br>*CRTAP*<br>*P3H1*<br>*PPIB* | Structural defects<br>3-Hydroxylation defects |
| V | Hypercallus formation<br>Joint contractures (Bruck syndrome)<br>Prenatal cortical hyperostosis (Caffey disease) | AD<br>AR<br><br>AD | | *IFITM5*<br>*PLOD2*<br>*FKBP10*<br>*COL1A1* | |

*Sillence classification: original osteogenesis imperfecta classification.
AD, Autosomal dominant; AR, autosomal recessive.

them to have has as many as twenty fractures by the age of five.

The disorder is autosomal dominant, and in many cases, the individual is the first affected in their family. Their sclerae are blue that becomes gray to pale blue in adulthood. Arcus senilis not related to lipid abnormalities may occur in some patients. Other reported ocular defects include scleromalacia, keratoconus, and retinal hemorrhage.[48] Their teeth frequently show dentinogenesis imperfecta (DI) because of the effects of mutation on the tooth dentin.[49] The deciduous and permanent teeth have an opalescent and translucent appearance, which tends to darken with age. The enamel is normal, but the dentin is dysplastic; chipping of enamel occurs, and the teeth are subjected to erosion and breakage. Affected individuals' teeth appear discolored or gray; this finding is variable in the disorder but does cosegregate in families with OI. DI can be seen in all forms of OI.

During the second and third decades of life, a characteristic high-frequency sensorineural or mixed hearing loss can be detected.[50] The incidence of mitral valve prolapse is not increased in these patients compared with the population at large, but individual kindreds with increased diameter of the aortic root or patients with aortic regurgitation have been reported.[51] Many patients complain of easy bruising, and this may result from the effects of mutation on both skin and the vessels below.

Mildly affected patients may not have fractures at birth, although there is occasionally a fracture of a clavicle or extremity during delivery. Radiographically, affected newborns have wormian bones seen on lateral views of the skull, with significant osteopenia seen through the skeleton, especially the spine. The frequency of fracture thereafter depends on the child's activity, the need for immobilization after lower extremity fractures, and the attitude of the family toward independent activity. In general, these patients may experience 5 to 15 major fractures before puberty, as well as several minor traumatic fractures of the digits or the small bones of the feet. Characteristically, the fracture rate falls dramatically after puberty, only to increase during later life. Mild scoliosis approximating 20 degrees is common. Osteopenia is observed in vertebral bodies and the peripheral skeleton and progresses with age. It is remarkable that in type I OI the long bones usually heal with no significant deformity. When compared with more severe phenotypes, children with type I OI only infrequently require the insertion of intramedullary rods and almost never experience nonunion at a fracture site.

Although osteopenia with rarefaction of the medullary space and cortical thinning are observed in radiographs, many type I cases are so mild as to be missed on routine radiographic examination. Measurement of bone mineral density (BMD) by dual-energy x-ray absorptiometry (DEXA) at any age discloses a significant decrease in bone mass. T scores (i.e., standard deviation from the young-adult mean BMD) are frequently in the range of −2.5 to −4.0 at the lumbar spine or proximal femur, consistent with the diagnosis of osteoporosis as defined by the World Health Organization (WHO). Low BMD in children with recurrent fractures may assist in identifying those with OI.

### Molecular Pathology

Type I OI results from mutations affecting the COL1A1(I) and COL1A2(I) polypeptide chains of type I collagen. Cultured fibroblasts from individuals with mild OI synthesize low amounts (approximately one-half) of the expected amounts of type I collagen. Most of the reported mutations in type I OI are nonsense and frameshift mutations and thus are predicted to lead to premature termination codons, although there are some exceptions.[52,53]

## Lethal Osteogenesis Imperfecta (Type II OI)

Approximately 10% of patients with OI have the severe neonatal form of the disease, lethal osteogenesis imperfecta. Most cases result from sporadic mutations, however, recessively inherited forms of the disease has recently been documented.[54-56] These infants are seen with severe bone fragility, multiple intrauterine fractures at various stages of healing, deformed extremities and, occasionally, hydrops fetalis. Radiographic features include wormian bones, multiple fractures, crumbled bones and characteristic beading of the ribs caused by healing callus formation.

### Molecular Pathology

Most cases occur de novo as new dominant mutations; however, autosomal recessive forms have been established, as has recurrence based on germline mosaicism.[57] The biochemical abnormality in lethal OI is the inability to synthesize and secrete normal type I collagen.[57] As a result, the amount of type I collagen in bone is low, much of the secreted collagen is abnormally overmodified, and the quantity of the minor collagens, types III and V, is relatively high. Bone collagen fibers are thinner than normal, and at the intra-cellular level type I collagen in retained within dilated endoplasmic reticulum.

Similar to most cases of OI, mutations in the genes encoding COL1A1 and COL1A2A lead to the dominant or de novo form of lethal OI.[58] Single glycine substitution with the Gly-X-Y triplet of either *COL1A1*and *COL1A2* lead to this form of OI, as do some small deletions, all producing severe effects on the triple helix. Recessive form accounts for a small number of these cases and the involved genes are listed in Table 105-2. Some of these recessive forms result from mutations in the genes encoding either *CRTAP* (cartilage-associated protein), *P3H1* (prolyl-3-hydroxylase 1), or *PPBI* (Cyclophilin B). These molecules form a complex that hydroxylate (add an –OH group) to a third-position residue at proline 986 (Pro986). This modification of a single residue is thought to stabilize the collagen helix, although the mechanism of disease production is probably much more complicated, and this complex may act as a chaperone complex.

## Severely Deforming Osteogenesis Imperfecta (Type III OI)

The deforming variant of osteogenesis imperfecta is the classic form of OI. Similar to lethal OI (type II OI), most cases are inherited as autosomal dominant (or de novo), although recurrent cases based on autosomal recessive inheritance resulting from mutations in numerous recessively inherited genes have been recently demonstrated (see Table 105-2). This variant of OI is characterized by severe deformity of the limbs and marked kyphoscoliosis, thorax deformity, and significant short stature. The extent of growth retardation is remarkable, and in many adults the height may not surpass 3 ft (90 to 100 cm). Abnormal cranial molding occurs in utero and during infancy, producing frontal bossing and a characteristic triangular-shaped facies. Radiographically, wormian bones and delayed closure of the fontanelles may be observed well into the first decade.

Pulmonary function can be diminished because of distortion of the spine and thorax and can progress with time and lead to restrictive lung disease and sleep apnea. Unfortunately, because of diminished vital capacity, pulmonary insufficiency is a leading cause of death in subjects with type III OI. Respiratory compromise develops in many patients with scoliosis greater than 60 degrees, creating a need for pulmonary investigations. Many of these individuals need supplemental oxygen.

Platybasia resulting from soft bone at the base of the skull may cause the external ear canals to slant upward while the base of the skull sinks on the cervical vertebrae. This distortion may lead to communicating or obstructive hydrocephalus, cranial nerve palsies, and upper and lower motor neuron lesions. Headache, diplopia, nystagmus, cranial nerve neuralgia, decline in motor function, urinary dysfunction, and respiratory compromise are complications of basilar invagination.[59] As opposed to type I OI, most affected patients with type III OI have white sclera as adults. Approximately 25% of patients with type III OI have dentinogenesis imperfecta, necessitating constant dental care throughout childhood. Severe hearing impairment occurs in 10% of patients, although milder degrees of hearing loss are more common.

The skeleton in these patients has significant osteopenia, leading to multiple fractures in the upper and lower extremities and vertebral bodies, particularly before puberty. Unlike type I OI, in which fractures tend to heal without deformity, fractures in type III OI frequently lead to skeletal deformity. Radiographs of the skeleton reveal marked osteopenia, thinning of cortical bone, narrowing of the diaphysis, widening of the metaphysis, which merges into a dysplastic epiphyseal zone filled with whorls of partially calcified cartilage (i.e., popcorn deformity).[60] Osteoporosis leads to collapse of vertebral end plates contributing to worsening kyphoscoliosis. Pectus excavatum or pectus carinatum adds to thoracic deformity.[61] In addition, lack of weight bearing increases the severity of osteoporosis and increases the risk of fracture. Many of the individuals become wheelchair bound at an early age or walk with mechanical assistance.

### Molecular Pathology

The molecular basis of type III OI is very similar to type II OI. Most of the cases result from heterozygosity for mutations in *COL1A1* and *COL1A2*. These mutations are glycine substitutions scattered through the triple helix, as well as in-frame deletions. As in type II OI, familial

recurrences result from recessively inherited mutations in numerous genes listed in Table 105-2.

## Osteogenesis Imperfecta of Moderate Severity (Type IV OI)

Clinically, the phenotype of patients with moderately severe OI (type IV OI) falls between the milder type I and type III OI, and this diagnosis is somewhat subjective. In most cases, this type is usually inherited in an autosomal dominant fashion. Fractures are rarely at birth, and some patients may not have the initial fracture until later in the first decade. The extent of skeletal deformity involving the spine, thorax, and extremities is usually intermediate between that of types I and III OI, and these patients have short stature and frequently have scoliosis. The patients may have some mild facial dysmorphisms but not as severe as type III OI. Hearing loss occurs but less than in types I and III OI.

Most fractures occur during childhood and may reoccur during the post-menopausal period in women or in men older than age 50 years.[61] Long-bone deformity tends to develop after fractures, which may lead to a difficulty in ambulation. Radiographs of the long bones and vertebral bodies demonstrate marked osteopenia with vertebral collapse. Although there is marked cortical thinning, bowing, and coarsening of trabeculae, the overall architecture of the bone is normal.

### Molecular Pathology

The molecular mechanisms for type IV OI are similar to the other forms. The mutations are in *COL1A1* and *COL1A2* and include glycine substitution and in-frame deletions. At the present time, there is no evidence for an autosomal recessive form of type IV OI.

## Type V Osteogenesis Imperfecta

This phenotype was reported by in 2000 as a variant within the heterogeneous group classified under type IV OI.[62] In the initial report of seven patients with OI, the phenotype was distinguished by the following criteria: moderate fracture history, hyperplastic callus formation, limitation in forearm pronation and supination due to intramembraneous bone formation at the joint, normal sclera, and no DI. Bone biopsy showed a meshlike appearance of irregularly spaced lamellae, different from the woven bone seen in types II, III, and IV OI. This etiology of this rare form has been established and results from heterozygosity for mutations in *IFITM5*.[63]

## Histopathology of Bone in Osteogenesis Imperfecta

The range of histologic appearances of bone in the different OI phenotypes is as variable as the clinical phenotypes. Undermineralization and overmineralization of bone have been recognized within the same specimen.[47] Bone histomorphology appears relatively normal in type I OI, but osteopenia caused by thin lamellar plates and diminished cortical width is evident. Immature woven bone and lamellar disarray are characteristic of more severe OI phenotypes.[47]

## Treatment

During the years, there have been multiple attempts to treat OI with a variety of vitamins, hormones, and drugs, none of which has been successful. The list includes administration of mineral supplements, fluoride, androgenic steroids, ascorbic acid, and vitamin D. During the last 15 years, bisphosphonates administered parenterally to children has demonstrated favorable results. Intravenously administered bisphosphonate pamidronate increased bone mass, decreased skeletal pain, and decreased fracture incidence in children with severe OI.[64] Dosage regimens in different series for children and adults have varied from 1 mg/kg to 3 mg/kg, administered intravenously at 2- to 4-month intervals, and lower-dosage regimens also have been reported.[64] In general, reports indicate a significant increase in bone mass in children and a decrease in fracture rate. The effect is most marked in the spine, where vertebral remodeling may improve vertebral height. Metabolic studies by have demonstrated a decrease in serum ionized calcium and increase in serum parathyroid hormone (PTH). The major side effects of intravenous (IV) bisphosphonate treatment involve the acute phase response (24 hours after infusion) and the occurrence of otitis and vestibular imbalance in a small number of patients. The currently recommended treatment regimen includes the use of a bisphosphonate with adequate calcium and vitamin D supplementation to avoid the occurrence of hypercalciuria and to maintain serum vitamin D levels within normal limits. Recently, the use of anabolic treatment to build bone has been tested by using teriparatide in adult patients in OI, and the study showed that patients with mild OI, but not severe OI, benefited from treatment.[65]

The use of surgery to correct deformities and to facilitate weight bearing has been the subject of several reviews.[66] Multiple osteotomies and realignment of a deformed bone over intramedullary rods is an option for many children with severe bowing.[67] Indications include frequent fractures at the apex of the bow, impaired standing, or limb-length inequality resulting from bowing.[68] Expanding (telescoping) rods are best for growing children, for they require fewer revisions. Spinal deformities are common and usually progressive. Surgical stabilization is most advisable in the teen years or early adulthood when patients are best able to tolerate these complex reconstructions.[69] Early basilar invagination may be halted with prophylactic posterior fusion of the occipital-cervical junction with plate fixation.[70] Patients with severe brain stem compression may require anterior transoral decompression, as well as posterior instrumented fusion. Patients with various types of OI appear to be at increased risk of premature OA, and the reasons for this are not clear. Total joint arthroplasty is usually successful in these patients, thus referral is appropriate.[71]

Every child with OI benefits from appropriate rehabilitative therapy.[72] Bracing with lightweight plastics while the child begins to walk can minimize microfracture and bowing of the upper femurs. Muscle-strengthening exercises are essential as primary care and after immobilization for fracture. Perhaps the most beneficial programs have been

developed around swimming, preferably in heated pools, and as part of continuous rehabilitative medical care.

## EHLERS-DANLOS SYNDROME

The heterogenous group of disorders grouped together as Ehlers-Danlos syndrome (EDS) illustrates the genetic and clinical variability characteristic of the heritable disorders of connective tissue. The most cardinal feature of these disorders is the presence of joint hypermobility, associated with an increase in skin elasticity and skin fragility. There are more than 10 recognized types of EDS, and in 1997, a simplified classification was proposed, dividing EDS into six major clinical types. The classification includes the types of classic, hypermobility, vascular, kyphoscoliosis type, arthrochalasia, and dermosparasix, and several rarer EDS types, grouped into "other forms."[73] However, clinically EDS can be difficult to separate because of considerable overlap in phenotype findings.

### Ehlers-Danlos Syndrome (Classic Type)

#### Clinical Features

This autosomal dominant type of EDS accounts for approximately 80% of the reported cases[74] and is inherited as an autosomal dominant trait. Originally, EDS was classified as types I and II, and now they are classified as the classic form, although these subclassifications are still in use by some clinicians. Previously, they were distinguished from each other based on joint laxity and skin fragility, which are less severe than in type II EDS. Most prototypic forms of EDS are characterized by varying degrees of hyperextensibility of large and small joints and are classic findings in EDS. Thus it is critical that hyperextensibility be defined, and differentiating mild "normal" laxity from hyperextensibility can be challenging. Beighton has presented a clinically useful classification of joint laxity.[74]

1. Passive dorsiflexion of the fifth digit beyond 90 degrees = one point for each hand
2. Passive apposition of the thumbs to the flexor surface of the radius = one point for each hand
3. Hyperextension of the elbows beyond 10 degrees = one point for each side
4. Hyperextension of the knees beyond 10 degrees = one point for each knee
5. Flexion of the trunk forward so that the palms are placed flat on the ground = one point

A score of five or more points is defined as joint hypermobility.

Large-joint hyperextensibility is seen in varying degrees in the classic form and decreases with age. Recurrent joint dislocations, periodic joint effusion related to trauma, and the eventual appearance of OA pose significant management problems. Bilateral synovial thickening has been observed in EDS, along with the accumulation of small masses of crystalline material in synovial villi. It was observed that patients with EDS constituted 5% of cases in a pediatric arthritis clinic population.[75] There is debate about whether affected infants may be born prematurely to affected mothers because of early rupture of amniotic membranes.[76] Patients with EDS have characteristic facies, with a broad nasal root and epicanthal folds. They may have large, lax ears, and traction on the ears or elbows reveals skin hyperextensibility. Another sign of hypermobility is the ability to touch the tip of the tongue to the nose (Gorlin's sign).[77,78] In addition, absence of the lingual frenulum is characteristic for this disorder.[77]

In EDS the skin has a characteristic soft or "velvety" feel that can be appreciated by stroking of the forearms. Thin, atrophic corrugated and hyperpigmented scars are found on the forehead, under the chin, and on the lower extremities (known as *cigarette paper* or *papyraceous* scars), although this is not a uniform finding. Typically, skin lesions heal slowly after injury or surgery. Molluscoid pseudotumors (violaceous subcutaneous tumors ranging in size from 0.5 to 3 cm) may be palpated in tissue over pressure points on the forearms and lower extremities and may be seen on radiographs. Although many patients claim to bruise easily, ecchymoses distributed on the extremities are found only in patients with the more severe forms of the disorder. Severe bilateral varicose veins are a common problem.

Associated pulmonary complications of EDS include spontaneous pneumothorax, pneumomediastinum, and subpleural blebs.[79] Mitral valve prolapse and tricuspid valve insufficiency may complicate the classic EDS, and aortic root dilatation has been reported, although the rate of progression is unknown.[80,81] The spectrum of skeletal abnormalities include thoracolumbar kyphoscoliosis, a long, giraffe-like neck, downward sloping of the ribs of the upper part of the thorax, and a tendency toward reversal of the normal cervical, thoracic, and lumbar curves. Anterior wedging of thoracic vertebral bodies occasionally is seen.

### Ehlers-Danlos Syndrome (Hypermobility Type)

The hypermobile type of EDS is a dominantly inherited disorder that presents as marked joint and spine hypermobility, recurrent joint dislocations, and the typical soft skin that is neither hyperextensible nor velvety. Individuals with EDS III may have virtually normal skin. Because of the extent of joint laxity affecting both large and small joints, these patients experience multiple dislocations and may require surgical repair. The shoulders, patellae, and temporomandibular joints are frequently sites of dislocation. Musculoskeletal pain may mimic that of the fibromyalgia syndrome, and patients frequently seek medical attention for symptoms consistent with chronic pain.

One of the difficulties in this subtype is differentiating it from benign hypermobility syndrome. Benign hypermobility syndrome was used to describe patients with generalized joint laxity, associated musculoskeletal complaints, but normal skin.[82] They do not have the classic stigmata of either EDS or Marfan syndrome. Many of these patients are seen in their twenties and thirties with rheumatologic symptoms that can pose problems in diagnosis and treatment. The precise approach and treatment for these patients is not clear.

### Structural and Molecular Pathology of the Classic and Hypermobile Type of Ehlers-Danlos Syndrome

Abnormally large, small, or frayed dermal collagen fibrils and disordered elastic fibers have been observed in the classic and hypermobile forms of EDS by electromicroscopy.[83]

Type V collagen is a heterotrimeric collagen composed of the products of three genes: *COL5A(V)*, *COL5A(V)*, and *COL5A(V)*. Type V collagen may stabilize type I collagen by co-assembling with that protein. It has been established that approximately 50% of patients with either the classic or hypermobility types of EDS have mutations in *COL5A1(V)* or *COL5A2(V)*.[84] Furthermore, in some cases of EDS classic type a heterozygosity for mutations in *COL1A1* has been seen.[85]

## Ehlers-Danlos Syndrome (Vascular Type)

This autosomal dominant disorder is one of the most severe forms of EDS and was formerly referred to as *EDS type IV*. It is associated with arterial rupture, commonly involving iliac, splenic, renal arteries, or the aorta, and resulting in either massive hematomas or death.[86] Arterial rupture may lead to stroke or intracompartmental bleeding in a limb. Patients with this disorder are also susceptible to rupture of internal viscera and may experience repeated rupture of diverticula on the antimesenteric border of the large bowel. Problems with pregnancy vary from pre-term delivery to uterine or vascular rupture, although delivery is uneventful in many instances.[87,88] Typical causes of death in families with EDS have included gastrointestinal rupture, peripartum uterine rupture, rupture of the hepatic artery, and vascular ruptures.

In contrast to the other forms of EDS, EDS IV is not associated with hyperextensibility of large joints, although small joints may be minimally hypermobile. These patients have thin, soft, transparent skin, through which a prominent venous pattern is seen, especially on their chest walls. Their skin is not "doughy" as in the classic form. Excessive bruisability may occur. Vascular EDS includes, as a subgroup, patients who have been described as *acrogyric*. These individuals have characteristic thin faces, prominent eyes, and extremities that lack subcutaneous fat, giving the appearance of premature aging. Peripheral joint contractures and acro-osteolysis have been described.[89] Spontaneous hemopneumothorax associated with hemoptysis and mitral valve prolapse occurs frequently.[90] Surgical repair of ruptured vessels or internal viscera is extremely difficult because of friable tissues. Anesthetic and surgical difficulties related to intubation, spontaneous arterial bleeding during surgery, and the ligation of vessels that tear under pressure complicate surgical maneuvers. Similarly, arteriography may be dangerous in these patients. These patients can be quite difficult to manage. Imaging studies may reveal normal-appearing aorta or other large vessels that rupture shortly after a "normal study."

### Molecular Pathology

Although EDS IV was clinically recognized as a disorder distinct from the other forms of EDS, the finding that tissues from these individuals were deficient in type III collagen clearly distinguished this as a separate form of EDS. Type III collagen is a homotrimer (1[III]3) found in skin, blood vessels, and the walls of hollow viscera. Heterozygosity for mutations in the gene encoding *COL3A1* leads to EDS vascular type and affects both the synthesis and secretion of type III collagen.[90-92] Various types of mutations have been identified, including missense, nonsense, and deletions, and there is no correlation between the clinical phenotype and type of type III collagen mutation. In this disorder biochemical abnormalities include decreased production of type III collagen or production of an abnormal homotrimer that is retained in the endoplasmic reticulum and, if secreted, contributes an an abnormal extra-cellular matrix. Biochemical and mutational analysis for this disorder is available. Optimal surveillance of these patients, particularly for vascular adverse events, has not been determined.

## Therapy in the Classic, Hypermobility, and Vascular Types of Ehlers-Danlos Syndrome

There are no specific treatments for these forms of EDS. Supportive therapy, however, is essential for preservation of normal joint function and alleviation of joint pain. Planned exercise programs and muscle-strengthening exercises are useful and do much to maintain a positive outlook in these individuals, who may have a poor prognosis if joint stability and articular surfaces are compromised by excessive activity or chronic trauma. Unfortunately, many children and young adults with large joint hypermobility are attracted to activities such as gymnastics and dance, and these activities promote hypermobility and joint damage. The presence of multiple ecchymoses raises concern about a bleeding diathesis, particularly at the time of elective surgery. Although no consistent basis exists for the hemorrhagic tendency in the classic and hyperextensibility forms of EDS, anecdotally these patients tend to have higher blood losses than expected at surgery. In our center, we discourage pregnancy in patients with the vascular form, because the mortality rate is increased.

## Arthrochalasia Type

Formerly known as *EDS VIIA* and *VIIB*, this is an another autosomal dominant form of EDS resulting from mutations that cause faulty processing of type I collagen at the N-terminus.[93,94] EDS, arthrochalasia type, is characterized by pronounced and generalized joint hypermobility, moderate cutaneous elasticity, moderate bruising, a characteristic round facies with midface hypoplasia, and significant short stature. Their skin has a doughy feel and is fragile and hyperelastic. Kyphoscoliosis and muscle hypotonia are frequently present. These patients experience multiple dislocations, particularly involving large joints, including the hips, knees, and ankles. These dislocations often present in the newborn period, especially at the hips and ankles. Patients frequently need orthopedic surgery for their joint dislocation, and their tissues are highly friable, which complicates orthopedic procedures.

## Molecular Pathology

The two disorders (EDS VIIA and EDS VIIB), termed *arthrochalasia type*, result from mutations involving the N-terminal propeptide cleavage site of type I collagen. The arthrochalasia type of EDS has provided insight into the process of normal type I collagen fiber formation. The initial observation was of an accumulation of unprocessed

procollagen within the dermis of affected individuals.[95] With subsequent recognition that procollagen has both N- and C-terminal extension propeptides and that separate enzymes are responsible for their removal, the syndrome became more sharply defined as an accumulation of procollagen with the N-terminal peptides still attached (pN collagen).[96] Of the two distinctly different genetic abnormalities resulting in procollagen accumulation, the more frequent form is the mutational resistance of a procollagen cleavage site to the action of the N-terminal procollagen peptidase. The resistance results from an amino-acid substitution or deletion in the proCOL1A1 or pro2COL2A1chain (type VIIA and VIIB, respectively), leading to a portion of the collagen chains containing an abnormal N-terminal extension. This results from mutations in *COL1A1* or *COL1A2* in exon 6 of the molecule, which alters the proteinase cleavage site. Those individuals with mutations in exon 6 of *COL1A1* are more severely affected than those with similar mutations in *COL1A2*.

### Ehlers-Danlos Syndrome (Dermatosparaxis Type)

This type, dermosparaxix type, was formerly known as *EDS VIIC* and is an autosomal recessive form of EDS. In this type, the skin is extremely fragile, soft, and doughy, with easy bruising. The phenotype includes blue sclerae, marked joint hypermobility, micrognathia, large umbilical hernia, epiphyseal delay, and mild hirsuitism.[93] The dermosparaxix type results from a deficiency of the procollagen-N-propeptidase, in contrast to the arthrochalastic form, which involves the enzyme cleavage site and individuals have been identified who are homozygous form mutations in the gene,. Interestingly, this defect is homologous to the defect in sheep and cattle termed *dermatosparaxis*.[97]

### Ehlers-Danlos Syndrome (Kyphoscoliosis Type)

This type of EDS, kyphoscoliosis type, formerly known as *EDS VI*, is inherited as an autosomal recessive disease. The findings in this disorder include severe kyphoscoliosis, noted-at-birth recurrent joint dislocations, hyperextensible skin and joints, poor tone, and reduced muscle mass.[98] The skin is grossly abnormal, and has been described as pale, translucent, velvety and, upon trauma, showing gaping wounds that heal poorly. One difference in this form of EDS is that there is significant ocular involvement. Affected individuals have microcornea, retinal detachment, and glaucoma leading to blindness in some individuals. In addition, respiratory and cardiac compromise and, ultimately, cardiorespiratory failure may develop in patients with severe kyphoscoliosis.

#### Molecular Pathology

This disorder results from lysyl hydroxylase deficiency.[99] A variety of mutations within the lysyl hydroxylase gene have been defined and include premature stop codons, amino-acid substitutions, internal deletions, and compound heterozygotes.[99] Defective lysyl hydroxylase impairs the conversion of lysyl residues to hydroxylysine on procollagen peptides. The consequence of deficient hydroxylysine content of collagen is the effect it has on cross-linking, which helps stabilize the mature collagen molecule.

### Other Ehlers-Danlos Syndrome Types

There are numerous other rare forms of EDS that have some overlap with other disorders or have only been reported in a small cohort of individuals, and thus will not be included in the discussion.

## MARFAN SYNDROME

One of the most common inherited disorders of connective tissue, Marfan syndrome (MFS) is an autosomal dominant disorder with a reported incidence of 1 in 10,000 to 20,000 individuals.[100] There is a wide spectrum of clinical presentations, from the severe infantile form to individuals who are only mildly affected. Although the most impressive findings are relative to the musculoskeletal, cardiac, and ocular findings, affected individuals also suffer from pulmonary, neurologic, and psychological complications. Marfan syndrome has also become one of the few genetic disorders for which there has been advocacy for treatment to slow the progression of the disease. Physicians need to recognize the phenotype because many of the affected individuals are seen with life-threatening emergencies.

### Clinical Features

Marfan syndrome can be difficult to diagnose in some individuals and families, and it also has been recognized that it has been overdiagnosed. Stringent criteria for this diagnosis were proposed in 1996 and revised in 2010.[100] The 1996 criteria rely on the recognition of both "major" and "minor" clinical manifestations involving the skeletal, cardiovascular, dura, and ocular systems. Major criteria include four of eight typical skeletal manifestation and ectopia lentis, aortic root dilatation involving the sinuses of Valsalva or aortic dissection, and lumbosacral dural ectasia by computed tomography (CT) or MRI. Major criteria for establishing the diagnosis in a family member include having a parent, child, or sibling who meets a major criterion independently, the presence of a *FBN1* mutation. To unequivocally establish the diagnosis in the absence of a family history requires a major manifestation from two systems and involvement of a third system. If a mutation known to cause MFS is identified, the diagnosis requires one major criterion and involvement of a second organ system. The reason for this is that there is a great deal of intrafamilial variability in this disorder, and there are individuals who harbor heterozygosity for mutations, yet do not meet criteria for Marfan syndrome and may have different prognoses.[101] Similar to other connective tissue disorders, there is wide variability in phenotypic expression.

Aortic disease leading to the formation of aneurismal dilatation and dissection is the main cause of morbidity and mortality in the MFS.[101] Dilatation of the aorta is found in 50% of children and progresses with time. Echocardiography demonstrates that 60% to 80% of adult patients have dilatation of the aortic root that may involve other segments of the thoracic aorta, the abdominal aorta, or even

the carotid and intracranial arteries. Dissection usually begins above the coronary ostia and extends the entire length of the aorta. Of patients with MFS, 60% to 70% have mitral valve prolapse with regurgitation. Heart failure and myocardial infarction may complicate the course of patients with MSF. Pregnant women are at particular risk for aortic dissection, particularly those who already have aortic root dilatation; this should be taken into consideration when treating women of reproductive age with MFS.[102]

Arachnodactyly occurs in 90% of patients. Listed below are techniques that aid in determining arachnodactyly.

- The thumb. The Steinberg test is positive when the thumb, enclosed in the clenched fist, extends beyond the hypothenar border.
- The wrist. The Walker-Murdoch sign is positive when there is overlap of the thumb and fifth digit when they encircle the opposite wrist.
- The metacarpal. The metacarpal index is done by radiographic determination and is the mean value of the lengths divided by the midpoint widths of the second, third, and fourth metacarpals. In healthy patients, the metacarpal index ranges from 5.4 to 7.9, whereas this range is 8.4 to 10.4 in patients with MFS.

Thoracic kyphosis may be associated with reduced lung capacity and residual volume that may lead to pulmonary insufficiency. Dural ectasia, which may occur in as many as 40% of patients, results from enlargement of the spinal canal caused by progressive ectasia of the dura and neural foramina and erosion of vertebral bone. This usually involves the lower spine.[103] Diminished BMD has been reported in several patients with MFS.[104] Ectopia lentis occurs in 50% to 80% of patients with MFS. Subluxation of the lens is usually bilateral and appears by the age of 5 years. Although the lens is typically displaced upward, displacement into any quadrant may occur. Visual acuity is diminished in many patients because of lens subluxation or secondary acute glaucoma. Secondary myopia, retinal detachment, and iritis with loss of vision contribute to most of the ocular-related morbidity.[105]

Large epidural venous plexuses in the lumbar and cervical regions have been found to develop in patients with MFS, a major diagnostic criterion for the syndrome. These engorged venous plexuses, which are visualized by MRI myelography have, in turn, been associated with the syndrome of spontaneous intracranial hypotension, which is also associated with dural tears. Clinical signs of this are severe headache, back and leg pain, radiculopathies, and incontinence secondary to cerebral displacement. Spinal abnormalities in MFS include increased interpedicle distance of nonrotated vertebrae, vertebral inversion (flattening of the normal kyphosis at the dorsal level and kyphosis or disappearance of the physiologic lordosis at the lumbar level), and vertebral dysplasia (dolichospondylia, elongated vertebral bodies with increased concavity). Scoliosis constitutes one of the major management problems in MFS. In one series, the average age of onset was 10.5 years (range of 3 to 15 years), with rapid progression during adolescence. If mechanical bracing or physical therapy fails to halt progression, spinal fusion should be considered, particularly when the curvature exceeds 45 to 50 degrees.[106,107]

## Differential Diagnosis

### Homocystinuria

Homocystinuria, which shares several skeletal and ocular features with MFS, is the prime diagnostic consideration.[108] This is an autosomal recessive disease. The characteristic features of this metabolic disorder of sulfur metabolism are marfanoid phenotype with joint laxity, scoliosis, lens dislocation, early-onset osteoporosis, vascular thrombosis affecting arteries and veins due to increased clotting activity and the cytotoxic effect of homocysteine on vascular endothelial cells, and mild mental retardation.[108]

Cystathionine β-synthase (CBS) deficiency is the most common cause of homocystinuria.[108] Affected individuals have elevated levels of homocystine and methionine levels, whereas cystathionine and cysteine levels in blood are decreased. Importantly, this disorder is differentiated from MFS because the direction of ectopia lentis is different than MFS and there is no progressive aortic root dilation.

## Molecular Biology of Marfan Syndrome

Fibrillin 1 (FBN1) protein is an important component of both elastic and nonelastic connective tissues throughout the body.[109] It is the main protein of a group of connective tissue microfibrils that are essential for normal elastic fibrillogenesis. In nonelastic tissues, the FBN1-containing microfibril functions as an anchoring fiber. *FBN1* is a large gene (65 exons).

*FBN1* mutations occur across a wide range of milder phenotypes that overlap the classic Marfan phenotype, including dominantly inherited ectopia lentis, Shprintzen-Goldberg syndrome, acromicric dysplasia, Weill-Marchesani syndrome, and familial or isolated forms of aortic aneurysms.[110-115] The majority of these are private mutations (occur genetically independent with no "hot spot" in the molecule). The one exception is the rare infantile MFS mutations, which cluster between exons 24 and 26 and exon 32.[116] Heterozygosity for missense, frameshifts, deletions and insertions, splice site alterations, and nonsense mutations have all been seen.[117] The clinical presentation of the fibrillinopathies caused by *FBN1* mutations range from isolated ectopia lentis, to short stature in acromicric dysplasia, to severe neonatal MFS, which generally leads to death within the first 2 years of life

## Treatment

In 1972, the life span of untreated patients with classic MFS was approximately 32 years. The early mortality in the MFS results primarily from complications associated with aortic dilatation. This symmetric dilatation of the sinuses of Valsalva is progressive throughout life and is often detectable in infancy. In the early 1970s, it was suggested that perhaps treatment and surveillance could lessen the complications of aortic dissection in MFS. There have been many treatment trials with β-blockers. When compared with the control group, the treated individuals had a significantly slower rate of dilatation of the aortic root, improved survival, and fewer treated patients reached a clinical end point (death, congestive heart failure, aortic regurgitation, aortic dissection, or cardiovascular surgery).[118,119]

Data generated from mouse models of MFS suggest excessive signaling by the transforming growth factor (TGF)-β family of cytokines.[120] A large randomized control trial on these findings suggested that losartan, an antihypertensive, and an angiotensin II blocker would have advantages over β-blocker use because it can attenuate TGF-β signaling. A randomized control study in MFS using atenolol versus losartan did not show any difference between the two drugs.[121]

Echocardiograms are the mainstay of management for the aortic root complications of MFS. Elective repair of aortic root disease before enlargement to 4.5 cm is typically suggested and is preferable to emergency repair required for marked dilatation or dissection.[122] Mitral valve replacement is frequently accomplished during the same procedure. Most importantly, repeated trials have shown that patients who undergo elective repair versus emergent repair do substantially better.

Correction of scoliosis may be attempted with bracing; however, surgical repair should be considered when the curve exceeds 40 degrees. Progressive scoliosis in MFS may require fixation with rods, and complications of joint laxity may require orthopedic correction. Arthropathy associated with excessive joint mobility may require orthopedic intervention. Dislocated lenses should not be removed surgically unless more conventional means of correcting vision are ineffective.

## LOEYS-DIETZ SYNDROME

In 2005, individuals with a previously undescribed autosomal dominant aortic aneurysm syndrome were described.[123] This disorder, now referred to as *Loeys-Dietz syndrome*, is also characterized by hypertelorism, bifid uvula and/or cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm and dissection. Other abnormal findings include craniosynostosis, structural brain abnormalities, mental retardation, congenital heart disease, and aneurysms with dissection throughout the arterial tree.

Some individuals with Loeys-Dietz syndrome have a clinical phenotype that overlaps with MFS, but none met diagnostic criteria set forth. Although MFS is associated with progressive arterial disease, in Loeys-Dietz syndrome, the aneurysms tend to be particularly aggressive and rupture at an earlier stage and size than seen in MFS.[123] Heterozygosity for mutations in *TGFBR1*, *TGFBR2*, *TGFB2*, and *SMAD3* have been identified.[123-125] From a management perspective, it is important to recognize these individuals because they are managed more aggressively than in MFS.[126] Aortic aneurysms are corrected at smaller sizes (4 cm), and complaints such as abdominal pain and headaches should be thoroughly investigated because they may be associated with aneurysms. The distinction from MFS is important because the management needs to be even more aggressive.

## CONGENITAL CONTRACTURAL ARACHNODACTYLY

Congenital contractural arachnodactyly is an autosomal dominant condition that includes tall stature, arachnodactyly, dolichostenomelia, and multiple contractures involving large joints.[127] There is a characteristic "crumpled ear" deformity caused by a flattened helix with partial obliteration of the concha. Marked deformity of the chest cage also occurs, and scoliosis may be progressive and severe. For unknown reasons, the contractures tend to become less severe with age. Radiographically, osteopenia can be seen. The ocular and typical cardiac lesions of classic MFS are absent. This disorders result from heterozygosity for mutation in *FBN2*.[127]

## CONCLUSION

The heritable disorders of connective tissues are a heterogenous group of disorders characterized by abnormalities in skeletal tissues, which include cartilage, bone, tendon, ligament, muscle and skin. They are fascinating because the clinical spectrum ranges from extreme short stature to excessively tall individuals, and the types of altered genes span all numerous gene families and pathways. Affected individuals usually need medical attention their entire lives and, unfortunately, have been victims of appearing different because they cannot mask their abnormalities. Understanding and appreciation for the unique set of medical issues in each disorder will hopefully improve these individuals' quality of life and their life span.


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PART 16 RHEUMATIC DISEASES OF CHILDHOOD

CHAPTER 106



# Etiology and Pathogenesis of Juvenile Idiopathic Arthritis

Lucy R. Wedderburn • David Bending • Kiran Nistala

**KEY POINTS**

- **Juvenile idiopathic arthritis (JIA) is a group of conditions with distinct clinical phenotypes and, likely, differing underlying pathogenic mechanisms.**
- **The heterogeneity of JIA at pathologic and immunologic levels is informative in driving our understanding of these diseases.**
- **An increasing number of genetic regions, outside of the major histocompatibility complex (MHC), have been linked to JIA. The majority of confirmed genetic hits are polymorphisms lying outside of coding DNA, many of them in enhancer regions.**
- **In JIA there is evidence to support abnormalities in both innate and adaptive immune systems. Successful treatments may require targeting of multiple cytokine pathways in a single patient.**
- **Strong links with MHC loci, threshold and signaling pathways, and the presence of inflammatory T cells within the inflamed joint supports a role for T cells in the pathogenesis of JIA.**
- **Systemic arthritis has an immune profile distinct from other types of JIA. Inflammatory mediators of the innate immune system, including IL-1β, IL-6, and myeloid-related protein, play a major role in disease pathogenesis.**

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of diseases that lead to a final common pathway: thickening and inflammation of the joint lining (or synovium membrane). Infiltrating inflammatory cells interact with each other and resident synovial fibroblasts, promoting a chronic inflammatory process in the synovial membrane and secreted synovial fluid (Figure 106-1). In this chapter, we discuss the genetic factors that predispose to JIA and the abnormalities that underlie synovial and systemic inflammation. As well as the established association between the major histocompatibility complex (MHC) loci and JIA, more recent studies have detected links to genes that regulate cellular activation, signaling thresholds, or cytokines.[1,2] The fact that immune-related genes make up the major risk alleles in JIA strongly supports the concept of JIA as a disease of dysregulated immunity.

Highly activated T cells, monocytes, and neutrophils are attracted to joints and secrete mediators that perpetuate inflammation and attenuate immune regulation. The relative importance of individual cytokines varies among disease subtypes. Results from therapeutic trials support a role for TNF in the pathology of polyarthritis forms of JIA, and IL-1β and IL-6 in systemic JIA (sJIA), as well as some patients with polyarticular JIA (Table 106-1). The last decade has seen a step change in the quality of treatments in JIA. To build upon this success, we need to be able to interrogate the vast array of biologic data that is emerging in field of JIA, and translate this knowledge into novel therapies and a more tailored treatment approach for our patients.

The term *juvenile idiopathic arthritis* refers not to one disease but to a group of conditions, defined under the International League of Associations for Rheumatology (ILAR) classification, which have the common features of beginning before the sixteenth birthday and characterized by arthritis of at least one joint that persists for 6 weeks or more.[3] Although this classification has proven highly valuable for both clinical and basic research, and has enabled more precisely defined subgroups and comparison of data from different studies, it does not encompass all aspects of the heterogeneity of childhood arthritis. Thus, for example, use of the classification criteria with strict exclusion rules may lead to as many as 30% of cases designated as "unclassified." In addition, certain common features may occur across several subtypes. For example, factors associated with risk of autoimmune uveitis of JIA include positivity of the anti-nuclear antibody (ANA), early age at onset, and female sex. Girls with young-age onset of arthritis and ANA positivity represent a group of patients who have been proposed to represent a relatively homogeneous group yet currently may fall into several subtypes within the ILAR classification.[4] Similarly, genes and immunologic processes that influence the likelihood of mild oligoarthritis to extend to more severe arthritis may well overlap with etiopathologic factors involved in polyarticular JIA.[5,6] Furthermore, an increasing understanding of the pathogenesis of systemic-onset JIA has led to the suggestion that this condition may be more akin to an autoinflammatory disorder and should perhaps be classified separately.[7] In the future, a full molecular and genetic analysis of the heterogeneity of childhood arthritis may permit the development of a more mechanism-driven classification, which could help inform on disease course, complications, or response to treatment.

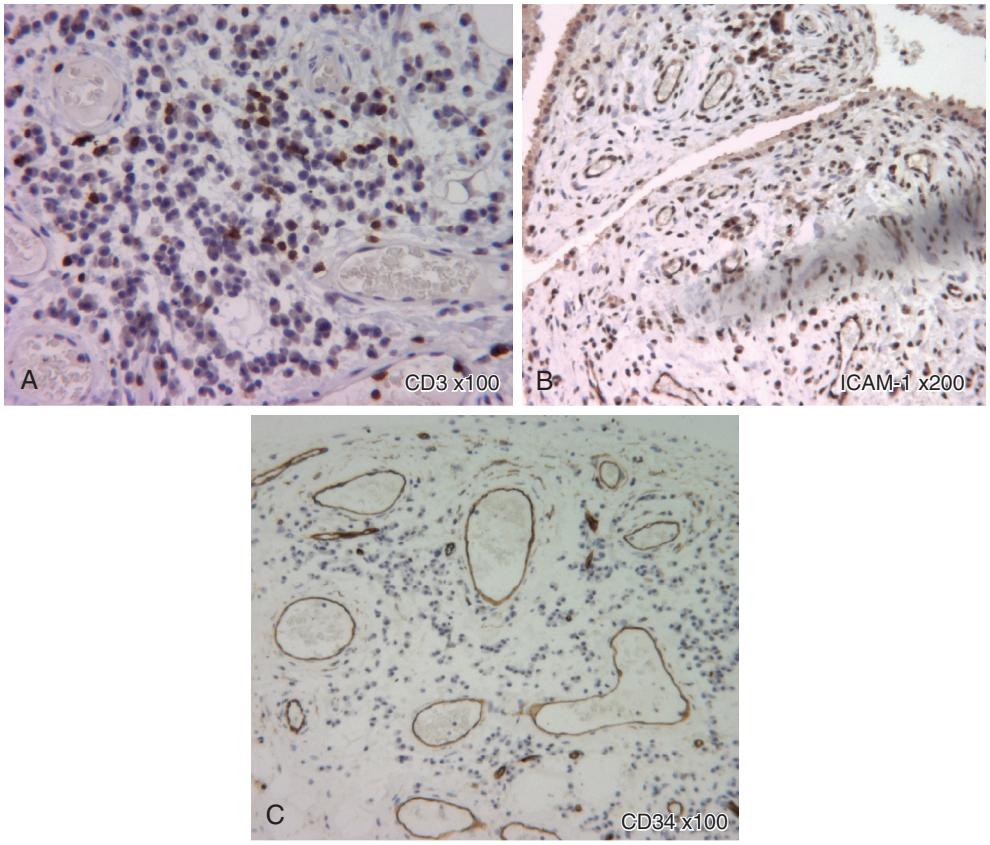



**Figure 106-1** Sections of synovium from the knee of a child with oligoarticular juvenile idiopathic arthritis showing intense inflammatory infiltrate and highly vascular hypertrophied tissue. **A,** Stained for CD3 (surface protein on T lymphocytes) (magnification, ×100). **B,** Stained for intra-cellular adhesion molecule 1, which is expressed on the endothelium and a proportion of the infiltrating cells (magnification, ×200). **C,** Stained for CD34, expressed on vascular endothelium (and hematopoietic stem cells) (magnification, ×100).

**TABLE 106-1** Summary of Cytokines and Inflammatory Mediators Implicated in Juvenile Idiopathic Arthritis

| Cytokine/ Mediator | Cell | Pathology |
|---|---|---|
| TNF | Monocytes, T, B cells, PMNs, mast cells, fibroblasts | Activates monocytes and neutrophils<br>Damages cartilage<br>↑Endothelial cell adhesion molecules<br>Inhibits regulatory T cells |
| IL-1β | Monocytes, B cells, fibroblasts | Activates osteoclasts (bone damage)<br>Fibroblast cytokine, chemokine release<br>↑Endothelial cell adhesion molecules |
| IL-17 | T cells (Th17), mast cells | Chemokine release (recruit PMNs)<br>Cartilage damage<br>Activates osteoclasts<br>Synergizes with TNF and IL-1β |
| IL-6 | Monocytes, fibroblasts, B cells | B cell activation<br>Inhibits regulatory T cells<br>Growth retardation<br>Acute phase response and anemia |
| IFN-γ | T cells ($CD4^+$ Th1, $CD8^+$, NK cells) | Activates monocytes<br>↑Endothelial cell adhesion molecules<br>May assist recruitment of Th17 cells |
| MRP 8/14 | Monocytes, PMNs | Activates monocytes<br>Promotes pathologic $CD8^+$ T cells<br>Secretion of IL-1β<br>↑Endothelial cell adhesion molecules |

IFN-γ, Interferon-γ; IL, interleukin; MRP, myeloid-related protein; NK, natural killer; PMNs, polymorphonuclear neutrophils; TNF, tumor necrosis factor.

Both genetic and environmental factors, as well as the manifestations of their interactions through inflammatory and immunologic mechanisms, are clearly implicated in the pathogenesis of JIA. Recent advances in our understanding of some clinical subtypes, most notably systemic JIA, have led to specific and distinct features being recently recognized that have led to novel treatment options. However, it is also increasingly clear that some underlying genetic risk associations and immune abnormalities may have effects across several types of childhood arthritis and, indeed, across many forms of autoimmune disease.

## HISTOLOGIC FEATURES OF SYNOVIUM IN JUVENILE IDIOPATHIC ARTHRITIS

The pathologic hallmark of juvenile inflammatory arthritis is the inflamed synovium. Histology of this tissue shows thickened synovium that is highly vascular and shows marked hyperplasia of synoviocytes in the lining layer, as well as a dense infiltrate of inflammatory cells comprising T cells, macrophages, dendritic cells and, in some cases, B cells and natural killer (NK) cells[8,9] (see Figure 106-1). The hypertrophied synovial layer is highly vascular and likely related to the increased production of proangiogenic factors, including vascular endothelial growth factor (VEGF), osteopontin, and the angiogenic chemokines,[10-12] whereas recruitment of the inflammatory infiltrate is likely mediated by multiple chemokines that are increased in JIA, including

CCL5, CXCL10, CCL20, IL-8, and membrane cofactor protein (MCP)-1, among others.[13,14] The proteolytic enzymes, matrix metalloproteinases (MMP)-1 and MMP-3, are abundantly expressed in synovial lining, and their levels correlate with degree of infiltration, in particular by myeloid $CD68^+$ cells.[14] The T cells that infiltrate the inflamed synovium, like those in the excess synovial fluid, are highly activated memory cells[15,16] of both CD4 and CD8 populations.[17]

## Genetics of Juvenile Idiopathic Arthritis

**KEY POINTS**

- There is strong evidence for a genetic component to the etiology of JIA.
- The strongest genetic associations of JIA are with genes of MHC.
- Specific class I MHC alleles are associated with enthesitis-related arthritis.
- Distinct class II MHC alleles are associated with oligoarthritis and polyarthritis subtypes.
- Many genes that alter the threshold of immune cell activation and signaling, or relate to cytokines and their receptors, are linked with JIA.
- Recent evidence suggests that many of the genetic differences associated with JIA fall in enhancer regions that affect gene expression rather than in coding sequences.

There is strong evidence for a genetic component to the etiology of the JIA diseases. Twin studies have shown concordance rates in monozygotic twins of between 20% and 40%.[18] A study of 164 affected sibling pairs with JIA showed a 70% concordance for gender, 73% for age at disease onset, and 66% for disease course.[19] Estimates suggest that the recurrence risk for siblings of a proband with JIA are between 15 and 30 times that of the general pediatric population, a figure that is as high as for insulin-dependent diabetes mellitus (IDDM) or multiple sclerosis.[20] In addition, first-degree relatives of children with JIA have a higher rate of other autoimmune disease than controls.[21] Despite this, families of multiple affected siblings are rare. One caveat in comparing early studies is that some were performed using the American College of Rheumatology (ACR) classification criteria (at that time called *JRA*), and so may not be directly comparable to studies that have used the ILAR classification of JIA.

Genetic influences on JIA susceptibility and phenotype are polygenic so that JIA is thought of as a complex genetic trait.[2,20] Although some studies have analyzed all forms of JIA together, the heterogeneity of childhood arthritis suggests distinct genetic risk associations for different phenotypes; this has been confirmed in recent adequately powered studies. The strongest genetic associations with JIA are of genes that lie within the MHC or human leukocyte antigen (HLA). These were the first to be documented as associated with childhood arthritis.[22] The MHC region, located on chromosome 6 of the human genome, includes more than 200 genes, many of which are central to functions of the immune system. Numerous associations between HLA genes and JIA have been reported, and these involve many different populations.[2,23] The best characterized associations are with genes of the class I and class II MHC loci, genes that code for heterodimeric proteins that present peptide antigen to the specific antigen receptor of T cells (the TCR).

Class I loci include HLA-A, -B, and -C, of which the earliest report was of the association between HLA-B27 and the form of JIA known as *enthesitis-related arthritis* (ERA), which includes children whose disease has parallels with adult ankylosing spondylitis (AS) (see Chapters 74 and 75). The HLA-A2 allele *HLA-A*0201* is increased in several types of JIA, especially oligoarthritis and children with early-age onset.[24,25]

Multiple studies have revealed associations of class II MHC loci, (HLA-DR, -DP, and -DQ), with JIA, of which the strongest are DRB1*0801 and *11 with oligoarticular JIA, as well as DRB1*1301, in particular in $ANA^+$ cases.[25] Because of inheritance of genes in the MHC region together in haplotypes because of linkage disequilibrium (LD), observed associations may in fact be the result of genes within the haplotype, distinct from the locus first implicated. Several haplotypes across the MHC confer increased risk for all types of JIA, such as DRB1*08-DQA1*0401-DQB1*0402, which confers an odds ratio of 6.1 and 10.3 for persistent and extended oligoarticular JIA, respectively. Frequency of the DRB1*1301-DQA1*01-DQB1*06 haplotype distinguishes persistent from extended oligoarticular JIA, whereas DRB1*0801 and *1401 are associated with polyarticular JIA.[25] Some associations closely mirror those of the corresponding adult disease, such as the strong association of the HLA-B27 allele with spondyloarthropathy and the HLA-DRB1*0401 allele with rheumatoid factor $(RF)^+$ polyarticular JIA. Although sJIA shows less strong associations with HLA alleles, even in this subtype, specific haplotypes (such as DRB1*11-DQA1*05-DQB1*03) are increased compared with control participants. A study with fine allele-specific typing across eight HLA loci in a large cohort and analyzing by haplotype, confirmed that within class II loci, HLA-DR is the driving association, rather than HLA-DP.[26] Some HLA allele/JIA subtype associations show[24] age-specific effects, in that they confer risk over a specific age range only. Another study confirmed both age- and gender-specific effects.[26] Thus, for example, in polyarticular JIA the HLA-DRB1*0801 allele has risk effect in those who are older than 6 years when their arthritis begins, whereas in younger-onset polyarticular JIA, the risk alleles are more closely related to those of patients with oligoarticular JIA: HLAB1*1103/1104. Some HLA alleles, notably DRB1*0401, *0701, and *1501, are consistently reduced in frequency in JIA cases compared with controls, suggesting a protective effect for some or even all subtypes. These associations among genes that code for proteins, the central function of which is the presentation of antigenic peptides to T cells, implicate a T cell–driven process in at least part of the etiopathogenesis of JIA.

In addition to genes coding for MHC proteins, a large number of other candidate genes (now totaling more than 100) have been the focus of research in JIA.[2,27] Inflammatory cytokines have been an important target for drug development in JIA, and their gene polymorphisms have been an area of scrutiny. Genes studied have included cytokine

and chemokine genes such as those for IL-1, IL-6, TNF, macrophage inhibitory factor (MIF), IL-10, and their receptors (including IL-1R, IL-2RA, CCR5), and key signaling molecules, including CTLA4 and PTPN22. However, only a few of these loci have been independently validated in candidate gene studies. These include genes for the PTPN22, MIF, SLC11A6, WISP3, and TNF and, in a meta-analysis, CCR5, proteins.[28]

Genetic association at the TNF locus is complex to study, given the position of the TNF locus within the MHC, but some data suggest that several specific TNF haplotypes are significantly associated with JIA, although the functional consequences of these alleles are not yet clear.[29] The hypothesis suggesting a link between sJIA and IL-6 was proposed many years ago because several clinical features in sJIA resembled the phenotype of IL-6 overexpression (e.g., fevers, stunted growth, anemia).[30,31] A polymorphism (−174G/C) in the regulatory region of the IL-6 gene alters transcription of IL-6 in response to IL-1 and lipopolysaccharide; sJIA patients have significantly lower frequency of the protective CC genotype, and the IL-6 −174G allele has been confirmed as a susceptibility gene for sJIA.[32,33]

Some conflicting studies on candidate gene loci reflect two problems that hindered progress: the heterogeneity of JIA, which means that combining all JIA into one study may lead to loss of detection of effects specific for a subtype, and, opposing this, the difficulty of reaching adequate power for genetic studies, if stratification by subtype is preferred. New insights have come from several large genetic studies. The Wellcome Trust Case Control Consortium performed genome-wide association studies in seven major common diseases, including rheumatoid arthritis (RA).[34] Extrapolation of loci implicated in RA to JIA has suggested that other key loci associated with JIA are IL-2/IL-21 and IL-2RA, the alpha chain of the IL-2 receptor, also known as *CD25*.[35,36] Another study focused upon specific subtypes of JIA and comparison with their adult disease counterparts. The genetic loci IL-23R and endoplasmic reticulum aminopeptidase-1 (ERAP1) have been identified as carrying risk associations with ankylosing spondylitis and psoriatic arthritis in adults.[37] Genotyping of these genes in a large cohort of JIA cases has shown associations of both with the ERA subtype of JIA but no association with JIA as a whole, again emphasizing subtype-specific genetic features and, likely, pathogenesis.[38] ERAP1 is of particular interest given its functional role in trimming of peptides within the ER, for presentation of peptide on, and folding of, class I MHC molecules,[39] and given the strong evidence for class I MHC folding abnormalities in patients with the HLA-B27 risk alleles (HLA-B*2705 and *2702, among others). ERAP1 is also thought to have a role in trimming of cytokine receptors at the cell surface. IL-23 is of great functional interest because of the central role of IL-23 in the Th17 pathways and the demonstration that Th17 cells play a role both in JIA and adult AS and psoriatic arthritis.

Significant insights into the pathogenesis of childhood arthritis have been gained by the largest ever genetic study to date on approximately 3000 JIA oligoarticular and polyarticular RF-JIA cases by using the Immunochip (Illumina, San Diego) platform. This provides single nucleotide polymorphism (SNP) genotyping of 186 known autoimmune loci. The study confirmed a total of 16 non-HLA loci as associated with JIA at genome-wide significance level.[40] New analysis modeling the most likely causal SNPs, and linking both genetic and epigenetic data, in JIA and other autoimmune diseases, has suggested that true causal SNPs frequently fall in enhancer or super enhancer regions that regulate gene expression.[41]

## ADAPTIVE IMMUNE SYSTEM

**KEY POINTS**

- **Effector T cells secreting pro-inflammatory cytokines are enriched in the joint membrane and synovial fluid.**
- **Regulatory T cells are found in the joints of JIA patients and are enriched in persistent oligoarthritis patients; however, they can have an atypical phenotype in synovial fluid.**
- **Immune regulation may be impaired as a result of cytokine-driven resistance of effector T cells to suppression.**
- **A B cell gene expression signature has been detected in the peripheral blood of patients with an early age at onset of JIA.**

### T Cells

The strong association of many JIA subtypes with genetic variants at HLA loci, as well as the central role of HLA proteins in presenting peptide antigens to T cells for recognition, which is central to T cell function, has led to intense investigation of the role of T cells in the pathology of JIA. Highly activated memory T cells make up a significant proportion of the inflammatory infiltrate in JIA, and express an "oligoclonal" or restricted set of TCRs.[17,42] Specific T cell clones can be long lived, and are detectable in different inflamed joints. Nevertheless, it is still unclear whether these clones represent autoreactive T cells specific for an "arthritogenic" self-epitope. Although immune activation leads to tissue damage in JIA, the role of self-antigen recognition in this process remains unclear. Recent probing of autoreactive T cells in a mouse model of arthritis identified a ubiquitously expressed ribosomal protein, RPL23A, as an autoantigen to which T cells and antibodies from rheumatoid arthritis patients could also react.[43] However, to date, no clear autoantigens have been identified in JIA. The association of JIA with genetic loci that influence the threshold of T cell activation, such as PTPN22,[44] cytokine receptors essential to T cell biology (IL-2RA, IL-2RB), as well as those central to recognition of antigen by T cells (HLA), supports the concept of JIA as a disease of dysregulated adaptive immunity.

Early work examining animal models of arthritis suggest that cells of the T helper (Th)1 lineage, secreting IFN-γ, are central to pathogenesis. Th1 cells are recruited to the joint by high levels of chemokines CCL5, MIP-1a, and IP-10,[45] and make up the majority of T cells in the JIA joint.[15] However, in both mouse models of arthritis and early clinical trials, blocking IFN-γ offered little clinical benefit, suggesting that other players may be more important to pathogenicity. TNF, the prototypic inflammatory cytokine in arthritis, is secreted by T cells and macrophages within the JIA joint and is detectable within inflamed synovial tissue,[46] and, to a lesser extent, in synovial fluid.[47] The success of TNF blockade in polyarthritis and extended

oligoarthritis subtypes implicates TNF in JIA pathology, but its mechanisms of action remain unclear. Some evidence suggests that TNF inhibitor therapy can improve the response of effector T cells to suppression by regulatory T cells, suggesting TNF may be pathologic because of its ability to impair regulation at the inflamed site.[48] Still, roughly 30% of patients do not respond adequately or relapse on TNF inhibitor therapy and the Th17 T cell population. Secretion of IL-17 and IL-22 may in part account for this recalcitrant disease.[49] IL-17 causes significant bony and cartilage damage in the joint by promoting neutrophil influx via the secretion of IL-8 and synergizing with IL-1β and TNF to drive metalloproteinase secretion and osteoclast activation. Th17 cells are enriched in the joints of JIA patients, and Th17 numbers correlate with severity of disease course in oligoarthritis.[14] Th17 cells within the JIA joint can also upregulate expression of granulocyte-monocyte colony-stimulating factor (GM-CSF).[50] Because both IL-17 and GM-CSF are potent inflammatory cytokines that are pathogenic in mouse models of autoimmunity,[51] this raises the hope that Th17 or GM-CSF blockade may be an effective treatment in some subtypes of JIA.

In addition to pro-inflammatory processes, there is strong evidence for ongoing immune regulation in JIA. *IL2RA*, the gene encoding CD25, and *RUNX1*, a transcription factor that interacts with FOXP3, are two genes intimately involved in regulatory T cell (Treg) biology that are significantly associated with JIA.[40] Children with persistent oligoarticular JIA have high numbers of Treg cells expressing CD25 and FOXP3 within the joint, and these are significantly higher than those with more severe extended oligoarticular disease.[52,53] In addition, $CD30^+$ T cells specific for the conserved self-antigens heat shock proteins (HSP) appear to play a regulatory role.[54]

Although synovial Treg cells suppress effector T cell functions in vitro, inflammatory cytokines such as IL-6 and TNF, present in the arthritic joint, may attenuate Treg cell function in vivo.[48,55] In addition, Treg cells in synovial fluid can have an atypical phenotype with low to absent expression of FOXP3, which is associated with defects in pathways that control FOXP3 expression.[56] Furthermore, some Treg cells expressing the innate lymphoid cell marker CD161 can secrete inflammatory cytokines, suggesting that perturbation of the Treg compartment may occur in JIA.[57] Treatment strategies that expand Treg cell numbers and augment function are currently under investigation[58,59] will need to take into account issues of Treg cell stability and their potential for pro-inflammatory phenotype.

## Antigen-Presenting Cells

Dendritic cells are specialized antigen-presenting cells (APCs) that activate T cells, as well as promote the differentiation of effector T cell functions. Comparatively little is known about their role in JIA. In the inflamed joint, myeloid dendritic cells (mDCs) are localized to the synovial lining layer, whereas IFN-α–secreting plasmacytoid dendritic cells (pDCs) are in T and B cell–rich aggregates.[47] Both subsets are found in high numbers within synovial fluid when compared with PB. Synovial mDC express high levels of co-stimulatory molecules, including CD80, CD86, and receptor activator of NF-κB (RANK),[60,61] which augment T cell activation and lead to the secretion of pro-inflammatory cytokines and chemokines. In contrast to mDCs, some studies suggest that synovial pDCs may provide a regulatory function. pDCs within the joint do express markers of activation,[62] but secrete high levels of granzyme B, which limits T cell proliferation.[63]

## B Cells

There has been a renewed interest in B cell–effector function in inflammatory arthritis following the efficacy of the anti-CD20 agent rituximab in RA. In JIA, the exact role of B cells as effector or regulatory players is still unclear. B cell–derived autoantibodies, ANAs, RFs, and anti-citrullinated protein antibodies (ACPAs) are common in some JIA subtypes, but none are considered to be directly pathogenic (see Chapter 107). Interestingly, patients who are $ANA^+$ are more likely to have T-B cell aggregates in their synovial membrane, suggesting a link between lymphoid neogenesis and autoantibody production.[64] Although lymphoid aggregates are seen in JIA synovium, mature germinal centers are rare compared with RA. B cells only make up small fraction of mononuclear cells within synovial fluid, but are skewed toward a memory phenotype, expressing high levels of the co-stimulatory molecules CD80 and CD86.[65] When cultured together with T cells in vitro, synovial B cells were more likely to activate T cells toward a Th1 phenotype than equivalent peripheral B cells.[66] Total B cell numbers do not vary between JIA patients and healthy controls, but oligoarthritis patients have higher numbers of immature transitional-type B cells in their blood compared with healthy controls (CD38 high, CD24 high).[65] However, treatment with methotrexate reduced the frequency of this immature population, whereas etanercept had no effect on B cell subsets.[67] Patients with an early onset of disease also have expression of B cell and immunoglobulin-related genes in peripheral blood.[68] The B cell signature, as well as frequent ANA positivity in patients with oligoarthritis, has led some authors to suggest a major role for B cells in the pathogenesis of this subtype.

## Innate Immune System

**KEY POINTS**

**Defects in the innate immune system have been most closely associated with sJIA.**

**Neutrophils and monocytes secrete myeloid-related protein 8/14, IL-6, IL-1β, and TNF, which drive systemic inflammation.**

Many cytokines detectable in the inflamed joint are the products of the innate immune system. The successful targeting of these cytokines with biologic agents (e.g., IL-1β and IL-6) heavily implicates the innate immune system in the pathology of JIA, particularly the sJIA subtype.

### Macrophages/Monocytes

Monocytes and their tissue counterparts, macrophages, are key effector cells of the innate immune system and have been linked to the pathogenesis of autoimmune arthritis for several decades.[69] In JIA, analysis of gene expression has

detected an activated macrophage gene expression signature in the cells within synovial fluid of early oligoarthritis patient at risk of extension[70] and a monocyte signature in the peripheral blood of patients with an older-onset oligoarthritis,[68] and RF+ polyarthritis patients.[71] Monocytes typically make up equivalent proportions of synovial fluid and peripheral blood mononuclear cells (approximately 10%), but they have a highly activated phenotype,[70] secreting high levels of IL-6, IL-1β, and TNF within the joint.[72] Synovial monocytes also secrete VEGF and osteopontin (OPN), which contribute to the vascularity of inflamed pannus,[73] and the chemokine CCL20, which drives Th17 cells recruitment to the joint.[14]

### Neutrophils

Neutrophils may make up the major fraction of the synovial infiltrate but have remained an infrequent research interest in JIA. sJIA patients have very high levels of the heterodimeric myeloid-related protein (MRP)-8/14, also known as S-100A8/9, secreted by activated monocytes and neutrophils.[74,75] Roth and colleagues have shown that MRP8/14, secreted by cells of the innate immune system, induces autoreactive CD8+ T cells, illustrating how crosstalk between the innate and adaptive immune system can contribute to chronic inflammation.[76]

Examination of neutrophils from JIA has found more than 700 differentially expressed genes between patients and control blood.[77] Genes linked to IL-8 and IFN-γ were prominent among these differences but failed to return to baseline levels after clinical remission. Rather, disease quiescence was associated with an increase in regulatory genes, such as those for TGF-β and retinoic acid, suggesting that remission reflects a balanced state of inflammation and regulation rather than true immunologic resetting.

### Stromal Cells

Resident tissue stromal cells are important targets of both the innate and adaptive immune system and may play an important role in defining the anatomic location of inflammation after systemic immune dysregulation. Synovial fibroblasts from JIA patients secrete metalloproteinases and chemokines in response to locally secreted proinflammatory cytokines[78] and may be an important therapeutic target of the future.

## DISEASE SUBTYPE–SPECIFIC PATHOGENESIS

**KEY POINTS**

**Excessive secretion of IL-1β and IL-6 contributes to many of the clinical features seen in patients with sJIA.**

**The balance between inflammatory and regulatory T cell populations may determine the severity of clinical course in oligoarticular forms of JIA, especially when there is Treg cell dysfunction or instability.**

**RF− polyarthritis shares immunopathologic features with oligoarthritis subtype, whereas RF+ polyarthritis patients are more closely aligned with RA.**

**In ERA, HLA-B27 protein is prone to misfolding, generating an unfolded protein response within cells, which leads to inflammation and arthritis.**

**Psoriasis and psoriatic arthritis have been very strongly linked to pathogenic T cells secreting IL-22 and IL-17.**

### Systemic Juvenile Idiopathic Arthritis

A key role for IL-1β, IL-6, and IL-18, cytokines of the innate immune system, as well as the absence of autoantibodies or a strong association with MHC, have led many to propose sJIA as an autoinflammatory disease. This hypothesis is supported by the close mirroring of these cytokines with the characteristic fever of sJIA, the high levels of IL-6 and IL-18 in serum from active sJIA patients,[79,80] and the enrichment of monocytes in sJIA blood,[81] secreting high levels of IL-1β after activation. Early studies reported a significant higher ratio of IL-1 receptor antagonist (IL-1ra) to IL-1 in these sJIA patients,[79,82] although this may be difficult to interpret because IL-1α and IL-1β are highly labile in serum. Successful treatment with soluble IL-1Ra, anakinra for some patients with sJIA, supports a role for IL-1.[83] Interestingly, IL-1β secretion in sJIA may be mediated by myeloid-related protein (MRP) 8/14 because once this protein is removed from JIA serum, the potential to induce IL-1β is almost completely attenuated.[75] This study confirmed that levels of serum MRP8/14 are very high indeed in active sJIA, and that this measure is both sensitive and specific in distinguishing sJIA from other important diagnoses such as infection or hematopoietic malignancy.

IL-18, a macrophage-derived pro-inflammatory cytokine with a similar signal transduction pathway to IL-1β, is also grossly elevated in sJIA, with serum levels correlating with disease activity. High IL-18 levels may account for the defective NK cytotoxic function found in sJIA.[84] Follow-up studies of anakinra have cast doubt on the primacy of IL-1β in sJIA because more than 50% of patients are either nonresponders or relapse on treatment. Even in responders, it is likely that other inflammatory mediators are involved because treatment response to anakinra fails to correlate with either pre-treatment IL-1β or IL-18 secretion or changes in gene expression of the IL-1β pathway.[85] A specific antibody to IL-1β, canakinumab, is highly effective in sJIA, including in those patients who do not respond to anakinra.[86]

IL-6 is another key player in the pathogenesis of sJIA, secreted by a range of cells, including monocytes. IL-6 levels are elevated in the serum and synovial fluid of sJIA patients, levels correlate with disease activity,[30,79] and clinical features (including growth failure and osteoporosis) can be explained by high levels of IL-6.[87] This evidence led to trials of IL-6 blockade with the monoclonal antibody to soluble IL-6 receptor, tocilizumab. Early trials of tocilizumab demonstrated impressive results in sJIA.[88] A recent multicenter, randomized placebo-controlled, double-blind clinical trial of tocilizumab showed clear efficacy in sJIA (TENDER study).[89]

Gene expression profiling from sJIA patients has confirmed pathways that involve IL-6 and IL-1 and that these can distinguish active from inactive patients.[90] In addition to these roles for monocytes in sJIA, defects of NK cell function, in particular perforin function, are well recognized.[91] The defect in perforin function in active sJIA has

been seen to reverse upon successful treatment with autologous stem cell transplantation.[92]

## Macrophage Activation Syndrome

Macrophage activation syndrome (MAS) is a potentially life-threatening complication of sJIA (as well as sometimes complicating other rheumatologic conditions, such as juvenile systemic lupus erythematosus) thought to result from immune activation of pathogenic T cells and hemophagocytic macrophages.[93] These macrophages express CD163, a scavenger receptor that recognizes haptoglobin-hemoglobin (HP-Hb) complexes. Increased uptake of these complexes within the macrophage leads to production of ferritin, explaining the hyperferritinemia associated with MAS. Soluble forms of CD25, the alpha chain of the IL-2 receptor expressed on T cells, and CD163 are useful biomarkers for MAS in JIA.[94]

MAS shares many similarities with a group of inherited disorders called hemophagocytic lymphocytic histiocytosis (HLH). Defects in the perforin gene and related genes involved in its cytosolic secretory pathway (MUNC13-4, Rab27a, and SH2D1A) have been identified as causes of HLH. When examined in sJIA, polymorphisms in MUNC13-4 and perforin have been associated with MAS in some studies but not others.[95,96] How defects in cytotoxic function lead to MAS is still unclear, but it has been proposed that appropriate clearance of microbial antigens or activated macrophages is prevented, leading to chronic immune stimulation. A gene expression profiling study of active sJIA showed that patients with active sJIA, and in particular those with MAS, have a signature in PBMC that is similar to patients with familial HLH and characteristic of immature erythropoeisis.[97]

## Oligoarthritis

There is increasing evidence that the balance between inflammation and regulation plays a role in driving the clinical phenotype of oligoarthritis patients.[98] The dominance of Treg cells within the joints of patients with persistent oligoarthritis may explain the relatively benign prognosis of this subgroup, in contrast to the extended subgroup who have a low ratio of Treg to Th17 cells. Those who remain in the mild prognosis group have higher numbers of Treg cells in the joint, defined by either CD25 or FOXP3 expression,[14,52] as well as Treg cells that are specific to the human self-antigen HSP60.[54]

Although patients with oligoarthritis JIA are seen with few joints involved, evidence suggests that underlying differences between the groups who remain mild and those who go on to extend are present even very early in disease. Thus genetic differences between persistent and extended oligoarticular phenotypes include variation in the class I MHC allele associations[25] and IL-10.[99] A study of gene expression and cellular and cytokine profiling showed that those children destined to go on to extend had a more heavily IFN-γ–driven and activated macrophage signature in synovial cells, as well as differences in levels of the chemokine regulated on activation, normal T cell expressed and secreted (RANTES) (CCL5), from the start of disease even before extension occurs.[70] Similarly, proteomic profiles are distinct, very early in disease, in those who go on to extended disease.[100] These data have led to the concept of a separate group of patients with oligoarthritis who are destined to progress to more severe disease, the so-called *extended-to-be* group. There is strong evidence to link Th17 cells with autoimmune uveitis, and so agents targeting the IL-17/IL-23 pathway, currently under trial in RA, may ameliorate both joint and eye disease in JIA (typically ANA-positive oligoarticular disease) with uveitis.

## Rheumatoid Factor–Positive Polyarthritis

The presence of RF and a severe erosive disease course suggests that children in this subgroup represent an early presentation of adult-onset RA. Indeed, this subtype of JIA shares genetic associations, in particular at the HLA-DRB1 locus, with adult rheumatoid, in particular, the association with the DRB1*0401 allele at this locus.[25] However, few studies have directly compared immunology between adult and childhood onset of disease. Studies of cyclic citrullinated protein (CCP) positivity in children with JIA have shown association with $RF^+$ polyarticular disease,[101] and there is evidence to support a pathogenic role for RF and autoantibodies against citrullinated peptides by fixation of complement on synovial endothelium. Children with $RF^+$ polyarthritis may share histologic features with adult patients with RA with lymphoid follicles and germinal centers, which may be more abundant than in $RF^-$ patients.[64,102]

## Rheumatoid Factor–Negative Polyarthritis

Patients with $RF^-$ polyarthritis represent a more heterogeneous group than patients with $RF^+$ polyarthritis. Analysis of gene expression data from patients with polyarthritis showed three separate signatures.[71] The first was associated with monocyte-related genes and had the highest proportion of $RF^+$ patients. The third signature found in $RF^-$ patients was associated with reduced $CD8^+$ T cells and increased plasmacytoid DCs. Interestingly, patients did not have overlap between signatures 1 and 3, suggesting distinct immunopathology. It is noteworthy that some patients (24 of 61), who were younger and had a higher rate of ANA positivity, did not fall into a clearly distinct category. A further gene expression study suggested these patients may overlap with $ANA^+$ patients with oligoarthritis, and that age of onset of disease is a more important distinguishing factor than the number of involved joints.[68] As discussed, there are similar immune pathways in $RF^-$ polyarthritis and oligoarthritis patients, and overall disease expression may depend on the balance between immune regulation and inflammation.

## Enthesitis-Related Arthritis

The HLA-B27 allele, recognized many years ago for its association with autoimmune arthritis, appears to play a particular role in pathogenesis through its molecular properties. The presence of a cysteine residue at position 67 of the HLA-B27 alpha1 helix heavy chain makes possible a disulphide bond that promotes the formation of homodimers of the B27 chain.[103] These homodimers, which can

form in absence of antigenic peptide and can induce endoplasmic reticulum stress, are pro-inflammatory and are ligands for NK cell receptors such as KIR3DL1 and KIR3DL2.[104] This abnormal folding of the HLA-B27 molecule, which in the context of microbial antigens drives very high expression of IL-23, may thus promote Th17 cells,[105] which are enriched in ERA synovial fluids.[106] This hypothesis may also explain the association between ERA and inflammatory bowel disease because Th17 cells also drive pathology in the latter condition, and pathogenic cells readily recirculate from gut to joint. Patients with ERA may be particularly prone to disease induction by bacterial pathogens because cells from patients have high expression of pathogen-recognition molecules Toll-like receptor (TLR)2 and TLR4 when compared with healthy controls.[107] It is interesting that ERAP1, which is strongly associated with adult AS and also associated with ERA JIA,[38] is thought to affect the repertoire of peptides available to bind class I MHC by cleavage of N-terminal amino acids from peptide precursors in the ER.[39]

### Psoriatic Arthritis

The etiology of psoriatic arthritis shares features with enthesitis-related arthritis, having genetic association with class I MHC loci, a clonal expansion of CD8 T cells in inflamed joints,[108] and a putative role for Th17 cells. In adult psoriasis and psoriatic arthritis, there is strong evidence that Th17 cytokines, IL-22, and IL-17 contribute to the disease process (see Chapter 77). The receptor for IL-23, a key cytokine that induces Th17 cells, is linked to the juvenile form of the disease, and IL-23 blockade appears promising in trials of skin and joint disease in adult patients.[109] Evidence of genetic associations with autoinflammatory genes in juvenile patients with psoriatic arthritis suggests that the innate immune system may also play a role.[110]

## CONCLUSION

The heterogeneity of childhood arthritis is complex but is gradually being characterized and harnessed through the application of novel methods, including gene expression profiling, proteomics, and high-throughput genetics and the major progress in our understanding of the immunologic processes involved. In this chapter, cellular processes at play in JIA have been considered individually; the immune system acts in concert to create vastly complex networks. The challenge is to understand the functional hierarchy of these networks and discover checkpoints that will be amenable to therapeutic targeting in the future.

As well as new treatments, better biomarkers are required for use in the clinic. The task in coming years will be to integrate the vast body of data that will be generated through these novel approaches to allow the development of more precise classification definitions, and perhaps more important, predictive tools with which to drive treatment choices for patients.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 107



# Clinical Features and Treatment of Juvenile Idiopathic Arthritis

Joyce J. Hsu • Tzielan C. Lee • Christy I. Sandborg

**KEY POINTS**

- Juvenile idiopathic arthritis (JIA) is the umbrella term for a family of heterogeneous childhood arthritides of unknown cause.
- JIA affects at least 1 in 1000 children. The goals of treatment in JIA are complete suppression of inflammation on medications and remission when possible.
- Oligoarticular JIA, characterized by early age of onset, female predominance, anti-nuclear antibody positivity, and subacute anterior uveitis, occurs only in childhood.
- Systemic JIA is distinct from other subgroups of JIA in its equal sex distribution, lack of autoantibodies and human leukocyte antigen associations, and increased responsiveness to IL-1 and IL-6 inhibition compared with TNF inhibition.
- Juvenile spondyloarthritis typically occurs in children older than 6 years, but sacroiliitis may not develop until adolescence.
- Because bone erosions on conventional radiographs are late radiographic findings in growing children, detection of early joint damage may require different imaging modalities.
- Considerable progress in understanding the genetics and pathogenesis of JIA has revealed subtype-specific associations as well as some common mechanisms of disease that will translate to more effective and targeted therapies.

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood, but actual estimates of prevalence and incidence vary remarkably in different geographic regions, ranging from 7 to 400 per 100,000 children, reflecting variations in disease reporting, classification, and racial/ethnic and environmental differences in disease expression.[1] Reasonable working estimates are 150 per 100,000 children, making JIA one of the most common chronic diseases of childhood. There are significant differences in the disease manifestations in children compared with adults, with some types occurring exclusively in children. This chapter discusses the current understanding of the key clinical and diagnostic features of the various forms of JIA, treatment approaches, and outcomes. The rapid growth in understanding the biologic basis for JIA and the ongoing development of targeted therapies for rheumatic diseases are likely to lead to enhancements in these recommendations. Data concerning long-term disease course and outcome in children with JIA in this modern treatment era remain limited, and there are international prospective inception cohorts of children with arthritis designed to address these gaps in knowledge.[2,3]

## CLASSIFICATION CRITERIA FOR JUVENILE IDIOPATHIC ARTHRITIS AND DIFFERENTIAL DIAGNOSIS

In the past, different groups used various nomenclature to classify children with persistent arthritis, including "juvenile rheumatoid arthritis" (American College of Rheumatology [ACR]) and "juvenile chronic arthritis" (European League against Rheumatism), which created problems in comparing research studies and outcomes. The goal of the International League of Associations for Rheumatology (ILAR) is to identify subtypes of JIA for research purposes that are homogeneous and mutually exclusive. JIA classification is currently based on predominant clinical and laboratory features and the number of involved joints at disease onset,[4] which are presented in Table 107-1. However, classification systems are ever changing, and categorization may evolve to more biologically and genetically similar subgrouping, especially with recent advances in etiology and pathogenesis.[5,6]

The patterns in children with rheumatic diseases can overlap with other concerning diagnoses, such as malignancies, infection, and trauma (especially nonaccidental). Therefore it is crucial to evaluate these diagnostic possibilities before accepting the diagnosis of JIA. The ILAR categories are meant to simplify classification and are useful for typical presentations of disease. However, if a patient does not easily fit into the ILAR classification system, clinicians must carefully consider all other possibilities, rheumatologic and nonrheumatologic. For example, among patients with acute lymphocytic leukemia (ALL), 15% to 30% are seen with musculoskeletal symptoms without blasts in the peripheral smear and may be misdiagnosed with JIA. In addition, anti-nuclear antibody (ANA) status, rash, radiographic abnormalities, and objective signs of arthritis were found to not be helpful in distinguishing between ALL and JIA.[7]

## OLIGOARTICULAR JUVENILE IDIOPATHIC ARTHRITIS

Oligoarticular JIA accounts for as many as 20% of all new rheumatic diagnoses in the general pediatric rheumatology clinic[8] and is the most prevalent of all the JIA subcategories, comprising 30% to 60% of all patients with JIA in

**TABLE 107-1** ILAR Classification Criteria for Juvenile Idiopathic Arthritis (JIA)

**General Definition of JIA**

Juvenile idiopathic arthritis is arthritis of unknown etiology that begins before the sixteenth birthday and persists for at least 6 weeks; other known conditions are excluded.

| Subcategory | Definition | Exclusions |
|---|---|---|
| **Oligoarticular JIA**<br>Two subcategories are recognized:<br>1. Persistent oligoarticular JIA: affecting ≤4 joints throughout the disease course<br>2. Extended oligoarticular JIA : affecting a total of >4 joints after the first 6 months of disease | Arthritis affecting 1-4 joints during the first 6 months of disease | a. Psoriasis or a history of psoriasis in the patient or a first-degree relative<br>b. Arthritis in a human leukocyte antigen (HLA)-B27$^+$ male beginning after the sixth birthday<br>c. Ankylosing spondylitis, ERA, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative.<br>d. The presence of IgM RF on at least 2 occasions, at least 3 months apart<br>e. The presence of systemic JIA in the patient |
| RF$^-$ polyarticular JIA | 1. Arthritis affecting ≥5 joints during the first 6 months of disease, **and**<br>2. Test for RF is negative | a, b, c, d, e |
| RF$^+$ polyarticular JIA | 1. Arthritis affecting ≥5 joints during the first 6 months of disease, **and**<br>2. ≥2 positive RF tests (as routinely defined in an accredited laboratory), at least 3 months apart during the first 6 months of disease | a, b, c, e |
| Psoriatic arthritis | 1. Arthritis and psoriasis, **or**<br>2. Arthritis and at least 2 of the following:<br>a. Dactylitis<br>b. Nail pitting (minimum of 2 pits on ≥1 nails at any time) or onycholysis<br>c. Psoriasis in a first-degree relative | b, c, d, e |
| Enthesitis-related arthritis | 1. Arthritis *and* enthesitis, **or**<br>2. Arthritis *or* enthesitis, with at least 2 of the following:<br>a. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain<br>b. The presence of HLA-B27<br>c. Onset of arthritis in a male >6 years of age<br>d. Acute (symptomatic) anterior uveitis<br>e. History of ankylosing spondylitis, ERA, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative | a, d, e |
| Systemic JIA | Arthritis in ≥1 joints with, or preceded by, fever of at least 2 weeks' duration that is documented to be daily and quotidian (fever that rises to ≥39° C once a day and returns to ≤37° C between fever peaks) for at least 3 days, and accompanied by ≥1 of the following:<br>a. Evanescent (nonfixed) erythematous rash<br>b. Generalized lymph node enlargement<br>c. Hepatomegaly and/or splenomegaly<br>d. Serositis | a, b, c, d |
| Undifferentiated arthritis | Arthritis that fulfills criteria in no category or in ≥2 of the above categories | |

ERA, Enthesitis-related arthritis; ILAR, International League of Associations for Rheumatology; RF, rheumatoid factor.

From Petty RE, Southwood TR, Manners P, et al: International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 31(2):390–392, 2004.

North America and Europe.[9] Oligoarticular JIA has no adult equivalent. The peak age of onset occurs in Caucasian children ages 2 to 4 years from the United States and Europe, and the female to male ratio is 3:1.[10] It is rare in African-Americans,[11] Native Americans,[12] and southern Asians. Two general subgroups are recognized within oligoarticular JIA: first, persistent oligoarticular JIA, in which the number of joints affected by arthritis remains less than five throughout the disease course; second, extended oligoarticular JIA, in which arthritis develops in additional joints 6 months after disease onset. Extension occurs in 30% to 50% at 4 to 6 years after onset of disease.[13-15] Symmetric disease, ankle and/or wrist involvement, and an elevated erythrocyte sedimentation rate

(ESR) in the first 6 months of disease might be associated with extension.[13,15,16]

Oligoarticular JIA usually manifests as an asymmetric arthritis affecting one or two large joints, especially of the lower extremities, with the knee the most commonly affected, followed by the ankle, wrist, and digits. Involvement of the hip and back, especially in young children, is so unusual that extensive evaluation is warranted to rule out other conditions such as infection or malignancy. Significant constitutional and systemic symptoms are unusual in oligoarticular JIA and, if present, should raise concern regarding the accuracy of the initial diagnosis. Pain in an obviously inflamed joint is surprisingly minimal compared with septic arthritis, and in as many as 25% of cases, the symptoms may be subtle with parents only noticing a limp and joint swelling. In a young child, there is reluctance to walk and bear weight with a return to crawling after a period of immobility, such as in the morning or after a nap. There is a high risk for a relatively asymptomatic chronic uveitis, especially in $ANA^+$ individuals, requiring regular ophthalmology examinations to detect early changes (see Uveitis).

Among children with oligoarticular JIA, a positive ANA in low to moderate titers (1:40 to 1:320) is seen in 70% to 80% of children with persistent oligoarticular JIA and 80% to 95% with extended oligoarticular JIA.[10] The rate of ANA positivity is even higher in girls with early onset disease.[17] The typical child with oligoarticular JIA will have normal white blood counts, normal or mild-moderately elevated acute phase reactants, and, in some cases, mild anemia.

The differential diagnosis of oligoarticular JIA includes other JIA subtypes, especially juvenile spondyloarthritis (JSpA) and juvenile psoriatic arthritis (JPsA); other rheumatic diseases of childhood, or other inflammatory conditions such as inflammatory bowel disease (IBD). Nonrheumatic causes of joint pain and swelling should also be ruled out, such as septic arthritis, benign or malignant tumors, reactive arthritis, foreign body synovitis, pigmented villonodular synovitis, arterial-venous malformation, bleeding disorders (such as hemophilia), or bony fractures, including nonaccidental injury. Mild trauma is rarely a cause of joint swelling in young children because serious sprains or orthopedic problems such as meniscal tears or ligamentous injury are exceedingly rare. In general, ligaments and tendons are stronger than growing bone in children, and the bone-ligament or bone-tendon junction is the weakest link. Therefore fractures are more common in children than adults. Transient joint effusions can develop in children with hypermobility after exercise.[18] Lyme disease (in an endemic area) can cause recurrent monoarticular arthritis (typically involving the knee and often with popliteal cysts), usually for less than 6 weeks.

## RHEUMATOID FACTOR–NEGATIVE POLYARTICULAR JUVENILE IDIOPATHIC ARTHRITIS

Rheumatoid factor $(RF)^-$ polyarticular JIA makes up 10% to 30% of all JIA cases, with a bimodal distribution of age of onset, with the first peak at 1 to 4 years of age and the second peak at 10 to 12 years. Girls are more commonly affected than boys, with a ratio of 3.2:1, and subacute anterior uveitis occurs in 4% to 25% of cases.[10] Polyarticular JIA is the most common JIA subtype in African-Americans and Native Americans, who often have the most severe disease with the worst prognosis.[11,12] Patients with $RF^-$ polyarticular JIA have more involvement of hip, shoulder, cervical spine, and distal interphalangeal joints than in adults. Some authors distinguish between two clinical subgroups based on ANA status: (1) an $ANA^+$ form that resembles oligoarticular JIA, consisting of young girls aged 6 years or less, with an asymmetric arthritis in more than four joints and high risk of uveitis, and (2) an $ANA^-$ form that is similar to adult-onset $RF^-$ rheumatoid arthritis (RA), characterized by older patients, symmetric synovitis of large and small joints, a greater cumulative number of affected joints, and less uveitis. The similarities between $ANA^+$, $RF^-$ polyarticular JIA and oligoarticular JIA have led to the hypothesis that these two entities are actually in the same disease spectrum.[6] $RF^-$ polyarticular JIA may be associated with low-grade fevers, fatigue, poor growth, weight loss, elevated acute phase reactants, mild anemia, and ANA positivity in as many as 40% of patients. Even though RF is negative, 50% to 80% of patients are anti-citrullinated protein antibody $(ACPA)^+$,[10] using high-sensitivity but low-specificity testing methods.[19]

The differential diagnosis of $RF^-$ polyarticular JIA includes the other JIA subtypes, such as JSpA which should be considered particularly in boys older than 6 years because sacroiliac (SI) involvement may not occur until adolescence. Other major considerations include other rheumatic conditions; malignancies such as lymphoma and leukemia; and infections such as *Neisseria gonorrhoeae*, rubella, parvovirus, and Lyme disease.

## RHEUMATOID FACTOR–POSITIVE POLYARTICULAR JUVENILE IDIOPATHIC ARTHRITIS

$RF^+$ polyarticular JIA is a well-characterized JIA subcategory and is part of the same disease spectrum as adult $RF^+$ RA,[20] sharing immunogenetic and serologic factors. This subcategory makes up 5% to 10% of cases of JIA[10] with reported female-to-male ratios between 5.7-12.8:1.0.[8,21] Age of onset occurs in late childhood or adolescence, about ages 10 to 13 years, and typically is an aggressive, symmetric polyarthritis affecting many small and large joints. Hip involvement is common and may be debilitating. The arthritis can be quite severe, often resulting in bony erosions and joint destruction. Radiologic changes tend to take place early,[22] especially in hands and feet. With active disease, patients may have mild systemic signs and symptoms. Rheumatoid nodules occur in as many as 10% of cases, most frequently around the elbow. Other extra-articular manifestations are reported less often than in adults. Uveitis is unusual, seen in less than 2% of patients.[10]

Polyarticular JIA may be associated with mild to moderate inflammation, such as elevated acute phase reactants and a normocytic, normochromic anemia. By definition, all patients have immunoglobulin (Ig)M–anti-IgG RF. The ANA test is positive in approximately 55% of patients,[10] and ACPAs have been reported in 57% to 73%.[23,24] The prevalence of ACPAs varies in polyarticular JIA but usually coincides with RF status.[25]

## JUVENILE SPONDYLOARTHRITIS/ ENTHESITIS-RELATED ARTHRITIS

The current classification criteria for JSpA has been split into two forms: undifferentiated and differentiated. The undifferentiated form is made up of seronegative enthesopathy and arthropathy syndrome (SEA) and enthesitis-related arthritis (ERA; JIA subtype in the ILAR classification), whereas the differentiated form includes juvenile ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis, and arthritis associated with IBD. Unfortunately, the clinical features vary from adult spondyloarthritis, making adult classification criteria inaccurate for this pediatric group. Because of the significant heterogeneity of this disease phenotype, it has been difficult to propose classification criteria.[26] JSpA accounts for approximately 20% of JIA, with onset usually after age 6 years and higher prevalence in boys (male : female ratio 7 : 1), although girls may be underrecognized because of milder disease and less axial involvement.[27] In many cases, JSpA is diagnosed initially as oligoarticular JIA.[8] These patients rarely have sacroiliitis until adolescence, and instead usually are seen with lower-limb arthritis and enthesitis.[4] The articular manifestations of JSpA include hip and peripheral arthritis with an asymmetric oligoarthritis most commonly in the lower limb. As many as one-third of JSpA children will have the classic finding of tarsitis, which is inflammation of the subtalar joint and the surrounding tendon sheaths. Approximately 25% of JSpA patients have polyarthritis presentation at onset.[26]

Enthesitis (inflammation of the tendon insertion site) is seen in 60% to 80% of JSpA patients. It is an early manifestation and occurs more frequently in children than in adult-onset AS.[28] The most frequent locations include the inferior pole of the patella (50%); plantar fascial insertion into the calcaneus (38%) or metatarsal head (22%); and Achilles tendon insertion site into the calcaneus (22%).[29] In addition, enthesitis may be seen at the 2 o'clock and 10 o'clock positions on the patella, greater trochanter, tibial tuberosity, and the base of the fifth metatarsal. Children may also report vague buttock pain, groin pain, or heel pain. At onset, spinal symptoms are rare, but a subgroup of children with ERA will progress to features more typical of adult AS, with SI joint and spinal inflammation during adolescence. This progression is more likely in boys who are human leukocyte antigen (HLA)-B27$^+$ and have spinal or SI pain within 1 year of diagnosis.[30] The modified Schober's test can be used to evaluate lower lumbar flexibility, with a change less than 6 cm considered abnormal.[31] Because thoracic excursion varies greatly in a growing child, only sequential measurements are helpful. In the adolescent, any thoracic excursion less than 5 cm should be regarded as abnormal.[28]

JSpA is associated with an acute anterior uveitis in 6% to 27% of children, which typically presents as an acutely red, painful, and photophobic eye that needs immediate medical attention because, if left untreated, it may lead to blindness.[28,32,33] It is more common in HLA-B27$^+$ boys. Cardiovascular disease, although uncommon, can be severe in patients with juvenile AS, with inflammatory aortic regurgitation reported in as many as 10% of patients.[34] Subclinical gut inflammation has been known to be associated with JSpA, with findings of as many as two-thirds of patients observed to have changes similar to patients with Crohn's disease.[35] Arthritis associated with IBD is also seen in children with two distinct patterns of joint involvement: (1) peripheral arthritis more common than (2) sacroiliitis and spondylitis. Additional clinical manifestations unique to arthritis associated with IBD include clubbing, periostitis, erythema nodosum, pyoderma gangrenosum, osteoporosis, and rarely, hypertrophic osteoarthropathy. The peripheral arthritis activity correlates with gut disease activity, unlike the SI joint activity, which tends not to correlate.[31]

Reactive arthritis is categorized under JSpA, following infection with organisms from the gastrointestinal tract (i.e., *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Clostridium difficile*) or genitourinary tract (i.e., *Chlamydia* or *Ureaplasma*) without actual infection in the joint tissues. Usually self-limited, the arthritis can persist and transform into a more chronic arthropathy.[36] In addition to the arthritis, urethritis and uveitis have been documented in children to complete the classic triad for reactive arthritis. The clinical infection usually precedes the arthritis, enthesitis, or extra-articular disease by 1 to 4 weeks.

Compared with adults, in whom radiologic evidence of sacroiliitis is the diagnostic hallmark of AS, radiologic evaluation in children with JSpA is less often diagnostic because sacroiliitis is rare as a presenting symptom. Interestingly, the presence of hip disease in a patient with JSpA increased the likelihood of future sacroiliitis 11-fold, whereas the presence of dactylitis decreased it.[37] MRI with contrast is increasingly used to detect evidence of acute sacroiliitis without chronic changes in children. MRI has also been used to evaluate enthesitis.[38]

HLA-B27 is present in 60% to 80% of patients with JSpA, depending on ethnicity.[26] The ESR may be mildly or markedly raised, and there may be a mild anemia, but these should also raise the suspicion that the child may have subclinical IBD. RF is negative, and ANA may be positive.

The differential diagnosis of JSpA includes true infectious or postinfectious arthritides (viral, rheumatic fever, post-streptococcal, Lyme). More benign entities such as toxic synovitis and benign limb pains of childhood (growing pains), as well as more concerning entities such as malignancies and solid tumors, should also be considered. Orthopedic diagnoses, such as Legg-Calvé-Perthes disease, slipped capital femoral epiphyses disease, and Osgood-Schlatter disease, and less common rheumatologic diseases, such as SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteomyelitis), Kawasaki's disease, and vasculitis, can share similar articular and extra-articular manifestations with JSpA. Both Behçet's disease and familial Mediterranean fever can have sacroiliitis features and therefore could be mistaken for JSpA.[39] Last, children with widespread amplified musculoskeletal pain may have very tender entheses that can be mistaken for enthesitis.

## JUVENILE PSORIATIC ARTHRITIS

Juvenile psoriatic arthritis (JPsA) was initially defined as juvenile-onset arthritis associated with psoriasis occurring at some point during the disease course. This definition has been further expanded to arthritis in the presence of

psoriasis or at least two minor criteria—nail pits/onycholysis, dactylitis, or a first-degree family history of psoriasis—in the absence of specific exclusions.[4,39,40] JPsA represents 2% to 5% of all JIA, with a slight female preponderance and a bimodal pattern of onset (2 to 4 years and 10 to 12 years).[35,39,41,42] There are noted differences between the two onset groups, with the younger-onset cohort more likely to be ANA$^+$, female, and have chronic uveitis, and the older-onset cohort more likely to have HLA-B27 positivity, enthesitis, and axial disease.[35]

The psoriasis typically occurs within 2 years of the onset of arthritis, and for the majority of children, the skin symptoms follow the arthritis symptoms.[28] As many as 80% of children have a classic psoriatic vulgaris or plaque psoriasis characterized by well-demarcated, erythematous, scaly lesions occurring over extensor surfaces (elbows and knees), scalp, and trunk.[28,43,44] However, in small children younger than 2 years, the most common finding is psoriatic diaper rash.[44] Additional areas that should be evaluated include the hairline behind the ears, the navel, the groin region, and superior to the gluteal cleft.[28]

Because JPsA shares similar manifestations with multiple subtypes of juvenile arthritis, there is not one specific articular presentation. Patterns of joint involvement can be oligoarthritis or polyarthritis, with 55% to 70% of patients with JPsA having an oligoarthritis onset (<5 joints), although evolution to polyarthritis can occur in as many as 52%.[45,46] In one study, as many as one-third of patients were diagnosed with another JIA subtype before the diagnosis of JPsA after a mean of 4.2 years.[45] There were no differences in ANA status, HLA-B27 positivity, or uveitis frequency between oligoarticular JIA versus JPsA patients with fewer than 5 joints involved at onset. However, JPsA patients with fewer than 5 joints involved at onset did have more dactylitis, more small joint involvement, and less large joint involvement than oligoarticular JIA patients.[39,47-49] Children with psoriatic arthritis may have mild elevated acute phase reactants and mild anemia of chronic disease. However, as many as one-third have no laboratory evidence of inflammation.

## SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

Systemic JIA (sJIA), which makes up 10% of all JIA, has unique characteristics that include fever, specific rash, and significantly elevated inflammatory markers in addition to arthritis. Recent research has identified biologic differences between sJIA and the other JIA subcategories, including prominent involvement of components of the innate immune system (in particular, inflammatory cytokines IL-1, IL-6, and IL-18; neutrophils; and monocytes/macrophages), suggesting that sJIA may be in the autoinflammatory disease spectrum.[50]

sJIA occurs at any age, with a tendency toward patients younger than 5 years, peaking around 2 years of age.[51,52] Rarely, it occurs in adults and is called *adult-onset Still's disease*. The gender predilection has stayed neutral. The systemic features of this disease are striking and are always present at onset, often predominating the clinical presentation. The arthritis may not be clinically present at onset, although arthralgias and myalgias are almost universally present at onset.[28] The fever is typically spiking in character with a peak of at least 39° C daily, occurring once or twice a day. Interestingly, the temperatures can be subnormal between fevers. The child appears unwell and irritable during the fever but often recovers when afebrile. In 80% of patients, the fevers are accompanied by an evanescent, migratory, salmon pink, and sometimes urticarial macular rash (Figure 107-1).[51] The rash typically spares the face and can occur without specific pattern on any part of the trunk or extremities. It can be subtle and scattered or diffuse and almost confluent. A helpful diagnostic feature is that the rash can be elicited by the Koebner phenomenon.



**Figure 107-1** Typical rash of systemic juvenile idiopathic arthritis, with small 1 to 5 mm flat or slightly raised salmon pink macules.

Other systemic features include lymphadenopathy, hepatosplenomegaly, serositis (pleural/pericardial involvement/abdominal pain), headaches, and sore throat. Pericarditis and pericardial effusions are the most common organ system manifestations, occurring in as many as 10% of patients.[51] A serious life-threatening systemic manifestation called *macrophage activation syndrome* (MAS) is discussed in more detail later. The arthritis in sJIA can vary from minimal to oligoarthritis to polyarthritis presentation[51] and typically does not correlate with the severity of the systemic manifestations.[53] Tenosynovitis and synovial cyst formation can also be seen. Although the systemic manifestations can be serious initially, the long-term morbidity is the result of severe articular damage and adverse effects of medications, especially chronic corticosteroids.

There are no diagnostic tests for sJIA, but there are characteristic patterns of laboratory abnormalities, including high inflammatory markers, significant leukocytosis with neutrophilia and bandemia, thrombocytosis, and anemia. Liver transaminases, aldolase, ferritin, fibrin split products, and coagulation screen may be abnormal in severe cases and can be signs of early or impending MAS. ANA or other autoantibodies are rarely present. Inflammatory cytokine gene expression profiles distinguish sJIA from other febrile inflammatory diseases seen in children, correlate with response to IL-1 inhibitor therapy, and may lead to more specific tests in the future.[54,55]

Because of the nonspecific nature of the characteristics of sJIA, the diagnosis can be difficult and should be approached as a diagnosis of exclusion. Infections, Kawasaki's syndrome, malignancy, and other autoimmune diseases can have similar symptoms; therefore it is necessary to

screen for infectious agents and malignancies, especially leukemia and neuroblastoma, with appropriate tests including cultures, bone marrow aspiration and biopsy, and urinary vanillylmandelic acids. The recurrent fever syndromes are often mistaken for sJIA, but the character of the fevers and the fixed rashes associated with these syndromes should alert the clinician to a different diagnosis.

### Special Considerations: Macrophage Activation Syndrome

Overt life-threatening MAS will develop in approximately 10% of patients with sJIA, and a milder form will develop in as many as 30%. If inadequately treated, the milder form could lead to full-blown MAS.[54,56] MAS is a form of secondary or acquired hemophagocytic lymphohistiocytosis (HLH) seen with rheumatic disease.[57] The mortality rate can be 20% to 30%, accounting for the majority of the mortality seen in all patients with JIA.[58] The main manifestations include unrelenting high fevers, hepatosplenomegaly, lymphadenopathy, severe cytopenias, liver dysfunction, central nervous system (CNS) involvement (seizures/coma), and coagulopathy. Ferritin often exceeds 10,000 ng/mL, and coagulopathy can be impressive, with elevated prothrombin and partial thrombin times, hypofibrinogenemia, petechiae, mucosal bleeding, epistaxis, and hematemesis. Often MAS is heralded by decreased leukocyte and platelet counts, falling ESR, liver dysfunction, hyperferritinemia and elevated D-dimers.[56] Histologically, most patients with MAS show expansion of well-differentiated macrophages, exhibiting hemophagocytosis in the bone marrow, lymph nodes, and other organs such as the liver and lungs. There is no gender, age, or race predilection for MAS in sJIA. Half of MAS cases studied were associated with active disease, one-third with infectious triggers (Epstein-Barr virus, varicella, coxsackie, parvovirus B19, hepatitis A, *Salmonella*, and *Pneumocystis*), 4% with medication exposures (aspirin, NSAIDs, sulfasalazine [SSZ], methotrexate [MTX], etanercept, anakinra, and gold salts), and the rest unidentified.[56] Because many patients who are receiving these medications have active disease, it is challenging to attribute MAS to disease activity versus medications.[59,60] Current understanding of pathophysiology includes excessive inflammatory cytokines, activation of T cells and macrophages, poor natural killer and CD8 cell cytolytic activity, and low levels of perforin expression.[54,61] Soluble IL-2Rα receptors and soluble CD163 are increased several-fold in MAS.[62,63] Recently, *MEFV* mutations seen in Familial Mediterranean fever have been identified in 18.5% of patients with sJIA, and these patients were noted to have the most resistant disease.[64]

## TREATMENT

With the current development of increasingly more effective biologic treatment for arthritis, pediatric rheumatologists now aim to achieve complete disease remission. Several studies support the paradigm of treating aggressively to reach inactive disease as early as possible in the disease course, which may ultimately lead to better outcome, such as improved quality of life, shorter periods of time spent in active disease, and less long-term joint damage.[65] Based on ACR published guidelines for treatment of JIA,[66,67] a comprehensive picture of each patient's clinical features, JIA subtype, disease activity, and prognostic factors should drive treatment escalation. Comparative effectiveness studies based on consensus treatment plans developed by rigorous consensus methodology, are currently underway for systemic and polyarticular JIA.[68-70] Integration of these published ACR guidelines, the consensus treatment plans, and randomized control trials (RCT) have been used to inform the treatment recommendations in this chapter. There are now validated definitions of core sets, disease activity, clinical inactive disease (CID), disease severity, improvement, and remission that can be used in individual practice and research trials to determine treatment changes.[71,72] The following paragraphs summarize general information about different therapies used in children, followed by specific treatment approaches for the different subtypes of JIA. Table 107-2 shows medications, dosing, route, and safety monitoring recommendations.

### Nonsteroidal Anti-inflammatory Drug Use in Children

NSAIDs may help control symptoms of pain and stiffness, but do not alter the natural history of JIA. NSAIDs are frequently used for symptom control as an adjunctive therapy to more definitive therapies. The overall use of NSAIDs is declining in the era of biologic treatment.[73] In general, NSAIDS should only be considered as monotherapy in initial therapy in patients with low disease activity. If control is not achieved in 1 to 2 months, additional therapy should be considered.[66]

**TABLE 107-2** Commonly Used Medications in Juvenile Idiopathic Arthritis[66,67,234-236]

| Medication | Typical Dose and Administration Route | Typical Frequency |
|---|---|---|
| Abatacept<br>*FDA approved for poly JIA ages >6 years | 10 mg/kg (max 1000 mg) IV | Load at 0, 2, 4 wk, then every 4 wk |
| Adalimumab<br>*FDA approved for poly JIA ages ≥2 years | 24 mg/m$^2$ SQ<br><30 kg: 20 mg SQ<br>≥30 kg: 40 mg SQ | Every 2 wk (arthritis)<br>Every wk (uveitis) |
| Anakinra | 1-4 mg/kg (max 100 mg) SQ | Daily |
| Canakinumab<br>*FDA approved for sJIA ages ≥2 years | 4 mg/kg (max 300 mg) SQ | Every 4 wk |

**TABLE 107-2** Commonly Used Medications in Juvenile Idiopathic Arthritis[66,67,234-236]—cont'd

| Medication | Typical Dose and Administration Route | Typical Frequency |
|---|---|---|
| Certolizumab pegol | Initial adult RA dose: 400 mg SQ loading dose on wk 0, 2, 4, then<br>Maintenance dose with either:<br>a. 200 mg SQ<br>b. 400 mg SQ | Every 2 wk or<br>Every 4 wk |
| Cyclosporine | 6 mg/kg/day orally | Divided twice a day |
| Diclofenac | 2-3 mg/kg/day (max 200 mg/day) orally | Divided 2-4 times a day |
| Etanercept<br>*FDA approved for poly JIA ages ≥2 years | 0.8 mg/kg/wk (max 50 mg) SQ, *or*<br>0.4 mg/kg/dose (max 25 mg/dose) SQ | Once weekly, or<br>twice a week |
| Golimumab | 30 mg/m$^2$ (max 50 mg) SQ | Every 4 wk |
| Ibuprofen | ≥6 mo of age: 30-40 mg/kg/day | Divided 3-4 times a day |
| Indomethacin | >1 mo of age: 1-4 mg/kg/day | Divided 2-4 times a day |
| Infliximab | 5-10 mg/kg/dose IV | Load at 0, 2, 6 wk, then every 4 wk |
| IV immunoglobulin | 2 g/kg/dose IV | Every 2 wk |
| Leflunomide | <40 kg: 10 mg orally<br>>40 kg: 20 mg orally | Daily |
| Meloxicam | 0.125-0.25 mg/kg (max 15 mg/day) | Daily |
| Methotrexate<br>*FDA approved for JIA | 15 mg/m$^2$/dose or SQ (0.6 mg/kg; max 25 mg) | Weekly |
| Naproxen | >2 yr of age: 20 mg/kg/day (max 1 g/day) | Divided twice a day |
| Piroxicam | 0.2-0.4 mg/kg/dose (max 20 mg/day) | Daily |
| Rilonacept | 4.4 mg/kg (max 320 mg) loading dose, then 2.2 mg/kg (max 160 mg) SQ | Once weekly |
| Rituximab | 375 mg/m$^2$ IV, or<br>750 mg/m$^2$ (max 1000 mg) IV | Weekly × 4 doses, or<br>every 2 wk × 2 doses |
| Sulfasalazine | 50 mg/kg/day (max 2 g/day) orally | Divided twice a day |
| Sulindac | 4-6 mg/kg/day (max 400 mg/day) orally | Divided twice a day |
| Thalidomide | 5 mg/kg/dose orally | Daily |
| Tocilizumab<br>*FDA approved for sJIA and poly JIA ages ≥2 years | sJIA: <30 kg: 12 mg/kg IV<br>≥30 kg: 8 mg/kg IV (max 800 mg)<br>Polyarticular JIA/uveitis: <30 kg: 10 mg/kg IV<br>≥30 kg: 8 mg/kg IV (max 800 mg) | Every 2 wk for sJIA<br><br>Every 4 wk for polyarticular JIA/uveitis |
| **Summary of Recommendations for Medication Safety Monitoring** | | |
| **Nonsteroidal Anti-inflammatory Drugs**<br>Complete blood count, liver enzymes, serum creatinine, and urinalysis<br>Before or soon after initiation of routine use<br>Repeat approximately twice yearly for chronic daily use<br>Repeat approximately once yearly for routine use (3-4 days/wk) | | |
| **Methotrexate**<br>Complete blood count, liver enzymes, serum creatinine<br>Before initiation<br>Approximately 1 mo after initiation<br>Approximately 1-2 mo after increase in dose<br>Repeat approximately every 3-4 mo if prior results are normal and dose stable | | |
| **TNF Inhibitors**<br>Complete blood count, liver enzymes, serum creatinine<br>Before initiation<br>Repeat approximately every 3-6 mo<br>Tuberculosis screening<br>Before initiation<br>Repeat approximately once yearly (controversial) | | |

*ACR has not established monitoring guidelines for other biologics besides TNF inhibitors. Please refer to drug product insert for monitoring recommendations.

ACR, American College of Rheumatology; FDA, U.S. Food and Drug Administration; IV, intravenous; poly, polyarticular; RA, rheumatoid arthritis; sJIA, systemic JIA; SQ, subcutaneous; SR, sustained release.

## Intra-articular Steroid Injections

The use of intra-articular triamcinolone hexacetonide (THA), 1 mg/kg in large joints, such as the knee, and 0.5 mg/kg in smaller joints, such as the ankle, has been found to be superior to triamcinolone acetonide (TA),[74] betamethasone, and methylprednisolone acetate in RCTs.[75-80] Early treatment is associated with better outcome,[81] and intra-articular steroids (IAS) are expected to result in clinical improvement of arthritis for as long as 4 months. Therefore, if arthritis recurs, joint injections can be repeated as many as three times in a 12-month period. Difficult-to-reach joints, such as the hip, SI joint, temporomandibular joint (TMJ), and subtalar joint, may be injected by using ultrasound or fluoroscopy. Multiple IAS injections in the knee, ankle, or wrist do not appear to change the mean cartilage thickness.[82] IAS should be administered under sedation or general anesthesia for the young. The use of IAS for active arthritis has been recommended by the ACR guidelines, regardless of concurrent therapy, JIA subtype, disease activity, prognostic features, or joint contracture. If the duration of clinical improvement is shorter than 4 months, systemic treatment (e.g., MTX) may be indicated.[66]

## Corticosteroid Use in Children with Juvenile Idiopathic Arthritis

In general, systemic corticosteroids should be used sparingly in the treatment of any subgroup of JIA because of the severe morbidity associated with chronic use, even at low doses. Newer therapeutic modalities such as biologics may eliminate the need for any corticosteroids or limit the doses needed. The evidence for use of corticosteroids for the synovitis of JIA is controversial and conflicting, and the ACR was unable to make any specific recommendations for JIA treatment regarding corticosteroid use, except in sJIA for severe systemic features.[66] However, in some cases, low doses of corticosteroids (0.1-0.5 mg prednisone equivalent/kg/day) or brief high-dose regimens (intravenous [IV] methylprednisolone 30 mg/kg/day, maximum dose 1000 mg daily for 1 to 3 days) may be used in polyarticular JIA to bridge constitutional features of pain and fatigue while waiting for disease-modifying anti-rheumatic drugs (DMARDs) or biologic therapies to reach therapeutic effect.

## Methotrexate

MTX is effective, safe, and the most commonly used DMARD in JIA.[83,84] In a retrospective cohort study involving all JIA subtypes, the strongest predictor of response to MTX at 6 months of treatment was the time from diagnosis to start of MTX, suggesting that starting MTX early will lead to a better response.[85] The ACR guidelines support a maximum dose of 0.6 mg/kg once weekly (equivalent to 15 mg/m$^2$/wk, maximum 25 mg/week) of parenteral MTX.[66] In patients with lower disease severity, lower doses (8-12.5 mg/m$^2$/wk, oral or parenteral) may be effective, and these doses are similar in safety.[86] Most pediatric rheumatologists start folic acid at 1 mg/day, but daily folate supplementation remains controversial. Liver biopsies are not indicated except in unusual circumstances. Some children develop an intolerance to MTX, and leflunomide may be used as an alternative.[87,88] Both MTX and leflunomide are associated with teratogenic effects.

## Biologic Agents

### Tumor Necrosis Factor Inhibitors

TNF inhibitor agents are effective in many children with polyarthritis of any JIA subtype, although less in sJIA.[89] In RCTs, etanercept and adalimumab have high efficacy and safety in patients with JIA with polyarthritis.[90,91-96,97,98] Combination MTX with etanercept or adalimumab seems to be more effective than TNF monotherapy.[91,97]

Infliximab did not reach statistical significance for efficacy in an RCT in polyarticular-course JIA patients[99]; however, there was a trend for a higher dose 6 mg/kg/infusion to be more effective than the adult dose of 3 mg/kg/infusion. Higher doses of infliximab can be given[100] if loss of efficacy with time occurs, possibly from the formation of anti-infliximab antibodies. Low-dose MTX given concomitantly may reduce the risk of neutralizing antibody development, as it does in adults.

In August 2009, the U.S. Food and Drug Administration issued a black box warning on the increased risk of cancer, particularly lymphoma, in children and adolescents receiving TNF inhibitors for arthritis or IBD.[101] The other adverse events involved with TNF inhibitors are similar to that of adults and have been reported in children as well, including serious infections, demyelinating processes, optic neuritis, injection-site reactions or infusion reactions, and development of autoimmune conditions. A recent cohort study showed that patients with JIA who were not taking MTX or TNF inhibitors still had a twofold increased rate of serious bacterial infections requiring hospitalization. Children with JIA did not have an increased rate of infection with MTX or TNF inhibitor use but had three times more infections with high-dose steroid use (≥10 mg/day of prednisone or equivalent).[102] In most, but not all, of the recent epidemiologic studies to approximate malignancy risk, children with JIA appeared to have a twofold to fourfold increased risk of malignancy compared with children without JIA, which did not appear to be significantly associated with treatment, including TNF inhibitors.[103,104] Efforts are underway in the United States and Europe to capture post-marketing serious adverse events (SAE) with all biologics using disease registries or within the context of an established multicenter research network.[105,106]

### Abatacept

Abatacept, a fusion protein of the naturally occurring co-stimulatory inhibitory molecule cytotoxic T lymphocyte–associated protein 4 and the Fc portion of IgG, is currently the only T cell co-stimulatory inhibitor used in inflammatory arthritis. It is safe and efficacious in a RCT of polyarticular-course JIA patients who had inadequate response or intolerance to DMARD or TNF inhibitor in the past.[107] Abatacept continued to be clinically significant with durable effectiveness in the 3-year open-label extension study. Some patients require longer periods for optimal response (>3 to 4 months) compared with TNF inhibitors.[108]

### IL-1 Inhibitors

Anakinra was the first IL-1 inhibitor developed from the endogenous IL-1 receptor antagonist (IL-1Ra). Although anakinra has not been approved for use in JIA, a series of studies suggested that anakinra was effective in sJIA,[109-111] leading to extensive use in this difficult-to-treat disease subtype. Two additional IL-1 inhibitors, canakinumab and rilonacept, have been developed and are highly effective and safe in RCTs of patients with sJIA.[112-114] Canakinumab is a fully human monoclonal antibody against IL-1B that can be administered monthly. Rilonacept is a fusion protein of the IL-1 receptor molecules and the Fc portion of IgG that can bind to IL-1α, IL-1B, and IL-1Ra, and is given every 2 weeks. The safety profile of IL-1 inhibitors is less understood compared with other agents because of the small numbers of patients in the phase III trials and the overall smaller numbers of patients with sJIA. In addition, patients with sJIA can have severe disease complications, making it difficult to attribute SAEs to a specific treatment versus underlying disease. Tuberculosis, opportunistic infection, demyelinating disease, or malignancy have not been noted, but clearly, more long-term studies and more patients are required to evaluate the actual safety concerns.

### IL-6 Inhibitors

Tocilizumab is a fully human monoclonal antibody directed against the IL-6 receptor, and it is highly effective in sJIA and polyarticular-course JIA patients.[115-117] Similar to canakinumab and rilonacept, tocilizumab showed remarkable efficacy in sJIA. In a RCT, tocilizumab was safe and efficacious in biologic-naïve and biologic-refractory patients, comparable to the efficacy of etanercept and adalimumab, in polyarticular-course JIA patients.[90-118] Those patients with polyarthritis receiving concomitant MTX and tocilizumab had better responses compared with those without MTX. Patients who had previously taken biologics had poorer ACR responses than biologic-naïve patients, showing more treatment-resistant disease. As with the other newer biologics, the numbers of patients exposed are small, making it difficult to assess the safety profile. Infection, neutropenia, and reversible transaminitis were noted in both trials, with mild to moderate elevation of low-density lipoprotein and cholesterol levels in the trial with polyarticular-course JIA patients.

## Other Disease-Modifying Anti-rheumatic Drugs and Biologics

Rituximab may be considered as a treatment option for patients who have not responded to other therapies and continue to have high, refractory disease activity based on adult RA studies, limited observational studies, and case reports in children.[66,119] An open-label, multicenter RCT comparing combination DMARDs (MTX, SSZ, and hydroxychloroquine) versus MTX and infliximab versus MTX alone in early diagnosed polyarticular-course JIA patients showed that MTX and infliximab was the most effective.[120] The following paragraphs discuss specific JIA subtypes and their recommended treatments.

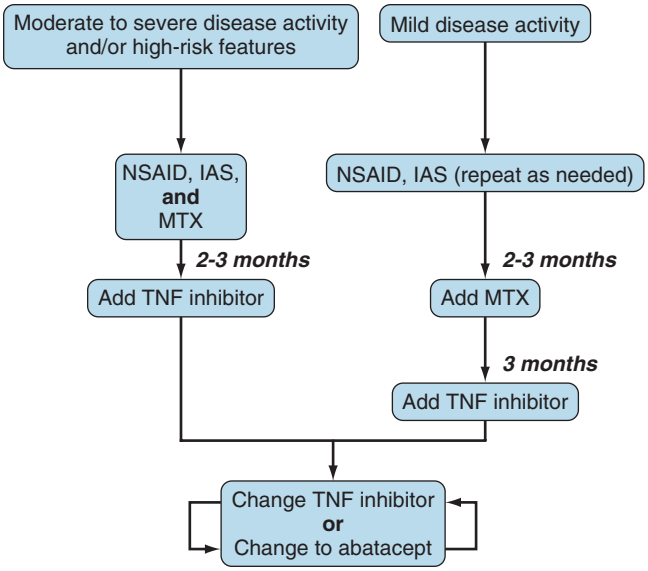



**Figure 107-2** Treatment algorithm for patients with juvenile idiopathic arthritis (JIA) with oligoarthritis or arthritis in four or fewer joints. IAS, Intra-articular steroid; MTX, methotrexate.

# TREATMENT OF JUVENILE IDIOPATHIC ARTHRITIS SUBTYPES

## Treatment of Oligoarticular Juvenile Idiopathic Arthritis

Treatment of patients with arthritis in four or fewer joints is usually approached in a stepwise fashion (Figure 107-2).[66,121] Poor prognostic features include involvement of the hip or cervical spine; ankle or wrist involvement with high inflammatory markers; or radiographic changes, such as erosions and joint space narrowing.[66]

## Treatment of Polyarticular Juvenile Idiopathic Arthritis

Treatment approaches are similar for patients with arthritis in five or more joints, regardless of JIA subtype with the exception of sJIA (see Treatment of sJIA section). Children with polyarthritis require a DMARD such as MTX as soon as practically possible after the diagnosis has been confirmed, if they have high disease activity or if there is moderate activity with poor prognostic features. Poor prognostic features include arthritis of the hip or cervical spine, positive RF or ACPAs, and radiographic damage. If NSAIDs are initially used as monotherapy, with or without IAS, continued disease activity for no more than 1 to 2 months should prompt escalation of therapy, as shown in Figure 107-3.[66] Per the ACR guidelines, MTX is the first DMARD of choice, and if there is an inadequate response to MTX by 3 months with continued moderate or high disease activity, TNF inhibitors should be added corresponding to the "Step Up" arm of the consensus treatment protocol.[66,86,90] The different TNF inhibitors have not been directly compared, so it is not possible to determine which might be most effective and safe in a given patient. After 4 months of TNF inhibition, if there is still moderate or high disease activity, the ACR guidelines recommend switching to

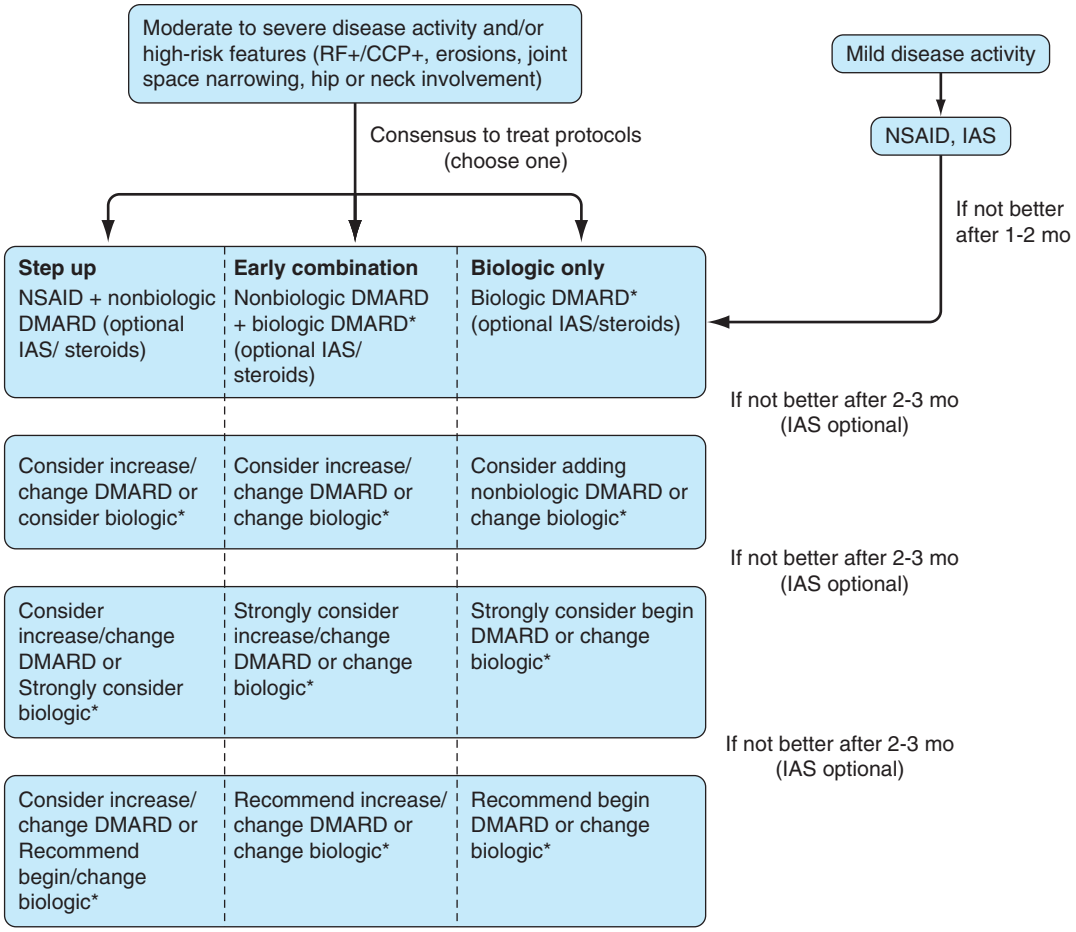



**Figure 107-3** Treatment algorithm for patients with juvenile idiopathic arthritis (JIA) with polyarthritis or arthritis in five or more joints. ACPA, Anti-citrullinated protein antibody; CCP, cyclic citrullinated peptide; DMARD, disease-modifying anti-rheumatic drug; IAS, intra-articular steroid; IL, interleukin; MTX, methotrexate; RF, rheumatoid factor.

another TNF inhibitor or to another biologic, such as abatacept.[66,121] However, with sequential use of biologics, the response rate is attenuated.[122] Rituximab, tocilizumab, abatacept, or different combinations of various DMARDs and biologics have all have been used after failure of response to TNF inhibitors, with varying degrees of success.[66,121]

Because children with $RF^+$ polyarticular JIA are at higher risk of prolonged erosive arthritis compared with other subtypes of JIA, these children should be considered to be in the more severe disease category, requiring rapid escalation of treatment if even mild disease activity persists.[66]

SSZ and leflunomide can be used before a TNF inhibitor in mild disease, although evidence suggests that leflunomide may be slightly less effective than MTX.[123] SSZ is more typically used with HLA-B27–associated arthritis and JSpA.

In 2012, an RCT called Trial of Early Aggressive Therapy (TREAT) compared two aggressive treatment arms (combination of corticosteroids and etanercept, and MTX vs. MTX alone) for patients with active polyarticular JIA within 1 year of diagnosis. There was a trend toward improvement in the MTX, etanercept, and prednisone arm, and it showed that CID[124] at 6 months was attainable by using either of the two early aggressive treatment arms. This study also suggested a treatment window of opportunity: The shorter the disease duration before treatment, the higher the likelihood of reaching CID at 6 months (odds ratio [OR] per month, 1.3, $P < 0.011$).[125,126] However, 65 % of children were not able to sustain CID beyond 6 to 12 months, especially with medication taper,[127] suggesting that longer therapy may be helpful.

## Treatment of Juvenile Spondylitis

To date, there have been few studies or trials on JSpA. One study of SSZ showed no significant effects in JSpA, whereas another showed good response in JIA patients with oligoarticular and polyarticular course.[128,129] For severe symptoms or evidence of potential joint damage (such as erosive sacroiliitis), the use of TNF inhibitors has been effective based on small pediatric studies and adult studies.[130,131,26,132] The 2011 ACR treatment recommendations for JIA as described previously can be applied to JSpA, taking special note that evidence of active sacroiliitis should be considered a feature of poor prognosis, thus potentially progressing to biologic initiation more quickly.[66]

## Treatment of Juvenile Psoriatic Arthritis

There have been only two open-label prospective studies examining the efficacy of biologics in JPsA, both of which showed improved response within 3 months.[96,133] A study comparing treatment regimens of patients with JPsA versus oligoarticular JIA patients and polyarticular JIA patients found no difference in use of NSAIDs, MTX, and TNF inhibitors.[48] In general, the treatment of JPsA should follow the treatment approaches for patients with oligoarthritis or polyarthritis. MTX is of benefit for both the skin psoriasis and arthritis and in children.[43] In children with more aggressive or axial disease, TNF inhibitors (which have also been successful in treating psoriasis) is indicated and may significantly limit bony destruction.[44,134,135]

## Treatment of Systemic Juvenile Idiopathic Arthritis

sJIA is an active area of research with increasing understanding of the autoinflammatory biology and the potential therapeutic roles for targeted biologics. Therapies such as MTX and TNF inhibitors are less helpful in sJIA compared with other forms of JIA.[52,136-138] An approach to treating sJIA is shown in Figure 107-4, separating this complex disease into different severities and manifestations. This algorithm is consistent with the recently published 2013 ACR guidelines for sJIA,[67] consensus treatment plans for sJIA,[68] and published RCTs.[114,115] Mild sJIA, defined as mild systemic symptoms and synovitis without organ system involvement, can be successfully treated with anti-inflammatory doses of NSAIDs. Certain NSAIDs are helpful for different aspects of the disease (e.g., indomethacin can be used for fever control and serositis symptoms). In addition, if there are only a few large joints involved, IASs are an additional option.

In the more severe cases with persistent fevers, cardiopulmonary symptoms, significant anemia, and significantly elevated inflammatory markers, corticosteroids may be used, often given as pulsed IV methylprednisolone (30 mg/kg/dose, maximum 1 g/day) daily for 3 consecutive days, followed by tapering doses of oral prednisolone.[139] The newer biologics that inhibit IL-6 (tocilizumab) and IL-1 (anakinra, canakinumab, and rilonacept) have been shown in clinical trials to be very effective in treatment of sJIA.[112-115] The IL-1 signature found in active sJIA and subsequent response to IL-1 inhibition demonstrates a key feature in the pathogenesis of sJIA.[54,55,111] Increasingly, IL-1 or IL-6 inhibition is instituted earlier to avoid steroid toxicity, and in some recent studies, is used as initial therapy before corticosteroids.[140,141] Use of MTX and TNF inhibitors may be more helpful for more significant articular disease.[67,89] Cyclosporine, as well as monthly IV immunoglobulin, have shown some benefit in systemic symptoms and can be used for

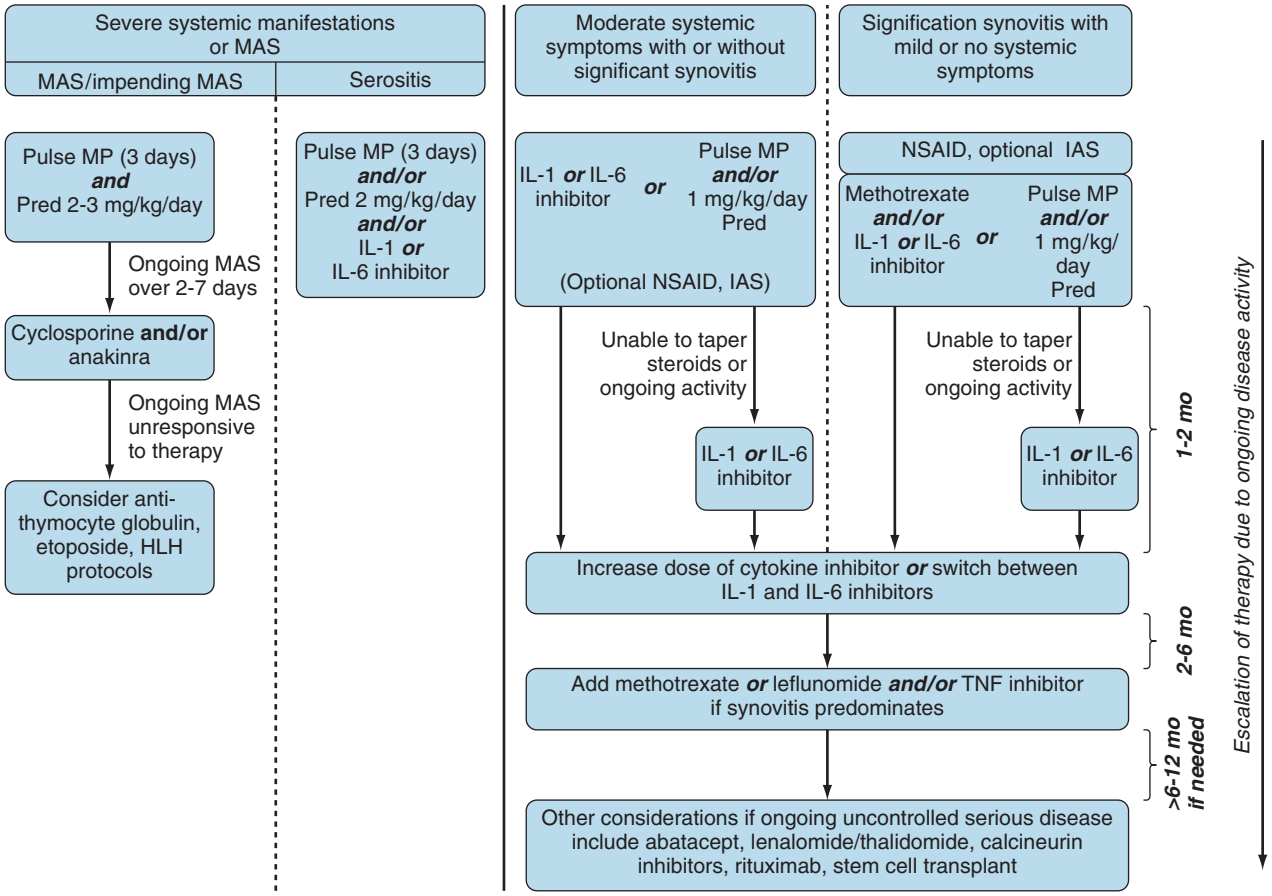



**Figure 107-4** Treatment algorithm for systemic juvenile idiopathic arthritis and macrophage activation syndrome (MAS). HLH, Hemophagocytic lymphohistiocytosis; IAS, intra-articular steroid; IL, interleukin; MP, methylprednisolone; Pred, prednisone.

**TABLE 107-3** Recommended Ophthalmologic Screening Frequency for Asymptomatic Uveitis in Juvenile Idiopathic Arthritis Patients*

| JIA Subgroup, According to ILAR Criteria | Anti-nuclear Antibody Status | Age of JIA Onset (yr) | JIA Duration (yr) | Eye Examination Frequency (mo) |
|---|---|---|---|---|
| Oligoarticular JIA, RF⁻ polyarticular JIA, PsA, other arthritis | + | ≤6 | ≤4 | 3 |
| | + | ≤6 | >4 | 6 |
| | + | ≤6 | ≥7 | 12 |
| | + | >6 | ≤2 | 6 |
| | + | >6 | >2 | 12 |
| | − | ≤6 | ≤4 | 6 |
| | − | ≤6 | >4 | 12 |
| | − | >6 | NA | 12 |
| Enthesitis-related arthritis | NA | NA | NA | 12 |
| RF⁺ polyarticular JIA, systemic JIA | NA | NA | NA | 12 |

*Patients with uveitis should have ophthalmologic screenings according to their uveitis course.

ILAR, International League of Associations for Rheumatology; JIA, juvenile idiopathic arthritis; mo, months; NA, not applicable; PsA, psoriatic arthritis; RF, rheumatoid factor.

From Heiligenhaus A, Niewerth M, Ganser G, et al: German uveitis in childhood study group: prevalence and complications of uveitis in juvenile idiopathic arthritis in a population-based nation-wide study in Germany: suggested modification of the current screening guidelines. *Rheumatology* 46(6):1015–1019, 2007.

steroid-sparing effects.[142] Abatacept has been used in patients with sJIA who have failed previous treatments. In the open-label portion of an RCT of abatacept therapy in polyarticular-course JIA patients, 65% of patients with initial sJIA subtype at onset had a response comparable with the other JIA subtypes evaluated in that study.[107] For very severe refractory cases, stem cell transplantation (SCT) has been performed, and some cases have shown benefit.[143]

The 2013 ACR guidelines for JIA treatment divided sJIA patients into two groups: (1) active systemic features, and (2) active arthritis, but the guidelines did not include patients with MAS, impending MAS, or life-threatening manifestations (e.g., cardiac tamponade).[67] The algorithm shown in Figure 107-4 also addresses severe systemic disease and MAS. The optimal treatment for MAS continues to be controversial. Therapy should be aggressive and usually starts with high-dose steroids and disease-modifying agents such as cyclosporine and IL-1 blockade.[57,144,145] Almost 50% of patients in one study showed response to steroids alone.[60] If the patient is not responding, then etoposide can be used based on the current treatment protocol for HLH.[146,147] In an attempt to avoid severe bone marrow suppression, antithymocyte globulin has been used.[57,148]

## UVEITIS

Chronic anterior uveitis, defined as inflammation of the anterior uveal tract, including the iris and ciliary body, is the most frequent extra-articular manifestation of JIA. Uveitis is most common in patients with oligoarticular JIA (17% to 26%), patients with RF⁻ polyarticular JIA (4% to 25%), and patients with JPsA (10%). It is rare in patients with RF⁺ polyarticular JIA and sJIA.[10,149] Overall, uveitis is observed in 30% of ANA⁺ patients with JIA.[150] Risk factors for uveitis include ANA positivity, age at JIA onset 6 years or younger, disease duration 4 years or less, female sex, oligoarticular JIA subtype, and JPsA subtype.[151] ANA positivity and age of JIA onset are larger risk factors in girls compared with boys.[152,153]

Chronic anterior uveitis is typically nongranulomatous, recurrent, and asymptomatic and, if unrecognized, can lead to serious visual deficits. Because of its insidious onset, regularly scheduled screening by an experienced ophthalmologist with slit lamp examination is required for early diagnosis and treatment with specific guidelines (Heiligenhaus guidelines) as shown in Table 107-3.[154] Newly diagnosed patients are ideally screened within 6 weeks of diagnosis[155] because approximately 30% of uveitis cases have been found at the onset of JIA,[156] and 5% to 10% have occurred prior to diagnosis.[157] Ninety percent of uveitis cases are diagnosed in the first 4 years after JIA diagnosis,[150] but uveitis has been reported even into adulthood.[150,158] In children with acute anterior uveitis, as can be seen in JSpa, the uveitis is usually highly symptomatic and therefore requires only annual screening. The clinical course of the uveitis and arthritis may not parallel one another.

The differential diagnoses include infectious causes, such as tuberculosis, toxoplasmosis, cytomegalovirus, herpes simplex virus, syphilis, human immunodeficiency virus, Lyme disease, cat scratch disease, and fungus. Uveitis can occur secondary to a systemic illness, such as in Behçet's disease, sarcoidosis, autoinflammatory syndromes, multiple sclerosis, TINU (tubulointerstitial nephritis and uveitis syndrome), and Vogt-Koyanagi-Harada syndrome. It is also important to not miss a malignancy such as lymphoma or retinoblastoma.[151]

The goal of uveitis treatment is to control the acute inflammation and complications, treat the underlying systemic disease, minimize medication side effects, prevent recurrences, and preserve vision. Inflammation should be controlled to less than 0.5+ cells per eye.[154] Initial treatment is to use high-potency topical corticosteroids, as well as mydriatics to dilate the eye in an attempt to prevent synechiae.[159] If there is impending vision loss, severe inflammation, ocular hypotony, or macular edema, ocular steroid injections or systemic steroids can be used. In approximately 40% of patients, the uveitis remains active, and if topical or systemic corticosteroids cannot be weaned,

alternative regimens need to be considered to prevent cataracts and glaucoma as well as ongoing inflammatory damage.[154,160,161] Although there are no RCTs on the use of immunosuppressive medications in children with uveitis, several observational studies suggest the effectiveness of these treatments.[154,162] MTX is usually the first choice [163-165] with an estimated response rate to MTX in 74%.[166] Alternative immunosuppressives include azathioprine[154,167] and mycophenolate mofetil (MMF).[168,169] Cyclosporine has limited value.[170] In cases of refractory inflammation after 3 to 4 months of MTX, TNF inhibitors are effective.[171] Infliximab and adalimumab appear to be equally effective, whereas etanercept is less effective.[171-180] Adalimumab is currently preferred over infliximab because of a higher probability of persistent remission.[175,181] There have been a few reports investigating golimumab at 50 mg SQ monthly dosing for uveitis.[182-185] For cases refractory to treatment with TNF inhibitors, there have been case series examining the use of abatacept,[186-188] tocilizumab,[189,190] and rituximab.[191] Uveitis specialists recommend tapering immunomodulatory therapy after 12 to 24 months of disease quiescence.[162-192] Treatment of uveitis requires close collaboration between the child's rheumatologist and ophthalmologist. In 2012, Heiligenhaus and colleagues. published treatment guidelines for uveitis in JIA based on the current evidence and consensus.[154]

The reported rate of visual loss due to JIA-related uveitis has decreased from 22% to 66% in studies before 1990 to 3% to 25% in newer studies, suggesting that increased screening and newer, more aggressive therapeutic approaches, including biologics and ophthalmologic surgery, are effective.[2] Risk factors for poor visual acuity include presence of posterior synechiae (Figure 107-5), band keratopathy, and glaucoma; oral steroid use; active uveitis, especially with 1+ or higher-grade anterior chamber cells; and prior intraocular surgery.[193,194]




**Figure 107-5** Chronic anterior uveitis demonstrating posterior synechiae and absence of significant scleral inflammation.

## IMAGING

Imaging is important in JIA to confirm the diagnosis—to exclude other diseases such as infection, malignancy, osteoid osteoma, or avascular necrosis; to monitor therapy; and to track disease activity. Determining the presence of cartilage loss and erosions is complicated by the anatomically changing joints during the normal growth process. Because children have a large amount of cartilage in their joints, a significant amount of destruction can occur before erosions are identified on plain radiographs. Local growth disturbances can also be seen in children, including bony overgrowth, particularly in the small joints of the hands and feet and in the knees, likely because of chronic hyperemia. In contrast, premature fusion of the epiphyses can lead to shortening of certain joints, most notably TMJ leading to micrognathia.[195-197] Interestingly, the articular cartilage of growing children has unique regenerative qualities, and studies have shown that children with JIA can have improvement in their radiographic joint damage.[197]

Plain radiographs in patients with JIA show soft tissue swelling as an early but nonspecific finding. The most common form of radiographic damage in JIA is joint space narrowing caused by erosion, thinning, and loss of articular cartilage.[193,195] Periarticular osteopenia caused by hyperemia of inflamed joints is frequently seen. Erosions and ankylosis can be seen as late findings in radiographs.[195] Joint protection is important in pediatric patients, and damage can be as frequent and as severe as in adult patients with RA, with a higher risk for bilateral hip damage in patients with JIA.[198] The trend toward early aggressive treatment to prevent erosive disease shifts the imaging need away from plain radiographs and toward other imaging modalities that are more sensitive in detecting early disease activity,[196] such as MRI and ultrasound.

Ultrasound is reliable, safe, and relatively inexpensive but not as well standardized in children as in adults. It is more sensitive than plain radiography in the detection of effusions, synovial thickening, and synovial cysts. In experienced hands, cartilage thinning and bone erosions can also be seen. When compared with healthy controls, all patients with JIA have been found to have decreased cartilage thickness in the knee and wrist joints even if no signs of active arthritis on examination. Patients with polyarticular JIA or sJIA appear to have thinner cartilage than patients with oligoarticular JIA.[82] Inflammatory involvement of the hip, shoulder, and elbow is more frequently accurately detected by ultrasound compared with clinical examination.[199,200] Color and power Doppler ultrasound can facilitate the evaluation of hyperemia and vascular abnormalities in affected joints and tendon involvement.[201]

MRI is the most sensitive imaging modality currently available for detecting synovial inflammation; however, its use is limited by expense and the need for anesthesia in younger children. With the use of contrast, accurate differentiation between active and inactive states can be established. Synovial enhancement and thickening can easily be seen,[202] and pannus volume measurements can be tracked.[203]

For evaluating bony erosions, MRI was found to be the most sensitive imaging modality in wrists of JIA patients, revealing more than twice as many erosions compared with plain radiography and ultrasound.[204]

## OUTCOMES

With earlier, more aggressive, targeted therapies, as well as the goal of achieving CID, the overall outcome of JIA is clearly improving. However, even with these new approaches, JIA can persist through adulthood and cause significant morbidity, functional disability, joint damage, and growth impairment. Although there is wide within-patient and between-patient variability, children with early disease onset and a greater number of restricted joints have the highest risk of developing long-term functional physical disability.[233]

In general, use of MTX and biologics are improving outcomes. Prebiologic cohorts suggest that 34% to 60% of patients continued to have active disease.[205,206] Children younger than 5 years had worse outcomes in achieving clinical remission, independent of ILAR category.[207] Recent reports during the biologic era showed overall improved probability of achieving CID in 2 years, yet one-third of patients transferring to adult services still required biologics for an average duration of 4.2 years.[208,209] Growth delay, a major issue for children with polyarticular JIA and sJIA, improves with TNF inhibitors.[210] The following paragraphs detail outcomes of the major JIA subtypes.

### Oligoarticular Juvenile Idiopathic Arthritis

In general, patients with oligoarthritis have a better overall outcome than patients with polyarthritis. Patients with persistent oligoarticular JIA generally have the best outcome, with 68% to 80% of patients achieving clinical remission.[13,206,211] Extended oligoarticular JIA patients usually follow a similar chronic disease course as $RF^-$ polyarticular JIA and have higher cumulative duration of active arthritis,[211] more erosive disease, and a higher risk of chronic disability.[13] Partial or complete remission on MTX can be induced in 60% to 70%,[86,212,213] whereas complete remission off medications was seen in 31% to 50% of children with extended oligoarticular JIA.[206,211] Relapses occur with disease flares occurring 2 years after remission in 45% to 47% of patients with persistent oligoarticular JIA and 56% to 67% with extended oligoarticular JIA.[207,211]

Ongoing disease activity and extension of joint involvement in oligoarticular JIA patients are related to a poor outcome and radiographic damage,[214] and therefore emphasis on control of disease activity is critical. Morbidity from long-term inflammation can cause growth problems, such as leg-length discrepancy with knee arthritis, muscle atrophy, bony overgrowth, joint contractions, and micrognathia.

### Rheumatoid Factor–Negative Polyarticular Juvenile Idiopathic Arthritis

$RF^-$ polyarticular JIA patients have variable outcomes,[9] but the overall prognosis appears to be improving.[22] Early response to treatment is an important predictor of long-term outcome.[215,216] Symmetric arthritis and early hand involvement predicts future disability and poorer overall well-being.[216,217] Approximately 30% to 52% of children will achieve complete remission, especially in the first 5 years.[206,211,218] However, flare of disease after 2 years of remission occurred in 52% to 69% of patients.[207,211]

### Rheumatoid Factor–Positive Polyarticular Juvenile Idiopathic Arthritis

Children with $RF^+$ polyarticular JIA have the most severe articular outcome, especially in the era prior to biologic therapies.[22,219,220] Inactive disease is difficult to achieve, with 84% of the disease course consisting of active disease, and only 5% to 17% of patients were able to reach clinical remission.[206,211] Despite treatment with nonbiologic DMARDs, a radiographic study showed that 9 years after disease presentation, $RF^+$ JIA polyarticular patients had more joint space narrowing and erosions (75%) compared with $RF^-$ polyarticular JIA (39%), sJIA (63%), and oligoarticular JIA patients (25%).[214]

### Juvenile Spondyloarthritis

The long-term outcome of JSpA is not well-studied, but as many as 40% of these children appear to progress to the adult form of AS. Predictors of sacroiliitis include HLA-B27 positivity, absence of DPB1*02, hip joint involvement within the first 6 months, and disease onset after 8 years of age.[221] When compared with AS, juvenile AS had less severe axial involvement but worse hip involvement and worse functional impairment.[222,223] Before the use of TNF inhibitors, which appear to be effective in severe JSpA and AS, the probability of remission was low. According to one study, if the arthritis had persisted for more than 5 years, the chance of remission was only 17%.[219] Recent studies have also shown that patients with JSpA have poorer patient-reported outcomes of pain intensity, physical function, and health status.[161,224-225]

### Juvenile Psoriatic Arthritis

Because of the heterogeneity in the patterns of arthritis in JPsA, the outcomes are variable and tend to track with the pattern of joint involvement. In one study, erosions were less common in JPsA compared with polyarticular JIA (23% vs. 46%, respectively).[40] Older reports note that JPsA patients had persistent disease activity and more evidence of physical limitations continuing into adulthood. However, in recent cohort studies, the outcomes appeared improved, with as many as 88% of patients achieving inactive disease on therapy and 50% achieving inactive disease off therapy.[40,226,25,35] This may be a result of more recent aggressive use of immunomodulatory therapy because as many as 74% of patients have been treated with at least one DMARD or biologic.[39]

### Systemic Juvenile Idiopathic Arthritis

sJIA is heterogeneous in severity, disease course, and outcome. Studies of the natural history of sJIA before biologic therapies demonstrate that the course can be (1) monocyclic, with remission within 2 to 4 years; (2) relapsing,

characterized by flares of systemic features with mild arthritis; or (3) continuous with persistent destructive arthritis, often more prominent after the regression of systemic features.[52,227] Patients with severe disease can have flares of extra-articular features at any time and may have active arthritis into adult life despite standard therapies.[227] Evidence now shows that IL-1 and IL-6 inhibitors have permitted significant disease control with no or much lower doses of corticosteroids.

Predictors of poor articular outcome in sJIA include systemic features 6 months after onset, thrombocytosis, and the presence of polyarthritis with hip involvement.[53,228,229] The mortality rate for sJIA is still perceived to be higher than other subtypes.[230] The severe growth delay seen before the use of IL-1 and IL-6 therapies is significantly ameliorated with biologic therapies.[231]

Amyloidosis was previously a major cause of death in sJIA but is now less common, most likely because of the use of more aggressive therapy to better control inflammation. Pulmonary hypertension and interstitial lung disease has been reported in case series.[232]

## SPECIAL CONSIDERATIONS IN CHILDREN

Physiotherapy (PT) and occupational therapy (OT) are crucial for all children with JIA, for range of motion, strengthening, joint protection, and endurance. Children with hand involvement need OT assessment and input regarding writing and school accommodations. Children who are seen later in their disease may already have flexion contractures that may require splinting and serial casting. Some children with marked leg-length discrepancies (resulting from overgrowth of the affected joint) may even require a shoe lift. Strict limitations from physical education at school and sports are generally not encouraged because physical activity plays an important role in rehabilitation and therapy; however, impact sports should be limited in the presence of joint effusions or when there is a concern for joint instability (i.e., C1/C2 atlantoaxial subluxation). A modified physical education program and sports activities to patients' own tolerance are recommended instead. Education is considered the child's main job, and trying to keep the child in school is of utmost importance for social and emotional growth. Children may be inconsistent in their report of pain; therefore, the physical examination becomes important to understand the true extent of disease. Growth can also be affected in children with chronic inflammation; therefore, one must be vigilant to watch for children falling off their growth curve as a sign of uncontrolled disease activity. Adolescents begin to think independently yet rely heavily on peer influences. This is a crucial time that can be extremely dangerous with impulsive decisions such as stopping medication or refusing medical care.

## CONCLUSION

The modern treatment of JIA has significantly decreased the long-term burden of disease but requires early recognition, diagnosis, and aggressive treatment to extinguish disease activity. Pediatric rheumatologists have in their favor the remarkable capacity for childhood growth and development to allow repair and restoration of function. The advent of new biologic therapies and rapid translation of basic research into therapeutic strategies as well as collaborative multicenter research networks should continue to improve the outlook for children with arthritis.



The full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 108



# Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis, Scleroderma, and Vasculitis

Stacy P. Ardoin • Angela B. Robinson • Kathryn S. Torok • Heather Van Meter • Ann M. Reed

## PEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

**KEY POINTS**

- Compared with adult-onset systemic lupus erythematosus (SLE), pediatric-onset SLE is more severe, with more organ involvement and higher mortality.
- Renal and central nervous system involvement cause significant morbidity and mortality and warrant aggressive therapy.
- As survival has improved, it has become important to identify and prevent long-term complications of SLE and its therapy, including osteoporosis, infections, cardiovascular disease, and malignancy.
- Neonatal lupus is a passive autoimmune condition distinct from SLE. Infants born to mothers with circulating anti-Ro/SS-A and anti-La/SS-B antibodies are at risk for characteristic rash, hepatitis, cytopenias, cardiac conduction disease, and cardiomyopathy.
- Drug-induced lupus can occur in children and adolescents and is treated by withdrawal of offending medication and immunosuppression if needed.

### Definition and Classification

Pediatric systemic lupus erythematosus (SLE) is a chronic, incurable, multisystem autoimmune disorder with potential to involve any organ in the body, most commonly affecting the skin, joints, kidneys, lungs, cardiovascular system, hematopoietic cells, muscles, and central and peripheral nervous systems. The clinical presentation of SLE in both adults and children is heterogeneous, and disease manifestations can range from mild to life-threatening. The disease is characterized by periods of disease quiescence and flare. Approximately 20% of SLE patients are diagnosed before age 18 years and, compared with adults, onset of SLE in childhood is associated with higher mortality and more widespread and severe organ involvement.[1]

Pediatric SLE is usually defined as age of onset younger than 18 years.[2] No diagnostic criteria specific for pediatric SLE exist. Existing SLE classification criteria were developed for use in adults participating in clinical trials and are often used, albeit with limitations, in clinical practice. The American College of Rheumatology (ACR) Classification Criteria for SLE (Table 108-1)[2,3] and the more recently developed Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria (see Table 108-1) have been validated in pediatric SLE populations.[4-6] The ACR nomenclature for neuropsychiatric SLE (NPSLE; see Chapter 80), primarily derived from adult data, is used in pediatric SLE, also with acknowledged limitations.

### Epidemiology

Pediatric SLE occurs worldwide, and SLE prevalence is lower in children (2 to 26/100,000) compared with adults (20 to 150/100,000).[7,8] Reported incidence ranges from 0.4 to 0.9/100,000 per year.[9] As in adults, pediatric SLE is more common in Hispanics and non-Caucasian populations.[7,8] SLE is more likely to develop in females than males, and the ratio of female to male involvement varies across age groups. The female to male ratio is highest between puberty and menopause (ranging from 5 to 10:1), whereas before puberty and after menopause, the ratio is lower, estimated at 3 to 4:1.[10] Diagnosis of SLE at age younger than 5 years is unusual, whereas diagnosis in adolescence (between 12 and 16 years of age) is most common.[10]

### Genetics and Pathogenesis

Adult and pediatric-onset SLE share complex pathophysiology (see Chapter 79) with genetic, epigenetic, sex, and environmental factors interacting to produce widespread immune system dysregulation. Immune system abnormalities in adult and pediatric SLE include dysregulated apoptosis and clearance of nuclear debris, decreased clearance of immune complexes, abnormal function of Toll-like receptors, aberrations of interferon (IFN)-α and nuclear factor-κB (NF-κB) pathways, abnormal neutrophils and neutrophil function, disrupted B and T cell function, and production of autoantibodies.[7] SLE in both adults and children is considered a multigenic disorder. However, rare single-gene disorders can cause SLE or lupus-like disorders and usually are seen in childhood. Of these single-gene disorders, C1q deficiency is the rarest but most strongly associated with SLE. Deficiencies of C1s, C2, and C4 also increase the risk.[11] Males with Klinfelter's syndrome (XXY) have a substantially increased risk for SLE.[12] Multiple candidate genes implicated in adult and pediatric SLE are depicted in Table 108-2[13] (see Chapter 79). Earlier age of onset may suggest enhanced genetic susceptibility, and *STAT4* and *ETS1* gene mutations are associated with early age at SLE onset.[13] In addition to specific gene variants, the copy number, or "dose," of certain genes also likely influences disease susceptibility and severity.[14]

**TABLE 108-1** Classification Criteria for Systemic Lupus Erythematosus

| American College of Rheumatology (ACR) 1997 Revised Classification Criteria |
|---|
| Malar rash |
| Discoid rash |
| Photosensitivity |
| Arthritis (nonerosive, affecting ≥2 joints) |
| Serositis (pleuritis, pericarditis, or peritonitis) |
| Seizure or psychosis |
| Hematologic manifestations (hemolytic anemia, leukopenia [<4000 leukocytes/mm$^3$], lymphopenia [<1500 lymphocytes/mm$^3$], thrombocytopenia [<100,000 platelets /mm$^3$] |
| Immunologic abnormalities (positive anti–double-stranded DNA or anti-Smith antibody) |
| False-positive rapid plasma regain (RPR), positive lupus anticoagulant test result in elevated anti-cardiolipin immunoglobulin (Ig)G or IgM anti-cardiolipin antibody |
| Anti-nuclear antibody |

*For ACR criteria, the presence of 4 or more of 11 criteria is needed.

| Systemic Lupus International Collaborating Clinics (SLICC) Classification Criteria |
|---|
| **Clinical Criteria** |
| Acute cutaneous lupus |
| Chronic cutaneous lupus |
| Oral or nasal ulcers |
| Nonscarring alopecia |
| Synovitis (≥2 joints) |
| Serositis |
| Glomerulonephritis |
| Neurologic disorder (seizures, psychosis, mononeuritis multiplex, myelitis, peripheral or cranial neuropathy, acute confusional state) |
| Hemolytic anemia |
| Leukopenia (<4000 leukocytes/mm$^3$) or lymphopenia (<1000 lymphocytes/mm$^3$) |
| Thrombocytopenia (<100,000 platelets/mm$^3$) |
| **Immunologic Criteria** |
| Anti-nuclear antibody (ANA) |
| Anti–double-stranded DNA antibody |
| Anti-Smith antibody |
| Anti-phospholipid antibody (false-positive RPR, medium or high-titer IgA, IgG, or IgM anti-cardiolipin antibody, IgG, IgA, or IgM anti–β2 glycoprotein I antibody |
| Low complement (C3, C4, CH50) |
| Positive direct Coombs' test in the absence of hemolytic anemia |

†For SLICC criteria, the presence of 4 or more criteria (at least 1 clinical and 1 laboratory criterion) is needed or a biopsy demonstrating lupus nephritis with a positive ANA or anti–double-stranded DNA is needed.

Genetic inheritance alone is not sufficient to cause SLE, as suggested by the fact that only a minority (approximately 24%) of monozygotic twins are concordant for SLE.[10] Thus other factors, including epigenetics and hormonal and environmental exposures, are important in SLE susceptibility. In both adult and pediatric SLE, epigenetic changes, including histone modifications, methylation of DNA, and microRNA pattern modulate gene transcription and expression.[15]

**TABLE 108-2** Candidate Systemic Lupus Erythematosus Genes by Immune System Pathway

| Immune System Pathway | Candidate Genes |
|---|---|
| Apoptosis, DNA breakdown, debris clearance | *FCGR2B, ACP5, TREX1, DNASE1, DNASE1L3, ATG5* |
| Type 1 interferon and Toll-like receptor signaling | *TLR7, IRF5, IRF7/PHRF1, IRF8, IRAK1, IFIH1, TYK2, PRDM1, STAT4, TREX1, ACP5* |
| Nuclear factor-κB signaling | *IRAK1, TNFA1P3, TNIP1, UBE2L3, SLC15A4, PRKCB* |
| Immune complex processing | *C1Q, C1R/C1S, C2, C4A/B, FCGR2A/B, FCGR3A/B* |
| B cell function | *FCGR2B, BLK, LYN, BANK1, PRDM1, ETS1, IKZF1, AFF1, RASGRP3, IL10, IL21, NCF2, PRKCB, HLA-DR2, HLA-DR3, MSH5, IRF8* |
| T cell function | *PTPN22, TNFS4, CD44, ETS1, IL10, IL21, TYK2, STAT4, PRDM1, AFF1, IZKF1, HLA-DR2, HLA-DR3* |
| Neutrophil/monocyte function | *ITGAM, ICAMS, FCGR2B, FCGR3A/B, IL20, IRF8* |

Modified from Rullo OJ, Tsao BP: Recent insights into the genetic basis of systemic lupus erythematosus. *Ann Rheum Dis* 72S:56–61, 2013.

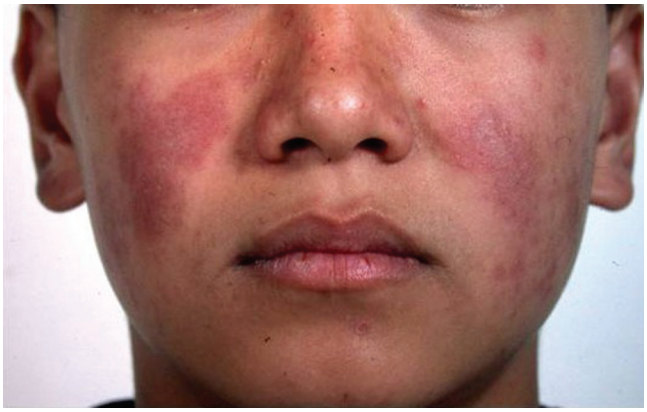


**Figure 108-1** Malar rash of a 14-year-old patient with pediatric systemic lupus erythematosus. The disease's typical "butterfly" or malar rash is an erythematous rash in the malar distribution, which includes the cheeks and crosses the nasal bridge but spares the nasolabial folds.

## Clinical Features

Pediatric-onset SLE can manifest with a vast array of clinical symptoms and findings. Renal disease can be insidious, sometimes appearing with few or no symptoms. Several common clinical manifestations of pediatric SLE are summarized in Table 108-3, and some common mucocutaneous findings are shown in Figures 108-1 and 108-2. Despite the similarities in SLE pathophysiology and genetics across the age spectrum, the clinical presentations of pediatric and adult SLE differ substantially. Children and adolescents with SLE tend to be seen with more widespread and aggressive organ involvement. Specifically,

**TABLE 108-3** Clinical Manifestations of Pediatric Systemic Lupus Erythematosus

| | |
|---|---|
| General | Fevers, fatigue, weight loss, lymphadenopathy, poor growth |
| Mucocutaneous | Alopecia<br>Acute cutaneous lupus erythematosus (malar or diffuse rash)<br>Subacute cutaneous lupus<br>Chronic cutaneous lupus erythematosus (discoid lupus, hyperkeratotic lupus, lupus tumidus, lupus profundus, chilblain lupus)<br>Cutaneous vasculitis<br>Livedo reticularis<br>Palmar erythema<br>Petechiae<br>Nasal and oral ulcers (often painless) |
| Musculoskeletal | Arthralgias, arthritis, tendonitis, myositis, loss of or reduced peak bone mass, avascular necrosis |
| Cardiovascular | Raynaud's phenomenon, cutaneous and systemic vasculitis, myocarditis, pericarditis, nonbacterial valvular vegetations (Libman-Sacks endocarditis), thrombi, and emboli |
| Pulmonary | Pleuritis, pleural effusion, interstitial lung disease, alveolar hemorrhage, shrinking lung syndrome, pulmonary embolism, pulmonary hypertension |
| Gastrointestinal | Hepatosplenomegaly, enteritis, protein-losing enteropathy, hepatitis |
| Renal | Glomerulonephritis, renal vein thrombosis, renal microthrombi, edema, hypertension |
| Neuropsychiatric | Seizures, psychosis, mood disorders, cerebritis, stroke, transverse myelitis, cognitive impairment, headaches, pseudotumor, peripheral or cranial neuropathy, chorea, optic neuritis |
| Ophthalmic | Retinitis, optic neuritis, scleritis, episcleritis |
| Hematologic | Immune-mediated hemolytic anemia, thrombocytopenia, leukopenia, hypercoaguability, thrombocytopenic thrombotic microangiopathy, anemia of chronic inflammation |
| Other | Delayed puberty, menstrual cycle disturbances, dyslipidemia |



**Figure 108-2** Oral ulcers in a 16-year-old girl with active pediatric systemic lupus erythematosus. Characteristic oral ulcers and hyperemia in children with lupus can be found on the hard palate. These ulcers are typically painless.

renal, neurologic, and hematologic involvement are more common at presentation in pediatric versus adult SLE.[16-18] Lupus nephritis is present in as many as 80% of children with SLE, but the distribution of histologic classes of lupus nephritis is similar to adults.[10] NPSLE is challenging to define, especially in developing children and adolescents. The reported prevalence of NPSLE in children and adolescents ranges from 22% to 95% and is at least as high as adult SLE rates.[19] The most common presenting findings in pediatric SLE include fever, malar rash, arthritis, glomerulonephritis, neuropsychiatric disease, and hematologic abnormalities.[10] Compared with adults, children, and adolescents with SLE typically are seen with overall higher SLE disease activity scores and are treated more frequently with moderate- to high-dose corticosteroids (CSs) and cyclophosphamide.[10,20,21]

## Diagnosis and Diagnostic Tests

Because of the heterogeneous and systemic nature of pediatric-onset SLE, the differential diagnosis is broad and often includes multiple infections (including Epstein-Barr virus, cytomegalovirus, histoplasmosis, coccidiomycosis, and tuberculosis), systemic-onset juvenile idiopathic arthritis, vasculitides, macrophage activation syndrome, malignancies (especially leukemia and lymphoma), other systemic causes of glomerulonephritis, neurologic and cardiopulmonary disease, and drug-induced lupus.

The diagnosis of pediatric SLE is clinical and is made on the basis of detailed history, meticulous physical examination, laboratory studies, and tissue biopsy when required.[22] The diagnostic approach is similar to that used in adult SLE (see Chapter 80) and usually includes assessment of complete blood count, hepatic and renal function, direct Coombs' assay testing, assessment for hemolysis and low complement levels, and measurement of anti-nuclear antibodies (ANA), anti–double-stranded DNA (dsDNA), antibodies to the extractable nuclear antigens (Ro/SSA, La/SSB, Smith, and ribonucleoprotein [RNP]), and antiphospholipid antibodies. In pediatric SLE, certain autoantibodies are detected more frequently than in adults, including anti–dsDNA, anti-cardiolipin immunoglobulin (Ig)G or IgM, anti-histone, and anti-ribosomal P antibodies.[10] As in adults, ANA-negative lupus is exceedingly rare in children and adolescents.

In the presence of abnormal urinalysis (proteinuria or cellular casts) or reduced renal function, prompt kidney biopsy is recommended. Skin biopsies often show abnormal immunofluorescence in cutaneous SLE manifestations. Diagnosis of NPSLE is challenging in children and adolescents, given normal developmental changes and increased risk of mood disorders in teens. As in adults (see Chapter 80), neuroimaging may include standard and functional MRI and positron-emission tomography (PET) imaging with technetium. Analysis of cerebrospinal fluid (CSF) may reveal nonspecific signs of intrathecal inflammation, including elevated protein levels and white blood cell counts. No serum or CSF serologic tests are diagnostic of NPSLE. Developmentally appropriate neurocognitive batteries, including pediatric automated neuropsychological assessment (peds ANAM) are used to detect and follow neuropsychological abnormalities.[19,23,24]

## Treatment

As in adult SLE, treatment of pediatric SLE is tailored to the severity of specific manifestations (see Chapter 81). Photoprotection, including regular use of sunscreen and avoidance of sun exposure, is advised for all. Hydroxychloroquine (5 to 7 mg/kg/day) is recommended for all who tolerate it because of known effects on flare reduction, lipid lowering, and potential protective effects on mortality.[25] Glucocorticoids are the mainstay for initial treatment of significant organ involvement; however, the substantial toxicity associated with long-term therapy mandates careful use. In children and adolescents, the steroid side effects of growth failure, acne, striae, and mood fluctuations can be particularly problematic. NSAIDs are used in pediatric SLE, primarily in treatment of musculoskeletal symptoms. It is not clear whether the cardiovascular toxicity associated with NSAID use in adults extends to the pediatric population. When steroid-sparing therapy is indicated, options include methotrexate (MTX), leflunomide, azathioprine, mycophenolate mofetil (MMF), cyclosporine, belimumab, and cyclophosphamide. Rituximab is sometimes used in severe or refractory pediatric SLE, despite the negative randomized clinical trials in adults. Plasma exchange and intravenous immunoglobulin are sometimes used for severe pediatric SLE. Thus far, there are no drugs specifically U.S. Food and Drug Administration approved for pediatric SLE, and treatment decisions are often inferred from adult trials. Because of its potential toxicity, cyclophosphamide is reserved for severe or refractory pediatric SLE disease. Fortunately, the risk of gonadal failure with cyclophosphamide is lower in children and adolescents compared with adults; however, many pediatric rheumatologists offer gonadotropin-releasing hormone agonists, such as leuprolide, to females to limit this risk.[26,22]

Randomized, controlled trials in pediatric SLE are rare; thus treatment approaches are often inferred from adult studies. The Childhood Arthritis and Rheumatology Research Alliance (CARRA), a network of from pediatric rheumatologists across North America, developed consensus treatment plans for newly diagnosed proliferative lupus nephritis. As outlined in Figure 108-3, these consensus treatment plans utilize oral and/or intravenous glucocorticoids, MMF, or cyclophosphamide.[27] Consensus treatment recommendations for maintenance therapy of proliferative lupus nephritis, treatment of membranous lupus nephritis, and treatment of severe central nervous system (CNS) disease and other SLE manifestations have not been published. Renal replacement therapy—dialysis or renal transplantation—is necessary in the setting of renal failure. Unfortunately, children with SLE-related end-stage renal disease (ESRD) on hemodialysis are twice as likely to die as children with ESRD from

**Select one induction immunosuppressive agent**

| Cyclophosphamide IV monthly for 6 months | Mycophenolate mofetil 600 mg/m$^2$/dose PO bid (max 3000 mg/day) or Mycophenolate sodium 400 mg/m$^2$/dose PO bid (max 2160 mg/day) for 6 months |
|---|---|

**Select one steroid regimen**

| Primarily oral* | | | Primarily IV | | | | Combined oral and IV | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Week | Daily dose | | Week | # Steroid pulses† | Daily dose | | Week | # Steroid pulses† | Daily dose | |
| | >30 kg | ≤30 kg | | | >30 kg | ≤30 kg | | | >30 kg | ≤30 kg |
| 1-4 | 60-80 mg | 2 mg/kg | 1 | 3/wk | 20 mg | 10 mg | 1 | 3/wk | 60 mg | 1.5 mg/kg |
| 5-6 | 60 mg | | 2 | 1-3/wk | 20 mg | 10 mg | 2 | 1/mo | 60 mg | 1.5 mg/kg |
| 7-10 | 50 mg | ↓ by 5-10 mg | 3 | 1-3/wk | 20 mg | 10 mg | 3 | 1/mo | 50 mg | 1.2 mg/kg |
| 11-12 | 40 mg | ↓ by 5 mg | 4 | 1-3/wk | 20 mg | 10 mg | 4 | 1/mo | 40 mg | 1 mg/kg |
| 13-14 | 40 mg | ↓ by 5 mg | 5-7 | 1-3/wk | 20 mg | 10 mg | 5-8 | 1/mo | 35 mg | 0.9 mg/kg |
| | | | | | | | 9-12 | 1/mo | 30 mg | 0.8 mg/kg |
| 15-18 | 30 mg | ↓ by 5 mg | 8-11 | 1/mo | 20 mg | 10 mg | 13-16 | 1/mo | 25 mg | 0.7 mg/kg |
| 19-22 | 25 mg | ↓ by 2.5-5 mg | 12-18 | 1/mo | 15 mg | 7.5 mg | 17-20 | 1/mo | 20 mg | 0.6 mg/kg |
| 23-24 | 20 mg | ↓ by 2.5-5 mg | 19-24 | 1/mo | 10 mg | 5 mg | 21-24 | 1/mo | 15 mg | 0.5 mg/kg |

**Figure 108-3** Childhood Arthritis and Rheumatology Research Alliance consensus treatment plans for induction therapy of newly diagnosed proliferative lupus nephritis. *Optional three steroid pulses in first week permitted. †Steroid pulse = methylprednisolone, 30 mg/kg/dose (maximum 1000 mg/dose). bid, twice a day; IV, intravenous; po, by mouth. *(From Mina R, von Scheven E, Ardoin SP, et al: Consensus treatment plans for induction therapy of newly diagnosed proliferative lupus nephritis in juvenile systemic lupus erythematosus.* Arthritis Care Res *64:375–383, 2012.)*

other causes.[28] When pediatric SLE is complicated by antiphospholipid antibody syndrome, lifelong anti-coagulation is advised.

Glucocorticoid-induced osteoporosis and failure to achieve peak bone mass are worrisome complications of pediatric SLE. Ensuring adequate vitamin D and calcium intake, counseling regarding weight-bearing exercise, and avoiding risk factors, including smoking, are universally recommended. However, because of the unknown potential impact on skeletal development and future fetal development, bisphosphonates are not routinely prescribed for children and adolescents with SLE treated with steroids.[9]

Because atherosclerosis begins in childhood and is a major long-term source of morbidity and mortality in SLE, anticipatory counseling concerning cardiovascular risk reduction and screening for modifiable cardiovascular risk factors need to start at a young age. Control of blood pressure and lipids with dietary and pharmacologic interventions is indicated. The Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial results did not suggest that statins should be routinely offered to all children and adolescents with SLE, but post hoc analyses suggested that higher-risk subgroups may benefit.[29,30]

Because of the use of teratogenic medications and the risk of SLE flare or clotting during pregnancy, teens require counseling regarding pregnancy risks and contraceptive options. Given estrogen's potential to incite flare or clotting, contraceptive medications containing significant doses of estrogen are often avoided. The results of the Safety of Exogenous Estrogens in Lupus Erythematosus National Assessment (SELENA) trial, which included adult women with low disease activity and excluded those with lupus anti-coagulant or moderate to high-titer anti-cardiolipin antibodies, suggested that oral contraceptives with low-dose estrogen increase the risk of clotting or flare in this limited population.[31]

Because infections are an important source of morbidity and mortality in pediatric SLE, immunization is critically important for prevention. The American Academy of Pediatrics recommends withholding live, attenuated vaccines from children on high-dose, chronic prednisone (defined as prednisone dose equivalent to >2/mg/kg/day or >20 mg/day if child's weight is >10 kg). Otherwise, routine vaccinations are recommended for all children and adolescents with SLE. Although data to support use in lupus are lacking, many pediatric rheumatologists used prophylactic trimethoprim/sulfamethoxazole, dapsone, or pentamidine to prevent *Pneumocystis jiroveci* pneumonia in patients on substantial immunosuppression.[22]

## Outcome

Fortunately, advances in understanding of SLE pathogenesis, diagnosis, treatment, and supportive care have improved, leading to longer survival. The 5- and 10-year survival rates, which were less than 40% in the 1960s, have increased to 80% to 90% in the 1990s. Although there is no cure for SLE, most children and adolescents with SLE are now expected to survive into adulthood. However, mortality rates are higher in pediatric versus adult-onset SLE. Across the age spectrum, early in the course of SLE, death most often occurs because of infection or severe SLE organ involvement (particularly renal and CNS disease). In the longer term, causes of mortality shift to infection, atherosclerosis, or malignancy.[9,10,22]

Because of longer exposure to disease and treatment, pediatric-onset SLE is associated with more cumulative damage as measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index score. In particular, development of cataracts, avascular necrosis, and renal damage are more likely to accrue in pediatric-onset SLE.[16,18] The risk of lupus-related damage is inversely related to age at onset, with prepubertal-onset SLE patients having higher risk of damage than peri-pubertal or post-pubertal.[32] Premature atherosclerosis is clearly linked to SLE, and increased carotid intimal medial thickness is evident even in children and adolescents with SLE.[30] Growth failure and delayed puberty are SLE-related morbidities unique to childhood and adolescence. Adolescents are at particularly high risk for mood disorders and poor adherence to medications, and this can influence outcomes. The transition from child-centered to adult-centered care is a vulnerable period during which adolescents and young adults with SLE are at risk for gaps in care and higher morbidity; thus efforts to improve this transition are needed. In general, poor prognostic factors for pediatric SLE include male gender, earlier age at diagnosis, Hispanic ethnicity or non-Caucasian race, lower socioeconomic status, and presence of renal or CNS disease.[9,10,22]

## DRUG-INDUCED LUPUS

Drug-induced lupus is the presence of SLE manifestations occurring after exposure to specific medications, listed in Table 108-4. Specific classification criteria for drug-induced lupus have not been published, and no published data address differences between adult and pediatric drug-induced lupus. Thus drug-induced lupus is presumed not to differ across the age spectrum. As in adults (see Chapter 80), drug-induced lupus affects males and females equally and is unlikely to cause significant renal or CNS disease. Hepatic involvement in drug-induced lupus is common. Laboratory abnormalities can include circulating antihistone antibodies, although these antibodies can also be present in SLE unassociated with drug exposure. Anti–dsDNA antibodies are less likely to occur in drug-induced

**TABLE 108-4** Drugs Implicated in Drug-Induced Lupus*

| Definite | Probable/Possible | |
|---|---|---|
| Hydralazine | Phenytoin | Hydrochlorothiazide |
| Procainamide | Ethosuximide | Sulfasalazine |
| Minocycline | Carbamazepine | Amiodarone |
| Isoniazid | Sulfonamides | Ticlodipine |
| Quinidine | Rifampin | Hydrazine |
| Methyldopa | Lithium | Docetaxel |
| TNF inhibitors | Captopril | Statins |

*This list is not exhaustive.

lupus than in SLE. Epigenetic abnormalities are important in drug-induced lupus, and, for example, hydralazine and procainamide can cause DNA hypomethylation. Treatment of drug-induced lupus involves withdrawal of offending medication and immunosuppressive therapy if indicated. Resolution of clinical findings may take months to years.[33]

## NEONATAL LUPUS

### Definition and Classification

Neonatal lupus (NLE) is a separate entity from SLE and is one of the few rheumatic disorders presenting in neonatal period or early infancy. NLE is a passive autoimmune condition caused by transfer of circulating maternal autoantibodies to the fetal circulation. No diagnostic criteria are available for NLE.

### Genetics and Pathogenesis

The maternal autoantibodies associated with the vast majority of NLE cases are anti-Ro/SS-A (or anti-SSA) and anti-LA/SS-B (or anti-SSB); however, anti-UA RNP antibodies have been described in NLE. Because NLE develops in only 1% to 2% of children born to mothers with these circulating antibodies, these antibodies are not sufficient to cause NLE. Although the exact pathogenesis of NLE remains unknown, a popular hypothesis based upon in vivo studies postulates that Ro and La antigens are exposed on the surface of cardiac myositis during fetal cardiac development and become a target for maternal autoantibodies. The ensuing inflammatory cascade causes damages to the conduction system and, in some cases, leads to cardiomyopathy.[34,35]

Inflammatory changes related to NLE resolve when maternal autoantibodies are cleared from the infant's circulation, typically within 6 months. Noncardiac NLE manifestations are reversible and usually do not require any treatment. Topical CSs are sometimes used to treat the rash. Because congenital heart block and cardiomyopathy can cause death and morbidity, there has been interest in finding therapies that can prevent or retard the development of cardiac involvement. Clinical trials are challenging to perform in rare diseases affecting fetuses in which placebo treatment is unpalatable or unethical. A multicenter, open-label study of 30 pregnancies and 31 fetuses showed that dexamethasone did not reverse congenital heart block. In another multicenter, prospective, open-label study, use of dexamethasone monitored 56 pregnancies in which first-degree heart block developed in 6 of 70 fetuses, 6 were treated with dexamethasone, and aterioventricular (AV) conduction system abnormalities resolved in all treated fetuses.[36,37] Two multicenter prospective studies showed that preventive treatment with intravenous Ig in mothers with circulating anti-Ro/SS-A or La/SS-B antibodies and prior pregnancy complicated by NLE/congenital heart block did not prevent congenital heart block.[38,39]

### Clinical Features

The clinical manifestations of NLE include a nonscarring malar or annular rash commonly affecting the face, scalp, and trunk (Figure 108-4). The rash usually appears in the first 6 weeks of light and can be triggered by exposure to ultraviolet (UV) light. Histologically, the cutaneous findings are similar to those in subacute cutaneous lupus. Other findings can include immune-mediated cytopenias and hepatitis (transaminitis, hepatomegaly). The most serious complication of NLE is cardiac involvement. Cardiac conduction abnormalities can present in utero (starting at 16 weeks of gestation when maternal Igs can cross the placenta) or shortly after birth and include conduction system abnormalities ranging from prolongation of the PR interval to complete heart block. In addition, endocardial fibroelastosis and progressive cardiomyopathy can develop.[34]



**Figure 108-4** A nonscarring malar or annular rash commonly affecting the face, scalp, and trunk in neonatal lupus.

### Treatment

Recent retrospective studies suggest that maternal use of hydroxychloroquine during pregnancy may reduce the risk of NLE/congenital heart block in offspring.[40,41] Given the risk for NLE, it is recommended that pregnant women with circulating anti-Ro/SSA or anti-La/SSB antibodies undergo monitoring of fetal P-R interval and cardiac function during pregnancy beginning at 16 weeks. Cardiac pacing is used in some children with complete heart block. Overall mortality in congenital complete heart block is approximately 17% to 19%. Recurrence of NLE congenital heart block in future offspring is estimated at 17%. Children and adults with congenital heart block are at risk for exercise limitations and heart failure. Given their family histories, children with NLE may be at risk for autoimmune disease.[34]

## Conclusion

Although the pathophysiology of pediatric SLE is likely similar to adult SLE, clinical presentations, prognosis, and some aspects of management differ. Children and adolescents with SLE have higher disease activity at presentation, are at substantial risk for organ damage, including renal and CNS complications, and have overall higher mortality compared with adult counterparts. Especially because children and adolescents face a lifetime burden of disease and effects of therapy, early intervention is indicated to reduce risk of long-term complications, including cardiovascular disease and bone loss. Randomized controlled clinical trials for pediatric SLE are lacking, and therefore therapeutic approaches are inferred from adult studies. However, children and adolescents require special attention to uniquely pediatric concerns, including impact of therapies on growth and body image, compliance concerns, and psychosocial and transition needs.

# JUVENILE DERMATOMYOSITIS

**KEY POINTS**

- **Juvenile dermatomyositis (DM) is an inflammatory, immune-mediated vasculopathy affecting predominantly skin and muscle.**
- **Children with DM have more vasculopathy and a greater likelihood of calcinosis or ulceration but have a better long-term prognosis with improved survival.**
- **Adults with DM are more likely to have myositis-specific antibodies, experience interstitial lung disease, and have a marked association with malignancy.**
- **Both have similar features on muscle biopsy and IFN gene signature, although subtle differences may exist in pathogenesis and pathology.**
- **Initiatives are underway to improve our classification, markers of disease activity, and ability to predict outcome of juvenile DM.**

## Definition and Classification

Bohan and Peter criteria for dermatomyositis include[42] typical skin rash of dermatomyositis, including a heliotrope rash, Gottron sign, and Gottron papules plus three of the following (two of the following would be classified as probable juvenile DM and one as possible juvenile DM):

- Symmetric proximal muscle weakness determined by physical examination.
- Elevation of serum skeletal muscle enzymes (particularly creatine kinase and aldolase), serum glutamate oxaloacetate, pyruvate transaminases, and lactate dehydrogenase.
- The electromyographic triad of short, small, polyphasic motor unit potentials; fibrillations, positive sharp waves, and insertional irritability; and bizarre, high-frequency repetitive discharges.
- Muscle-biopsy specimen abnormalities of degeneration, regeneration, necrosis, phagocytosis, and interstitial mononuclear infiltrate.

Bohan and Peter criteria date back to 1975, before the routine use of MRI, and so MRI findings are not part of the original criteria. MRI using T2-weighted images and fat suppression may localize active sites of disease for biopsy and may improve diagnostic yield. Muscle biopsy often demonstrates evidence of disease activity and chronicity that is not suspected from the levels of the serum enzymes alone; findings may be useful in grading the extent of disease and predicting disease severity or refractory disease.[43] Currently, an international multidisciplinary collaboration, the International Myositis Classification Criteria Project (IMCCP), has been working to update classification criteria for multiple myositis subtypes, including juvenile DM. The new criteria involve probability modeling for classification but have not been published yet.[44]

## Epidemiology

Juvenile DM is an autoimmune vasculopathy characterized clinically by proximal muscle weakness, muscle inflammation, characteristic rash, and frequently, the presence of autoantibodies. Juvenile DM is by far the most common form of idiopathic inflammatory myopathy in children and adolescents; therefore other types of childhood myositis are not addressed in this chapter. Although there is some overlap, DM and juvenile DM are separate diseases with variable features. Before corticosteroid treatment, mortality for juvenile DM approached 33%. Currently, juvenile DM has a 5-year survival of greater than 95%, whereas DM 5-year survival is 75% to 90%.[45-47] Incidence is 3.2 cases/million children/year in juvenile DM.[48-50] Females are affected more commonly than males in a ratio of approximately 2 : 1.[51,52] The average age of onset is 7 years of age in juvenile DM and the fourth to sixth decades in DM.[53-56]

## Genetics

The major immunogenetic risk factor for DM and juvenile DM in Caucasians is the human leukocyte antigen (HLA) 8.1 ancestral haplotype (HLA-B*08/DRB1*03/DQA1*05/DQB1*02).[57,58,59] The *HLA-DQA1*0501* allele is not only increased in Caucasians, but also in other populations and clinical disease subsets in children.[60] Protective alleles have been described in large genetic association studies in both adult and juvenile DM. Gene variation has been seen within an IFN regulatory factor 5 (IRF5) gene sequence, which may be associated with higher IFN-regulated gene expression in patients with juvenile DM.[61] Polymorphisms in TNF gene (*TNF-308A* allele) have been associated with greater disease severity in patients with juvenile DM.[62] The *TNF-308A* allele may be increased in both adult and juvenile DM; however, disease significance is lost in DM when adjusting for HLA-B, but not HLA-DB.[63] Interleukin (IL)-1α polymorphisms may also convey risk from juvenile DM for Caucasians.[64] A weak association may exist between an R620W variant of the *PTPN22* gene in juvenile idiopathic inflammatory myopathies.[65]

## Etiology and Pathogenesis

### Maternal Microchimerism

Juvenile DM has been postulated to resemble chronic graft-versus-host disease (GVHD), and maternally derived

chimeric cells have been found more often in children with juvenile DM than in either siblings or healthy controls.[66-69] These chimeric cells may be present in lymphoid aggregates in muscle tissue of affected children with juvenile DM, and chimeric stem cells may be seen in peripheral blood.[43,66] Studies in adult DM are lacking; interestingly, PM was seen in 8% of patients who underwent hematopoietic stem cell transplantation, in which 33% to 64% of patients develop chronic GVHD.[70]

### Infectious and Environmental Triggers

Many children will report symptoms consistent with infection before the development of juvenile DM; however, viral illness is common in this age group and retrospective analyses of infectious history before the development of juvenile DM is prone to recall bias. Multiple retrospective studies have been performed, indicating high frequency of infectious symptoms within a few months before onset. Searches for infectious agents in tissue or serum have been negative.[71,72] There has been some association of birth seasonality, especially in Hispanic patients, with risk of juvenile DM, but this was not reproduced in other ethnic groups within the same cohort.[73] Adult and juvenile DM also share a number of suspected environmental triggers that have been temporally associated with the onset of myositis, including exposure to excessive UV light, certain drugs (such as D-penicillamine), and infectious agents. A retrospective multicenter cross-sectional study in 2009 found that UV radiation intensity was associated with increased odds of adult DM compared with other inflammatory myopathies (odds ratio [OR], 2.3 [95% confidence interval [CI], 0.9 to 5.8]), and increased odds of anti–Mi-2 antibodies (OR, 6.0 [95% CI, 1.1 to 34.1]), and this effect was stronger in women.[74] A brief report in 2013 indicated that the highest UV index in the month before the development of symptoms was associated with development of juvenile DM versus polymyositis (PM), and development of anti-p155/140 antibodies.[75] There is also some evidence that maternal inhaled pollutants and tobacco exposure during fetal development may be associated with development of juvenile DM.[76]

## Clinical Features

Children with DM typically present with rash, proximal weakness, or both.

### Characteristic Rash

Typical rashes include a generalized photosensitive erythema, Gottron's papules, which occur over extensor




**Figure 108-5** Fifteen-year-old boy with newly diagnosed juvenile dermatomyositis demonstrating Gottron's papules over the metacarpophalangeal and proximal interphalangeal joints **(A),** heliotrope rash with periorbital swelling **(B),** and nailfold capillary dropout, dilatation, and tortuosity **(C). D** shows a 4-year-old boy with juvenile dermatomyositis with a cutaneous ulcer adjacent to his right axilla. He died 3 months after presentation.

surfaces (Figure 108-5A), and a periorbital heliotrope rash (Figure 108-5B). Multiple rashes have been described in adult and juvenile DM, but heliotrope rash and Gottron's papules are pathognomonic for DM. Adults may develop a thickened, erythematous, and scaly rash over the tips and lateral aspects of the fingers known as *mechanic's hands*; this occurs rarely in children. Evidence of small-vessel inflammation may be seen in the nailfolds, eyelids, and gums as telangiectasias of the capillary loops (Figure 108-5C). The number of end row nailfold loops can be quantified, and normal numbers of end-row loops in juvenile DM have been correlated with earlier diagnosis and less severe skin disease (Table 108-5).[77] Conversely, baseline nailfold abnormalities may predict slower time to improvement.[78] Persistent capillary abnormalities and Gottron's papules at age 6 months may also be associated with longer time to remission in children.[79] Severe vasculopathy may cause livedo reticularis and cutaneous ulcerations and may be an indicator of increased severity and poor prognosis; adults (4%) are less likely than children (24%) to develop ulceration.[80] Ulcerative lesions are more common over extensor surfaces and the inner canthi of the eye (Figure 108-5D).

### Proximal Muscle Weakness

Weakness is often insidious and difficult to recognize at onset. It is typically symmetric and affects proximal muscles. Parents may report fatigue and difficulty climbing stairs, combing hair, or getting out of bed rather than weakness. Children and toddlers may want to be carried more frequently. On examination, clinical weakness may be seen with difficulty performing sit-ups in school-aged children, development of head lag in a child after infancy, Gower's sign (use of hands on thighs to stand from a sitting position), or difficulty raising the arms straight above the head. Weakness may affect esophageal and respiratory muscles in severe cases. Respiratory weakness may lead to acute respiratory failure with hypercarbia rather than hypoxemia.

### Systemic Manifestations

Lipodystrophy and calcinosis are thought to be associated with long-standing or undertreated disease in juvenile DM and are uncommon in adults (see Table 108-5). Calcinosis may occur in as many as 40% of patients with juvenile DM; occasionally, it is found at the time of diagnosis but is typically thought to be a result of long-standing or severe disease.[81] An unusually high percentage of children with calcinosis (71%) was seen in a sub-Saharan African cohort, which was irrespective of time to diagnosis or use of steroids,[82] and there has been some suggestion that calcinosis may be more prevalent in African-American patients with juvenile DM.[83] Presence of calcinosis may predict higher disease activity in long-term follow-up.[84] Calcinosis may take several forms: superficial plaques and nodules, tumoral calcinosis, fascial plane deposition, or, rarely, an exoskeleton of calcium deposition (Figure 108-6).[85,86] Calcinosis may present with mixed forms as well. These lesions may be asymptomatic or may appear clinically similar to cellulitis with induration and erythema with pain, cause skin breakdown, or extrude calcium through the skin. Large deposits can impair function, cause contractures, or serve as a nidus for infection. These lesions are often associated with active ongoing disease, requiring aggressive treatment for the underlying condition.

Lipodystrophy has been reported in as many as 40% of patients, and may be localized, partial, or generalized. Subclinical lipodystrophy and metabolic abnormalities may be under-reported. Lipodystrophy clinically results in a progressive loss of subcutaneous and visceral fat and may be associated with a metabolic syndrome similar to polycystic ovarian syndrome, with insulin resistance, acanthosis, hypertriglyceridemia, and abnormal glucose tolerance.[87,88] Only a few cases of adults with myositis and lipodystrophy are reported in the literature (see Table 108-5).[89]

Fevers, dysphagia or dysphonia, arthritis, muscle tenderness, and fatigue are also commonly reported at diagnosis.

**TABLE 108-5** Clinical Characteristics of Juvenile Dermatomyositis (DM) and Adult DM

| | Juvenile DM | Adult DM |
|---|---|---|
| Peak age of onset | 7 years[48,55,54,51,56] | 30 to 50 years |
| Proportion of idiopathic inflammatory myositis cases | 80% to 95%[52,157] | 35% to 50%[157] |
| Proximal weakness | 85% to 95%[52,54,56] | 88%[158] |
| Characteristic rash | | |
| Gottron's papules | 73% to 91%[49,139] | 54%[158,159] |
| Heliotrope rash | 62% to 83% | 74% |
| Malar rash | 42% to 57% | N/A |
| Abnormal nailfold capillaries | 80% | 43% |
| Calcinosis or ulceration | 26% to 70%[80,82,139,160] | 2% to 16%[139] |
| Refractory or chronic disease | 59% to 63%[54,161] | 63%[160] |
| Mortality | <5%[54,80,160] | 21%[160] |
| Malignancy | 1%[54,160] | 15% to 24%[160,162] |
| Myositis-specific antibodies | 2% to 70%[90] | 40% to 80%[101,163] |
| Interstitial lung disease | 7% to 19%[90] | 35% to 40%[91] |
| Gastrointestinal disease | 2% to 3%[80] | 1%[80] |
| Raynaud's phenomenon | 10%[164] | 11%[165] |

N/A, Data unavailable.




**Figure 108-6** **A,** Elbow and forearm of a 14-year-old girl with chronic continuous juvenile dermatomyositis showing multiple sites of tumoral calcinosis. Several of these, particularly over pressure points, have ulcerated, extruding a chalklike material. **B,** Solitary site of tumoral calcinosis posterior to the knee resulted in limited range of motion. **C,** Fascial and tumoral calcinosis affecting the lower extremity. **D,** Same patient as in **A** demonstrating marked calcification, giving an exoskeleton-like appearance across the chest wall.

Rarely, vasculitis of the gastrointestinal (GI) tract with crampy abdominal pain, pancreatitis, GI bleeding, and potential for intestinal perforation or infarction may develop in patients.

### Lung Disease

Fatal pulmonary complications rarely develop in patients with juvenile DM. Interstitial lung disease (ILD) has been described in 7% to 19% (see Table 108-5).[90] In adults, ILD is a major contributor to morbidity and mortality in myositis, with ILD developing in 35% to 40% of patients during the course of their disease, associated with anti-synthetase antibodies such as anti–Jo-1.[91] Rapidly progressive ILD has also been associated with clinically amyopathic dermatomyositis and anti–CADM-140 autoantibodies.[92] Pulmonary function tests will show a restrictive pattern with decreased diffusing capacity for carbon monoxide (DLCO); high-resolution chest CT should be used for confirmation because pulmonary function test abnormalities may result from respiratory weakness alone. Imaging abnormalities include nodules, linear opacities, irregularity, ground-glass opacities, and fibrosis.

### Heart Disease

Involvement of the cardiac muscle with pericarditis, myocarditis, conduction defects, or heart block has been reported in patients. Cardiovascular complications in juvenile DM are rare (see Table 108-5). In a case-control study,

patients with juvenile DM had more evidence of diastolic dysfunction, pericarditis, and hypertension compared with healthy controls, although disease was largely subclinical.[93] More recent studies have implicated chronic inflammation in the pathogenesis of cardiovascular disease: Patients with adult dermatomyositis may have increased risk of acute myocardial infarction and ischemic stroke.[94] Given the fact that juvenile dermatomyositis involves chronic inflammation and, commonly, subclinical dyslipidemia and lipodystrophy, it would be logical to assume increased cardiovascular disease risk in this population, but this topic has not been well studied. One study has described evidence of increased endothelial dysfunction and worsened cardiovascular risk profile years after treatment of juvenile DM compared with healthy controls, despite decreased body mass index and younger age.[95]

### Amyotrophic Dermatomyositis

Some patients may be seen with classic rash of DM but no sign of muscle weakness or inflammation. This has been described clinically as *amyotrophic dermatomyositis* (CADM). In children, it is controversial whether these patients have mild undiagnosed muscle inflammation or will progress to more severe muscle involvement and/or long-term sequelae, such as calcinosis and lipodystrophy if untreated. There have been case reports of CADM associated with aggressive interstitial lung disease in Japanese children, and there may be some overlap with scleroderma.[96-99]

### Malignancy

An association with malignancy at disease onset is observed in adults with dermatomyositis but rarely in children (see Table 108-5).[100]

## Diagnosis and Diagnostic Tests

Elevated serum levels of muscle-derived enzymes (creatine kinase, aldolase, aspartate aminotransferase, and lactate dehydrogenase) reflect muscle inflammation or damage. In juvenile DM, elevation of creatine kinase becomes less sensitive as a marker with chronic disease. Evidence of ANAs is seen in more than 70% of juvenile DM.[101,102] However, myositis-specific antibodies are more prevalent in adults with DM and suggest a disease subset more than in juvenile DM. Recently, reports of new autoantibodies in juvenile DM may allow prediction of disease subsets. Lymphocyte enumeration may show an increased or decreased percentage of $CD19^+$ B cells via peripheral blood flow cytometry in juvenile DM or DM.[103,104] Neopterin and von Willebrand factor products have been reported to be elevated in a subset of patients with active inflammation from juvenile DM or vasculitis but are not specific for juvenile DM.[105,106] Because of the low sensitivity of the available laboratory testing, further gene expression, cytokine, and chemokine markers are under investigation.

Multiple studies have reported overexpression of type I IFN-regulated genes, cytokines (IL-6 and IL-1) and chemokines (IFN-inducible protein-10 [IP-10], monocyte chemoattractant protein [MCP-1], and myxovirus resistance protein [MRP-2]) in peripheral blood of adult and juvenile DM patients. These appear to be sensitive markers of disease activity and may differ between DM and juvenile DM.[107,108,109]

### Autoantibody Profiles

*Myositis-associated antibodies (MAAs):* ANA is present commonly in juvenile DM; however, tests for antibodies to SSA, SSB, Sm, RNP, and DNA are typically negative. MAAs (especially anti-SSA) are more commonly found in the adult population clinically thought to have DM. Antibodies to polymositis-scleroderma (PM-Scl) identify a small, distinct subgroup of myopathies with a protracted disease course, often complicated by pulmonary interstitial fibrosis and/or cardiac involvement as well as sclerodactyly. This subset in juvenile DM has many presenting features of scleroderma, such as severe Raynaud's phenomenon (RP). In adults, PM-Scl is associated with scleroderma myopathy and with cardiac involvement.

*Myositis-specific antibodies (MSAs):* MSAs are divided into three main groups: anti-synthetase antibodies, anti–signal recognition particle (anti-SRP) autoantibodies, and anti–Mi-2 autoantibodies. Each group has unique clinical features and outcomes. Anti-synthetase antibodies (anti-ARS, anti–Jo-1, anti–PL-12, anti–PL-7, anti-Ej, anti-Oj, anti-KS, anti-Ha, and anti-Za) have been associated with anti-synthetase syndrome, consisting of mechanic's hands, Raynaud's syndrome, ILD, arthritis, and fever.[110] Anti-synthetase antibodies are relatively rare in juvenile DM (2% to 4%). Anti–Mi-2 antibodies have been described in a low proportion of children with juvenile DM who typically have classic cutaneous juvenile DM rash with mild disease but elevated creatine kinase (CK) levels.[111] Recently, anti-p155/140 and anti-p140 autoantibodies have been described in 20% to 30% of children with juvenile DM.[112] The anti-p155/150 antibodies may be associated with extensive photosensitive skin rashes, capillary changes, ulceration, and lipodystrophy with chronic illness course. Anti-p140 antibodies may be associated with increased odds of calcinosis (OR, 7.0 [3.0 to 16.1]).[113] In adults, anti-p155/140 and anti-p155 autoantibodies may be associated with malignancy.[114] Another autoantibody, labeled *anti-MJ antibody*, also recognizes a 140 kDa protein and was associated with severe disease with contractures and atrophy in Argentinian children.[115] There is also an anti–CADM-140 autoantibody, which has been reported in Japanese patients with CADM who experienced rapidly progressive ILD.[92] It is unclear whether these autoantibodies target similar proteins or are epitopically distinct. Anti-SRP antibodies have been described in DM but are more commonly described with severe acquired necrotizing myopathies, which are refractory to treatment. The presence of MSAs is rare in children compared with adults; positive tests for anti–Jo-1, anti–Mi-2 and other MSAs may portend more significant disease. With increasing knowledge of new autoantibodies, currently, as many as 40% of children and 70% of adults may carry myositis-specific or -associated antibodies.[116]

### Muscle Pathology

Muscle biopsy is indicated particularly for an atypical presentation or when diagnosis is in doubt. The presentation

of clinical and muscle findings may be discordant, and recent evidence suggests a muscle biopsy may be useful for grading extent of disease and staging potential long-term severity.[117,118] Adult and juvenile DM are characterized pathologically by varying degrees of perifascicular atrophy, vasculopathy, and perivascular inflammation (composed predominantly of plasmacytoid dendritic cells [pDCs], B and $CD4^+$ T lymphocytes, and macrophages), focal necrosis and phagocytosis of muscle fibers, fiber regeneration, inflammatory cell infiltrates and vasculitis, and tubuloreticular inclusion bodies within endothelial cells. Evidence of vasculopathy includes muscle capillary thrombosis and deposition of complement and the membrane attack complex on muscle capillaries and reduction in number of and enlargement of remaining endomysial capillaries (capillary dropout). Capillary dropout has been found more prominently in areas of perifascicular atrophy, although capillary remnants may still be found in areas of endothelial cell loss.[119] Intermediate-sized vessels may also be affected. A Chinese study comparing muscle biopsies from adult and pediatric patients with DM found increased evidence of vasculopathy in pediatric biopsies compared with adults; this is also consistent with the descriptions from Bohan and Peter in 1975 and 1977.[120,121] Formation of neolymphoid structures in juvenile DM muscle, in which immature and mature $CD4^+$ T cells are seen with B cells and pDCs, suggest local maturation of T cells[122] structures were reported in adult DM with $CD4^+$ cells surrounded by B cells.[123] One of the earliest findings in adult and juvenile DM muscle tissue is the upregulation of class I major histocompatibility complex (MHC), which is almost universal in juvenile DM and, to a lesser extent, in adult DM. It is unclear whether the upregulation of class I MHC molecules is one of the precipitating events in myositis; however, animal models suggest a role in the unfolded protein response, which correlates with upregulation of IFN-β.[124]

In both juvenile and adult DM, the predominance of $CD4^+$ cells are not only $CD3^+$ (traditional $CD4^+$ cells); a subset are positive for CD123 and CD83 (markers for mature pDCs), and IL-17 staining, suggestive of T helper (Th)17 cells.[125-128] Increased frequency of Th17 cells have been reported in the peripheral blood mononuclear cells of adults with DM.[129]

Type 1 IFNs (IFN-α and IFN-β) are important in the pathogenesis of juvenile and adult DM. IFNs upregulate genes critical in immunoregulation and class I MHC expression, activate natural killer cells, support dendritic cell maturation, and promote the activation and survival of T cells. Muscle and skin biopsies in DM have shown increased pDCs and upregulation of IFN signaling.[130] It is thought that the class I MHC upregulation by IFN-α in myofibers may cause activation of the endoplasmic reticulum stress response in DM, which could lead to muscle-tissue injury and resulting myositis.[131] It has been shown that nucleic acid antibodies such as Ro/La and Sm/RNP can induce the production of IFN-α in children with juvenile DM.[132] Damage could also be caused by the cytokines upregulated by type 1 IFNs, including CXC chemokines (IP-10, MIG, and IFN-inducible T cell–α chemoattractant [I-TAC]). An anti–IFN-α antibody, sifalimumab, has been shown to neutralize IFN signature in patients with adult DM and PM, but larger-scale studies of its efficacy in this disease have not been conducted.[133] Tubuloreticular inclusion bodies are seen in DM and juvenile DM but rarely seen in other forms of myositis; they have been reported in circulating blood cells of other patients treated with IFN-α and in cultured endothelial cells in response to treatment with type I IFNs.[134,135,136] It is possible that these inclusion bodies may represent viral infection or increased IFN signaling.

### Peripheral Blood Phenotyping in Juvenile and Adult Dermatomyositis

Phenotyping of the peripheral immune blood cells, although not diagnostic, are dysregulated in a subset of individuals with juvenile DM and are proposed to be reflective of active disease. Untreated, early disease (<3 months) is suggested to have a lower B cell (CD20) numbers in the peripheral blood in both adult and juvenile DM; however, studies on recovery of these cells is lacking, and there are few data to suggest usefulness in monitoring of disease activity.[137,138] This is in contrast to adult DM, in which there are reports of total lymphocyte ($CD3^+$) decrease (CD19, CD4, CD8) before the onset of treatment, which recovers shortly after treatment. However, additional reports suggest that an increase of $CD19^+$ and $CD19^+CD23^+$ cells are seen, along with a decrease of $CD4^+CD45RO^+$ cells, are seen in the peripheral blood.[125]

## Differential Diagnosis

The most important differential diagnosis for patients seen with rash and muscle weakness is SLE, particularly for those patients seen with significant arthritis and a malar rash. Characteristic autoantibodies of SLE, cytopenias, renal disease, and hypocomplementemia help to differentiate these disorders. Patients with systemic sclerosis and prominent myositis may be difficult to differentiate from patients with JDM. Patients with mixed connective tissue disease and other overlap syndromes may have features of JDM in addition to those of other autoimmune connective tissue diseases.

Other idiopathic inflammatory myopathies are extremely rare in children. These include inclusion body myositis, granulomatous myositis, and macrophagic myofascitis.

Patients who are seen with either no rash or mild rash and predominant muscle weakness may need to be differentiated from patients with primary myopathies and muscle biopsy to help with the differentiation. Patients with muscular dystrophies often have positive family histories and an insidious onset of disease with characteristic muscle groups involved. Congenital myopathies usually are seen in infancy with hypotonia. Metabolic myopathies may be associated with developmental delay. Cramping and weakness after exertion may also be signs of metabolic myopathy.

Various infections may lead to an acute myositis. Perhaps the best recognized is influenza B, presenting with acute calf pain, weakness, and raised muscle enzyme levels. *Trichinella* infection may be associated with periorbital edema and significant peripheral eosinophilia. Many other bacteria, viruses, and parasites may cause myositis; they should be suspected in the appropriate clinical circumstance.

**TABLE 108-6** Treatment of Juvenile Dermatomyositis (DM)

| | |
|---|---|
| First-line therapy | Corticosteroids (2 mg/kg oral therapy or pulse intravenous 30 mg/kg therapy for more severe disease)<br>Methotrexate (oral or subcutaneous)<br>Intravenous gammaglobulin 2 g/kg monthly may be used in more refractory or severe disease |
| Second-line therapy | Intravenous gammaglobulin<br>Rituximab<br>Cyclosporine A<br>Azathioprine<br>Tacrolimus<br>Mycophenolate mofetil |
| Third-line therapy | Bone marrow or stem cell transplant<br>Cyclophosphamide<br>Other biologics (TNF inhibitor, anakinra, alemtuzumab, sifalimumab) |
| Adjunct therapy | Sunscreen and sun avoidance<br>Hydroxychloroquine<br>Calcium and vitamin D for osteoporosis prevention |

## Treatment

### Traditional Therapies

Standard medications to treat juvenile DM are shown in Table 108-6. However, there have been no prospective case-control double-blinded studies of immunosuppressive therapy in juvenile DM. After the inception of corticosteroid treatment of juvenile DM, the mortality rate decreased markedly compared with historic controls. Prolonged corticosteroid treatment is associated with significant morbidity, including decreased height velocity in children, weight gain, osteoporosis, immunosuppression, avascular necrosis, adrenal suppression, cataracts, diabetes, and more. With the use of MTX in juvenile DM, the mean time of corticosteroid use was 10 months compared with historic controls who were treated for a mean time of 27 months.[139] Surveys of North American pediatric rheumatologists indicate that most practitioners will treat typical juvenile DM with CSs and MTX, although there is great discrepancy between dosing regimens. Adjunct medications and medications for refractory disease vary but include hydroxychloroquine, intravenous immunoglobulin, rituximab, cyclophosphamide, cyclosporine A, azathioprine, tacrolimus, and MMF.[140] In a North American registry of juvenile DM patients, almost all subjects were treated at some point with MTX (7.5 to 15 mg/m$^2$ or 1 mg/kg to a max of 40 mg weekly) and CSs; approximately 50% had been treated with hydroxychloroquine or intravenous immunoglobulin (IVIG).[51] In a large cohort of European and South American patients with juvenile DM, European patients were more likely to receive cyclophosphamide, cyclosporine, and azathioprine as treatment, suggesting some cultural or national variations in prescribing practices.[56]

In adults, CSs and multiple immunosuppressive agents have been used for treatment of DM and PM. In retrospective studies, MTX has been postulated to be effective as a steroid-sparing agent, but comparisons with other immunosuppressive agents have shown no superiority. A randomized trial of azathioprine versus MTX in adults showed no significant difference in effect.[141] Similarly, a randomized controlled trial of cyclosporine A versus MTX showed no difference in efficacy or toxicity.[142] MTX and azathioprine have also been tried in combination.[143] There has been no evidence that MTX is associated with ILD in adult DM, but this may be a rationale for the increased use of azathioprine in adults compared with children.

### Therapy for Refractory Disease

In open-label studies, IVIG has been helpful for patients with refractory disease and may help decrease steroid toxicity and improve skin and muscle disease, but placebo-controlled trials of this are not available in children.[144] In adults, a double-blind placebo-controlled trial showed improvement in skin and muscle disease, with improvement in biopsy results after treatment.[145] IVIG may help with severe dysphagia and esophageal weakness in adults.[146] Adverse reactions in juvenile DM patients have been associated with the IgA content in IVIG infusions.[147] In retrospective studies, MMF has been used as a steroid-sparing agent and may benefit patients with refractory skin and muscle disease.[148,149] Similarly, cyclosporine has been reported to have beneficial results on juvenile DM in small case series.[150,151] A pilot study of etanercept in patients with refractory juvenile DM showed no appreciable improvement.[152] There is no specific evidence basis for treatment of calcinosis, although multiple case reports of various treatments have been reported, but it is felt that control of disease activity is important in controlling calcinosis. Anti-TNF agents, bisphosphonates, and calcium channel blockers have been used for this purpose. Surgical excision may be indicated in areas of recurrent infection, chronic pain, or impedance of function from calcinosis. Treatment of resistant skin disease is controversial: Topical CSs and pimecrolimus may help with symptomatic itching or redness, but it is possible that resistant skin disease reflects ongoing systemic disease and should be treated with increasing immunosuppression.

Biologic therapies are beginning to emerge for the treatment of myositis, and given the findings of increased serum CD19$^+$ B cells and lymphoid follicles in muscle biopsies of affected patients, rituximab has been proposed as a possible therapy. Initial case series noted clinical improvements in skin and muscle that paralleled B cell depletion.[153] A large randomized controlled crossover trial of rituximab in refractory juvenile DM and adult DM/PM (the Rituximab in Myositis Study) was conducted. All patients received rituximab, and the outcome was time to response. The study found 83% of refractory adult and juvenile patients with myositis met the definition of improvement, but there was no significant difference in time to improvement.[154] Subanalysis of this group suggests that there may be better response to rituximab in the juvenile cohort within this study.[155]

### Consensus Protocols

CARRA has developed consensus protocols for therapy of patients with moderately severe juvenile DM, which include

combinations of oral CSs, pulse IV CSs, MTX, and IVIG.[156] Within the CARRA Registry cohort, almost all patients have been treated with CSs, and approximately half have been exposed to pulse CSs or IVIG.[51] Use of consensus protocols with time will hopefully lead to a more evidence-based rationale for treatment in the future.

### Outcome

In children, prognosis is generally favorable, although work needs to be done in minimizing treatment toxicity and long-term morbidities of disease (calcinosis, lipodystrophy, cardiac, and pulmonary complications). Patients may follow one of three disease courses: monocyclic, polycyclic (flares of disease while off treatment), or chronic continuous disease. Delay in recognition or undertreatment may result in prolongation of disease course. In the modern era, two large series showed that 40% of patients pursued a monophasic course, and the remainder followed a polycyclic or chronic continuous course.[84,156]

### Conclusion

Although the causes of juvenile DM are still elusive, recent developments point to the involvement of IFN and lymphoid follicles in the pathogenesis of DM. International and national collaborators are working on developing standard protocols for assessment and treatment of juvenile DM. An initially aggressive approach with CSs and disease-modifying anti-rheumatic drugs (DMARDs) such as MTX may result in better long-term outcomes and less calcinosis and steroid toxicity. Work in using IFN and cytokine signaling as a biomarker and using findings in pathogenesis as a rational basis for treatment and therapy will be a challenge for dermatomyositis researchers in the future.

## PEDIATRIC SCLERODERMA: SYSTEMIC AND LOCALIZED FORMS

**KEY POINTS**

- **Pediatric scleroderma includes two different clinical phenotypes: systemic sclerosis (SSc) and localized scleroderma (LS). Despite clinical differences, both share a common pathophysiology, with an initial inflammatory phase associated with endothelial activation and a later fibrotic phase evident by collagenization of the tissue and appreciable skin thickness.**
- **Juvenile SSc (jSSc) has similar subtypes and organ manifestations compared with adult-onset SSc, except that there is an increased frequency of overlap SSc and associated antibodies and clinical features, such as arthritis and myositis.**
- **Despite similar organ involvement and manifestations, survival rates in jSSc are substantially higher in jSSc than SSc.**
- **Although LS is typically limited to the skin and underlying connective tissue, at least one-quarter of the patients have extracutaneous manifestations, especially those with linear scleroderma of the extremities or head.**
- **Both SSc and LS are best treated systemically if identified during the earlier inflammatory stage when progression can be halted, whereas advancement is still needed in developing anti-fibrotic therapy to help reverse disease damage.**

The scleroderma disorders in children encompass both systemic sclerosis (SSc) and localized scleroderma (LS), also termed "morphea." In childhood-onset scleroderma, SSc is much less common than LS and comprises less than 10% of all SSc cases.[166,167] Both share the general features of an earlier inflammatory state, followed by collagen deposition and atrophy. Skin biopsies of these patients are typically indistinguishable, and they may share some common pathophysiology; however, their clinical manifestations are markedly different with unique morbidities and prognoses. LS is typically confined to the skin and underlying tissue, and although without the fatal potential of SSc, as many as a quarter of the patients may have extracutaneous disease manifestations, such as joint contractures and uveitis.[168] Although any organ may be affected in SSc, vascular (RP), cutaneous (skin thickening), GI, pulmonary, and musculoskeletal involvement are most commonly seen in children. Both SSc and LS can be further classified by subtype (Table 108-7; see Table 108-11).

### Epidemiology

Both conditions are uncommon in children; the estimated annual incidence of LS is 1 to 3.4 per 100,000 children[169,170] and of SSc is 0.27 to 1 per million children.[169,171] The mean age of onset for both forms of pediatric scleroderma is between 7 and 9 years of age. Unfortunately, there is significant delay in diagnosis, with an average of 0.7 to 2.8 years for SSc[166,167,169,172] and 0.9 to 1.6 years for LS.[168,172,173] For the few cases of congenital involvement of LS, the mean time to diagnosis is longer, 3.3 to 3.9 years.[173,174] The approximate female to male ratio of pediatric SSc is 4:1,[166,167] and for pediatric LS it is 2:1. There is no clear evidence for racial predilection for either form of pediatric scleroderma.

### Genetics

Genetic studies are lacking in pediatric-onset SSc and LS, although some HLA investigations are currently underway. Familial history of autoimmune disease, as in other connective tissue diseases, is not uncommon in either LS or SSc, with a general range of 10% to 25% positive family history of first-degree relative with conditions such as psoriasis, RA, SLE, Sjögren's syndrome, thyroiditis and, less commonly, SSc and LS.[167,168,173,175]

### Pathogenesis (Shared Between LS and SSc)

The skin histology findings of these two conditions are indistinguishable. Mononuclear cell populations identified in both SSc and LS skin specimens are predominantly T cells, both CD4 (Th) and $CD8^+$.[176,177] Th cell–associated cytokines and chemokines, such as IL-2, -4, -6, -8, -13, and TNF, have been identified in the circulating sera and

**TABLE 108-7** Clinical Subtypes of Systemic Sclerosis (SSc)

| Subtype | MAIN CLINICAL FEATURES | | Antibody Association |
|---|---|---|---|
| | Organ System | Organ System Features | |
| Diffuse cutaneous (dc) | Skin | Thickness proximal to elbows and knees<br>Rapid progressive thickening | Topoisomerase (Scl-70) |
| | Cardiac | Congestive heart failure<br>Conduction abnormalities | |
| | Renal | Scleroderma renal crisis | |
| | Pulmonary | Interstitial lung disease | |
| Limited cutaneous (lc) | Skin | Thickness limited to distal extremities (and face)<br>Restricted and nonprogressive thickening | Centromere |
| | Gastrointestinal | Esophageal dysmotility<br>Gastrointestinal strictures<br>Malabsorption | |
| | Pulmonary | Pulmonary arterial hypertension | |
| Overlap syndrome | Skin | Either dcSSc or lcSSc pattern<br>Skin manifestations of other CTD, such as Gottron's papules (DM) and malar rash (SLE) | PM-Scl or U1-RNP |
| | Musculoskeletal | Arthritis<br>Myositis | |
| | Cardiac<br>Renal<br>Pulmonary<br>Gastrointestinal | Can have any of the above dcSSc or lcSSc manifestations<br>Additional organ involvement in association with other CTD feature, such as lupus nephritis | |

CTD, Connective tissue disease; DM, dermatomyositis; PM-Scl, polymyositis-scleroderma antibody; SLE, systemic lupus erythematosus; U1-RNP, U1 ribonucleoprotein antibody.

stimulated peripheral blood mononuclear cells (PBMCs) from LS and SSc patients.[178-183] Other chemokines, such as those associated with the IFN-γ pathway and suggest a Th1 profile—CXCL-10 and MCP-1—have been associated with both LS and SSc.[184,185] These T cell–associated chemokines and cytokines in turn further stimulate fibroblasts and endothelial cells to produce TGF-β and connective tissue growth factor (CTGF), which are thought to be the main stimulators of tissue fibrosis (via increased collagen production) and endothelial cell damage (via influence of adhesion molecules ICAM-1 [intercellular adhesion molecule-1] and VCAM-1 [vascular cell adhesion molecule-1]).[186-189]

The Th and regulatory T cell (Treg) immunophenotypes of patients with jSSc have been recently reported by Reiff and colleagues[190] and demonstrate some similarities and differences with adult-onset SSc. Both SSc[191] and jSSc demonstrate a decrease in Treg population while disease duration increases, and the chemokine receptor, CCR7, has increased RNA expression on both,[192] although only patients with jSSc have increased expression on the surface of T lymphocytes.[190] In jSSc there was an increase in frequency of the CD45RA-expressing effector memory T cell subpopulation,[190] but not in SSc studies.[193] Some of these differences may be attributable to reduced thymic function, with defective thymopoiesis in jSSc.[194]

Microchimerism is another area of interest under investigation regarding a shared pathogenesis between LS and SSc. Clinically and histologically, LS and SSc share characteristics with chronic GVHD, also termed *sclerodermatous GVHD*, a condition in which microchimerism plays a role in pathogenesis. Chimeric cells have been identified in patients with LS and SSc by using real-time polymerase chain reaction (PCR) techniques.[195,196]

## Systemic Sclerosis

### Classification

The three main subtypes of SSc—diffuse cutaneous (dc), limited cutaneous (lc), and overlap syndrome—which are present in adult-onset SSc, occur in pediatric-onset SSc with similar organ manifestations and antibodies associated with these subtypes (see Table 108-7). However, there are some differences in distribution of clinical subtype and associated antibodies between adult and pediatric patients (Table 108-8). There are only a few "large" cohort studies evaluating jSSc,[166,167,197-199] and in those that included the evaluation of overlap syndromes,[198] it is apparent that this subtype constitutes a significant proportion (approximately one-third) of patients with jSSc. The antibodies associated with overlap syndrome, PM-Scl and U1-RNP, and their affiliated clinical features of myositis and arthritis are more commonly observed in jSSc.[166] Another main difference is the lack of anti-centromere antibody–positive patients with jSSc (≤5%),[166,198,199] especially with pre-pubertal patients (<10 years at onset).[199] This is true despite approximately 30% to 50% of patients with jSSc classified as lcSSc (see Table 108-8). Because of some of these differences and the more indolent onset of symptoms in jSSc, provisional classification criteria were developed for jSSc to enable detection of early disease, such as the inclusion of positive ANA, Raynaud's phenomenon, and nailfold capillaroscopy changes. These criteria were developed through international efforts among PRES (Pediatric Rheumatology European Society), European League against Rheumatism (EULAR), the ACR through the Delphi survey, and nominal group techniques[200] and are displayed in Table 108-9. Some patients with undifferentiated or mixed connective tissue disease may have shiny or full-appearing

**TABLE 108-8** Comparison between Adult- and Pediatric-Onset Systemic Sclerosis (SSc) Subtype and Antibody Distribution

| | Pediatric-Onset SSc | Adult-Onset SSc |
|---|---|---|
| **Clinical Subsets (%)** | | |
| dcSSc | 30-45 | 35-45 |
| lcSSc | 30-50 | 40-55 |
| **Overlap** | **10-39*** | **9-18** |
| **Antibody Positivity (%)** | | |
| ANA | 90-97 | 90-99 |
| Scl-70 | 20-40 | 20-40 |
| **Centromere** | **2-8†** | **20-30** |
| **U1-RNP** | **15-20*** | **5** |
| **PM-Scl** | **15*** | **5** |
| **RNA polymerase III** | **2-4†** | **10-30** |

*Boldface* text highlights a difference between pediatric and adult SSc.

*Significantly higher in pediatric onset SSc compared to adult-onset SSc group

†Significantly lower in pediatric onset SSc compared to adult-onset SSc group

ANA, Anti-nuclear antibody; dc, diffuse cutaneous; lc, limited cutaneous; PM-Scl, polymyositis-scleroderma antibody; Scl-70, topoisomerase antibody; SSc, systemic sclerosis; U1-RNP, U1 ribonucleoprotein antibody.

Data from references 166, 198, and 199.

skin of the distal fingers, but they must have the major criterion, "skin induration or sclerosis proximal to the metacarpophalangeal (MCP) or metatarsophalangeal (MTP)," in addition to two minor criteria, to be classified with jSSc (see Table 108-9).[200]

## Clinical Features

Please refer to Chapter 84 for detailed organ manifestations in SSc. Here we will emphasize the similarities and differences between childhood-onset SSc and SSc.

In general, larger cohort studies of juvenile-onset SSc have been followed up into adulthood and compared with adult-onset SSc cohorts in that geographic region to compare disease course, organ complications, and outcome. The four largest studies are summarized in Table 108-10 and support similar frequency of the main SSc organ manifestations, such as ILD and pulmonary arterial hypertension (PAH), but there are differences with increased musculoskeletal involvement (because of overlap prominence in jSSc) and less scleroderma renal crisis in juvenile-onset SSc.[166,167,198,199] Despite this, jSSc was found to have significantly better survival rates compared with adult-onset SSc in these cohort studies.

### Vascular Manifestations

Common features at the onset of jSSc are RP (70%) and skin changes of hands (60%), including edema, sclerodactyly and induration proximal to the MCPs (Figure 108-7).[167] In the largest cohort of pediatric SSc (153 patients) studied, 53% presented with both skin induration of hands and RP, and 10% of those presenting with RP also had digital infarcts.[167] Throughout the course of the disease, almost all will have RP (97%) and skin changes of the hands (96%),

**TABLE 108-9** Provisional Criteria for the Classification of Juvenile Systemic Sclerosis (SSc)*

| |
|---|
| **Major Criterion (Required)** |
| Proximal skin sclerosis/induration of the skin |
| **Minor Criteria (at Least Two Required)** |
| Cutaneous |
| Sclerodactyly |
| Peripheral vascular |
| Raynaud's phenomenon |
| Nailfold capillary abnormalities |
| Digital tip ulcers |
| Gastrointestinal |
| Dysphagia |
| Gastroesophageal reflux |
| Cardiac |
| Arrhythmias |
| Heart failure |
| Renal |
| Renal crisis |
| New-onset arterial hypertension |
| Respiratory |
| Pulmonary fibrosis (HRCT/radiography) |
| Decreased DLCO |
| Pulmonary arterial hypertension |
| Neurologic |
| Neuropathy |
| Carpal tunnel syndrome |
| Musculoskeletal |
| Tendon friction rubs |
| Arthritis |
| Myositis |
| Serologic |
| Anti-nuclear antibodies |
| SSc-selective autoantibodies (anti-centromere, anti–topoisomerase I [Scl-70], anti-fibrillarin, anti–PM-Scl, anti-fibrillin or anti–RNA polymerase I or III) |

*The presence of one major and at least two minor criteria in a patient younger than 16 years classifies the patient as having juvenile systemic sclerosis, with a sensitivity of 90% and a specificity of 96%.

DLCO, Diffusing capacity for carbon monoxide; HRCT, high-resolution computed tomography.

From Zulian F, Woo P, Athreya BH, et al: The Pediatric Rheumatology European Society/American College of Rheumatology/European League Against Rheumatism provisional classification criteria for juvenile systemic sclerosis. *Arthritis Rheum* 57:203–212, 2007.



**Figure 108-7** Early skin changes in a patient with juvenile systemic sclerosis (jSSc) patient, including edema, sclerodactyly, and skin induration, demonstrated as shiny skin and puffy hands. Her fingers have also started to taper.

**TABLE 108-10** Summary of Frequency of Clinical Features from Four Cohort Studies Comparing Juvenile-with Adult-Onset Systemic Sclerosis (SSc)

| Clinical Feature | Pediatric-Onset SSc (% during the Course) | Adult-Onset SSc (% during the Course) |
|---|---|---|
| **Vascular** | | |
| Raynaud's phenomenon | 84-100 | 91-100 |
| Digital ulcers | 29-35 | 22-41 |
| **Pulmonary** | | |
| Any pulmonary involvement | 42-55 | 44-64 |
| Pulmonary arterial hypertension | 2-13 | 14 |
| Pulmonary fibrosis | 9-23 | 22-36 |
| **Cardiovascular** | | |
| Hypertension | 3-8 | 11-17 |
| Cardiac abnormalities | 2-17 | 3-20 |
| **Renal** | | |
| Renal crisis | 0-4 | 2-13 |
| Proteinuria | 3-5 | 6 |
| **Gastrointestinal** | | |
| Any gastrointestinal involvement | 74 | 78 |
| Esophageal | 24-60 | 65 |
| Gastric | 16-30 | 26 |
| Intestinal | 10-15 | 10-22 |
| **Musculoskeletal** | | |
| Synovitis | 10-27 | 15-17 |
| Joint contracture | 30 | 37 |
| Muscle weakness | 20-32 | 15-27 |
| Tendon friction rub | 8-11 | 12-23 |
| **Cutaneous** | | |
| Modified Rodnan skin score | 9-19 | 9-26 |

Data from references 166, 167, 198, and 199.

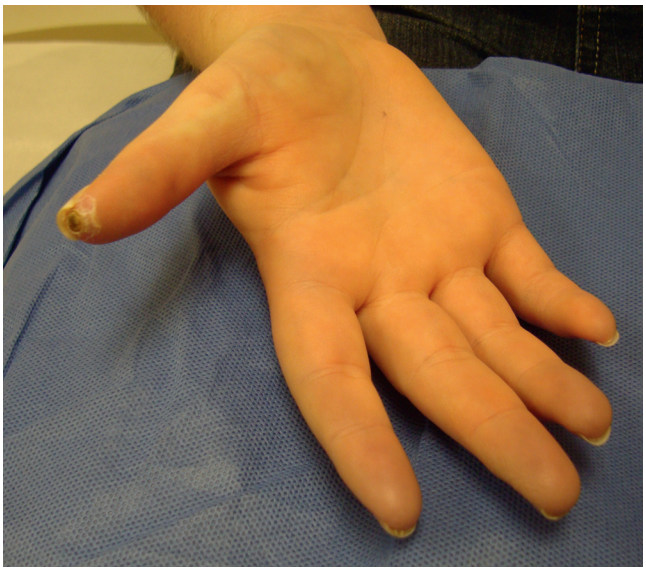


**Figure 108-8** Patient with overlap systemic sclerosis (polymositis-scleroderma positive) with several years of Raynaud's phenomenon. A digital nonhealing ulcer has developed on her thumb and has bluish discoloration of distal fingers secondary to moderate-severe Raynaud's phenomenon.

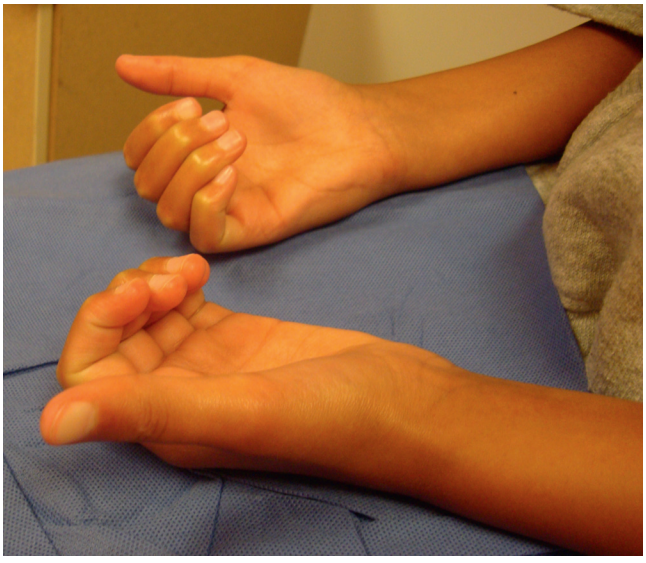


**Figure 108-9** Maximum finger flexion in a patient with juvenile systemic sclerosis with diffuse cutaneous disease (scleroderma-70 positive). Overlying skin thickening and deeper collagen infiltration of the tendons causes finger joint contracture with both limited flexion and extension.

and digital ulcers will develop in approximately one-third (Figure 108-8; Table 108-10).[166,167,198,199] Nailfold capillaroscopy changes, including dilatation, tortuosity, hemorrhage, drop-off (avascularization) and, later, arborization of the capillaries,[201] are observed in children as they are in adult SSc, and, if identified in context with RP, should raise suspicion for diagnosis of jSSc. The prevalence of nailfold capillary changes in jSSc is reported to be 50%[166,167] but is likely higher if standardized microscopy is performed.

### Cutaneous Manifestations

Cutaneous manifestations are similar to adult-onset SSc, in which there is an edematous, sclerodactyly phase with associated increase in skin thickness (see Figure 108-7), followed by an atrophic phase. There is a distal tapering of fingers, with some jSSc having shortened middle and distal phalanges and loss of digital pads in adulthood.[166] Joint contractures, especially of the hands, are common in jSSc due to overlying skin and deep tendon collagen deposition and tightening (see Figure 108-9; see Table 108-10). The typical scleroderma facies of tight atrophic skin produces a pinched nose, thin pursed lips, small mouth, and prominent teeth occurs in children but may be less dramatic. Skin thickness is measured using the modified Rodnan Skin Score (mRSS),[202] and in general mirrors that found in adult-onset SSc (see Table 108-10).[199] Other skin findings in jSSc include those found in adult-onset SSc, such as subcutaneous calcium deposits (calcinosis cutis) and telangiectasias.

Other organ manifestations in jSSc (in order of decreasing frequency) include GI, pulmonary, musculoskeletal, cardiac, renal, and neurologic symptoms (see Table 108-10).

### Gastrointestinal Manifestations

GI symptoms occur in approximately half of the children, although more detailed investigation often indicates the presence of abnormalities in a larger percentage.[166] Esophageal dysmotility and gastroesophageal reflux often leads to dysphagia and esophagitis, and also increases the risk of aspiration pneumonia. Gastroparesis with delayed gastric emptying, in addition to esophageal dysfunction, can lead to increased reflux symptoms. Evaluation of the GI tract by using manometry and intraesophageal 24-hour pH monitoring have been reported as sensitive indicators of lowered esophageal tone and reflux.[203] However, these modalities are used less often in pediatrics. Instead, a swallow study with speech therapy and radiology to evaluate for aspiration and dysmotility, followed by an upper GI study with small bowel follow-through (SBFT), are typically used. If the small bowel is involved, cramps, diarrhea, and constipation may result from peristaltic dysfunction. Episodes of pseudo-obstruction with post-prandial abdominal distension, pain, and nausea can occur because of a functional ileus. Bacterial overgrowth, steatorrhea, weight loss, volvulus, and, even perforation, can occur.

### Pulmonary Involvement

Pulmonary involvement in jSSc ranges from 30% of 70% in the literature and includes ILD, PAH, and abnormal pulmonary function tests (PFTs). PFT abnormalities especially of concern are decreased forced vital capacity (FVC) and DLCO.[204] The clinical presentation of ILD with slowly progressive dyspnea on exertion (DOE) and that of PAH with a more rapid progression of DOE is similar between adults and children with SSc. Moderate to severe lung disease from progressive pulmonary fibrosis appears to be less frequent in children, with 9% and 13% of the patients with jSSc having an FVC less than 50% in two large cohorts.[166,198]

### Musculoskeletal Involvement

Compared with adult-onset SSc, musculoskeletal involvement is more common in pediatric SSc. Approximately one-third of patients with jSSc experience inflammatory arthritis (joint effusions) in addition to the typical dry synovitis of scleroderma, reflected by the fibrosis of tendons transversing the joints, which limits their range of motion (ROM).[166,167] Early in the disease process, especially in those with dcSSc, tenosynovitis/bursitis causes palpable tendon friction rubs (a "leathery crepitus") when the joint is extended or flexed,[205] demonstrated in approximately 10% of patients with jSSc.[167,199] Myopathy typically results in symmetric proximal weakness and muscular atrophy. Muscle weakness has been reported to range between 20% to 32% in jSSc cohorts (see Table 108-10),[166,167,199] and was markedly higher than the adult comparison cohort in one of the studies.[166] Myositis also occurs, more commonly in the overlap SSc subtype, and presents similarly juvenile DM. Children with dcSSc and myositis are particularly at risk for severe cardiac involvement, including myocardial perfusion deficits and dilated cardiomyopathy.[206]

### Cardiac Involvement

Cardiac involvement, although infrequent (see Table 108-10), is the major cause of scleroderma-related mortality in children, as evidenced by 10 out of 15 deaths in the Martini and colleagues cohort[167] and 5 out of 32 in the Scalapino and colleagues cohort.[166] Cardiac manifestations result from the combination of myocardial fibrosis, vascular insufficiency, and inflammation and include heart block, arrhythmia, congestive heart failure, cardiomyopathy, and pericarditis. In contrast to pediatric SSc, scleroderma-related mortality in adults is primarily caused by pulmonary involvement, both ILD and PAH.[207]

### Renal Dysfunction

Mild renal dysfunction is not uncommon in SSc and is secondary to vasculopathy instead of glomerulonephritis and occurs with similar frequency in jSSc and SSc. In contrast, the more severe and morbid form of renal disease, scleroderma renal crisis (SRC), occurs in approximately 15% of adult patients with SSc but only rarely in children (<5%, only five patients among the four cohorts).[166,167,198,199] SRC is characterized by accelerated arterial hypertension, renal insufficiency, microangiopathic hemolytic anemia, and thrombocytopenia. SRC is most frequently seen early in disease course of dcSSc RNA polymerase III-positive patients, which is an antibody rarely detected in jSSc (see Table 108-8), possibly explaining the low frequency of SRC in jSSc.

### Neurologic Involvement

Neurologic involvement in jSSc is rare (<5% in all studies) and can include CNS changes, such as seizure, abnormal brain MRI findings, and peripheral neuropathy.[167]

## Diagnosis and Diagnostic Tests

The diagnosis of jSSc relies on distinctive clinical features (see Table 108-9), and subtypes are further defined by both skin thickening distribution and scleroderma-related serum autoantibody profiles, which predict organ involvement in both adult- and childhood-onset SSc (see Table 108-7).[166,167,197,208,209] See Tables 108-7 and 108-8 for antibody relationship to clinical features and disease subtype. Although many scleroderma-related autoantibodies have been identified in adult SSc, there is a high proportion of pediatric patients (20% to 23%) who have a positive ANA without a specific autoantibody identified.[166,197]

### Evaluation/Disease Monitoring of the Patient with jSSc

For most children with SSc or suspected SSc, initial cardiopulmonary assessment, especially evaluating for evidence of ILD and PAH, includes chest radiograph, PFTs with DLCO, electrocardiogram, and an echocardiogram. For pediatric patients, it is important that the DLCO is calculated by using a specific pediatric mean predictive DLCO based on the patient's weight, height, age, sex, and race. This will avoid the unwanted anxiety of a falsely low reading if unmatched norms are used. Additional cardiopulmonary

testing is pursued if abnormalities are detected or the patient is too young to appropriately complete testing (i.e., PFTs). Abnormal PFTs prompt evaluation with high-resolution computed tomography (HRCT) of the chest and, possibly, bronchoalveolar lavage (BAL) if further evidence of inflammation (alveolitis) is required or evaluation of infection is needed before starting therapy.[204,210] Evidence of PAH on echocardiogram, elevated right-side-of-the-heart pressures, is more formally assessed by right-heart catheterization as in adults. A 6-minute walk test may also be used, but the standardized values in children are still being determined and depend on ethnicity, weight, height, and age, and nonpulmonary issues may contribute to decreased values, such as musculoskeletal involvement.[211,212] After initial assessment, ongoing cardiopulmonary screening with an annual echocardiogram and quarterly to twice a year PFTs with DLCO are recommended by most pediatric rheumatologists, at least in the first few years of disease.[210] There is a lack of consensus regarding screening for GI involvement in jSSc. Most practitioners will screen at some point in time for evidence of silent aspiration and dysmotility with a swallow study and upper GI with SBFT; additional imaging is more dependent on symptoms (see discussion under GI symptoms above).

There are no standardized biomarkers available, although a study has suggested serum KL-6, a mucin-like protein strongly expressed in type II pneumocytes, as a promising noninvasive pulmonary marker of fibrosis in jSSc.[213]

## Treatment

Treatment of SSc is dependent on organ involvement, but general measures such as antacid medication for GI protection, prokinetic agents for dysmotility, rotating antibiotics for malabsorption, as well as vasodilators for RP (may also help with PAH), NSAIDs and physical therapy (PT) for arthritis or tendonitis are generally recommended. CSs for myositis, arthritis, and other inflammatory clinical features must be used with caution because they can prompt SRC. More specific treatment recommendations for different organ manifestations have been agreed upon by a consensus conference of international scleroderma experts and should be considered for all patients with SSc.[214] The reader is referred to Chapter 84 for a more in-depth discussion of SSc treatment. As in adult SSc, autologous stem cell transplant is an option and may be the best opportunity for survival in selected severe patients, with a number of successful outcomes reported.[215]

## Outcome

Despite all the potential organ involvement in pediatric SSc, it has a far more favorable prognosis at 5, 10, and 15 years compared with adult SSc, with a lower frequency of severe organ involvement. Foeldevari and colleagues[198] recently reported Kaplan-Meier survival rates at 10 years for children to be 98% versus 75% in adults. In jSSc cohorts, the most common cause of death was cardiac (including PAH) and respiratory failure.[166,167,197] Death within a few years of onset of disease (majority ≤5 years) was reported in two jSSc cohorts.[167] Martini and coleagues proposed a model for prediction of poor outcome in jSSc that included fibrosis on chest radiograph, raised creatinine levels, and pericarditis.[216]

More recently, the research group at Padua University coordinated a study that developed and validated the jSS Severity Score (J4S), to objectively establish the degree of jSSc severity in daily practice using a comprehensive score.[217] This is not a mortality predictor but a gauge to possibly use longitudinally to follow the patients' disease course and trajectory, and will enable comparisons of patients across observational cohorts and assist in the stratification of patients for clinical trials. It is mirrored from the Medsger Severity Score,[218] ranging from normal, mild, moderate, to severe involvement of different organ manifestations and general health parameters. The J4S is a promising measure and should be validated in prospective studies.

## Localized Scleroderma

### Classification

Localized scleroderma (LS) has a different pattern of skin involvement than systemic sclerosis (SSc) and encompasses several subtypes classified by depth and pattern of the lesion distribution. The group of disorders is characterized by fibrosis and atrophy that is mainly confined to the skin and subcutaneous tissue; however, deeper forms also involve the fascia, muscle, tendon, and joint capsule. Certain subtypes of LS have unique associated extracutaneous manifestations (ECMs). The "Padua criteria" have been used more recently to describe the LS subtypes, which include a "mixed morphea" subtype; the prevalence of this category is 15% in juvenile LS (jLS) (Table 108-11).[219] The most common subtype in jLS is linear scleroderma (LiScl) (50% to 60%), which is characterized by a linear streak or band involving the dermis, subcutis, and often the underlying muscle, tendons, and bone. LiScl typically occurs as a single, linear, unilateral band on the extremities, trunk, or head.[168,173] Linear lesions crossing the joint may cause joint contracture and limb dysfunction (Figure 108-10A). Of particular concern in pediatric patients with LiScl is involvement of the underlying tissues, including muscle, bones, growth plates, and organs, which can lead to permanent shortening or atrophy of the limb (Figure 108-10B). Orthopedic complications are reported in approximately 30% to 50% of patients with LiScl of the limb(s), causing mild to moderate disability.[170,173,220,221]

Linear lesions affecting the head affect approximately 25% of patients with jLS.[168] Those with more obvious cutaneous findings, displayed as a dyspigmented linear streak or depression of the scalp and forehead, are termed "en coup de sabre" (ECDS), resembling a saber wound scar (Figure 108-11). Those with more atrophy of the subcutis, with associated hemifacial atrophy of the mandible, maxilla, tongue, and muscle tissue of the face are classified with Parry-Romberg syndrome (PRS). These disorders can occur together, either at the same location (side of face) or opposing sides (Figure 108-12).[222] PRS and ECDS are associated with neurologic, ophthalmologic, and dental ECMs. Neurologic involvement is demonstrated in 8% to 20% of the patients with these conditions, including CNS involvement expressed as seizures, chronic headaches, optic neuritis, and, less frequently, neuropsychiatric changes or ischemic

**TABLE 108-11** Proposed Subtypes for Juvenile Localized Scleroderma: Padua Preliminary Classification*

| | |
|---|---|
| Circumscribed morphea | Oval/round lesions<br>(a) Superficial lesions (*Plaque morphea*)—limited to epidermis and dermis<br>(b) Deep lesions (*Deep morphea*)—involve subcutaneous tissue |
| Linear scleroderma | Linear lesions can involve dermis, subcutaneous tissue, muscle, bone<br>(a) Trunk/limbs<br>(b) Head-*En coup de sabre* or *Parry-Romberg syndrome* |
| Generalized morphea | ≥4 large plaques (>3cm) on at least 2 of 7 anatomic areas: head/neck, Right upper extremity, LUE, RLE, LLE, anterior trunk, posterior trunk |
| Pansclerotic morphea | Circumferential involvement of limb (spares fingertips/toes)<br>All depths skin/subcutaneous tissue/muscle/bone |
| Mixed morphea | Combination of two or more of above subtypes |

*Modified from Laxer RM, Zulian F: Localized scleroderma. *Curr Opin Rheumatol* 18:606-613, 2006.
LLE, Left lower extremity; LUE, left upper extremity; RLE, right lower extremity.

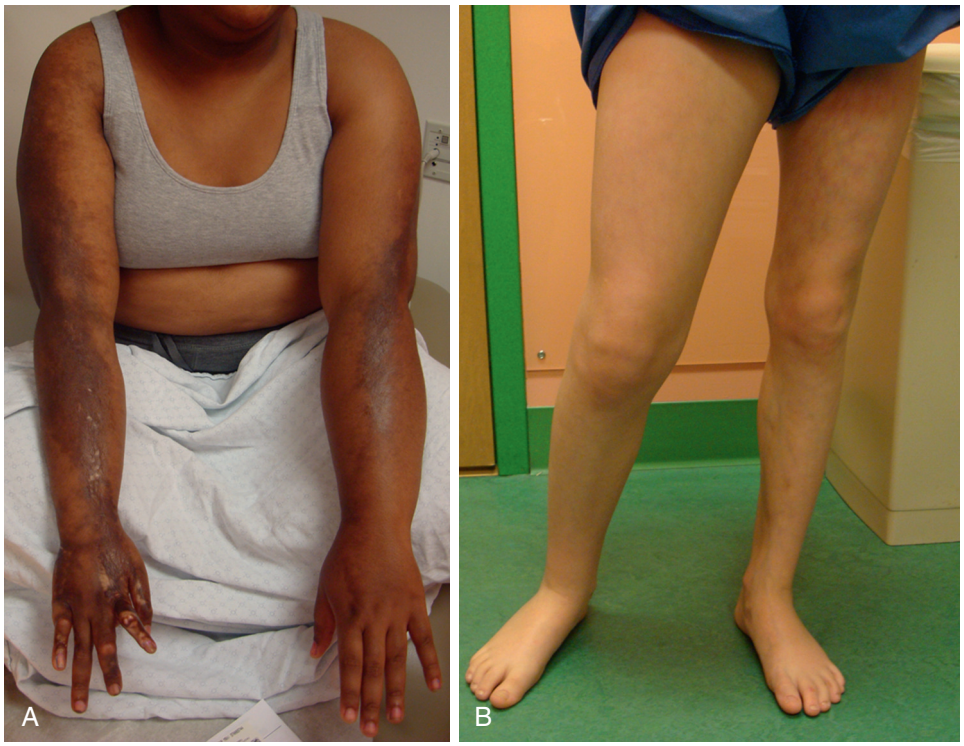



**Figure 108-10** **A,** Linear scleroderma (LiScl) affecting bilateral upper extremities with more severe involvement of the right, causing moderate to severe finger and wrist limitation. **B,** LiScl affecting the entire left limb with associated leg-length discrepancy and limb-girth circumference difference.

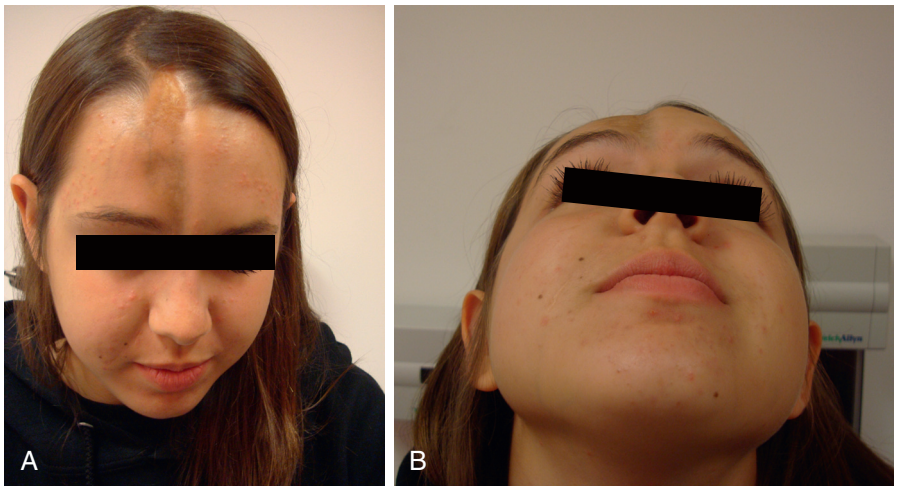



**Figure 108-11** Pediatric patients with localized scleroderma with typical "sword of the saber," or "en coup de sabre" lesion affecting the forehead and scalp with resultant skull depression.




**Figure 108-12** Patient with long-standing juvenile localized scleroderma, linear scleroderma of the head, both en coup de sabre variant (affecting the right lower face) and Parry-Romberg subtype (left hemifacial atrophy). She also has hemitongue atrophy, skewed palate, malocclusion, and other dental abnormalities.

stroke.[222,223] Orthodontic (i.e., malocclusion) and ophthalmologic (i.e., uveitis) abnormalities also have been reported with similar prevalence in both conditions.[223]

The less common subtypes of jLS are superficial and deep circumscribed LS, generalized morphea and pansclerotic morphea. Most superficial circumscribed lesions (epidermis and upper dermal involvement) affect the trunk, are more common in adults, and are usually treated topically (do not come to rheumatology attention). Deep circumscribed morphea affects the dermis and subcutis with variable penetration into the fascia and muscle. Typically, there are only subtle cutaneous changes, such as slight erythema and thinning of the dermis, but secondary to deep infiltrate causes a scooped-out appearance of the subcutis. Generalized morphea (GM) is more typical in the adult LS population, occurring in less than 10% of patients with jLS,[168,173] and is associated with systemic symptoms such as myalgia, arthralgia, and fatigue.[173,175] Lesions typically are widespread throughout the trunk and coalesce. This subtype is often associated with several autoimmune phenomena, including positive antibodies and concomitant and family history of autoimmune disorders.[175] Although pansclerotic morphea is a rare subset of LS, affecting 1% to 2% of patients, it is the most disabling. It initially involves the extremities but later may migrate to the trunk, only sparing the face and distal areas of fingers and toes (Figure 108-13). There is a rapidly progressive fibrosis of the deep dermis, subcutis, fascia, and muscles, with occasional bone involvement. This leads to significant contracture of joints, muscle atrophy, cutaneous ulcerations and sometimes restrictive pattern of respiratory insufficiency if the truck is involved. The development of squamous cell carcinoma is an additional complication in pansclerotic morphea, especially in areas of chronic wounds.

Eosinophilic fasciitis (EF), once considered a type of deep morphea by Peterson criteria, is not considered a subtype of jLS by Padua criteria, although it often behaves as a mixture of deep morphea and linear scleroderma, resulting in deep infiltration and joint contractures, and is treated the same as LS.[224]



**Figure 108-13** Pansclerotic morphea subtype affecting bilateral upper and lower extremities and some of this patient's trunk, only sparing the face and very distal finger and toe skin. There is notable atrophy of the subcutis and muscles as well as diffuse joint contractures.

## Clinical Features

### Cutaneous Manifestations

**Disease Activity and Damage Features: Clinical and Histologic Findings.** Similar to SSc both clinically and histologically, LS exhibits an earlier more active phase of disease and a later, more fibrotic, phase. Early active lesions in LS are characterized by a violaceous inflammatory border or "lilac ring" and skin induration throughout the lesion that may appear waxy or white (Figure 108-14). During this active state, lesions may also expand and new lesions can accumulate.[225] Histologic findings in the early stages of disease consist of a perivascular and perineural infiltrate of predominantly lymphocytes with admixed rare plasma cells and eosinophils in the deep reticular dermis and subcutis, accompanied by thickened collagen bundles, decreased elastic fibers, and swollen endothelial cells.[226-228] With time, disease damage accumulates and is represented by an increase in skin thickness, especially at the center of the lesion, combined with dermal and subcutaneous atrophy,



**Figure 108-14** Typical active inflammatory localized scleroderma lesion with white, waxy center and violaceous halo ("lilac ring").

appearing as visible veins, "cliff drop" appearance of the dermis, and flattening or concavity of the subcutis, respectively. Histologic evaluation of the skin at this stage reveals thickened hypocellular (homogenized) collagen that replaced the previous inflammatory infiltrate around the dermal appendages, leaving behind atrophic eccrine glands and hair follicles. In addition, capillaries are decreased in number with a fibrotic wall and narrowed lumen.[226,227] Post-inflammatory hyperpigmentation and hypo-pigmentation also are a result of previous inflammation and are often the features that bring the lesion to the attention of a medical provider.

**Extracutaneous Manifestations.** ECMs are not uncommon in LS and occur in approximately one-quarter of the patients throughout the course of disease.[229] According to a large international study of 750 children with LS, ECMs are more frequent in patients with LiScl and consist essentially of orthopedic complications (19% in the entire group, 50% in those with LiScl of the extremity), and neurologic or ocular findings (24 patients, 5% in the entire group, 60% in those with LiScl of the head).[229] Less common ECMs are GI, respiratory, and renal, all of which occurred in less than 2% of the 750 patients examined.[229] These estimates may be lower than the true percentage of ECMs, because only symptomatic patients were included in the analysis. Interestingly, of the 168 patients with ECMs, 25% had articular, neurologic, and ocular manifestations unrelated to the site of skin lesions, further supporting the hypothesis that localized scleroderma is more of a systemic process.[229]

## Diagnosis and Diagnostic Tests

**Diagnosis.** LS remains a clinical diagnosis by physical examination features, and when there is uncertainty, a skin biopsy is supportive (findings described in previous text). There are no diagnostic laboratory tests for LS, but there is a high prevalence of autoantibodies, including ANA, anti-histone antibody (AHA), and anti–single-stranded DNA antibody (ssDNA Ab), which could be supportive of the diagnosis.[230] A positive ANA is found in 42% to 73% of patients with LS and has been associated with an increased risk for ECMs.[229,231] The prevalence of ssDNA and AHA in LS is approximately 50%[180,232] and both are associated with disease severity features, such as deep muscle involvement, joint contractures, and increased number of lesions.[221,230,231] In addition, RF is present in one-third of patients, particularly those who have GM, and also correlates to disease severity (number of lesions).[168] These antibodies (ssDNA and AHA) and RF may help in stratifying patients during the earlier stages of disease, suggesting the possible initiation of more aggressive therapy and increased surveillance.

General markers of inflammation, including erythrocyte sedimentation rate (ESR) and serum immunoglobulin levels (IgG and IgE), may be useful markers of disease activity in select patients with LS, particularly those with deep or eosinophilic-like variants. However, the majority of patients seen with active LS lesions do not have elevation of these markers. A recent review of the CARRA Registry data of 259 patients with jLS supports elevated creatine phosphokinase (CPK) or aldolase to reflect disease activity through their association with new lesions. CPK elevation was also associated with muscle atrophy and limb shortening and may predict muscle involvement.[233]

Differential diagnosis is limited and includes lichen sclerosus et atrophicus and atrophoderma of Pasini and Pierini; superficial dermal and epidermal inflammatory conditions that are thought by some to be on the milder spectrum of LS; cutaneous T cell lymphoma, especially if there is a deeper purple skin discoloration; and collagenoma, usually presenting with induration of the skin and subcutis without pigmentary changes. Other possible conditions are associated with certain exposures and generally have more widespread lesions, such as GVHD (status, post-transplant), nephrogenic fibrosing dermatopathy (gadolinium administered in renal compromised state), and porphyria cutanea tarda (sun exposure).

**Diagnostic Tests.** Several tools have been used for the assessment of skin and subcutaneous involvement in LS, such as clinical skin scores, measurement tools, and imaging. Clinical skin scores are commonly used by physicians and include the modified Rodnan Skin Score[202] and the Localized Scleroderma Cutaneous Assessment Tool (LoSCAT), which combines disease activity and damage parameters.[225,234] A computerized skin score (CSS) can be used to determine the size of lesion in an objective manner.[235] Measurement tools, including a durometer, which measures the degree of skin hardness,[236] and a cutometer, which measures skin elasticity,[237] have also been proposed as useful. In addition, several types of imaging can be used for diagnostic or assessment purposes, such as thermography,[238,239] laser Doppler flowmetry,[240] ultrasound,[241,242] and MRI.[243] Some of these techniques have been validated in LS, including the LoSCAT, the CSS, ultrasound using higher frequencies (10 to 25 MHz), and MRI. However, currently, standard measures are not widely used, and assessment depends on center-specific modalities that rely on the investigator's experience. Recent efforts through CARRA used expert opinion to outline clinical criteria for the assessment of disease activity, damage, and severity features of LS, which include cutaneous, laboratory, and imaging features, to stratify patients and monitor disease status as they enter a consensus treatment plan (CTP).[244]

In general, there is not any recommended internal organ screening for a patient with LS because they tend not to have typical SSc features; however, the exception is LiScl of the head. A baseline brain MRI, ophthalmology examination, and dental evaluation are considered standard by experts in the field.[212] Because of the high incidence of odontostomatologic abnormalities in LiScl of the head, a recent study suggests cone-beam CT as a useful technique to evaluate and monitor hard and soft tissue changes.[245]

### Treatment

Approach to the treatment of LS initially depends on the severity of the disease, which is characterized by the subtype of LS, potential involvement of deeper tissue and structures (such as joints), location of LS, and disease activity state. If the diagnosis has been significantly delayed and only disease damage parameters remain, simply monitoring the lesion is acceptable. However, if there is any sign of active disease, such as erythema or lesion expansion, both rheumatologists and dermatologists agree treatment is warranted. For a single superficial circumscribed lesion in a noncosmetically concerning location, topical treatment with CSs, calcineurin inhibitors, imiquimod, vitamin D, or recurrent phototherapy with UV light is acceptable. All modalities have had clinical success in case series or uncontrolled trials exhibited by lightening of hyperpigmentation and decreased skin thickness. UVA-1 was successful in two randomized control trials, especially in regard to early inflammatory and superficial dermal lesions.[246,247] There is growing consensus among both rheumatologists and dermatologists that systemic therapy is warranted for more moderate to severe LS, which is defined as lesions with deep tissue involvement (subcutis, fascia, and muscle) that transverse a joint and potentially cause functional impairment, affect the face or scalp, are rapidly progressive or widespread active disease, and for those who have failed topical or UV therapy.[248,249] The most commonly used systemic therapy is MTX in conjunction with CSs.[249]

The first double-blind randomized control trial in jLS with objective measures was performed by Zulian and colleagues comparing MTX and oral CS versus placebo and oral CS. MTX was found to be effective and safe, confirming the community's support for the use of MTX in LS. In addition, those treated with MTX significantly improved and had significantly fewer relapses compared with those on CS alone.[250] An open-label extension (average follow-up, 40 months) supported continued MTX efficacy, with 74% responders and 73% maintaining remission after weaning off of medications.[251] Relapses were documented during this longitudinal study, and the authors concluded that is beneficial to continue MTX treatment for at least 24 months to ensure a prolonged and sustained disease remission.[251] This was supported in a retrospective study, reporting higher relapse rates in those with shorter MTX treatment duration.[252]

A subcommittee of CARRA derived four CTPs for the first 12 months of induction of therapy for those with moderate to severe jLS. Three protocols involve subcutaneous MTX (1 mg/kg, with a maximum dose of 25 mg/week), and the fourth was derived for those who either fail to respond to MTX or are intolerant to MTX. The protocols are divided into MTX alone, MTX with oral CS and taper, MTX with IV CS, and MMF in addition to either oral or IV CS.[244] MMF was included as an alternative because it has been shown to be effective in severe MTX-resistant jLS (panscleroticc, GM, LiSCl subtypes).[253] Patients have been enrolled into the juvenile LS CTP and data collection, and analysis is currently ongoing.

**Other Therapies.** Intensive physical and occupational therapy in conjunction with systemic immunosuppressive therapy is recommended for those with linear or deep scleroderma of the extremity to help minimize joint contractures. Also, for those with ECDS and/or PRS, plastic surgery intervention is felt to be reasonable after the disease has been in remission without any signs of clinical activity. Augmentation includes fat grafting with microdroplets from the patient's abdominal subcutis to assist in filling in some of the subcutaneous atrophy to yield a more symmetric facial contour. For more severe disease, fasciocutaneous flaps and facial bone reconstruction are also options, although they are typically reserved for the late teenage years after the skeleton has matured.[254]

### Outcome

The largest obstacle to good outcomes is awareness of LS among primary care physicians, and timely referral to academic dermatology or rheumatology to enable correct diagnosis and treatment while the disease is still in active phase. Once months or years have passed and fibrosis and atrophy dominate, current therapies that target inflammation have little usefulness. Two recent European studies demonstrated an average 11- to 13-month delay from onset to correct diagnosis of LS.[172,255] In one of the studies, all 50 cases were originally misdiagnosed or undiagnosed at first provider visit.[255] A North American dermatology longitudinal morphea cohort confirmed that prolonged interval between onset and definitive diagnosis adversely affects the final outcome.[172,256]

Once systemic immunosuppressive therapy is initiated in patients with moderate to severe LS, the signs of disease activity typically resolve within months, and some of the damage features mildly to moderately improve.[250,252,257-260] After this initial improvement, the detection of subclinical disease is important so as to not wean off of immunosuppression too early, potentially resulting in relapse.[250,257,260] In general, it is recommended to treat for 2 to 3 years with MTX with continued remission before stopping systemic therapy. After this initial course, it is not uncommon for the patient to relapse, and despite prior notions that LS "burns out," approximately one-third of patients will relapse, requiring reinitiation of systemic therapy. In the author's opinion, theses relapses occur in the same region or at the site of the original lesion, and most respond to reinitiation of systemic therapy.

## Conclusion: LS and SSc

Although the underlying pathogenesis of jLS and jSSc remain understudied and mostly rely on adult SSc studies, newer data are available, suggesting a shared immunophenotype and associated cytokine profiles between juvenile

and adult patients and between LS and SSc. Treatment is most effective during the active inflammatory state in both jLS and jSSc; therefore it is imperative to raise awareness to general providers for prompt referral, diagnosis, and management. CARRA is further refining disease activity, damage, and responsiveness measures of LS through consensus treatment plans to offer more defined guidelines for therapy management and to be used in future clinical trials of LS. The J4S is a promising tool for similar purposes in jSSc.

# PEDIATRIC VASCULITIS

**KEY POINTS**

- **Henoch-Schönlein and Kawasaki disease represent the majority of childhood vasculitis, following a self-limited course in the majority of children with excellent outcomes when recognized and treated appropriately.**
- **Specific pediatric criteria for diagnosing several of the vasculitides more commonly seen in adult populations have been defined.**
- **Primary CNS vasculitis of childhood has modified diagnostic criteria and unique categories of disease compared with adult populations.**
- **Prompt recognition and initiation of therapy are essential and can result in remarkable recovery.**

Childhood vasculitis encompasses a broad spectrum of diseases, from predominantly self-limited disease to chronic, progressive disease with devastating end-organ damage. Pediatric vasculitis is defined similarly to adult vasculitides with disease categories based on the size and distribution of vessel involvement as well as histology. They can be associated with infection, medication exposure, and other systemic disease. Although the incidence of the vasculitic subtypes varies across patient populations and ethnicity, the overall incidence of childhood vasculitis is 23/100,000, with Henoch-Schönlein (HSP) and Kawasaki's disease (KD) representing the majority of cases.[261] Although other forms of childhood vasculitis are rare, they tend to be more chronic, with a greater potential for significant morbidity.

A classification system for childhood vasculitis takes into account vasculitic conditions predominantly seen in children and reflects the clinical features particular to pediatric populations.[262] This section will focus on the types most commonly seen in the pediatric age range and highlight the unique features of childhood-onset vasculitis.

## Small-Vessel Vasculitis: Henoch-Schönlein Purpura

### Definition and Classification

HSP, the most common pediatric vasculitis, is characterized by an IgA-mediated leukocytoclastic vasculitis.[263] Self-limited in most cases, it is associated with significant morbidity in a notable minority who experience persistent renal impairment and end-stage renal disease.

### Epidemiology

HSP has an incidence of 10 to 20 per 100,000 children and affects boys more commonly than girls.[261,264-266] The highest incidence is seen in young children, with a population study reporting an incidence of 70 per 100,000 children aged 4 to 6 years.[261] HSP is much less common in adult populations but may be associated with more aggressive disease.[267,268]

### Etiology and Pathogenesis

Infectious triggers have long been recognized, with more than half of affected children having a preceding upper respiratory infection.[269] IgA is thought to play a pivotal role in the pathogenesis of HSP, with $IgA_1$ found to complex with IgG and deposit in the kidney.[270] IgA-bound streptococcal M protein deposits have been found in the skin and kidneys of patients with HSP, serving as a link between infection and vasculitis.[271]

### Clinical Features

Nonthrombocytopenic purpuric skin lesions are the hallmark of HSP, and their presence is required for the diagnosis.[265,266,272] Palpable purpura and petechiae appear predominantly over the lower extremities and in dependent areas. The rash may initially manifest as a maculopapular or urticarial rash before evolving into the more classic purpura, and may even progress to areas of necrosis. Lesions are seen in various stages and sizes during the course of the disease, often occurring in waves, with skin manifestations lasting 4 to 8 weeks.[266,272]

Arthritis and arthralgias are common, reported in approximately 60% to 75% of cases.[265,266,272] The arthritis is usually painful, nonerosive, and nonmigratory. Periarticular swelling, without clear arthritis, is also well described. Knees and ankles are the most frequently affected joints, although wrists and small joints of the hands may also be involved. Scrotal swelling and swelling of the hands and feet are commonly seen, especially in children younger than 2 years of age.[272]

GI symptoms occur in 50% to 75% of children, manifesting predominantly as abdominal pain.[265,266,272] The pain can be quite severe and may be seen before the characteristic rash appears. This complicates the diagnosis, and these children may initially undergo evaluations for appendicitis or other GI diseases. Occult GI bleeding may occur; although gross bleeding and intussusception are rare, they pose significant morbidity.

Renal disease is described in 40% to 50% of children with HSP.[265,266,272,273] Although it is most often mild and self-limited, it is the major cause of morbidity and requires careful follow-up. The majority of cases involve microscopic hematuria or low-grade proteinuria presenting in the first 6 weeks, with only a 2% risk of developing nephritis more than 2 months after diagnosis.[273-276] Nephrotic-range proteinuria or nephritis will develop in some children, with older children having potentially higher rates of nephritis.[275] Renal biopsies performed in those with marked renal disease demonstrate IgA nephropathy. The degree of damage on biopsy as well as higher levels of proteinuria confer a

worse outcome.[277] End-stage renal disease develops in 1% to 3% of children with HSP.[272,278,279]

### Diagnosis and Diagnostic Tests

There are no specific diagnostic markers of HSP, and the diagnosis is largely made on clinical features.[263] Elevated sedimentation rates (57%), elevated serum IgA (37%), and proteinuria (42%) are the most common laboratory abnormalities.[265,266,272,273] Urinalysis should be obtained serially in all children given the potential for developing nephritis with time. Skin biopsy may be performed to confirm the diagnosis but is not required in most cases. Skin biopsy demonstrates a leukocytoclastic vasculitis with IgA deposition.[277] Stool guaiac and imaging should be considered in those with abdominal complaints to evaluate for GI bleeding and intussusception.

### Treatment

Treatment recommendations vary depending on the severity of disease. Most children with HSP have self-limited disease that requires only supportive care, including hydration, NSAIDs (use with caution if significant GI complaints are present), and pain management.[280] For those with more severe disease, there are no clear treatment recommendations. Prednisone has been found to reduce abdominal pain and arthritis symptoms, but there is no indication it reduces purpura or prevents the progression of nephritis.[274,281-283] The largest benefit from CSs seems to be in preventing the GI complications of HSP, with fewer abdominal surgeries and endoscopies.[282] For children with more severe disease, particularly nephritis, high-dose IV pulse methylprednisolone, azathioprine, and cyclophosphamide are at times used, although there are no clear recommendations for immunosuppressive therapy.[284]

### Outcomes/Prognosis

Overall, the majority of children with HSP have a self-limited disease resolving within 4 to 6 weeks, with only 1% to 3% progressing to end-stage renal disease.[277] Of patients in whom nephritis develops during their course, 30% will have some degree of ongoing renal impairment. Recurrence is commonly seen, affecting a third of children, especially in those older than 8 years and those with nephritis.[265,274,276,278,279] More persistent disease will develop in some children, and they will require a chronic DMARD to control their disease.

## Medium-Vessel Vasculitis: Kawasaki's Disease

KD is the second most prevalent form of pediatric vasculitis. It is frequently triggered by an infectious or proposed environmental exposures.[261,285,286] First described as infantile polyarteritis nodosa based on histologic findings, KD is now recognized as a distinct disease with characteristic clinical and laboratory features.[287] Although KD is a monophasic, self-limited inflammatory condition, the cardiac complications are the leading cause of acquired heart disease in childhood in Western countries.[288-290]

### Classification

The diagnosis of KD is made based on clinical features, with supporting laboratory and imaging studies (Table 108-12). Since initially described, a spectrum of disease with fewer than four criteria, with or without cardiac involvement, has been recognized and termed "incomplete" or "atypical" KD.[288,291] In 2004, the American Heart Association proposed an algorithm to assist in the diagnosis of incomplete KD (see Figure 108-15) that was subsequently found to increase the detection of KD.[292-294]

### Epidemiology

KD is predominantly seen in young children, with the majority (80%) of cases in those between 1 and 5 years of age. Males are affected more frequently than females, with reported ratios of 1.4 to 1.9:1.[285,288] Incomplete disease is more common in children younger than 1 year and older than 9 years of age, with as many as one-third of children in these age groups seen with incomplete disease. Incidence rates vary greatly across ethnicity; Asian children have the highest rates, with 218/100,000 in children younger than 5 years in Japan and 113/100,000 in Korea, compared with 5 to 13/100,000 in Western countries.[285,288,295,296] Genetic variation in pro-inflammatory genes and matrix metalloproteinases are thought to play a role in susceptibility and disease severity.[297,298]

### Clinical Features

KD progresses through three phases: acute (as long as 14 days), subacute (2 to 4 weeks), and convalescent (as long as several months). The acute phase is characterized by high fever, ocular findings, and rash. Continuous, high-grade fever is the hallmark of KD but is less consistently seen in children younger than 1 year and older than 9 years. By definition, the fever should last at least 5 days and be minimally responsive to anti-pyretics.[292] Ocular manifestations include nonpurulent conjunctivitis with limbic sparing (more than 90% of cases) and asymptomatic uveitis. Rashes are reported in more than 90% of cases, and although nonspecific, commonly affect the perineal area and axilla in the acute phase of the disease. The characteristic sheetlike desquamation described in KD starting at the fingertips is seen in the subacute phase and after the period of maximal benefit from IVIG. Mucocutaneous changes include marked erythematous, swollen and cracked lips, strawberry tongue and, less frequently, oral ulcers. Hands and feet often appear swollen. Cervical lymphadenopathy is the least common of the five classification criteria, and is asymmetric with a single lymph node greater than 1.5 cm.[285,288,292,299] Although not a classification criterion, neurologic manifestations are common, including irritability, lethargy, headaches, and aseptic meningitis. Nondestructive polyarticular or oligoarticular arthritis, dysuria, sterile pyuria, scrotal pain, abdominal pain, hydrops of the gallbladder, and diarrhea are also well described in children with KD. Macrophage activation syndrome has been described as a complication.[300]

Cardiac disease can be seen as myocarditis, pericarditis, and/or endocarditis. The most concerning feature of KD is

**TABLE 108-12** Classification Criteria of Pediatric Vasculitis

| | Required Criteria | Additional Criteria |
|---|---|---|
| **Small-Vessel Vasculitis** | | |
| Henoch-Schönlein purpura | Purpura or petechial with lower limb predominance plus one additional criteria | 1. Arthritis/arthralgia<br>2. Abdominal pain<br>3. Histopathology with IgA deposition<br>4. Renal involvement (hematuria or proteinuria) |
| **ANCA-Associated** | | |
| Granulomatous polyarteritis | No required criteria; need 3 of 6 criteria | 1. Histopathologic evidence of granulomatous inflammation<br>2. Upper airway involvement<br>3. Laryngotracheal-bronchial involvement<br>4. Pulmonary involvement (radiograph or CT)<br>5. Positive ANCA<br>6. Renal involvement (proteinuria, hematuria, necrotizing pausi-immune glomerulonephritis |
| **Medium-Vessel Vasculitis** | | |
| Kawasaki's disease | Fever for ≥5 days plus 4 of the additional criteria | 1. Bilateral conjunctivitis (nonpurulent)<br>2. Changes in the lips or oral cavity<br>3. Cervical lymphadenopathy<br>4. Polymorphous exanthema<br>5. Changes in peripheral extremities/perineal area |
| Polyarteritis nodosa | Systemic inflammation with biopsy evidence of necrotizing vasculitis or angiographic abnormalities of medium sized arteries plus at least one of the additional criteria | 1. Skin involvement (livedo reticularis, modules, infarcts)<br>2. Myalgia/muscle tenderness<br>3. Hypertension (>95th percentile)<br>4. Peripheral neuropathy<br>5. Renal involvement (proteinuria, hematuria, impaired function) |
| **Large-Vessel Vasculitis** | | |
| Takayasu's arteritis | Characteristic angiographic abnormality of the aorta or its major branches and the pulmonary artery plus one of the additional criteria | 1. Absent peripheral pulses or claudication<br>2. Blood pressure discrepancy in any limb<br>3. Bruit<br>4. Hypertension(>95th percentile)<br>5. Increased acute phase reactants (ESR >20, any elevation in CRP) |

ANCA, Anti-neutrophil cytoplasm antibody; CT, computed tomography; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin.
Modified from Ozen S, et al: EULAR/PRINTO/PRES criteria for Henoch-Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: Final classification criteria. *Ann Rheum Dis* 69:798–806, 2010.

coronary artery disease that results in the majority of morbidity and mortality. The coronary changes tend to appear in the subacute and convalescent stages, although they can be seen in the acute stage, and include dilatation, ectasia, and frank aneurysms.[285,288,292,299,301]

## Diagnosis and Diagnostic Tests

Characteristic laboratory studies include elevated inflammatory parameter, with markedly elevated sedimentation rates (ESR) and C-reactive protein (CRP) (Table 108-13). Complete blood counts reveal elevated white blood cells and platelets. Platelets are usually markedly elevated, often more than a million, but low platelets can also be seen and indicate higher risk for coronary disease.[302] Hemoglobin is usually low, even at onset of disease. Urinalysis may reveal sterile pyuria caused by urethritis. Mild elevations in alanine aminotransferase (ALT) and low albumin are commonly seen.[285,288,292,299,301] A high level of suspicion is needed to ensure those with possible incomplete KD are evaluated per the current recommendations (Figure 108-15). Infectious studies should be performed, given the association with infectious triggers, but it is important to recognize KD can develop in the setting of infections, and a concurrent infection does not preclude the need to treat patients who meet the clinical and laboratory criteria for KD with IVIG and other recommended therapies while also treating active infections.[286]

**TABLE 108-13** Laboratory Findings in Acute Kawasaki's Disease

| |
|---|
| Leukocytosis with neutrophilia that can be immature forms |
| Elevated erythrocyte sedimentation rate and C-reactive protein |
| Thrombocytosis after 7+ days |
| Anemia |
| Elevated plasma lipids |
| Hypoalbuminemia |
| Hyponatremia |
| Sterile pyuria |
| Elevated serum transaminases and gamma-glutamyl transferase |
| Pleocytosis of cerebrospinal fluid |
| White cell elevation in synovial fluid if arthritis |

An echocardiogram should be performed at diagnosis, after 2 weeks, and again at 6 weeks. ECG should be done at presentation. Abdominal ultrasound should be considered for those with abdominal pain and evaluation of hydrops of the gallbladder. The American Heart Association guidelines should be referenced to evaluate for incomplete disease and prognostic indicators.[292] Given that macrophage activation syndrome has been described in some children with KD, additional studies, including ferritin, triglycerides,

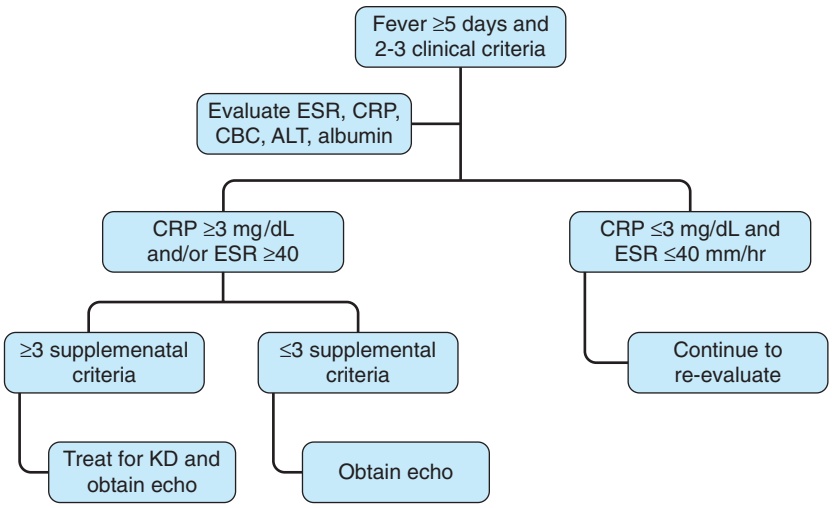



**Figure 108-15** Evaluation of incomplete Kawasaki's disease (KD). Supplemental criteria: albumin 3.0 g/dL or less, anemia for age, elevated alanine aminotransferase (ALT), platelet count 450,000 or higher after 7 days of illness, white blood cell (WBC) count 15,000 or higher, urine with higher than 10 WBC/high-powered field. CBC, Complete blood cell count; CRP, C-reactive protein; echo, echocardiogram; ESR, erythrocyte sedimentation rate. *(Modified from Newburger JW, et al: Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association.* Pediatrics *114:1708-1733, 2004.)*

fibrinogen, and D-dimers, should be considered in children with more severe disease.[303]

### Treatment

IVIG and aspirin are considered first-line therapy for the treatment of KD.[288,290,292] Studies have demonstrated a dose-dependent response to IVIG, with dosing of 2 gm/kg given during 12 hours having greater efficacy than both lower-dose IVIG and divided doses.[304] High-dose aspirin (80 to 100 mg/kg) is recommended until the patient becomes afebrile. Low-dose aspirin should be continued at 3 to 5 mg/kg until the 6-week repeat echocardiogram and normalization of the platelet count.[292] However, it should be noted that the evidence for the use of aspirin is limited.[305,306]

Incomplete response to IVIG with persistent or recurrent fevers or continued elevation of CRP is common and should be treated with a second course of IVIG.[307-310] Patients with continued refractory disease may be treated with steroids or infliximab.[311,312] A recent study reported no added benefit of adding methylprednisolone to the initial dose of IVIG.[313] TNF is noted to be elevated in patients with KD and thought to be an important mediator of disease. Infliximab has shown improved efficacy versus repeated doses of IVIG for children with continued refractory disease, although there are no data to support infliximab as first-line therapy.[309,312,314]

### Outcome

Overall, the 5-year survival rate is 99%, although coronary artery damage will develop in 1 in 20 children, including aneurysms and stenosis.[315,316] In addition, although the long-term impact of transient coronary dilatation is unclear, there is concern for potential endothelial dysfunction and early atherosclerosis, and these children require ongoing follow-up.[317-321]

## Anti-neutrophil Cytoplasm Antibody–Associated Vasculitis, Polyarteritis Nodosa, and Takayasu's Arteritis

### Definition and Classification

Other forms of pediatric vasculitis are rare and present with similar manifestations as adult-onset disease.[262,322-326] Given the paucity of these vasculitides in children, much of the information on pathophysiology, clinical manifestations, and treatment is based on adult studies. This section will highlight some of the differences between adult and pediatric disease. Please refer to the individual chapters on each disease for more in-depth review.

## Anti-neutrophil Cytoplasm Antibody-Associated Vasculitis

### Epidemiology

Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis is a small-vessel necrotizing vasculitis and includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (previously known as Churg-Strauss syndrome). Overall, these are very rare conditions in the pediatric population, with GPA the most prevalent.[262,322-327] The EULAR/PRES-endorsed criteria are listed in Table 108-6. Given the paucity of both MPA and eosinophilic

granulomatosis with polyangiitis in children, there are no pediatric-specific classification criteria.

### Clinical Features

The clinical features of the various ANCA-associated vasculitic conditions overlap significantly and predominately involve the kidneys, lungs, and upper airway. Mean duration from onset of symptoms to diagnosis is approximately 2 months in children. Constitutional symptoms with fever, fatigue, and weight loss are seen in the majority of cases, with renal and pulmonary involvement seen in more than 80%.[262,322-329] Upper airway disease, including sinusitis, mastoiditis, subglottic stenosis, and nasal septum perforations are common in children and may be a more prominent feature than in adult disease, as is an increase in thrombosis.[323,330,331]

### Diagnosis and Diagnostic Tests

Laboratory studies are similar to adults. ANCA is positive in the majority of children, with 66% having a positive cytoplasmic (c)ANCA and 22% a positive perinuclear (p)ANCA (Table 108-14).[323,330] A positive test for proteinase 3 (PR3) antibodies and a positive cANCA are seen primarily in patients with active granulomatosis with polyangiitis. Positive MPO antibodies and pANCA are seen primarily in microscopic polyangiitis but also are reported in glomerulonephritis, eosinophilic granulomatosis with polyangiitis, and Goodpasture syndrome. MPO and pANCA may be seen in other autoimmune disorders, such as Sjögren syndrome, systemic lupus erythematosus, and rheumatoid arthritis as well as (rarely) in nonrheumatic disease.

### Treatment

Treatments for childhood-onset disease are largely based on adult studies and include cyclophosphamide, B cell–depleting agents, high-dose CSs and, at times, plasmapheresis, as well as MTX and azathioprine for maintenance or milder disease. The use of B cell–depleting therapies provides promise for those with refractory disease and recurrent disease, with more recent studies suggesting use as first-line therapy for systemic disease.[322,332-334] ANCA-associated vasculitis has a chronic, relapsing-remitting pattern in children, similar to adult disease.

**TABLE 108-14** ANCA Antibodies and Disease Classification

| | Patients with cANCA and PR3 | Patients with pANCA and MPO |
|---|---|---|
| Granulomatosis with polyangiitis | 90% inactive disease, 60%-70% inactive disease | Less than 10% |
| Microscopic polyangiitis | 30% | 60% |
| Eosinophilic granulomatosis with polyangiitis | Rare | 50%-80% |

ANCA, Anti-neutrophil cytoplasm antibody; cANCA, cytoplasmic ANCA; MPO, myeloperoxidase; pANCA, perinuclear ANCA; PR3, proteinase 3.

## Polyarteritis Nodosa

### Classification

Polyarteritis nodosa (PAN) is the third most prevalent pediatric vasculitis, although it only affects 3% to 6% of children with vasculitis.[335-337]

The diagnostic criteria for pediatric systemic PAN was modified by the EULAR/PRES consensus group to require biopsy evidence of necrotizing vasculitis or angiographic abnormalities (see Table 108-12).[262] Overall, cutaneous PAN is believed to be more common and characterized by periodic flares often associated with streptococcal infections.[335,338]

### Clinical Features

The clinical features are similar to adult-onset disease, with systemic manifestations of inflammation, including fever, weight loss, fatigue and malaise, classic skin findings of painful subcutaneous nodules tracking along vessels, as well as abdominal pain, neuropathy, and hypertension.[335-337,339-342]

### Diagnostic Testing and Treatment

The diagnostic evaluation and treatment mirror adult disease as well, with the use of CSs, azathioprine, MTX, biologics (anti-TNF and B cell–depleting therapy), and cyclophosphamide all reported.[334,335,342,343] Prophylactic antibiotics should also be considered if streptococcal infection was a trigger. There are limited data on the long-term survival of children, with reports of 5-year survival rates across populations of 60% to 90%.[340,342,344,345]

## Takayasu's Arteritis

### Classification and Epidemiology

Takayasu's arteritis (TA) is the most prevalent large-vessel vasculitis in children.[346] Pediatric diagnostic criteria require angiographic abnormalities as listed in Table 108-12.[262,338,347-351] Although rates of TA are so low the incidence and prevalence in children are not known, children in Africa, Asia, and Latin America seem to be at increased risk. The association with *Mycobacterium tuberculosis* may play a role in some of the differences seen across populations.[347-349,352-356]

### Clinical Features

As with other forms of systemic vasculitis, children with TA are seen with constitutional symptoms as well as signs of organ ischemia. Headaches, strokes, chest and abdominal pain, and claudication are common complaints.[354,357,358] Cardiovascular evaluations may reveal hypertension, absent pulses, and bruits. Arterial wall disease can manifest as vessel wall thickening, stenosis, occlusion, or even aneurysms. Conventional angiography provides information on distribution of vessels involved, perfusion, collateralization, and stenosis. Doppler ultrasound correlates well with angiogram in many children and may be a useful modality especially for follow up.[359-362] Children are more likely to experience aneurysms than adults (41% vs. 11%), although

given the fact that aneurysms frequently heal with fibrosis, there is a lower risk of rupture.[363]

### Treatment and Outcome

Treatment is similar to adults, with a broad range of agents used, including high-dose CSs, cyclophosphamide, MTX, TNF inhibitors, and IL-6 inhibitors.[355,356,364-366] Although the overall survival for TA is 70% to 93%, children may have lower rates of remission than adults (24% compared with 56%) and have significant limitations and morbidity.[315,363,367,368]

## Central Nervous System Vasculitis

CNS vasculitis can affect small and medium vessels and may be a primary or secondary disease. Pediatric primary CNS vasculitis is categorized differently than adult disease, with three pediatric categories of disease that are based on angiography and the distribution of involvement: (1) angiographic-positive nonprogressive disease, (2) angiographic-positive progressive disease, and (3) angiographic-negative disease.[322,369-374]

### Definition and Classification

Primary CNS vasculitis is diagnosed based on the initial criteria of Calabrese, including newly acquired focal or diffuse neurologic deficit, angiographic and/or histologic evidence of CNS vasculitis, and exclusion of other systemic disease (systemic vasculitis, infection, oncologic disease), with the additional pediatric criteria of new-onset psychiatric manifestations in patients 18 years and younger.[373,375,376]

### Epidemiology

The incidence of primary CNS vasculitis is unknown because this condition and its various phenotypes continue to be recognized.

### Clinical Features

New-onset neurologic and psychiatric manifestations can range from an acute stroke to isolated psychosis. Angiographic-positive disease typically is seen with headache and focal deficits, such as acute hemiparesis, motor and sensory deficits, loss of speech, and seizures. Children with progressive angiographic-positive disease and angiographic-negative disease are more likely to be seen with more diffuse deficits, including headaches, seizures, memory impairment, and psychiatric features such as psychosis and personality and behavior changes. The initial neurologic deficits may be mild or intermittent, but with time tend to become more prominent and disabling.[372,376,377]

Angiographic-negative disease is associated with more systemic signs of inflammation, including fever and malaise along with neurocognitive decline, behavioral changes, and often intractable seizures. Patients can also have focal deficits, optic neuritis, and myelitis. Disease can progress rapidly during days or be more insidious, with progression of neurologic and/or psychological manifestations during weeks to months.[370,372,376,377]

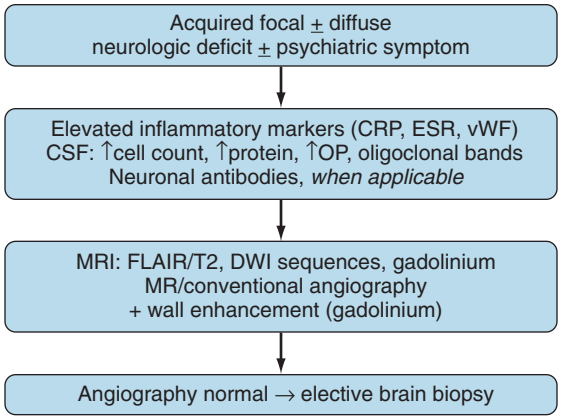



**Figure 108-16** Proposed diagnostic algorithm for children with suspected central nervous system vasculitis. CRP, C-reactive protein; CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; ESR, erythrocyte sedimentation rate; FLAIR, fluid-attenuated inversion recovery; OP, opening pressure; vWF, von Willebrand factor.

### Diagnosis and Diagnostic Tests

Recommendations for evaluating children with possible CNS vasculitis have been proposed (Figure 108-16). Imaging should be obtained in all children presenting with signs and symptoms concerning for an inflammatory brain disease. MRI with and without gadolinium may identify areas of ischemia, diffusion-restricted lesions, parenchymal lesions, and leptomeningeal disease.[378-381] Magnetic resonance angiography and angiography may reveal classic signs of vasculitis, such as stenosis, beading, and collateralization, with conventional angiography considered the gold standard.[382]

Extensive laboratory studies should be performed to both confirm the diagnosis of CNS vasculitis and to rule out other systemic diseases, such as systemic vasculitis, infections, metabolic, oncologic, and other neurologic conditions. Children with angiographic-negative disease and progressive angiographic-positive CNS vasculitis commonly have elevated inflammatory markers, whereas those with nonprogressive disease usually have normal studies. von Willebrand factor antigen may be a marker of disease and correlates with disease activity in some patients. CSF studies may reveal mild pleocytosis, elevated protein, oligoclonal bands, and elevated opening pressure.[322,369,370,372,374,376,377,380,383-385] However, patients with all forms of CNS vasculitis can also have normal or near-normal markers of inflammation despite extensive disease.

Brain biopsy is required to confirm a diagnosis of angiographic-negative CNS vasculitis but is not required in children with angiographic disease in the right clinical setting. Lesional biopsies are preferred when possible, but given the diffuse nature of angiographic-negative disease, nonlesional biopsies in the nondominant hemisphere are also acceptable. The yield of brain biopsies in pediatrics is 90%, with nondiagnostic biopsies most commonly the result of inadequate samples.[369,371,386]

### Treatment

The treatment of primary CNS vasculitis varies based on the disease subtype. There are no randomized controlled

trials for pediatric primary CNS vasculitis, but a prospective cohort study showed the efficacy and safety of a 6-month course of cyclophosphamide and high-dose steroids, followed by maintenance therapy with MMF or azathioprine for angiographic-negative disease.[387,388] Similar therapy is considered in those with progressive angiographic-positive disease. Nonprogressive disease may be successfully managed with CSs and anti-coagulation.[369,370,380,389]

### Outcome

There are limited data regarding long-term outcomes. Although two-thirds of children with nonprogressive angiographic-positive disease have a monophasic course, because they often are seen with strokes, they also have higher residual deficits. Progressive disease and angiographic-negative disease have more diffuse inflammation and reversible deficits when identified early and treated aggressively, allowing the majority of children to have good outcomes. Disease flares are common, and the long-term outcome of children with CNS vasculitis is not known.[369,370,380,389]



Full references for this chapter can be found on ExpertConsult.com.

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PART 17 INFECTION AND ARTHRITIS

CHAPTER 109



# Bacterial Arthritis

Paul P. Cook • Dawd S. Siraj

## KEY POINTS

- Acute bacterial arthritis is a medical emergency that warrants rapid, accurate diagnosis and immediate treatment along with appropriate consultation with specialists as needed.
- Most cases of native joint infection are the result of bacteremic seeding.
- *Staphylococcus aureus* is the most frequent microorganism in adult nongonococcal septic arthritis.
- The initial selection of an antibiotic regimen should be broad enough to take into account host factors, clinical characteristics, likely causative microorganisms, and regional antibiotic sensitivity data pending confirmation of bacteria by culture and sensitivities.
- An infected joint must be adequately drained, and an antibiotic course that is sufficiently long to cure the infection must be instituted. Surgical drainage should be considered, but only if needle aspirations are unsuccessful or impractical.
- Poor prognostic factors in bacterial joint infection include old age, underlying rheumatoid arthritis, and infection in a prosthetic joint.
- Débridement, antibiotics, and implant retention (DAIR) is a reasonable option for selected patients with early prosthetic joint infections.
- Late prosthetic joint infections require antibiotic treatment directed at the isolated microorganism and the complete removal of the infected prosthesis before reimplantation of a new prosthesis in a one-stage or two-stage operation.
- Reducing the risk of a prosthetic joint infection involves a thorough pre-operative evaluation, peri-operative use of antibiotics, and the careful use of antibiotic prophylaxis when a patient with a prosthesis is exposed to transient bacteremia. Clinical evidence does not support the use of antibiotic prophylaxis for most dental procedures.

## EPIDEMIOLOGY

Bacterial infections of the joint are usually curable with treatment, but morbidity and mortality are still significant in patients with underlying rheumatoid arthritis (RA), patients with prosthetic joints, elderly patients, and patients who have severe and multiple comorbidities. Incremental knowledge of the pathogenesis of septic arthritis caused by two common organisms, *Neisseria gonorrhoeae* and *Staphylococcus aureus*, and understanding of the pathobiology of prosthetic devices may lead to innovations in the management and prevention of bacterial joint infections.

The normal diarthrodial joint is resistant to bacterial infection because of local and systemic host defenses. Bacteria can reach the synovial-lined joint, however, via the hematogenous route and result in septic arthritis. The large joints are affected more commonly than the small joints, and monoarticular infection is the rule, with polyarticular infection (more than one joint involved) in less than 20% of cases. A prospective series from a community-based population in the Netherlands reflected a representative distribution of joint involvement: knee, 55%; ankle, 10%; wrist, 9%; shoulder, 7%; hip, 5%; elbow, 5%; sternoclavicular joint, 5%; sacroiliac joint, 2%; and foot joint, 2%.[1]

The incidence of septic arthritis ranges from 4 to 10/100,000/year in the general population, 5.5 to 12/100,000/year in children, 28 to 38/100,000/year in patients with RA, and 40 to 68/100,000/year in patients with joint prostheses.[2,3] The incidence appears to be increasing, probably related to orthopedic procedures, an aging population, and the increased use of immunosuppressive therapy.[4] The organisms causing bacterial arthritis depend on the epidemiologic circumstances (Table 109-1). Monoarthritis of a prosthetic joint in an elderly man is likely the result of *Staphylococcus*, whereas a migratory arthritis in a sexually active woman with skin lesions is likely caused by disseminated gonococcal infection. Septic arthritis caused by methicillin-resistant *S. aureus* (MRSA) is common in the elderly, in people who use intravenous drugs, and in individuals with prosthetic joints.[5]

## ETIOLOGY

The three main etiologies to consider when a patient is seen with acute monarticular arthritis are trauma, infection, and crystal-induced synovitis such as gout or pseudogout. Acute bacterial arthritis is most commonly monarticular. Most cases of septic arthritis result from hematogenous seeding of the synovial membrane. The abundant vascular supply of the synovium and the lack of a limiting basement membrane allow organisms to target joints during bacteremia. Less common causes of septic arthritis include direct inoculation after joint aspiration or corticosteroid injection of a joint; animal or human bites; nail puncture wounds or plant

**TABLE 109-1** Organisms Causing Joint Infection in Various Hosts

| Adults<br>*Common* | Children ≤5 yr old<br>*Common* | Children >5 yr old<br>*Common* | Neonates<br>*Common* | Prosthetic<br>*Common* |
|---|---|---|---|---|
| *Staphylococcus aureus*<br>*Streptococcus pneumoniae*<br>β-Hemolytic streptococci (mainly Lancefield groups A, G, and B) | *S. aureus*<br>*Haemophilus influenzae**<br>Group A streptococci<br>*S. pneumoniae* | *S. aureus*<br>Group A streptococci | *S. aureus*<br>Group B streptococci<br>Enterobacteriaceae | Coagulase-negative staphylococci *(Staphylococcus epidermidis; Staphylococcus lugdunensis)*<br>*S. aureus* |
| *Neisseria gonorrhoeae* (adults and sexually active adolescents) | | | | |
| Enterobacteriaceae (age >60 years or predisposing condition) | | | | |
| Salmonella | | | | |
| **Adults**<br>***Rare*** | **Children ≤5 yr old**<br>***Rare*** | **Children >5 yr old**<br>***Rare*** | **Neonates**<br>***Rare*** | **Prosthetic**<br>***Less Common*** |
| *Pseudomonas*<br>*Mycobacterium tuberculosis*<br>*H. influenzae* | *Salmonella*<br>*H. influenzae*<br>*N. meningitidis* | *N. meningitidis*<br>*N. gonorrhoeae*<br>*K. kingae* | *Pseudomonas*<br>*H. influenzae*<br>*N. gonorrhoeae* | Corynebacterium<br>Enterococci and streptococci |
| *Neisseria meningitidis* | *N. gonorrhoeae* | *M. tuberculosis* | | *Pseudomonas aeruginosa* |
| *Pasteurella* | *Kingella kingae* | *B. burgdorferi* | | Enterobacteriaceae |
| *Anaerobes* | *M. tuberculosis* | | | *Propionibacterium* |
| *Mycoplasma/Ureaplasma* | *B. burgdorferi* | | | Other anaerobes |
| Fungi (*Sporothrix*, dimorphic fungi, *Cryptococcus*) | | | | *Candida*<br>*M. tuberculosis* |
| *Borrelia burgdorferi* | | | | |

*Rare in children immunized with Hib vaccine.
Modified from Atkins BL, Bowler IC: The diagnosis of large joint sepsis. *J Hosp Infect* 40:263–274, 1998.

thorn injury; joint surgery, especially hip and knee arthroplasties; and spread by contiguous osteomyelitis, cellulitis, or septic bursitis.

Table 109-1 lists the common organisms that cause joint infections according to the age of the patient and whether the joint is native or prosthetic.[4] Overall, *S. aureus* is the most common etiologic agent among children of all age groups, followed by group A streptococci and *Streptococcus pneumoniae*.[6,7] Neonates and infants younger than 2 months old are more susceptible to group B streptococci and Gram-negative enteric bacilli than older children. Rarely, *Pseudomonas*, *N. gonorrhoeae*, and *Candida albicans* may be responsible in very young children. Since the introduction of the *Haemophilus influenzae* type B vaccine, the incidence of septic arthritis caused by *H. influenzae* has declined dramatically.[8] In sexually active adolescents, *N. gonorrhoeae* must be considered. *Pseudomonas aeruginosa* and *Candida* are potential pathogens in adolescent intravenous drug abusers. Patients with sickle cell anemia are prone to *Salmonella* arthritis, and immunocompromised children are at higher risk for infection with Gram-negative bacilli. Other unusual joint pathogens in children include *N. meningitidis*, anaerobes, *Brucella*, and *Kingella kingae*.

The organisms causing nongonococcal septic arthritis in adults are 75% to 80% Gram-positive cocci and 15% to 20% Gram-negative bacilli.[9] *S. aureus* is the most common organism in native and prosthetic joint infections. *Staphylococcus epidermidis* is common in prosthetic joint infections but is a rare cause of native joint infections. *Staphylococcus lugdunensis* is a unique coagulase-negative *Staphylococcus* that can cause infection in prosthetic and native joints (particularly following arthroscopy). Unlike other coagulase-negative staphylococci, *S. lugdunensis* frequently causes severe systemic infections similar to what is seen with *S. aureus* infection. The streptococci, including *S. pneumoniae*, are the next most common group of Gram-positive aerobes. *Streptococcus pyogenes* is followed by groups B, G, C, and F in frequency. Patients with non–group A streptococcal disease often have comorbidities such as immunosuppression, diabetes mellitus, malignancy, and severe genitourinary or gastrointestinal infections.[10] Group B streptococcal arthritis in adults is uncommon, but it can be a serious infection in adult diabetics and patients with late prosthetic hip infections.[11] Aggressive polyarthritis caused by group B streptococci may result in serious functional damage and permanent morbidity. Patients predisposed to Gram-negative bacillary infections include patients with a history of intravenous drug abuse, very young and very old patients, and immunocompromised patients.[12] The most common Gram-negative organisms are *Escherichia coli* and *P. aeruginosa*.

Anaerobes account for 5% to 7% of septic arthritis.[1,2,13] Common anaerobes include *Bacteroides* sp., *Propionibacterium acnes*, and various anaerobic Gram-positive cocci. Predisposing factors include wound infections, joint arthroplasty, and immunocompromised hosts. Foul-smelling synovial fluid or air in the joint space should raise the suspicion of anaerobic infection, and appropriate cultures should be

obtained and held for at least 2 weeks. Anaerobes and coagulase-negative staphylococci are more common in prosthetic joint infections.

Polyarticular (two or more joints) septic arthritis is much less common than monoarticular infection.[14] Polyarticular septic arthritis is usually seen in patients with systemic inflammatory disorders, such as the spondyloarthropathies, RA, systemic lupus erythematosus, and other connective tissue diseases or in patients with overwhelming sepsis.[14,15] Approximately one-fourth of septic arthritis in patients with RA are polyarticular.[16] Intravenous drug abusers have also a high prevalence of polyarticular septic arthritis. Although *S. aureus* is the most common pathogen, group G streptococci, *H. influenzae*, *S. pneumoniae*, or mixed aerobic and anaerobic bacteria have been responsible for polyarticular infections. Involvement of more than one joint also can occur in certain patient populations such as neonates and patients with sickle cell anemia, or with certain organisms, such as *N. gonorrhoeae*, *N. meningitidis*, and *Salmonella*.[17]

Polymicrobial (two or more bacterial species), polyarticular septic arthritis is a rare clinical entity.[18] Large joints are usually affected. Among five reported cases, the knee was affected in four cases (bilaterally in two); the elbow and wrist were affected in three cases, and the shoulder was affected in two cases. The mean number of joints infected was three. Bacteremia was present in all but one case (80%) and always involved the same organisms that were in the synovial fluids. Most bacterial species isolated were the usual organisms seen in septic arthritis. Combinations of Gram-positive aerobic and anaerobic organisms were common. A characteristic of most cases (80%) was the extension of locally destructive processes as a result of the contiguous spread of infection from the affected joints, such as osteomyelitis, fasciitis with compartment syndrome, and abscess or sinus tract formation. Systemic complications, including septic shock, multiorgan failure, and toxic shock syndrome, were noted in 60% of cases. The mortality rate of polymicrobial, polyarticular septic arthritis in this small series was 60%.[18]

Arthrocentesis is a common procedure frequently used in conjunction with corticosteroid administration in patients with various forms of joint diseases. Septic arthritis after joint aspiration and injection is extremely rare, occurring in 4 cases per 10,000 injections.[3] Arthroscopic surgery is also a common procedure that is complicated by a low incidence of septic arthritis (<0.5% of procedures).[19] Coagulase-positive and coagulase-negative staphylococci account for more than 87% of these infections. In rare cases of septic arthritis of the knee related to anterior cruciate ligament repair, the tissue allografts were identified as the source of the infection.[20] Cultures yielded Gram-negative organisms such as *Pseudomonas aeruginosa*, *Citrobacter*, *Klebsiella oxytoca*, and mixed infection with *S. aureus*, *Enterococcus faecalis*, and *P. aeruginosa*.

## PATHOGENESIS

Acute bacterial arthritis is usually designated gonococcal or nongonococcal. In the case of gonococcal arthritis, *N. gonorrhoeae* possesses a variety of virulence factors on the cell surface. *N. gonorrhoeae* is able to attach to cell surfaces via filamentous outer-membrane appendages, or pili. Another outer membrane protein, protein I, has forms IA and IB. Protein IA binds the host factor H and inactivates complement component C3b, circumventing the host's complement system.[21] Protein IA also prevents phagolysomal fusion in neutrophils, enabling survival of the organism within the phagocytes. Lipo-oligosaccharide is a gonococcal molecule similar to lipopolysaccharide of other Gram-negative bacteria and possesses endotoxin activity, which contributes to the joint damage seen in gonococcal arthritis.[22]

**TABLE 109-2** Virulence Factors of *Staphylococcus aureus* and Their Mechanisms of Action

| Virulence Factor | Mechanism of Action |
|---|---|
| Collagen-binding protein | Binds collagen |
| Clumping factor A and B | Binds fibrinogen |
| Fibronectin-binding protein A and B | Binds fibronectin |
| Capsular polysaccharide | Anti-phagocytic |
| Protein A | Binds fragment crystallizable portion of IgG |
| Toxic shock syndrome toxin-1 | Superantigen |
| Enterotoxins | Superantigens |

*S. aureus* is the most common organism that causes nongonococcal arthritis. The virulence of *S. aureus* is associated with its ability to attach to host tissue within the joint, evade host defenses, and cause damage to the joint. Table 109-2 lists some of these virulence factors and their mechanisms of action. The attachment of *S. aureus* to the joint tissues is facilitated by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). MSCRAMMs are embedded in the cell wall peptidoglycan of *S. aureus* (Figure 109-1).[23] They bind to host matrix proteins, including collagen, fibrinogen, elastin, vitronectin, laminin, and fibronectin. Gene knockout experiments in animal models showed that the gene coding for the protein that binds collagen is an important virulence factor for *S. aureus* joint infections.[24] Most *S. aureus* isolates also express the fibronectin-binding proteins, FnbpA and FnbpB. Disruption of the respective genes, *fnbpA* and *fnbpB*, by knockout gene experiments completely obliterates adherence of *S. aureus* to fibronectin-coated surfaces (e.g., prosthetic joints).[25]

The genes of several *S. aureus* cell surface proteins (e.g., protein A, fibronectin-binding proteins, coagulase) and exotoxins (e.g., toxic shock syndrome toxin [TSST]-1, enterotoxin B, proteases, and hemolysins) are regulated by the accessory gene regulator *agr*.[26] At low cell numbers, such as at the time of infection, production of cell surface proteins for attachment to host tissues is facilitated by the *agr* gene. When the cells have attached to tissue or an orthopedic device and have passed from the exponential to stationary phase of growth, *agr* represses the expression of genes coding for cell surface proteins and activates genes coding for exotoxins and tissue-destroying exoenzymes. Because of this complex effect on the different stages of infection, inhibitors of *agr* may reduce tissue destruction but enhance tissue colonization. This effect could have implications for chronic infections such as occur with prosthetic joints.

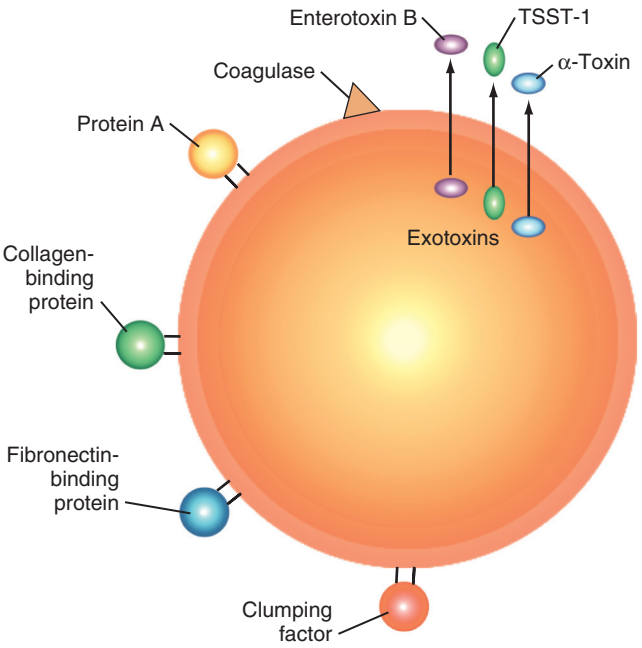



**Figure 109-1** Schematic diagram of *Staphylococcus aureus*. Many of the cell surface proteins are regulated by the agr locus (see text in Pathogenesis section). At low cell concentrations, agr facilitates the production of the cell surface proteins, which facilitate attachment to tissue. At higher cell concentrations, as occurs with establishment of infection, agr downregulates production of the cell surface proteins and activates genes coding for exotoxins. TSST, Toxic shock syndrome toxin.

Adherence receptors may allow intra-cellular movement of *S. aureus* into host cells (e.g., osteoblasts, endothelial cells, neutrophils).[27] The ability of *S. aureus* to switch from a normal colony phenotype to a slow-growing, antibiotic-resistant, small-colony variant phenotype may partially explain why this organism causes persistent and recurrent infections.[28] When internalized, the organism is protected from the host's immune system and from anti-microbial agents. After adherence to the joint tissue, the bacteria activate the host immune response. Opsonization and phagocytosis are key defenses to eradicate the organism. *S. aureus* possesses two virulence factors, protein A and capsular polysaccharide, which interfere with these defenses. Protein A interferes with binding of complement by binding to the fragment crystallizable (Fc) portion of immunoglobulin (Ig)G. Protein A has been termed a *superantigen* for B cells because 30% of human B cells show Fab-mediated binding of the protein A molecule.[29] Binding of protein A by B cells leads to activation and, subsequently, to depletion of B cells through apoptosis.[30] This process may have implications regarding the ability of the immune system to control infection with *S. aureus*. The gene coding for protein A had been experimentally disrupted, and joint infection caused by the altered strain in a mouse model resulted in less joint destruction than infection caused by the wild-type strain.[31]

Capsular polysaccharide interferes with opsonization and phagocytosis. Of the 11 reported capsule serotypes of *S. aureus*, types 5 and 8 account for 85% of clinical infections.[32] The capsule of these two serotypes is thinner, which facilitates the attachment to host fibronectin and fibrin.[33] When attached to these host proteins, capsule production is upregulated to form a thicker capsule, which makes the bacteria more resistant to opsonization and phagocytosis. The thicker capsule can also conceal the highly immunogenic adherence proteins (MSCRAMMs).[34] A mutant of the type 5 capsule in a murine model had a lower rate of infection and resulted in less severe arthritis compared with mice infected with the wild-type strain.[35] A vaccine consisting of types 5 and 8 polysaccharide reduced *S. aureus* bacteremia by more than half in hemodialysis patients.[36] The duration of protection was approximately 40 weeks after a single vaccination.

*S. aureus* exotoxins (e.g., TSST-1 and enterotoxins) act as superantigens that bind to host class II major histocompatibility complex (MHC) molecules and T cell receptors, resulting in clonal expansion and activation of some T cells. This activation triggers the release of numerous cytokines, including IL-2, interferon (IFN)-γ, and TNF.[37] Induction of these cytokines results in systemic toxicity and joint damage. The stimulated T cells initially proliferate but later disappear, likely because of apoptosis, and result in immunosuppression.[38] Internalized organisms that had been protected from this inflammatory response may cause fulminant or persistent infection. Mice injected with strains of *S. aureus* lacking TSST-1 and enterotoxins rarely develop arthritis; when arthritis is induced, it is much milder compared with arthritis in animals injected with the wild-type strain.[37] Vaccination of mice with a mutated, recombinant form of enterotoxin A devoid of superantigen function was associated with a significant reduction in mortality.[39]

In response to bacterial infection of the joint space, the host releases a variety of cytokines and inflammatory mediators. Initially, IL-1β and IL-6 are released into the joint space, leading to an influx of inflammatory cells. These neutrophils and macrophages engulf invading bacteria and release additional cytokines, including TNF, IL-1, IL-6, and IL-8. Blocking TNF with a monoclonal antibody and IL-1 with an IL-1 receptor antagonist inhibited leukocyte infiltration into the joint by 80% in a rabbit model of *S. aureus*–induced arthritis when the cytokine inhibitors were given simultaneously with *S. aureus*.[40] When the same inhibitors were given 24 hours after infection, however, there was no effect on leukocyte infiltration, suggesting the crucial roles of TNF and IL-1 in the early stages of *S. aureus*–induced arthritis. Release of IFN-γ is associated with the influx of T cells, which occurs a few days after infection. In a mouse model of *S. aureus* septic arthritis, IFN-γ has been associated with a worsening of the severity of arthritis while protecting the animals from septicemia.[41] The host's early cytokine response may aid the clearance of organisms and limit infection in the host. A late cytokine response may amplify the destructiveness of an established infection.

## CLINICAL FEATURES

The classic presentation of nongonococcal septic arthritis is the acute onset of pain, swelling, and decreased range of motion in the affected joint. Large joints are affected most commonly. In adults, the knee is involved in more than 50% of cases; hip, ankle, and shoulder infections are less common.[41] In infants and small children, the hip is more often involved.[42] Patients with septic arthritis often have underlying illnesses and predispositions to infections. Many

**TABLE 109-3** Risk Factors for Development of Septic Arthritis

| |
|---|
| Age >80 yr[2] |
| Diabetes mellitus[2] |
| Presence of a prosthetic joint in the knee or the hip[2] |
| Recent joint surgery[2] |
| Skin infection[2] |
| Previous septic arthritis[15] |
| Recent intra-articular injection[5] |
| HIV or AIDS |
| Intravenous drug abuse |
| End-stage renal disease on hemodialysis |
| Advanced hepatic disease |
| Hemophilia with or without AIDS |
| Sickle cell disease |
| Underlying malignancy |
| Hypogammaglobulinemia (susceptible to *Mycoplasma* infections)[46] |
| Late complement-component deficiency (susceptible to *Neisseria* infections)[45] |
| Low socioeconomic status with high rate of comorbidities[15] |

are immunocompromised, are intravenous drug abusers, have prosthetic joints, and have diseases such as neoplasia, renal failure, and RA. Table 109-3 lists the risk factors that predispose to septic arthritis.[2,4,43]

Most patients with bacterial arthritis are febrile, although chills are unusual. Fever may be absent in elderly patients. In children, septic arthritis usually is accompanied by fever, malaise, poor appetite, irritability, and progressive reluctance to use the affected limb. Physical examination typically reveals warmth and tenderness of the affected joint, joint effusion, and limited active and passive range of motion. Septic arthritis among patients with RA has been a special challenge to clinicians because in many cases, it is difficult to differentiate septic arthritis in a joint already affected by RA from rheumatoid flare. Septic arthritis in patients with RA is associated with poor joint outcome and high mortality.[44] Whenever bacterial arthritis is suspected, the most important diagnostic procedure is arthrocentesis and examination of the synovial fluid. For joints that are deep and more difficult to aspirate, ultrasound-guided or fluoroscopy-guided needle aspiration should be done.

Disseminated gonococcal infection (DGI) occurs in 1% to 3% of patients infected with *N. gonorrhoeae*. DGI is three times more common in women than men. Women are more commonly affected because they are more likely to have asymptomatic and untreated primary infections. Bacterial dissemination has been associated with intrauterine devices and has occurred during menstruation, pregnancy, and pelvic operation.[45] Gonococcal arthritis develops in approximately 42% to 85% of patients with DGI.[46]

Gonococcal arthritis is the most common cause of acute monarthritis in sexually active young adults. In the 1970s it accounted for almost two-thirds of all septic arthritis and tenosynovitis cases in North America. Clearance of gonococcal infection is dependent on a potent complement-mediated immune response. Individuals with terminal complement (C5-8) deficiency are at greater risk for infection.[47]

Patients with gonococcal joint disease typically are seen with one of two forms. The first form is seen in patients with DGI and is characterized by fever, shaking chills, vesiculopustular skin lesions, tenosynovitis, and polyarthralgias. Blood cultures are frequently positive, whereas synovial fluid cultures are rarely positive. *N. gonorrhoeae* can be cultured from genital, rectal, and pharyngeal sites. Tenosynovitis of multiple tendons of the wrist, fingers, ankle, and toes is a unique feature of this form of DGI and distinguishes it from other forms of infectious arthritis. In the second form, patients have localized joint infection without the systemic manifestations classic to DGI. Clinical findings are similar to those seen in nongonococcal septic arthritis. Patients have purulent arthritis, most commonly of the knee, wrist, or ankle, and more than one joint can be infected simultaneously. *N. gonorrhoeae* frequently can be cultured from the synovial fluid.[46]

## DIAGNOSIS AND DIAGNOSTIC TESTS

Whenever bacterial arthritis is suspected, the most important diagnostic procedures are arthrocentesis and examination of the synovial fluid. Arthrocentesis and synovial fluid analysis should be performed in patients who are seen with an inflamed joint for which alternative explanations are not immediately apparent. For example, a patient with a history of gout or evidence of a generalized flare of rheumatoid arthritis might not require arthrocentesis. However, the clinician should follow up carefully and reassess for an infectious process if the decision is made to treat for a diagnosis other than infection. For joints that are deep and more difficult to aspirate, ultrasound-guided or fluoroscopy-guided needle aspiration should be done.

Normal joints contain a small amount of synovial fluid that is clear, is highly viscous, and has few white blood cells (WBCs). The protein concentration is approximately one-third that of plasma, and the glucose concentration is similar to that of plasma. Infected synovial fluid is usually purulent with an elevated leukocyte count typically greater than 50,000 WBC/mm$^3$ and often exceeding 100,000 WBC/mm$^3$ with polymorphonuclear cell predominance. Synovial fluid levels of glucose, lactate dehydrogenase, and total protein have limited value in the diagnosis of septic arthritis. Although a low synovial fluid glucose (<40 mg/dL or less than half the serum glucose concentration) and an elevated lactate dehydrogenase suggest bacterial infection, they are not sufficiently sensitive or specific for the diagnosis.[48] Figure 109-2 is an algorithm for synovial fluid analysis, and Table 109-4 lists the differential diagnoses of septic arthritis and the known causes of pseudoseptic arthritis.[49]

A definite diagnosis of bacterial arthritis can be made only by visualizing bacteria on a Gram-stained smear or by culturing bacteria from the synovial fluid. In patients not previously treated with antibiotics, synovial fluid cultures are positive in 70% to 90% of cases of nongonococcal bacterial arthritis.[4,50] Blood cultures are positive in 40% to 50% of cases of septic arthritis and are the only method of

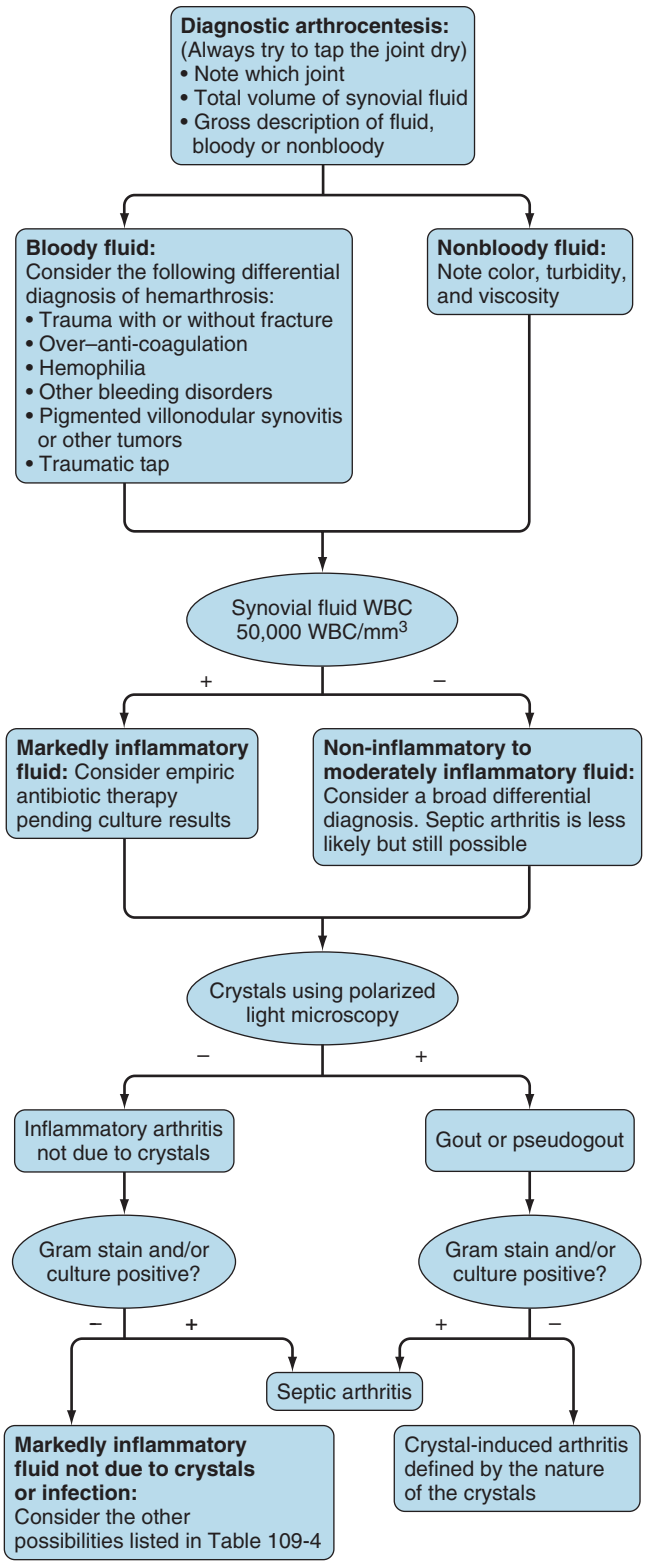



**Figure 109-2** Algorithm for synovial fluid analysis in septic arthritis. WBC, white blood cells.

**TABLE 109-4** Differential Diagnosis of Septic Arthritis and Reported Causes of Pseudoseptic Arthritis*

| |
|---|
| Partially treated septic arthritis |
| Rheumatoid arthritis |
| Juvenile rheumatoid arthritis |
| Gout |
| Pseudogout |
| Apatite-related arthropathy |
| Reactive arthritis |
| Psoriatic arthritis |
| Systemic lupus erythematosus |
| Sickle cell disease |
| Dialysis-related amyloidosis |
| Transient synovitis of the hip |
| Plant thorn synovitis |
| Metastatic carcinoma |
| Pigmented villonodular synovitis |
| Hemarthrosis |
| Neuropathic arthropathy |
| Synovitis after injection of hylan |

*Extremely inflammatory synovitis with negative culture is referred to as *pseudoseptic arthritis.* Typically, synovial fluid analysis shows ≥50,000 white blood cells (WBC)/mm$^3$. Often the WBC count is >100,000 WBC/mm$^3$.

Data from Dubost JJ, Soubrier M, De Champs C, et al: No changes in the distribution of organisms responsible for septic arthritis over a 20 year period. *Ann Rheum Dis* 61:267–269, 2002; Weston VC, Jones AC, Bradbury N, et al: Clinical features and outcome of septic arthritis in a single UK health district 1982–1991. *Ann Rheum Dis* 58:214–219, 1999; and Coakley G, Mathews C, Field M, et al; British Society for Rheumatology Standards, Guidelines and Audit Working Group: BSR & BHPR, BOA, RCGP and BSAC guidelines for management of the hot swollen joint in adults. *Rheumatology (Oxford)* 45:1039–1041, 2006.

identifying the pathogen in approximately 10% of cases.[51,52] Occasionally, an extra-articular site of infection offers a clue to the etiologic organism infecting the joint. Examples include septic arthritis in association with pneumococcal pneumonia, *E. coli* urinary tract infection, and cellulitis caused by staphylococci or streptococci. Gram-positive cocci are identified in 50% to 75% of synovial fluid Gram-stained smears, but Gram-negative bacilli are identified less than 50% of the time in culture-proven cases.[50]

Inflammatory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and WBC are usually raised, but the sensitivity is low, and their absence does not exclude the diagnosis of septic arthritis.[43,53] Procalcitonin, the peptide precursor of calcitonin, has been extensively investigated as a biomarker for bacterial infection.[54] Procalcitonin levels are elevated in bacterial infections, and the levels return to normal relatively rapidly once the bacterial infection is controlled. Unlike the ESR and CRP, procalcitonin levels are not elevated in many other inflammatory conditions, such as gout, systemic lupus erythematosus, or Still's disease.[55] Also, the levels are not affected by NSAIDs or glucocorticoids. It is postulated that cytokines and endotoxins released from bacteria inhibit conversion of procalcitonin to calcitonin. Viral infections are not associated

with procalcitonin elevations because viruses stimulate release of IFN, which blocks procalcitonin production in human cells. The majority of procalcitonin is produced in nonthyroidal tissue. Therefore procalcitonin elevations may occur in post-thyroidectomy patients who have a bacterial infection. In healthy individuals, serum procalcitonin levels are less than 1 ng/mL. A meta-analysis of studies on the use of procalcitonin for the diagnosis of septic arthritis recommended that procalcitonin be used as a rule-in test at the cutoff value of 0.5 ng/mL and as a rule-out test at the cutoff value of 0.3 ng/mL, with specificity and sensitivity in the range of 90%.[56] In this meta-analysis, procalcitonin was more sensitive and specific than CRP for diagnosing or ruling out septic arthritis.

Culture for *N. gonorrhoeae* is almost always negative in skin lesions and is positive in less than 50% of synovial fluid samples and in less than one-third of blood cultures; this phenomenon may be the result of the fastidious growth requirements of *N. gonorrhoeae*. The organism can often be easily recovered from other sites, such as urethral, cervical, rectal, or pharyngeal specimens (i.e., the genitourinary tract). In culture-negative septic arthritis in which *N. gonorrhoeae* is suspected, polymerase chain reaction techniques can be used to detect gonococcal DNA in the synovial fluid. Unfortunately, the technique is not standardized and is not widely avilable.[57]

When culturing the synovial fluid, it should be brought directly to the laboratory and placed on conventional broth and solid media or into aerobic and anaerobic blood culture bottles. Inoculating blood culture bottles with 5 to 10 mL of joint fluid or smaller volumes into isolator tubes may increase the yield of positive cultures beyond that of standard techniques.[58,59] Synovial fluid culture can use specialized collection and detection systems that can identify significantly more pathogens and fewer contaminants than culture by the agar-plate method.[60]

Plain radiographs in septic arthritis are usually normal early in the course of the infection, but baseline films should be obtained to look for evidence of other disease and contiguous osteomyelitis. Radiographs often show nonspecific changes of inflammatory arthritis, including periarticular osteopenia, joint effusion, soft tissue swelling, and joint space loss. In more advanced infection, periosteal reaction, marginal or central erosions, and destruction of subchondral bone may be seen. Bony ankylosis is a late sequela of septic arthritis. Dislocation or subluxation of the femoral head is unique to hip infection of neonates.[61]

Ultrasound of the hip is the modality of choice to detect fluid collections in this deep joint and can serve as a guide in its aspiration. Ultrasound can be similarly used in other joints, such as the popliteal cyst of the knee, shoulder, acromioclavicular, or sternoclavicular joints. Triple-phase bone scan using technetium 99m is often done in children to identify an associated metaphyseal osteomyelitis or avascular necrosis of the femoral head. Whole-body bone scan is preferred in young children because, despite focal symptoms, septic arthritis and osteomyelitis may be multifocal in this age group.[62] In septic arthritis of all age groups, the periarticular distribution of increased uptake is seen on the early "blood-pool" phase and the delayed images of the joint. Bone scans provide only nonspecific information, however, and cannot differentiate septic from noninfectious causes of joint inflammation. Bone scans are more sensitive than standard radiography in the diagnosis of arthritis because radionuclide uptake precedes morphologic bone changes that are seen on radiograph. A suggestive bone scan must be interpreted in the proper clinical context and supported by microbiologic data for a definitive diagnosis of joint or bone infection.

In joints that are difficult to evaluate otherwise or that have complex anatomic structures, computed tomography (CT) and MRI can provide useful images to delineate the extent of the infection.[63] MRI is highly sensitive in early detection of joint fluid and is superior to CT in the delineation of soft tissue structures and soft tissue abscess. These images can show early bone erosion, reveal soft tissue extension, and facilitate arthrocentesis of joints such as shoulders, hips, acromioclavicular,[64] sternoclavicular, sacroiliac, and facet joints of the spine. MRI findings such as reactive bone marrow can suggest the presence of secondary osteomyelitis, which can complicate septic arthritis. When multiple joint involvement is suspected, triple-phase bone scintigraphy is the preferred modality of investigation.

## TREATMENT

Treatment of septic arthritis must begin immediately after the clinical evaluation is complete and all appropriate cultures are taken. A serious clinical suspicion of a joint infection warrants the initiation of empiric antibiotic therapy before culture confirmation is available. Delays in treatment allow the infection to become more established in the joint and permanently damage the articular cartilage. Untreated, there is the opportunity for the joint infection to spread to other body sites via the hematogenous route and become more widespread and more difficult to cure.

The principles of treatment of an infected joint, whether natural or prosthetic, follow those of treatment of an infected body cavity in which antibiotics must be used in conjunction with adequate drainage of the infected closed space. The clinical circumstances and the preliminary laboratory data aid the selection of antibiotic agents. Host factors, any extra-articular sites of infection, and the Gram-stained smear of the synovial fluid are the best early guides for the antibiotic agents with which to start. Table 109-5 lists current antibiotic agents for adults,[53] and Table 109-6 lists agents for children.[65]

Narrow antibiotic coverage is indicated if Gram-positive cocci are found in the synovial fluid, and the clinician suspects a primary source of staphylococcal infection from the skin. Appropriate monotherapy in this case may be a penicillinase-resistant penicillin or vancomycin if methicillin resistance is likely. If Gram-negative bacilli are noted in the synovial fluid, and the patient has a kidney infection, specific agents (e.g., ampicillin or a cephalosporin) against *E. coli* and other common urinary tract pathogens may be used. In healthy, young, sexually active individuals with community-acquired septic arthritis and a negative synovial fluid Gram-stained smear, ceftriaxone is a reasonable option to cover *N. gonorrhoeae*. If synovial fluid Gram stain shows Gram-positive cocci, vancomycin should be the empiric therapeutic option, considering the fact that a significant proportion of community-acquired *S. aureus* infections are now methicillin resistant.[66] A reasonable initial empiric

**TABLE 109-5** Antibiotic Agents Used in Adults

| Synovial Fluid Gram Stain | Organism | Antibiotic | Dose |
|---|---|---|---|
| Gram-positive cocci (clusters) | *Staphylococcus aureus* (methicillin-sensitive) | Nafcillin/oxacillin<br>or<br>Cefazolin | 2 g IV q4h or 12 g qd continuous infusion<br><br>1-2 g IV q8h |
| | *S. aureus* (methicillin-resistant) | Vancomycin<br>or<br>Daptomycin<br>or<br>Linezolid | 15 mg/kg IV q12h* (serum trough level of 15-20 µg/mL)<br>6-8 mg/kg q24h<br><br>600 mg IV q12h |
| Gram-positive cocci (chains) | *Streptococcus* | Penicillin<br>or<br>Cefazolin | 2-4 million units IV q4h or 18-24 million units qd continuous infusion<br>1-2 g IV q8h |
| Gram-negative diplococci | *Neisseria gonorrhoeae* | Ceftriaxone<br>or<br>Cefotaxime<br>or<br>Ciprofloxacin | 2 g IV q24h<br><br>1 g IV q8h<br><br>400 mg IV q12h |
| Gram-negative bacilli | Enterobacteriaceae *(Escherichia coli, Proteus, Serratia)* | Ceftriaxone<br>or<br>Cefotaxime<br>or<br>Ertapenem | 2 g IV qd<br><br>2 g IV q8h<br><br>1 g IV qd |
| | *Pseudomonas* | Cefepime<br>or<br>Piperacillin-tazobactam<br>or<br>Imipenem<br>or<br>Meropenem<br>plus<br>Gentamicin/tobramycin | 2 g IV q12h<br><br>3.375 g IV q6h or 3.375 g IV q8h infused over 4 h or 14 g qd continuous infusion<br>500 mg IV q6h<br>1-2 g IV q8h<br><br>7 mg/kg IV qd |
| Polymicrobial infection | *S. aureus, Streptococcus,* Gram-negative bacilli | Nafcillin/oxacillin*<br>plus<br>Ceftriaxone<br>or<br>Cefotaxime<br>or<br>Ciprofloxacin | 2 g IV q4h or 12 g qd continuous infusion<br><br>2 g IV q24h<br><br>2 g IV q8h<br><br>400 mg IV q12h |

*If patient is penicillin allergic, use vancomycin plus third-generation cephalosporin or ciprofloxacin.
IV, Intravenous; q4h, every 4 hours; q6h, every 6 hours; q8h, every 8 hours; q12h, every 12 hours; q24h, every 24 hours.

**TABLE 109-6** Antibiotic Agents Used in Children

| Age | Likely Pathogen | Antibiotic | Dosage (mg/kg/day) | Doses/day |
|---|---|---|---|---|
| Neonate | *Staphylococcus aureus*; group B streptococci; Gram-negative bacilli | Nafcillin<br>plus<br>Cefotaxime<br>or<br>Gentamicin | 100<br><br>150<br><br>5-7.5 | 4<br><br>3<br><br>3 |
| Child <5 yr old | *S. aureus*; *Haemophilus influenzae**; group A streptococci; *Streptococcus pneumoniae* | Nafcillin†<br>plus<br>Cefotaxime<br>or<br>Ceftriaxone<br>or<br>Cefuroxime | 150<br><br>100-150<br><br>50<br><br>150-200 | 4<br><br>3-4<br><br>1-2<br><br>3-4 |
| Child >5 yr old | *S. aureus*; group A streptococci | Nafcillin†<br>or<br>Cefazolin | 150<br><br>75 | 4<br><br>3-4 |
| Adolescent (sexually active) | Previous organisms; *Neisseria gonorrhoeae* | Ceftriaxone | 50 | 1-2 |

*Decreased incidence in children fully immunized with Hib vaccine.
†If patient is penicillin allergic, alternatives include vancomycin (40 mg/kg/day divided into four doses) or clindamycin (20-40 mg/kg/day divided into four doses).
Modified from Gutierrez KM: Infectious and inflammatory arthritis. In Long SS, Pickering LK, Prober CG, editors: *Principles and practice of pediatric infectious diseases,* ed 2, New York, 2002, Churchill Livingstone, pp 475–481.

therapy to cover gonococci, *S. aureus*, and streptococci is ceftriaxone plus vancomycin, pending final culture results. In elderly debilitated patients or adults with low risk for sexually transmitted disease as well as a negative Gram-stained smear of synovial fluid, broad antibiotic coverage against a wide variety of organisms, including *S. aureus*, streptococci, and Gram-negative bacilli should be given initially. A typical regimen includes an anti-staphylococcal agent (e.g., vancomycin) plus a third-generation cephalosporin (e.g., ceftriaxone).

When the identity and the sensitivities of the organism are known, antibiotic therapy should continue with the most efficacious agent that has the best safety profile and narrowest spectrum. The parenteral route of antibiotic administration is the preferred initial treatment. Continued antibiotic therapy may be switched to oral agents if adequate blood levels can be achieved and maintained by this route. There is no evidence that the direct intra-articular instillation of drugs is necessary or preferable in septic arthritis because there is no barrier against the free diffusion of antibiotic agents from the blood to the synovial fluid. In cases in which uncertainty exists, serum and synovial fluid levels of antibiotic drugs can be measured to ensure that therapeutic levels are reached.

Most penicillins, cephalosporins (excluding ceftriaxone), and carbapenems (excluding ertapenem) have short half-lives necessitating frequent dosing (i.e., every 4 to 8 hours). Extended or continuous infusion therapy maintains levels of the drug above the minimal inhibitory concentration (MIC) for a longer period of time, thus improving clinical outcomes and providing for more convenience for patients and caregivers, particularly in the outpatient or home health setting. Selected extended and continuous infusion dosing is reflected in Figure 109-3. Use of once-daily drugs, such as ceftriaxone and ertapenem, is increasingly used in the outpatient management of septic arthritis. These drugs, because of high protein binding, may not be appropriate for morbidly obese patients.

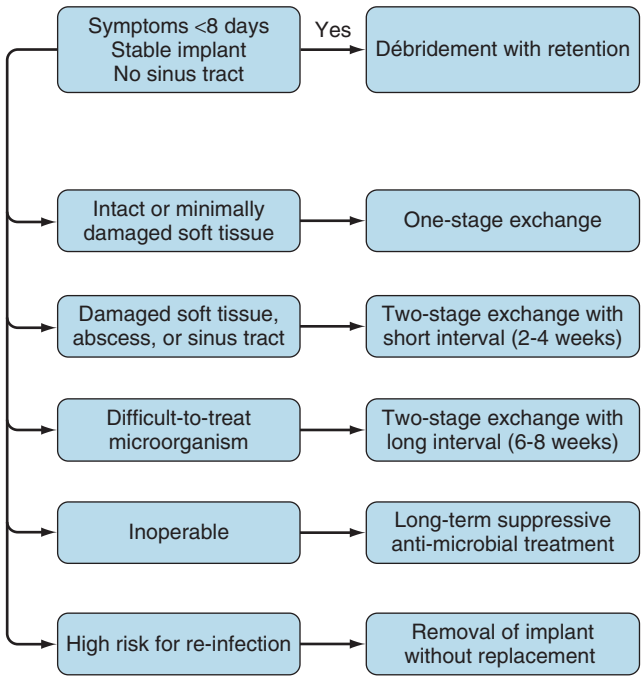



**Figure 109-3** Algorithm for the management of an infected joint prosthesis. *(Modified from Trampuz A, Zimmerli W: Prosthetic joint infections: update in diagnosis and treatment.* Swiss Med Wkly *135:243–251, 2005.)*

Most individuals with septic arthritis respond adequately to appropriate anti-microbial agents after initial joint aspiration for fluid analysis. In experimental infectious arthritis cases, early antibiotic therapy reduced the loss of collagen and erosion of articular surface, which minimizes the need for open surgical drainage.[67] It is generally accepted that prompt and adequate drainage of the septic joint is essential to decrease the risks of substantial loss of articular function; however, the best approach to drain the joint remains controversial.[68]

From retrospective studies, daily aspiration of an infected joint showed better functional outcome than open surgical drainage, although the former had higher overall mortality.[69,70] An explanation for higher mortality could be the higher comorbid conditions of patients who had daily aspirations than the ones who were more fit and underwent open surgical drainage.[69] If the synovial fluid cell count and polymorphonuclear percentage decrease with successive aspiration, the anti-microbial therapy is probably effective.[13,71] If needle aspiration is technically difficult (as in the hip or the shoulder) or does not provide thorough drainage of the joint, if the joint effusion does not resolve promptly, if sterilization of the joint fluid is delayed, if the infected joint is already damaged by pre-existing rheumatoid disease, or if infected synovial tissue or bone needs débridement, surgical drainage should be considered sooner rather than later.[13,50,72] Arthroscopy is emerging as an alternative to arthrotomy with the advantage of reduced surgical morbidity. Wound healing is faster, and rehabilitation time is shortened.[73] A retrospective analysis from the United Kingdom suggested that most patients can be treated medically (with repeated aspirations) and do not require surgical drainage (either by arthroscopy or arthrotomy).[74] Although statistically not significant, medical therapy resulted in more complete cure and less deterioration of functional status at time of discharge. Another study, which included 20 adults with native hip joint septic arthritis, concluded that symptom duration, especially if it is longer than 3 weeks before presentation, was a statistically significant predictor of the need for excision arthroplasty.[75] These results highlight the need for careful case selection for surgical intervention.

The optimal duration of antibiotic treatment has not been prospectively studied. For native joint infections caused by *N. gonorrhoeae*, a 1-week course of ceftriaxone should be adequate. For septic arthritis caused by organisms other than *N. gonorrhoeae*, therapy ranging from 2 weeks to 6 weeks is recommended depending on type, sensitivity of microorganism, and presence of osteomyelitis. If long-term antibiotics are chosen (4 to 6 week duration), parenteral antibiotics may be switched to oral antibiotics after 2 weeks, provided that there is clinical improvement, inflammatory markers are trending down, and oral antibiotics are available to which the microorganism is susceptible.[52] The duration of antibiotic administration can be 2 weeks for uncomplicated infection by susceptible microorganisms or 4 to 6 weeks for more extensive infection in an immunocompromised host. For septic arthritis caused by *H. influenzae*, streptococci, or Gram-negative cocci, 2 weeks of

antibiotic therapy is usually adequate. Staphylococcal septic arthritis usually requires 3 to 4 weeks of therapy, and for pneumococcal or Gram-negative bacillary infections, therapy should be continued for at least 4 weeks.[76,77]

During the first few days of management, immobilization of the infected joint by external splinting and adequate analgesic administration ensure patient comfort. Physical therapy, starting with passive then graduating to active motion, should be instituted as soon as the patient can tolerate mobilization of the inflamed joint because early active range-of-motion exercises are beneficial for ultimate functional recovery. Involving the orthopedic surgeon and the physical therapist early on in the course of treatment facilitates the best choice of drainage procedure and results in the best functional outcome.[78]

## PROSTHETIC JOINT INFECTIONS

Total joint replacement for advanced arthritis is one of the major advances in medicine in the 20th century and continues to improve in the 21st century. Infection of prosthetic joints is an uncommon but devastating complication of joint replacement surgery. More than 700,000 total knee arthroplasties (TKA) and 332,000 total hip arthroplasties (THA) were done in the United States in 2010.[79] Arthroplasties are increasing, and projections are that nearly 4 million arthroplasties (3.5 million TKA and 572,000 THA) will be done each year in the United States by 2030. In addition to hip and knee arthroplasties, shoulder, elbow, and ankle replacements are becoming more common, particularly in patients with RA. The infection rate of arthroplasties varies considerably among industrialized countries. Data from the United States indicate arthroplasty infection rates of 2% to 2.2%,[80] whereas European countries report infection rates of 0.8% and 1.2% for knees and hips, respectively.[81] In the United States, the infection rate is higher for elbow (3.3%) and lower for shoulder (1.3%) arthroplasties compared with hip and knee arthroplasties.[82,83] Infection rates are much higher in patients undergoing revision of a prosthetic joint compared with primary arthroplasty (3% for hips and 6% for knees).[84] The risk of infection is approximately twofold higher in patients with RA compared with patients with osteoarthritis.

The risk of prosthetic joint infection (PJI) is related to many factors. In a retrospective study of 56,216 total knee arthroplasties, the most important risks for infection included (1) a diagnosis of osteonecrosis (hazard ratio [HR], 3.65), (2) a diagnosis of post-traumatic arthritis (HR, 3.23), (3) male sex (HR, 1.89), (4) an American Society of Anesthesiologists (ASA) score of 3 or more (HR, 1.65), (5) body mass index of 35 or more (HR, 1.47), and (6) diabetes mellitus (HR, 1.28). Surgical risk factors included quadriceps-release exposure (HR, 4.76) and the use of antibiotic-laden cement (HR, 1.53).[85] Certain patient populations are at increased risk of infection because of comorbid conditions (e.g., diabetes mellitus and RA). Other risk factors include long operative time, use of disease-modifying anti-rheumatic drugs, and smoking.[85,86,87]

Orthopedic implants adversely affect host defenses. Prosthetic devices impair opsonic activity and diminish the ability of neutrophils to kill bacteria. Polymorphonuclear leukocytes release lysosomal enzymes and superoxide into the area surrounding the prosthesis, resulting in tissue damage and local devascularization. Phagocytes may be focused on removal of the foreign body such that fewer cells are available to fight infection. Finally, polymethylmethacrylate bone cement can inhibit neutrophil and complement functions, and the heat produced by the polymerization of polymethylmethacrylate can damage adjacent cortical bone and result in a devascularized necrotic area, which is ideal for bacterial growth. After implantation, prosthetic joints are immediately coated by host proteins, including albumin, fibrinogen, and fibronectin. *S. aureus*, which possesses numerous host protein-binding receptors (MSCRAMMs), is a common pathogen in infection of prosthetic joints. Patients with a prosthetic joint in whom *S. aureus* bacteremia develops have an approximate one in three chance of experiencing an infection of the implant.[88,89]

Another phenomenon crucial to development of infection is the ability of organisms to form biofilms on the surface of the prosthetic device. A biofilm is defined as "an assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material."[90] Biofilm formation is a natural process. Organisms grow on indwelling medical devices, potable water system pipes, and living tissues. *S. epidermidis* is particularly adept at attaching to and forming biofilms on foreign bodies such as prosthetic joints. Small numbers of these organisms from the patient's skin or mucous membranes, or from the hands of the surgeons or clinical staff, contaminate and colonize the orthopedic device at the time of implantation. Staphylococcal surface proteins (SSP)-1 and SSP-2, are fimbria-like polymers that facilitate adherence of *S. epidermidis* to polystyrene. *S. epidermidis* produces a polysaccharide/adhesin substance crucial to the formation of this extra-cellular matrix known as *slime*. Polysaccharide/adhesin mutants are less virulent than the wild-type strain in a rabbit model of endocarditis.[91]

PJIs are divided into early onset (<3 months after placement), delayed (3 to 24 months post-surgery), and late onset (>24 months after placement). Early and delayed infections are usually related to surgical contamination at the time of the implantation, whereas late infections usually result from hematogenous seeding of the joint. Most infections occur within the first 2 years of implantation. Because of its high virulence, *S. aureus* accounts for most early and late infections (see Table 109-1). So-called *small-colony* variants of *S. aureus* may be responsible for persistent and recurrent infections of prosthetic implants.[92] These subspecies of *S. aureus* are difficult to treat because they grow slowly and are relatively resistant to cell-wall–active antimicrobial agents, anti-folate drugs (trimethoprim and sulfamethoxazole), and aminoglycosides. There is some concern that the practice of using gentamicin-impregnated polymethylmethacrylate beads to treat prosthetic joint infections may actually select for the small-colony variant species.[92] Delayed infections are usually caused by less virulent microorganisms such as coagulase-negative staphylococci and *P. acnes*. Because these low-virulence organisms are common skin contaminants, it is important to interpret culture results carefully.

Clinically, the most common symptom in patients with prosthetic joint infection is pain of the affected joint. Differentiating pain from mechanical loosening of the

prosthesis from pain related to infection can be difficult. Typically, a patient with mechanical loosening but no infection has pain only with motion, whereas a patient with infection experiences pain at rest and with motion. Warmth at the implant site, effusion, erythema, and fever are frequently associated with early and late, but not delayed, infections. This difference in clinical presentation likely represents the virulence of the most common organisms associated with the three categories of infection. The presence of a sinus tract with purulent discharge suggests involvement of the implant and is an indication for removal of the prosthesis. The Infectious Diseases Society of America (IDSA) has published guidelines regarding the diagnosis and management of patients with prosthetic joint infections.[93] Criteria for diagnosis of PJI are given in Table 109-7.

Inflammatory blood laboratory markers have variable sensitivity and specificity in the diagnosis of prosthetic joint infection. In a meta-analysis, elevated levels of IL-6 and CRP had a much higher sensitivity (97% and 88%, respectively) than an elevation of the WBC count or ESR (45% and 75%, respectively).[94] The specificity of an elevated level of IL-6 (91%) was the highest of these markers with elevation of WBC (87%), CRP (74%), and ESR (70%) somewhat lower. Inflammatory markers increase following arthroplasty, and the return to normal levels is quite variable. IL-6 will return to normal within a matter of a few days following surgery, whereas the CRP level may remain elevated for as long as 3 weeks and the ESR for several months following arthroplasy.[94] Therefore a low or normal CRP or IL-6 level in a patient with suspected prosthetic joint infection has a good negative-predictive value. Current IDSA guidelines recommend the use of CRP and ESR in the management of patients with proven or suspected PJI.[93] IL-6, TNF, and procalcitonin testing look promising but have not been incorporated into existing guidelines.[95] Because serum procalcitonin levels are easily obtainable from local hospitals or reference laboratories, it may be more practical to obtain procalcitonin levels. Serial plain radiographs may be helpful; the presence of subperiosteal bone growth and transcortical sinus tracts is specific for infection. Bone scans with technetium 99m–labeled methylene diphosphonate are sensitive but lack specificity because the bone scan is typically positive for 6 to 12 months after the original implantation. Bone scans may be a useful screening test for patients with suspected late prosthetic joint infection. CT has limitations because of the imaging artifacts caused by the metal implant. MRI can be performed only in patients with titanium or tantalum implants. IDSA guidelines do not recommend bone scans, leukocyte scans, MRI, CT scans, or positron-emission tomography for the routine management of PJI.[93]

Aspiration of the joint is essential in differentiating infection from noninfectious causes of joint pain, particularly in patients without RA.[93] In one study, a synovial fluid leukocyte count of greater than 1700/mm$^3$ had a sensitivity of 94% for determining infection, whereas a differential count of greater than 65% neutrophils had a sensitivity of 97%.[96] The specificities of these two measurements were 88% and 98%, respectively, in patients without underlying inflammatory diseases such as RA. Gram-stained smear of the synovial fluid has a low sensitivity (<20%) but a high specificity (>97%).[93] Cultures of drainage from a sinus tract are not helpful, unless the culture grows *S. aureus*.[97]

Generally, at least three tissue specimens should be taken at the time of surgery, including tissue from the joint capsule, synovial lining, bone-cement interface, and samples from purulent material or sequestrum.[93,98,99] Swabs of the joint have a low sensitivity and should be avoided. Unless the patient is septic or otherwise systemically ill, anti-microbial therapy should be discontinued a minimum of 2 weeks before the revision surgery, and peri-operative antibiotics should not be administered until all of the tissue cultures have been obtained.[93] Using this methodical approach, there is a direct correlation between the number of tissue specimens positive for a particular microorganism and the probability of infection. The probability of infection is estimated at less than 5% if all tissue specimens are negative and greater than 94% if three or more tissue specimens are positive for growth.[99] Finally, the location of the prosthesis is helpful in the interpretation of the positive culture. The isolation of *P. acnes* from a single tissue culture from a knee prosthesis is more likely to be a contaminant than if the same organism is obtained from a shoulder prosthesis.

For patients who have received recent antibiotics, there may be a role for sonication of the prosthesis at the time of explantation.[100] Culturing of the explanted prosthesis that has been sonicated to remove adherent bacteria had a higher sensitivity (75%) as compared with culture of periprosthetic tissue (45%) in patients who had received antibiotics in the past 14 days.[101]

Medical therapy of patients with prosthetic joint infections is challenging. Organisms existing in biofilms are much more resistant to anti-microbial agents for several reasons. Most anti-microbial agents penetrate the biofilm layer adequately but do not inhibit growth of bacteria within the biofilm matrix. Biofilm-associated organisms, including the small colony variants of *Staphylococcus aureus*, grow much slower (i.e., are in stationary-phase growth) than organisms in suspension. As a result, anti-microbial agents such as vancomycin, penicillins, and cephalosporins, which act on rapidly dividing organisms, are not effective in treating device-related infections.[102] Rifampin and fluoroquinolones (particularly moxifloxacin) may be more effective because they are active against organisms in the stationary phase of growth.[102] IDSA guidelines recommend rifampin in combination with another agent for the

**TABLE 109-7** Criteria for Diagnosis of Prosthetic Joint Infection (PJI)

| |
|---|
| The presence of a sinus tract that communicates with the prosthesis is definitive evidence of PJI (B-III). |
| The presence of acute inflammation on histopathologic examination of the periprosthetic tissue at the time of surgical débridement or prosthesis removal is highly suggestive evidence of PJI (B-II). |
| The presence of purulence without another known etiology surrounding the prosthesis is definitive evidence of PJI (B-III). |
| Two or more intra-operative cultures or combination of pre-operative aspiration and intra-operative cultures that yield the same organism may be considered definitive evidence of PJI. Growth of a virulent microorganism (e.g., *Staphylococcus aureus)* in a single specimen of a tissue biopsy or synovial fluid may also represent PJI (B-III). |

treatment of rifampin-susceptible strains of *S. aureus*.[93] There is evidence that clinical outcomes are improved in patients receiving rifampin, although the data are based on retrospective analysis.[103,104] The role of newer anti-staphylococcal antibiotics such as linezolid, daptomycin, telavancin, ceftaroline, dalbavancin, oritavancin, and tigecycline, is unclear at this time.[105] There is some evidence that oritavancin may be active against biofilm-associated small-colony variants of *Staphylococcus aureus*.[106]

Treatment of late prosthetic joint infections is complex. In most patients, effective therapy requires a combination of antibiotics with the removal of the orthopedic device. Failure to remove the infected prosthesis is frequently associated with an unacceptably high rate of relapse, probably related to biofilm formation on the orthopedic implant. Removal of the joint prosthesis, débridement of infected bone, and placement of a new prosthesis during the same operation has been associated with a high rate of recurrence of infection, but studies indicate that single-stage revision or débridement with retention of the prosthesis may be effective in certain situations.[107] Patients who have had symptoms of pain and swelling of the joint for less than 8 days[107] or less than 3 weeks[108] and who have a stable prosthesis with little soft tissue damage and no sinus tract are candidates for débridement, antibiotics, and implant retention (DAIR) if the pre-operative synovial fluid cultures are negative or if the cultures grow an easily treatable organism (Figure 109-3).[93,107,109] The treatment of choice for most patients is a two-stage process involving removal of the infected prosthesis and débridement of infected bone, stabilization of the joint by using an antibiotic-impregnated methyl-methacrylate spacer, and 6 weeks of intravenous antibiotics (first stage), followed by reimplantation of a second orthopedic implant (second stage). Using this approach, the success rate is approximately 72% to 95%; the success rate is slightly higher for hip versus knee arthroplasties. Risk factors for treatment failure include prior joint revision, presence of lymphedema with infection, rheumatoid arthritis, presence of a sinus tract, and infection with MRSA.[110] Rarely, antibiotic treatment is continued indefinitely in a patient in whom the risk of removing the infected prosthesis is too great, the prosthesis is not loose, and the organism responsible for the infection can be reasonably suppressed by the use of an oral antibiotic agent.[111]

The pathogenesis of bacterial infection in prosthetic joints is complex. Anatomic, virulence, and host factors affect prognosis and approach to therapy. An understanding of these interactions may lead to novel therapeutic and preventive strategies, such as vaccines against capsule antigens or surface adhesins in patients undergoing elective joint replacements.

## Prevention of Prosthetic Joint Infections

There is consensus that pre-operative evaluation of a patient for occult infection, such as periodontal disease, is warranted, and corrective steps to eradicate any infection are essential before joint replacement. There is also consensus that perioperative antibiotic prophylaxis significantly reduces the rate of early post-operative infection, and this practice is routine. Until recently, the role of antibiotic prophylaxis to prevent late prosthetic joint infection before diagnostic or therapeutic procedures that lead to transient bacteremia, especially dental treatment, has been controversial. Two case-controlled studies determined that there was no increased risk of infection of prosthetic joints following low- or high-risk dental procedures.[112,113] A position paper from the American Dental Association and the American Academy of Orthopedic Surgeons published in 2013 stated that there was no convincing evidence to support the routine use of prophylactic antibiotics in patients with prosthetic joints who undergo dental procedures.[114]

The incidence of late infection of a prosthetic joint as a result of procedure-related bacteremia is extremely low—10 to 100 cases per 100,000 patients with total joint replacement per year. The cost of providing antibiotic prophylaxis to all patients with prosthetic joints before all procedures that are associated with transient bacteremia is substantial. The efficacy of such antibiotic prophylaxis is not proven. Cost-effective analyses had mixed results.[115] These discrepancies are due to the lack of reliable data and the different assumptions used in the calculations. In the patient with the greatest risk of infection, an invasive procedure that leads to bacteremia sometimes can result in an infected total joint replacement. Counseling these patients on the risks and benefits of antibiotic prophylaxis would lead to an informed decision on which the patient and the physician can agree.

There is substantial evidence that there is an increased risk of orthopedic infection associated with the use of biologic agents that interfere with TNF inhibitors and IL-6. A retrospective analysis of 10 cases of post-operative infections showed the use of a TNF inhibitor was significantly associated with the development of a serious infection (odds ratio, 4.4).[116] A meta-analysis of the clinical literature on TNF inhibitors through 2005 found an increased risk of

**TABLE 109-8** Factors That Might Portend a Poor Outcome in Septic Arthritis

| |
|---|
| Older age |
| Pre-existing arthritis, especially rheumatoid arthritis, but also osteoarthritis and tophaceous gout |
| Presence of synthetic material (e.g., total joint replacement) |
| Delay in diagnosis or long duration of symptoms before seeking medical attention |
| Polyarticular infection, especially if >3 joints and small hand joints are affected |
| Presence of bacteremia |
| Infection caused by virulent or difficult-to-treat organisms (e.g., *Staphylococcus aureus, Pseudomonas aeruginosa,* or some Gram-negative bacilli) |
| Patients receiving immunosuppressive therapy |
| Serious underlying comorbidities (e.g., liver, kidney, or heart diseases) |
| Peripheral leukocytosis at presentation |
| Worsening renal function |

Data from Christodoulou C, Gordon P, Coakley G: Polyarticular septic arthritis. *BMJ* 333:1107-1108, 2006; Shinefield H, Black S, Fattom A, et al: Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *N Engl J Med* 346:491–496, 2002; and Shmerling RH, Delbanco TL, Tosteson ANA, et al: Synovial fluid tests: what should be ordered? *JAMA* 264:1009–1014, 1990.

serious infection.[117] Guidelines from both the American College of Rheumatology and the British Society for Rheumatology recommend discontinuing TNF inhibitors around the time of elective arthroplasty surgery revision.[118,119] The risks and benefits must be weighed carefully, and the patients must be fully informed on how these agents should be used on a case-by-case basis.

## CONCLUSION

In the 21st century, patients with septic arthritis as a group are becoming older, with more risk factors for infection and more comorbidities. The number of patients with prosthetic joints is increasing while the population ages and people live longer. It is thus not surprising to see more cases of native and prosthetic joint infections. The organisms causing those infections have not changed significantly, however. Staphylococci (44% to 66%) are still the dominant organisms, followed by streptococci (18% to 28%) and Gram-negative bacilli (9% to 19%).[5] The emerging challenges in the treatment of septic arthritis are how to improve outcome, how to deal with resistant organisms, and how to overcome host factors that portend a poor prognosis.

The outcome of treated septic arthritis can be measured as mortality, as the functional outcome of the infected joint, or as short-term and long-term outcomes. Among the survivors, loss of articular cartilage, loss of motion, or increase in pain in the affected joint would be considered poor functional outcomes. Loss of the limb to infection and need for surgery to fuse the joint or restore function are also poor outcomes. Most studies report the outcome at the time of hospital discharge, and long-term data on adults with septic arthritis are unavailable. The rate of development of degenerative joint disease, the rate of relapse or recurrence of infection, and the rate of progression of functional impairment in the affected joint over time have not been well studied.

Many retrospective studies have characterized features that may increase the chance of a poor outcome at the time of hospital discharge (Table 109-8).[14,43,120] One prospective community-based study of adults and children found poor joint outcome in 33% of survivors among 154 patients with bacterial arthritis and noted older age, pre-existing joint disease, and an infected joint containing synthetic material as negative prognostic factors by univariate analysis.[120] These investigators noted no association between poor outcome and young age, comorbidity, immunosuppressive medication, functional class, multiple infected joints, type of organism, or treatment delay. In a large retrospective study from the United Kingdom on the outcome of 243 patients, 11.5% died secondary to septic arthritis, and additional morbidity was noted in 31.6% of patients. Multivariate analysis suggests that important predictors of death are confusion at presentation, age 65 years or older, multiple joint sepsis, and involvement of the elbow joint. Predictors of morbidity were age 65 years or older, diabetes mellitus, open surgical drainage, and Gram-positive infections other than *S. aureus*.[51]


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 110

# Lyme Disease



Linda K. Bockenstedt

**KEY POINTS**

- Lyme disease is caused by infection with tick-transmitted spirochetes of the genus *Borrelia burgdorferi* sensu lato.
- Lyme disease has a characteristic pattern of signs and symptoms and usually begins with the expanding macular skin lesion erythema migrans.
- Earlier recognition and treatment has led to a decline in the incidence of carditis, acute neurologic disease, and late disease manifestations.
- Musculoskeletal manifestations occur in more than 50% of person and at all stages of infection, but frank arthritis is a sign of late disease.
- The diagnosis of Lyme disease should be suspected when a person who lives, works, or vacations in an endemic area is seen with signs and symptoms of *B. burgdorferi* infection.
- Two-tiered serologic tests (enzyme-linked immunosorbent assay and immunoblot) can be negative with early infection but become positive in most patients with infection of longer than a 1-month duration.
- Most patients are cured with 2 to 4 weeks of antibiotic therapy, although the time to disease resolution may extend beyond the duration of therapy, and irreversible tissue damage may occur.
- Antibiotic-refractory arthritis occurs in less than 10% of patients with Lyme arthritis, responds to disease-modifying anti-rheumatic drugs, and typically resolves within 5 years.
- Post–Lyme disease syndrome after antibiotic treatment for Lyme disease occurs in a minority of patients. *B. burgdorferi* cannot be detected in these patients, and controlled treatment trials show no benefit of prolonged antibiotic therapy over placebo.

Lyme disease is a multisystem disorder caused by the tick-borne spirochete *Borrelia burgdorferi*.[1] The disease first came to medical attention in the United States in the late 1970s with the investigation of a clustering of cases of juvenile arthritis in the region of Lyme, Connecticut.[2] A characteristic skin rash, described as single or multiple expanding red macules, often heralded the onset of arthritis. This rash, termed *erythema migrans* (EM), had been linked in Europe to the bite of *Ixodes* ticks and the subsequent development of neurologic abnormalities.[3] Further investigation revealed that arthritis was one manifestation of a systemic disorder affecting the skin, heart, joints, and nervous system. In 1982, Burgdorfer isolated the causative agent, the spirochete *B. burgdorferi*, from *Ixodes* ticks.[4] Demonstration that antibodies to this organism developed in patients with Lyme disease and its eventual culture from skin and cerebrospinal fluid (CSF) confirmed the infectious etiology of the disorder.[5] It is now the most common vector-borne disease in the United States.[1]

## ECOLOGY AND EPIDEMIOLOGY OF LYME DISEASE

Lyme disease has a worldwide distribution, with most cases reported in North America, Europe, and Asia.[6,7] On each of these continents, hard-shelled ticks of the *Ixodes* family are the only known vectors for the disease. Other arthropods and blood-sucking insects such as mosquitos cannot transmit the infection. The incidence of Lyme disease varies geographically and is determined by the prevalence of *B. burgdorferi*–infected ticks. In the United States, cases of Lyme disease have been reported in all 50 states and the District of Columbia, but most are clustered in the Northeast and mid-Atlantic region, upper Midwest, and northern California. Cases of Lyme disease outside these regions are mostly acquired after travel to highly endemic areas. In 2014, 25,359 confirmed and 8102 probable cases were reported to the Centers for Disease Control and Prevention, with 96% of confirmed cases originating from 14 states: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and Wisconsin.[8] The number of reported cases is approximately 10-fold less than the actual case incidence, which is estimated to be approximately 300,000 cases annually.[9]

The spirochetes associated with Lyme disease reside within the genus *B. burgdorferi* sensu lato (sl), and the vast majority of cases are caused by *B. burgdorferi* sensu stricto (ss), *Borrelia garinii*, and *Borrelia afzelii*.[6,7] All three genospecies can be found in Europe, whereas *B. burgdorferi* ss is the main species found in North America. Variation among the genospecies may account for the differences in clinical expression of Lyme disease between the two continents: *B. garinii* is associated with neurologic disease, *B. afzelii* is associated with late skin involvement, and *B. burgdorferi* ss is associated with arthritis.[1,6] Because of the prominence of musculoskeletal manifestations with *B. burgdorferi* ss infection, this chapter focuses mainly on Lyme disease in North America.

## TICKS AND LYME DISEASE

Lyme disease is found primarily in temperate climates where an enzootic cycle maintaining *B. burgdorferi* in *Ixodes* ticks

is established.[6,7] *Ixodes* ticks have a 2-year life span in which they pass through three developmental stages—larva, nymph, and adult—feeding only once per stage.[10] *B. burgdorferi* is not passed transovarially and is maintained by passage between reservoir hosts and ticks. Small rodents are the main reservoirs for *B. burgdorferi* ss and *B. afzelii*, whereas birds are the principal haven for *B. garinii*.[6]

Larvae acquire *B. burgdorferi* after feeding on an infected mammal in early spring and then molt to nymphs, which lay dormant until the following late spring and summer. The peak incidence of Lyme disease is in the summer months, when humans come in contact with questing nymphs.[11] Engorged nymphs molt into adult ticks, which feed mainly on deer. *B. burgdorferi* does not persist in deer, which serve to maintain and propagate the tick population.

## PATHOGENESIS

### *Borrelia burgdorferi* Invasion of the Mammalian Host

The onset of tick feeding signals *B. burgdorferi* residing in the tick midgut to undergo global changes in gene expression, multiply, and migrate to the salivary glands, where they pass with saliva into the blood meal host.[12,13] Although spirochetes expand greatly in numbers in the tick midgut, relatively few spirochetes—estimated in the hundreds—are deposited at the bite site, a process that takes at least 24 hours.[14] After establishing infection in the skin, spirochetes can disseminate via the bloodstream to infect any organ system. Spirochete virulence, growth conditions that allow persistence at a particular site, and host factors that modulate the inflammatory response determine disease expression.

*B. burgdorferi* has no known virulence factors common to other bacterial pathogens that may explain the pathogenesis of Lyme disease.[15,16] Its genome is remarkably rich in genes encoding putative lipoproteins, only a handful of which have been studied in detail. Outer surface protein (Osp) A is a midgut adhesin required for spirochete infection of ticks.[17] Osp C is essential for initial infection of the mammal but is dispensable after spirochetes have disseminated and colonized other tissue.[18] To do so, *B. burgdorferi* harnesses host plasmin to move through tissues[19,20] and expresses adhesins, including decorin-binding proteins A and B, BBK32, and p66, which allow it to bind to extracellular matrix proteins (ECM) and integrins on cells.[21] *B. burgdorferi* expresses a protease with aggrecanase activity and induces the host expression of matrix metalloproteinases, which degrade ECMs.[22] Expression of VlsE, an Osp that undergoes antigenic variation, is required for infection to persist.[23,24]

### Pathology of Lyme Disease

Because few spirochetes can be seen in infected tissues, and *B. burgdorferi* secretes no known toxins, the host inflammatory response is believed to underlie the pathology seen in Lyme disease. Engagement of adaptive immunity is present at the earliest clinical sign of Lyme disease (EM).[25,26] Histopathologic studies of EM lesions, cardiac tissue, synovial biopsy specimens, and limited nervous system tissue (meninges, spinal cord, and nerve roots) reveal varying degrees of monocytic and lymphoplasmacytic infiltrates, especially perivascular, that stain positively for cell surface markers for macrophages, T cells, and B cells.[27,28] The proportions of responding immune cells differ among EM, CSF, and synovial fluid samples, suggesting a tissue-specific immune response to *B. burgdorferi* infection. In particular, synovial fluid exhibits elevated leukocyte counts, whereas a lymphocytic pleocytosis is seen in CSF of patients with central nervous system (CNS) involvement. Lyme arthritis synovium resembles that of rheumatoid arthritis, with chronic inflammation mediated by mononuclear cell infiltration and pseudolymphoid follicles formed by T cells, B cells, and plasma cells. In the synovium and, less commonly, the epineural area, perivascular infiltrates can be associated with endarteritis obliterans.

### Immune Response to *Borrelia burgdorferi*

Innate immune cells respond to *B. burgdorferi* through engagement of the Toll-like receptor (TLR) family of pattern recognition receptors, especially TLR2/TLR1 heterodimers (lipoproteins), TLR5 (flagellin), TLR7 and TLR8 (RNA), and TLR9 (spirochete DNA).[29-33] As a consequence, pro-inflammatory cytokines, including IL-1β, TNF, and type I interferons, are produced that recruit inflammatory cells to the site of infection.[33] *B. burgdorferi* also induces matrix metalloproteinase expression in tissues through TLR-dependent and non–TLR-dependent pathways.[34] Other pattern recognition receptors may be engaged by *B. burgdorferi* after its ingestion by phagocytes, including the intra-cellular NOD2 receptors, which respond to peptidoglycan and potentiate the inflammatory response in vitro.[35]

Humoral immunity is a key host defense against *B. burgdorferi* infection. *B. burgdorferi* lipoproteins are B cell mitogens, and antibodies that arise in the absence of T cell help are sufficient to resolve inflammation and prevent challenge infection in the mouse model of Lyme borreliosis.[36,37] With the induction of adaptive immunity, immunoglobulin (Ig)G-containing immune complexes can be found in the serum of patients with Lyme disease and are concentrated in the joints of patients in whom Lyme arthritis develops.[38] The B cell–recruiting chemokine CXCL13 can be found in the CSF of patients with neuroborreliosis[39]; B cells constitute approximately 15% of the nucleated cells, an order of magnitude higher than in other infectious and inflammatory neurologic diseases.[40] Intrathecal production of *B. burgdorferi*–specific antibodies is found and some of these antibodies can also bind neural antigens.[41-45]

*B. burgdorferi* infection primes $CD4^+$ and $CD8^+$ T cells, and the predominance of T helper (Th) type 1 responses correlates with more severe arthritis and neuroborreliosis.[46,47] Th17 cells are also involved. In the CSF, T cells represent more than 60% of the responding cells with $CD4^+$ Th1 cells a majority; approximately half of patients with neurologic involvement have elevated levels of IL-17.[48] *B. burgdorferi* neutrophil-activating protein A is abundant in synovial fluid and can elicit IL-17 from synovial fluid T cells ex vivo.[49] Synovial fluid also contains other T cell subsets, including $CD25^+Foxp3^+$ T cells, γδ T cells, and natural killer (NK) T cells, which may regulate the inflammatory response.[28,50-52]

## Mechanisms of Spirochete Persistence

When visualized in vivo, *B. burgdorferi* resides primarily in the ECM in connective tissue.[27,53] Despite occasional sightings of spirochetes inside cells,[54] an intra-cellular phase of the *B. burgdorferi* life cycle has not been shown. *B. burgdorferi* uses immune evasion strategies of an extracellular pathogen, which are directed toward deterring phagocyte ingestion and antibody- and complement-mediated lysis.[1] *B. burgdorferi* expresses Erp and complement regulator–acquiring surface proteins that bind host factor H to prevent complement-mediated lysis.[55-57] To impede antibody-mediated clearance, *B. burgdorferi* undergoes antigenic variation[23,24] and reduces expression of lipoproteins as infection progresses.[58] The *vlsE* gene undergoes random rearrangement of its expression locus, producing antigenically distinct variants of VlsE.[23] In the chronic phase of *B. burgdorferi* infection in mice, spirochetes can be visualized in the ECM of connective tissue, especially in the skin, without an associated inflammatory response.[59]

# CLINICAL FEATURES

Lyme disease occurs in stages that reflect the immune response to the spirochete while it establishes infection in the skin and later disseminates to distant organ sites (Table 110-1). Presenting clinical manifestations depend on the stage of the illness in which patients first seek medical attention. A characteristic feature of Lyme disease is that clinical signs can resolve without specific therapy, and patients may be seen in later stages of the illness without exhibiting signs of early disease.

## Early Localized Infection

The hallmark of Lyme disease is the skin lesion EM, which is present in approximately 80% of patients (Figure 110-1).[60] The lesion arises within 1 month (median, 7 to 10 days) at the tick-bite site, which is often in skin folds or where clothes bind in adults and around the hairline in children. EM begins as a red macule that expands at the rate of 2 to 3 cm/day, enlarging to more than 70 cm in diameter. Characteristic lesions greater than 5 cm in diameter in an appropriate clinical setting are sufficient for establishing the diagnosis of Lyme disease.[61] EM most often manifests with uniform erythema, but central clearing can occur in larger lesions, producing a "bull's eye" appearance (see Figure 110-1B). Vesicular or necrotic centers are rarer (see Figure 110-1D), but even these EM lesions have relatively few symptoms other than a tingling or burning sensation. Intense pruritus or pain is unusual and should raise concern for alternative diagnoses.

EM may be accompanied by systemic flulike symptoms, including low-grade fever, malaise, neck pain or stiffness, arthralgias, and myalgias.[62,63] Particularly severe systemic symptoms should alert the physician to possible coinfection with another tick-borne pathogen such as *Babesia microti* or *Anaplasma phagocytophilum* (the agent of human granulocytic anaplasmosis, formerly known as *human granulocytic ehrlichiosis*). Lyme disease can also manifest with systemic symptoms alone.[64,65] Absence of upper respiratory or gastrointestinal symptoms may help distinguish Lyme disease from common viral infections. Musculoskeletal complaints and debilitating fatigue associated with Lyme disease should be distinguished from fibromyalgia and chronic fatigue syndrome, which are typically more insidious in onset and are not associated with objective findings or laboratory abnormalities.

Southern tick–associated rash illness (STARI) can produce a skin lesion similar to the bull's eye form of EM.[66] STARI is associated with the bite of the Lone Star tick, *Amblyomma americanum*, which is endemic to the southeastern and south-central states, but also can be found as far north as Maine or west as central Texas and Oklahoma. Systemic symptoms can accompany the STARI rash, but extracutaneous disease does not occur. The etiology of STARI is unknown; studies indicate it is not caused by *B. burgdorferi*. Antibiotics resolve EM and STARI, but STARI patients recover more quickly from systemic symptoms than patients with Lyme disease.

**TABLE 110-1** Clinical Manifestations of Lyme Disease

| |
|---|
| **Early Localized Infection** |
| Occurs 3 to 30 days after tick bite |
| EM in 80%-90% of patients; single lesion, occasionally associated with fever, malaise, neck pain or stiffness, arthralgias and myalgias |
| Systemic symptoms noted above in the absence of EM during summer months |
| Borrelial lymphocytoma (rare, seen primarily in Europe) |
| **Early Disseminated Infection** |
| Occurs weeks to months after tick bite |
| Profound malaise and fatigue common |
| Multiple EM lesions with systemic symptoms similar to early localized infection |
| Musculoskeletal<br>Migratory polyarthralgias and myalgias |
| Cardiac<br>Carditis (<3% of untreated patients)<br>Varying degrees of atrioventricular nodal block<br>Mild myopericarditis |
| Neurologic (<10% of untreated patients)<br>Cranial neuropathies (especially facial nerve palsy)<br>Lymphocytic meningitis<br>Radiculoneuropathies<br>Encephalomyelitis |
| **Late Disease** |
| Occurs months to years after tick bite |
| Arthritis (<10% of patients)<br>Acute monoarticular or migratory pauciarticular inflammatory arthritis, usually involving the knee<br>Chronic antibiotic-refractory arthritis (<10% of patients with arthritis) |
| Neurologic (rare)<br>Peripheral neuropathies<br>Mild encephalopathy<br>Encephalomyelitis (primarily seen in Europe) |
| Skin<br>Acrodermatitis chronica atrophicans (primarily seen in Europe) |

EM, Erythema migrans.

## Early Disseminated Infection

Disseminated *B. burgdorferi* infection occurs within weeks of the tick bite. At this stage, clinically apparent disease is




**Figure 110-1** Erythema migrans rash of Lyme disease. **A,** Typical macular lesion on left shoulder. **B,** Bull's eye lesion on lateral thigh with central punctum. **C,** Multiple lesions on back. **D,** Lesion with vesicular center on posterior thigh. *(Courtesy Juan Salazar, MD, University of Connecticut Health Center.)*

usually seen in the skin, heart, or nervous system. Patients with disseminated infection have debilitating fatigue and can appear ill. Specific localizing signs and symptoms may fluctuate, but profound fatigue is a consistent complaint.

### Skin Disease

Fifty percent of patients with untreated Lyme disease experience multiple EM lesions, a sign of disseminated infection (see Figure 110-1C).[60] Secondary lesions are typically smaller and can occur anywhere on the body, but they are most noticeable on the trunk. The lesions usually appear as flat macules and can develop partial central clearing. EM lesions may be accompanied by migratory muscle, joint, and periarticular pain that lasts hours to days, but arthritis does not appear until months after the initial infection.

### Cardiac Disease

Cardiac involvement is clinically apparent in less than 1% of patients with Lyme disease.[1] It most often occurs within the first 2 months of infection and manifests as varying degrees of atrioventricular block, occasionally accompanied by mild myopericarditis.[67,68] Sudden death can occur.[69-71] Electrophysiologic studies have mapped the conduction defect to the area above the bundle of His and involving the atrioventricular node, although multiple levels can be affected. Overt congestive heart failure is rare, and chronic cardiomyopathy, reported in Europe, has not been documented in the United States.[72] Patients with Lyme carditis often have a history of EM and may have concomitant arthralgia and myalgia at the time of presentation. Absence of valvular heart disease helps distinguish Lyme carditis from acute rheumatic fever, and prominent myocardial dysfunction or pericardial involvement should suggest other infectious etiologies.

### Nervous System Involvement

Acute neurologic Lyme disease occurs in less than 10% of patients and most commonly manifests as cranial nerve palsy or meningitis, although radiculopathy and encephalomyelitis are also occasionally seen.[73-77] Cranial palsy occurs in 8% of cases and usually affects the seventh nerve, resulting in unilateral or bilateral facial palsy. Even in endemic areas, however, onset of seventh nerve palsy in the nonwinter months is caused by *B. burgdorferi* infection in only 25% of cases.[78] Bilateral facial palsy is seen in only a few other conditions—Guillain-Barré syndrome, HIV infection, sarcoidosis, and other causes of chronic meningitis—all of which are readily distinguished from Lyme disease. Rarely, other cranial nerves (III, IV, V, VI, or VIII) may be involved. Lyme meningitis manifests with fever, headache, and stiff neck similar to viral meningitis, along with a CSF lymphocytosis and elevated protein.[76] In children, meningitis may occur with EM, cranial nerve involvement, and increased

intracranial pressure (papilledema), which is rare in adults.[75,79] Lyme radiculopathy typically manifests as pain, weakness, numbness, and reflex loss in a dermatomal distribution, resembling mechanical radiculopathies.[74] Untreated Lyme radiculopathy can progress to become bilateral, which helps distinguish it from mechanical disease. When truncal involvement causes unilateral chest or abdominal pain, Lyme radiculopathy is often mistaken for visceral disease or early herpes zoster before the development of vesicular lesions.

### Other Organ System Involvement

A variety of other organs can exhibit pathology with disseminated *B. burgdorferi* infection, including the eye (keratitis), the ear (sensorineural hearing loss), the liver (hepatitis), the spleen (necrosis), skeletal muscle (myositis), and subcutaneous tissue (panniculitis).[80] In general, more classic manifestations of Lyme disease are present concurrently or have been present in the recent past to suggest the diagnosis.

## Late Disease

Months after the onset of infection, untreated patients can develop late manifestations of Lyme disease, usually involving the joints (discussed separately later), nervous system, and the skin. At this stage of infection, two-tier (enzyme-linked immunosorbent assay [ELISA] and IgG immunoblot) serologic testing for *B. burgdorferi* should be positive.

### Neurologic Disease

Late neurologic Lyme disease is now rare; patients may be seen with encephalomyelitis, peripheral neuropathy, or encephalopathy.[81,82] Encephalomyelitis, seen predominantly in Europe with *B. garinii* infection, is a slowly progressive, unifocal, or multifocal inflammatory disease of the CNS, with increased T2 signals in the white matter on MRI. CSF examination often reveals a lymphocytic pleocytosis, elevated protein, and normal glucose, and serum IgG to *B. burgdorferi* and intrathecal antibody production can be found. These findings help distinguish Lyme encephalomyelitis from multiple sclerosis, which may rarely be associated with positive IgG reactivity to *B. burgdorferi* in serum and CSF samples, but there is no intrathecal antibody production.[82,83] Multiple sclerosis patients with positive Lyme serologies do not respond to antibiotics used for neurologic Lyme disease.

Late peripheral nervous system involvement manifests as a mild sensorimotor neuropathy in a "stocking and glove" distribution, with evidence of a mild confluent mononeuritis multiplex on electrophysiologic studies.[74] Patients may have intermittent limb paresthesias and occasionally radicular pain. The most common finding on physical examination is reduced vibratory sensation in the lower extremities. Serum IgG to *B. burgdorferi* should be present, but CSF examination is normal. Patients with this form of neuropathy should be evaluated for other infectious diseases (syphilis, human immunodeficiency virus, and hepatitis C virus); metabolic disorders (especially vitamin $B_{12}$ deficiency, diabetes mellitus, and thyroid disease); and autoimmune diseases.

Patients with Lyme encephalopathy complain of memory impairment and cognitive dysfunction that are best shown by formal neuropsychological testing.[81,84,85] Occasionally, patients may have CSF abnormalities with elevated protein, lymphocytic pleocytosis, and intrathecal antibody to *B. burgdorferi*, but CSF examination can also be normal. Serum IgG to *B. burgdorferi* should be present, however, to consider the diagnosis. The mild cognitive dysfunction seen in patients with Lyme encephalopathy must be distinguished from neurocognitive deficits secondary to chronic stress, sleep deprivation, fibromyalgia, chronic fatigue syndrome, or aging. As for any chronic encephalopathy, toxic-metabolic causes should be excluded. Brain imaging studies are generally normal or show only nonspecific abnormalities and are not useful in establishing a diagnosis of encephalopathy associated with Lyme disease.

### Skin Disease

The late-skin lesion acrodermatitis chronica atrophicans (ACA) is found mainly in Europe because of its association with *B. afzelii* infection, although any *B. burgdorferi* species can cause the lesion.[86] ACA develops insidiously over years and is most often found on an extremity. It begins as a unilateral bluish red discoloration and swelling, which evolves to atrophic, cellophane-like skin with prominent appearance of the blood vessels. Allodynia is considered a characteristic symptom of the skin lesion. Approximately 60% of patients also have a peripheral sensory neuropathy affecting the involved extremity. A prominent lymphoplasmacytic infiltrate is shown on the skin biopsy specimen. Antibiotics can lead to improvement in pain and swelling, but atrophic skin remains.

## LYME ARTHRITIS AND OTHER MUSCULOSKELETAL MANIFESTATIONS OF LYME DISEASE

Musculoskeletal symptoms are common in all stages of Lyme disease and include migratory pain in joints, tendons, bursae, and muscles.[1,87] Typically, musculoskeletal pain affects one or two sites at a time, lasts only hours to a few days at any one location, and is associated with significant fatigue. The incidence of frank arthritis has declined from 50% in early studies to less than 10% in more recent years.[28] ELISA and IgG immunoblot for *B. burgdorferi* are positive when arthritis appears, and *B. burgdorferi* DNA can be detected by polymerase chain reaction (PCR) in synovium and synovial fluid even though cultures are negative. Although Lyme arthritis can resemble pauciarticular juvenile arthritis or reactive arthritis, patients generally test negative for anti-nuclear antigen (ANA), rheumatoid factor, and anti-citrullinated protein antibodies and do not have an increased frequency of human leukocyte antigen (HLA)-B27 alleles. Joint fluid analysis and synovial histopathology cannot distinguish these entities. Axial and sacroiliac joint involvement is not a feature of Lyme disease, but enthesitis can be seen.

Arthritis usually begins months or years after *B. burgdorferi* infection (average 6 months) and is pre-dated by migratory arthralgias in half of patients.[87] The most typical pattern is a monoarticular or oligoarticular arthritis involving one

or a few large joints (≤5 total), with the knee affected in 80% of cases. Joints are warm with large effusions, often greater than 100 mL in the knee, but comparatively little pain. Synovial fluid is usually inflammatory with white blood cell counts ranging from approximately 500 to more than 100,000/mm$^3$ (median ~ 25,000/mm$^3$), with a predominance of neutrophils. Children with Lyme arthritis may present more acutely with fever and a painful, swollen joint resembling septic arthritis.[88,89] Large effusions can lead to Baker's cyst formation and rupture. The temporomandibular joint is also frequently involved.[87] Other joints commonly affected include the shoulder, ankle, elbow, wrist, and hip. Lyme arthritis is often intermittent, with episodes lasting a few weeks to months. Recurrent episodes are notable for smaller effusions and progressive synovial hypertrophy, bony erosion, and cartilage destruction. A small percentage (<10%) of patients with intermittent arthritis settle into a pattern of chronic arthritis, affecting only a single joint. Inflammation of a single joint that persists for more than 12 months would be an unusual presenting manifestation of Lyme arthritis, as is the prominent involvement of small joints.

The natural history of Lyme arthritis suggests that it is a self-limited disorder. In the late 1970s, before the use of antibiotics for Lyme disease, 21 patients who were seen with EM and later were diagnosed with Lyme arthritis were followed up for 1 to 8 years without anti-microbial therapy.[87] Six patients had only a single episode of arthritis, and the remaining 15 had recurrent episodes that decreased in frequency during the study period. On average, the number of patients who continued to experience episodes of arthritis decreased by 10% to 20% each year. Similar results were found in children in whom antibiotic treatment for arthritis was delayed 4 years.[90]

### Antibiotic-Refractory Lyme Arthritis

Approximately 10% of patients treated with standard antibiotic regimens for Lyme arthritis have persistent joint inflammation and proliferative synovitis that does not respond to further anti-microbial therapy.[28] The pathogenesis of "antibiotic-refractory" Lyme arthritis is unknown but may be to the result of persistent spirochetes or their antigens, infection-induced autoimmunity, or inadequate regulation of the inflammatory response.[1] Spirochete strains that have a greater propensity to become blood-borne have been found at higher rates in patients in whom antibiotic-refractory arthritis develops.[91-93] Although spirochete DNA can be detected in synovial fluid for months after treatment, its presence does not predict arthritis relapse rate or persistence.[94] Experiments in the mouse model of Lyme borreliosis suggest that spirochete debris, including *B. burgdorferi* DNA, can persist near cartilage and in the entheses for extended periods after infectious spirochetes have been killed with antibiotics, particularly when the initial pathogen burden was high.[53] Genetic polymorphisms may contribute to the rate at which inflammation subsides after viable spirochetes have been eliminated. A single nucleotide polymorphism in TLR1 (TLR1 1805 GG) was found to be more prevalent among patients who developed antibiotic-refractory Lyme arthritis.[95] Recently, deficiency in the lysosomal enzyme β-glucuronidase (GusB) was found to underlie the genetic susceptibility of the C3H mouse strain to more severe Lyme arthritis; deficiency in GusB also exacerbates arthritis in a serum transfer model of inflammatory arthritis.[96] GusB functions to degrade glycosaminoglycan (GAGs) that are produced in higher abundance during inflammation. GAGs directly activate TLRs, and their accumulation could potentiate and prolong inflammation.

A genetic predisposition to antibiotic-refractory Lyme arthritis had been suggested in earlier studies that found an association with the rheumatoid arthritis–related alleles HLA-DRB1*0401, HLA-DRB1*0101, and HLA-DRB1*0404.[97] A search for *B. burgdorferi* antigens that could be presented by these HLA molecules and potentiate inflammation through molecular mimicry initially centered on OspA because of the high prevalence of OspA-specific B cell and T cell responses in patients with antibiotic-refractory arthritis[28,97,98] Although an Osp A peptide was found to have homology with the adhesion molecule human leukocyte function–associated antigen 1 (LFA-1),[99] the corresponding LFA-1 peptide did not elicit the Th1 cytokine IFN-γ normally found in antibiotic-refractory arthritis.[100] More recently, linked T and B cell responses to peptides of epidermal cell growth factor have been found in about 50% of patients with antibiotic-refractory Lyme arthritis.[101] Antibodies to endothelial cell growth factor (ECGF) correlate with the presence of obliterative vasculopathy.[102] Deficiencies in regulation of the immune response have been reported. CD25$^+$FoxP3$^+$ T regulatory cells are reduced in number in the synovial fluid of patients with antibiotic-refractory Lyme arthritis.[50,103] Invariant NK T cells, which may have immunodulatory properties, were notably absent in one study.[52] If autoimmunity is responsible for antibiotic-refractory Lyme arthritis, it must eventually succumb to immune regulation because even this form of Lyme arthritis generally resolves within 4 to 5 years.[87,104]

## DIAGNOSIS

The diagnosis of Lyme disease should be considered in individuals who are seen with an appropriate clinical history and who have a reasonable risk of exposure to *B. burgdorferi*–infected ticks (Figure 110-2).[41] Supporting serologic evidence is necessary to secure the diagnosis at all stages of infection except when EM is present.[61] Routine laboratory tests are nonspecific, with some patients exhibiting mildly elevated white blood cell (neutrophil) counts, erythrocyte sedimentation rates, and liver function tests. Culture or microscopic visualization of spirochetes in clinical samples is not sensitive enough for routine use in diagnosis. Culture of a skin biopsy specimen taken from the leading margin of an EM lesion is an exception, with *B. burgdorferi* detected in more than 40% of samples. The validity of a novel serum culture assay for detecting *B. burgdorferi* in blood samples has been questioned and should not be used.[105,106]

### Serologic Testing

Detection of antibodies to *B. burgdorferi* is the mainstay of laboratory testing for Lyme disease.[41] Presence of antibodies to *B. burgdorferi* at best indicates previous exposure to the organism, however, and should not be considered evidence of active infection. In nonendemic areas, approximately 5%

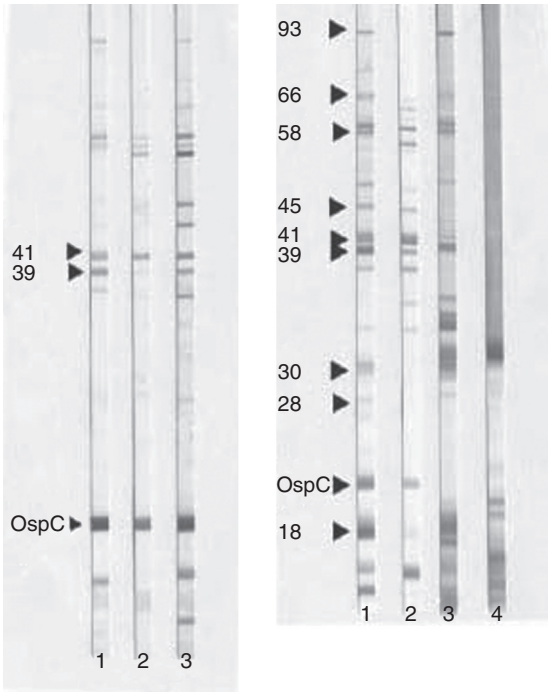



**Figure 110-2** *Left,* Selected IgM immunoblot reactivities. *Lane 1,* Serum band locator control showing several bands including the significant 41 kDa protein, 39 kDa protein, and OspC *(arrowheads). Lane 2,* Serum sample from a patient with early Lyme borreliosis with erythema migrans. *Lane 3,* Serum sample from a patient with early disseminated Lyme borreliosis with multiple erythema migrans lesions. Note the larger number of bands observed in a serum sample of the patient with early disseminated Lyme borreliosis. *Right,* Selected IgG immunoblot reactivities. *Lane 1,* Serum band locator control showing several immunoreactive bands, including those considered significant in the IgG blot criteria *(arrowheads). Lane 2,* Serum sample from a patient with early disseminated Lyme borreliosis with neurologic involvement. *Lane 3,* Serum sample from a patient with Lyme arthritis. *Lane 4,* Serum sample from an individual who received three doses of OspA vaccine; note the strong reactivity with OspA (31 kDa) and other antigens below OspC. *(From Aguero-Rosenfeld ME, Wang G, Schwartz I, et al: Diagnosis of Lyme borreliosis.* Clin Microbiol Rev *18:484–509, 2005.)*

of normal human serum samples yield positive results on serologic tests for Lyme disease. In endemic areas, asymptomatic IgG seroconversion to *B. burgdorferi* has been found in approximately 7% of patients.[107]

A two-tiered approach is recommended for detection of *B. burgdorferi*–specific antibodies.[108,109] An ELISA or an indirect immunofluorescence assay to detect IgM and IgG reactivity to *B. burgdorferi* should be used as an initial screening test, followed by an immunoblot (Western blot) to confirm that positive or equivocal results are due to antibodies that bind *B. burgdorferi* antigens. ELISA and immunofluorescence assays are highly sensitive tests but lack specificity because of cross-reactivity of antibodies to *B. burgdorferi* with other bacterial pathogens.[41] A positive or equivocal ELISA or immunofluorescence assay should be confirmed by immunoblot analysis of *B. burgdorferi* proteins (Table 110-2). Banding patterns characteristic of early infection include antibodies to the 41 kilodalton (kDa) flagellin protein and Osp C, which has a molecular weight ranging from 21 to 24 kDa depending on the *B. burgdorferi* strain used (see Figure 110-2). With disseminated infection, and especially with late Lyme disease, IgG reactivity to an expanding array of *B. burgdorferi* proteins can be seen (see Figure 110-2).

**TABLE 110-2** Criteria for Western Blot Interpretation in the Serologic Confirmation of Lyme Disease

| Duration of Disease | Isotype Tested | Criteria for Positive Test |
|---|---|---|
| 1 month or less | IgM | 2 of the following 3 bands are present: 23 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla) |
| Greater than 1 month | IgG | 5 of 10 bands are present: 18 kDa, 21 kDa, 28 kDa, 30 kDa, 39 kDa, 41 kDa, 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa |

Modified from Centers for Disease Control and Prevention: Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 44:590–591, 1995.

A peptide-based ELISA that uses a highly conserved invariant region of the VlsE protein, termed C6 (IR6), can be used in lieu of ELISA based on whole bacterial cell lysates.[110,111] This assay has increased sensitivity but slightly lower specificity in early infection when compared with the whole-cell lysate ELISA.[111] A positive or equivocal test should be confirmed with IgM and IgG immunoblots.

Two-tier testing for IgM and IgG should be performed for individuals with suspected Lyme disease and signs and symptoms of less than 1 month duration, whereas only IgG results should be considered for illnesses of longer duration.[108] Positive IgM serologies alone after 1 month of illness are most often false-positive tests, which occur in the setting of other infectious diseases (especially infectious mononucleosis and other spirochetal and tick-borne infections), rheumatoid arthritis (with or without rheumatoid factor), and conditions associated with a positive ANA (systemic lupus erythematosus).[112] No further testing is recommended if ELISA or immunofluorescence assay results are negative. Two-tier testing has overall sensitivities of 29% to 40% in EM during the acute phase; 29% to 78% for EM in the convalescent phase; and greater than 95% in neurologic, arthritis, and other manifestations of late disease.[41]

Some commercial laboratories have developed assays using recombinant antigens or use criteria for the interpretation of immunoblots that have not been validated.[113] For this reason, the Centers for Disease Control and Prevention advises using only validated tests approved by the U.S. Food and Drug Administration (FDA) for serologic diagnosis of Lyme disease.

After antibiotic therapy, IgM and IgG titers to *B. burgdorferi* measured by either whole cell ELISA or the C6 peptide ELISA (IgG only) generally decrease slowly but can remain positive for years.[114,115] Repeat serologic testing is not recommended as a means for assessing response to treatment.

## Antibodies to *Borrelia burgdorferi* in Cerebrospinal Fluid

In suspected cases of neuroborreliosis, intrathecal antibody production is usually assessed by measuring the ratio of IgG to *B. burgdorferi* in CSF and serum.[41,116] Intrathecal antibody

production is more commonly found in European neuroborreliosis than in North American Lyme disease, which may be the result of the higher prevalence of *B. garinii* in CNS infection than *B. burgdorferi* ss. Antibodies to *B. burgdorferi* may persist in CSF after treatment for Lyme disease and should not be used to assess efficacy of therapy.

### Polymerase Chain Reaction

PCR has been used to detect *B. burgdorferi* DNA in a variety of clinical specimens with variable success.[41,117] The greatest utility of PCR clinically is in Lyme arthritis, in which the sensitivity of PCR for detection of *B. burgdorferi* DNA is 85%.[118] In contrast, the sensitivity of PCR for detecting *B. burgdorferi* DNA in CSF is low and most often positive in patients with a CSF pleocytosis. PCR of urine specimens is not recommended because of documented nonspecific amplification of non–*B. burgdorferi* DNA targets.[41,113] Recently a novel two-step method for amplifying *B. burgdorferi* DNA was reported to detect the pathogen in two-thirds of blood specimens from patients with EM[119]; additional studies are needed to validate the method. PCR testing is useful for establishing exposure to *B. burgdorferi* but positive results are not necessarily a reflection of active infection, as *B. burgdorferi* DNA can persist for months after spirochetes are killed with antibiotics in vitro and in vivo.[53,94] There are currently no FDA-approved tests for PCR-based molecular techniques for detecting *B. burgdorferi* DNA in patient specimens.

### Other Tests

A urine antigen test, immunofluorescent staining for cell wall–deficient forms of *B. burgdorferi*, and lymphocyte transformation assays are offered by some commercial laboratories to aid in the diagnosis of Lyme disease. These tests have not been adequately validated for accuracy or clinical usefulness, and should not be used.[113]

### Imaging

Imaging studies have a limited role in the evaluation of patients with Lyme disease because no feature is sufficiently distinctive to confirm the diagnosis. Plain radiographs of arthritic joints show changes consistent with an inflammatory arthropathy, including joint effusions, synovial hypertrophy, periarticular osteoporosis, cartilage loss, bony erosions, and calcified entheses.[120] MRI of arthritic joints can confirm the radiographic findings and reveal associated myositis and adenopathy, which may be useful in distinguishing Lyme arthritis from septic arthritis in children.[121]

Cranial and spinal MRI findings in neuroborreliosis can reveal focal nodular lesions or patchy white matter lesions on T2-weighted images, consistent with inflammatory or demyelinating processes.[122-124] These lesions typically resolve after treatment for Lyme disease,[124] in some cases only after several years.[125] In patients with post–Lyme disease syndrome, cerebral MRI and the more sensitive technique of fluid-attenuated inversion recovery are normal in approximately 50% of cases or show nonspecific findings of small white-matter lesions.[126] Positron-emission tomography and single-photon emission computed tomography studies are often normal or show only nonspecific changes with subcortical and cortical hypoperfusion.[127,128]

## TREATMENT AND OUTCOME

Guidelines for the clinical assessment (Figure 110-3) and treatment (Table 110-3) of Lyme disease have been published.[129] Because many of the manifestations of Lyme disease resolve without specific therapy, the goal of antibiotic treatment is to hasten resolution of signs and symptoms and to prevent later clinical manifestations. Generally, oral antibiotics are sufficient therapy for EM, disseminated EM, uncomplicated facial palsy, mild carditis (first-degree atrioventricular block), and arthritis. Disseminated infection and late manifestations of Lyme disease may require longer courses of antibiotics, and there is often a greater lag time to symptom resolution compared with early disease. Doxycycline is the antibiotic of choice in non-pregnant adults and children aged 8 years and older because it is also effective against *A. phagocytophilum*, which may occur with early Lyme disease.[129] Amoxicillin and cefuroxime axetil are acceptable alternatives for the treatment of EM, facial palsy, and other non-neurologic manifestations of Lyme disease. Macrolide antibiotics are less effective than other antimicrobials and should be used only in individuals who cannot take doxycycline, amoxicillin, or cefuroxime axetil. First-generation cephalosporins are not effective therapy for Lyme disease.

Documented nervous system involvement (other than isolated facial palsy) and symptomatic cardiac involvement are the two main indications for intravenous antibiotic therapy.[129] Ceftriaxone administered intravenously for 2 to 4 weeks is the preferred anti-microbial, with parenteral cefotaxime or penicillin G acceptable alternatives. There is increasing evidence, however, that oral doxycycline, which is well absorbed and has a high CNS penetration, may be effective for meningitis or radiculopathy.[130-132] Lumbar puncture is recommended in individuals with cranial nerve palsies who have symptoms of meningeal irritation because a CSF pleocytosis would be an indication to treat with intravenous therapy. Asymptomatic CSF pleocytosis can occur in the setting of facial palsy and is not an indication for intravenous therapy. Repeat treatment is not recommended for chronic neurologic abnormalities, unless objective signs of relapse are present.

Patients with symptomatic cardiac involvement (chest pain, shortness of breath, syncope) or with significant conduction system disease (first-degree atrioventricular block with P-R intervals ≥0.3 msec, or second-degree or third-degree block) should be hospitalized for cardiac monitoring and intravenous antibiotic therapy. Consultation with a cardiologist is recommended, and placement of a temporary pacemaker may be necessary. Oral antibiotics can be substituted for intravenous antibiotics at the time of hospital discharge to complete the course of therapy.[129]

For arthritis, a 1-month course of oral doxycycline or amoxicillin is recommended, with a repeat course of oral therapy if inflammation does not resolve.[129] For patients with moderate-to-severe joint swelling after a 1-month course of oral antibiotics, intravenous ceftriaxone for 2 to 4 weeks can be used.[133] Longer courses of antibiotics for unresolved inflammation provide no additional benefit.[104] In this

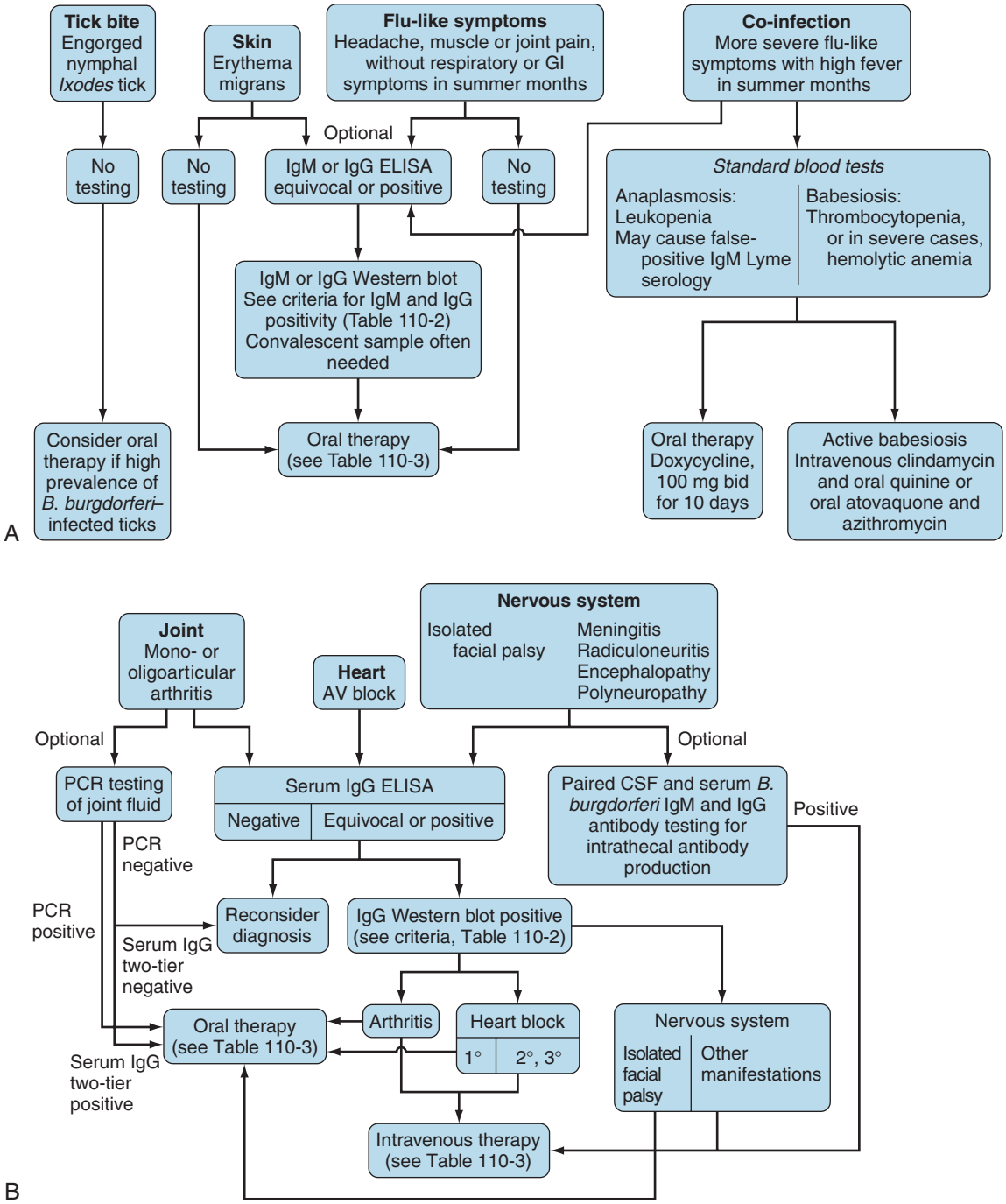



**Figure 110-3** Algorithms for diagnosis and treatment of Lyme disease for people living in or with recent (≤1 month) travel to endemic areas. **A,** Algorithm for evaluation and management of early Lyme disease. **B,** Algorithm for evaluation and management of later organ involvement in Lyme disease. AV, Atrioventricular; ELISA, enzyme-linked immunosorbent assay; GI, gastrointestinal; PCR, polymerase chain reaction. *(From Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease.* J Clin Invest *113:1093–1101, 2014.)*

situation, NSAIDs and hydroxychloroquine are recommended for treatment of antibiotic-refractory Lyme arthritis. In rare patients who do not respond to this approach, other disease-modifying anti-rheumatic drugs such as methotrexate and TNF inhibitors have been used with success. Arthroscopic synovectomy is curative in two-thirds of patients who do not respond to medical management.[134] Intra-articular corticosteroids may be associated with a higher rate of antibiotic unresponsiveness if instituted before antibiotic therapy. Use of intra-articular steroids once antibiotic therapy has commenced can accelerate resolution of inflammation and reduce the incidence of antibiotic-refractory arthritis in children.[135]

## Pregnancy and Lyme Disease

Pregnant and lactating women with Lyme disease can be treated with the same antibiotic regimens recommended for non-pregnant patients except that doxycycline should be avoided.[129] Maternal-fetal transmission of *B. burgdorferi*

**TABLE 110-3** Recommended Treatment of Lyme Disease*

| Manifestation | Drug | Adult Dosage | Pediatric Dosage | Duration (Range) |
|---|---|---|---|---|
| Erythema migrans (recommended) | Doxycycline† | 100 mg orally twice a day | <8 yr—not recommended<br>≥8 yr—4 mg/kg/day in 2 divided doses (max 100 mg/dose) | 14 days (10-21 days) |
| | Amoxicillin | 500 mg orally three times a day | 50 mg/kg/day in 3 divided doses (max 500 mg/dose) | 14 days (14-21 days) |
| | Cefuroxime axetil | 500 mg orally twice a day | 30 mg/kg/day in 2 divided doses (max 500 mg/dose) | 14 days (14-21 days) |
| Erythema migrans (alternative)‡ | Azithromycin | 500 mg orally daily | 10 mg/kg qd (max 500 mg/day) | 7-10 days |
| | Clarithromycin | 500 mg orally twice a day (if patient is non-pregnant) | 7.5 mg/kg bid (max 500 mg/dose) | 14-21 days |
| | Erythromycin | 500 mg orally four times a day | 12.5 mg/kg qid (max 500 mg/dose) | 14-21 days |
| **Acute Neurologic Disease** | | | | |
| Cranial nerve palsy§ | Same as oral regimens for erythema migrans | | | 14 days (14-21 days) |
| Meningitis or radiculopathy‖ | Ceftriaxone | 2 g IV daily | 50-75 mg/kg IV daily in single dose (max 2 g/day) | 14 days (10-28 days) |
| (Alternative IV) | Cefotaxime | 2 g IV every 8 hrs | 150-200 mg/kg/day IV in 3-4 divided doses (max, 6 g/day) | 14 days (10-28 days) |
| | Penicillin G | 18-24 million U/day, divided every 4 hrs | 200,000-400,000 U/kg/day divided every 4 hr (max 18-24 million U/day) | 14 days (10-28 days) |
| Cardiac disease¶ | Same as for erythema migrans *OR* | | | 14 days (14-21 days) |
| | IV regimen as for neurologic disease | | | 14 days (14-21 days) |
| **Late Disease** | | | | |
| Arthritis without neurologic involvement | Same as erythema migrans | | | 28 days (28 days) |
| Recurrent arthritis after oral regimen | Repeat oral regimen *OR*<br>IV regimen as neurologic disease | | | 14 days (14-28 days) |
| Central or peripheral nervous system disease | IV regimen as acute neurologic disease | | | 14 days (14-28 days) |

*Complete response to treatment may be delayed beyond the treatment period, regardless of the clinical manifestation, and relapse may recur. Patients with objective signs of relapse may need a second course of treatment.

†Tetracyclines are relatively contraindicated in pregnant or lactating women and in children younger than 8 years.

‡Due to their lower efficacy, macrolides are reserved for patients who are unable to take or who are intolerant of tetracyclines, penicillins, and cephalosporins.

§Patients without clinical evidence of meningitis may be treated with an oral regimen. The recommendation is based on experience with seventh cranial nerve palsy. Whether oral therapy would be as effective for patients with other cranial neuropathies is unknown; the decision between oral and parenteral therapy should be individualized.

‖For non-pregnant adult patients intolerant of β-lactam agents, doxycycline 200-400 mg/day orally (or IV if unable to take oral medications) in 2 divided doses may be adequate. For children aged 8 years and older, the dosage of doxycycline for this indication is 4-8 mg/kg/day in 2 divided doses (maximum daily dosage of 200-400 mg).

¶A parenteral antibiotic regimen is recommended at the start of therapy for patients who have been hospitalized for cardiac monitoring; an oral regimen may be substituted to complete a course of therapy or to treat outpatients. A temporary pacemaker may be required for patients with advanced heart block.

does occur,[136] but in contrast to syphilis in pregnancy, there is no evidence that the organism causes a congenital syndrome.[137,138] Pregnant patients should be reassured that with recommended therapy for Lyme disease, *B. burgdorferi* infection in the mother should not cause harm to the fetus.[138]

## OUTCOME

Most patients treated for Lyme disease with recommended courses of antibiotics resolve the disease.[139,140] Some patients may experience a Jarisch-Herxheimer reaction, a self-limited worsening of symptoms within 24 to 48 hours of initiation of antibiotic therapy.[141] Within the first week of treatment, patients may rarely show evolution of disease, such as the development of new EM lesions or facial nerve palsy, but these signs should improve while therapy progresses. Most patients with Lyme arthritis experience resolution of joint inflammation after a 1-month course of oral antibiotics, and less than 10% of patients progress to antibiotic-refractory arthritis.[104] Two recent reports from Europe evaluated the short-term (6 months) and long-term (median, 5 years) outcomes of children who had Lyme neuroborreliosis.[142,143] Overall, complete clinical resolution was seen in the majority during the long term, although mild residual deficits occurred in some (incompletely resolved facial palsy or mild motor or sensory deficits). Importantly, nonspecific symptoms, such as fatigue, headache, and cognitive issues, were no more frequent in patients who had a

history of neuroborreliosis than in age-matched controls, and residual deficits did not affect school performance.

Subjective complaints of fatigue and musculoskeletal pain may persist for months after treatment for Lyme disease.[144] Objective, nonprogressive signs are likely to be the result of irreversible tissue damage, and further antibiotic therapy does not seem to be beneficial.[145-148] When patients complain of persistent pain and fatigue, evaluation for coinfection with *B. microti* or *A. phagocytophilum* should be performed.[149] Patients with coinfection tend to be more symptomatic at presentation and can have a delayed resolution of symptoms compared with patients with Lyme disease alone. The impact of coinfection with the newly emergent *Ixodes* tick-borne relapsing fever spirochete *Borrelia miyamotoi* on Lyme disease is not yet known.[150,151]

## Chronic Lyme Disease and Post–Lyme Disease Syndrome

There is a great deal of controversy over the potential for Lyme disease to cause life-altering chronic morbidity in patients. It is unusual to have objective signs after recommended antibiotic regimens for Lyme disease diagnosed according to the guidelines discussed earlier, and when such signs (e.g., Lyme arthritis) are present, further antibiotic therapy does not alter outcome.[139] Recurrent EM after treatment for an initial episode of Lyme disease is caused by reinfection rather than resurgence of the original infection.[152] Even when objective signs are present, they are usually nonprogressive (e.g., residual facial weakness after facial palsy) or resolve with time (as is the case for Lyme arthritis). Use of the term *chronic Lyme disease*, which implies ongoing infection, for nonprogressive signs and symptoms after Lyme disease is not valid.

A few patients treated for Lyme disease may have fatigue, musculoskeletal pain, and complaints of memory impairment despite conventional or prolonged courses of antibiotic therapy, a condition referred to as *post–Lyme disease syndrome*.[145,153] In several controlled, population-based cohort studies that used validated standardized measures of outcomes (e.g., Short Form-36), patients with Lyme disease had more joint pain, symptoms of memory impairment, and worse functional status because of pain compared with controls.[154-156] These complaints could not be documented by abnormalities on physical examination or by neurocognitive testing, however, and a follow-up study showed that quality-of-life measures improved with time.[157] Psychological factors and the presence of psychiatric comorbidity in patients with Lyme disease correlate with poor functional outcomes after treatment.[158] A European study demonstrated that the frequency of nonspecific symptoms at 6 and 12 months after treatment for early Lyme disease was no greater than that of the control group, which included family members without a history of Lyme disease.[159,160] Children are less likely than adults to have persistent complaints after treatment for Lyme disease.[156]

Two randomized, double-blind, placebo-controlled trials of antibiotic therapy were conducted on seropositive and seronegative patients with chronic symptoms (>6 months) after treatment for Lyme disease.[146] Patients were randomly assigned to receive either intravenous ceftriaxone for 1 month followed by 2 months of oral doxycycline or matched intravenous and oral placebos. An interim analysis resulted in termination of the study because no differences in outcome between groups receiving antibiotics or placebo were found, and evidence of ongoing infection could not be documented. Another trial of antibiotics for post-treatment Lyme disease symptoms found that fatigue improved in the group receiving intravenous ceftriaxone, but cognitive dysfunction did not.[147] Individuals who had positive IgG immunoblots for Lyme disease and who had not received prior treatment with intravenous antibiotics were more likely to have improvement in fatigue. Subsequently, a randomized, placebo-controlled trial of a 10-week course of intravenous ceftriaxone for memory impairment after treatment for Lyme disease did not show sustained improvement in cognitive function.[148] An open pilot study provided evidence that gabapentin may be effective in the treatment of chronic pain syndromes after Lyme disease.[161]

A growing number of patients with similar subjective complaints are being treated for months or years with antibiotics for presumed *B. burgdorferi* infection.[162-164] These patients are diagnosed with *chronic Lyme disease* even though they often have no serologic evidence of *B. burgdorferi* exposure or are considered as testing positive on the basis of IgM reactivity on immunoblot despite years of not feeling well. They typically experience only partial resolution of their symptoms with antibiotics. Occasionally, patients may have other conditions such as rheumatoid arthritis or fibromyalgia, for which therapy has been delayed because of a misdiagnosis of Lyme disease.[164] Musculoskeletal pain is common in the general population; 20% to 30% of adults complain of chronic fatigue.[165] In the absence of a clinical history with objective manifestations of Lyme disease or positive two-tiered serologic tests, definitive attribution of symptoms to *B. burgdorferi* infection cannot be made. Caution should be exercised when attributing symptomatic response to the anti-microbial effects of antibiotics. Ceftriaxone and other β-lactam antibiotics can modulate neurotransmitter activity,[166] and tetracyclines inhibit matrix metalloproteinases.[167,168] Prolonged antibiotic use is not without risk. Minor side effects are common, and serious adverse events such as biliary complications from ceftriaxone therapy or indwelling catheter–related infections occur at high enough rates to warrant only judicious use of antibiotics.[146,148,162]

# PREVENTION

The most effective way to prevent Lyme disease is to reduce exposure risk to *B. burgdorferi*–infected ticks through personal protective measures and environmental controls.[169] These measures include avoidance of tick habitats such as wooded areas, stone fences, woodpiles, and tall grass, wearing protective clothing, and performing daily surveillance and prompt removal of ticks (within 24 hours of feeding). Other effective measures include use of DEET-containing insecticide sprays, yearly application of acaricides to property to kill ticks, construction of four-poster bait stations that apply acaricides onto deer as they feed, and tall fences to prevent deer from incidentally transporting ticks to an area.

A single 200 mg dose of doxycycline (or 4 mg/kg to as much as 200 mg for children aged 8 years and older) reduces the incidence of Lyme disease after a recognized tick bite[170]

but is not routinely recommended because of the low rate of infection.[129] An FDA-approved recombinant Osp A–based vaccine to prevent Lyme disease was withdrawn because of low market demand and concern for potential vaccine-related side effects.[171,172]

## CONCLUSION

Lyme disease is a localized or systemic infection that usually manifests with skin and musculoskeletal signs and symptoms, but it can involve other organ systems, especially the nervous system and more rarely the heart. The diagnosis should be based on objective clinical findings consistent with Lyme disease and supporting serologic tests. Most patients are cured with 2 to 4 weeks of antibiotic therapy, although the time to disease resolution may be prolonged, especially for individuals in whom therapy was delayed; irreversible tissue damage may occur. A poor response to antibiotic therapy should raise concern for alternative diagnoses or coinfection with other tick-borne pathogens. Arthritis becomes refractory to antibiotics in less than 10% of patients with Lyme arthritis. Treatment with NSAIDs, hydroxychloroquine, or other disease-modifying anti-rheumatic therapies usually resolve arthritis within 4 to 5 years. Some patients treated for Lyme disease experience a post–Lyme disease syndrome of fatigue, headaches, mild memory impairment, and musculoskeletal pain. Ongoing infection cannot be shown, and controlled treatment trials show no benefit of prolonged antibiotic therapy over placebo. Referral to an academic medical center with experience in the diagnosis and treatment of Lyme disease should be considered when patients do not respond as expected to therapy.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 111

# Mycobacterial Infections of Bones and Joints



Eric M. Ruderman • John P. Flaherty

**KEY POINTS**

- Global rates of tuberculosis disease have declined in recent years because of improvements in case detection and expansion of treatment, but resistance to anti-tuberculous drugs remains a threat. Rheumatologists must remain vigilant for tuberculosis disease in an era of expanded use of TNF inhibitors and other biologic agents.
- Musculoskeletal tuberculosis typically is seen as a chronic localized infection, most commonly involving the spine, less often the hip or knee.
- Diagnosis can be very difficult and often requires biopsy for histopathology and culture of the bone or synovium; rapid diagnostic test techniques are revolutionizing the early detection of pulmonary tuberculosis and, although promising, have not yet proved reliable in bone and joint specimens.
- The tuberculin skin test (TST) can be helpful in identifying latent tuberculosis before treatment with TNF inhibitors, but it is limited by false-positive and false-negative results. IFN-γ release assays might be as sensitive as and even more specific than TSTs, but they are expensive and associated with false-positive and false-negative test results.
- Treatment requires multiple agents selected on the basis of susceptibility testing for 6 to 9 months and has been complicated by drug resistance.
- Nontuberculous mycobacteria are important pathogens to recognize in the face of biologic therapy for rheumatic diseases.

The recognition of tuberculosis (TB) and other mycobacterial infections of the musculoskeletal system has become a major challenge for rheumatologists in the United States and other developed countries. Before 1999, most rheumatologists could easily go an entire year without seeing a single case of mycobacterial infection. Such cases were rare even at academic centers, where they would likely be presented as unusual teaching cases at clinical conferences.

However, 1999 introduced the routine clinical use of TNF inhibitor therapy in the United States, and with it came an unexpected increase in TB cases seen by rheumatologists. Fortunately, the initiation of routine screening with tuberculin skin test (TSTs) and interferon gamma release assays (IGRAs) has led to a sharp decline in the number of new cases, particularly those associated with reactivation of latent infection. However, the risk of primary infection with new exposure mandates continued vigilance in these patients and potentially those on other biologic therapies. Moreover, for non-TB mycobacterial infections, there are no screening tests available, making vigilance on immunosuppressive therapy even more important.

Another major force driving the increase in mycobacterial infections is the ongoing HIV epidemic. In the pre-highly active anti-retroviral therapy era, 21% of extrapulmonary TB cases in the United States were associated with underlying HIV infection. In developing countries, the HIV pandemic has led to marked increases in osteoarticular TB coinfection.[1] These cases are distinguished by disseminated multifocal disease suggestive of hematogenous spread, rapid progression, and more common coexistence with pulmonary infection.[2,3] HIV testing should be a routine part of the workup of any patient presenting with a musculoskeletal infection secondary to *Mycobacterium tuberculosis*. The good news is that since the introduction of effective anti-retroviral therapy, the incidence of TB and nontuberculous mycobacterial infection in patients co-infected with HIV has declined sharply in the United States.

TB is an uncommon disease in the developed world. In the United States, the incidence of TB has been steadily declining since 1990, and the majority of TB cases now may be considered imported (i.e., occurring in foreign-born individuals).[4] Worldwide, TB remains one of the world's deadliest infectious diseases.[4,5] In 2013, an estimated 9 million people developed TB, and 1.5 million people died of the disease. Worldwide, it is the number two infectious disease killer after HIV. Someone in the world is newly infected with TB bacilli every second. Approximately one-third of the world's population is infected with TB, providing a reservoir that will continue to complicate its global control.[6] More alarming are increasing rates of multidrug resistant TB (MDR-TB) and emergence of extensively drug-resistant TB (XDR-TB).[7] These strains fail to respond to all first-line and most second-line TB agents. Globalization of the world economy promotes contact between populations of the developed and developing worlds. Immigrants to the United States from areas endemic for TB constitute an ongoing reservoir of patients with latent TB.

The challenge of diagnosing musculoskeletal mycobacterial infection reaches beyond the rarity of the disease. Such infections are often indolent and may lack the pain, fevers, chills, and other prominent symptoms that typically accompany bacterial infections of the musculoskeletal system. In addition, routine culture techniques will not isolate the organism, so unless the diagnosis of TB is a consideration from the outset, the diagnosis may be missed. Delayed diagnosis of mycobacterial infections of the musculoskeletal system is common and will remain without a heightened index of suspicion for these types of infections.

For all these reasons, 21st century rheumatologists need to be well informed about a group of diseases with which they may have had limited experience.

## CLINICAL SCENARIOS

To appreciate the entire spectrum of clinical problems a rheumatologist may encounter in dealing with mycobacterial infections, it is useful to think in terms of several distinct categories. Franco-Paredes[8] and colleagues provide a clinically useful division of mycobacterial infections into four major categories. The following sections reflect that division.

### Direct Involvement of the Musculoskeletal System

Musculoskeletal infections caused by mycobacteria typically present as chronic, indolent, localized involvement of the bones, spine, peripheral joints, or soft tissues that produces a focus of nonspecific pain and, less often, swelling. In infections initiated by direct tissue inoculation, the traumatic event is often trivial or remote in time from the onset of clinical disease. Diagnosis may be delayed for months to years, in part because of minimal early symptoms and attribution of those symptoms to a noninfectious disorder until disease progression and disability prompt a more aggressive diagnostic investigation.

Constitutional symptoms are typically subtle or absent, and laboratory indicators of inflammation are often normal. Synovial effusion is frequently minimal, and the fluid, if it is obtainable, shows nonspecific inflammation. Radiographic abnormalities may be delayed, although newer imaging techniques have allowed the earlier detection of abnormalities and the distinction of TB from other infections and neoplasm.[9,10] Characteristic pulmonary or extrapulmonary findings are not always present; for example, less than 50% of osteoarticular TB presents with evidence of active or past pulmonary disease. TSTs and IGRA testing may provide useful clues to the cause, but results are not invariably positive, especially in debilitated or immunosuppressed patients. Correct diagnosis is highly dependent, in most cases, on demonstration of the infectious agent by microscopic examination and culture of affected tissue.

In HIV-infected patients, mycobacterial infections are often diagnosed before the patients' $HIV^+$ status is known, sometimes leading to its recognition.[11] Atypical pulmonary TB and extrapulmonary (often multifocal) infection are common; extrapulmonary infection occurs in 60% to 70% of such cases, compared with 16% of all patients with TB.

The clinical patterns of musculoskeletal TB include spondylitis, osteomyelitis, peripheral joint infection, and soft-tissue abscess. In a series of 230 consecutive cases of TB from the pre-antibiotic era, 5.2% had skeletal involvement; the spine was affected in 60% of these cases.[12] TB of the bones and joints is spread hematogenously. The sites most commonly affected are the spine and hips, followed by the knees and wrists; other joint involvement is rare. Constitutional symptoms are unusual in musculoskeletal TB and, when present, suggest TB in other organs. Vertebral collapse because of spinal TB may initially be attributed to the more common osteoporosis-caused spinal compression fracture.

**TABLE 111-1** Causes of False-Negative Purified Protein Derivative Test

| |
|---|
| Increased age (>70 yr) |
| Steroid use (prednisone ≥15 mg/day) |
| Hypoalbuminemia (<2 g/dL) |
| Azotemia |
| Impaired cellular immunity |
| HIV infection |
| Other debilitating diseases |

TB only rarely involves skeletal muscle but must be considered in the differential diagnosis of an enlarging muscle lesion.[13-17] Isolated cases involving tendons, trochanteric bursa, and fascia lata illustrate the variety of possibilities.[18-21] Biopsy and culture are required for diagnosis. Imaging studies do not reliably distinguish TB from neoplasm.

In nonendemic areas, skeletal TB usually occurs in elderly, debilitated patients, most often in the form of solitary osteolytic lesions in the axial skeleton. The development of skeletal disease is often remote from the initial infection, which strongly implies reactivation of previous subclinical disease. Patients may have false-negative TSTs for several reasons, including long-term corticosteroid use or coexisting debilitating diseases, such as rheumatoid arthritis (RA) or chronic renal failure, that compromise resistance and produce anergy (Table 111-1).[22,23] The occurrence of spinal disease in children in nonendemic regions has mostly been eliminated by effective medical therapy of pulmonary infection.

In contrast, in endemic areas with high infectivity rates, those infected are more commonly children and young to middle-aged adults. These individuals have a higher incidence of multifocal skeletal involvement in the ribs, pelvis, vertebrae, feet, and long-bone diaphyses.[24] Bone seeding occurs through hematogenous spread, sometimes secondarily from another extrapulmonary site. When pulmonary findings are present, a miliary pattern is typical. Spread to bone may also occur from infected nodes, either by direct extension or through draining lymph channels.[25]

#### Spondylitis

The spine is the dominant site of involvement in skeletal TB, accounting for 50% to 60% of cases.[26] Between 48% and 67% of lesions occur in the lower thoracic and thoracolumbar spine in $HIV^-$ patients, whereas the lumbar spine is most commonly involved in $HIV^+$ patients.[3] Infection usually begins in the anterior subchondral bone of a single vertebra adjacent to the intervertebral disk (Figure 111-1 and Figure111-2). Progression occurs during 2 to 5 months and begins with extension from cancellous to cortical bone, and then across the disk space to adjacent vertebrae (Figure 111-3). Bone destruction may lead to vertebral collapse, which typically occurs anteriorly, resulting in gibbous deformity. Isolated neural arch involvement and intraspinal abscess may also occur.

Paravertebral abscess begins with the extension of infection under the anterior longitudinal ligament. In the

thoracic spine, this may extend into the pleural space and lung parenchyma. In the cervical region, it may spread to the posterior cervical triangle or retropharyngeal space. In the lumbar spine, a cold abscess characteristically produces lateral displacement of the psoas muscle and may dissect along its length to present as a mass in the inguinal triangle, gluteal muscle, or upper thigh. In isolated cases, a cold abscess occurs without apparent bone involvement.

A particular variant of this presentation is subligamentous TB, in which infection spreads up and down the spine beneath the longitudinal ligament, producing scalloping of multiple anterior vertebral bodies without disk involvement. This pattern is more common in the cervical spine.[27]

The clinical presentation of spinal TB usually consists of localized pain, which may be accompanied by low-grade fever, weight loss, chills, and nonspecific constitutional symptoms. Paraparesis and paraplegia have been reported in 1% to 27% of patients in various series. In comparison with pyogenic vertebral osteomyelitis, patients with spinal TB more often are seen with a prolonged clinical course, thoracic segment involvement, spinal deformity, neurologic deficit, and paravertebral or epidural masses, and more often without fever, chills, and constitutional symptoms.[27] On occasion, patients with tuberculous spondylitis are seen with chronic inflammatory-type back pain more typical of a spondyloarthropathy.[28]

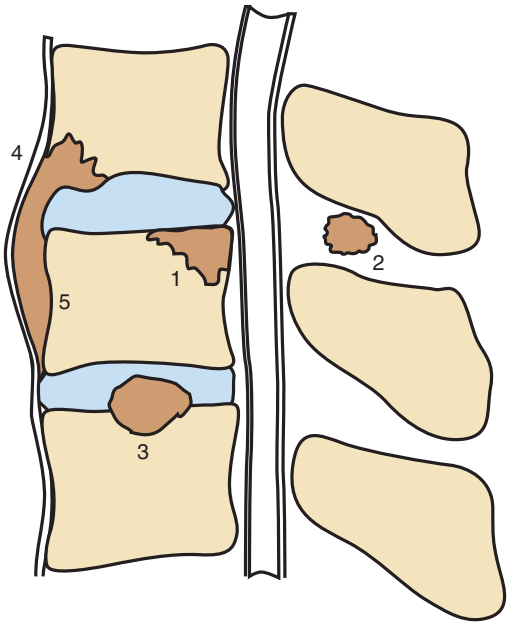



**Figure 111-1** Tuberculous spondylitis: sites of involvement. Tuberculous lesions can localize in the vertebral body *(1)* or, more rarely, in posterior osseous or ligamentous structures *(2)*. Extension to the intervertebral disk *(3)* or pre-vertebral tissues *(4)* is not infrequent; subligamentous spread *(5)* can lead to erosion of the anterior vertebral surface. *(From Resnick D:* Diagnosis of bone and joint disorders*, ed 3, Philadelphia, 1995, WB Saunders, p 2464.)*



**Figure 111-3** Tuberculous spondylitis with spinal cord compression. MRI of the lumbar spine shows destruction of contiguous vertebral bodies and an inflammatory mass pressing on the spinal cord. This patient was successfully treated with medical therapy alone.

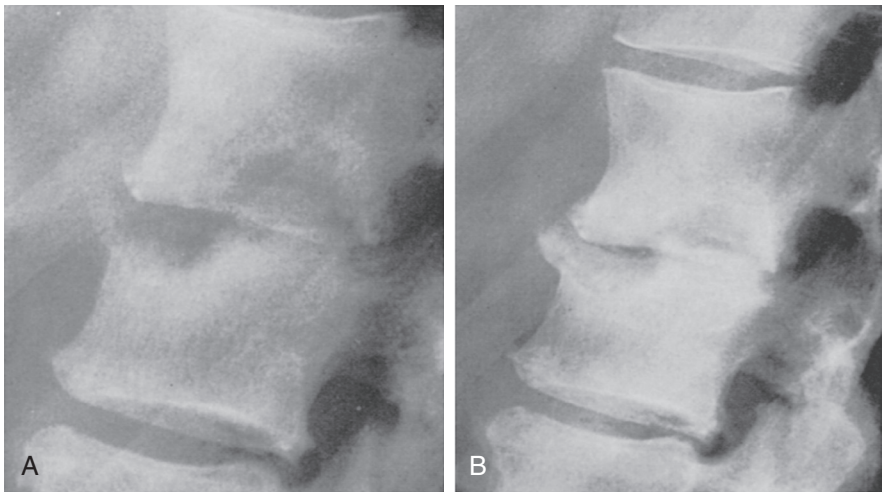



**Figure 111-2** Tuberculous spondylitis with discovertebral lesion. **A,** The initial radiograph reveals subchondral destruction of two vertebral bodies, with mild surrounding eburnation and loss of intervertebral disk height. The appearance is identical to that in pyogenic spondylitis. **B,** Several months later, an osseous response is evident. Note the increased sclerosis. Osteophytosis and improved definition of the osseous margins can be seen. *(From Resnick D:* Diagnosis of bone and joint disorders*, ed 3, Philadelphia, 1995, WB Saunders, p 2465.)*

Mycobacterial colony counts in bone biopsy specimens are relatively low. Only 40% of smears and cultures from psoas abscesses are positive. Among patients meeting strict clinical and radiographic criteria in one series, between 73% and 82% had compatible histologic features on biopsy; of these, 80% to 95% had positive culture results.[25] The differential diagnosis, which is extensive, includes pyogenic and fungal osteomyelitis, primary and metastatic tumors, sarcoidosis, multiple myeloma, and eosinophilic granuloma.

Cervical spine involvement is relatively rare, accounting for only 0.4% to 1.2% of cases of extrapulmonary TB in the United States.[29] The most common presenting symptoms are neck pain and stiffness, although hoarseness, dysphagia, torticollis, fever, anorexia, and neurologic disorders may also occur. Spinal involvement can progress to myelopathy because of delays in diagnosis. Radiographs may show characteristic osteolysis of the anterior vertebral body with sparing of the posterior portion, gibbus deformity, disk involvement, and a partially calcified paraspinous mass. CT or MRI is useful for assessing compromise of the spinal canal. Retropharyngeal infection may extend into the craniocervical junction and, if not promptly recognized, may cause atlantoaxial dislocation and neurologic complications.[30,31]

The sacroiliac joint is involved in as many as 10% of cases of skeletal TB, often without other evidence of disease.[32] Infection, and TB in particular, should be considered in all cases of unilateral sacroiliitis, particularly when other features of a spondyloarthropathy are absent. Emigration from an endemic area and a past history of TB increase the likelihood of this cause. Buttock pain on the involved side is the patient's presenting symptom and is often accompanied by proximal leg or radicular pain. Examination reveals sacroiliac tenderness to palpation and stress maneuvers. Sacroiliac films show joint widening and erosion. An elevated erythrocyte sedimentation rate (ESR) and anemia are common, and a positive tuberculin reaction is typical. Biopsy of the sacroiliac joint shows granulomatous histologic features or nonspecific inflammation and a positive culture in most cases.

Atypical spinal lesions, which occur in approximately 10% of cases, may lead to delayed diagnosis and treatment. Atypical radiographic presentations in a single vertebra include concentric collapse, sclerotic foci, and selective involvement of the vertebral arches and costotransverse joints. Multiple vertebrae may be involved, either in continuity or as skipped lesions. Atypical clinical presentations may suggest a herniated intervertebral disk, failed back syndrome, spinal tumor, or a meningeal granuloma.[26,33]

### Tuberculous Osteomyelitis

Bone lesions begin with hematogenous implantation of organisms in the medullary area. Metaphyseal involvement is most common, and lesions may spread through the growth plate to involve the adjacent joint, usually late in the disease course. Lesions are typically destructive. Lytic lesions in unusual areas, such as the pubic symphysis, sacroiliac joint, and elbow, can be misdiagnosed as malignancy.[34] Osteomyelitis may occur in a bone or joint that has been previously exposed to trauma.

Tuberculous osteomyelitis occurs in both children and adults.[35] Although any bone can be involved, the femur and tibia are most commonly affected. Dactylitis may also occur in children. In one large series from an endemic area, such cases represented 19% of bone and joint TB and 15% of cases of osteomyelitis of hematogenous origin.[36] Bone pain was the most common presentation; a draining sinus, abscess formation, and local swelling and tenderness were also common. The average delay before diagnosis was 28 months.

Multifocal osteoarticular TB is a less common variant of the disease,[36,37] but TB should be considered in all patients from endemic areas who are seen with multiple destructive skeletal lesions.

For a definitive diagnosis of osteoarticular TB, a biopsy specimen of an affected site must be obtained.[19] Soft-tissue lesions characteristically demonstrate rim enhancement on CT examination. CT may also facilitate percutaneous needle biopsy or abscess drainage.[38] Histologic examination generally reveals granulomatous inflammation. In one series of 121 cases, biopsy showed a positive culture in 33%, granulomatous histologic features in 46%, and both in 21%.[39] Radiographic findings include cavity formation with a thin adjacent layer of sclerosis in about 50% of cases, sometimes containing a sequestrum. The true extent of bone involvement may be difficult to detect because of clinically silent lesions. Bone imaging with technetium 99, although more sensitive than conventional radiographs, may provide false-negative results in early, indolent, or highly destructive disease. Tuberculin test reactions are positive in more than 80% of cases.[40]

Treatment with anti-tuberculous therapy is generally effective. In a minority of cases, surgical debridement is required for healing. Initiation of therapy on the basis of histologic findings is appropriate pending culture results. Sinus cultures are commonly positive for pyogenic bacteria both before and after anti-tuberculous therapy, but these are presumed to be contaminants. Healing is associated with sclerosis at the margin of lesions. Misdiagnosis of the condition as pyogenic osteomyelitis may lead to unnecessary surgery or to delayed anti-tuberculous treatment, resulting in extension of infection into the joint and chronic disability.

### Septic Arthritis

The typical pattern of tuberculous joint involvement is a monoarticular arthritis involving the large joints, most commonly the hip and knee (Figure 111-4 and Figure 111-5).[41,42] Other joints less commonly involved include the sacroiliac, shoulder, elbow, ankle, carpal, and tarsal joints. Infection begins in the synovium, with progression of destructive changes being slower than in pyogenic septic arthritis. Prosthetic joint infection with M. *tuberculosis* has been reported, and is usually caused by local reactivation of latent disease.[43]

The diagnosis of tuberculous arthritis is often missed. A consecutive series spanning the years 1970 to 1984 emphasized typical features.[44] Of 23 cases of musculoskeletal TB, 9 involved the spine, 1 the hip, and the remaining 13 the peripheral joints. Most patients were men older than 50 years. The history of TB or exposure was generally

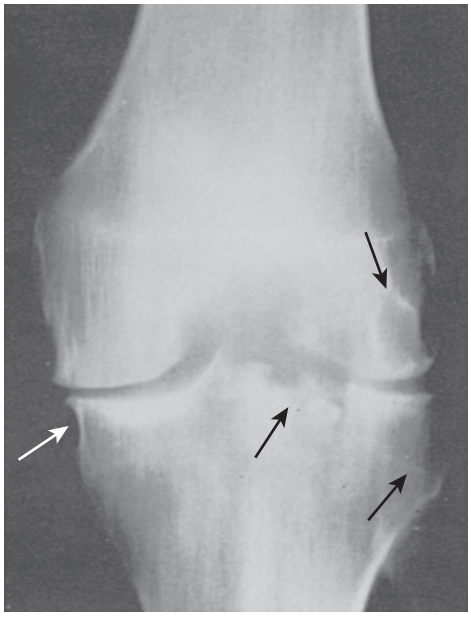


**Figure 111-4** Tuberculous arthritis of the knee. On conventional computed tomography, typical marginal and central osseous erosions *(arrows)* accompany tuberculous arthritis. Osteoporosis is not prominent. *(From Resnick D:* Diagnosis of bone and joint disorders*, ed 3, Philadelphia, 1995, WB Saunders, p 2480.)*



**Figure 111-5** Tuberculous arthritis following total hip replacement. A sinus tract emerges from the scar following total hip arthroplasty for childhood destructive arthritis of unknown cause. The young man was originally from Vietnam.

forgotten. In all cases, presenting symptoms were joint pain and swelling. Four patients had evidence of active pulmonary TB, and in two patients with sterile pyuria, M. *tuberculosis* grew from the urine. Only 5 of 10 patients tested had a positive TST. Radiographs showed changes of erosive arthritis in 7 and no changes in 4 of 11 joints studied. The median delay in diagnosis was 8 months.

Arriving at a correct diagnosis requires vigorous pursuit and usually a synovial biopsy and culture. Initial studies are often misleading and may contribute to delayed diagnosis or misdiagnosis. Synovial fluid findings are variable and do not distinguish this arthropathy from other inflammatory or septic arthritides.[45] Cell counts more often suggest bland inflammatory, rather than septic, arthritis and may contain a preponderance of neutrophils. The diagnosis may be facilitated if the organism is observed on an acid-fast smear of synovial fluid, but only 10% to 20% of reported cases are positive. In contrast, synovial fluid cultures are frequently positive. Radiographic changes are similar to those seen in other septic arthritides, beginning with juxta-articular bone demineralization and progressing to marginal bone erosion and articular cartilage destruction (see Figure 111-4).

With the open-biopsy technique, granulomatous histologic features and positive cultures are present in more than 90% of cases.[45] Experience with direct amplification tests in mycobacterial arthritis is limited and suggests that they have limited sensitivity but good specificity (63% and 100% in one small case series.[46] The histologic examination alone may be confusing, because granulomatous synovitis can also be found in nontuberculous mycobacterial infection, sarcoidosis, erythema nodosum, brucellosis, Crohn's disease, and foreign-body reaction. As noted, synovial acid-fast smear is of limited value. Tuberculous arthritis is also reported in children, sometimes early in the disease course; synovial biopsy and culture are recommended in children with monarthritis and a TST or IGRA.[47]

TB must be considered among the possible causes of septic arthritis occurring in patients with pre-existing RA, although it is not widely seen in developed countries.[48,49] Conversely, rheumatoid factor may be present in TB, leading to diagnostic confusion in the presence of chronic monarthritis.[48]

## Emergence of Tuberculosis During the Treatment of Rheumatic Diseases

Many patients with systemic rheumatic disease have dysregulated immune systems that are treated with immunosuppressive drugs. Such an impaired immune response may permit the reactivation of latent TB. TNF plays a key role in granuloma formation and stabilization, which promotes the containment of M. *tuberculosis*. The rate of TB among patients with RA before the widespread use of TNF inhibitors was approximately 6 cases per 100,000,[50] although some studies have suggested that the rate in RA is higher than in the general population.[51] In one large patient registry, the estimated incidence of TB associated with infliximab in RA patients was in excess of 1000 per 100,000 person-years of exposure during the years 2000 to 2001.[52] In the United States, the rate was 52 per 100,000 person-years during the same time period.[50] Etanercept may be associated with a lower risk for TB reactivation than infliximab: 10 cases per 100,000 person-years of exposure with etanercept, versus 41 per 100,000 person-years with infliximab, according to an analysis of Food and Drug Administration data.[53] Similarly, British and French registries have identified a greater risk for TB associated with the use of adalimumab and infliximab than with etanercept.[54,55] There are fewer data available for the newer TNF antagonists, and comparisons are

complicated by the widespread use of pre-treatment screening instituted after the first agents in this class were approved for use. Monoclonal antibodies to TNF may play a greater role in destabilizing granulomas than soluble TNF receptors. Nonetheless, similar precautions should be taken with any TNF antagonist and all should be presumed to increase the risk of both new infection and reactivation of latent infection.

Tocilizumab (which blocks IL-6), golimumab, and certolizumab have been associated with reports of TB disease, but because these cases occurred in TB-endemic countries, infection may have been acquired during treatment. Nevertheless, the labeling for all three agents recommends screening before therapy. Other biologic agents may pose a lower risk for reactivation of TB, because the mechanisms of action are not intimately involved with host defense related to intra-cellular organisms. Animal studies have suggested that abatacept does not impair the ability of mice to respond to M. *tuberculosis*, but this has not been confirmed in humans.[56] Little or no evidence indicates that B cells play a major role in containing TB, and there does not appear to be an increased risk of TB following treatment with rituximab.[57]

Glucocorticoid use also poses a significant hazard for patients with latent TB. Many mechanisms account for this effect, including impairment of cellular immune responses and monocyte chemotaxis and function, including the monocyte's production of TNF. The use of glucocorticoids has been associated with a fivefold increased risk for TB in a case-control study involving a large general practice database in the United Kingdom.[58] The risk appeared to be dose related but was seen even at the physiologic dose of prednisone, 7.5 mg/day. Just as with TNF inhibitors, the risk for TB was greatest early in the course of treatment. In a French study, prednisone at 10 mg/day or more but not lower doses, was associated with an increased risk for TB.[55]

Reactivation of TB in the face of TNF inhibitor therapy typically occurs within 6 months of treatment and often manifests as extrapulmonary disease. Institution of a protocol to screen and treat for latent TB infection (LTBI) in patients with RA before TNF antagonist administration resulted in a 78% decrease in TB disease.[59] Treatment of LTBI can effectively and safely lessen the likelihood of TB disease in patients treated with TNF antagonists. Indeed, it has been suggested that much of the blame for the development of TB disease in this situation can be attributed to incomplete compliance with screening protocols.[60] Conversely, in a report of 84 patients at a single center who were treated with etanercept after demonstrating a positive TST, no cases of TB disease were seen during a mean of 2 years of follow up despite the fact that 78 of 84 patients were prescribed treatment and only 52 completed the prescribed treatment regimen.[61] Although screening for LTBI has reduced the incidence of TB disease, false-negative TSTs and false-negative and indeterminate IGRAs can undermine such good intentions (see Table 111-1).

The approach to a patient with latent TB who needs a TNF inhibitor has not been standardized. Many authorities recommend that patients with positive TSTs or IGRAs and normal chest radiographs who have never been treated for TB receive at least 1 to 2 months of therapy before beginning a TNF inhibitor, if for no other reason than to be certain that they will be able to tolerate the full course of latent TB therapy. Appropriate regimens include 9 months of isoniazid, 4 months of rifampin, or 3 months of directly observed weekly isoniazid plus rifapentine. Patients discovered to have active TB should receive a complete course of standard anti-tuberculous therapy before initiating a TNF inhibitor.

Although TNF inhibitors and steroids are predominant risk factors for TB, all patients with rheumatic disease treated with immunosuppressive therapy that impairs cellular immunity should be considered at risk. This is especially true in the elderly, the malnourished, and immigrants from countries with high endemic rates of TB.

## Rheumatic Disorders Precipitated By the Treatment of Tuberculosis

A variety of rheumatic conditions may be precipitated by the drugs used in the treatment of TB. These include drug-induced lupus caused by isoniazid (INH) and rifampin. As with other cases of drug-induced lupus, they are associated with positive anti-nuclear antibodies and the presence of anti-histone antibodies. Typically, these patients follow a benign course, with resolution of disease after the drug is discontinued.

Arthropathy and tendinopathy have been described with the use of fluoroquinolones, especially ciprofloxacin and levofloxacin. The risk of tendon rupture (usually the Achilles tendon) is greatest in patients older than 50 years and increases with the concomitant use of corticosteroids.

Pyrazinamide interferes with the renal tubular excretion of uric acid and has been associated with the development of hyperuricemia and gout in adults.

Paradoxical reactions have been described in patients withdrawn from infliximab following the diagnosis and initiation of treatment for TB disease.[62] These reactions present as a worsening of pre-existing TB lesions or the development of new lesions not attributable to the normal course of disease in a patient initially improving on treatment. The mechanism for such reactions is not completely understood but has been categorized within the spectrum of immune reconstitution inflammatory syndromes. Such reactions are more common in patients with HIV following the initiation of anti-retroviral therapy. After infliximab therapy is discontinued, its anti-inflammatory effects persist for 3 or 4 weeks which may explain the timing of this delayed reaction. No changes in anti-tuberculous therapy are necessary when a paradoxical reaction occurs; corticosteroids may ameliorate this response.

## Reactive Immunologic Phenomena in the Setting of Tuberculosis

A variety of reactive immunologic phenomena have been associated with M. *tuberculosis* infection. These are uncommonly found in clinical practice.

Poncet's disease is an aseptic inflammatory polyarthritis that occurs in the presence of TB disease. Although any joints can be involved, the most commonly affected are the knees, ankles, and elbows.[63] The mechanism is thought to be similar to other forms of reactive arthritis secondary to remote infection. Most cases resolve after satisfactory

treatment of the TB. Reactive arthritis has been described following the intravesicular instillation of bacille Calmette-Guérin (BCG) vaccine for bladder cancer.[64]

Other seldom-encountered immune reaction patterns described with *M. tuberculosis* include erythema nodosum, erythema induratum, and amyloidosis (AA type).

# DIAGNOSIS

## Tuberculin Skin Test

The TST—also called the *purified protein derivative* (PPD)—has been in use for nearly a century and remains the most widely used screening test for TB. It is routinely administered in rheumatology offices that prescribe TNF inhibitors. The test represents a crude mix of antigens from *M. tuberculosis* and is plagued by both false-positive and false-negative results. It is unable to distinguish latent infection from active disease and may be negative in the face of severe active TB. Corticosteroids (≥15 mg/day of prednisone) may render the TST negative in people with latent TB. Elderly and malnourished patients may not exhibit a positive TST. Table 111-1 provides a partial list of causes for a false-negative TST that are of special interest to rheumatologists.

False-positive results may likewise occur in the case of infection with nontuberculous mycobacteria or previous BCG vaccination. TST positivity degrades with time in BCG-vaccinated patients, at a variable rate. The degree of positivity is influenced by a number of factors, including the number of BCG vaccinations and the number of subsequent TSTs performed. Some patients may retain a response as long as 15 years after BCG vaccination. However, a TST result of 20 mm or greater is rarely the result of BCG. In addition, if faced with a high-risk situation, such as the initiation of TNF inhibitor therapy, one should probably make a presumption of LTBI even if the diameter of induration measures as little as 5 mm.

The important message for rheumatologists is that the TST is an important but imperfect screening tool for latent TB infection, with a sensitivity and specificity in the range of 70%. A negative TST should not eliminate the clinician's vigilance in monitoring patients treated with TNF inhibitors for reactivated or new-onset TB, especially in high-risk populations.

### Interferon-γ Release Assays

The limitations of the traditional PPD skin test in terms of specificity and sensitivity in identifying latent TB have led to the development of new T cell–based testing. These assays measure the production of IFN-γ by whole-blood mononuclear cells stimulated by specific *M. tuberculosis* antigens. The IGRAs, including the Quanti-FERON Gold In-Tube (Cellestis, Inc; Brisbane, Australia) and TSPOT. TB (Oxford Immunotec, Inc; Oxford, England) assays, appear to have equivalent sensitivity and improved specificity when compared with TST.[65,66] These assays contain specific *M. tuberculosis* antigens and are less likely to result in a positive test in people exposed to nontuberculous mycobacteria, including *Mycobacterium avium* complex or the attenuated *Mycobacterium bovis* strain in the BCG vaccine. They also offer the opportunity to circumvent operator error with TST administration and do not require patients to return in 48 to 72 hours to have the test interpreted. IGRAs have replaced the TST in many centers for routine screening for LTBI. Studies of IGRAs in patients with RA and other inflammatory disorders have found them to have comparable or better accuracy than the TST.[67-79]

IGRAs are not without concerns. Indeterminate results from IGRAs in this setting have ranged from 1.2% to 28.6%, raising concerns about the performance of the assay from center to center.[80,81] Within-subject result variability is an issue, especially when the TB antigen response is near the cut-off value.[82,83] Factors that might contribute to IGRA result variability include reagent variability, blood volume, whether the blood tube is shaken gently or vigorously, delays in processing, and PPD-induced boosting of IFN-γ response.[84] Positive IGRAs are more closely related to TB risk factors than are positive TSTs.[85] Discordant $IGRA^-/TST^+$ results are associated with prior BCG vaccination, and discordant $IGRA^+/TST^-$ results are associated with corticosteroid therapy, suggesting that the former represents a false-positive TST and the latter represents a false-negative TST. Nevertheless, the meaning of a discordant result is uncertain enough that patients with a positive test result with either IGRA or TST should be considered for therapy if possible LTBI.[86] Additional experience will be required to provide confidence that a negative IGRA in the setting of a positive TST reliably excludes LTBI. If only one testing modality is utilized, one recent analysis found that use of an IGRA was more cost effective than the TST for patients screened before TNF inhibitor therapy.[87]

IGRAs have also been studied in patients receiving a TNF inhibitor to identify newly acquired TB infection. Many authorities recommend ongoing annual monitoring for new TB infection in patients treated with TNF inhibitors, especially in highly endemic areas. Rates of TST conversion in patients on TNF inhibitor therapy have been reported to be 33% and 37% from centers in Korea and Taiwan; corresponding rates of IGRA conversion were 14% and 11%, respectively, and appeared to better correlate with the risk of active TB.[88,89] Pending formal guidelines, in regions where ongoing exposure to infectious TB may occur, routine monitoring for TB on therapy—perhaps annually—is wise and the use of an IGRA rather than a TST may limit the number of patients requiring preventative treatment.

## Imaging

Although imaging patterns suggestive of TB have been discussed, there are no pathognomonic skeletal radiographic features that can establish the diagnosis. Early features on radiographs may be equivocal or nonexistent. Chest radiographs are often normal or fail to show the typical posterior upper lobe cavitary infiltrates characteristic of TB.

Conventional radiography is generally a useful approach for defining bone destruction, the extent of disease, and adjacent soft-tissue lesions.[52] MRI is more effective in identifying early disease; it may help distinguish TB from other infections and neoplasm and can aid in evaluating the extent of disease.[10,90] Scintiscans with technetium and gallium may also be helpful in localizing bone and

soft-tissue lesions, but early false-negative findings are not uncommon.[91] CT can be helpful in guiding diagnostic needle biopsy. Fine-needle biopsy is an acceptable alternative to core-needle biopsy and open biopsy for the diagnosis of osteoarticular TB in both the axial and peripheral skeleton, and it has the advantage of obviating general anesthesia.[92,93] Both CT and MRI may also be helpful in monitoring therapy.[94]

## Culture

Nearly all species of *Mycobacteria* are slow growing, with M. *tuberculosis* the slowest. Other bacteria may rapidly outgrow mycobacteria if the specimen is not inoculated on special isolation media. The small number of mycobacteria found in clinically infected areas further challenges the ability to confirm mycobacterial infection and accounts for the low yield of positive Ziehl-Neelsen staining (10% to 20%) in synovial fluid and other biologic fluids.

Synovial fluid and other body fluids are less likely than tissue to yield a positive culture. If a joint is suspected of harboring TB, a synovial biopsy should be obtained. Arthroscopically derived tissue yields higher positive cultures than needle biopsies. CT-guided needle aspiration and biopsy can provide invaluable information in the case of spinal involvement. Characteristic features on tissue pathology—caseating or noncaseating granulomas ± acid-fast bacilli on special stains—may allow an early presumptive diagnosis of TB pending culture results, which may take 4 to 6 weeks to be finalized.

### Polymerase Chain Reaction

Molecular diagnostics by using polymerase chain reaction (PCR) have proved very helpful in the rapid diagnosis of pulmonary TB, demonstrating a high yield even in patients with sputum smear-negative pulmonary TB, and are now endorsed for routine use by the WHO.[95] The role of molecular diagnostic assays in extrapulmonary TB is still being defined, but the limited preliminary experience has demonstrated promise. In a small cohort of patients with vertebral osteomyelitis, multiplex PCR testing of bone biopsy specimens demonstrated 90% sensitivity and 100% specificity for tuberculous infection, illustrating the potential for this technology to provide rapid and accurate diagnosis. Similarly, multiplex real-time PCR successfully distinguished TB from brucellosis as the cause of vertebral osteomyelitis, demonstrating a sensitivity 87% and specificity of 100% in the diagnosis of TB.[96] Additional experience is necessary before these assays are the standard for diagnosis of bone and joint TB.

# TREATMENT

Appropriate management of tuberculous infections of bones and joints is complex. Selection of appropriate antituberculous agents and ongoing disease monitoring should involve comanagement with an infectious disease consultant. Nonetheless, rheumatologists should be familiar with the basic treatment principles.

Treatment of M. *tuberculosis* infections that involves the musculoskeletal system consists of the same combination chemotherapy regimens that are effective in pulmonary TB. Treatment regimens for infections of the bone and spine are typically administered for longer durations than those recommended for lung disease and other extrapulmonary sites. These longer treatment courses for musculoskeletal disease have been recommended based on poor tissue penetration of anti-tuberculous agents into osseous tissues and the higher rates of relapse associated with these infections.

Current guidelines on the treatment of TB published jointly by the U.S. Centers for Disease Control, American Thoracic Society, and Infectious Diseases Society of America recommend a 6-month course of therapy for all sites except the bone (6 to 9 months) and the central nervous system (9 to 12 months).[97] Rifampin is the critical component that allows shorter-course therapy.

The standard approach to TB therapy (both pulmonary and extrapulmonary) in the United States currently includes four drugs to start: isoniazid, rifampin, ethambutol, and pyrazinamide—also known as *IREZ* therapy. Once the bacillus is confirmed to be sensitive to INH, the ethambutol can be discontinued. Pyrazinamide is administered for 2 months, and rifampin and INH are continued for the duration of therapy.

Longer courses of therapy are required for patients who are slow to respond. Radiographic features of mycobacterial disease may not change much after 6 months of treatment, and response to treatment is based mainly on clinical features, including reduction in pain, resolution of constitutional symptoms, and emergence of increased mobility. Longer courses are also advised for cases of relapse and resistant organisms.

Surgical intervention is seldom indicated for the initial management of osteoarticular TB. Possible exceptions at presentation include patients with advanced or progressive disease or spinal kyphosis of 40 degrees or greater. *Multidrug resistant tuberculosis* (MDR-TB), which refers to isolates that are resistant to isoniazid and rifampin, may also provide a relative indication for surgical debridement. Patients who have extensive joint destruction and immobility after an adequate course of chemotherapy may also be candidates for surgery.

Following successful antibiotic therapy, arthroplasty of the hip and knee may be undertaken and is usually successful. Recurrence of disease in the prosthetic joint is less likely if the surgery is performed years after the infection, and the tissue obtained at surgery is culture negative. This may be impractical, however, when a patient is unable to ambulate following an adequate course of therapy. In such cases, the TB therapy is continued through surgery and for at least 3 months post-operatively. If TB recurs in the prosthetic joint, it can sometimes be managed with antibiotic therapy alone. However, in many cases, removal of the prosthesis is necessary for complete resolution of the infection.

The increasing incidence of drug-resistant TB complicates the selection of appropriate drugs.[98,99] Primary monoresistance to isoniazid occurs in approximately 8% of TB isolates in the United States. When identified, this does not substantially impact the treatment outcome. The rate of MDR-TB in the United States has been relatively stable for the past decade, 1.2%. Worldwide, 5% of TB cases were MDR-TB in 2013; 3.5% of new cases and 20.5% in previously treated individuals. The treatment of MDR-TB is

complex and often requires the use of multiple (often toxic) second-line agents for 18 to 24 months or longer. XDR-TB—that is, strains resistant to all first-line TB drugs and at least three second-line agents—has been reported by 100 countries in 2013, with particularly high rates in Kazakhstan, Iran, and South Africa.[100] On average, an estimated 9% of individuals with MDR-TB have XDR-TB. Options for treatment of XDR-TB are very limited, and the risk of treatment failure and death is high.[101]

Incomplete adherence with treatment is a particularly important risk factor for secondary drug resistance, and the likelihood of drug resistance increases following relapse after treatment. Directly observed therapy is strongly advocated to reduce the spread of infection and the frequency of drug-resistant TB.[102]

## OSTEOARTICULAR INFECTIONS CAUSED BY NONTUBERCULOUS MYCOBACTERIA

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment, including soil, water, and animal reservoirs. They are not typically spread from human to human, and infections with these organisms have been reported with increasing frequency.[103] Although the majority of infections caused by these organisms are pulmonary, skin and soft-tissue infections may be seen in healthy individuals; a localized lymphadenitis may develop in children.[104] Osteoarticular infections are usually caused by either direct inoculation of the organism or spread of contiguous infection.[104] Blunt trauma has been implicated as a risk factor in cases of vertebral osteomyelitis due to NTM.[105] Nontuberculous mycobacterial infections of the musculoskeletal system may develop in immunosuppressed individuals, but these infections are much less common than TB. In contrast to TB, nontuberculous infection is more likely to cause tenosynovitis, synovitis, or osteomyelitis and less likely to cause spinal infection. A review identified only 31 cases of vertebral osteomyelitis due to NTM reported between 1965 and 2003.[106] Although there are more than 120 species of nontuberculous mycobacteria, the majority of musculoskeletal infections are caused by *Mycobacterium marinum*, *Mycobacterium kansasii*, and *Mycobacterium avium-intra-cellulare* (also called *M. avium* complex, or MAC), but cases of musculoskeletal infection with *Mycobacterium haemophilum*, *Mycobacterium chelonae*, *Mycobacterium xenopi*, and *Mycobacterium conceptionense* have also been reported.[107-111]

Three distinct patterns of musculoskeletal involvement are reported: tenosynovitis, synovitis, and osteomyelitis.[112,113] Tenosynovitis typically is seen as chronic unilateral hand and wrist swelling (Figure 111-6A-C).[114] Synovitis typically is seen as chronic indolent asymmetric swelling in a knee, hand, or wrist. A number of species have been associated with these syndromes, and the number isolated from immunosuppressed patients is growing.[11] Predisposing factors, in addition to immunosuppression and direct inoculation, include environmental exposure and pre-existing joint disease.[115]

In one recent case series of 32 cases of mycobacterial arthritis and tenosynovitis, *M. tuberculosis* was isolated exclusively from 11 patients with arthritis of large joints, whereas NTM were isolated primarily from 17 patients with tenosynovitis and only 4 patients with arthritis of large joints.[116] But musculoskeletal infections with atypical mycobacteria may be indistinguishable from *M. tuberculosis* infections, so that correct diagnosis usually requires tissue biopsy and culture. Synovial fluid, when obtainable, is typically inflammatory, and a culture may be helpful only if specific mycobacterial cultures are requested. Identification of acid-fast bacilli on smear and granulomatous inflammation from a tissue biopsy specimen often provides direction for an appropriate microbiologic investigation, but histologic features do not consistently demonstrate granuloma formation. With a compatible clinical presentation and histologic findings, mycobacterial culture, including special techniques for

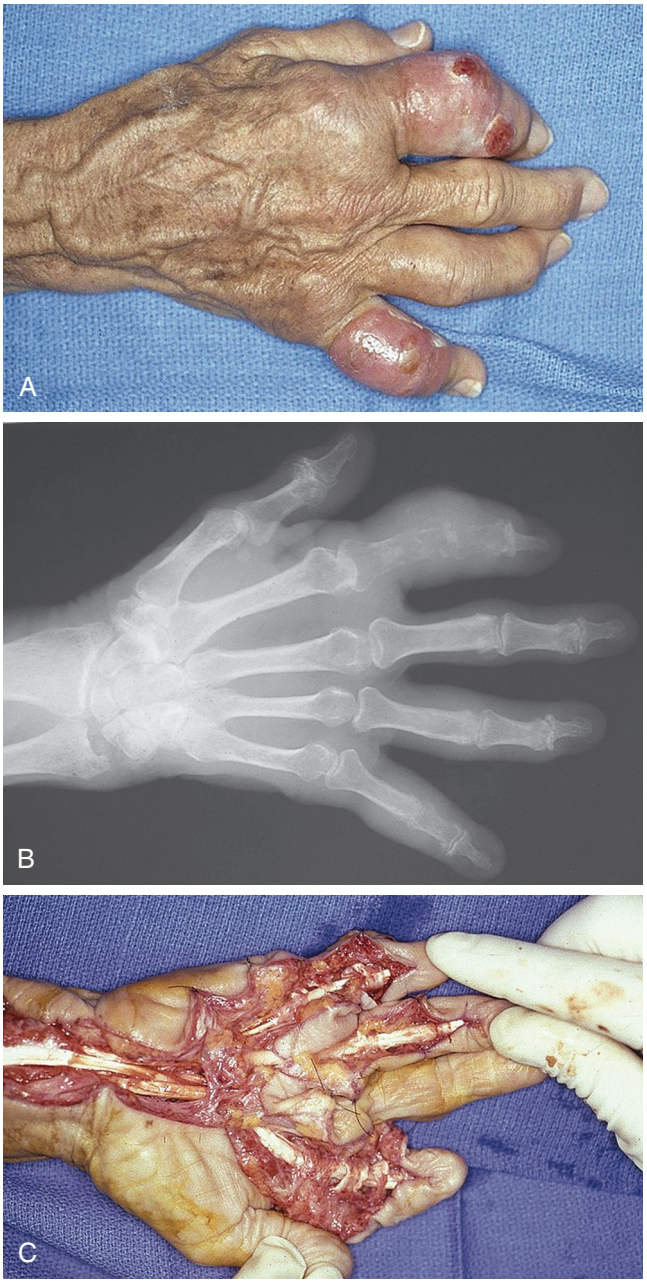



**Figure 111-6** **A,** Hand demonstrating tenosynovitis and synovitis secondary to *Mycobacterium marinum*. **B,** Radiograph of the hand in **A** demonstrates joint destruction secondary to *M. marinum*. **C,** Hand depicted in **A** and **B** shows extensive tenosynovitis at the time of synovectomy.

*M. marinum*, should be requested. Direct amplification testing may be useful for more rapid identification of mycobacterial species in tissue specimens, but data from musculoskeletal cases are limited.[117] In addition to mycobacteria, other causes of granulomatous synovitis include fungi, brucellosis, sarcoidosis, inflammatory bowel disease, and nonmetallic foreign bodies. *Mycobacteria* other than *M. tuberculosis* are responsible for a significant proportion of these cases.[118]

MAC has become the most common mycobacterial infectious agent affecting patients with HIV/AIDS, in whom it has a tendency to cause disseminated disease.[11] Fortunately, there has been a sharp decline in the incidence of MAC following the introduction of highly active antiretroviral therapy and MAC prophylaxis.

Isolation of *M. tuberculosis* is always clinically significant. In contrast, when dealing with a nontuberculous mycobacterial isolate, the clinician must judge whether it is a contaminant, represents insignificant colonization, or is the cause of disease. Certain guidelines have proven useful in this respect.[45,115,119,120]

- The illness should be consistent with one or more syndromes associated with mycobacterial infection.
- Other causes of disease, such as TB and fungi, should be excluded.
- The mycobacterial species isolated is one associated with human disease. The most significant are those that are not common environmental contaminants (*M. kansasii*, *M. marinum*, *Mycobacterium simiae*, *Mycobacterium szulgai*, *and Mycobacterium ulcerans*).
- The site of isolation of the organism should favor true infection versus contamination or colonization. (Isolation from bone biopsy, synovial tissue, or synovial fluid strongly suggests infection; isolation from the respiratory tract may represent infection or colonization.)
- Heavier growth suggests significant infection.
- Repeatedly positive cultures suggest significant infection.

Because laboratory identification of the organism and sensitivity testing may take weeks to months, initial therapy often includes multiple drugs to cover both TB and other mycobacteria. One common approach to initial empiric therapy is to link standard IREZ therapy for TB with clarithromycin until final culture results are available. The most recent American Thoracic Society guidelines for the treatment of nontuberculous mycobacteria were published in 2007.[121] Surgical debridement of infected tissue may play an important role in the treatment of selected patients, especially for resistant organisms. The most efficacious drugs remain controversial, prolonged treatment is often necessary, and relapses are not uncommon. Therapy customized to the individual patient is crucial with nontuberculous mycobacteria infection. The key to effective therapy lies in the unique characteristics of the culture and sensitivity of the particular isolate.

## EMERGENCE OF NONTUBERCULOUS MYCOBACTERIAL INFECTIONS DURING THE TREATMENT OF RHEUMATIC DISEASES

No published data exist on the risk of nontuberculous mycobacterial infections in the setting of therapy with nonbiologic disease-modifying anti-rheumatic drugs, including methotrexate. The decision to interrupt or continue therapy in such instances should be made on a case-by-case basis and should involve an infectious disease specialist, recognizing that alternative treatments, such as steroids and biologics, may carry an even greater risk.

Nontuberculous mycobacteria are increasingly recognized as important pathogens in patients receiving a TNF inhibitor. Indeed, these infections now occur twice as frequently as TB in the United States, presumably because of the use of screening tests for the latter.[122] The complexity of these infections was illustrated by a review of 239 cases of nontuberculous mycobacterial infection reported to the MedWatch database, in which only 105 were established to be probable or confirmed according to established disease criteria,[123] The most commonly reported presentation was in elderly women with RA, possibly because both nontuberculous mycobacterial infections and RA are more common in this group; most patients were also taking concomitant steroids and/or methotrexate. Infections with several different species within this group have been reported, with MAC the most common. As with TB, the risk appears to be associated with all drugs in the class, rather than a specific agent, although more infections have been reported with infliximab.[123] Extrapulmonary disease is a common finding, which is unusual for these organisms. A paradoxical response to therapy, similar to that seen with TB, has been reported with treatment of MAC following infliximab.[124] Not all patients with NTM lung disease will deteriorate on TNF inhibitor therapy, but some patients worsen despite administration of anti-NTM therapy.[125] Nevertheless, cessation of TNF inhibitor therapy should be strongly considered following the development of an atypical mycobacterial infection. Continuation or reintroduction of TNF blockers during treatment of NTM infections has been reported.[125,126]

Managing the risk of nontuberculous mycobacterial infections in the setting of TNF inhibitors treatment can be challenging. Although screening has been helpful in reducing the risk of TB infection in this setting, there is no currently available method of screening for atypical mycobacteria. Because these infections are more common in individuals with pre-existing pulmonary disease, clinicians may wish to consider more extensive evaluation of RA patients with bronchiectasis about to initiate TNF inhibitor therapy. Although MAC prophylaxis is recommended for $HIV^+$ patients with CD4 counts of 50 or less, there is no experience with, and no guidelines for, such prophylaxis with TNF inhibitor therapy. The complex nature of these infections has been further demonstrated by a case of *M. marinum* mimicking RA, with synovitis and subcutaneous nodules developing during infliximab therapy.[127]



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 112



# Fungal Infections of Bones and Joints

Eric M. Ruderman • John P. Flaherty

**KEY POINTS**

- Fungi are an infrequent but clinically important cause of bone and joint infections.
- Fungal infections are often indolent in onset and can masquerade as other disorders.
- Travel and immigration have affected the geographic localization of several important fungal infections, which may be seen in nonendemic areas.
- Although diagnosis may be assisted by clinical presentation and serologic testing, histologic examination and culture of infected tissue are critical.
- New antifungal therapies have broadened the effective options, but choice of drugs, duration of treatment, and combined surgical débridement must be carefully considered to achieve optimal outcomes.
- Immunocompromise, including anti-rheumatic biologic therapies, may predispose to fungal infections, often resulting in more acute and widely disseminated disease.
- Screening and/or prophylactic therapy have not proven useful for patients on immunosuppressive therapy, so a high index of suspicion should be maintained when such patients are seen with an acute illness.

Fungal infection is a relatively infrequent but important cause of osteomyelitis and arthritis. Fungal diseases that commonly cause osteomyelitis include coccidioidomycosis, blastomycosis, cryptococcosis, candidiasis, and sporotrichosis (Table 112-1). Fungal arthritis is less common and is most often associated with sporotrichosis, cryptococcosis, coccidioidomycosis, blastomycosis, candidiasis, and, occasionally, other species. The epidemiology of these fungal infections, their musculoskeletal presentations, and their treatment are considered in this chapter.

The epidemiology and clinical features of individual deep mycoses may suggest the diagnosis in some cases, but their indolent presentation, which often resembles that of other noninfectious diseases, may be misleading. Travel and immigration have blurred their geographic localization. Infection may be acute and overwhelming in immunocompromised patients, for whom disseminated fungal infections are a major risk. Anti-cytokine and other immunosuppressive treatments for rheumatic diseases, particularly those targeting TNF, are associated with disseminated fungal infection,[1,2] as are HIV infection, pregnancy, and treatments for transplantation and malignancies.[3] Fungal arthritis has also been reported following intraarticular injection of contaminated preservative-free methylprednisolone.[4] For rheumatologists, disseminated fungal infections are an important diagnostic consideration in some patients and must be considered before starting biologic treatments in those at risk; they may also complicate the clinical course of other arthritides. The presentation of fungal infections as a consequence of rheumatic disease therapy will also be considered in this chapter.

Fungal infections are generally diagnosed by histologic examination or culture of involved tissues. Improved molecular diagnostic techniques may facilitate the diagnosis, provided the possibility of fungal infection is considered and proper studies are requested. Synovial fluid leukocyte counts and culture results vary among fungal infections and in individual cases and may be misleading. Serologic testing may also assist in diagnosing and staging several fungal infections. Detecting fungal antigens and DNA in blood, urine, other body fluids, and tissue may contribute to diagnosis in selected circumstances.[5]

## COCCIDIOIDOMYCOSIS

The soil fungi *Coccidioides immitis and Coccidioides posadasii* generally cause a primary respiratory illness after spores are inhaled. A self-limited acute pneumonia may result, associated with systemic manifestations such as arthralgia and erythema nodosum (valley fever), but infection is often inapparent and only infrequently becomes chronic or disseminated.[6] Coccidioidomycosis is endemic to the southwestern United States and areas of Central and South America, but cases are increasingly diagnosed in nonendemic areas because of travel and reactivation of remote infection. Cases increase when soil is disturbed and in windy conditions. Direct human-to-human transmission is rare. Extrapulmonary infection is almost always caused by hematogenous spread from an initial pulmonary focus. The bones and joints are frequent sites of dissemination, particularly in immunocompromised hosts.

Septic arthritis of the knee is common, generally arising from direct infection of the synovium. Other joint infections are caused by spread from a contiguous osteomyelitis involving the vertebrae, wrists, hands, ankles, feet, pelvis, and long bones.[7] The onset is characterized by gradually increasing pain and joint stiffness, with little swelling but early radiographic changes. In one series, arthritis was the only manifestation of disseminated coccidioidomycosis in 51 of 57 patients and was an aspect of more generalized disease in the remaining 6 patients.[8]

**TABLE 112-1** Drug Treatment of Osteoarticular Mycotic Infections

| Infection | Drug Recommended | Infection-Specific References |
|---|---|---|
| Coccidioidomycosis | Itraconazole | 3, 8, 15, 16 |
| Blastomycosis | Itraconazole | 20, 31, 133 |
| Cryptococcosis | Fluconazole | 38, 40, 133 |
| Candidiasis | Fluconazole | 50-52, 133, 134 |
| Sporotrichosis | Itraconazole | 56, 63, 64 |
| Aspergillosis | Voriconazole | 67, 72, 133 |
| Histoplasmosis | Itraconazole | 79, 86 |
| Scedosporiosis | Voriconazole | 88, 91, 92 |

Diagnostic confusion is common in osteoarticular coccidioidomycosis because of the delayed dissemination (months to years) after primary infection and because of atypical clinical presentations. The criteria for diagnosis include compatible clinical features, serologic studies, histologic examination, and culture. Enzyme-linked immunoassays for IgM and IgG are the most sensitive screening tests; immunodiffusion assays are less sensitive but more specific. Quantitative complement fixation antibody levels are useful for monitoring the effect of treatment. Serological testing for *Coccidioides* may have negative results early in the infection and in immunosuppressed patients. A specific enzyme-linked immunosorbant assay (ELISA) to *Coccidioides* galactomannan antigen in urine has shown promise in the diagnosis of severe coccidioidomycosis infection.[9,10] The definitive diagnosis is most commonly made by the demonstration of granulomatous synovitis and typical spherules in a biopsy specimen, confirmed by positive culture and direct amplification testing. Synovial fluid, when obtainable, does not necessarily demonstrate septic leukocyte counts, and may have a lymphocyte predominance. Synovial fluid is rarely culture positive; culture of synovial tissue may be more helpful. Radioisotope bone scans may be helpful to identify areas of infection.[11]

With early diagnosis of effusive synovitis, antifungal treatment alone is appropriate. Treatment is usually initiated with oral azole antifungal agents, most commonly fluconazole or itraconazole.[12] Posaconazole and voriconazole may be useful alternatives.[13,14] Amphotericin B is recommended if lesions are worsening rapidly or are in particularly critical locations, such as the vertebral osteomyelitis. Lipid formulations of amphotericin B have demonstrated less nephrotoxicity and infusion-related side effects than conventional deoxycholate amphotericin B, and they may be given at doses higher than those tolerated with conventional amphotericin, but they have never been formally studied in clinical trials. With more widely disseminated infection or involvement of critical areas such as the spine, and in high-risk hosts, the choice and duration of treatments are often complicated. Factors that favor surgical intervention are large size of abscesses, progressive enlargement of abscesses or destructive lesions, presence of bony sequestrations, instability of the spine, or impingement on critical organs or tissues (e.g., epidural abscess compressing the spinal cord).[8,15,16] Newer antifungal agents such as voriconazole and posaconazole show promise as alternative therapy.[12,14,17,18] Long-term prophylaxis with fluconazole can limit the risk for reactivation in immunosuppressed patients.

Coccidioidal synovitis can also occur as a consequence of immune complex–mediated inflammation, a presentation that may complicate either primary pulmonary or disseminated disease and is typically polyarticular. It is accompanied by fever, erythema nodosum or multiforme, eosinophilia, and hilar adenopathy. It usually abates in 2 to 4 weeks.[6,19]

## BLASTOMYCOSIS

Blastomycosis, caused by *Blastomyces dermatitidis*, is endemic in the north-central and southern United States. Infection is acquired by inhalation of conidia associated with decaying vegetation or decomposing wood and typically results in sporadic or clustered cases of acute or chronic pneumonia.[20] Affected individuals do not appear to have any distinguishing or predisposing characteristics except for exposure to the organism during work or recreation. Clinical presentation includes pulmonary, skin, and bone involvement. Approximately half of affected patients will present with fever; leukocytosis and an elevated sedimentation rate are frequently seen.[21] Bone pain, swelling, and soft-tissue abscesses are the most common manifestations of osteoarticular disease.[22] Hematogenous dissemination is common; skin disease and osteoarticular disease occur most frequently. Bone involvement occurs in 25% to 60% of disseminated cases, and arthritis is estimated to occur in 3% to 5%.[21] In a study of 45 patients with skeletal blastomycosis, 41 had osteomyelitis, whereas 12 were seen with septic arthritis.[22] In another series of 14 cases at a single institution, 12 presented as a bone mass; 3 of 4 cases of septic arthritis in this series developed because of direct extension of osteomyelitis.[23] The skeletal areas most commonly affected are the long bones, vertebrae, and ribs (Figure 112-1).[23-26] Blastomycosis often mimics malignancy with subtle constitutional symptoms, nodular lung opacities, and lytic bone lesions.

Arthritis is usually monoarticular in the knee, ankle, or elbow but may, rarely, be polyarticular.[21,27,28] Joint infection is an isolated skeletal disorder in only a few cases; joint radiographs more commonly show punched-out bone lesions (Figure 112-2A). Synovial fluid is commonly purulent, and organisms are evident on microscopic examination as well as by culture. The synovial histologic examination shows granulomas and abundant neutrophils—a so-called *pyogranulomatous tissue response*—with broad-based budding yeast forms (Figure 112-2B). The yeast can be difficult to visualize with routine hematoxylin and eosin stains, but special stains, such as the methenamine silver stain or periodic acid-Schiff (PAS) stain, can be helpful. The diagnosis is also commonly made from involved nonarticular sites. Urinary antigen testing appears to be sensitive but cross-reacts with antigens present in other endemic fungal infections.[29] For moderately severe or severe disease, treatment with amphotericin B for 1 to 2 weeks or until improvement is noted, followed by oral itraconazole for a total of at least 12 months, is recommended. For mild to moderate disease, oral itraconazole for 12 months is recommended. Serum levels of

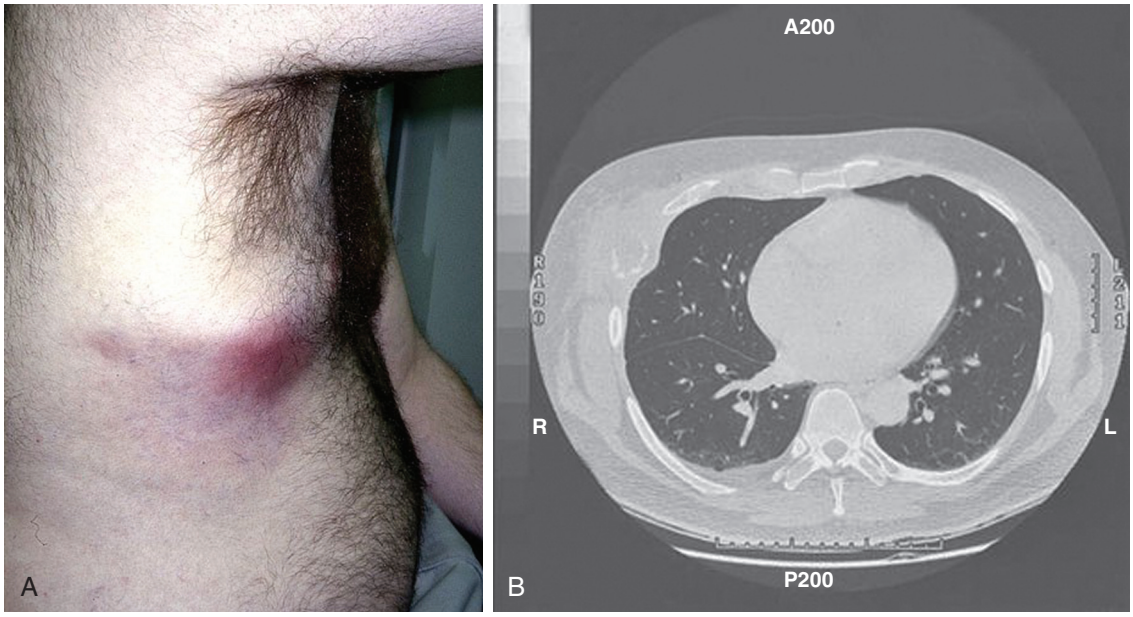



**Figure 112-1** **A,** Blastomycosis osteomyelitis of a rib and chest wall. **B,** Computed tomography scan appearance. Primary infection in the blood may spread to the skeleton.

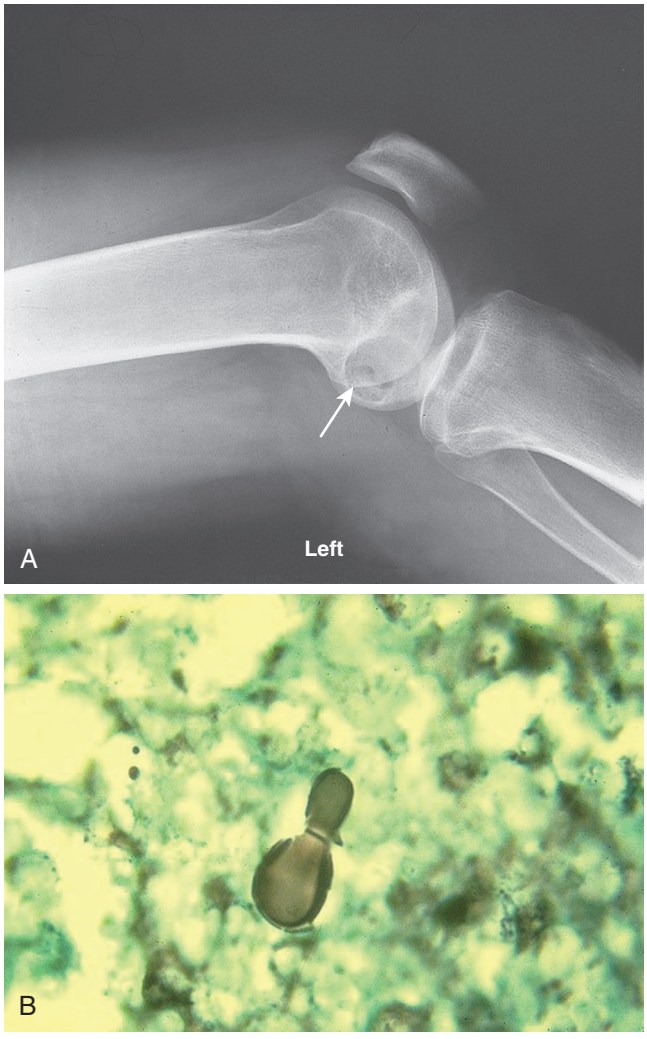



**Figure 112-2** Blastomycosis joint infection. **A,** Radiographs commonly show punched-out bone lesions. **B,** Synovial histology shows epithelioid granulomas with budding yeast forms.

itraconazole should be determined after the patient has been on treatment for at least 2 weeks to ensure adequate drug exposure.[20,30-32]

## CRYPTOCOCCOSIS

*Cryptococcus neoformans*, the fungus causing cryptococcosis, is geographically ubiquitous and is found in soil contaminated by pigeon feces; a related species, *Cryptococcus gattii*, is associated with certain types of eucalyptus trees in tropical climates and has been associated with an ongoing outbreak of disease in British Columbia, Canada, and the United States Pacific Northwest. It is a common pathogen only in association with defects in cell-mediated host defense, including HIV infection, organ and stem cell transplantation, lymphoreticular malignant neoplasms, TNF antagonist treatment,[33,34] and corticosteroid therapy.

Cryptococcosis varies in acuity, usually affecting the lungs in its primary form, but it sometimes disseminates hematogenously to a wide variety of sites, including the central nervous system and skin. Although bone infection is common, causing osteolytic lesions in 5% to 10% of cases, articular involvement is rarely reported.[35,36] As in blastomycosis, bone lesions may mimic metastatic neoplasm (Figure 112-3).

Cryptococcal arthritis is an indolent monoarticular arthritis in approximately 60% of reported cases and a polyarthritis in the remainder.[37,38] The knee is most commonly involved. A single case of tenosynovitis with carpal tunnel syndrome has been recognized. The majority of cases reported from the pre-AIDS era also demonstrated radiographic evidence of periarticular osteomyelitis. These patients were young adults, did not have debilitating disease or other evidence of dissemination, and had pulmonary involvement in only 50% of cases. Synovial tissue showed acute and chronic synovitis, multinucleate giant cells, prominent granuloma formation, and large numbers of budding yeast with special stains. Most recently reported

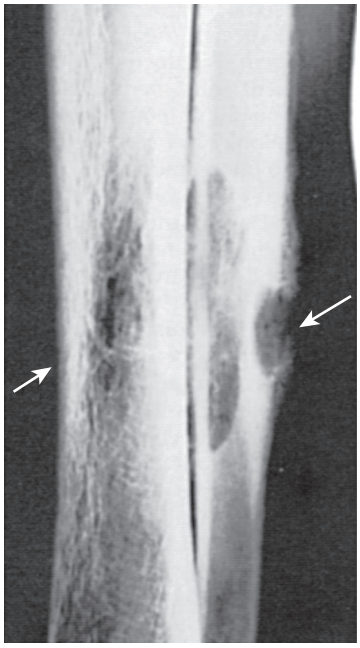


**Figure 112-3** Cryptococcosis (torulosis). Discrete osteolytic foci with surrounding sclerosis and, in some places, periosteal reaction are seen *(arrows)*. This involvement of bone protuberances such as the calcaneus is not unexpected in this disease. The resulting appearance simulates that of other fungal diseases, especially coccidioidomycosis, as well as neoplastic disorders. *(From Resnick D:* Diagnosis of bone and joint disorders*, ed 3, Philadelphia, 1995, WB Saunders, p 2507.)*

cases are associated with immunosuppression and disseminated infection. Interestingly, osteoarticular cryptococcal infections have been linked to sarcoidosis, although it is unclear whether this association relates to the immunologic impact of the sarcoidosis or to the immunosuppressive therapies used to treat it.[39] Serum cryptococcal antigen testing appears to be very sensitive, in part because osteoarticular infection results from hematogenous dissemination. The choice of treatment for cryptococcal disease depends on both the anatomic sites of involvement and the host's immune status, with amphotericin B and fluconazole considered most effective.[38,40] 5-Flucytosine is typically added to amphotericin B or fluconazole for the first 2 to 4 weeks (induction therapy) in cases of severe cryptococcal infection.[41]

## CANDIDIASIS

*Candida* species are widely distributed in nature. *Candida albicans* is a normal commensal of humans, and other species can probably live in nonanimate environments such as soil. Since the advent of antibiotic therapy in the 1940s, and related to the common use of immunosuppression and parenteral lines, candidiasis has been responsible for an increasing incidence of mucocutaneous and deep-organ infections.[42] Osteomyelitis, although uncommon, is a potentially serious complication of hematogenous dissemination in both adults and children.[43-45] A recent review identified a history of preceding candidemia in half of osteomyelitis cases, whereas it was the first proven site of infection in the other half.[46] It may also occur from direct tissue inoculation during surgery or by injection of contaminated heroin,[47] and bone infection may emerge after successful treatment of infection at other sites. Bone infection is commonly located in two adjacent vertebrae or in a single long bone. Surgical inoculation has occurred in the sternum, spine, and mandible. A few patients have had multiple sites of involvement. Candidal prosthetic joint infections may also occur as a late consequence of total joint replacement.[48]

The usual clinical presentation is localized pain. Other symptoms and laboratory abnormalities vary. It can present months after an episode of fungemia, and clinical manifestations tend to be milder than bacterial infections at the same sites. This can lead to long delays in diagnosis. Bone changes of osteomyelitis are commonly demonstrated in radiographs of the symptomatic site. The diagnosis is established when culture of involved bone obtained by either open or needle biopsy has identified a variety of *Candida* species. *Candida albicans* causes the majority of infections, but the role of nonalbicans species has been increasing in recent years.[45] Polymerase chain reaction (PCR) testing of blood may allow earlier diagnosis of disseminated Candida infection.[49] Treatment with azoles (fluconazole, itraconazole, voriconazole or posaconazole), echinocandins (caspofungin, micafungin, or anidulafungin) or amphotericin B formulations can be effective.[50,51] Species identification and susceptibility testing assist antifungal therapy selection. For example, *Candida glabrata* frequently demonstrate reduced susceptibility to azole antifungal agents and amphotericin but remain susceptible to echinocandins; *Candida krusei* are often resistant to azoles and may show reduced susceptibility to amphotericin but also remain susceptible to echinocandins; *Candida lusitaniae* are often resistant to amphotericin; and *Candida parapsilosis* may demonstrate reduced susceptibility to echinocandins.[51] The use of surgical débridement must be individualized. With vertebral involvement but no neurologic complications, medical therapy alone has been effective.

Candidiasis is an uncommon cause of monoarticular arthritis.[52] *Candida albicans* is the most common pathogen, although septic arthritis due to other candidal species has been reported.[53] Reported cases commonly involve a knee, occur in the context of multifocal extra-articular Candida infection, and are accompanied by constitutional symptoms. Both children and adults have been affected. Predisposing conditions include gastrointestinal and pulmonary disorders, narcotic addiction, intravenous catheters, leukopenia, immunosuppressive treatment (including TNF antagonists), broad-spectrum antibiotics, and corticosteroids. Some involved joints were previously affected by arthritis, and infection has followed arthrocentesis in isolated cases. In most cases, radiographs reveal coincident osteomyelitis. Synovial fluid leukocyte counts may vary; *Candida* species have been cultured from synovial fluid in all cases but are not commonly identified on smear. Histologic studies of synovium show nonspecific chronic inflammation rather than granulomas.

## SPOROTRICHOSIS

Sporotrichosis is caused by *Sporothrix schenckii*, a saprophyte found widely in soil and plants. Infection in humans occurs through inoculation of the skin or, rarely, inhalation into the respiratory tract; it is a source of infection among

agricultural workers in tropical and subtropical areas. It most commonly involves the skin and lymphatics but may disseminate from the lungs to the central nervous system, eyes, bones, and joints.[54] In immunocompetent hosts, a single site is typically involved; in immunocompromised hosts, including patients on anti-cytokine therapy, multifocal disease may occur.[55]

In contrast to the relatively common occurrence of skin infection, articular sporotrichosis is a rare disorder.[56,57] In 84% of patients in one series, there was no accompanying skin involvement, suggesting entry through the lungs. Sporotrichosis most often occurs in individuals with a chronic illness that alters host defense, such as alcoholism or a myeloproliferative disorder. *Sporothrix* arthritis is most often indolent and infects a single joint or multiple joints in equal proportions. The knee, hand, wrist, elbow, and shoulder are most frequently involved; hand and wrist involvement distinguishes this from other fungal arthritides. Articular infection shows a propensity to spread to adjacent soft tissues, forming draining sinuses. Constitutional symptoms are unusual.

Radiographic changes vary from juxta-articular osteopenia to the commonly observed punched-out bone lesions. When it can be obtained, synovial fluid is inflammatory. Synovitis is characterized on gross evaluation by destructive pannus and on microscopic examination by granulomatous histologic features or, less frequently, by nonspecific inflammation. Organisms are difficult to identify in tissue, and the diagnosis is often made by positive culture of joint fluid or involved tissue. Incubation at room temperature facilitates growth of the mycelial phase of *S. schenckii*. Serologic testing is not useful in the diagnosis of sporotrichosis. In a small number of cases, sporotrichosis may disseminate to cause a potentially fatal infection characterized by low-grade fever, weight loss, anemia, osteolytic bone lesions, arthritis, skin lesions, and involvement of the eyes and central nervous system.[58-61] These infections occur in immunosuppressed patients with either hematologic malignancies or HIV infection.

In 44 cases reported in 1979, treatment was optimal with combined joint débridement and high-dose intravenous amphotericin B (11 of 11 cured) and slightly less effective with amphotericin alone (14 of 19 cured).[56] More recently, itraconazole has proven effective for initial therapy of most patients,[62] with amphotericin B reserved for those with extensive involvement and for itraconazole failures. In contrast, fluconazole has demonstrated only modest success in osteoarticular sporotrichosis.[63,64]

## ASPERGILLOSIS

*Aspergillus* species are ubiquitous, but infection occurs only rarely in immunocompetent individuals. In contrast, invasive infection is an important life-threatening complication in immunocompromised adults and children.[65-69] It may spread directly from the lung to adjacent vertebrae, disk spaces, and ribs (more often in children) or through the bloodstream (Figure 112-4).[70-72] Rare cases of monoarthritis with adjacent osteomyelitis are also reported. The knee is the most commonly involved joint. The organism may be observed in infected tissue (see Figure 112-4B). The galactomannan ELISA has been validated as a surrogate marker

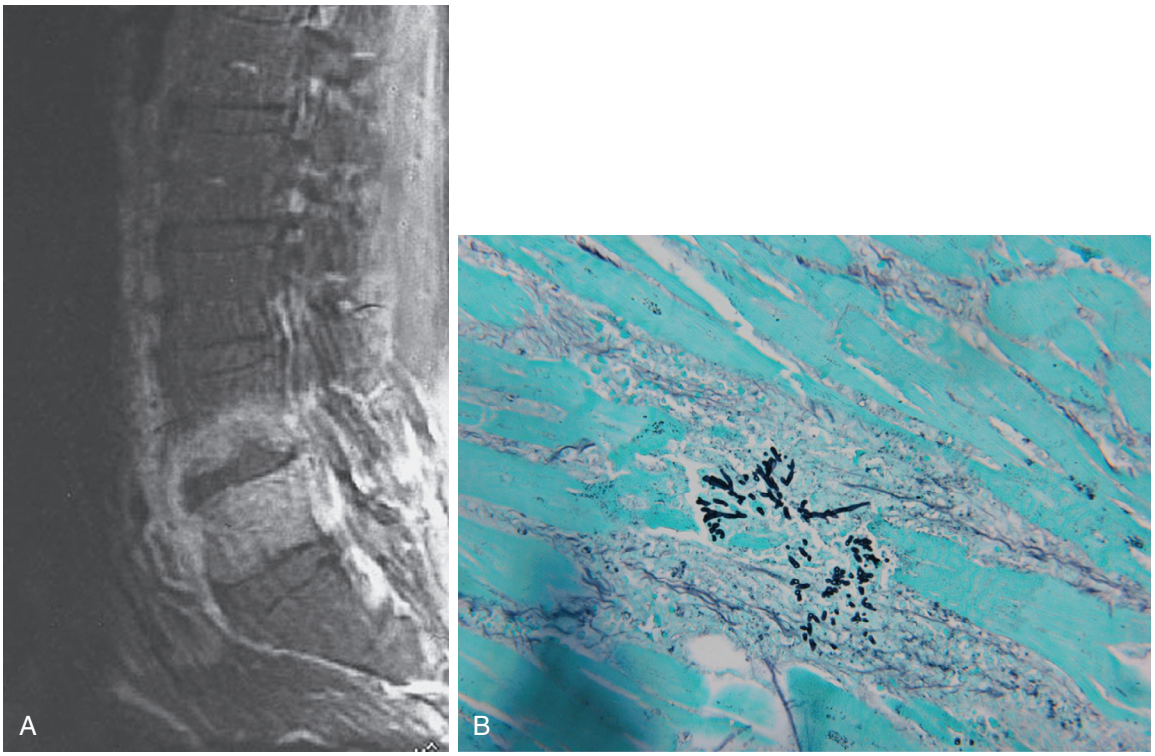



**Figure 112-4** Aspergillosis vertebral osteomyelitis and diskitis. **A,** *Aspergillus* may spread directly from the lung to adjacent vertebrae, disk spaces, and ribs (more often in children) or through the bloodstream. **B,** Infected tissue may show characteristic organisms.

for invasive aspergillosis, and the (1→3)-β-D-glucan assay similarly may provide support for the diagnosis of invasive fungal infection. Treatment with combined surgical débridement and antifungal therapy is an ongoing challenge.[66,71,73] Voriconazole has proven superior to amphotericin for the treatment of invasive aspergillosis and is now recommended for initial therapy.[74,69,73] Liposomal amphotericin B, posaconazole, caspofungin, and micafungin have demonstrated efficacy in salvage situations.[75] Combination therapy has not demonstrated clear improvement in outcomes versus monotherapy. In 2012, an outbreak of fungal infections, initially believed to be due to *Aspergillus fumigatus* but later attributed to the rarely pathogenic fungus *Exserohilum rostratum*, was traced to contaminated injectable methylprednisolone produced by a single compounding pharmacy in Massachusetts.[4,76] The index case involved *A. fumigatus* meningitis following an epidural injection; subsequent cases included meningitis, stroke, and musculoskeletal infections, including vertebral osteomyelitis, diskitis, and facet joint infections.[77,78]

## HISTOPLASMOSIS

*Histoplasma capsulatum* is a soil fungus that causes endemic disease in the Midwest and Southeast United States.[37,79] Bone and joint involvement is rare but has been reported in the knee, wrist, and ankle. Immunosuppression, including the use of TNF inhibitors predisposes to disseminated histoplasmosis in adults and children, which may be confused clinically with sarcoidosis, tuberculosis, and reactive inflammatory conditions.[80-83] Diagnosis depends on appropriate use of fungal staining and culture methods and antigen detection.[84] A case report emphasizes the rare occurrence of fungal prosthetic joint arthritis.[85] The more common osteoarticular involvement with histoplasmosis is a hypersensitivity syndrome accompanying acute pulmonary infection; it is characterized by self-limited polyarthritis, erythema nodosum, and erythema multiforme. Liposomal amphotericin B followed by itraconazole is the preferred treatment for severe infection and itraconazole for less severe cases.[79,86,87]

## SCEDOSPORIOSIS

*Scedosporium* species are environmental molds that have been identified as fungal pathogens in both immunocompetent and immunocompromised hosts. They may cause focally invasive and disseminated infection after cutaneous inoculation. *Scedosporium prolificans* has a predilection for bone and cartilage, leading to both septic arthritis and osteomyelitis. Infections are difficult to eradicate with surgery and antifungal agents, and the organism is resistant to amphotericin.[88,89] Case reports suggest improved infection control with voriconazole or voriconazole combined with terbinafine.[90-92]

## TREATMENT OF FUNGAL INFECTION

Antifungal chemotherapy has improved over the past several decades, first with the introduction of amphotericin B and then with the oral antifungal agents flucytosine, ketoconazole, fluconazole, and itraconazole. More recent advances include the development of less toxic formulations of amphotericin B, liposomal amphotericin B, and amphotericin B lipid complex. Voriconazole and posaconazole, which are broad-spectrum azole antifungals, have demonstrated improved activity against aspergillosis and mucormycosis, respectively. The echinocandin antifungal agents caspofungin, micafungin, and anidulafungin have emerged as alternative therapies for aspergillosis and as the treatments of choice for some *Candida* infections. For detailed treatment guidelines, several excellent reviews are available.[93-103] In choosing an appropriate drug (Table 112-2) and course of treatment, the clinician must consider the infecting agent, clinical manifestations of the disease, immune status of the host, anti-microbial resistance, drug side-effect profile, and direct and indirect costs of treatment. Treatment has become more complex because of immune system compromise in infected patients treated for transplant rejection, autoimmune disorders, malignant disease, and AIDS.[104]

Itraconazole has become the first choice for treatment of the endemic mycoses—blastomycosis, histoplasmosis, and sporotrichosis. A loading dose of itraconazole of 200 mg three times a day for 2 days is recommended, followed by 200 mg to 400 mg daily. Absorption of itraconazole is unpredictable and blood levels of itraconazole should be measured to ensure adequate drug exposure. Absorption of itraconazole requires stomach acid, so concurrent administration of drugs that reduce the acidity of the stomach such as proton pump inhibitors and $H_2$ blockers should be avoided. At least 6 months of treatment is required, and some patients may need as long as a year of therapy. For cryptococcosis, fluconazole is the recommended azole.[38,40] Amphotericin B is the preferred drug for meningeal and life-threatening infections. Specific treatment protocols and detailed side effect profiles are presented in reviews,[93-102] Infectious Diseases Society of America guidelines,[12,32,41,51,75,87] and infection-specific references (see Table 112-2). Surgical débridement is critically important in the treatment of mucormycosis and may be of value as adjunctive treatment for certain fungal infections, including selected patients with invasive aspergillosis and vertebral osteomyelitis caused by coccidioidomycosis, especially when there is evidence of spinal cord impingement or progression on repeat imaging on antifungal therapy. Surgery does not play a major role in the treatment of blastomycosis, histoplasmosis, or *Pneumocystis jiroveci* infection.

**TABLE 112-2** Disseminated Fungal Infections Reported with TNF Inhibitor Therapy

| Organism | References |
|---|---|
| Aspergillosis | 1, 55, 115 |
| Candidiasis | 1 |
| Coccidioidomycosis | 111, 113 |
| Cryptococcosis | 33, 116 |
| Histoplasmosis | 80, 83 |
| Pneumocystosis | 124, 135 |
| Scedosporosis | 117 |
| Sporotrichosis | 55 |

## FUNGAL INFECTION AS A CONSEQUENCE OF ANTI-RHEUMATIC THERAPY

Many of the same fungal infections associated with osteoarticular disease may cause infection in patients treated with anti-rheumatic therapy, particularly biologic therapy. Animal models of infection point to an important role for TNF in host defense against many of these organisms, including *Aspergillus*,[105] *Candida*,[106] *Cryptococcus*,[107,108] *Coccidioides*,[109] and *Sporothrix*.[110]

Coccidioidomycosis infection following TNF inhibitor therapy has been reported in endemic areas in the Southwest United States. In the largest published series, 12 of 13 cases followed therapy with infliximab, and 1 was associated with etanercept.[111] All but 2 of these cases were believed to represent new infection rather than reactivation. In one medical center included in this series, the relative risk of coccidioidomycosis infection was 5.23 with infliximab therapy compared with other anti-rheumatic therapy. In all cases, coccidioidomycosis infection developed in the absence of other known risk factors, including diabetes, pregnancy, and HIV infection. Updated data from the same institutions, however, has suggested that biologic or nonbiologic disease-modifying anti-rheumatic drug (DMARD) treatment can potentially be reinstituted during or after antifungal therapy.[112] Coccidioidomycosis infection has also been reported in nonendemic areas, presumably as a consequence of fomite exposure.[113]

Disseminated histoplasmosis infections have been reported following TNF inhibitor therapy in endemic areas in the Ohio-Mississippi River valley regions of the central United States; as with coccidioidomycosis infections, these have been reported more frequently following infliximab therapy than with the other agents.[80] The reason for the larger number of cases with infliximab is unclear; possibilities include a unique mechanism of action for infliximab, larger numbers of patients treated, patient selection, concomitant immunosuppressive therapy, or some combination of all of these. In most cases, patients in whom disseminated histoplasmosis with TNF antagonist therapy develops have been taking additional immunosuppressive therapy. In a series of 26 patients with histoplasmosis and rheumatoid arthritis (RA), TNF inhibitors (including adalimumab, etanercept, and infliximab), methotrexate, and corticosteroids were all implicated as potential contributing factors.[114] Patients typically are seen with cough, dyspnea, fever, and malaise, and can rapidly become quite ill. Histoplasma antigen may be identified in the urine in 92% of patients with disseminated histoplasmosis, and this test may facilitate rapid diagnosis.[82] Exposure to *Histoplasma* may result in asymptomatic latent infection; it has been difficult, however, to determine whether symptomatic infections following TNF inhibitor therapy represent reactivation or new infection. Both pulmonary and disseminated infections with cryptococcal infections have been reported with TNF antagonist therapy, as have infections with *Aspergillus, Candida, Scedosporium, and Sporothrix*.[1,33,55,115-118] Fungal infections have not been reported as a consequence of abatacept or rituximab therapy in rheumatic diseases. Whether this relates to decreased risk with these agents or smaller numbers of patients treated remains to be seen.

*Pneumocystis jiroveci* pneumonia (PCP) is caused by a fungus originally classified as a protozoan (*Pneumocystis carinii*). PCP, an opportunistic infection seen in association with HIV infection, has been reported with a number of anti-rheumatic therapies, including cyclophosphamide and low-dose methotrexate, often in combination with corticosteroid therapy.[119,120] Interestingly, PCP as a consequence of low-dose methotrexate therapy has been reported as a particular concern in Japan, where the prevalence of asymptomatic carriage of pneumocystis has been reported to be as high as 18.8% in the elderly.[121] These same authors have suggested that PCR testing may identify carriers at increased risk for PCP during therapy.[122] The use of biologic therapy, including rituximab[123] and TNF inhibitors,[124] has been associated with the development of PCP. Patients with PCP are seen with fever, dry cough, and dyspnea. The clinical presentation in patients receiving immunosuppressive therapy is typically more acute than in HIV-associated cases. Organisms may be identified in sputum from patients with HIV but are seen less commonly in rheumatologic patients. A presumptive diagnosis may be made on the basis of PCR testing for *P. jiroveci* DNA. Elevated serum levels of β-D-glucan, a common component of fungal cell walls, may aid in the diagnosis. One series of 21 patients identified older age, pre-existing pulmonary disease, higher corticosteroid doses, and low serum albumin and IgG levels as potential risk factors for PCP in patients treated with infliximab.[124] Chest radiographs may show diffuse infiltrates, whereas CT scans demonstrate ground-glass opacities (Figure 112-5 and Figure 112-6). Radiographic findings may be difficult to distinguish from methotrexate pneumonitis. Treatment includes supplemental oxygen and trimethoprim/sulfamethoxazole (TMP/SMX) or pentamidine isethionate. High-dose corticosteroids are commonly used as adjunctive therapy in the treatment of HIV$^+$ patients with PCP but are less well studied in patients taking immunosuppressives.

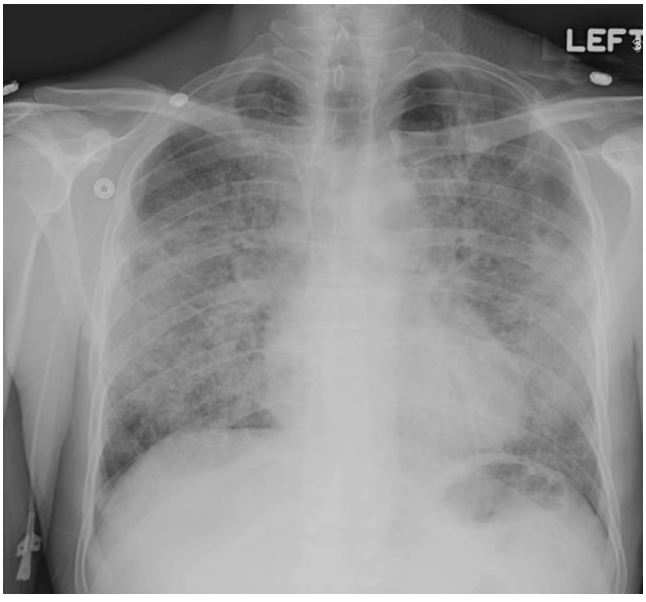



**Figure 112-5** Chest radiograph showing diffuse interstitial infiltrate in a patient with *Pneumocystis jiroveci* pneumonia.




**Figure 112-6** Computed tomography scan of the chest demonstrating ground-glass opacity in a patient with *Pneumocystis jiroveci* pneumonia.

PCP infection is also of concern with nonbiologic immunosuppressive therapy. In particular, the use of cyclophosphamide to treat systemic lupus, vasculitis, and other autoimmune diseases has been associated with a risk for PCP, although the overall risk appears to be low. In a recent review of published data on 76,156 patients with SLE, the risk of PCP was 15.88 per 10,000 patients, or 0.158%.[119] Potential risk factors include high-dose corticosteroid use, lymphopenia (especially low CD4 counts), renal disease, and overall high disease activity.[119,125] Patients with vasculitis with pulmonary involvement may be at increased risk, and diagnosis may be difficult in both these patients and patients with lupus with pulmonary disease. Unfortunately, there are no published guidelines to address the use of PCP prophylaxis in autoimmune diseases, nor a clear consensus regarding the standard of care in this situation. Recent clinical trials in vasculitis have routinely used PCP prophylaxis as part of the protocol, suggesting it is becoming standard of care.[126] Two recent surveys of U.S. rheumatologists, however, found that just 50.4% and 69.5%, respectively, routinely prescribed prophylactic antibiotics.[119,127] Academic rheumatologists and more recent graduates are more likely to prescribe prophylactic therapy. TMP/SMZ appears to be more effective than dapsone or aerosolized pentamidine, despite an apparent increased risk of sulfonamide allergy in the lupus population.[128,129] There are no consistent standards, or recommendations, for the use of prophylaxis for fungal infection other than *Pneumocystis*.

Unlike mycobacterial infection, biologic therapy of rheumatic disease has not been definitively associated with an increased overall risk of fungal infection, although the lack of a statistically significant association with these rare infections cannot disprove a possible connection.[130] Indeed, fungal infections have been reported in a post-marketing biologic registry in France.[131] In general, patients with a history of fungal infection, or those with significant exposure to these organisms, should receive biologic therapy only when alternatives are not available or appropriate. Patients should be cautioned to minimize exposure to sources of infection during therapy (e.g., avoid exposure to histoplasmosis in old buildings [demolition, remodeling, cleaning], chicken coops, bird roosts, wood piles, or caves [spelunking] and avoid exposure to outdoor dust in regions endemic for coccidioidomycosis). There is, as yet, no practical role for screening for latent infection or for prophylactic therapy.[132] Serologic tests and delayed-type hypersensitivity testing do not distinguish past, resolved infection from latent infection, although repeat coccidiomycosis serologies has been proposed as part of an algorithm for determining the potential safety of reinstituting biologic therapy after treatment of an acute, nondisseminated infection.[112] Chest radiographs may identify calcified granulomas consistent with prior histoplasmosis infection, but this is a nonspecific finding. In Japan, it has been suggested that TMP/SMX therapy might normalize PCR testing in asymptomatic *P. jiroveci* carriers treated with methotrexate, and that this might reduce the risk of developing pneumonia, but this has not been studied in a U.S. population.[122] The lack of useful screening tests makes early recognition of infection and institution of therapy critical. Indeed, the FDA has mandated a "black box" warning regarding the risk of fungal infections on the product labels of TNF inhibitors, reflecting the potentially fatal consequences of delayed recognition of such infections.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 113

# Rheumatic Manifestations of Human Immunodeficiency Virus Infection



John D. Reveille

**KEY POINTS**

- Because patients with HIV living longer as a result of more effective treatments, the challenges of HIV-associated rheumatic manifestations are growing.
- Certain diseases are specific to HIV infection (i.e., HIV-associated arthritis, diffuse infiltrative lymphocytosis syndrome [DILS], HIV-associated polymyositis).
- Other diseases, such as diseases that involve $CD4^+$ T cells including rheumatoid arthritis and systemic lupus erythematosus, tend to go into remission with HIV activity and to flare with anti-retroviral treatment.
- Effective anti-retroviral therapy decreases the prevelance of certain diseases (i.e., DILS, late opportunistic infections) but also is associated with side effects (e.g., osteonecrosis, myopathy, rhabdomyolysis).
- With immune reconstitution after anti-retroviral therapy, a new spectrum of autoimmune and autoinflammatory diseases has emerged that requires careful attention.

In the years since AIDS was initially described in 1981, the HIV pandemic has become one of the leading global health crises. According to new data in the Joint United Programme on HIV/AIDS (UNAIDS) 2013 report[1] the AIDS epidemic seems to be slowing down globally, with people living longer and new infections decreasing, although still occurring at alarming rates in certain regions such as southern Africa, eastern Europe, and central and eastern Asia. An estimated 35.3 million people worldwide are living with HIV (Figure 113-1). Approximately 2.3 million people became newly infected with HIV in 2012, and 1.6 million people died.

Progress in dealing with the HIV epidemic, including progress in education and in public health awareness, has undoubtedly influenced the decrease in new infections seen among young people in some countries in recent years. While availability of newer treatment strategies and better access to health care resulting in increased life expectancy has ensued, infection will be managed increasingly as a chronic illness, and complications such as musculoskeletal and rheumatic conditions associated with HIV infection and its treatment are expected to increase (Table 113-1 and Table 113-2).

Among the rheumatologic disorders, clinicians face the challenge of treating potentially disabling inflammatory disorders with immunosuppressive therapy in the face of ongoing viral-induced immunocompromise. Diagnosing infection is especially important in an immunocompromised patient because the probability of an opportunistic infection as a cause for a musculoskeletal complaint increases with advancing stages of the patient's HIV disease. At early stages ($CD4^+$ count >300/μL), opportunistic infections are unlikely, although bacterial infections (especially tuberculosis) still can occur. There should be a very high threshold for using immunosuppressive drugs in this population.

## HIV-ASSOCIATED BONE AND JOINT DISEASE

### HIV-Associated Arthralgia

As many as 25% of $HIV^+$ patients in recent series may have otherwise unexplained arthralgia.[2,3] Arthralgias and myalgias also form a part of the constitutional symptoms of HIV seroconversion. Whether arthralgia can be attributed to circulating viral and host immune complexes caused by HIV infection per se or to other infections (e.g., hepatitis C) has not been determined. The pathogenesis is unclear but may involve cytokines or transient bone ischemia.[4] However, patients presenting with arthralgia alone rarely progress to inflammatory joint disease. The most appropriate treatment consists of non-narcotic analgesics and reassurance.

### Painful Articular Syndrome

Painful articular syndrome is a self-limited syndrome lasting less than 24 hours, associated with few objective clinical findings, and characterized by severe bone and joint pain.[2-4] It occurs predominantly in the late stages of HIV infection. Its cause is unknown, and no evidence of synovitis has been found in these patients. The knee is most commonly affected, but the elbow and shoulders also can be involved. Radiographic features are nonspecific; occasionally, periarticular osteopenia is seen. Treatment is symptomatic. Given the abrupt nature of this disorder's onset, these patients are more likely to be encountered in emergency rooms and not in HIV clinics.

### HIV-Associated Arthritis

The first reports of a seronegative arthritis associated with HIV infection appeared in 1988,[4] with frequencies of 12%. HIV-associated arthritis is most common in sub-Saharan Africa, where HIV infection is pandemic. In the Congo, where the seroprevalence of HIV infection is 7% to 8%, AIDS is the leading cause of aseptic arthritis (60% of

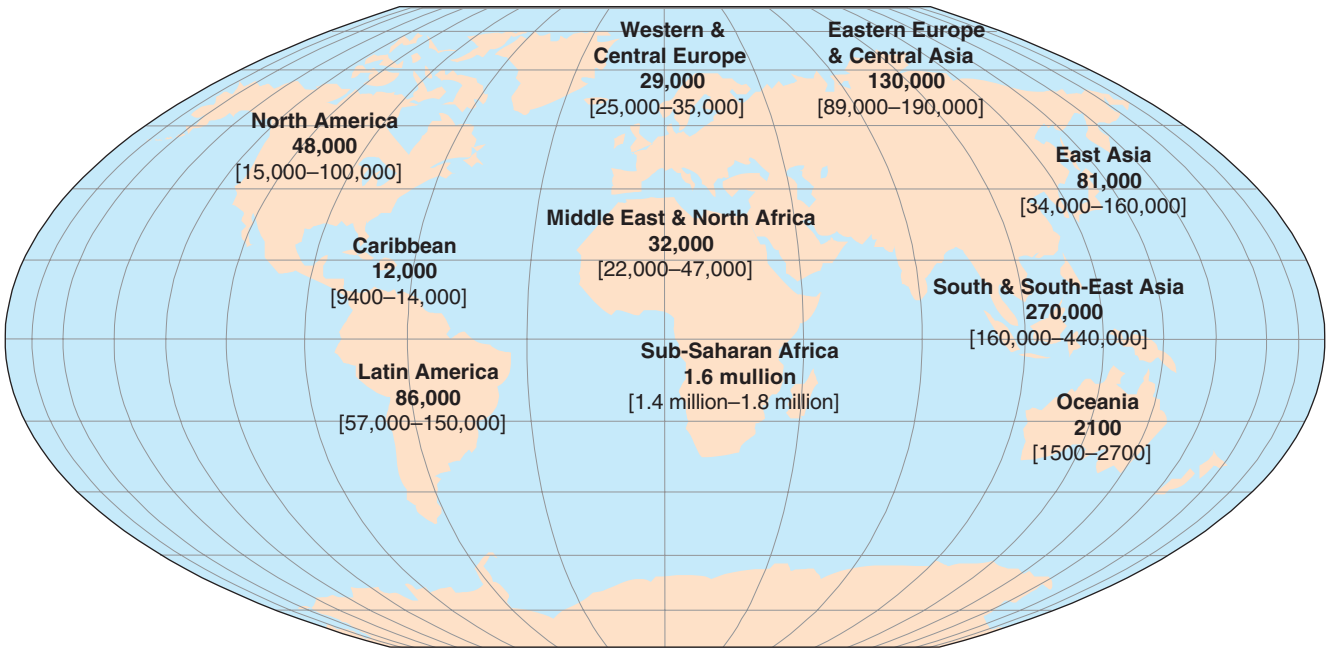



**Figure 113-1** A global view of human immunodeficiency virus (HIV) infection—33 million living with HIV in 2012. *(From UNAIDS: 2013 Report on the Global AIDS Epidemic [website]:* http://www.unaids.org/en/resources/campaigns/globalreport2013/globalreport/*.)*

**TABLE 113-1** Rheumatic Diseases Associated with or Occurring in Patients with HIV Infection

| |
|---|
| **Unique to HIV Infection** |
| Diffuse infiltrative lymphocytosis syndrome |
| HIV-associated arthritis |
| Zidovudine-associated myopathy |
| Painful articular syndrome |
| **Encountered in HIV-Infected Patients** |
| HIV-associated reactive arthritis |
| Polymyositis |
| Psoriatic arthritis |
| Polyarteritis nodosa |
| Giant cell arteritis |
| Hypersensitivity angiitis |
| Granulomatosis with polyangiitis |
| Henoch-Schönlein purpura |
| Behçet's disease |
| Infectious arthritis (bacterial, fungal) |
| **Ameliorated by HIV Infection but Worsening or Reappearing with IRIS** |
| Rheumatoid arthritis |
| Systemic lupus erythematosus |
| Sarcoidosis |

IRIS, Immune reconstitution inflammatory syndrome.

cases).[5] This is usually an oligoarthritis (Table 113-3), predominantly involving the lower extremities, and tends to be self-limited, lasting less than 6 weeks.[4,6] Most commonly involved are the knees (84%), ankles (59%), and metatarsophalangeal joints (23%) in the lower limbs, and the wrists (41%), elbows (29%), and metacarpophalangeal and interphalangeal joints (25%) in the upper limbs, similar to other viral arthritides. Some patients have been reported as having a longer course, with joint destruction.[7,8]

The origin is unclear; no association has been noted with human leukocyte antigen (HLA)-B27 or any other known genetic factor. Synovial fluid cultures are typically sterile, although one report described the presence of tubuloreticular inclusions, suggesting a viral origin, possibly HIV itself.[4] Radiographs of affected joints are usually normal, except in uncommon cases with prolonged symptoms, in which joint space narrowing and destruction can occur. Treatment includes NSAIDs and, in more severe cases, low-dose glucocorticoids. Hydroxychloroquine and sulfasalazine also have been used.[9]

## Reactive Arthritis Occurring in HIV Infection

Early reports in the United States suggested that reactive arthritis occurred more commonly in the setting of HIV infection; however, later studies showed that this may be reflective of the sexually active nature of the population at highest risk for HIV infection.[10] This contention is not borne out in studies from sub-Saharan Africa, where HLA-B27 is rare, as were reports of spondyloarthritis before the HIV epidemic. With the arrival of AIDS, a dramatic upsurge in the prevalence of reactive arthritis and undifferentiated spondyloarthritis and, less often, psoriatic arthritis,[8,11] was seen, suggesting a pathogenic role of HIV infection.

The typical presentation is a seronegative lower-extremity peripheral arthritis, usually accompanied by enthesitis (dactylitis, Achilles tendinitis, and plantar fasciitis). Mucocutaneous features are common, especially keratoderma blennorrhagicum (Figure 113-2) and circinate balanitis. Extensive psoriasiform skin rashes can occur. The clinical overlap makes it difficult sometimes to distinguish HIV-associated reactive arthritis from psoriatic arthritis.[12] Urethritis occurs in similar frequency as in HIV$^-$ reactive arthritis. Axial involvement and uveitis seem to be less common but do occur. Longitudinal studies from Africa have described an aggressive course with a poor prognosis.[13,14]

**TABLE 113-2** Distribution of Various Rheumatic Diseases in HIV$^+$ Patients from Various Sites

| Feature | Cincinnati, Ohio (until 1993) | Houston, Texas (1994-2002) | Bangkok, Thailand (2005) | Taiwan (1993-2013) | Darjeeling, India (2010-2011) |
|---|---|---|---|---|---|
| No. of patients | 1100 | 4467 | 178 | 3623 | 300 |
| HIV-associated arthralgia | 0.81% | 0.7% | 13.4% | NR | 26.7% |
| Myalgia | 0.7% | 0.6% | 10.2% | NR | 46.7% |
| PsA/Reactive arthritis | 0.45% | 0.6% | NR | 0.15% | 2.3% |
| HIV-associated arthritis | 0% | 0.5% | NR | NR | 0% |
| DILS/Sjögren's syndrome | NR | 3%-4% | NR | 0.03% | 0% |

*From Solinger AM, Hess EV: Rheumatic diseases and AIDS—is the association real? *J Rheumatol* 20:678-83, 1993.
†From Louthrenoo W: Musculoskeletal manifestations of HIV infection in Thailand: an analysis of 100 cases. *J Clin Rheumatol* 3:258-68, 1997.
‡Medical Record review only, not confirmed by clinical evaluation.
DILS, Diffuse infiltrative lymphocytosis syndrome; NA, not applicable; NR, not reported; PsA, psoriatic arthritis.

**TABLE 113-3** Contrasting Features of HIV–Associated Arthritis and Reactive Arthritis

| Feature | HIV-Associated Arthritis | HIV-Associated Reactive Arthritis |
|---|---|---|
| Joint involvement | Asymmetric oligoarthritis/ polyarthritis | Asymmetric oligoarthritis/ polyarthritis |
| Mucocutaneous involvement | Absent | Present |
| Enthesopathy | Absent | Frequent |
| Synovial fluid white blood cell count | 500-2000/µL | 2000-10,000/µL |
| Synovial fluid cultures | Negative | Negative |
| Microorganisms in synovial membranes | HIV (?) | Chlamydia* |
| Human leukocyte antigen-B27 association | Absent | 70%-90%† |

*Shown in non–HIV-associated reactive arthritis. Reports of such infections in patients with HIV-associated reactive arthritis are lacking.
†In whites.



**Figure 113-2** Keratoderma blennorrhagicum in a patient with reactive arthritis and HIV infection.

HLA-B27 is found in 80% to 90% of patients with HIV-associated reactive arthritis, at least among whites.[12] Studies from Africa have found most patients to be HLA-B27$^-$, however.[8,11] It has been known for more than 20 years that the presence of HLA-B27 antigen is associated with slower progression to AIDS.[13] In asymptomatic HIV-infected, HLA-B27$^+$ individuals, cytotoxic T lymphocyte responses are dominated by recognition of a gag-encoded p24 protein epitope that is not seen in HIV$^+$, HLA-B27$^-$ individuals.[14,15] Other class I HLA antigens that have been associated with a better outcome in HIV infection have been implicated in psoriasis and psoriatic arthritis and include HLA-B13 and HLA-B57.[13,14] Approximately half of Caucasian long-term nonprogressors have either HLA-B57 or HLA-B27.[15] Other genetic and innate immune factors associated with nonprogression include delta32 CCR5 allelic associations HLA-C/KIR interactions.[15] HLA-B*5703 was protective against HIV progression in a Zambian population but conferred susceptibility to spondyloarthritis.[16]

## Treatment

Treatment is similar to that for HIV$^-$ patients with reactive arthritis. NSAIDs are the mainstay; in particular, indomethacin is recommended, not only for its efficacy, but also for its inhibition of HIV replication that has been observed in vitro, which seems to be unique to this NSAID.[17] Patients frequently have an inadequate response to NSAIDs alone. Sulfasalazine was effective in some studies at doses of 2 g/day, and one study suggested that it ameliorated HIV infection.[18] Methotrexate was initially believed to be contraindicated because of its immunosuppressive effect, but with careful monitoring of HIV viral loads, CD4$^+$ counts, and the patient's clinical status, more recent studies have suggested a place for methotrexate in the treatment of reactive arthritis and psoriatic arthritis occurring in HIV infection.[19]

Hydroxychloroquine has been reported to be efficacious not only in treating HIV-associated reactive arthritis, but also in reducing HIV replication in vitro and in reducing HIV viral loads in vivo.[20] Arthritis and the cutaneous

lesions of HIV-associated reactive arthritis and psoriatic arthritis have been found to respond to etretinate (0.5 to 1 mg/kg/day),[21] although because of the side effects of this drug, its use should be reserved for patients unresponsive to other treatments. TNF blockers have been used,[22,23] although these agents should be used with extreme caution and only in patients with $CD4^+$ counts greater than 200/μL and HIV viral load less than 60,000 copies/$mm^3$.[24,25] One prospective study of eight HIV patients with spondyloarthritis or rheumatoid arthritis found TNF blockers effective and safe for as long as 5 years when these precautions were followed at initiation of therapy.[25]

## Psoriasis and Psoriatic Arthritis

The extent of skin involvement with psoriasis can be extensive (Figure 113-3) in $HIV^+$ patients, especially in patients not on anti-retroviral treatment. Of note, cutaneous T cell lymphoma can resemble psoriasis and should be considered in the differential diagnosis of psoriasis in $HIV^+$ individuals.[25] Psoriasis and psoriatic arthritis were infrequently reported in sub-Saharan Africa before the HIV pandemic, with prevalences of psoriasis (in hospital-based dermatology departments) ranging from 0.05% to 0.9% in West Africa and 2.8% to 3.5% in South Africa.[27] However, this has increased significantly in the HIV era, with more than 5% of $HIV^+$ individuals reported with psoriasis. Similarly, there were few data on psoriatic arthritis prevalence in sub-Saharan Africa. Psoriatic arthritis was reported before the beginning of the HIV pandemic (41.6% among 61 patients with psoriasis) and many feel this too has increased with the growing HIV prevalence.[26,27]

In general, the arthritis in HIV-associated psoriatic arthritis is predominantly polyarticular, lower limb, and progressive. Psoriasis is commonly an extensive guttate-plaque admixture and, in contrast to the articular disease, was nonremittive with the onset of AIDS.[27,28] Anti-retroviral treatment is effective in treating HIV-associated psoriasis and its associated arthritis.[29,30] Phototherapy may improve the skin rash but also may enhance viral replication, worsen HIV disease, and increase the risk of skin cancer. Other agents reported to be efficacious include cyclosporine (although renal function must be monitored carefully) and etretinate. Methotrexate also can be used, albeit with caution.[19] TNF blockers can be used in patients with refractory disease, and a number of patients have had dramatic improvements in skin lesions and in arthritis,[25,31] although with the usual precautions (see preceding text) because frequent polymicrobial infections while on the drug resulted in its discontinuation in some patients.[31]



**Figure 113-3** Disseminated psoriasis vulgaris in a patient with HIV–associated psoriasis.

## Undifferentiated Spondyloarthritis

Symptoms of reactive arthritis or psoriatic arthritis such as enthesopathy (plantar fasciitis, Achilles tendinitis) are observed in patients in whom full-blown disease does not otherwise develop. Treatment is symptomatic (e.g., NSAIDs, intralesional corticosteroid injections), although sulfasalazine should be considered in patients with more extensive disease.

## Avascular Necrosis of Bone

Case control studies have shown an association of osteonecrosis after the introduction of highly active anti-retroviral therapy (HAART), especially in those who have received corticosteroids.[32,33] A recent meta-analysis revealed a more than twofold increased odds of avascular necrosis (AVN) among HIV-infected patients exposed to protease inhibitors.[33] Other contributing factors, include dyslipidemia (associated with protease inhibitor usage), as well as alcohol abuse, use of megestrol acetate, the presence of antiphospholipid antibodies,[34] and intravenous drug abuse,[35] as well as HIV itself.[36] The most common presenting symptom of osteonecrosis is pain on weight bearing and activity. Some patients may be asymptomatic, and the diagnosis is made based on incidental findings in radiologic studies. Most patients tend to present when subchondral collapse already has occurred. Radiographs, computed tomography (CT), MRI, and nuclear medicine studies have been used successfully to diagnose osteonecrosis, as in $HIV^-$ patients.

## Hypertrophic Pulmonary Osteoarthropathy

Hypertrophic pulmonary osteoarthropathy affects bones, joints, and soft tissues and can develop in HIV-infected patients with *Pneumocystis jiroveci* pneumonia. It is characterized by severe pain in the lower extremity; digital clubbing; arthralgia; nonpitting edema; and periarticular soft tissue involvement of the ankles, knees, and elbows. The skin over the affected areas is glistening, edematous, and warm. Radiography reveals extensive periosteal reaction and subperiosteal proliferative changes in the long bones of the lower extremity. A bone scan shows increased uptake along the cortical surfaces. Treatment of *P. jiroveci* pneumonia usually alleviates this condition.[37]

### Osteopenia and Osteoporosis

Osteopenia and osteoporosis occur more than three times as commonly in HIV-infected patients regardless of antiretroviral treatment[38] and can result in pathologic fractures.[39] One meta-analysis found 15% of HIV$^+$ patients to have osteoporosis and 52% to have osteopenia.[40] Risk factors for osteopenia include use of protease inhibitors, advanced stage of HIV infection, high viral load, low body mass, low bicarbonate levels, increased alkaline phosphatase levels, and especially, smoking and Vitamin D deficiency.[41,42] Clinicians should have a low threshold to screen for vitamin D deficiency, should provide adequate repletion as necessary, and (if not done already for many other reasons) counsel against smoking. Bisphosphonates and, in patients with HIV wasting syndrome, testosterone, have been used to preserve bone density.[43]

## HIV-ASSOCIATED MUSCLE DISEASE

Muscle involvement in HIV infection varies from uncomplicated myalgias or asymptomatic creatine kinase (CK) elevation to severe, disabling, HIV-associated polymyositis or pyomyositis (Table 113-4). HIV seroconversion also can coincide with myoglobinuria and acute myalgia, suggesting that myotropism for HIV may be present early in the infection.

### Myalgia and Fibromyalgia

One-third of HIV$^+$ outpatients report myalgias,[2,44] and 11% describe fibromyalgia.[45] Fibromyalgia is associated with longer disease duration and a history of depression. Treatment is similar to that for fibromyalgia in the non-HIV setting.

**TABLE 113-4** Myopathies Associated with HIV Infection

| HIV-Associated Myopathies | Myopathies Secondary to Anti-retrovirals | Others |
|---|---|---|
| HIV polymyositis<br>Inclusion body myositis<br>Nemaline myopathy | Zidovudine myopathy<br>Toxic mitochondrial myopathies related to other NRTIs | Opportunistic infections involving muscle (toxoplasmosis)<br>Tumor infiltrations of skeletal muscle |
| Diffuse infiltrative lymphocytosis syndrome | HIV-associated lipodystrophy syndrome | Rhabdomyolysis |
| HIV wasting syndrome<br>Vasculitic processes | Immune reconstitution syndrome related to HAART | Pyomyositis |
| Myasthenia gravis and other myasthenic syndromes | Raltegravir-associated myopathy | |
| Chronic fatigue and fibromyalgia | | |

HAART, Highly active anti-retroviral therapy; NRTIs, nucleoside reverse transcriptase inhibitors.

### Non-inflammatory Necrotizing Myopathy and HIV-Related Wasting Syndrome

Severe wasting from chronic infection, malignancy, malabsorption, and nutritional deficiency often accounts for weakness and disability in patients with AIDS.[46] This wasting leads to loss of lean body and muscle mass. Cachexia and muscle wasting associated with HIV constitute "slim disease" in Africa. A non-inflammatory necrotizing myopathy of unclear pathogenesis has been described, accounting for 42% of patients diagnosed with myopathy.[45] Even in patients without significant wasting, muscle biopsy specimens have shown diffuse atrophy, mild neurogenic atrophy, or thick filament loss without conspicuous inflammation. Whether this condition is immune mediated, as some have suggested,[47] or whether it is the result of metabolic or nutritional factors remains unclear. Corticosteroids have been reported to restore muscle strength and mass.[47]

### Nemaline Myopathy

Nemaline myopathy is a rare disorder that has been described in some HIV$^+$ patients in addition to its occurrence as a congenital disorder. Nemaline myopathy represents a nonspecific myofibril alteration resulting from Z band disruption.[49] Muscle-biopsy specimens disclose prominent, randomly distributed, atrophic type 1 fibers with numerous intracytoplasmic rod bodies in the centers of the fibers, corresponding to nemaline rods at electron microscopy. Necrotic fibers and inflammatory infiltrates usually are not found. Some patients have been described to have associated monoclonal gammopathy.[48] Although no inflammation is noted, corticosteroids may be useful. In addition, two cases of successful treatment with intravenous immunoglobulin (IVIG) have been reported.[50]

### HIV-Associated Polymyositis

HIV-associated polymyositis most typically manifests early in the course of HIV infection and may be the presenting feature. In one large series of HIV$^+$ outpatients from a county clinic in Texas, the frequency was 2.2 per 1000.[51]

The pathogenesis of HIV-associated polymyositis is unclear—possibly stemming from direct viral invasion (leading to a cytopathic effect and subsequent muscle necrosis), as suggested in one pathologic study,[52] or from an autoimmune response of the HIV host, as suggested by another study.[53]

The most common manifestation is a subacute, progressive proximal muscle weakness occurring in the setting of an elevated CK. Myalgia is not a prominent presenting feature. Skin involvement is unusual, as is involvement of extra-ocular muscles and facial muscles. In a recent report from Africa, HIV polymyositis cases were all female and, compared to the non-HIV polymyositis cases, were younger and with fourfold lower serum CK.[54] On the other hand, only a handful of cases of dermatomyositis in HIV have been reported, usually in the setting of advanced immunodeficiency.[55] CK levels may be less elevated or even normal in some HIV-associated polymyositis patients.[51,54]

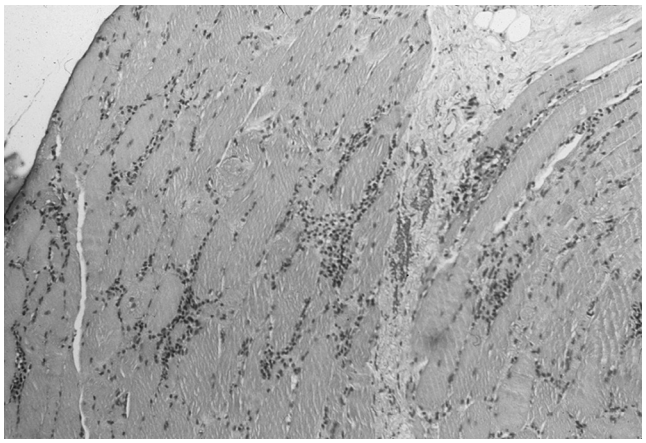


**Figure 113-4** Muscle biopsy specimen from a patient with HIV–associated polymyositis.

On MRI, T2-weighted studies with or without fat saturation show high signal intensity without rim enhancement, in contrast to pyomyositis, in which rim enhancement is seen.[56] MRI also is helpful in guiding muscle biopsy—the definitive diagnostic test. Electromyographic studies reveal myopathic motor unit potentials with early recruitment and full interference patterns and fibrillation potentials, positive sharp waves, and complex repetitive discharges indicative of an irritative process. Light microscopy of muscle biopsy specimens shows interstitial inflammatory infiltrates of variable intensity accompanied by degenerating and regenerating myofibrils, similar to those seen in polymyositis without HIV (Figure 113-4). Concomitant vasculitis rarely occurs. In specimens from $HIV^+$ and $HIV^-$ patients with myositis, the predominant cell populations were $CD8^+$ T cells and macrophages invading or surrounding healthy muscle fibers that express class I major histocompatibility complex (MHC) antigens on their cell surfaces.[57] Endomysial infiltrates in specimens from $HIV^+$ patients differed from those of patients with polymyositis without HIV infection only by a significant reduction of $CD4^+$ cells.[53]

Treatment is similar to that provided for other inflammatory myopathies. CK elevation and muscle weakness respond to moderate-dose glucocorticoids.[51,54] Refractory cases may require immunosuppressive agents, such as methotrexate, azathioprine, or mycophenolate mofetil. IVIG has been used with some success. These agents should be used with caution, however, with careful monitoring of the patient's clinical status, $CD4^+$ counts, and HIV mRNA levels.

CK elevation is commonly encountered in outpatients with HIV infection, secondary to HIV per se, behaviors associated with higher risk for HIV infection (e.g., cocaine use), or HIV treatment.[51] In most patients, these elevations are transient and are of little consequence, but they require careful follow-up for any sign of clinical deterioration before electrodiagnostic and biopsy studies are undertaken.

## Inclusion Body Myositis

Inclusion body myositis has been recognized as a complication of HIV infection.[58] This condition is clinically, histologically, and immunologically identical to sporadic inclusion body myositis. Muscle biopsy specimens suggest two concurrently ongoing processes—an autoimmune process mediated by cytotoxic T cells and a degenerative process manifested by vacuolated muscle fibers and deposits of amyloid-related proteins. Of particular interest has been the finding of elevated mRNA levels and constitutive expression of Toll-like receptor 3, which is known to mediate inflammatory stimuli from pathogens and endogenous danger signals, and to link the innate and adaptive immune systems, in muscle fibers of patients with HIV-associated inclusion body myositis in close proximity with infiltrating mononuclear cells.[59] One review of four cases of HIV-associated inclusion body myositis found that involved $CD8^+$ cells surrounding muscle fibers were virus-specific and may cross-react with antigens on the surface of muscle fibers, suggesting that HIV may trigger a viral-specific inflammatory response that can lead to inclusion body myositis.[60]

## Myopathy Associated with Treatment

A reversible toxic mitochondrial myopathy occurring in patients who received high doses of zidovudine has been described, which manifests as myalgias, muscle tenderness, and proximal muscle weakness mimicking HIV polymyositis.[61] Reports have also described mitochondrial toxicity manifesting as ptosis or ophthalmoplegia in $HIV^+$ patients on zidovudine and other nucleoside reverse transcriptase inhibitors (NRTIs) such as didanosine.[62,63] Histologically, it is characterized by the presence of *ragged red fibers*, a term coined to designate atrophic ragged red fibers with marked myofibril alterations, including thick myofilament loss and cytoplasmic body formation,[64] and minimal inflammatory infiltrates. Symptoms tend to improve while the drug is discontinued, with CK levels returning to normal within 4 weeks of discontinuing the drug and muscle strength returning within 8 weeks. In any HIV-infected patient seen with an elevated CK level, especially when symptoms of myalgia or muscle weakness are present, zidovudine should be discontinued for 4 weeks and the patient re-evaluated before electromyography or muscle biopsies are undertaken. However, with decreasing use of zidovudine and new medications, other myopathic complications are being seen. The use of raltegravir is associated with a higher prevalence of symptomatic skeletal muscle toxicity, which does not seem to be concentration or time dependent, nor associated with elevated CK. Proximal myopathy may be an uncommon but significant side effect of raltegravir exposure.[65]

## Rhabdomyolysis

Rhabdomyolysis can occur at all stages of HIV infection and may be separated into three groups: (1) HIV-associated rhabdomyolysis, including rhabdomyolysis in primary HIV infection, recurrent rhabdomyolysis, and isolated rhabdomyolysis; (2) drug-induced rhabdomyolysis; and (3) rhabdomyolysis at the end stage of AIDS, associated or not with opportunistic infection of muscle. Drugs implicated in rhabdomyolysis in HIV patients include didanosine, lamivudine, trimethoprim-sulfamethoxazole, ritonavir, indinavir, and raltegravir.[64,66]

## DIFFUSE INFILTRATIVE LYMPHOCYTOSIS SYNDROME

Diffuse infiltrative lymphocytosis syndrome (DILS), found exclusively in $HIV^+$ patients, is characterized by salivary gland enlargement and peripheral $CD8^+$ lymphocytosis often accompanied by sicca symptoms and other extraglandular features. The prevalence of DILS is declining since the introduction of HAART.[67] Using parotid enlargement as a criterion, the prevalence in Houston, Texas, was 4% in the pre-HAART era, declining to 0.8% after the introduction of aggressive HIV therapy.[67,68] In another study from Greece, using xerophthalmia and xerostomia as the defining criteria (and requiring confirmatory minor salivary gland biopsy specimen and technetium scintigraphy),[69] the prevalence of DILS was 7.8% and has decreased dramatically there and elsewhere after the introduction of HAART.[2,67,70]

The primary immunogenetic association has been with HLA-DRB1 alleles expressing the ILEDE amino acid sequence in the third diversity region—usually HLA-DRB1*11:02, DRB1*13:01, and DRB1*13:02.[67,71] Delayed progression to AIDS in patients with DILS has been attributed to delay in the evolution of HIV from the less aggressive M-tropic strain to the more rapidly replicating T-tropic strain by a more effective $CD8^+$ lymphocyte response.[72] This response has been attributed in part to the finding of sequence homology of a six-residue epitope shared by HLA-DRB1 alleles associated with DILS with a V3 loop on M-tropic HIV strains. Studies of immunophenotypes of circulating and tissue-infiltrating lymphocytes and salivary gland T cell-receptor sequence analysis suggested that DILS represents an MHC-restricted, antigen-driven, oligoclonal selection of $CD8^+$, $CD29^-$ lymphocytes that express selective homing receptors and infiltrate the salivary glands, lungs, and other organs, where they are postulated to suppress HIV replication.[72]

Minor salivary gland biopsy specimens show a focal sialadenitis, similar to that observed in Sjögren's syndrome, although destruction of the salivary glands tends to be lessened (Figure 113-5). $CD8^+$ lymphocytes constitute most of the inflammatory infiltrate,[73,74] in contrast to that seen in primary (non–HIV-associated) Sjögren's syndrome. Lymphoepithelial cysts are seen frequently in the parotid glands of patients with DILS, leading to inspissated salivary secretions that may be painful.

The characteristic, if not defining, presentation of DILS consists of painless parotid enlargement, often massive (Figure 113-6). This enlargement is accompanied by sicca symptoms in more than 60% of patients. Although parotid and submandibular enlargement is nearly universal in this disorder, certain extraglandular features also are prominent (Table 113-5). DILS and Sjögren's syndrome share some similarities and differences (Table 113-6).

Diagnostic criteria have been proposed for DILS as follows:

- HIV seropositive by enzyme-linked immunosorbent assay and Western blot analysis.
- Bilateral salivary gland enlargement or xerostomia persisting for longer than 6 months.
- Histologic confirmation of salivary or lacrimal gland lymphocytic infiltration in the absence of granulomatous or neoplastic enlargement.

Minor salivary gland biopsy specimens are usually positive (see Figure 113-5). Gallium-67 scintigraphy (Figure 113-7) of the salivary glands has been used when lip biopsy was not feasible or equivocal. Tc99m pertechnetate scanning offers little diagnostic help. Scintigraphy is used as a primary diagnostic aid in patients on protease inhibitors because minor salivary gland biopsy specimens are rarely positive in patients on these drugs. CT also has been used to determine the extent of glandular swelling and to evaluate parotid cysts and possible salivary glandular malignancy.

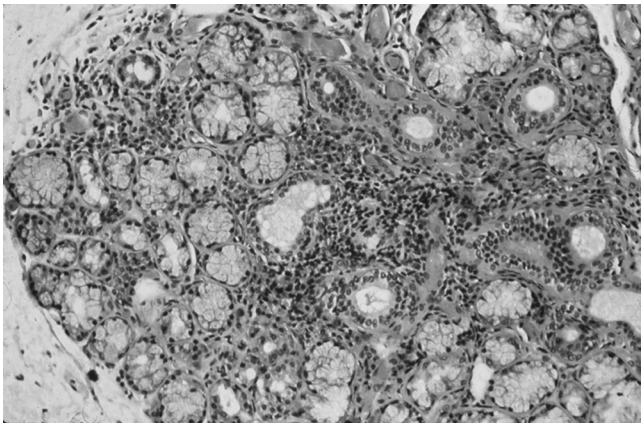


**Figure 113-5** Minor salivary gland biopsy specimen from a patient with diffuse infiltrative lymphocytosis syndrome. Note the relative preservation of the glandular architecture, even with significant interstitial inflammation.

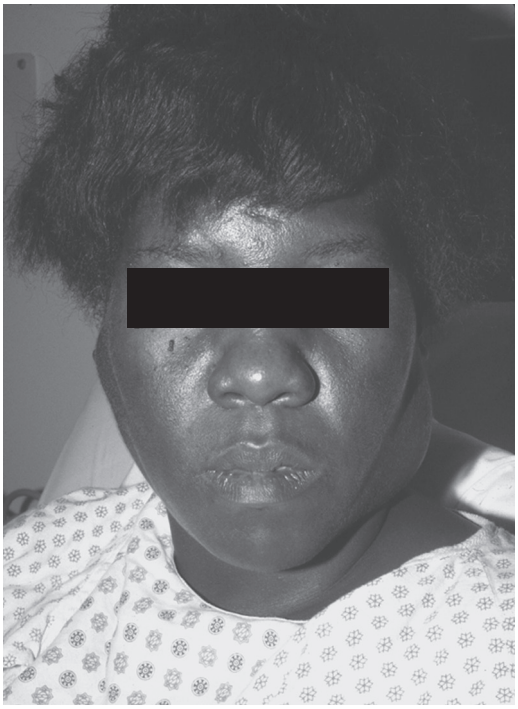


**Figure 113-6** Massive bilateral asymmetric salivary gland enlargement in a patient with diffuse infiltrative lymphocytosis syndrome. This was the presenting feature of human immunodeficiency virus infection in this patient. Computed tomography revealed this to be a solid mass. At follow-up 2 years after this photograph was taken, the gland had not changed in size.

**TABLE 113-5** Extraglandular Features of Diffuse Infiltrative Lymphocytosis Syndrome

| |
|---|
| **Pulmonary** |
| Lymphocytic interstitial pneumonitis* |
| **Neurologic** |
| Cranial nerve VII palsy† |
| Aseptic lymphocytic meningitis |
| Peripheral neuropathy |
| **Gastrointestinal** |
| Lymphocytic hepatitis |
| **Renal** |
| Renal tubular acidosis |
| Interstitial nephritis |
| **Musculoskeletal** |
| Peripheral arthritis |
| Polymyositis |
| **Hematologic** |
| Lymphoma‡ |

*25% to 50%, but decreasing.
†Due to mechanical compression by inflamed parotid tissue.
‡Poor prognostic indicator.

**TABLE 113-6** Similarities and Differences between Diffuse Infiltrative Lymphocytosis Syndrome (DILS) and Sjögren's Syndrome

| Feature | DILS | Sjögren's Syndrome |
|---|---|---|
| Parotid swelling | Universal | Uncommon |
| Sicca symptoms | Common | Very common |
| Extraglandular symptoms | Common | Uncommon |
| Autoantibodies (anti-nuclear antibodies, anti-Ro/La) | Rare | Common |
| Class II Human leukocyte antigen association | DRB1*11:02, DRB1*13:01, DRB1*13:02 | DRB1*03:01, DQA1*05:01, DQB1*02:01 |

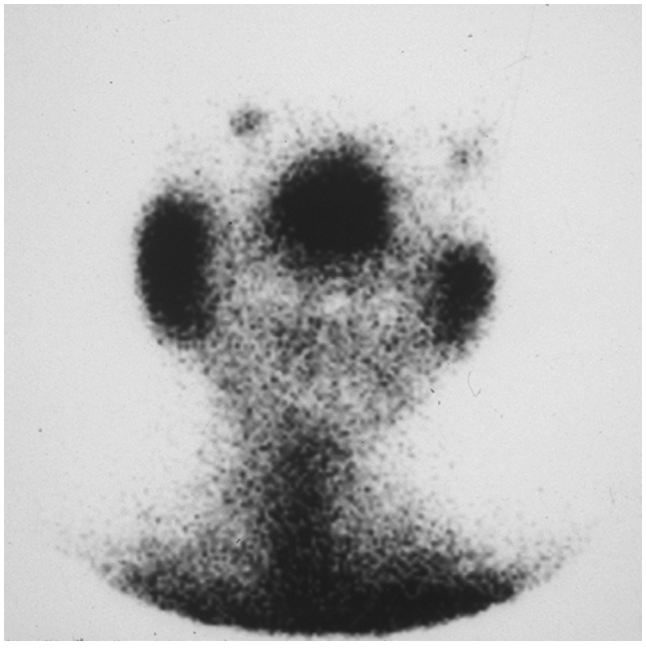


**Figure 113-7** The "snowman" sign. Gallium-67 scintigraphy of the parotid glands of a patient with diffuse infiltrative lymphocytosis syndrome occurring in the setting of hemophilia.

**TABLE 113-7** Treatment of Diffuse Infiltrative Lymphocytosis Syndrome

| |
|---|
| Reassurance and education |
| Regular dental care |
| No specific treatment for asymptomatic individuals |
| Effective anti-retroviral treatment |
| Pilocarpine or cevimeline for sicca symptoms |
| Systemic glucocorticoids |
| Drainage and instillation of corticosteroids into parotid lymphoepithelial cysts |
| Radiation of parotid cysts |



**Figure 113-8** Aspiration of a parotid epithelial cyst in a patient with diffuse infiltrative lymphocytosis syndrome.

Patients with asymptomatic glandular swelling and mild, if any, sicca symptoms can be observed over time (Table 113-7). Anti-retroviral treatment is effective in treating the glandular swelling and sicca symptoms associated with DILS and complications such as neuropathy.[67] We have found also that moderate doses of corticosteroids (30 to 40 mg/day of prednisone) are effective in treating the glandular swelling and sicca symptoms of DILS without adversely affecting the frequency of opportunistic infections, increasing the viral loads, or depressing the $CD4^+$ counts, although the effect is transient. Lymphocytic interstitial pneumonitis may require higher doses of corticosteroids (60 mg/day of prednisone), sometimes for extended periods. Radiation therapy should be avoided. Cranial nerve VII palsy tends to respond poorly to any treatment. Combination anti-retroviral therapy has been reported to be effective in resolving parotid epithelial cysts, although when refractory, the cysts can be managed by aspiration and instillation of 1 mL (40 mg) of either methylprednisolone or triamcinolone suspension into the cyst (Figure 113-8). Frequent recurrence may necessitate surgical excision.

## VASCULITIS ASSOCIATED WITH HIV INFECTION

A wide spectrum of vasculitis has been described in patients with HIV infection.[75,76] Fever, malaise, weakness, rash,

headaches, and neurologic symptoms are common in HIV$^+$ patients, and triggers of vasculitis range from specific infectious agents and drugs to idiopathic causes. Among infectious causes, cytomegalovirus and tuberculosis are probably the most common. Inflammatory vasculitides are less common rheumatologic diseases that occur in less than 1% of HIV patients.

One series found 34 (23%) of 148 "symptomatic" HIV$^+$ patients to have vasculitis.[77] Of these patients, 11 met American College of Rheumatology criteria for a distinct category of vasculitis, including hypersensitivity vasculitis in 6, polyarteritis nodosa in 4, and Henoch-Schönlein purpura in 1. Another series of 98 Chinese patients found that 20% had vasculitis, including 15 with Behçet's-like disease, 2 cases of Henoch-Schönlein purpura, 2 cases of digital gangrene, and 1 case of CNS vasculitis.[78] Granulomatosis with polyangiitis and pulmonary microscopic polyangiitis can occur in patients with high CD4$^+$ counts and during immune reconstitution. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome) has also been described.[79] Behçet's disease and relapsing polychondritis occur in HIV infection[79,80] and respond to HAART.[81]

Rapidly progressive focal necrotizing vasculitis of the aorta and large arteries with occlusion and occasionally aneurysm formation and rupture has been described in Africans with HIV infection. In one recent study, 90% of HIV$^+$ patients, but no HIV$^-$ patient, had evidence of adventitial leukocytoclastic vasculitis of the vasa vasorum.[82] Giant cell arteritis likewise has been described in patients with HIV infection with aortic root dilation.[83] Kawasaki's disease has been reported in HIV$^+$ children and adults.[84] Cryoglobulinemic vasculitis with associated lymphocytic interstitial pneumonia occurs with and without hepatitis C coinfection.

Patients with isolated CNS angiitis usually are seen with organic brain syndromes and neurologic deficit.[85] In children and in one case report in an adult, HIV-associated cerebral aneurysmal arteriopathy was described as causing multiple fusiform aneurysms in the circle of Willis.[86] CNS vasculitis may manifest as recurrent strokes. Although imaging studies (MRI, angiography) may be helpful, brain biopsy may be necessary to establish the diagnosis. Necrotizing granulomatous vasculitis not limited to the CNS has been reported in patients with low CD4$^+$ counts that respond to anti-retroviral therapy.[75] More recently, a case report has described leukocytoclastic cerebral vasculitis treated with anti-CD25 antibody.[87]

Diagnosis is based on a high degree of suspicion and on angiography and biopsy of specific organ beds. Similar to immunocompetent patients, perinuclear anti-neutrophil cytoplasmic antigen (pANCA) and cytoplasmic anti-neutrophil cytoplasmic antigen (cANCA) may be useful with granulomatosis with polyangiitis or microscopic polyangiitis. However, biopsy with cultures is important to rule out infectious mimics.

Corticosteroids are the mainstay of treatment for HIV-associated vasculitis, although cytotoxic agents such as cyclophosphamide, IVIG, and plasmapheresis have been used in refractory cases. Painful neuropathy secondary to vasculitis responds well to high-dose glucocorticoids, in contrast to HIV-associated peripheral neuropathy.[87,88]

## PRIMARY PULMONARY HYPERTENSION

Pulmonary hypertension is a severe life-limiting disease that often affects younger patients. Patients with AIDS and primary pulmonary hypertension present with a higher degree of pulmonary hypertension than non-AIDS patients.[89,90] The predominant histopathologic finding has been a plexogenic pulmonary arteriopathy, although thromboembolic changes also have been reported. One group found an association with HLA-DRB1*13:01 and HLA-DRB1*13:02 and with the linked allele HLA-DRB3*03:01.[90]

Symptoms include progressive shortness of breath, pedal edema, nonproductive cough, fatigue, syncope or near-syncope, and chest pain. Pulmonary function tests show mild restrictive patterns with variably reduced diffusing capacities. In a review of 131 cases of pulmonary hypertension associated with HIV infection, the interval between the diagnosis of HIV disease and the diagnosis of pulmonary hypertension was 33 months. The median length of time from diagnosis to death was 6 months.[91] However, with improving treatment of pulmonary hypertension, the prognosis has improved considerably. Responses to vasodilator agents—calcium-channel blockers, sildenafil, intravenous and inhaled prostanoids, and endothelin antagonists—and to HAART vary, and recent studies show improved mortality.[92,93]

## HIV-ASSOCIATED MUSCULOSKELETAL INFECTION

### Pyomyositis

Pyomyositis is a primary infection of skeletal muscle that does not arise from contiguous infection; it is presumably hematogenous in origin and often is associated with abscess formation. Rarely seen in developed countries, infectious myositis nonetheless is an important complication of HIV infection in areas most endemic for HIV, such as Africa and India. It tends to occur in later stages of the infection, with CD4$^+$ counts less than 200/μL. *Staphylococcus aureus* is the most common pathogen.[94] Other organisms that have been implicated include *Streptococcus pyogenes*, *Cryptococcus neoformans*, *Mycobacterium tuberculosis*, *Mycobacterium avium-intra-cellulare*, *Nocardia asteroides*, *Salmonella enteritidis*, *Escherichia coli*, *Citrobacter freundii*, *Morganella morganii*, *Pseudomonas aeruginosa*, and group A streptococci.

The clinical course of pyomyositis can be roughly divided into three stages: invasive, suppurative, and late. The first stage, which typically lasts 1 to 3 weeks, is characterized by localized cramplike pain and induration in conjunction with a low-grade fever. Large muscle groups, particularly those of the lower extremities, are most often affected. The degrees of pain and fever increase in the second stage, which is characterized further by the development of edema and pus in the affected muscle. Untreated, the disease progresses to the third stage; within 3 weeks of onset, sepsis and death can occur.[95] The mortality rate associated with pyomyositis has been estimated to range from 1% to 20%. Ultrasound and MRI with contrast enhancement are effective in localizing infection, although sometimes tagged

white blood cell scans may be needed. Oral and intravenous antibiotics in conjunction with surgical drainage are often required.

## Bacterial Arthritis and Osteomyelitis

No data suggest that bacterial infection of bones or joints occurs more frequently in patients with HIV infection.[96] *S. aureus* is the most common infectious agent encountered, but parenteral drug use and not HIV infection per se may account for this. Many other organisms have been reported to cause osteomyelitis in HIV-infected patients, including *Mycobacterium tuberculosis*, *Salmonella*, *Nocardia asteroides*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, cytomegalovirus, invasive *Aspergillus*, *Toxoplasma gondii*, *Torulopsis glabrata*, *Cryptococcus neoformans*, and *Coccidioides immitis*. Osteomyelitis is associated with mortality rates of greater than 20% in HIV-infected patients. The most frequently involved bones are the wrist, tibia, femoral heads, and thoracic cage, but other rare sites, such as the patella and the mandible, have been reported.

## Musculoskeletal Tuberculosis

Musculoskeletal involvement, the fourth most common extrapulmonary manifestation of tuberculosis, is found in approximately 1% to 5% of patients with tuberculosis. It can mimic many skeletal diseases and can manifest in various locations. Less than 50% of reported patients with musculoskeletal tuberculosis have radiographic evidence of pulmonary tuberculosis. *M. tuberculosis* disseminates hematogenously after an acute or reactivated pulmonary infection. Usually, skeletal tuberculosis lesions in immunocompetent patients are solitary, but in AIDS patients, they may have a multicentric distribution in approximately 30% of cases.[95] The vertebrae are the most common site involved, mostly the lower thoracic or upper lumbar segments. The frequency of tuberculous spondylitis is 50% to 66%; peripheral arthritis, 20% to 30%; osteomyelitis, 10% to 20%; and tenosynovitis and bursitis, approximately 1% to 3%. Treatment includes four-drug antitubercular therapy and, often, surgical intervention.

## Atypical Mycobacterial Infection

Musculoskeletal infection caused by atypical mycobacterial species is unusual in immunocompetent individuals.[97] Atypical mycobacterial species most commonly implicated in causing septic arthritis or osteomyelitis in HIV include *M. avium-intra-cellulare* complex, *Mycobacterium kansasii*, *Mycobacterium haemophilum*, *Mycobacterium terrae*, and *Mycobacterium fortuitum*. *M. haemophilum* has been most frequently implicated in skeletal infection, accounting for more than half of cases, and *M. kansasii* is second, accounting for an additional 25%. These are systemic infections that have involved several joints or skeletal sites. Cutaneous lesions, such as nodules, ulcers, and draining sinus tracts, occur in approximately 50% of patients.[97] These infections tend to occur late in the course of HIV, usually when the $CD4^+$ T lymphocyte count is less than 100/μL. *M. avium-intra-cellulare* complex osteomyelitis has also been associated with immune reconstitution inflammatory syndrome after initiation of HAART.[98] Along with standard antituberculosis therapy, clarithromycin is effective.[97]

## Bacillary Angiomatosis Osteomyelitis

Bacillary angiomatosis is a multisystem infectious disease caused by two closely related organisms: *Bartonella henselae* and *Bartonella quintana*. It seems to be a disease unique to HIV-infected patients and, to a lesser degree, to other immunocompromised patients.[99] Clinical characteristics associated with bacillary angiomatosis and bacillary peliosis (BAP) in patients with HIV infection include fever (temperature, >37.8° C; 93%), cutaneous or subcutaneous vascular lesions, lymphadenopathy, osteomyelitis, and abdominal symptoms associated with profound depression of CD4 lymphocyte counts, anemia, and elevated serum level of alkaline phosphatase.[99,100]

Osteomyelitis is found in approximately one-third of patients in association with skin disease. These lesions usually are characterized by extensive destruction of the cortical bone, periostitis, medullary invasion, and an overlying soft-tissue mass that might resemble cellulitis. Complete remission of bacillary angiomatosis after doxycycline or erythromycin therapy occurs, although bone lesions may need surgical drainage.

## Fungal Infections

In addition to bacterial infection, patients with advanced HIV infection ($CD4^+$ T lymphocyte count <100/μL) are at high risk for fungal musculoskeletal infections, particularly infections caused by *Candida albicans*[101] and *Sporothrix schenckii*.[102] *S. schenckii* can manifest with oligoarticular or even polyarticular involvement and tenosynovitis (Figure 113-9) and can be particularly difficult to eradicate, requiring long-term suppressive antifungal therapy. Various disseminated fungal infections, such as histoplasmosis, cryptococcosis, and blastomycosis, occur in HIV and often cause osteomyelitis.

## Parasitic Infections

Muscle toxoplasmosis is found in profoundly immunodepressed patients, typically manifesting with a painful subacute myopathy and concurrent multivisceral toxoplasmosis.[103] *Toxoplasma* cysts are observed mainly in muscle fibers in muscle biopsy specimens, and identification of cysts as *Toxoplasma* may be easier when specific antibodies or electron microscopy is used. Muscle weakness such as in polymyositis can occur in muscle toxoplasmosis. Treatment is based on a combination of drugs acting synergistically against *T. gondii*, including pyrimethamine and sulfadiazine or trisulfapyrimidines.

# RESPONSE OF OTHER RHEUMATIC DISEASES TO HIV INFECTION

Early reports suggest that rheumatoid arthritis went into remission in the face of HIV infection. Early reports likewise suggest that HIV infection might reduce the activity of systemic lupus erythematosus, particularly at times of low $CD4^+$ T cell counts. With emergence of the immune

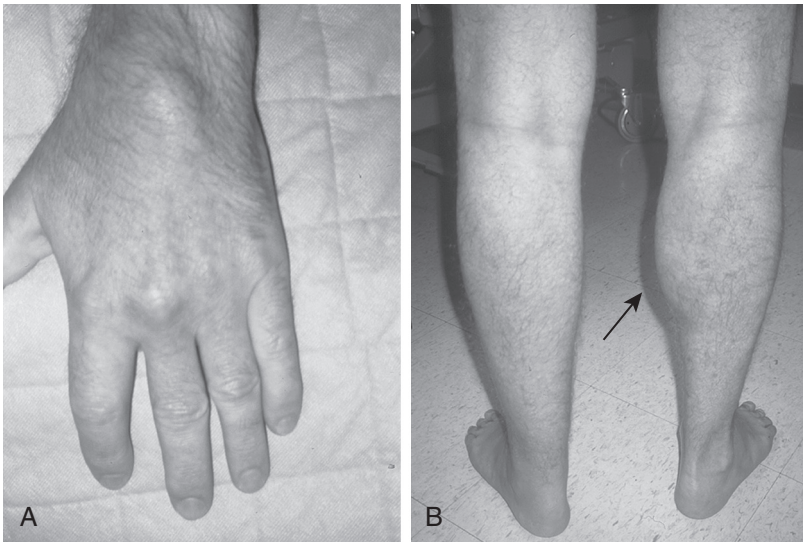



**Figure 113-9** **A,** Third metacarpophalangeal synovitis and common extensor digitorum longus tendon sheath effusion at the dorsal surface of the wrist. **B,** Dissecting Baker's cyst *(arrow)* in the same patient with disseminated *Sporothrix schenckii* infection (organism cultured from synovial fluid obtained from both sites).

reconstitution syndrome, most autoimmune diseases appear de novo or recur with institution of HAART and increased $CD4^+$ counts.[7,104]

## HAART-RELATED IMMUNE RECONSTITUTION SYNDROME

Starting HAART may be initially accompanied by aberrant inflammatory responses termed immune reconstitution inflammatory syndrome (IRIS), wherein patients experience paradoxical deterioration in response to HAART, despite efficient control of HIV viral replication and no apparent drug toxicity.[105,106] It was initially described with recrudescence of infections, but now autoinflammatory and autoimmune phenomena are being described. A meta-analysis of more than 13,000 HIV patients starting HAART found that IRIS developed in 13%.[107] The mean time of onset of IRIS from the time of HAART initiation is approximately 9 months.[107]

Shelburne and co-workers[105] put forth four criteria required for the diagnosis of immune reconstitution inflammatory syndrome, as follows:

1. Patient has been diagnosed with AIDS.
2. Treatment with anti-HIV therapy results in increased $CD4^+$ counts and decreased HIV viral load.
3. Infectious and inflammatory symptoms appear during therapy.
4. Symptoms cannot be explained by a new cause.

Improved understanding of the immunology of IRIS has helped elucidate how atypical, hyperaccentuated inflammatory host responses to pre-existing or co-existing infection can occur in HIV patients taking HAART. HIV infection causes a relentless decline in $CD4^+$ memory and naïve cells, an increase in activated T cells in the peripheral blood, and thymic dysfunction. HAART can lead to sustained suppression of HIV and concomitant repopulation of T cell counts in a biphasic mode.[107] The first phase represents the release of predominantly memory $CD4^+$ cells and lasts a few weeks to months. The second phase, from approximately 6 months on, represents the main phase of naïve T cell proliferation and is accompanied by changes in T helper cytokine production profiles.[108,109] IRIS can occur during both phases of immune recovery, and different infections and autoimmune phenomena occur in phase 1 compared with phase 2.

Organ-specific autoimmune phenomena have been described more often than generalized systemic autoimmune disease and tend to occur later during reconstitution. These phenomena may be a manifestation of naïve T cell release as opposed to memory T cell reconstitution. Graves' thyroiditis occurring approximately 21 months after initiation of HAART has been described in approximately 17 cases.[110] Terminal ileitis, alopecia universalis, cerebral $CD8^+$ lymphocytosis, and Guillain-Barré syndrome[111] have been reported. Polymyositis,[112] rheumatoid arthritis,[7] systemic lupus erythematosus,[113] autoimmune hepatitis, adult Still's disease, and sarcoidosis[114] newly appearing after initiation of HAART also have been described. These conditions tend to occur earlier during reconstitution compared with organ-specific autoimmunity. In addition, IRIS has been reported in the setting of TNF blocker discontinuation, with cryptococcal pneumonia occurring in an $HIV^+$ patient after stopping adalimumab.[115]

If a diagnosis of IRIS is made, HAART is continued, and most symptoms resolve with little or no therapy. If inflammatory symptoms involve areas where significant damage secondary to uncontrolled inflammation is likely to occur, such as in the CNS or eye, HAART should be stopped, and careful use of corticosteroids should be considered. IRIS is less likely to occur if the $CD4^+$ count is greater than 200/μL when HAART is initiated.[106,107] Other risk factors for IRIS include being HAART naïve and the presence of a high antigenic burden with opportunistic infection when HAART is begun.[115,116] Continued systematic analysis is needed, however, and guidelines need to be established for

defining the autoimmunity associated with immune reconstitution. The prognosis for most IRIS cases is favorable because a robust inflammatory response may predict an excellent response to HAART in terms of immune reconstitution and, perhaps, improved survival.

## RHEUMATOLOGIC COMPLICATIONS OF HIV TREATMENT

The myopathy associated with nucleoside transcriptase inhibitors such as zidovudine and the osteonecrosis and parotid lipomatosis associated with the use of protease inhibitors have been discussed previously. In addition to these conditions, cases of adhesive capsulitis, Dupuytren's contracture, tenosynovitis, and temporomandibular joint dysfunction have been reported as a consequence of indinavir treatment.[116]

## LABORATORY ABNORMALITIES ASSOCIATED WITH HIV INFECTION

Humoral immunologic abnormalities are frequent in patients with HIV but are rarely associated with severe clinical signs. The most common laboratory abnormality is polyclonal hyperglobulinemia, found in 45% of HIV$^+$ individuals.[117] Rheumatoid factor and anti-nuclear antibodies, usually in low titer, have been described in 17% of patients with HIV infection in some series,[117,118] although anti-dsDNA antibodies and hypocomplementemia are rare. Increased titers of anti-citrullinated protein antibody (ACPA) and rheumatoid factor occur in advanced HIV infection. Although more specific than rheumatoid factor, before immune reconstitution, ACPA is an unreliable diagnostic marker for RA and does not necessarily predict future RA. Immunoglobulin (Ig)G anti-cardiolipin antibodies are found in 95% of untreated patients with AIDS, particularly in patients with advanced disease, and overall in 20% to 30% of HIV$^+$ individuals.[119] They are rarely associated with thrombotic events. cANCAs and pANCAs have been described in the serum of HIV$^+$ individuals, as have anti–glomerular basement membrane antibodies.[120] Cryoglobulinemia is decreasing in this population since the introduction of HAART.[121] Indeed, with HAART, many of these serologic abnormalities tend to decrease or resolve.

## CONCLUSION

The impact of the global HIV pandemic continues to grow, and rheumatologists need to be aware of the wide spectrum of rheumatic diseases that occur in HIV$^+$ patients. HAART has changed the natural history of HIV infection. It has modified the frequency and expression of some HIV-related clinical syndromes and has been associated directly (toxicity) and indirectly (immune reconstitution) with the development of new ones. With longer survival and newer refinements in treatment, the spectrum of rheumatic disease seen in HIV$^+$ patients is very much a moving target for rheumatologists and is likely to continue to evolve.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 114

# Viral Arthritis



Philippe Gasque

**KEY POINTS**

- Viral infection can lead to chronic arthralgia and symmetric polyarthritis, particularly in elderly patients.
- Exposure, travel, occupation, and vaccination histories should be considered.
- Parvovirus B19 is one of the most common viral arthritides.
- Rubella arthritis can occur in young adults. Rubella vaccination has reduced the overall incidence of infection but can provoke arthritis.
- Alphaviruses are African/Asian mosquito-borne causes of arthritis and rash. Outbreaks are emerging worldwide (including in Europe, the Caribbean islands, and the United States). For example, Chikungunya can cause chronic arthralgias evolving to arthritis, months to years postinfection and in more than 50% of adult patients.
- Hepatitis C virus, Epstein-Barr virus, and many other viruses that find sanctuaries in synovial tissues can cause arthritis.
- Robust humoral and cellular anti-viral responses may not control for viral persistence. Viruses can interfere with the innate immune response.
- Nonconventional autoimmune responses may orchestrate arthritis. Anti-citrullinated antibodies are rare.
- Synovial macrophages and fibroblast-like synoviocytes that carry viral remnants orchestrate the inflammatory response and are associated with angiogenesis and cell hyperplasia.
- NSAIDs, methotrexate, and preventive vaccination measures are beneficial

Many viruses have been claimed to be commonly associated with arthritogenic diseases and reminiscent of rheumatoid arthritis (RA): old world alphaviruses such as Chikungunya (CHIKV) and Ross river virus (RRV)[1-5] and, more rarely, parvovirus B19, Epstein-Barr virus (EBV), rubella, and hepatitis B/C virus (HBV/HCV)[6-9] (Table 114-1). Other viruses such as adenoviruses, coxsackie viruses, HIV, influenza, measles, mumps, and varicella zoster are more likely to be linked to autoimmune responses such as type I diabetes, and may also affect the joints, leading to reactive arthritis.[1,10]

The phenotypes of these diseases may be distinct in terms of the primary cells and tissues initially targeted, the specific humoral and cellular immune responses elaborated, and the inflammatory effector pathways that predominate. Despite the different nature of each viral disease, however, they clearly share some common pathogenic mechanisms involved in arthralgias (acute nociception at the nerve endings), macrophage activation and polarization, as well as fibroblast hyperplasia and invasion sustained by major angiogenesis in the synovial tissues as described in RA (Figure 114-1).[11-13]

Early and robust systemic innate and adaptive immune anti-viral responses are important events to protect the host, including the contribution of the epithelia, a major portal of entry for many of these viruses at the lung and skin.[14] However, in the context of a major infection associated with high viremia and a failure to mount a robust anti-viral response, it has been proposed that the virus would escape from immune surveillance and find protective niches in tissues such as synovial tissues.[12] Indeed, the acute virus infection might, in a genetically predisposed or otherwise susceptible host (e.g., association with human leukocyte antigen [HLA] or immune senescence in elderly patients), be able to recapitulate a polarized inflammatory response as described in RA and other autoimmune diseases.[15] Viruses could also polarize host-cell defense mechanisms such as type I interferon (IFN) signaling pathways, apoptosis, and autophagy to its own advantage.[16,17]

As discussed below, it is increasingly recognized that synovial tissue is a sanctuary for many arthritogenic viruses, which can recruit reactive T and, more rarely, B cells to the joints to drive chronic inflammation and, ultimately, contribute to hyperplastic synovial pannus tissue formation, and finally to the destruction of cartilage and bone as found in RA.[13,18] Viruses could be brought into the synovial tissue by infected monocytes, fibrocytes, or lymphocytes before being transferred to resident cells such as synovial fibroblasts.[12,19,20]

Autoimmunity against self-antigens prominently expressed by infected synovial fibroblasts may be exceptionally engaged through bystander activation and the expression of cryptic antigens and more rarely through well-described molecular mimicry mechanisms.[10]

Surprisingly, many of the self-antigens are nuclear or mitochondrial proteins released by dying cells as a consequence of the infection but which are also critically involved as natural adjuvants (also called *alarmins*) to alert innate and adaptive immune cells.[21,22,22]

Paradoxically, the events involved in the clearance of viruses will contribute to sustained chronic inflammation and will instigate arthralgia and ultimately leading to destructive arthritis. Regulatory mechanisms to control chronic inflammation have been recently emphasized, but

**TABLE 114-1** Examples of Viruses Implicated in Human Chronic Arthralgia and Arthritis

| | PRIMARY INFECTION (PI) AND ACUTE RESPONSES | | CHRONIC RESPONSES (MONTHS TO YEARS AFTER PI) AND FACTORS THAT MAY BE INVOLVED | | | | |
|---|---|---|---|---|---|---|---|
| **Virus (Clinical Hallmarks)** | **Primary Target Cells** | **Cellular and Humoral Immunity Against Virus** | **Tissue Sanctuaries** | **Systemic, Synovial Tissues and Articular Chronic Inflammations/ Injuries** | **Chronic Arthralgia** | **Humoral/Cellular Autoimmune and Inflammatory Responses** | **Therapies of Arthralgia and Arthritis** |
| **RNA Viruses** | | | | | | | |
| CHIKV (Arthralgia) (Myalgia) (Tenosynovitis) | Fibroblast (skin)[16,55,76] Receptor-mediated entry is unknown | Robust activation of NK cells[107] Robust activation of T/B immunity against virus[107] Poor expression of type I IFN by dendritic cells[76] Monocytes can be infected[73] and carry the virus to sanctuary | Localization of virus (RNA, proteins) in synovial perivascular macrophages and few fibroblasts months after infection in one patient[107] and in nonhuman primates[71] and in mouse model[57] Ballooned macrophage NK cells, T cells, but no PMN, are found in synovial fluids as in RRV patients | High levels of interferon type I (alpha) High levels of Apoptosis in synovium Complement activation and cytotoxicity may be involved[141] High levels of angiogenesis in synovium and may assist fibroblast aggressive phenotype (proliferation and invasion)[107] | May be associated with high levels of COX2 CHIKV-induced high levels of PGE2 by fibroblasts[138] Roles of other molecules involved in pain remain unknown (NGF, Substance P, kinins, anaphylatoxins[133] | Canonical anti-citrullinated antibodies are rare. CHIKV-infected osteoblast and fibroblasts promote osteoclast differentiation through RANKL and possibly to joint damage[146] | Antalgics NSAIDs Methotrexate[24] TNF inhibitors CCL2 inhibitors[149] |
| **DNA Viruses** | | | | | | | |
| Parvovirus B19 (Anemia) (Arthralgia) | Precursors of red blood cell P glycoprotein and other coreceptors ($\alpha 5\beta 1$ and Ku80 nuclear antigen) | Robust activation of adaptive immunity against virus[8,34] Antibody specificity against some viral proteins may be compromised and favoring viral persistence[33] | Detection of viral DNA in synovial tissue of patients with RA[38] B and T cells are carriers of the virus to joints | In vitro, B19 can increase the invasive phenotype of fibroblast through poorly-characterized mechanisms[42] | Experienced by patients through unknown mechanisms | Molecular mimicry between viral proteins and proteins of the extra-cellular matrix (e.g., type II collagen)[40] Autoantibodies to nuclear, mitochondria and smooth muscle actin[39] | Methotrexate |

CHIKV, Chikungunya; IFN, interferon; NGF, nerve growth factor; NK, natural killer; PGE, prostaglandin; PMN, poly-morpho-nuclear cells (or neutrophils); RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor ligand; RRV, Ross river virus.

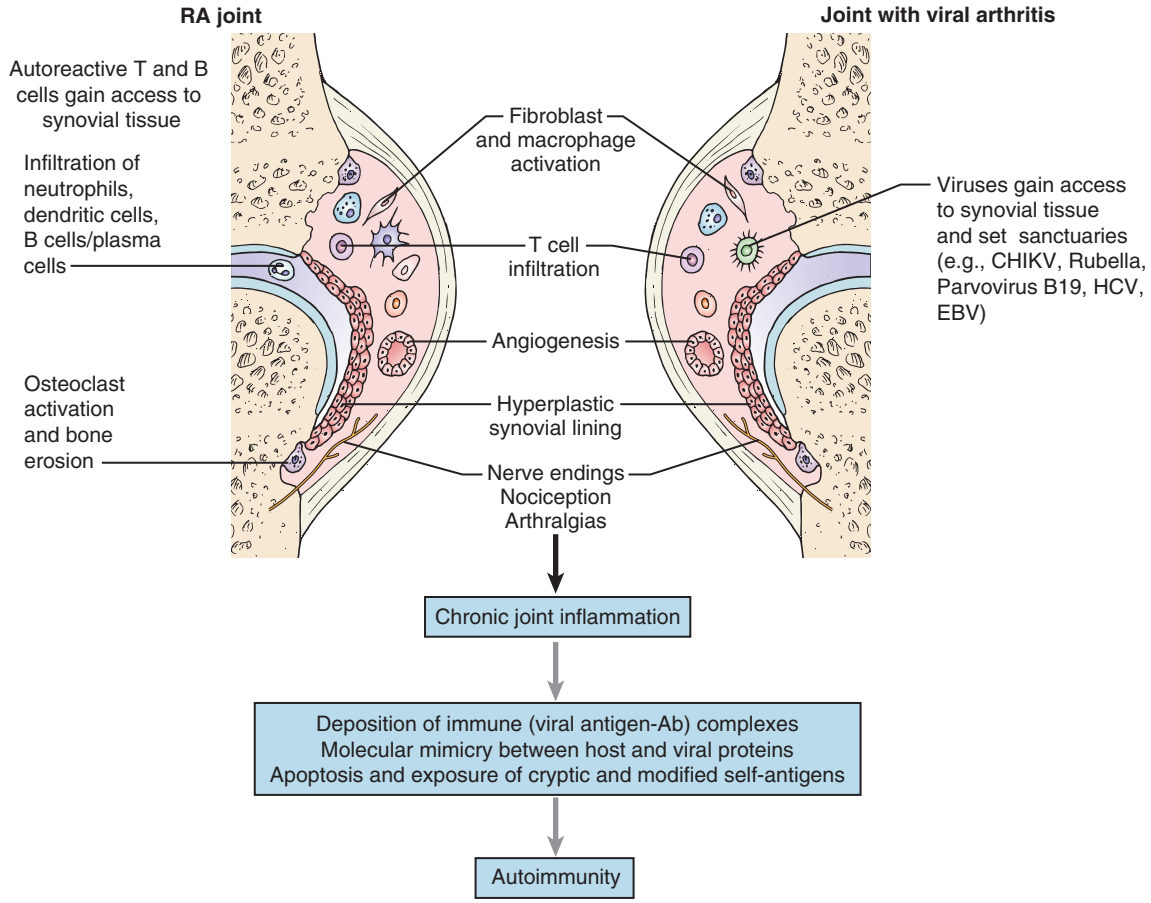



**Figure 114-1** Common and unique inflammatory pathways are involved in viral arthritis and reminiscent of autoimmune rheumatoid arthritis (RA). In the context of a major infection associated with high viremia and a failure to mount a robust anti-viral response, it has been proposed that several DNA and RNA viruses would escape from systemic immune surveillance and find protective niches in tissues such as synovial tissues. Infected monocytes, fibrocytes, or lymphocytes may transfer these viruses to resident cells such as synovial fibroblasts and perivascular macrophages but through ill-characterized mechanisms. Viruses may hide into apoptotic vesicles, allowing a Trojan horse–like scenario to subvert and infect resident cells. Despite the different nature of each viral disease, however, they clearly share some common pathogenic mechanisms involved in arthralgias (acute nociception at the nerve endings and involving for instance prostaglandins), macrophage activation and polarization (M2≫M1), as well as fibroblast hyperplasia and invasion sustained by major angiogenesis in the synovial tissues as described in RA. The T and B lymphocyte cell responses may help to thwart off viral challenge and persistence but could also contribute to bystander cell injuries. Dying resident cells may release cryptic and modified self-antigens, such as nuclear proteins or extra-cellular cell matrix (ECM) proteins (e.g., collagen, vimentin) which, in some individuals, may instigate a local autoimmune response. In RA and, to a much lesser extent, in viral arthritis, there is a major recruitment of neutrophils (releasing peptidyl arginine deiminase enzyme [PAD]), plasma cells, and dendritic cells, which will orchestrate a more robust autoimmune response characterized by antibodies against citrullinated proteins. Osteoclasts are involved in bone erosion. EBV, Epstein-Barr virus; HCV, hepatitis C virus.

little is known about their contributions in synovial tissues and whether or not they could favor viral persistence and chronic arthritis.[23]

The prevention measures should include vaccinations whenever possible in endemic regions, and particularly for vulnerable individuals such as the elderly. Other treatment regimens include analgesics and NSAIDs for pain relief and methotrexate (MTX) to control actual arthritis, although this may compromise patients' immune surveillance to prevent viral reactivation.[24,25]

This chapter describes key examples of some of the major DNA and RNA viruses associated with arthritis and proposes common pathogenic mechanisms that may be the driving forces of the chronic viral arthritic diseases. It should be emphasized that the mechanisms may involve exceptionally autoreactive T and B cells but more commonly involves a crosstalk between persistently infected synovial fibroblasts and macrophages, contributing to chronic inflammation as described in several chronic fibrotic disorders.[26]

## KEY EXAMPLES OF ARTHRITOGENIC VIRUSES

### Parvovirus B19

Parvovirus B19 has long been known to be associated with arthritis.[27,28] B19 is the smallest single-stranded DNA virus, of 6 kb and 20 to 25 nm in diameter. The genome encodes for two structural proteins, VP1 (nucleotides 2444–4786) and VP2 (nucleotides 3125–4786), and for the major non-structural protein NS1 (nucleotides 436–2451).

Parvovirus B19 infection may be particularly deleterious during pregnancy because of the consequences on the fetus.

The critical time of infection is during the first 20 weeks of gestation, when the fetal immune system is thought to be immature and red cell mass increases more rapidly, resulting in inhibition of erythropoiesis. B19 is the cause of erythema infectiosum (also known as *fifth disease*), a common childhood rash, and of various joint complications especially in adult women, although many B19 infections remain subclinical. The seroprevalence increases with age, and more than 70% of adults are seropositive.[29] Parvovirus B19 is mainly transmitted by respiratory aerosol spread from individuals with acute infection.

Parvovirus B19 replicates in human red blood cell precursors of the bone marrow.[30] This leads to transient arrest of red cell formation. Hence, B19 infection can lead to transient aplastic crisis manifesting as severe anemia. B19 infection also affects other hematopoietic cell lineages and causes cytopenias of various types. B19 DNA or antigen has furthermore been found in the fetal heart, liver, spleen, kidney, lung, muscle, and brain, and in the heart, lung, liver, kidney, testes, cerebrospinal fluid, and synovium of children or adults. The main cellular receptor of B19 has been identified as the P antigen of the blood group P system.[31] P antigen, also known as *globoside*, is present on erythrocytes, erythroblast, megakaryocytes, endothelial cells, fetal liver, and heart skeletal muscle cells. B19 also has two coreceptors, $\alpha 5\beta 1$ integrin and KU80 nuclear autoantigen.[32]

Immunity after infection with B19 is conferred by a B cell immune response with development of neutralizing antibodies.[33] T cell–mediated responses are also involved and directed essentially against the capsid proteins VP1 and VP2.[34]

B19 is the object of intense investigations to clarify whether it is able to trigger autoimmune diseases. It has been suggested that it might be a major contributing factor or the precipitating agent of several autoimmune disorders including RA, systemic lupus erythematosus (SLE), anti-phospholipid syndrome, systemic sclerosis, and vasculitides. The joint manifestations sometimes mimic those of RA and as many as approximately 50% of patients with chronic B19 arthropathy meet the criteria of the American College of Rheumatology (ACR) for RA, suggesting that the persistence of the virus may be involved in the initiation and perpetuation of the disease.[35,36] The involved joints, mainly wrists, knees, and the small joints of the hands, are painful, swollen, and often symmetrically affected.

The onset of arthralgias coincides with the detection of circulating immunoglobulin (Ig)M and IgG antibodies against the viral structural proteins, suggesting that the deposition of immune-complexes in the synovial membranes may be responsible for the onset of the symptoms, as happens for erythema infectiosum. Parvovirus B19 DNA can be detected in synovial fluid and cells, and synovial biopsy specimens of affected joints revealed infection of $CD20^+$ B cells or $CD3^+$ T cells by double immunostaining.[37,38]

Molecular mimicry between host and viral proteins seems to be the main mechanism involved in the induction of autoimmunity. B19 infection has been associated with production of antibodies directed against a plethora of autoantigens including nuclear antigens, rheumatoid factor (RF), neutrophils, cytoplasmic antigens (CA), mitochondrial antigens (MA), smooth muscle actin (SMA), gastric parietal antigens, and phospholipids.[39] For instance, Lunardi and colleagues reported that antibodies against B19 viral proteins reacted specifically with human keratin, type II collagen, single-stranded DNA, and cardiolipin.[40] More importantly, levels of reactivity against keratin and type II collagen were correlated with clinical hallmarks, and the main autoantigen specificity in which Igs from patients with arthritis reacted preferentially with type II collagen, whereas Igs affinity purified from patients with skin rashes reacted preferentially with keratin. To confirm a role of the virus in inducing an autoimmune response, BALB/c mice were immunized with the viral proteins and autoantibodies against keratin, type II collagen, and cardiolipin, as well as single-stranded DNA, were detected in the majority of the mice that developed a strong antivirus response.

In addition to RA, parvovirus B19 infection has been associated with a variety of other autoimmune diseases, including juvenile idiopathic arthritis, SLE, progressive systemic sclerosis, Sjögren's syndrome (SS), primary biliary cirrhosis, polymyositis, and dermatomyositis.[8] In patients with systemic sclerosis, the presence of parvovirus B19 in bone marrow and/or skin biopsy specimens has been reported from a significant number of patients. Interestingly, the same authors found high levels of B19 DNA and TNF expression in endothelium and fibroblasts of scleroderma patients.[41] The degree of viral transcript expression correlated with active endothelial cell injury and perivascular inflammation, features considered to be important in the initial phases of the disease.

B19 can induce in vitro an invasive phenotype in normal human synovial fibroblasts, but mechanisms involved in cell infection and cell polarization remain largely ill characterized.[42] These experiments are now highly warranted to better establish biologic links between the capacities of viruses to polarize directly the aggressive phenotype of the resident cells of the joints.

## Rubella Virus

Rubella virus is the sole member of the genus *Rubivirus* and belongs to the Togaviridae family together with alphaviruses (see subsequent text). Rubella is an enveloped single-stranded RNA virus of 9757 nucleotides in length, which contains two long open reading frames, the 5′ proximal, which encodes nonstructural proteins, and the 3′ proximal, which encodes the structural proteins (capsid, E1, and E2). The virion is spherical and measures 50 to 70 nm in diameter, with a 30 nm dense core.[43]

Transmission is by nasopharyngeal secretions. Most cases of infection lead to a mild, self-limiting, measles-like disease, but major concern arises when rubella infects the fetus, which can lead to miscarriage or congenital rubella syndrome (CRS). In CRS, rubella virus is able to infect the placenta, spread to the fetus, and alter the function of many organs and causing systemic inflammation.[44]

Rubella E1 protein binds to myelin oligodendrocyte glycoprotein (MOG), and ectopic expression of MOG on nonpermissive cells will contribute to infection.[45] Moreover, there is a high level of homology between rubella E2 protein and MOG, which could explain the ability of antibodies against rubella to cause demyelination at least in vitro.[46]

Joint symptoms are a complication of naturally acquired rubella and have been reported to occur in more than 50% of adult females with rubella but are rare among children and adult males with rubella.[47] Symmetric or migratory arthralgias are more common than synovitis. Morning stiffness is prominent, and symptoms usually resolve during a few days to 2 weeks. Proximal interphalangeal, metacarpophalangeal, wrist, elbow, ankle, and knee joints are most frequently affected. Periarthritis, tenosynovitis, and carpal tunnel syndrome may be seen. In some patients, symptoms may persist for months to years. Following vaccination, joint symptoms occur less frequently and are usually less severe and of shorter duration than those that occur following naturally acquired rubella.[48] Live attenuated rubella vaccines have also caused a high frequency of postvaccination myalgia and paresthesia—symptoms similar to those in natural infection—beginning 2 weeks after inoculation and lasting less than a week. However, in some patients, symptoms may persist for more than a year. RA27/3, the vaccine strain in current use, may cause postvaccination joint symptoms in 15% or more of recipients.

Persistent rubella infection may be established in synovial cell cultures and, as described, in tissues.[49,50] Rubella-specific antibody titers in the synovial fluid of some patients with chronic arthritis are higher than those in serum, suggesting local synthesis of specific antibody. Persistence of rubella virus is also suggested by previous studies, which demonstrated that virus-specific IgM may persist for as long as 4 years following both natural infection and vaccination.[48] The presence of rubella RNA in synovial tissues from patients may be the result of reactivation and a result of immunodeficiency or declining immunity in old age.[51]

Rubella arthritis needs to be differentiated from other viral arthritides and from inflammatory arthritides, including RA. It may be clinically confused with parvovirus B19 infection, but serologies differentiate the two infections. NSAIDs are useful to control symptoms.

## Chikungunya Alphavirus

CHIKV[11] is an enveloped RNA positive-strand alphavirus of 12 kb belonging to the Togaviridae family, transmitted by *Aedes (Ae)* mosquitoes.[52] Aedes albopictus, the main competent vector of CHIKV, has nowadays become indigenous to many places throughout the world. The alphavirus group comprises 29 viruses, six of which can cause human joint disorders (arthralgias evolving into arthritis), namely CHIKV, O'Nyong-Nyong virus, Semliki forest virus (Africa, Asia, Europe), RRV (Australia and the Pacific), Sindbis virus (SINV; cosmopolitan), and Mayaro virus (MAYV; South America, French Guyana).

Several million CHIKV cases in humans have now been described in Africa, Southeast Asia, islands of the western and eastern Pacific and the Indian Ocean, and the Middle East (for review see[53]). Recent CHIKV outbreaks have been reported since November 2013 and have spread to several parts of Central and Latin America.[54]

CHIKV infection of humans results in a brief, usually 5 to 7 day viremia, which is in principle controlled primarily by type I IFN and antibodies.[55-57] However, CHIKV disease is often debilitating and associated with persistent arthralgias and arthritis that can last from weeks to months.[12,58] Many of the chronic patients do not respond well to usual analgesics, suggesting that the nature of chronic pain may be not only nociceptive but also neuropathic.[59] The mean pain impact on life activities calculated from the Brief Pain Inventory (BPI) was significantly higher in patients with chronic pain than in patients without it. Persistent symptoms, mainly joint and muscular pain, have been reported several months after CHIKV infection.[60,61] Long-term CHIKV rheumatic manifestations are frequent underlying postepidemic conditions. Predictors of nonrecovery include patient age older than 45 years, severe initial joint pain, and presence of underlying osteoarthritis.[61,62] Rheumatic manifestations in as many as 50% of the adult patients typically consisted of a febrile arthritis mainly affecting the extremities (ankles, wrists, phalanges).[61-67] Typically, the joint symptoms occurred in a fluctuating manner (often perceived as infectious relapses by the patients) but did not change anatomic location. Pain within or around tendons was also a common trait and evolved to tenosynovitis. Chopra et al.[68] reported high levels of CHIKV IgM antibodies in a cohort of Indian patients with post-CHIKV RA-like illnesses. These patients were clearly naïve for musculoskeletal disorders prior to CHIKV infection. Surprisingly, few were positive for RF or anti-citrullinated protein antibodies (ACPAs), and these findings were confirmed in a more recent survey of CHIKV patients with chronic arthralgias for 36 months postinfection.[61] Tenosynovitis/enthesopathy rather than synovitis was evidenced by ultrasonography in several cases, and these unique observations are in agreement with our radiograph imaging of enthesitis in post-CHIK RA patients in la Réunion.[24] Patients with a diagnosis of RA following CHIKV infection were given a classical disease-modifying anti-rheumatic drug therapy with MTX (as much as 0.3 mg/kg/week), sulfasalazine (1.5 g to 3 g/day), leflunomide (10 to 20 mg/day), or hydroxychloroquine (200 to 600 mg/day). In case of inadequate response to MTX, treatment was successful with TNF blockers (etanercept, adalimumab).[69] While the chronic disease progresses, more severe arthritis was reported 1 to 2 years post-CHIKV infection. Some patients with chronic rheumatic symptoms manifested progressive erosive arthritis.[63,67,70] In Manimunda's study, it was confirmed that the levels of RF ACPAs were not elevated in contrast to what is known in traditional RA.[67] In the same study, one-third of the patients who had joint pain met the ACR criteria to classify them with RA. The MRI findings were joint effusion, bony erosion, marrow edema, synovial thickening, tendinitis, and tenosynovitis.

CHIKV infection has been reported in multiple organs of the body and particularly the synovial tissues in humans and animal models.[55,57,71-73] In vitro studies have been instrumental for the comprehension of CHIKV tissue tropisms and to provide insights into the causal pathogenic mechanisms. The main target cells for CHIKV infection and replication are fibroblasts, as described in 1970s.[74] Recent studies support this finding, and CHIKV readily infects human fetal lung fibroblasts (MRC-5 cells) as well as human synovial and skin fibroblasts.[16,75,76] Not all fibroblasts are equally susceptible to CHIKV infection. Resting fibroblasts, as well as a subpopulation of synovial fibroblasts ($CD141^+$/$CD90^+$), manifest some resistance through mechanisms still largely unknown.[16,75] CHIKV can also infect epithelial cells

(e.g., HeLa, HEK293, but not A549), endothelial cells, hepatocytes (carcinoma HuH7), neurons, astrocytes, neuroblastoma cell lines (e.g., SH-SY5Y), and muscle satellite cells.[72,75,77,78] The nature of the receptor that mediates virus entry remains to be characterized. Evidence from biopsies of infected human patients showed that CHIKV antigens were detected mostly in cells located in the skeletal muscle epimysium, of the joint capsule, and of the dermis.[55] On the contrary, immune cells, such as monocytes, lymphocytes, dendritic cells, natural killer (NK), and their transformed cell line counterparts (e.g., THP1, U937, H9) are not permissive to infection by CHIKV.[75,77] However, human monocyte and macrophage infection remains controversial, and it is likely that they could be infected but with a poor capacity to replicate CHIKV.[73,75]

There are very few studies to support the idea that alphaviruses will contribute to chronic RA-like diseases through the production of autoantibodies against self-antigens, as described for B19, EBV, or HCV. One study from Sane and colleagues reported that Finnish patients with Pogosta disease following SINV infection had autoantibodies to NA, MA, and RF at a higher prevalence compared with healthy Finnish patients at 3 years postinfection.[79] Interestingly, the ACPAs were detected only in 1 patient out of 49, who was also positive for RF. It will remain to be determined whether this autoimmune profile is more prominent in recovered Pogosta patients versus those with long-term arthritis.

## Epstein-Barr Virus

EBV has been found in association with several common autoimmune diseases, like RA, SLE, and multiple sclerosis.[7,80] EBV belongs to the genus lymphocryptoviruses within the subfamily gammaherpesvirus of the herpesvirus family. It is the fourth member of this family. Herpesviruses are large DNA viruses with a linear double-stranded DNA genome inside a membrane-coated icosaedrical capsid. The EBV genome has a length of 172 kb. EBV has strong tissue tropism for B lymphocytes. The virus is associated with a wide variety of neoplasms, including lymphoid tumors like Burkitt's lymphoma, Hodgkin's disease, NK T cell lymphoma, and epithelial malignancies, especially undifferentiated nasopharyngeal carcinoma, gastric carcinoma, and carcinomas of the salivary glands. The virus is shed into the saliva of persistently infected individuals who spread the virus to uninfected individuals. When transmitted orally, EBV will initially infect either epithelial cells or resting tonsillar B lymphocytes. EBV binds through its viral envelope glycoprotein gp350 to the complement receptor type 2 (CR2/CD21) that is primarily expressed on B cells.[81] This receptor can also bind to IFN-α, but the role of such molecular interactions in the IFN-mediated anti-viral response is unknown.[82] B cell–bound virions can also efficiently transferred to $CD21^-$ epithelial cells, which act as helpful enhancers for transferring the virus to others cells (possibly to fibroblasts), and for establishing latency in B and other cells.[83]

Both the latent and lytic EBV proteins are potent immunogens and elicit vigorous B and T cell responses. In immunosuppressed and immunodeficient patients, or in individuals with a functional deficit of EBV-specific T cells, lytic EBV replication is regularly activated, and an increased viral load can be detected in the blood.[7] Enhanced lytic replication results in new infection events and EBV-associated transformation events, and seems to be a risk factor both for malignant transformation and the development of autoimmune diseases. It is conceivable that an increased load or altered presentation of a limited set of lytic or latent EBV proteins that cross-react with cellular self-antigens triggers and perpetuates the pathogenic processes that result in arthritis, multiple sclerosis, and SLE.

Of critical note, $EBV^+$ B cell lymphomas have been reported to develop in patients with RA when MTX is used for immunosuppression, which is not the case for other immune-modulating drugs.[84] It has been proposed that MTX could induce lytic EBV replication in latently infected $EBV^+$ cell lines in vitro. Thus MTX may promote the generation of lymphomas in RA and polymyositis patients by its immunosuppressive properties, combined with a specific reactivation of latent EBV genomes.

Although a causal role of EBV in the generation of malignancies is established, a causal role in the generation of autoimmune diseases is circumstantial. However, cross-reactivity between viral antigens and self-antigens and the immortalization of pre-existing autoreactive B cells through infection with EBV with the consequent production of autoantibodies may be plausible viral mechanisms for triggering autoimmunity. Strong genetic risk factors for RA are the alleles that contain peptide sequence homologies to EBV glycoprotein gp110 (BALF4).[85] BALF4 is normally one of the most abundant EBV proteins in late lytic infection and also serves as a target of antibody-dependent cell-mediated cytotoxicity against EBV-infected cells. The BALF4 open reading frame contains the sequence QKRAAQRAA, which is highly homologous to the RA susceptibility determinant. QKRAA is found in certain class II HLA DRβ chains, such as DR4. Carriers of this shared epitope have significantly higher RA risk through smoking compared with nonsmoking shared epitope carriers and develop autoantibodies against citrullinated self-antigens.[86]

## Hepatitis C Virus

HCV[6] is a positive-strand RNA virus of the family Flaviviridae. Studies of this virus have made huge leaps forward since the implementation of efficient cell culture systems. Worldwide, more than 130 million people are chronically infected with HCV. The viral genome consists of a single open reading frame that is less than 10 kb long. The proteins of this virus are arranged along the genome in the following order: N terminal-core-envelope (E1)–E2–p7-nonstructural protein 2 (NS2)–NS3–NS4A–NS4B–NS5A–NS5B–C terminal.

Acute infection goes along with mild and generalized symptoms, and therefore mostly remains undiagnosed. In more than half of the patients, however, the infection persists and, during the years, can cause liver damage such as fibrosis, cirrhosis, or hepatocellular carcinoma. Viral antigen is detectable within the first weeks of exposure together with a rise in serum liver transaminases. Antibodies develop and clear HCV in some patients; however, in chronic HCV infection, viral antigenemia and HCV RNA persist despite

humoral and cellular immune responses. There is also some evidence to suspect a role for HCV-associated autoimmunity for a broad spectrum of autoimmune diseases, including RA.[6] Although this association may be sporadic or indicate an increased incidence of HCV infection in patients with autoimmune disease, case reports documenting the occurrence of acute and chronic HCV infection before the development of autoimmune disease suggest a causal role in individuals who may be predisposed to the development of autoimmunity. Liver biopsy in HCV-infected patients is useful to establish disease severity and to exclude other causes of hepatitis such as alcoholism or hemochromatosis. A probable role for HCV infection in autoimmune hepatitis is emerging and with the detection of autoantibodies such as ANA and anti-SMA.

Polyarthralgias and polyarthritis have been associated with HCV infection, and HCV RNA has been isolated from synovial fluid.[87] Patients with chronic HCV associated with polyarthralgias/myalgias will have elevated levels of RF and ANA without anti-DNA antibodies.[87,88] The optimal treatment for HCV-associated polyarthritis has not been determined, but MTX has been used with some success and minimal complications.[89]

Diverse receptors or factors are involved in HCV infection and include CD81, scavenger receptor class B type I (SR-BI, also known as *SCARB1*), and claudin-1. Occludin is also an essential HCV cell entry factor.[90] We don't currently know whether synovial fibroblasts, which express many of the aforementioned receptors, can be infected by HCV and whether this could affect cell behavior.

## Arthritogenic Viruses Use the Innate Interferon-Type I Response to Favor Persistence

The role of our immune system (innate and adaptive) is to rapidly control infection by viruses, for instance, at the major portals of entry (skin, nasopharynx) and before dissemination to other organs.[91] To initiate an effective antiviral immune response, all cells should be capable of sensing and responding appropriately to pathogens. This coordinated response should in principle limit viral spreading and persistence through the mobilization of adaptive immune T and B cells.[92]

A growing number of germ-line pattern recognition receptors (PRRs) have been characterized and are expressed on immune as well as nonimmune structural cells. These receptors are capable of recognizing a plethora of conserved pathogen-associated molecular patterns (PAMPs) that do not occur under normal circumstances in the host.[93] The PRRs sense specific viral structures and induce the production of type I IFNs. Two types of PRR exist: ubiquitously expressed cytoplasmic RNA-sensing molecules and membrane-bound Toll-like receptors (TLRs). The expression of TLR is more limited than that of the cytoplasmic receptors.

The retinoic-acid-inducible gene (RIG)-I–like RNA helicase receptors (RLRs), which include RIG-I and melanoma differentiation-associated gene 5 (MDA5), are a ubiquitously expressed group of cytosolic receptors that detect viral RNA structures and induce type I IFN-α and -β production.[94] Binding of viral RNA enables interaction between the RLRs and downstream signalling molecules such as IPS-1 (IFN-β promoter stimulator-1; also known as MAVS, CARDIF, and VISA), which activate transcription factors (e.g., IFN regulatory factor [IRF]3) and regulate the production of type I IFNs. The expression of RLRs is low in the steady state but is highly induced by type I IFNs, which enhances their capacity to recognize viruses and to propagate the anti-viral response. At least ten TLRs have been identified in humans, of which three (TLR3, TRL7, and TLR9) recognize nucleic acid viral components and stimulate type I IFN production.[95] Interestingly, TLR expression is also regulated by IFNs. TLR3 is triggered by dsRNA, whereas TLR7 recognizes guanosine-rich and uridine-rich ssRNA, and TLR9 senses unmethylated cytosine–phosphate–guanine (CpG) motifs. TLR4, which is expressed on the cell surface, recognizes lipopolysaccharide from bacteria but also viral proteins and can stimulate type I IFN production.[96]

Release of type I IFN from infected cells results in autocrine and paracrine stimulation of the IFN-α/β receptor (IFNAR), which leads through associated tyrosine (TYK) and Janus kinases (JAK) to the phosphorylation of STAT (signal transducers and activators of transcription) 1 and 2. STAT1/2 heterodimers associate with IRF9 and bind to IFN-stimulated response elements (ISREs) present in the promoter region of the IFN-stimulated genes (ISGs).[97]

ISG-encoded proteins represent the anti-viral effector molecules that directly inhibit molecular and biochemical activities required for virus replication. Expectedly, type I IFNs are essential for the anti-viral activities against many arthritogenic viruses as demonstrated in vitro and in vivo.[55,75,98,99] However, many viruses (including CHIKV) have been reported to block nearly all steps of the IFN regulatory pathway. This includes the disruption of dsRNA and IFN receptor/JAK–STAT signaling events, the inhibition of IRF and nuclear factor-κB (NF-κB) functions, and other mechanisms that target the anti-viral actions of specific ISG products.[100-102]

The anti-viral molecules induced by IFN include members of the apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC) family and which can mutate viral genomes, the Mx GTPase, an inhibitor of viral polymerases, and members of the tripartite motif family (including the autoantigen Ro/SS-A 52 kDa [Ro52, also known as TRIM21]), which can inhibit viral replication at multiple stages in the life cycle. Other anti-viral molecules are, for example, RNA-activated protein kinase (PKR).[103]

Immune recognition is enhanced in cells exposed to type I IFNs and controlling class I major histocompatibility complex (MHC) expression and resulting in enhanced presentation of viral but also of self-antigens for recognition by the immune system. Other changes include the induction of molecules (such as p53) that increase susceptibility to cell-death–inducing stimuli, thus enhancing the killing of virally infected cells by the immune system. Type I IFNs regulate pathways in target cells, antigen-presenting cells (such as Dc and macrophages), and effector cells (which mediate anti-viral effects as well as tissue damage).

pDCs have the unique capacity to rapidly secrete large quantities of type I IFNs upon recognition of nucleic acid structures by TLR7 or TLR9.[104] These cells are ideally suited to respond rapidly to viruses in the absence of prior IFN

exposure. pDCs are, therefore, thought to have a central role in the self-amplifying nature of autoimmune diseases through the production of large quantities of type I IFNs, which mediate numerous downstream functional effects on target cells and cells of the immune system.[105]

Surprisingly, it was found recently that some parvoviruses did not induce an IFN response in normal human fibroblasts, glia cells, or melanocytes.[106] Furthermore, the authors found that parvovirus infectivity was unaffected by IFN treatment of human normal or tumor cells. The IFN-resistant phenotype of parvoviruses may give them an advantage, including the capacity to persist in tissue sanctuaries.[106] In contrast, a great deal of information has been gathered during the recent years about the innate and adaptive immune responses to clear CHIKV. Very high levels of circulating CHIKV can be reached in acute settings, ranging from $10^5$ to $10^{12}$ virus RNA particles per mL of blood during the first days of infection.[107] CHIKV is efficiently cleared within a matter of days and the symptoms usually subside within a couple of weeks, suggesting a robust and efficient innate immune response that limits systemic viral replication. CHIKV is a single-stranded RNA virus; therefore, it may be detected by TLR7 and/or TLR8 (in mice). The helicase RIG-I and MDA-5 may also be involved in the recognition of CHIKV. Moreover, CHIKV can have a double-stranded RNA intermediate during the replication cycle, which may be detected by TLR3. Recently, White et al. showed that CHIKV infection leads to activation of the transcription factor IRF3 and subsequent transcription of IRF3-dependent anti-viral genes, including IFN-β. IRF3 activation occurred via the engagement of the adaptor molecule IFN promoter stimulator 1 (IPS-1).[108] Recently, M. Alberts's team showed that CHIKV does not stimulate directly type I IFN production in immune cells such as DC. Instead, infected nonhematopoietic cells sense viral RNA in an IPS-1-dependent manner and participate in the control of infection through their production of type I IFNs.[76] Although IPS-1 signaling pathway contributes to the immune response, they also find evidence for an MyD88-dependent PRR pathway that may be critical for preventing viral dissemination.[76] CHIKV-induced type I IFN response seems to be protective in vitro and possibly induces the expression of restriction factors such as IFN-stimulated genes (ISGs) and oligoadenylate synthetase (OAS).[109,110] OAS is active against CHIKV replication in HeLa cells, and its role in other cell types needs to be further established.[109]

In patients and in the nonhuman primate (NHP) model, the secreted IFN-α response was very transient and dropped to basal levels within 24 hours, while the peak of viremia persisted for several days.[110] These data suggest that CHIKV may be capable of controlling the IFN response. Infection and replication of alphavirus usually cause a general host translational shutoff, leading to severe cytopathic activities in mammalian cells. White et al. confirmed this paradigm, demonstrating that translation of cellular (but not viral) genes is blocked during CHIKV infection.[108] CHIKV was found to trigger inactivation of the translational molecule eukaryotic initiation factor subunit 2α (eiF2α) through a PKR-dependent pathway. Moreover, Fros et al. showed that CHIKV replication is resistant to inhibition by IFN once RNA replication has been initiated, and that CHIKV actively suppresses the anti-viral IFN response by preventing IFN-induced gene expression. Indeed, CHIKV infection efficiently blocked STAT1 phosphorylation and/or nuclear translocation in mammalian cells induced by either type I or type II IFN. Nonstructural protein 2 (NSP2) was a potent inhibitor of IFN-induced JAK-STAT signaling.[101] Hence, CHIKV, like many viruses, has evolved to evade or counteract the anti-viral responses, hence, favoring viral persistence.

An additional anti-viral molecular response is reported during CHIKV infection. Macro-autophagy, referred herein as *autophagy*, is a fundamental homeostatic process that leads to the degradation and recycling of long-lived proteins and organelles.[111,112] Autophagy is first a fundamental cell-surviving process during starvation conditions, but it does also play a role in both innate and adaptive immunity in response to pathogens.[113] We have recently explored the role of the autophagy machinery in CHIKV infection and showed that cellular autophagy was promoted within CHIKV-infected cells and, furthermore, contributed to enhance CHIKV replication.[17]

A robust protective adaptive immune response develops after CHIKV infection,[114] and this is also the case for all arthritogenic viruses listed above. IgM antibodies against CHIKV can be detected in the sera of infected patients during the acute phase of the infection, and anti-CHIKV IgG antibodies, especially neutralizing, may prevent virus reinfection.[114,115] The kinetic of appearance of the IgG, sometimes within the first days postinfection, is in favor of a rapid development of a protective adaptive immune response. At the cellular levels, a major immune response in the acute stage is characterized by a rapid mobilization of $CD4^+$ and $CD8^+$ T cells. Surprisingly, the level of TNF and IL-1β was rather weak during the acute phase and not significantly elevated when compared to control groups.[107,110] Other T helper (Th)1 cytokines were elevated in acute settings (IFN-γ and IL-12), although the response seemed to be skewed toward a Th2/Th17 response as indicated with high levels of IL-4, IL-13, and IL-17.[110] Another study demonstrated a production of IL-4, IL-10, and IFN-γ in the acute phase of CHIKV associated with a robust $CD8^+$ T lymphocyte response in the early stages of the disease, and a $CD4^+$ T lymphocyte-mediated response in the later stages.[116] Of note, a specific CHIKV IgM response persisted several months after infection[117] in patients suffering from chronic arthralgias/arthritis. This observation may be related to viral persistence.

## Arthritogenic Viruses Engage Trojan Horse Scenario to Avoid Adaptive Immunity

Many studies have searched for DNA or RNA of viruses in synovial tissue or fluid samples, mostly of arthralgic patients. Many of the arthritogenic viruses aforementioned can be found in synovial tissue or fluid. The diagnostic significance of these findings depends both on the particular microbe and on the clinical context, whereby the findings should be interpreted with caution. Furthermore, for establishing the etiology of chronic arthropathy induced by latent viral infection, the concentration and the location of the pathogen (i.e., in the synovial membrane, in synovial fluid, or in the cells of the latter), might be of importance. Even

if the pathogen is found by PCR or it can be cultured from the arthritic joint, its causal role is not easy to prove.

One of the emerging paradigms is that synovial tissue cells are infected and display an altered phenotype involved in tissue injuries.

Focusing on alphaviruses, it was found that persistent symptoms, such as joint pain (arthralgia) and arthritis (not necessarily associated with bone erosions) were reported several months after CHIKV or RRV infection.[61,118] Indeed, rheumatic manifestations affect as many as 50% of the adult patients 6 to 15 months postinfection.[119,120] There is a strong body of evidence that persistent joint damage is linked with the persistence of arthritogenic alphaviruses by mechanisms still poorly characterized.[1,121,122] This hypothesis is now thoroughly tested for CHIKV; it is plausible that CHIKV hides in immune-privileged niches and that those sanctuaries contribute directly to synovial tissue damage.[12] The study of P. Roques and colleagues showed the presence of viral antigens and RNA, 3 months postinfection, in joints, muscles, lymphoid organs, and liver.[71] Of note, they found high levels of macrophage/mononuclear cell infiltration in lymphoid organs and large amounts of CHIKV antigen and viral RNA in spleen macrophages for prolonged periods (as long as 3 months), demonstrating that macrophages act as the cellular reservoirs for CHIKV persistence.[71] The same conclusions were drawn from a study in humans. Indeed, using a longitudinal study of hospitalized patients in la Réunion, the authors showed that CHIKV (RNA and protein) was found in perivascular synovial macrophages in one chronic patient 18 months postinfection.[107] Surhbier et al. confirmed this in a mouse model of acute infection.[57] Indeed, rheumatic disease was associated with a prolific infiltrate of monocytes, macrophages, and NK cells and the production of MCP-1, TNF, and IFN-γ.

As one plausible cytopathic mechanism, the authors found that CHIKV can induce apoptosis in vitro and in synovial tissues, and that completion of the apoptotic process was important for viral propagation. Furthermore, CHIKV is able to hide in apoptotic blebs to enhance viral spreading to neighboring cells, especially macrophages that will engulf the bleb.[16] This Trojan horse scenario may take place in tissues, allowing CHIKV to escape the immune response and cause chronic pathologies, such as arthritis. These infected macrophages may recapitulate humoral and cellular inflammatory processes to drive arthritis (angiogenesis, tissue remodeling, and injuries).[107] This Trojan horse scenario is a possible immune evasion strategy reported for many viruses (B19, HBV, poxvirus, HIV, vaccinia).[123-126]

## Other Mechanisms Favoring Viral Persistence Inside Synovial Tissues

Historically, it has been thought that many viruses, including B19, are by the actions of the host immune system fully removed from the body within a few weeks or months of infection. With new sensitive detection tools, such as PCR and in situ hybridization, it is becoming obvious that eradication of a virus (if not all pathogens) from the human body is seldom complete. It is, however, critically important to study also healthy controls in order to avoid misinterpretations of disease causality.

Defective interfering (DI) particles may play a role in establishing or maintaining persistent infections. DI particles are replication-deficient deletion mutants that interfere with the growth of the homologous wild-type virus. DI particles have been observed recently also in natural infections.[97,95] It has been speculated that a potentially latent B19 infection could, under certain conditions such as stress or immunosuppression (following MTX treatment), be reactivated and cause arthralgic symptoms.[48] B19 might be carried around inside macrophages or perhaps could be attached as a full virion to the surface of follicular dendritic cells.

Another possible reason for viral persistence is that the synovial tissue cells constitute a good hiding place from the host's immune defense. The dominant local cell populations in joints are synovial and cartilage cells. Synovial cells can be divided into fibroblast-like and macrophage-like synoviocytes. These cells present several distinct phenotypical characteristics compared with classic fibroblasts and tissue macrophages.[18] It may be that these cells have major immunoregulatory mechanisms to control tissue injuries but these may also be mobilized during viral infection to favor viral persistence.[127] A positive correlation was detected between $CD4^+CD25^+$ T regulatory (Treg) cell frequency and HCV chronic infection as detected by high levels of RNA titers in the liver of patients.[128] It has been proposed that Tregs may play a role in viral persistence by suppressing HCV-specific T cell responses in a cell-cell contact manner. Chronic HCV infection may also be favored, given the concomitant presence of virus-specific $CCR7^-$ $CD8^+$ T cells.[129] These $CD8^+$ Tregs are antigen specific because they can be stimulated by HCV epitopes and suppress T cell responses through the production of considerable amounts of IL-10. It remains to be addressed whether these Tregs are mobilized by other arthritogenic viruses.

## Mechanisms Involved in Chronic Arthralgias

As a testament to the major involvement of excruciating joint pains in many alphaviral arthritides in humans, the native African name for CHIKV means "that which bends up." These arthralgias can be observed through the acute phase of the infection and which will last over months and years in chronic patients.[61] Joint pains are also clinical hallmarks associated with chronic B19 or HCV infections.[130-132]

The joint is a densely innervated organ, and its sensory innervation is geared predominantly toward nociception, indicating how vital positioning sense and awareness of potentially injurious movement are to proper joint function.[133] Chronic pain pathways are subject to complex levels of control and modulation, both in the periphery and in the central nervous system. In humans, articular branches of the tibial nerve that innervate the posterior knee joint capsule contain 70% to 80% unmyelinated C fibers and sympathetic nerves and which have also been associated with pain.[134] Nociceptors (nerve endings involved in pain) are abundant in the joint capsule, ligaments, periosteum, menisci, subchondral bone, and synovium. Because cartilage is abneural, it is not involved in nociception of the joint.

Nociceptors express a broad range of receptors for ligands that will induce action potentials when the receptors are engaged and transmitting nociception to the brain.[135] These

ligands include cytokines, chemokines, neuropeptides, and prostaglandins (PG), which can all form part of the biochemical milieu in the joint persistently infected by arthritogenic viruses.[12] As a result of this peripheral sensitization, joint movement within the normal range becomes painful.

TNF, IL-17, and IL-6 are inflammatory cytokines abundantly expressed in response to infection by many of the arthritogenic viruses.[5,12] High concentrations of pro-inflammatory factors such as IFN-α, IL-6, and IP-10 were also found at higher levels in serum of patients with CHIKV-induced polyarthritis than in noninfected healthy individuals, indicating their potential causative role in chronic joint and muscle pains.[116,136,137] These molecules can cause prolonged mechanical hypersensitivity and sensitizing joint nociceptors to mechanical stimuli.[135] The pro-inflammatory cytokines IL-1β and TNF can directly affect sensory neurons and can also trigger pain via downstream mediators such as other cytokines, chemokines, PG, neurotrophins, nitric oxide (NO), kinins, lipids, adenosine triphosphate (ATP), and complement anaphylatoxins.[133] Interestingly, it has been reported that IFN produced by CHIKV-infected fibroblasts induced elevated expression of PG.[138] HCV, and particularly the NS3 viral protein, is also known to modulate cyclooxygenase 2 activity and hence PG synthesis.[139,140] All in all, PG may contribute to mechanisms of nociceptor activation and sensitization. In addition, the activation of complement system and the production of complement anaphylatoxins in RRV-infected joints may contribute to arthralgia.[141]

Inflammatory cytokines can also enhance local levels of nerve growth factor (NGF), a major contributor to peripheral hypersensitivity.[142] The binding of NGF to its high-affinity receptor, TrkA, on peripheral nociceptors causes rapid potentiation of the thermosensitive ion channel TRPV1 (transient receptor potential cation channel, subfamily V, member 1) and lowering pain threshold and contributing to pain hypersensitivity. The authors currently do not know whether arthritogenic viruses may affect the biology of NGF in the joint, but it has already been reported that alphaviruses such as SINV can increase NGF production by glial cells.[143]

Nociceptors have been reported to express TLRs, which recognize a variety of alarmins released during tissue injury and contribute to pain generation. The primary function of these alarmins is to boost innate and adaptive immune cell anti-viral activities, but could also contribute to chronic inflammation and joint pain.[21,22]

## Mechanisms Involved in Chronic Inflammatory Viral Arthritis Resembling Rheumatoid Arthritis

RA is an extremely widespread systemic autoimmune heterogeneous disorder that is characterized by an antigen-driven immune response against self-antigens with the destruction of host tissue with a highly disabling impact that affects approximately 1% of the world population.[13] A hallmark of RA is its self-sustaining and autoamplifying nature, in which immune-mediated tissue damage and tissue repair collaborate in a feed-forward loop to provide the destructive power and the fuel that sustain the inflammatory process.

CD4 and CD8 T cells, NK cells, and macrophages contribute to the local and chronic inflammation of the joints. Secretion of inflammatory cytokines such as IL-1β, IL-6, and TNF[13] play a pathogenic role in the disease process. Overproduction of pro-inflammatory cytokines is believed to be orchestrated predominantly by macrophage-like synoviocytes.

Fibroblast-like synoviocytes invade the cartilage and contribute to joint destruction. The activation and differentiation of resident macrophages into osteoclasts is a key process leading to bone erosion.

Women are three times more affected than men, which has been interpreted as the influence of the pro-inflammatory estrogens, but also additional hormonal influences (e.g., prolactin) and additional environmental factors (e.g., socioeconomic status and lifestyle). Cigarette smoking is the strongest environmental risk factor for RA, increasing the risk 3- to 13-fold, depending on the number of pack years.

The mode of pathogenesis in virally induced arthritis might be mediated through different routes, but the outcomes are remarkably similar, with chronic inflammation driven in the various syndromes including RA. Several studies have already suggested that macrophage recruitment and subsequent secretion of pro-inflammatory cytokines and chemokines play a major role in the development of arthritis in alphavirus infection.[71,107,144,145] Other cellular injuries could be direct cellular lysis of virally infected cells, indirect effects mediated by the immune response and/or enhancement of the invasiveness of synovial fibroblasts (acute or chronic arthritis), and production of immune complexes.[12]

More recently, it has been demonstrated that osteoblasts as well as synovial fibroblasts infected by CHIKV could drive osteoclastogenesis in vitro, and which, in essence, may contribute to joint damage.[146,147] Osteoclasts are specialized cells derived from the monocyte/macrophage hematopoietic lineage that develop and adhere to bone matrix, then secrete acid and lytic enzymes that degrade it in a specialized, extracellular compartment.[148] Osteoprotegerin (OPG), receptor activator of NF-κB (RANK) and RANK ligand (RANKL) together regulate osteoclast function. CHIKV and RRV patients demonstrated high levels of RANKL and low levels of OPG, which would favor the appearance of macrophage-derived osteoclasts in the joints.[5,149]

Chen et al. reported recently using a CHIKV infection mouse model that blood monocytes were recruited to sites of infection through the action of chemokines such as CCL-2 and differentiated into osteoclast-like cells, leading to bone erosion. Furthermore, they observed that treatment with an IL-6 neutralizing antibody reduced bone loss and RANKL/OPG ratios in sera of mice.[5] Bindarit, an inhibitor of CCL2 action, was also able to control for the osteoclastogenic activity of CHIKV.[149]

## Treatment and Prevention

Arthralgias and arthritis postviral infection are mainly treated with analgesics, NSAIDS, which provide some symptomatic relief but do not significantly modify the underlying disease process. Chronic arthritis lasting more than 3 months and treated with MTX showed good response,

probably according to its anti-proliferative action on invasive fibroblast or for its capacity to control the pro-inflammatory activities of alarmins such as HMGB1.[150,151] Whether chloroquine may be useful as a prophylactic treatment or in the case of chronic infection needs further investigations.[152] Several molecules are in development and have been tested in animal models. IFN-α treatment was able to prevent arthritis in mice but only if given before infection, suggesting that IFN-α could not be a potential therapy.[57] A high level of CCL2 is produced following alphaviral infection in humans,[107,110] as well as in mice suffering from polyarthralgia and polyarthritis postalphavirus infection.[57,153] By reduction of the inflammatory infiltrate in infected mice, bindarit, an inhibitor of CCL2, brings relief to disease symptoms related to tissue damage by reducing the influx of inflammatory cells.[153]

Vaccination remains the best strategy to prevent most vector-borne diseases. Ideally, vaccines should combine low cost, single-dose efficacy and rapid and long-lived immunity with negligible risk of serious adverse reactions. For example, recombinant CHIKV vaccines are under development in mouse or macaque models, using different new technologies.[154-156]

## CONCLUSION

Our understanding of the pathologic mechanisms involved in chronic arthralgias and arthritis postviral infection is still in its infancy, given that several intricate humoral and cellular pathways are involved. Causal links will need to be verified in animal models, which can be controlled for immune status, immune senescence, or any other comorbidities, such as chronic systemic inflammation (e.g., type II diabetes). The development of animal models of chronic and flaring viral arthritis will be instrumental for screening new drug treatments and vaccines and to evaluate the short- and long-term effects of immunomodulation therapies of these arthritides.[3] This is critical and will need intensive investigations to improve animal models currently reported in the literature that do not seem to develop chronic joint inflammation. Gene and protein profiling studies will be required to address the phenotypic polarization of the synovial cells (fibroblasts and macrophages) that are chronically infected and which will contribute to aggressive destruction of the joints. It may be possible that viruses may control many of the inflammatory pathways, which may be of further interest to understand RA and identify novel therapeutic avenues.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 115



# Rheumatic Fever and Post-streptococcal Arthritis

Luiza Guilherme • Pedro Azevedo Ming • Jorge Kalil

**KEY POINTS**

- Rheumatic heart disease, the most common cause of acquired valvular disease in the world, is responsible for approximately one in four episodes of heart failure in endemic countries.
- Acute rheumatic fever (ARF) follows nontreated group A streptococcus throat infection, is mediated by immune mechanisms, and is controlled by genetic makeup.
- ARF rarely occurs before age 3 years and occurs infrequently after age 15 years. The majority of cases occur between ages 4 and 9 years.
- According to the World Health Organization ARF criteria, migratory polyarthritis, carditis, erythema marginatum, Sydenham's chorea, and subcutaneus nodules are major manifestations and arthralgia, fever, first-degree heart block, and elevated inflammatory markers are minor manifestations.
- Symptoms begin approximately 2 to 3 weeks after group A streptococcus infection, with the exception of chorea, which normally begins after the fourth to sixth week.
- Inflammation tends to be more intense in small children, who often exhibit greater prostration, fever, and carditis, whereas adults tend to exhibit a greater incidence of arthritis.
- Autoimmune reactions are mediated by both humoral and cellular immune response.

Rheumatic fever (RF) and rheumatic heart disease (RHD) occur in young, susceptible people who have not received treatment for group A streptococcal (GAS) infection. In this chapter, we briefly describe the etiologic agent and review several genes involved in the development of inflammatory and autoimmune lesions. Of note, the role of class II human leukocyte antigen (HLA) alleles is described in association with the disease and its function in antigen presentation to T cell receptors, which activates the adaptive branch of the immune response. The mechanisms that underlie organ-specific lesions are also discussed. Both cellular and humoral immune responses are involved in the autoimmune reactions, and T helper (Th)1 and Th17 inflammatory cytokines are mediators of rheumatic heart lesions. Clinical spectra and treatment are presented, along with perspectives regarding a vaccine for *Streptococcus pyogenes*.

## RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

### Etiologic Agent *(S. pyogenes)*

RF is always preceded by infection with group A streptococcus. The streptococci were classified by Rebecca Lancefield in 1941, who identified carbohydrates on the cell walls of the bacteria and classified them as groups A, B, C, F, and G based on the types of cell wall polysaccharide.

Group A is characterized by the presence of N-acetyl-β-D-glucosamine and rhamnose in the cell wall and is responsible for the development of RF and RHD. These bacteria contain M, T, and R surface proteins and lipoteichoic acid, which are involved in bacterial adherence to epithelial cells of the throat.

M protein, the major antigen, is composed of approximately 450 amino acid residues that extend from the cell wall. The N-terminal portion of this protein is highly variable and defines the emm type that is responsible for the antigenic diversity of the bacteria. According to the Centers for Disease Control and Prevention, more than 200 emm genotypes currently exist worldwide, with a variable distribution depending on the region.

The diversity of *S. pyogenes* is also influenced by social and economic conditions. It is accentuated in low-income countries, likely because of the lack of treatment, resulting in the spread of bacteria and favoring genetic mutations. Similarities in the distribution of emm-type strains have been observed in high-income countries, Asia, the Middle East, and Latin America, in contrast to those found in Africa and the Pacific region.[1] It is interesting to note that most of the streptococcal strains identified and described in this study were associated with the development of RF and RHD. The most frequent strain in most countries was emm1, which accounted for approximately 20% of the strains compared with approximately 5% in Africa and the Pacific region.[1] Interestingly, the emm1 strain is also frequently associated with post-streptococcal glomerulonephritis and invasive disease.[2,3]

Recently, an analysis of 220 variants of the M protein revealed 48 emm clusters that contained closely related M proteins sharing binding and structural properties.[4] This classification could provide important information for new drug and vaccine designs for the treatment and prevention of diseases related to streptococcal infection.

The end of the C-terminal half, in contrast to the N-terminal portion, contains multiple repeat regions and

presents a high degree of conservation of approximately 70%. In addition, it is a target of vaccine development models.

## Epidemiology

The development of RF and RHD after *S. pyogenes* infection in the throat depends on the genetic background of the individual and is influenced mainly by low socioeconomic conditions that facilitate the agglomeration of people and the spread of bacteria. In poor regions of several low-income countries in which medical treatment is not available or is limited, as well as in developing nations, the burden of RF and RHD remains a challenge.[5] The risk of RF after untreated GAS pharyngitis in susceptible people ranges from 0.3% to 3%.[6]

Although recent epidemiologic information is not available, data from the past 5 to 10 years have shown that the incidence of acute RF (ARF) in some developing countries exceeds 50 per 100,000 children. The worldwide incidence of RHD is at least 15.6 million cases and is the major cause of approximately 233,000 deaths per year. The incidence of ARF varies from 0.7 to 508 per 100,000 children per year in different populations from several countries.[6] In Brazil, according to the World Health Organization (WHO) 2008 epidemiologic model and data from the Brazilian Institute of Geography and Statistics (IBGE), there are approximately 10 million cases of streptococcal pharyngitis infections. These cases could lead to 30,000 new cases of RF, among which approximately 15,000 could become cardiac lesions. One important aspect of the recurrence of RF episodes is the possibility of worsening valve damage.

## Clinical Manifestations

### Acute Rheumatic Fever

Manifestations of ARF include a constellation of symptoms that may occur in isolation or in any combination. The incidence of ARF manifestations varies in frequency and intensity among different age groups, individuals, and localities, as previously reported.[7]

ARF is rare before age 3 years and infrequent after age 15 years, with the majority of cases occurring between ages 4 and 9 years. Inflammation tends to be more intense in small children, who often exhibit greater prostration, fever, and carditis, whereas adults tend to exhibit a greater incidence of arthritis. Symptoms begin approximately 2 to 3 weeks after GAS infection (average, 18.6 days), with the exception of chorea, which normally begins after the fourth to sixth week. However, some patients appear to have a very short latent period as a result of clinically unapparent infection some weeks prior to the observed respiratory illness.

### Pharyngitis

Approximately half of streptococcus pharyngeal infections can be asymptomatic.[8] The proportion of patients in whom ARF will develop after a pharyngeal infection largely depends on characteristics of the bacteria, population, and individual. Data obtained from RF outbreaks in U.S. military camps from 1949 to 1950 suggested that ARF would develop in 2.5% of patients infected with a rheumatogenic streptococcus strain. In a similar situation in 1987, the rate of ARF per case of streptococcal pharyngitis was 1.6%.

### Arthritis

Arthritis is the most common and often the earliest major symptom of ARF, although rarely it may be preceded by carditis. The relative proportion of patients with ARF who have polyarthritis and the severity of this manifestation increase with age.[9] In the old series (1962), arthritis was present in nearly 100% of young adults, 82% of teenagers, and 66% of children.[10] Tenderness is often disproportionate to the physical findings. A significant proportion of patients exhibit an additive rather than the classic migratory pattern of involvement, which occurs mainly in adults. A monoarticular presentation occurred in only 17% to 25% of the cases in the old series,[10] but it is currently much more common, which has been attributed to the widespread use of anti-inflammatory treatments. Untreated patients typically have 6 to 16 affected joints. Knees (76%), ankles (50%), and elbows and wrists (12% to 15%) are the most frequently involved sites. The shoulder (7% to 8%) and phalangeal (7% to 8%), lumbosacral (2%), cervical (1%), sternoclavicular (0.5%), and temporomandibular (0.5%) joints are less frequently affected.[10] Articular symptoms should last a few days for each joint and not much longer than a week for each ARF attack.[6] They typically respond well to NSAIDs. Other diagnoses should be considered if this pattern is not followed.

Although arthritis is never radiographically destructive in people with RF, Jaccoud's arthropathy (chronic postrheumatic fever arthropathy) has been described in recurrent cases in the past.

### Acute Carditis

Carditis occurs in 15% to 91% of the first ARF episodes, depending on the patient selection and whether echocardiography is used for diagnosis.[10-12] Carditis affects children more often and more severely than adults, but the latter are not spared. Inflammation may involve the pericardium, myocardium, and endocardium (pancarditis), and it may be mild and silent (subclinical) or severe and lead to acute congestive failure and death. In the acute phase, endocarditis is associated with edema of the valves and possibly vegetations (marantic vegetations) that do not produce an embolism. The mitral valve is affected in almost all cases, which is attributed to the predominant migration of inflammatory cells through the papillary muscles. The tricuspid valve is also frequently affected, but rarely in a significant manner. The aortic valve is involved in approximately 20% to 30% of cases.[13] Valve involvement may lead to a new or changing murmur that is used clinically to define carditis. Mitral regurgitation is the most common acute valvular lesion and may be isolated or concomitant with aortic regurgitation. Isolated aortic regurgitation is infrequent. Typical acute-phase murmurs consist of the high-pitched, blowing, holosystolic, apical murmur of mitral regurgitation; the low-pitched, apical, mid diastolic, flow murmur (Carey-Coombs murmur); and a high-pitched, decrescendo, diastolic murmur of aortic regurgitation heard in the aortic

area. Mitral regurgitation can be caused by valvular inflammation and deformity and/or valve prolapse. The latter is caused by annular dilation and chordal elongation, and it typically involves the anterior leaflet with minimal leaflet redundancy. Rupture of the chordae tendineae is possible and often leads to acute heart failure (HF) and the need for emergency surgery. Aortic or mitral stenosis at presentation is atypical and may be a sign of previous undiagnosed RF attacks.

Congestive HF (CHF) is the most life-threatening feature of ARF and is a result of valvular damage along with heart muscle dysfunction due to myocarditis and chamber dilatation. It affects 5% to 10% of people experiencing their first ARF episode and is more frequent during recurrences. Myocarditis in the absence of valvulitis should not be considered rheumatogenic in origin. Variable degrees of heart blockage may be present, and a transient pacemaker may be required if CHF occurs.

Pericarditis is present in approximately 10% of patients and manifests clinically as pleuritic chest discomfort or pain and pericardial friction rub. Although effusion may be prominent, cardiac tamponade is rare.

### Transition from Acute to Chronic Heart Disease

Inflammatory markers normally subside within 3 months after the acute phase, but they may remain high for a much longer period in a subset of patients. The causes or prognostic implications of persistent inflammation are not known. According to the classic view, if the patient received appropriate prophylaxis and did not experience further GAS infections, then any deterioration observed after the acute attack would be a result of mechanical dysfunctions caused by the valve insufficiency (acute injury and scarification) or the slow progression of valve stenosis. It is now increasingly recognized that some local inflammation may persist in the myocardium and (mainly) the valves of some patients and that it may, to some extent, be responsible for the observed deterioration. Samples from the valve and papillary muscles of patients with chronic RHD that are collected during valve replacement procedures often show variable degrees of mononuclear or lymphocytic infiltrates and sometimes Aschoff nodules, even in patients considered to be in remission clinically and on the basis of laboratory results.[14,15] Figure 115-1 illustrates rheumatic valve lesions and infiltrating T lymphocytes. In most cases the fibrotic and neovascularization effects that are involved in healing processes do not affect the function of the valves. Nevertheless, scarring may progress over the years and affect cusps, chordae tendineae, subendocardial tissue, and the annulus fibrosus, leading to valvular thickening, stiffening, incompetence, or stenosis. The speed of scarification and progression of mitral stenosis vary between geographic areas. In countries with a low RF incidence, 20 to 40 years will pass until surgery is required, whereas in countries with a high incidence of RF, mitral stenosis is possible in patients younger than 5 years. Again, factors relating to this behavior are unknown but are believed to be linked to the degree of acute damage, recurrences, and the persistence of inflammation.

In the classic 1951 series by Bland and Duckett Jones,[11] clinical signs of rheumatic valvular disease disappeared in 17% of the patients after 20 years. However, murmurs later developed in another 44% of the patients who initially did not have clinical carditis. In total, 70% of the patients eventually evolved to chronic RHD.[11] More recently, 258 Brazilian children with ARF were followed up prospectively, and 72% and 37% of them eventually experienced chronic valvular disease based on echocardiography and clinical assessment, respectively. Severe aortic and/or mitral disease occurred in 16% of the patients. Risk factors for progression to severe chronic valvular diseases were moderate or severe acute carditis, recurrences of ARF, and a low educational level of the mother.[16]

### Indolent Carditis

Indolent carditis may be defined as carditis of insidious onset and slow progression (low acute-phase aggressiveness and absence of significant acute progression), especially in people presenting for a medical diagnosis months after the onset of RF.[17] Recognition is usually delayed, and prophylaxis is not introduced; therefore, multiple recurrences are likely to drive progression. Diagnosis is typically made by routine consultations or when symptoms of RHD are present. It is not a rare situation, especially in the developing world, but the exact incidence has not been ascertained.

### Subclinical Carditis

Subclinical carditis is defined as pathologic valvular regurgitation detected by echocardiography that is not clinically evident.[18] Its clinical importance is discussed in the Echocardiography section.

### Rheumatic Heart Disease

RHD, the most common cause of acquired valvular disease in the world, is responsible for approximately one in four cases of HF in endemic countries.[19] Chronic RHD is normally oligosymptomatic until HF is established. HF features include a third heart sound, tachycardia, dyspnea, rales, and edema. If the diagnosis is not established during the acute phase, RF often remains undetected for many people until the first symptoms of HF occur. In South Africa, 82.5% of affected children were previously undiagnosed.[20] Sixty percent of the patients who are oligosymptomatic at the time of diagnosis will not experience any progression of symptoms in 10 years. Survival is greater than 80% in such cases. After the development of New York Heart Association functional class III or IV disease, survival is estimated to be 0 to 15% over 10 years if surgery is not performed. In the presence of severe pulmonary hypertension, survival is expected to be no longer than 3 years without surgery.[21] Typically, young patients exhibit pure mitral regurgitation, middle-aged adults have mitral stenosis, and older patients present with mixed mitral valve disease.[13] RHD normally remains asymptomatic for a long period and frequently is not noticed if ARF does not manifest.

### Sydenham's Chorea

Sydenham's chorea, also known as St. Vitus dance and chorea minor, is a neurologic disorder characterized by

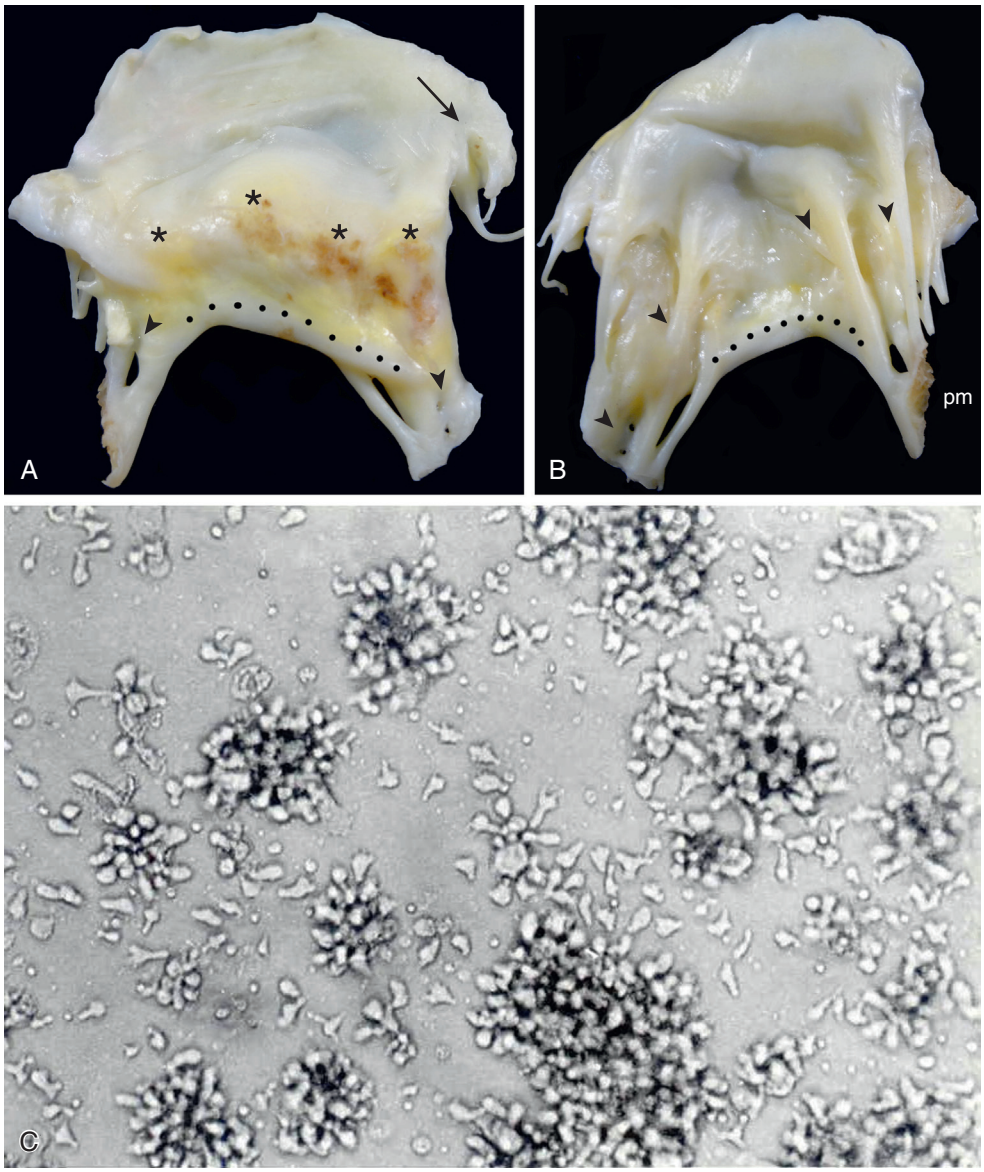



**Figure 115-1** Photograph of a mitral valve surgical specimen of a patient with rheumatic heart disease who underwent surgery for valve correction. Post-rheumatic mitral insufficiency and stenosis are shown as follows: (**A**) atrial and (**B**) ventricular aspects of the anterior mitral cusp show a thickened, calcified, and distorted leaflet with commissural adherence *(arrow)*, crescentic notching of the free edge *(dotted line)*, and calcification at the closure line (*). There is thickening, fusion, and retraction of the chords *(arrowheads)* and fibrosis in the papillary muscle (pm) tips. **C**, T cells (mainly $CD4^+$ cells) obtained by cell culture of valve fragments. Leukocytes infiltrate the myocardium and the left atrium. Later these cells go to the mitral valve, the most frequent site of valve damage, probably through papillary muscle; then they can reach the aortic valve and less frequently the tricuspid valve. A great number of infiltrating autoreactive T cells[65] that lead to the rheumatic lesions are present in the papillary muscle and valves. The inflammatory process is permanent as a result of a milieu composed mainly of inflammatory cytokines (TNF and IFN-γ) and low numbers of regulatory cytokines (IL-4 and IL-10).[77] *(**A** and **B**, Courtesy Dr. Demarchi, LMMF, Laboratory of Pathology, Heart Institute [InCor], School of Medicine, University of São Paulo, São Paulo, Brazil. **C**, Magnification 200×. Courtesy L. Guilherme, Laboratory of Immunology, Heart Institute [InCor], School of Medicine, University of São Paulo, São Paulo, Brazil.)*

emotional lability, personality changes, muscular weakness, reduced fine-motor skills, and uncoordinated abrupt, nonrhythmic, purposeless, jerky movements. Gait difficulties, headache, impaired cognition, dysarthric speech, and hypotonia are also possible features. Motor symptoms disappear during sleep; may affect the hands, arms, shoulders, feet, legs, face, and trunk; and are frequently more marked on one side. Other classic signs of chorea include tongue fasciculation ("bag of worms") and a relapsing involuntary hand grip ("milk maid's sign"). Although movements may be partially repressed by will, they can impair important daily activities. Emotional changes may precede motor symptoms and manifest through outbursts of inappropriate behavior, including crying and restlessness. In rare cases, psychological manifestations are severe and may result in transient psychosis.

In contrast to the other manifestations of RF, chorea generally occurs 6 to 8 weeks after the streptococcal infection is sustained; however, a latent period of up to 8 months has been described.[22] In the 1951 series of 1000 patients reported by Bland and Jones,[11] 51.8% had chorea. More recently, it has been reported to occur in 20% to 30% of

patients with ARF.[22] Females are affected more often than are males, and a first episode of chorea in adulthood is rare, although recurrences are more common during this period of life.

Chorea may occur in isolation or along with other RF manifestations. A recent series showed that 71% and 42% of 69 patients with chorea also had carditis based on an echocardiogram and on a clinical basis, respectively.[23] In the series by Bland and Jones,[11] chorea was associated with less severe carditis, and this manifestation is believed to have a better overall prognosis. Most patients with chorea recover spontaneously within 2 to 6 months. However, cases have been reported in which the condition persisted up to 3 years.

### PANDAS

In pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), antibodies from the sera of patients with chorea are able to bind to neuronal cells and induce the release of dopamine.[24] A subset of these patients will experience behavioral disturbances such as tics and obsessive-compulsive disorder (OCD) years after the initial manifestation of chorea. Symptoms may abruptly worsen after a new streptococcus infection. These data led to the hypothesis that this spectrum of mental disorders is a result of previous streptococcal infection in children. A strong association between OCD and the D8/17 antigen present on B cells has been described.[25] This antigen is believed to differentiate the clinical spectrum of patients who have RF with high specificity.[26] The PANDAS hypothesis remains controversial, although it is largely accepted that streptococcal infection can worsen symptoms of OCD in some patients.

### Fever

Fever may be absent, subtle, or high. Similar to subcutaneous nodules, erythema marginatum, and chorea, fever is extremely rare in adult-onset ARF. Malaise and myalgia are commonly associated with fever and may also be intense.

### Erythema Marginatum

Erythema marginatum is an evanescent, pink, nonpruritic, nontender rash that involves the trunk and sometimes the limbs but not the face. It is strongly associated with carditis. The lesion is also known as "erythema annulare" because its margins often assume the form of a ring. The border is irregular, serpiginous, and sharp on the outside but diffuse on the inside edge; it extends centrifugally while the center progressively clears. Lesions may appear, disappear, and reappear in a matter of hours and may be difficult to visualize on dark skin. A hot bath or shower may make them more evident. Erythema marginatum usually occurs early in the course of ARF, but it may occur at any time, occasionally in the chronic heart disease phase.

### Subcutaneous Nodules

Subcutaneous nodules in RF resemble those of rheumatoid arthritis in terms of having a predilection for bony surfaces or areas near tendons (elbows, knees, ankles, Achilles tendon, and occiput); however, they are smaller and more fleeting (they rarely persist for more than a month and normally subside within 1 or 2 weeks). Lesions range from a few millimeters to 2 cm in size and are firm, painless, and loose in relation to adjacent surfaces. The skin over the lesions is not inflamed. The number may vary from one to a few dozen (with an average of four). When they are abundant, the lesions tend to occur in crops and to have a symmetric distribution. Subcutaneous nodules are virtually always associated with significant chronic active carditis and are uncommon in early stages of the disease. They were described in 8.8% of the patients in the series by Bland and Jones[11] but only in 1.5% in a more recent series of 786 patients,[27] representing the least common RF manifestation.

## Genetic Associations

In autoimmune diseases, the activation of both innate and adaptive immune responses leads to tissue damage, probably as a result of a previous infection caused mainly by viruses or bacteria. In general, the control of pathogens and triggering of autoimmune reactions involve several genes.

The predisposition to develop both RF and RHD disease is associated with some class II HLA alleles, as well as single nucleotide polymorphisms in several genes related to activation of the immune response, initially against *S. pyogenes* and later against self-antigens.

The innate immune system detects pathogen-mediated injury, as well as other types of body injury, and represents the first line of immune defense. Both immune and somatic cells present receptors that are able to recognize microbial molecules called pathogen-associated molecular patterns and tissue injury-induced molecules as a sign of damage-associated molecular patterns.[28]

Most studies involve RF/RHD cohorts from diverse ethnicities and are representative of the genes involved in the development of the disease, as summarized in Figure 115-2. The genes associated with a predisposition to the disease are described, taking into consideration their role in both innate and adaptive immune responses.

### Innate Immunity

Toll-like receptors (TLRs) initiate host defense responses in multicellular organisms because they are sensors of foreign microbial products. A polymorphism of TLR-2 at codon 753 generally leads to the replacement of arginine with glutamine. The genotype 753Arg/Gln was more frequent in a Turkish ARF cohort when compared to controls and probably interferes with the recognition of *S. pyogenes* and the innate immune response against the pathogen.[29]

Mannan-binding lectin (MBL) is an acute phase inflammatory protein that binds to a wide variety of sugars on the surface of pathogens to promote phagocytosis and complement cascade activation via the lectin pathway.[30] Different variants of the promoter and exon 1 regions of the *MBL2* gene, which encodes mannan-binding lectin, have been reported in patients with RF/RHD. These studies have shown that the A allele, which encodes a high level of MBL production, is associated with the development of mitral

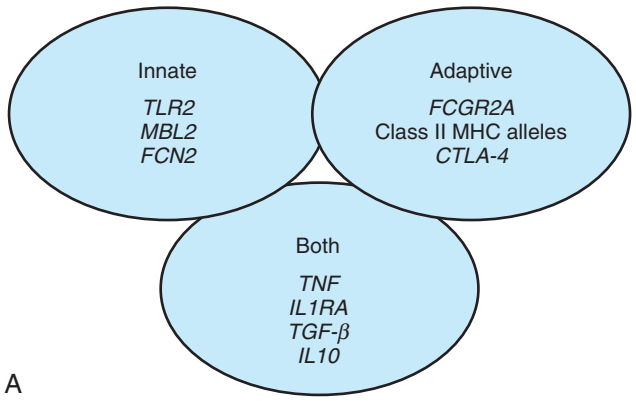



**Figure 115-2** Rheumatic fever (RF)/rheumatic heart disease (RHD) cohorts from diverse ethnicities are representative of the genes involved in the development of the disease. CTLA-4, Cytotoxic T lymphocyte–associated antigen-4; HLA, human leukocyte antigen; MHC, major histocompatibility complex; TGF, transforming growth factor.

stenosis,[31] whereas the O allele encoding a low level of production is related to aortic regurgitation.[32] Ficolins are proteins that bind to collectin cellular receptors and also initiate the complement lectin pathway. Three ficolin genes have been identified in humans with different functions, sequences, and specificities. Polymorphisms at –986, –602, and –4 within the promoter region of ficolin 2 (*FCN2*) encoding the ficolin-2 (L-ficolin) protein are associated with the serum levels of this protein. In Brazilian patients with chronic RHD, the haplotype G/G/A (–986/–602/–4) was shown to occur more frequently than in control subjects and was correlated with low levels of this protein and consequently a prolonged infection duration or repeated streptococcal infections.[33]

**TABLE 115-1** Class II HLA-DRB1 Alleles Associated with RF, RHD, and SC

| Class II HLA-DRB1 Alleles | Clinical Picture | Country | References |
|---|---|---|---|
| DR1 | RF/RHD<br>RHD<br>SC | South Africa<br>Martinique<br>Brazil | 35<br>36<br>37 |
| DR2 | RF/RHD<br>RHD | U.S.<br>Mexico | 38<br>39 |
| DR3 | RF/RHD<br>RHD | India<br>Turkey | 40<br>41, 48 |
| DR4 | RF/RHD<br>RF/RHD | U.S.<br>Saudi Arabia | 38<br>43 |
| DR5 (DR11) | RF/RHD | Turkey | 41 |
| DR6 | RF/RHD | South Africa | 35 |
| DR7 | RF/RHD<br>RHD<br>RF/RHD<br>RHD | Brazil<br>Egypt<br>Latvia<br>Turkey | 44, 45<br>46<br>47<br>41, 48 |
| DR9 | RF/RHD | U.S. | 42 |

HLA, Human leukocyte antigen; RF, rheumatic fever; RHD, rheumatic heart disease; SC, Sydenham's chorea.

## Adaptive Immunity

The *FCGR2A* gene controls the adaptive immune response and plays a role in the clearance of immune complexes by macrophages, neutrophils, and platelets. In patients with ARF, a polymorphism that typically encodes arginine (codon 131) instead yields histidine and encodes a protein with a low binding capacity to the immune complex, favoring the inflammatory response.[34]

Susceptibility to the development of RF and Sydenham's chorea or RHD was first associated with diverse class II HLA alleles (DR and/or DQ) encoded by the *DRB1* gene of the major histocompatibility complex, which is located in the short arm of human chromosome six. Several HLA-DR and/or DQ alleles have been described as being associated with the development of the disease worldwide (Table 115-1).

Briefly, HLA-DR1 was found in patients with RF and RHD from South Africa[35] and Martinique[36] and in patients with Sydenham's chorea from Brazil.[37] HLA-DR2 was detected in the black population in the United States[38] and in Mexico,[39] and DR3 and DR5 have been described in northern Indian and Turkish patients with RF/RHD.[40-42] HLA-DR4 has been found in Caucasian patients with RHD in the United States[38,42] and in patients in Saudi Arabia,[43] whereas HLA-DR6 has been detected in South Africa,[35] and HLA-DR7 has been detected in the Brazilian Caucasian and Mulatto populations,[44,45] in Egyptians[46] and Latvians,[47] and in patients from some regions of Turkey.[41,48] DR9 has been found in Caucasian Americans.[42] These data are summarized in Table 115-1.

In Egyptian and Latvian patients with RHD, the association of HLA-DR7 with some DQ-A or DQ-B alleles, both encoded by the *HLA-DQ* gene, was linked to the development of multiple valvular lesions.[46,47] In Japanese patients with RHD, susceptibility to mitral stenosis seems to be controlled in part by the *HLA-DQA* gene or by genes in

close linkage disequilibrium with some HLA-DQA and DQB1 alleles.[49] An RHD cohort consisting of a Mexican Mestizo population displayed an association between HLA-DR2 and some DQA and DQB alleles.[39]

CTLA-4 is a protein that plays a role in the adaptive immune response as a negative regulator of T cell proliferation. A polymorphism (G/G) in exon 1 of the *CTLA4* gene with an inhibitory function was found in patients with RHD.[50]

Inflammatory and anti-inflammatory cytokines play a role in both innate and adaptive immune responses and are also involved in the development of RF tissue lesions.

The *IL1* gene cluster located on chromosome 2 includes the genes expressing the pro-inflammatory cytokines IL-1a and IL-1b and their inhibitor IL-1 receptor antagonist (IL-1ra). The absence or misrepresentation of two alleles of VNTR from the *ILRA* gene results in a strong inflammatory response. Patients with RHD who have severe carditis display low frequencies of one of these alleles, suggesting an absence of inflammatory control.[51,52]

The *TNF* gene encodes the inflammatory cytokine TNF, which contributes to the development of valvular lesions. An association of *TNF* polymorphisms with RF and RHD has been shown in four independent studies.[53-56]

The anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)-β are produced by activated immune cells, especially monocytes/macrophages and T cell subsets including regulatory T cells (Tr1 and Treg) and Th1 cells.[57] The development of multivalvular lesions is associated with some polymorphisms in the promoter regions of the *IL10* and *TGFB1* genes[51,52] and with the severity of RHD lesions.

The roles of these genes are to drive the immune response against infection, and as previously described, they lead to a predisposition of developing the disease. The major mechanisms leading to autoimmune reactions are discussed in the next section.

## Pathologic Effector Mechanisms and Autoimmunity

As previously mentioned, recurrent attacks of ARF lead to tissue damage that can affect the joints, heart, and brain in people who have susceptibility genes. Both humoral and cellular immune reactions are effectors of the autoimmune reactions that follow *S. pyogenes* infection, and the phenotype of the disease is under genetic control and depends on factors such as age and episodes of recurrence, as previously mentioned.

Diverse biologic mechanisms such as immune complex formation, molecular mimicry, and epitope spreading are involved in the development of autoimmune reactions in RF and RHD. To aid the reader, we briefly define the mechanisms followed by their implications in the pathologic process.

### Immune Complex

The generation of immune complexes occurs through the interaction between antigen and specific antibodies and facilitates the clearance of foreign antigens by phagocytic cells.

### Molecular Mimicry and Epitope Spreading

The molecular mimicry mechanism takes into consideration the existence of similar or identical antigens in microbes (viruses, bacteria, and other pathogens) and their hosts. This mechanism enables the microbe to evade the host immune response and facilitates the recognition of self-antigens after an infection by cross-reactivity. Both T and B lymphocytes can recognize pathogens and self-antigens through molecular mimicry by four different types of mimicry. They can recognize (1) identical amino acid sequences, (2) homologous but nonidentical sequences, (3) common or similar amino acid sequences of different molecules (proteins and carbohydrates), and (4) structural similarities between the microbe or environmental agent and its host.[58]

Diversification of the immune response against an immunodominant epitope from a pathogen, human tissue, or soluble protein leads to its amplification and has been defined as epitope spreading.[59] This process favors the recognition of several self-antigens and facilitates tissue damage in different organs.

The major manifestations of disease result from interactions between these mechanisms.

### Arthritis

In the joints, anti-streptococcal antibodies—likely against group A carbohydrates, components of the bacterial cell membrane, or the M protein—precipitate to form immune complexes and activate the complement cascade to induce transitory arthritis.[60]

### Sydenham's Chorea

The pathogenic mechanism leading to the development of Sydenham's chorea was clarified by Kirvan et al.[24,61] Briefly, these investigators showed that antibodies from the sera of patients with chorea were able to bind to neuronal cells. They also identified gangliosides as autoantigens that cross-react with N-acetyl-β-D-glucosamine, the major cell wall streptococcal antigen, and showed that autoantibodies mediate signal transduction by calcium/calmodulin-dependent protein kinase II, triggering dopamine release from neuronal cells.

### Rheumatic Heart Disease

Sera antibodies from patients with RF simultaneously recognize N-acetyl-glucosamine, a streptococcal carbohydrate antigen, and specific regions of cardiac myosin in the myocardium and tropomyosin and laminin in the valves.[62] These cross-reactive antibodies may bind to the valvular endothelia and basement membrane, generating inflammatory signals that may upregulate the expression of several adhesion molecules, facilitating lymphocyte extravasation into the valve. The expression of vascular cell adhesion molecule (VCAM)-1, an adhesion molecule present in the vascular endothelium, is activated after initiation of an inflammatory reaction by anti-myosin and N-acetyl-glucosamine antibodies. These molecules interact with very late activation antigen (VLA)-4, another adhesion molecule

expressed on $CD4^+$ T lymphocytes, and facilitate its infiltration into the heart tissue.[62] Recently we verified that intercellular adhesion molecule (ICAM), another adhesion molecule, as well as the integrin P-selectin and several chemokines and their receptors, were upregulated. Among the chemokines, the gene expression of CCL3/MIP1α was upregulated in the myocardium, whereas CCL1/I-309 and CXCL9/Mig were highly expressed in the valvular tissue of patients with RHD.[63] An in vitro assay demonstrated that valvular lesions infiltrating T cells migrated mainly toward a CXCL9/Mig gradient, suggesting that specific chemokines can mediate both $CD4^+$ and $CD8^+$ T cell infiltration to the site of inflammation in the heart.[63] Several cytokines contribute to the inflammatory process and cellular infiltration. These data are summarized in Table 115-2.

Cardiac myosin is the major antigen in the myocardium. Using affinity-purified anti-myosin antibodies, Cunningham et al.[64] identified an epitope of the N-terminal M5 and M6 proteins consisting of a five-amino acid residue (Gln-Lys-Ser-Lys-Gln) that cross-reacted with cardiac myosin.

Human heart intralesional T cells obtained from valve tissue from patients with RHD who underwent surgery for valve correction allowed evaluation of in vitro reactivity against heart-tissue proteins[65] and human light meromyosin (LMM)[66] streptococcal M5 peptides and mitral valve–derived proteins. The frequency of intralesional T cell clones that recognized LMM peptides was 63.2%. Thirty-four percent of the T cell clones presented different patterns of cross-reactivity: i, myosin and valve-derived proteins; ii, myosin and streptococcal M5 peptides; and iii, myosin, valve-derived proteins, and M5 peptides. In addition, it has been recognized that several LMM peptides simultaneously display multiple reactivity patterns in heart-infiltrating T cells and also against peripheral T cells of patients with RF/RHD.[67] The high percentage of reactivity against cardiac myosin strengthens its role as one of the major autoantigens involved in rheumatic heart lesions. T cell reactivity toward myosin epitopes in patients with RHD may also trigger the broad recognition of valvular proteins with structural or functional similarities.

Valvulitis follows myocarditis, and the mitral and aortic valves display the most damaged tissue. Similarities between N-acetyl-β-D-glucosamine, a polysaccharide present in both the streptococcal cell wall and heart valve tissue, are probably responsible for the autoimmune reactions, as suggested by the observation that antibodies against N-acetyl-β-D-glucosamine display cross-reactivity with laminin, an extracellular matrix α-helical coiled-coil protein that surrounds heart cells and is also present in valves.[62]

Vimentin, a structural protein that plays diverse roles, is important for the binding and stabilization of collagen messenger RNAs. In vitro, this protein has been detected by a proliferation assay in both peripheral blood and heart tissue–infiltrating T cells from patients with RHD, reinforcing its role as a putative target antigen of autoimmune rheumatic lesions.[65,68]

Recently, an evaluation of the protein profile of valvular tissue from patients with RHD based on two-dimensional electrophoresis and mass spectrometry analyses showed an imbalance of heart tissue protein expression, probably as a result of the autoimmune reactions that follow streptococcal infection. Briefly, several proteins displayed an elevated expression, including vimentin, lumican, and apolipoprotein-A1, whereas collagen-VI, haptoglobin-related protein, prolargin, biglycan, and cartilage oligomeric matrix protein had a reduced expression level.[69]

The prediction of protein interactions in valve damage has also been evaluated by in silico analysis. Among the interactions, the potential roles of TNF, an inflammatory cytokine, and TGF-β (anti-inflammatory) and metalloproteinase (MMP-25) were noteworthy.[69] MMP-25 is involved in matrix degradation, as previously described,[70] and probably also plays an important role in valve damage.

Table 115-3 summarizes the major events described herein leading to RF, Sydenham's chorea, and RHD.

## Cytokines and the Inflammatory Process

Cytokines are also key players in the inflammatory process during RF episodes and later in RHD heart lesions.

Four subsets of Th cells (Th1, Th2, Th17, and Tregs) are currently described. Cytokines are soluble mediators that regulate cell proliferation, differentiation, and survival. Antigen-activated $CD4^+$ T cells polarize to these subsets, depending on the cytokine secreted.

**TABLE 115-2** Molecules Recruiting and Expanding Immune Cells into Heart Tissue

| Integrins | Adhesion Molecules | Chemokines Receptors | Cytokines |
|---|---|---|---|
| P-selectin | VCAM | CCL3/MIP-1α | TNF* |
| α4/β1 | ICAM | CCL1/I-309 | IFN-γ* |
| | VLA-4 | CXCL9/Mig | IL-4, IL-10† IL-17, IL-23‡ |

*Inflammatory.
†Anti-inflammatory.
‡Pro-inflammatory related to autoimmunity.
CCL, Chemokine (CC motif) ligand; CXCL, chemokine (CXC motif) ligand; ICAM, intercellular adhesion molecule; MIP, macrophage inflammatory protein; VCAM, vascular cell adhesion molecule; VLA-4, very late activation antigen-4.

**TABLE 115-3** Autoimmunity Leading to Rheumatic Fever, Sydenham's Chorea, and Rheumatic Heart Disease

**Arthritis**
Autoimmune antibodies (immune complex) against group A carbohydrate and *Streptococcus pyogenes* cell membrane antigens

**Sydenham's Chorea**
*S. pyogenes*—N-acetyl-β-D-glucosamine cross-reacts with human neuronal gangliosides, inducing autoantibodies
Cross-reactive autoantibodies are mediators of signal transduction by calcium/calmodulin-dependent protein kinase II that triggers dopamine release

**Rheumatic Heart Disease**
Myocarditis antibodies and T cell immune response against S2 and LMM subfragments of human cardiac myosin
Valvulitis—altered expression of valve-tissue proteins
- High expression: vimentin, lumican, and apolipoprotein-A1
- Low expression: collagen VI, haptoglobin-related protein, prolargin, biglycan, and cartilage oligomeric matrix protein

Th1 cells are involved in the cellular immune response and produce IL-2, interferon (IFN)-γ, and TNF. Th2 cells mediate humoral and allergic immune responses and produce IL-4, IL-10, IL-5, and IL-13. Th17 cells are important for protection against bacterial and fungal infections and in the development of autoimmunity. This lineage is induced by TGF-β, IL-6, and IL-23 and secretes IL-17 to mediate a type of pro-inflammatory response.[71]

Tregs, a subset of cells that regulate the inflammatory and autoimmune process, are identified as $CD4^+CD25^+CD127^-CD45RO^+FoxP3^+$ T cells and display immunosuppressive activity.[72]

The roles of cytokines in both RF and RHD have been evaluated in the peripheral blood and in heart tissue lesions. Briefly, in patients with ARF and active RHD, increased production of IL-2 and elevated numbers of $CD4^+CD25^+$ cells have been observed, suggesting the expansion of activated $CD4^+$ T cells in the peripheral blood,[73] as well as increased plasma levels of TNF.[74-76]

The Th1/Th2 cytokine pattern of infiltrating mononuclear cells in the mitral valve and myocardial tissue in patients with ARF and chronic RHD reveal large numbers of IFN-γ and TNF-secreting mononuclear cells. Mononuclear cells secreting IL-10 and IL-4 (regulatory cytokines) were also found in the myocardial tissue, whereas in the valvular tissue, only a few cells secreted IL-4. These low numbers of IL-4–producing cells may contribute to the progression of valvular RHD lesions.[77] Recently, we identified large numbers of IL-17 and IL-23–producing cells in the valves. These cytokines belong to the Th17 subset of cytokines, which are frequently involved in the development of autoimmune diseases, as previously mentioned. In agreement with these data, a Turkish RHD cohort study recently reported increased numbers of Th17 cells in peripheral blood that correlated with IL-17A cytokine levels in the sera and decreased numbers of Tregs.[78]

## Diagnostic Tests

No single laboratory test can confirm the diagnosis of ARF. In a patient in whom ARF is suspected, the workup aims to detect evidence of recent streptococcal infection, documenting the presence or persistence of inflammation, and diagnosing and following the course of carditis.

### Throat Cultures

A rayon-tipped throat culture swab obtained by sampling the tonsils and back of the throat is the current gold standard for the diagnosis of GAS pharyngitis. Nevertheless, when ARF symptoms are present, approximately 75% of patients have negative throat culture findings.[21] A positive throat culture finding may be due to a new infection or to convalescent carriage of the rheumatogenic strain.

### Rapid Antigen Testing

Rapid antigen testing (RSAT) has a high specificity (≥95%) but a moderate sensitivity (65% to 90%).[79] A positive RSAT finding is useful in establishing a diagnosis of GAS pharyngitis, but a negative RSAT finding does not exclude it. In high-risk ARF locations, where high-stake decisions are being made, rapid antigen detection tests (RADTs) are not currently recommended.[80] If an RADT is used and is negative, additional costs are incurred because a follow-up throat swab is required.

### Streptococcal Antibody Tests

Antibody tests are directed against the extra-cellular products of streptococci and include anti-streptococcal lysine O (ASO), anti-DNAse B (ADB), anti-hyaluronidase, antinicotinamide adenine dinucleotidase, and anti-streptokinase. ASO and ADB are the most commonly available tests. ASO begins to rise at 1 week and peaks at 3 to 5 weeks after infection. It begins to decline after approximately 6 to 8 weeks, at a faster pace during the immediate subsequent months, and at a slower pace after 6 months. ADB begins to rise at 2 weeks and peaks at 6 to 8 weeks after infection. It begins to decline approximately 3 months after the precipitating infection. Antibody peaks normally coincide with the second or third week of ARF. Patients will have elevated ASO and ADB titers 80% and 85% of the time, respectively, when symptoms of ARF are present. When both ASO and ADB tests are conducted, 92% of patients will have abnormal test results.[81] The antibody tests must be interpreted with caution in areas with high rates of streptococcal infection and ARF because relatively high titers are commonly encountered in such populations. A second sample should be collected 2 weeks later to characterize a recent infection based on a significant rise in antibody titers. Asymptomatic pharyngeal streptococcus carriers usually have titers that are just above detectable, and therefore a high titer indicates a true infection. Increases in ASO occur not only in response to GAS infections but also in infections with groups C and G streptococci and other bacteria such as bacillus, *Clostridium tetani*, and *Listeria monocytogenes*.

### Acute-Phase Reactants

Acute-phase reactants, the erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels rise during an ARF attack, with the exception of chorea or erythema marginatum as a single manifestation. Acute-phase reactants aid in monitoring the evolution of inflammation and serve as minor manifestations based on the Jones criteria. Salicylates or other NSAIDs may mask the results of these tests. ESR may stay elevated for up to 2 months after an inflammation, whereas CRP normalizes within days.

### Other Laboratory Tests

Transient and asymptomatic abnormalities of liver function tests, bilirubins, and a mild normochromic normocytic anemia are observed in a significant portion of patients with ARF and usually normalize after cessation of inflammation. Blood cultures should be obtained to help rule out infective endocarditis, bacteremia, and disseminated gonococcal infection.

Depending on the clinical features, additional tests should be performed to rule out an alternative diagnosis, such as anti-nuclear antibody and head magnetic resonance assessments.

### Chest Radiography

If myocardial dilatation and/or pericardial effusion are significant, a chest radiograph may reveal an enlarged heart silhouette. Pulmonary edema may be observed in the presence of HF.

### Electrocardiography

Tachycardia and prolongation of the PR interval are the most common electrocardiographic findings of myocarditis. Rarely, third-degree heart blockage may be observed. Pericarditis can induce a diffuse elevation of the ST segments. Electrocardiographic abnormalities are present in approximately 21% of patients, 60% of whom have degrees of heart blockage.

### Echocardiography

Echocardiography is 10 times more sensitive than auscultation in identifying valvular involvement,[82] and the use of echocardiography results in a 16% to 47% increase in the diagnosis of carditis.[83] Echocardiography confirms the clinical findings for valvular lesions objectively, addresses progression, and helps dismiss nonrheumatic causes of valvular damage. Nevertheless, echocardiography was not included in the 2004 WHO criteria for the diagnosis of RF.[21] This decision was driven by concern that the diagnosis could be hampered by the lack of availability of echocardiographs in some locations and because the evolution of most cases of subclinical carditis is benign.[84]

The long-term follow-up of subclinical carditis diagnosed in patients with a previous or current history of ARF is better characterized than subclinical carditis in asymptomatic populations. In the first group, valve lesions persist in 30% of patients after 5 years, and half of the patients diagnosed with subclinical carditis at the time of ARF show persistence or deterioration of their carditis during the ensuing 2 years.[83] As a result, the New Zealand (NZ) and Australia (AU) criteria for RF diagnosis include subclinical carditis as a major criterion for the diagnosis of ARF in high-risk people and recommend that all patients with suspected or confirmed ARF undergo echocardiography.[85,86,87]

Subclinical RHD may be defined as features of RHD on echocardiography in the absence of symptoms and signs of heart valve disease. Studies reporting the short-term progress of subclinical RHD detected by echocardiography suggest that the severity of subclinical RHD is not progressive in 68% to 69% of children, whereas it worsens in 4% to 9% and regresses in 28% to 32% of children.[84] This evolution is similar to that observed in auscultation-only screening programs[86] and indicates that the spontaneous resolution of changes will take place in 30% to 40% of suspected RHD cases that are detected by either auscultation or echocardiography in asymptomatic populations. Techniques to identify "persistors and progressors" who may be eligible for secondary prophylaxis are lacking. Preliminary studies suggest that disease classified as definite by echocardiography (i.e., with morphologic and functional changes) is more likely to persist than are borderline or possible categories (with either minor morphologic or functional changes).[86] The additional costs and excess of people receiving treatment as a result of RHD screening protocols would be justifiable if the prognosis of RHD improved; however, it has not yet been demonstrated that penicillin prophylaxis for all children with subclinical rheumatic valve lesions is the best option in clinical practice. The question remains unanswered; however, some specialists consider these screening programs to be cost-effective in areas with a high prevalence.[88,89]

A standardized evidence-based guideline for the echocardiographic diagnosis of RHD has been developed by the World Heart Federation to improve the reliability, comparability, and reproducibility of echocardiographic screening studies.[89] It should be used to follow up on subclinical lesions to evaluate the progression of RHD and recurrences of ARF. It is envisioned that the new guideline may reduce the potential for overdiagnosis of RHD in screening studies by excluding physiologic valve regurgitation and congenital valve anomalies that may be present in at least 1% of normal children.[84]

## Diagnosis

The manifestations of ARF, as with its incidence, vary notably in frequency and intensity among different age groups, individuals, and localities.[7] The sensitivity and specificity of any criteria depend on the incidence of the disease and thus have the same variation. This phenomenon is relevant because there are no specific diagnostic tests for ARF, and a diagnostic mistake has severe consequences. The classic Jones criteria was first published in 1944 and revised in 1965 and 1992. In 2004, the WHO published an expert consultation report that included ARF criteria based on the previous revised Jones criteria (Table 115-4). Each criterion modification has resulted in more specific and less sensitive criteria, which is appropriate for most of the developed world but not for hot spots. NZ[85] and AU[86] have developed their own criteria, the main particularities of which are the use of echocardiograms for the diagnosis of carditis, aseptic monoarthritis as a major criterion (provided NSAIDs are used in NZ and only for high-risk individuals in AU), the use polyarthralgia as a major criterion for high-risk individuals (AU) and monoarthralgia as a minor criterion for high–risk individuals (AU). Utilization of NZ guidelines for the diagnosis of ARF result in a modest increase (16%) in cases classified as definite ARF compared with the American Heart Association (AHA) 1992 Jones criteria.[83] These guidelines may be more applicable to high-risk populations.

## Treatment

### Primary Prophylaxis

Adequate treatment of streptococcus pharyngitis reduces the likelihood of the subsequent development of ARF.[90] Nevertheless, streptococcus cannot be differentiated from other pharyngeal pathogens on a clinical basis, cultures take days to provide results, and RSAT lacks sensitivity and adds costs. The majority of sore throats are viral in origin. Fifteen percent to 30% of sore throats in children and 10% in adults are estimated to be due to GAS.[83] Whether all pharyngeal

**TABLE 115-4** 2004 World Health Organization Criteria for the Diagnosis of Rheumatic Fever and Rheumatic Heart Disease

| Diagnostic Categories | Criteria |
|---|---|
| Primary episode of RF[a] | 2 major* or 1 major and 2 minor** manifestations plus evidence of a preceding group A streptococcal infection*** |
| Recurrent attack of RF in a patient without established RHD[b] | 2 major or 1 major and 2 minor manifestations plus evidence of a preceding group A streptococcal infection |
| Recurrent attack of rheumatic fever in a patient with established RHD | 2 minor manifestations plus evidence of a preceding group A streptococcal infection[c] |
| Rheumatic chorea and insidious onset of rheumatic carditis[b] | Other major manifestations or evidence of group A streptococcal infection are not required |
| Chronic valve lesions of RHD (patients presenting for the first time with pure mitral stenosis or mixed mitral valve disease and/or aortic valve)[d] | Do not require any other criteria to be diagnosed as having RHD |
| *Major manifestations | Carditis, polyarthritis, chorea, erythema marginatum, subcutaneous nodules |
| **Minor manifestations | Clinical: fever, polyarthralgia; Laboratory findings: elevated acute-phase reactants (ESR or leukocyte count) |
| ***Supporting evidence of: electrocardiogram | Prolonged PR interval; preceding streptococcal infection; elevated or rising anti-streptolysin-O or streptococcal infection with another streptococcal antibody; infection in the past 45 days; a positive throat culture; rapid antigen test for group A streptococci; recent scarlet fever |

[a]Patients may present with polyarthritis (or with only polyarthralgia or monarthritis) and with several (three or more) other minor manifestations, together with evidence of recent group A streptococcal infection. Some of these cases may later turn out to be rheumatic fever. It is prudent to consider them as cases of "probable rheumatic fever" (once other diagnoses are excluded) and advise regular secondary prophylaxis. Such patients require close follow-up and regular examination of the heart. This cautious approach is particularly suitable for patients in vulnerable age groups in high-incidence settings.

[b]Infective endocarditis should be excluded.

[c]Some patients with recurrent attacks may not fulfill these criteria.

[d]Congenital heart disease should be excluded.

ESR, Erythrocyte sedimentation rate; RF, rheumatic fever; RHD, rheumatic heart disease.

Data from the revised Jones criteria.[21]

infections should be treated a priori with antibiotics strongly depends on the incidence of ARF in the evaluated population. The case of Costa Rica is often used by those who advocate the use of antibiotics for all sore throat cases in locations with a high incidence of ARF. This policy was instituted in Costa Rica in 1970, and the number of new cases of ARF fell from 94 new cases in that year to only 4 in 1991.[90] A similar decrease in ARF incidence occurred in Europe and in the United States before the availability of antibiotics, and critics attribute the decrease observed in Costa Rica, at least in part, to other socioeconomic improvements, including housing conditions, education, and health care access. NZ long ago introduced an efficient program of close surveillance and optimized treatment of pharyngitis for its high-risk populations, but to date, these measures have not been successful in reducing the incidence of ARF.[91] This outcome could be related to the observation that approximately half of streptococcus pharyngeal infections can be asymptomatic[8] and that only a small portion of patients with a sore throat will seek medical attention.[8] The NZ and AU Guidelines for sore throat management do not indicate throat swabs or antibiotics for low-risk people with mild symptoms, provided there is a low occupational risk of spreading GAS. People with a high risk of developing ARF should have a throat culture and be treated if positive results are obtained or should receive empiric antibiotics if follow-up is a concern.[82]

## Acute Rheumatic Fever Treatment

**Eradication of Streptococcus.** Immediate eradication of streptococcus is a standard practice for patients presenting with ARF, even before throat culture results are obtained. Although an improvement of 1-year outcomes was never demonstrated, it is conducted with the goal of minimizing prolonged exposition to the trigger factor and of reducing the spread of rheumatogenic strains. Household contacts should also have throat cultures, and people with positive results should be treated even if they are asymptomatic.[85]

**Acute Carditis.** The extent of acute cardiac involvement is probably the most important event in determining the long-term morbidity and mortality of RF. Nevertheless, currently no effective therapies have been demonstrated to alter the natural history of the disease.[92] Illingworth and colleagues reviewed 170 articles and concluded that aspirin was not beneficial in the treatment of acute carditis.[92] This finding was reiterated by a United Kingdom–United States joint report in 1955.[92] Studies of corticosteroid therapy for the treatment of ARF were performed largely in the 1950s and 1960s, before the availability of echocardiography. In 1995, a meta-analysis involving more than 130 studies concluded that corticosteroids were not statistically superior to salicylates in preventing a pathologic murmur at 1 year after treatment, and inflammation tended to recur once steroid therapy was withdrawn. However, given that the confidence intervals were wide and that there was significant heterogeneity in the results between these studies, the authors indicated that the precision of this meta-analysis was not high, and therefore marginal improvements may have been overlooked.[93] Three Cochrane reviews have been published on this subject, and similar conclusions were drawn.[94] Additionally, the clinical parameter used by the referenced studies does not allow accurate inferences about the severity of heart damage. As a result, doubt persists, and corticosteroids are still widely used to treat acute carditis worldwide. A new multicentric randomized clinical trial (Steroids in Acute Rheumatic Fever [STERARF]) is currently under way.

In the NZ and AU guidelines, glucocorticoids are indicated as optional for severe cases of carditis.[84,85] The 2004

WHO expert consultation panel recommended the use of corticosteroids for patients with pericarditis (HF) who do not respond to salicylates and continue to worsen and experience HF despite anti-inflammatory therapy. Prednisone (1 to 2 mg/kg per day to a maximum of 80 mg/day, administered once daily or in divided doses) is usually the drug of choice.[21] After 2 to 3 weeks of therapy, the dosage may be diminished weekly by 20% to 25%. Investigators who conducted two additional recent, small, randomized clinical trials[95,96] investigating the use of intravenous methylprednisone for the treatment of active rheumatic carditis have obtained inconsistent findings on the advantage of intravenous compared with oral corticosteroid therapy for valvular outcomes. Nevertheless, both the WHO and NZ/AU Guidelines recommend intravenous methylprednisolone in life-threatening circumstances. Salicylates or other NSAIDS may need to be resumed after discontinuation of glucocorticoids to avoid rebound joint symptoms or fever.

IVIG was evaluated prospectively in a randomized clinical trial involving 59 patients with ARF (39 of whom had carditis), and it was found that the treatment did not alter the natural history of ARF. No differences were observed in the clinical, laboratory, or echocardiographic parameters of the disease process during the 12 months of follow-up.[97]

Bed rest is traditionally advised, although this recommendation is not based on clinical trial results. In the prepenicillin era, it was associated with a shorter duration of carditis, fewer relapses, and less cardiomegaly.[98] Patients with mild or no carditis should avoid ambulation only as necessary to avoid joint pain. Persons with severe carditis are advised to rest, especially during the first 4 weeks or until serum CRP levels have normalized and the ESR has normalized or significantly diminished.[85]

**Heart Failure.** Although CHF is more frequent during recurrences, it also occurs in 5% to 10% of initial ARF episodes. If bed rest and steroids are not sufficient to manage HF, it must be treated early and aggressively with diuretics and a diet with restricted sodium content. Angiotensin-converting enzyme inhibitors and digoxin may also be used. Few studies have evaluated treatment with β-blockers in people with HF due to acute carditis, and currently the use of these treatments is not recommended.[85] Second- and third-degree heart blockages may require a transient pacemaker.

**Emergency Valve Surgery.** Surgery should be deferred until active inflammation has subsided, but the rupture of a valve leaflet or chordae tendineae may lead to severe regurgitation and the need for emergency procedures. During the acute phase, valve replacement is usually preferred compared with repair of the friable inflamed valve.[85]

**Arthritis and Fever.** Arthritis and fever often respond to NSAIDs within hours and almost always within 3 days. If not, the diagnosis should be challenged. Aspirin (80 to 100 mg/kg per day in children and 4 to 8 g/day in adults) is traditionally used and should be continued until all symptoms subside and inflammatory markers normalize. Most patients will need to take NSAIDs for only 1 to 2 weeks; however, some will need NSAIDs for up to 6 weeks, and in approximately 5% of cases, 6 months or more of salicylate therapy is required.[85] The use of other NSAIDs is a possibility, and naproxen has been reported to be effective and well tolerated. NSAIDs alter acute-phase reactants, and thus therapy for ARF manifestations should be withheld if the diagnosis is uncertain. In such cases, paracetamol can be used. The recurrence of joint symptoms during NSAID withdrawal does not indicate ARF recurrence and can be treated with another brief course of high-dose NSAIDs.

**Chorea.** Chorea is often self-limited and mild and does not require specific treatment aside from rest and a calm environment. In prolonged or incapacitating cases, neuroleptics, diazepam, and anti-convulsant agents have been reported to be effective. Aspirin and glucocorticoid therapy do not influence this manifestation. A prospective comparison of carbamazepine, valproic acid, and haloperidol in 18 patients with chorea concluded that valproic acid was the most effective treatment.[99] A reduction but not an eradication of symptoms should be expected after 1 to 2 weeks of therapy. Medication should be continued for 2 to 4 weeks after chorea has abated.

## Rheumatic Heart Disease Management

In 2014, the American College of Cardiology (ACC) and the AHA published an update of their former 2008 AHA/ACC guidelines for the management of patients with valvular heart disease (VHD).[100] The focus of this guideline is the diagnosis and management of adult patients with VHD through a combination of lifestyle modifications, medications, and medical procedures.

## Secondary Prophylaxis

Patients with RF are at a high risk for recurrences and progression of the severity of manifestations following a new GAS infection. Because streptococcal pharyngitis is often asymptomatic, prevention requires continuous antimicrobial prophylaxis rather than treatment of acute GAS pharyngitis episodes. Continuous administration of benzathine penicillin G (BPG) reduces the chances of a new streptococcal pharyngitis in 71% to 91% of cases, the chances of a new episode of ARF in 87% to 96% of cases, and the severity of RHD[102] and its associated mortality.[103]

Secondary prophylaxis is indicated for all patients with a documented history of ARF, Sydenham's chorea, or convincing evidence of RHD.

Intramuscular penicillin is more effective than oral penicillin in preventing recurrences.[101] BPG is indicated at 1.2 million units by intramuscular injection for people weighing more than 27 kg (60 lb) and 600,000 units for people weighing less than 27 kg[21] (20 kg in NZ/AU guidelines).[84,85] Serum penicillin levels may be undetectable or low 28 days after a 1,200,000-unit BPG dose.[104] For high-risk patients, intramuscular BPG injections every 3 weeks should be considered. Patients treated with this regimen exhibit fewer streptococcal infections and ARF recurrences[85,102] and a greater resolution of mitral regurgitation over the long term.[104] Nevertheless, prospective data from NZ showed that recurrences were rare (0.07 per 100 patient years) among patients who were fully adherent to a four-weekly BPG regimen, which is the current recommendation in the United States.

If an intramuscular route is not possible or refused, the second-line regimen is oral penicillin V, 250 mg twice

daily. Patients with a documented allergy to penicillin should receive oral erythromycin, 40 mg/kg (maximum of 1 g/day) daily divided into two to four doses for children and oral erythromycin, 400 mg twice daily for adults and adolescents.[21,85,86]

Secondary prophylaxis should last until the risk of ARF recurrence is irrelevant, and thus the discontinuation time should be individualized. Guidelines from the WHO, NZ, and AU Guidelines were developed on the basis of their respective data and differ slightly. In the NZ environment, maintenance of prophylaxis up to 21 years of age is considered safe and effective in cases with absent or mild heart disease.[85] In a review of data from the 1993-1999 Auckland (NZ) Acute Rheumatic Fever Register, only five recurrences occurred after the age of 30 years. Data from the Northern Aboriginal Territory of Australia showed that only approximately 1% of 259 ARF episodes recorded between 2005 and 2009 occurred after 40 years of age. Therefore, 30 and 40 years of age were considered the most reasonable cutoffs to cease secondary prophylaxis for patients with severe carditis in NZ and AU, respectively. In both countries, exceptions are made for individual circumstances warranting continuing prophylaxis (e.g., patients wanting to reduce even a small chance of a recurrence).

It is not clear whether these prophylaxis regimens should be followed in sporadic cases in locations with a low incidence of ARF, because these regimens have been largely developed in regions where ARF remains a significant burden. In a prospective study in modern Chile, 59 patients with ARF who were judged to be at a low risk for recurrences were permitted to receive no secondary prophylaxis. During the 3349 patient-months of follow-up, only two ARF attacks were observed, and the authors suggested that ARF prophylaxis could be discontinued safely in patients with a low risk of recurrence who undergo careful prospective surveillance.[105]

The efficacy of secondary prophylaxis depends completely on compliance. In a prospective international study involving 1790 patients, a prevalence of new ARF attacks was observed in 0.45% of the compliant patients compared with 11.5% of noncompliant patients.[106] Only one in five patients with RF from Aborigine communities of Australia received 80% or more of the scheduled doses.[107] Reasons for failed compliance were painfulness and a high frequency of injections, a long treatment period, and low socioeconomic and educational levels of the patients.

### Infective Endocarditis Prophylaxis

Infective endocarditis (IE) is a potentially lethal complication of RHD. Antibiotic prophylaxis prior to procedures has not been proven effective in preventing IE but is supported by animal models, empirical observations, and expert opinion.[86] All patients with RHD or prosthetic valves should receive IE antibiotic prophylaxis prior to dental procedures and other procedures expected to produce bacteremia. People with a history of ARF but no valvular damage do not require endocarditis prophylaxis. Transesophageal echocardiogram, esophagogastroduodenoscopy, colonoscopy, and cystoscopy are examples of procedures that do not require IE prophylaxis in the absence of active infection. For people receiving secondary prophylaxis for RF, a different antibiotic should be offered. Guidelines aimed at the optimal diagnosis and management (including prophylaxis) of IE are offered and constantly updated by specialized institutions.[101]

## Vaccine Development

Although RHD control programs have been implemented around the world, the disease remains a health problem in several countries. The epidemiologic growth of streptococcal diseases in undeveloped and developing countries has encouraged many groups to study vaccine candidates for the prevention of GAS infections.

The development of a vaccine against *S. pyogenes* would provide many benefits such as preventing streptococcal infections and their sequelae. Several vaccine development studies have been proposed in previous decades with no satisfactory results.

After the introduction of new methodologies, such as the synthesis of peptides and molecular biological techniques, some proposals have been under development for the past 20 to 25 years.

Four anti-GAS vaccine candidates target the M protein, and eight other candidates target alternative streptococcal antigens, including group A-CHO, C5a peptidase (SCPA), cysteine protease (Spe B), binding proteins similar to fibronectin, opacity factor, lipoproteins, Spes (super antigens) and streptococcal pili, as reviewed by Steer et al.[108]

Strain-specific vaccines based on recombinant N-terminal portions of the M protein serotypes that are most prevalent in the United States were entered into phase I/II clinical trials and, currently, a new approach based on the 30 most prevalent serotypes is being investigated in a phase I clinical trial.[109]

Two other promising vaccines incorporate C-terminal protective epitopes and differ with respect to their mechanisms of action. One is based on a minimal protective epitope that induces protective antibodies,[110-112] and the other is a long peptide (55 mers) identified as StreptInCor.[113] The advantage of the StreptInCor vaccine model is the possibility of the vaccine epitope undergoing processing by antigen-presenting cells (monocytes and/or macrophages) to generate several peptides that induce both B and T immune responses, resulting in a safe and robust immune response.[114] Experimental assays have demonstrated that the StreptInCor peptide induces high titers of opsonic and neutralizing and protective antibodies in outbred immunized mice.[115,116] Using class II HLA transgenic mice, it was possible to evaluate the immunogenicity and safety of the StreptInCor vaccine epitope for a period of 1 year. Specific and nonautoreactive antibodies were produced. No autoimmune or pathologic reactions were observed in the heart or other organs.[117]

Considering all the vaccine development approaches in progress, a safe and protective anti–*S. pyogenes* vaccine may be achieved within the next 5 years.

# POST-STREPTOCOCCAL ARTHRITIS

According to the current definition of reactive arthritis, only infections that affect the gastrointestinal or genitourinary tract are associated with this diagnosis.[118] However, the

GAS and *Chlamydophila pneumoniae* agents that affect the respiratory tract have also been implicated in reactive immune-mediated aseptic arthritis. Another disputed question is whether post-streptococcal reactive arthritis (PSRA) should be considered an atypical and incomplete form of ARF or a separate disease. The small number of available publications are predominantly based on small sample sizes and are hampered by a generally accepted definition of PSRA. These case series have suggested significant dissimilarities between the two conditions. Whereas ARF affects mainly children aged 4 to 9 years, PSRA seems to have a bimodal age distribution with peaks between 8 and 14 years and between 21 and 37 years.[118,119] RF symptoms commence 2 to 3 weeks after the streptococcal infection, whereas in PSRA, the latency period is only approximately 10 days. Arthritis in PSRA is often additive and symmetric. Knee and ankle joints are normally involved, but small joint and axial involvement also occurs. In PSRA, the mean duration of arthritis is approximately 2 months, but subsequent arthralgia may persist for 3 years. In up to 19% of patients, arthritis recurs after an interval of a few months. The response to salicylates and other NSAIDs is dramatic in people who have arthritis associated with ARF but less convincing in people with PSRA.[120]

A retrospective series involving 26 patients demonstrated that polytendinitis, tenosynovitis, and enthesitis are common presentations in PSRA and can be the only manifestation.[120] This finding, and the possibility of axial involvement, raises suspicion that what is currently known as PSRA is truly a heterogeneous group of disorders, with some disorders sharing features of spondyloarthropathies and other disorders being more similar to ARF. An animal model of rheumatoid arthritis may facilitate our understanding of this heterogeneity. In this model, rats are treated via intraperitoneal injection of streptococcus cell walls fragments, which are highly resistant to biodegradation. Within 24 to 48 hours of administration, complement-dependent acute inflammation develops in the peripheral joints of the animal together with dissemination of cell wall fragments in the blood vessels of the synovium and subchondral bone marrow. In some rats, the initial inflammation subsides; in others, it persists, or it subsides and recurs. The chronic, T cell–dependent erosive polyarthritis that affects some rats is believed to be initiated by an inflammatory process promoted by macrophages struggling to degrade the cell wall fragments. Chronic joint inflammation develops only in susceptible rats but is not dependent on specific streptococcus strains. Remarkably, chronic arthritis will not develop in rats raised in a germ-free environment, suggesting that streptococcus cell wall fragments do not elicit a specific autoimmune reaction but instead function as an adjuvant, promoting other cross-reactive immune processes involving other bacteria, perhaps in the gastrointestinal or genitourinary track. It is possible that in PSRA, the streptococcus infection has a similar adjuvant rule, promoting only an established autoimmunity. In such a case, the different clinical pictures of the PSRA spectrum would follow the different individual susceptibilities.

The most important practical question to be answered is whether patients with PSRA have an increased risk of RHD and require secondary prophylaxis. Three echocardiography-based studies in children with PSRA provided evidence of cardiac involvement in 6 of the combined number of 76 patients. Adults with PSRA were shown to be free of valvular abnormalities by echocardiographic screening in two case series and in one prospective study involving 75 patients who did not receive antibiotic prophylaxis and were followed up for almost 9 years.[118] Because the present literature on PSRA is mainly based on American and western European patients, the conclusions may not be applicable to people in other parts of the world. The WHO ARF guidelines state that "patients with arthritis following a streptococcal upper respiratory infection should be considered to have RF if they fulfill the Jone's criteria." The NZ and AU guidelines recommend that the diagnosis of PSRA rarely, if ever, should be made in high-risk populations and should be made with caution in low-risk populations. In those countries, patients diagnosed with PSRA who are at a high risk of developing ARF should receive secondary prophylaxis for at least 5 years. Low-risk patients should receive this treatment for at least 1 year. An absence of valve involvement should again be confirmed by echocardiography prior to the discontinuation of antibiotics.

## CONCLUSION

The data presented herein demonstrate that several genes play a role in the development of RF, RHD, and PSRA. These genes control both innate and adaptive immune responses. The autoimmune reactivity is due to molecular mimicry between human proteins and antigens of *S. pyogenes*. The M protein is the major antigen of the bacteria and consists of two regions. The polymorphic N-terminal region and differences in the A and B blocks of amino acids define the diverse types of bacteria. The C-terminal region is conserved. Some regions of the block B of the N-terminal region are involved in autoimmune reactions. An imbalance between inflammatory/anti-inflammatory cytokines that are produced in response to the infection leads to acute and chronic inflammation. In addition, the low numbers of Treg cells facilitate increased inflammatory reactivity and consequently chronic disease.

The treatment and prevention of new episodes of RF and RHD are based on penicillin-benzathine as a primary prophylaxis and other drugs, as previously described. The possibility of prevention/treatment by administration of a vaccine against *S. pyogenes* in the near future is also presented.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 116

# Amyloidosis



Ralph C. Budd • David C. Seldin†

**KEY POINTS**

- ***Amyloidosis*** **is the term for systemic disease in which aggregated proteins form extra-cellular fibrils in tissues of the body, eventually leading to organ failure and death if not effectively treated.**
- **Patients with amyloidosis can present with joint symptoms and soft tissue deposits that mimic rheumatologic disorders, and inadequately controlled rheumatologic disease or chronic infection can lead to secondary AA amyloidosis.**
- **The diagnosis of amyloidosis requires a tissue biopsy demonstrating green birefringent fibrils by polarization microscopy after staining with Congo red.**
- **Appropriate treatment depends on accurate biochemical or immunochemical identification of the amyloid type, distinguishing hereditary from acquired forms of amyloidosis.**

The term *amyloidosis* includes diseases that have in common the extra-cellular deposition of insoluble fibrillar proteins in tissues and organs. These diseases are a subset of a growing group of disorders recognized to be caused by misfolding of proteins; they include Alzheimer's and other neurodegenerative diseases, prion diseases, serpinopathies, some of the cystic fibroses, and others. A unifying feature of the amyloidoses is that the deposits share a common β-pleated sheet structural conformation that confers unique staining properties. The name "amyloid" is attributed to the pathologist Virchow, who in 1854 thought that such deposits in livers at autopsy were cellulose because of their peculiar staining reaction with iodine and sulfuric acid.[1] In the 20th century, amyloid was found to be a proteinaceous fibrillar deposit in tissues.[2] Biochemical characterization of fibril proteins from clinical cases have proven the amyloidoses to be diverse diseases, many with a potentially fatal outcome as a result of progressive deposition of amyloid fibrils in major organs. A growing number of treatments are available to target the source of the abnormal protein and, for some types, to inhibit the amyloidogenic protein misfolding process.

## CLASSIFICATION AND EPIDEMIOLOGY

Amyloid diseases are defined by the biochemical nature of the protein in the deposited fibril. The proteins are diverse and are unrelated in primary amino acid sequence, resulting in classification of amyloid diseases according to whether they are systemic or localized and acquired or inherited; their recognized clinical patterns also determine how they are classified (Table 116-1).[3] Each amyloid disease has a shorthand nomenclature, expressed as *A* for amyloidosis and an abbreviation for the biochemical nature of the fibrils; for example, *AL* is amyloid of Ig *light chain* origin. The discussion in this chapter is limited to the systemic amyloidoses because it is these diseases that can involve the joints and are potentially confused with autoimmune rheumatologic disorders.

The acquired systemic amyloidoses are AL (Ig light chain, or primary), AA (reactive, secondary), and $A\beta_2M$ (β2-microglobulin, dialysis-associated) types. The AL type is most common, although epidemiologic data are limited. One study based on National Center for Health Statistics data estimated the incidence as 4.5 per 100,000.[4] AL amyloidosis usually manifests after age 40 years and is associated with rapid progression, multisystem involvement, and short survival. AA amyloidosis is rare, occurring in fewer than 1% of patients with chronic inflammatory diseases in the United States and Europe, but it is more common in Turkey and the Middle East, where it occurs in association with familial Mediterranean fever.[5] As treatments for chronic inflammation and infection improve, the origin of AA amyloidosis appears to be shifting toward rare hereditary periodic fever syndromes or hematologic disorders associated with production of inflammatory cytokines such as Castleman's disease; workup should include genetic testing and imaging to screen for these conditions.[6] The onset of AA amyloidosis is variable and can occur as soon as 1 year after the onset of an inflammatory disease or many years later, often proportionate to the degree of inflammation. It is the only type of amyloidosis that occurs in children, such as children with juvenile rheumatoid arthritis.[7] $A\beta_2M$ amyloidosis, a chronic rheumatologic complication that occurs in some patients who undergo long-term dialysis, is related to formation of fibrils of β2-microglobulin in synovial tissues.[8]

The inherited amyloidoses are rare autosomal dominant diseases in which a variant plasma protein forms amyloid deposits beginning in mid life.[9] The most common form is caused by variant transthyretins (TTRs), of which more than 100 types are known to be associated with amyloidosis.[10] One variant, with a substitution of isoleucine for valine

†Deceased.

**TABLE 116-1** Classification of Amyloidosis

| Term | Fibril Composition | Systemic (S) or Localized (L) | Clinical Syndrome |
|---|---|---|---|
| AL | Immunoglobulin light chains (κ or λ) | S, L | Primary; myeloma-associated; systemic or localized in skin, lymph nodes, bladder, tracheobronchial tree |
| AA | Amyloid A protein | S | Secondary; reactive; familial Mediterranean fever |
| $A\beta_2M$ | β2-Microglobulin | S | Long-term hemodialysis or ambulatory peritoneal dialysis |
| ATTR | Transthyretin (one of >100 heritable mutations in ATTRm, or wild-type protein in ATTRwt) | S | ATTRm: familial amyloidotic polyneuropathy and/or cardiomyopathy; ATTRwt: senile systemic or age-related amyloidosis |
| AApoA | Apolipoprotein A-I (16 familial variants) or apolipoprotein A-II (4 familial variants) | S | Familial polyneuropathy with nephropathy |
| AGel | Gelsolin (variant Asn 187, Tyr 187) | S | Familial polyneuropathy with lattice corneal dystrophy, cranial neuropathy, nephropathy |
| AFib | Fibrinogen A alpha (9 familial variants) | S | Familial amyloidosis with nephropathy |
| ALys | Lysozyme (5 familial variants) | S | Familial amyloidosis with nephropathy |
| Aβ | Amyloid β protein | L | Alzheimer's disease; Down syndrome; cerebral amyloid angiopathy (Dutch) |
| ACys | Cystatin C (variant with N-terminal deletion and Glu 68) | S | Cerebral amyloid angiopathy (Icelandic) |
| AIAPP | Islet amyloid polypeptide | L | Type 2 diabetes mellitus; insulinoma |
| ACal | Calcitonin | L | Medullary carcinoma of the thyroid |
| AANF | Atrial natriuretic factor | L | Atrial amyloid, localized |

at position 122 (V122I), has a carrier frequency that may be 4% in the black population and is associated with late-onset cardiac amyloidosis. Among a large cohort of African-Americans older than age 65 years, the frequency of congestive heart failure and mortality was higher among those carrying the V122I variant of TRR than in age, gender, and ethnically matched control subjects.[11] In an amyloidosis referral population, African-Americans older than age 60 years who presented with amyloid cardiomyopathy were twice as likely to have TTR amyloidosis (ATTR) due to V122I variant of TRR as to have the more common AL type of amyloidosis.[12] Even wild-type TTR can form fibrils in older patients, predominantly involving the heart.[13] Formerly termed "senile systemic amyloidosis," the nomenclature has recently been revised to distinguish the familial form of ATTR in which a mutant allele is inherited (ATTRm) from the age-related form as a result of aggregation of the wild-type protein (ATTRwt).

## PATHOLOGY AND PATHOGENESIS OF AMYLOID FIBRIL FORMATION

### Pathologic Features

Amyloid deposits are widespread in AL amyloidosis and can be present in the extra-cellular spaces and blood vessels of all organs. Deposits in AA amyloidosis usually develop in the kidneys, liver, and spleen, although widespread deposits can be found late in the course of the disease. In $A\beta_2M$ amyloidosis, deposits tend to occur in synovial membrane, cartilage, and bone, but visceral organs are sometimes affected. In ATTR amyloidosis, the nervous system, heart, and thyroid are frequently affected organs, and only small deposits are found elsewhere.

All amyloid deposits stain with Congo red dye and exhibit a unique green birefringence by polarized light microscopy,[14] although the deposits also can be recognized on routine hematoxylin-eosin–stained sections. Electron microscopy shows that amyloid fibrils are 8 to 10 nm wide and of varying lengths, with a 2.5 to 3.5 nm filamentous subunit arranged along the long axis of the fibril in a slow twist.[15] Typing of amyloid deposits can be performed with conventional immunohistochemical staining. False-positive results can occur, however, because of the presence of non-amyloid serum proteins. Immunoelectron microscopy can provide more definitive immunologic identification of the protein in the fibril, or the composition of extracted fibrils can be identified by mass spectrometric proteomic analysis.[16,17]

### Pathogenesis of Amyloid Fibril Formation

The exact mechanism of fibril formation is unknown and may differ among the various types of amyloid.[18] Studies suggest a common underlying mechanism, however, in which a partially unfolded protein intermediate forms multimers and then higher order polymers. Factors that contribute to fibrillogenesis include variant or unstable protein structure, extensive β-conformation of the precursor protein, proteolytic processing of the precursor protein, association with components of the serum or extra-cellular matrix (e.g., serum amyloid P [SAP] component, amyloid enhancing factor, apolipoprotein E, and glycosaminoglycans), and physical properties such as the pH of the tissue site.

AL amyloidosis is a plasma cell dyscrasia with an excess of clonal plasma cells in the bone marrow; it can occur in isolation or in combination with multiple myeloma. Similar cytogenetic changes have been identified in these plasma

cell diseases, suggesting that they may have a common molecular pathogenesis.[19,20] By two-dimensional gel electrophoresis and mass spectrometry, it can be seen that the amyloid fibril deposits are composed of intact 23 kDa monoclonal Ig light chains and C-terminal truncated fragments.[16] Although all κ and λ light chain subtypes have been identified in amyloid fibrils, λ subtypes predominate, and the λ6 subtype seems to have unique structural properties that predispose it to fibril formation,[21] often in the kidney.[22] AL amyloidosis is usually a rapidly progressive disease with amyloid deposits in multiple tissue sites.

The AA type of amyloidosis is a complication of severe, long-standing inflammation, such as that which occurs in chronic inflammatory disorders or infections. AA amyloid fibrils usually are composed of an 8 kDa, 76 amino acid amino-terminal portion of the 12 kDa precursor, serum amyloid A (SAA).[23] SAA is a polymorphic protein encoded by a family of SAA genes, which are acute-phase apoproteins synthesized in the liver and transported by a high-density lipoprotein, HDL3, in the plasma.[24] An underlying inflammatory disease of several years' duration causing an elevated SAA usually precedes fibril formation, although infection can produce AA deposition more quickly. AA fibril formation can be accelerated by an amyloid-enhancing factor present in high concentration in the spleen (which may be early SAA aggregates or deposits), by basement membrane heparan sulfate proteoglycan, or by seeding with AA or heterologous fibrils.[25,26]

Factors related to β2-microglobulin fibril formation are under investigation. The high prevalence of $A\beta_2M$ disease in patients undergoing long-term dialysis provides an argument against an amyloidogenic variant β2-microglobulin molecule. The permeability of dialysis membranes may be a factor because the molecular weight of β2-microglobulin is 11.8 kDa, above the porosity of standard membranes. It has been hypothesized that dialysis membranes may be bioincompatible and may induce pro-inflammatory mediators that stimulate β2-microglobulin and contribute to fibril formation.[27]

In ATTRm and all other forms of familial amyloidosis, inherited mutations or polymorphisms in the genes encoding large serum proteins produce amyloid-prone variants. The process of fibrillogenesis has been best studied for TTR, in which variant TTR molecules are prone to dissociation from stable tetramers, leading to misfolding, polymerization, and fibril formation.[28] The role of aging is intriguing because patients with variant proteins do not have clinically apparent disease until midlife or later, despite the lifelong presence of the abnormal protein.[29] Further evidence of an age-related trigger is that ATTRwt, caused by the deposition of fibrils derived from normal TTR, is exclusively a disease of elderly people.[13]

## DIAGNOSIS

A tissue biopsy specimen showing amyloid fibrils is necessary for the diagnosis of amyloidosis (Figure 116-1). The least invasive biopsy is the abdominal fat aspirate, which is positive in 80% to 90% of patients with AL or ATTR amyloidosis and in 60% to 70% of patients with AA amyloidosis.[30,31] This biopsy is easy to perform after local injection of anesthetic and has a low rate of infectious or hemorrhagic

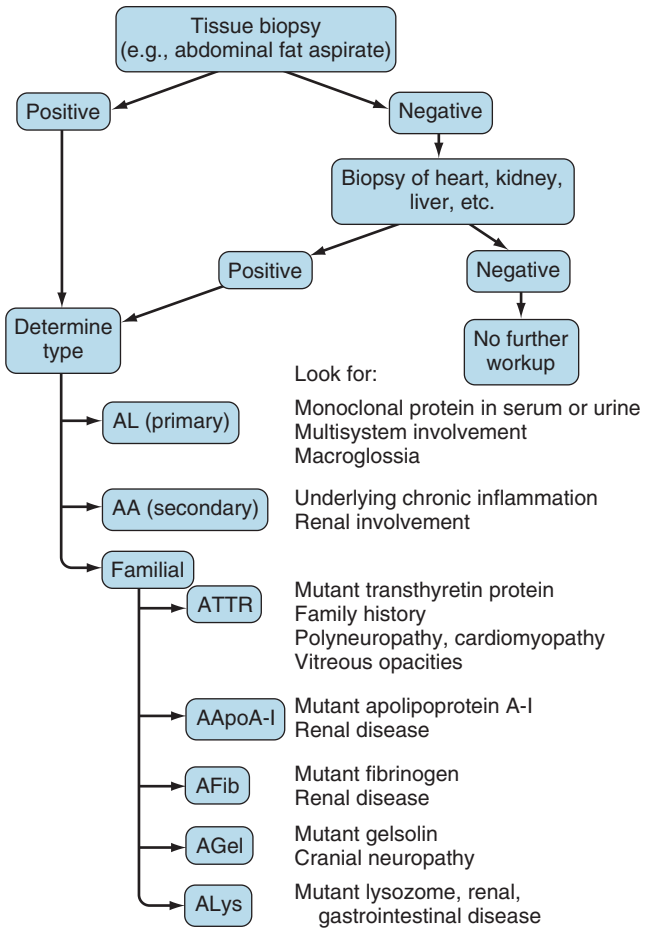



**Figure 116-1** Algorithm for the diagnosis of amyloidosis and determination of type.

complications (Figure 116-2). If the aspirate is negative but clinical suspicion for disease persists, a more invasive tissue biopsy should be performed. Although a biopsy specimen of a clinically involved organ is recommended, almost any tissue biopsy specimen is likely to be positive if the patient has systemic amyloidosis: In a series of 100 patients with AL amyloidosis, 85% of 249 tissue biopsy specimens were positive, including all samples from the kidney, heart, and liver.[32] When the diagnosis of amyloidosis is made, careful evaluation of the entire clinical picture, including the manner of presentation, organ system involvement, underlying disease, and family history, should provide a clue to the type of amyloidosis.

Identification of a plasma cell dyscrasia distinguishes AL from other types of amyloidosis (Figure 116-3). More than 90% of patients have a serum or urine monoclonal immunoglobulin protein or a free light chain on testing by immunofixation electrophoresis or by a recently available nephelometric assay for free light chains.[33,34] In addition, the percentage of plasma cells in the bone marrow is often increased; these are monoclonal on immunohistochemical staining (Figure 116-4).[35] A monoclonal serum protein by itself is not diagnostic of amyloidosis because monoclonal gammopathy of uncertain significance is common in older patients. When monoclonal gammopathy of uncertain significance is present in a patient with biopsy-proven

amyloidosis, however, the AL type is strongly suspected. Immunohistochemical staining by light or electron microscopy should be performed by a laboratory familiar with the techniques and able to perform appropriate controls. Mass spectrometry–based microsequencing of small amounts of protein extracted from fibril deposits ultimately may be the most reliable way to identify the components of the fibrils.[16,17] AA amyloidosis is suspected in patients with renal amyloidosis and a chronic inflammatory condition or infection. AL and ATTR amyloidosis must be ruled out. AA amyloidosis must be confirmed by immunohistochemical staining for AA protein.

Familial amyloidosis must be excluded in every patient who does not have a plasma cell dyscrasia or the AA type of amyloidosis. Although the disease has a dominant inheritance, family history may not be apparent when the disease occurs later in life; in addition, some cases occur through new mutations. Variant TTR proteins usually can be detected by isoelectric focusing (Figure 116-5).[36] With the use of polymerase chain reaction–based sequencing of genomic DNA, mutant TTR alleles, as well as amyloidogenic mutations in genes encoding variant proteins responsible for other types of hereditary amyloidosis, can be detected.[37]

A novel renal amyloid protein, leukocyte chemotactic factor 2, has been found in glomeruli, renal vessels, and interstitium in patients with isolated renal amyloidosis and should be considered in patients who are negative for the more common types of amyloidosis.[38]

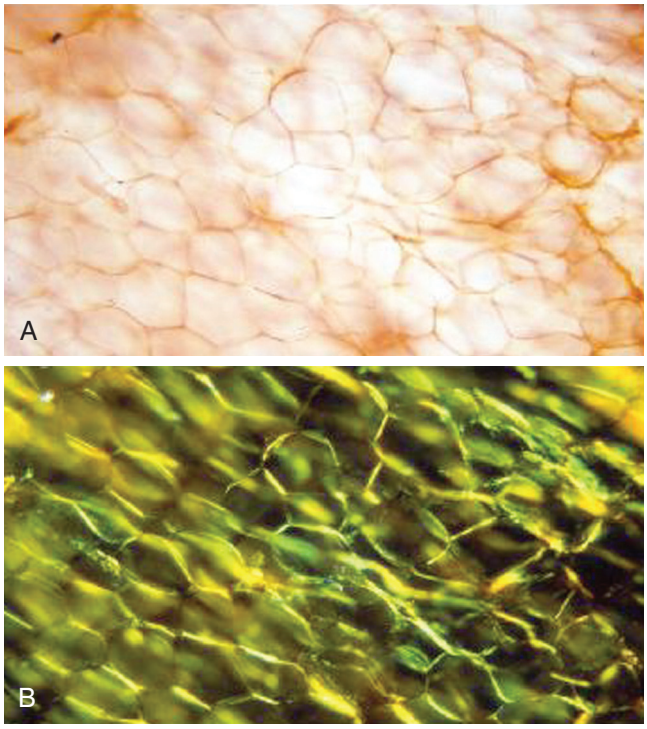



**Figure 116-2** Subcutaneous fat aspirate stained with Congo red viewed by light microscopy **(A)** and polarized light **(B)** (×200). Staining and birefringence are evident in the walls and connective tissues surrounding the adipose cells.

## CLINICAL FEATURES AND TREATMENT

### AL Amyloidosis

AL amyloidosis usually occurs in middle-aged or older people but also can occur in the third or fourth decade of life. It has a wide spectrum of organ system involvement,

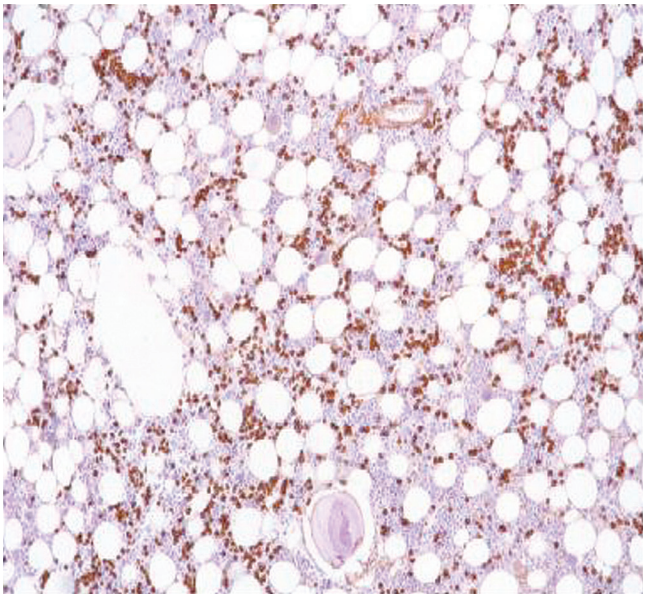


**Figure 116-4** Bone marrow biopsy specimen stained with antibody to λ light chain shows predominance of λ plasma cells and staining of amyloid deposit around a blood vessel (×400).

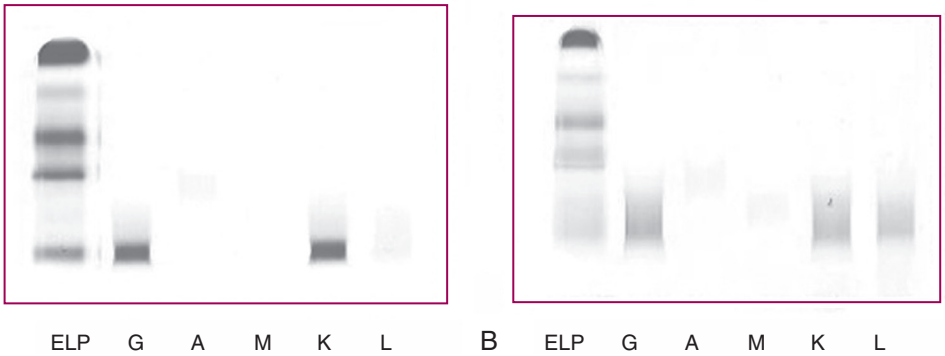



**Figure 116-3** **A,** Pre-treatment serum immunofixation electrophoresis (ELP) shows an IgG (G) κ monoclonal protein. **B,** Post-treatment serum immunofixation ELP shows absence of the monoclonal protein. A, IgA; K, κ chain; L, λ chain; M, IgM.

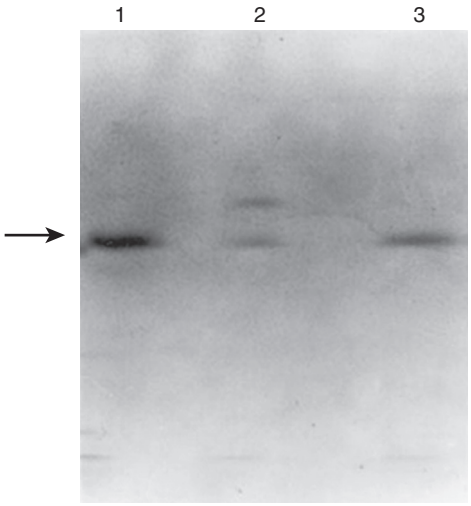



**Figure 116-5** Isoelectric focusing of serum samples shows two bands of variant and wild-type transthyretin (TTR) protein from a patient with TTR amyloidosis *(lane 2)* and a single band of wild-type TTR in normal subjects *(lanes 1 and 3, indicated with an arrow).*



**Figure 116-6** Enlarged tongue of a patient with AL amyloidosis.

and presenting features reflect the organs most prominently affected.[39,40] Initial symptoms of fatigue and weight loss are frequent, but the diagnosis is rarely made until symptoms referable to a specific organ appear.

The kidneys are commonly affected; renal amyloidosis is manifested by proteinuria, sometimes massive, with edema and hypoalbuminemia. Mild renal dysfunction is frequent, but rapidly progressing renal failure is rare. Cardiac involvement, often with congestive heart failure, is a common presentation.[41] An electrocardiogram may show low voltage with a pattern of myocardial infarction. An echocardiogram frequently shows concentrically thickened ventricles and a normal or mildly reduced ejection fraction. Nervous system features include peripheral sensory neuropathy, carpal tunnel syndrome, and autonomic dysfunction with gastrointestinal motility disturbances (early satiety, diarrhea, and constipation) and orthostatic hypotension. Macroglossia, a classic feature pathognomonic of AL amyloidosis, is found in 10% of patients (Figure 116-6). Hepatomegaly may be massive with mild cholestatic abnormalities of liver function, although liver failure is uncommon, even when hepatomegaly is massive. The spleen is frequently involved, and functional hyposplenism may occur, even in the absence of significant splenomegaly. Cutaneous ecchymoses are common, particularly around the eyes, giving the "raccoon-eyes" sign, and appear spontaneously or when provoked by minor trauma (Figure 116-7). Other findings include nail dystrophy (Figure 116-8), alopecia, and amyloid arthropathy with thickening of synovial membranes. The soft tissue and joint manifestations of AL amyloidosis were reviewed in a series of almost 200 patients.[42] Symptoms and signs mimicking rheumatologic diseases, with arthropathy, subcutaneous tissue deposits, muscle pseudohypertrophy, adenopathy, carpal tunnel syndrome, submandibular gland enlargement, and macroglossia, occur in more than 40% of patients with AL amyloidosis.



**Figure 116-7** Periorbital ecchymoses in a patient with AL amyloidosis.



**Figure 116-8** Fingernail dystrophy in a patient with AL amyloidosis.

Timely diagnosis of AL amyloidosis is crucial. Patients with any of these clinical syndromes should be screened for amyloid deposition and for a plasma cell disorder, as previously described. Note that the sensitivity of serum or urine protein electrophoresis without immunofixation is poor; these tests alone are not useful for excluding or diagnosing AL amyloidosis.

Extensive multisystem involvement typifies AL amyloidosis, and median survival with no treatment is usually only about 1 year from diagnosis. Current therapies target clonal bone marrow plasma cells using chemotherapy approaches employed for multiple myeloma (Table 116-2). Oral melphalan and prednisone constituted the first regimen used to treat AL amyloidosis; it was minimally effective[32,43] and is rarely used today because recent studies have shown that substitution of high-dose dexamethasone for prednisone seems to increase response rates significantly.[44]

**TABLE 116-2** Major Treatment Options for Amyloidosis

| |
|---|
| **AL Amyloidosis** |
| Intravenous melphalan with autologous stem cell rescue<br>• Granulocyte colony-stimulating factor–mobilized peripheral blood stem cell collection<br>• Intravenous melphalan, 140-200 mg/m$^2$<br>• Autologous stem cell reinfusion |
| Cyclic oral melphalan and dexamethasone<br>• Melphalan, 0.22 mg/kg/day × 4 days<br>• Dexamethasone, 20-40 mg/day × 4 days, or weekly<br>• Repeat administration every 4 weeks |
| Immunomodulators (including thalidomide, lenalidomide, and pomalidomide, for example)<br>• Oral lenalidomide, 5-15 mg/day × 21 days<br>• Dexamethasone, 20-40 mg weekly<br>• Repeat administration every 4 weeks |
| Proteasome inhibitors (including bortezomib, ixazomib, and carfilzomib, for example)<br>• Intravenous bortezomib, 1.6 mg/m$^2$ weekly × 4<br>• Dexamethasone, 20-40 mg weekly × 4<br>• Repeat every 5 weeks |
| **AA Amyloidosis** |
| Aggressive treatment of underlying inflammatory disease<br>Medical or surgical treatment of underlying infection<br>Colchicine, 1.2-1.8 mg/day for AA amyloidosis secondary to familial Mediterranean fever<br>Antifibril drug, eprodisate (investigational) |
| **ATTR Amyloidosis** |
| Orthotopic liver transplantation<br>Transthyretin stabilizers: diflunisal and tafamidis<br>RNA-based therapeutics: siRNA and antisense RNA (investigational) |

High-dose intravenous (IV) melphalan followed by autologous stem cell transplantation is highly effective. Of more than 500 patients treated with such protocols at Boston Medical Center, approximately 40% of evaluable patients had achieved a hematologic complete response when assessed 1 year after treatment; most patients experienced significant improvement or stabilization of organ function.[45] Median survival in treated patients exceeds 4.5 years and is durable.[46] Other referral centers have found similar results.[47-49] However, patients with amyloidosis and organ impairment have high rates of treatment-related morbidity and mortality with aggressive treatment. Factors that contribute to mortality include amyloid cardiomyopathy, poor performance or nutritional status, reduced pulmonary function, and amyloid-associated bleeding disorders. The only randomized multicenter study comparing oral melphalan chemotherapy with IV melphalan chemotherapy with stem cell support was plagued by high treatment-related mortality and failed to show benefit for the high-dose regimen.[50] At centers with experience in treating this disease, this treatment is still considered first line in low-risk patients. Age alone[51] or renal failure[52] does not exclude patients from such treatment. Cardiac biomarkers have been used to stratify risk for high-dose therapy.[53]

For patients with significantly impaired cardiac function or arrhythmias resulting from amyloid involvement of the myocardium, median survival is only about 6 months without treatment, and stem cell mobilization and high-dose chemotherapy are associated with great morbidity. In such patients, orthotopic cardiac transplantation followed by intravenous melphalan and stem cell rescue to prevent fibrillogenesis in the transplanted heart or other organs can be effective.[54]

New agents that are efficacious in reducing the plasma cell burden in multiple myeloma are being tested for AL amyloidosis. These agents include immunomodulators such as lenalidomide, a derivative of thalidomide[55-57] that affects the bone marrow microenvironment, as well as proteasome inhibitors to which plasma cells are particularly sensitive, such as bortezomib.[58-60] These agents generally have been tested for use in patients for whom alkylator chemotherapy has failed; current clinical trials are examining these novel agents in combination with alkylators, as induction before transplant, and for maintenance. Weekly rather than biweekly dosing of bortezomib appears to reduce the incidence of peripheral neuropathy and may be safer in patients with AL amyloidosis. Innovative approaches target the amyloid fibrils themselves or accessory binding proteins. The anthracycline derivative 4′-iodo-4′-deoxydoxorubicin was noted to cause resorption of amyloid deposits in model systems, but clinical trials have failed to show any clinical benefit.[61] The agent R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]-pyrrolidine-2-carboxylic acid binds serum amyloid P protein and accelerates clearance from the circulation and from amyloid fibrils in animal models.[62] The addition of an antibody against SAP appears to further accelerate clearance.[63] Thus far, trials using this approach have not shown a clear clinical benefit. Antibodies directed against the amyloidogenic proteins themselves are also in pre-clinical and clinical development as potentially useful agents for imaging and therapy. A humanized monoclonal antibody, NEOD001, which specifically targets the amyloid that accumulates in AL amyloidosis, is in early stage clinical trials (Prothena Corporation, Dublin, Ireland).

Supportive treatment is recommended for patients with all types of amyloidosis (Table 116-3). At times, supportive treatment may be lifesaving (e.g., heart or kidney transplantation, renal dialysis, cardiac pacemaker, and nutritional support). Digitalis, calcium channel blockers, and β-blockers are relatively contraindicated because toxicity has been observed at therapeutic levels.

## AA Amyloidosis

AA amyloidosis can occur at any age. The primary clinical manifestation is proteinuria, renal insufficiency, or both.[5] A study from Finland found AA amyloidosis to be the most common cause of nephrotic syndrome in patients with rheumatoid arthritis.[64] Hepatomegaly, splenomegaly, and autonomic neuropathy frequently occur as the disease progresses; cardiomyopathy occurs rarely. With chronic inflammatory diseases, amyloid progression is slow, and survival is often longer than 10 years, particularly with treatment for end-stage renal disease. In contrast, untreated infections, such as osteomyelitis, tuberculosis, or leprosy, can produce a more rapidly progressive amyloid syndrome, which remits with effective medical or surgical treatment of the infection.

The major therapy in AA amyloidosis is treatment of the underlying inflammatory or infectious disease. Treatment that suppresses or eliminates the inflammation or infection also decreases the SAA protein. For familial Mediterranean fever, colchicine, 1.2 to 1.8 mg/day, is the appropriate treatment. Colchicine has not been helpful for AA amyloidosis

**TABLE 116-3** Supportive Treatment for All Types of Amyloidosis

| Organ System | Symptom | Treatment Options |
|---|---|---|
| Cardiac | Congestive failure | Salt restriction of 1-2 g/day<br>Diuretics: furosemide, spironolactone, and metolazone |
| | Arrhythmia | Pacemaker<br>Automatic implantable cardiac defibrillator<br>Antiarrhythmics |
| Renal | Nephrotic syndrome | Salt restriction of 1-2 g/day<br>Elastic stockings and leg elevation<br>Maintaining dietary protein<br>Angiotensin-converting enzyme inhibitor, if blood pressure tolerates it |
| | Renal failure | Dialysis (long-term ambulatory peritoneal dialysis or hemodialysis) |
| Autonomic nervous | Orthostatic hypotension | Midodrine<br>Increased dietary salt or added fludrocortisone, depending on edema<br>Elastic stockings |
| | Gastric atony or ileus | Small frequent feedings (6/day), low in fat<br>Oral nutritional supplements<br>Jejunostomy tube feeding<br>Parenteral nutrition |
| Gastrointestinal | Diarrhea | Low-fat diet (≤40 g)<br>Psyllium hydrophilic mucilloid (Metamucil)<br>Loperamide hydrochloride (Imodium)<br>Tincture of opium<br>Parenteral nutrition |
| | Macroglossia | Soft solid diet<br>Partial glossectomy (rarely effective) |
| Peripheral nervous | Sensory neuropathy | Avoid trauma<br>Gabapentin (Neurontin), 100-300 mg 3 times daily<br>Amitriptyline, 25-50 mg at bedtime<br>Pregabalin (Lyrica), 50-100 mg 3 times daily |
| | Motor neuropathy | Ankle-foot orthotics for foot drop<br>Physical therapy |
| Hematologic | Intracutaneous bleeding<br>Factor X deficiency | Avoid trauma, anti-platelet agents<br>Factor replacement (recombinant factor VIIa and prothrombin complex concentrates)<br>Splenectomy for splenomegaly |

of other causes or for other amyloidoses. A multicenter randomized trial using a new antiamyloid drug, eprodisate, has been completed; the drug was found to significantly delay worsening of renal function in patients with AA amyloidosis.[65] A second multicenter trial requested by the Food and Drug Administration is now under way. Eprodisate interferes with the interaction of AA amyloid protein and glycosaminoglycans in tissues and prevents fibril formation and deposition.

## $A\beta_2M$ Amyloidosis

Several distinct rheumatologic conditions are observed in people with $A\beta_2M$ amyloidosis, including carpal tunnel syndrome, persistent joint effusions, spondyloarthropathy, and cystic bone lesions. Carpal tunnel syndrome is usually the first symptom of disease. Persistent joint effusions accompanied by mild discomfort occur in 50% of patients who undergo dialysis for longer than 12 years. Involvement is bilateral, and large joints (shoulders, knees, wrists, and hips) are more frequently affected. The synovial fluid is non-inflammatory, and β2-microglobulin amyloid deposits can be found if the sediment is examined with Congo red staining. Spondyloarthropathy with destructive changes in the intervertebral disks and paravertebral erosions have occurred in association with β2-microglobulin amyloid deposits. Cystic bone lesions sometimes leading to pathologic fractures have been described in the femoral head, acetabulum, humerus, tibial plateau, vertebral bodies, and carpal bones. Although less common, visceral β2-microglobulin amyloid deposits occasionally occur in the gastrointestinal tract, heart, tendons, and subcutaneous tissues of the buttocks.

Treatment for $A\beta_2M$ amyloidosis is difficult to provide because the 11 kDa β2-microglobulin molecule is too large to pass through a dialysis membrane. Consistent with a postulated role of copper in initiating $A\beta_2M$ fibrillogenesis,[66] copper-free dialysis membranes seem to reduce the incidence of disease. Patients undergoing continuous ambulatory peritoneal dialysis usually have lower plasma levels of β2-microglobulin than do patients undergoing hemodialysis and may not develop amyloid deposits as quickly. Symptoms of arthropathy are common, and prevalence may approach 100% of people undergoing dialysis for longer than 15 years. Patients who have received kidney transplants after developing $A\beta_2M$ report improvement in symptoms.

## ATTR Amyloidosis

The clinical features of ATTR and AL in patients presenting with involvement of heart and peripheral nerves overlap, such that the diseases cannot be reliably distinguished by clinical presentation alone. A family history makes ATTRm more likely, but many patients with ATTRm

seem to present sporadically with new mutations in the germline. Within each family, disease begins at nearly the same age, and symptoms usually include neuropathy, cardiomyopathy, or both. Peripheral neuropathy begins as a lower extremity sensory and motor neuropathy and progresses to the upper extremities. Autonomic neuropathy is manifested by gastrointestinal symptoms of diarrhea with weight loss and orthostatic hypotension. Cardiomyopathy and conduction system defects are similar to those caused by AL, although in people with ATTR amyloidosis, heart failure is less common, and the prognosis is better.[67] Vitreous opacities caused by amyloid deposits are pathognomonic of ATTRm.

The TTR variant, V122I, is a common allele in African-Americans and is associated with cardiomyopathy. In a large referral population, 25% of African-American patients with amyloidosis had this TTR variant.[11,12] ATTRm due to V122I is likely underdiagnosed because of lack of physician awareness and the difficulty of distinguishing amyloid and hypertensive cardiomyopathy without an endomyocardial biopsy.

Without intervention, survival after ATTR disease onset is 5 to 15 years. Orthotopic liver transplantation, which removes the major source of variant TTR production and replaces it with normal TTR, is the major treatment for ATTR amyloidosis.[68,69] Liver transplantation arrests disease progression, and some improvement in autonomic and peripheral neuropathy may occur.[70] Cardiomyopathy does not improve and in some patients seems to worsen after liver transplantation.[71] A beneficial long-term outcome depends on transplantation early in the disease course.[72]

Two international multicenter randomized placebo-controlled clinical trials have been conducted to test the efficacy of the NSAID diflunisal, a salicylic acid derivative, and an analog, tafamidis, for the treatment of ATTRm polyneuropathy, based on laboratory studies showing that these agents stabilize the normal circulating TTR tetramer, preventing dissociation into monomers, misfolding, and aggregation.[73] Diflunisal, which was tested in patients with a variety of TTR mutations for 2 years, met its primary endpoints, resulting in a reduction in the rate of progression of neurologic impairment and preserving quality of life.[74] Tafamidis, tested only in patients with V30M ATTRm for a shorter time, also showed some benefit.[75] These drugs are now being tested for efficacy in people with ATTR cardiomyopathy, including that caused by the wild-type protein. ATTRm is also the first disease being investigated with small interfering RNA therapeutics in studies sponsored by Alnylam Pharmaceuticals, Inc., Cambridge, Mass., as well as with antisense oligonucleotides developed by Ionis Pharmaceuticals, Inc., Carlsbad, Calif. In the future, patients may be treated with a combination of agents designed to downregulate TTR protein expression in the liver and to stabilize the residual TTR protein in the circulation.

## CONCLUSION

Timely and accurate diagnosis of amyloidosis is essential because effective treatments for some forms of amyloidosis are available or are undergoing clinical trials. The first step is recognition of a clinical syndrome consistent with amyloidosis, followed by an appropriate biopsy or fat aspirate to identify tissue fibrils. The next priority should be to determine whether an associated plasma cell disorder can be identified, because AL is the most rapidly progressive type of systemic amyloidosis, and therapy should be initiated before heart, kidney, or liver failure occurs. Definitive identification of the amyloid precursor protein is essential for appropriate therapy, and amyloid referral centers can provide specialized diagnostic techniques and access to clinical trials. An understanding of the biophysical properties of amyloid proteins and of the mechanisms of protein misfolding and tissue damage will enable the further development of more specific and less toxic antiamyloid therapeutics.


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CHAPTER 117

# Sarcoidosis



Nadera J. Sweiss • Peter Korsten • Robert P. Baughman

**KEY POINTS**

- Sarcoidosis is a heterogeneous multisystem inflammatory disease of unknown cause characterized by the development and accumulation of noncaseating granulomata in any organ system.
- Sarcoidosis occurs worldwide and affects people of all racial and ethnic backgrounds, but prevalence and severity are increased in African-American patients.
- The pathogenesis of sarcoidosis involves the interplay of different cells, cytokines, and other inflammatory mediators in a susceptible host.
- Rheumatologic manifestations are common in sarcoidosis but are often overlooked or misdiagnosed.
- No treatments have been approved by the U.S. Food and Drug Administration for extrapulmonary manifestations, including sarcoid arthritis.
- Current treatment options include first-line treatment with glucocorticoids, second-line treatment with disease-modifying antisarcoid drugs like methotrexate, and third-line treatment with TNF inhibitors, which may be used depending on the severity and the type of organ system involved.

Sarcoidosis is an orphan, systemic, and clinically heterogeneous disorder. Its cause has yet to be identified, but environmental, genetic, and infectious causes have been suggested. The hallmark of sarcoidosis is the development and accumulation of noncaseating granulomas in any organ system. Organ system involvement, which is unpredictable and varies between patients, is the major determinant of morbidity and mortality in sarcoidosis. Any organ system can be involved, but the lungs are affected in more than 90% of cases. Lymphatic, skin, and ocular findings are also common. Given the variability of sarcoidosis manifestations, diagnosing this disorder is often difficult. Patients may be asymptomatic or present with a range of nonspecific symptoms, or specific symptoms such as cough, dyspnea, burning of eyes, or a rash may suggest the diagnosis.[1] When extrapulmonary symptoms develop, they sometimes result in rheumatologic manifestations including but not limited to arthritis, skin lesions, arthralgias, and neuropathy.[2] Rheumatologists often are consulted when the presentation is atypical or when multiple organs are affected.

## CLASSIFICATION CRITERIA

No classification criteria for sarcoidosis have been adopted by the American College of Rheumatology or the European League Against Rheumatism for sarcoidosis. However, the American Thoracic Society, the European Respiratory Society, and the World Association of Sarcoidosis and Other Granulomatous Disorders suggest a diagnosis of sarcoidosis if the following criteria are fulfilled: (1) a compatible clinical picture, (2) histologic demonstration of noncaseating granulomas, and (3) exclusion of other diseases capable of producing a similar clinical picture.[1]

## EPIDEMIOLOGY

Sarcoidosis is a global disease. Because of its clinical heterogeneity and variable diagnostic criteria in different countries, the worldwide prevalence and incidence of sarcoidosis have been difficult to calculate. In Northern Europe, up to 40 cases per 100,000 people have been reported,[3,4] whereas in a study from Eastern Europe, only 3.68 cases of sarcoidosis per 100,000 people were found.[5] The incidence of sarcoidosis in Japan is also low, with one study estimating it to be 3.7 cases per 100,000 individuals.[6] Within countries, incidence rates may vary between races. In the United States, the annual incidence of sarcoidosis is more than three times higher in black people (35.5 per 100,000) than in white people (10.9 per 100,000).[7] Furthermore, the disease course of sarcoidosis is more progressive and mortality may be higher in African-Americans.[8,9] Despite numerous epidemiologic studies of sarcoidosis, many clinicians and researchers believe that estimates of prevalence and incidence are lower than actual rates of the disease because of inaccurate diagnoses or asymptomatic cases that are never diagnosed.[10]

Although sarcoidosis affects men and women of all ages and from diverse ethnic backgrounds,[4,11] some disparities in how it affects these groups have been noted. The fact that it affects slightly more women than men has been confirmed in studies from around the world; estimates indicate that 57% of patients with sarcoidosis are women.[4] Relative to men, women with sarcoidosis have a greater number of ocular and neurologic manifestations. People of any age may acquire the disease, but the median age of onset is around 40 years.[4] A second peak of incidence has been reported at around age 65 years, especially in women.[8,12]

## ETIOLOGY

The exact cause of sarcoidosis remains elusive despite years of research; however, multiple etiologic factors have been identified. The heterogeneity of the disease suggests that multiple causative agents may be responsible for the

Supplemental images available on the accompanying ExpertConsult website.

variable disease manifestations of sarcoidosis. Given the immunopathogenic mechanisms that underlie sarcoidosis, such a disease trigger might be a T cell antigen that stimulates the cascade of events leading to granuloma formation. Because the lungs are affected in more than 90% of sarcoidosis cases, it is likely that an environmental agent (including an infectious agent, potentially exposed via the pulmonary route) might contribute to the onset of sarcoidosis.

Both inorganic and organic environmental factors with antigenic capabilities have been implicated in the pathogenesis of sarcoidosis. Early studies on the causes of sarcoidosis suggested a link between sarcoidosis and agents associated with a rural lifestyle, such as the lumber industry and burning wood.[13,14] In a case-control etiologic study of sarcoidosis (ACCESS), it was found that burning of agricultural debris and wood is particularly associated with pulmonary sarcoidosis but not with systemic sarcoidosis.[15] In a different analysis of the ACCESS trial, radiation, insecticides, mildew, and mold were environmental factors associated with the systemic sarcoidosis phenotype.[16] More recently, a sarcoidosis-like phenotype has been reported in fire department rescue workers involved in the World Trade Center catastrophe.[17] These findings may indicate that each of these unique sarcoidosis subtypes has its own causes.

Because of clinical and histologic similarities, numerous methods have been used to look for an infectious agent as a cause of sarcoidosis, with most studies focusing on *Mycobacterium tuberculosis* or *Propionibacterium acnes*. When in situ hybridization was used, *Mycobacterium tuberculosis* catalase-peroxidase protein (mKatG) was found in nearly 40% of tissue samples from patients with sarcoidosis. Recombinant mKatG protein was then used to measure mKatG antibodies in patients with sarcoidosis, which were present in 50% of patients studied.[18] Other investigators have found evidence of an immunologic reaction to additional mycobacterial antigens such as ESAT-6.[19,20] *P. acnes* has been found more frequently in granulomas from patients with sarcoidosis but also can be found in people without sarcoidosis, and thus its primary role in pathogenesis remains unclear.[21-23] These studies suggest that sarcoidosis may represent overexposure of the patient to a commonly encountered microorganism associated with hyperreactive resultant immune responses. Because several types of bacteria have been associated with sarcoidosis, some clinicians have attempted to use antibiotics or antimycobacterial agents to manage the disease. Although skin sarcoidosis has on occasion responded to these treatments,[24,25] their usefulness in treating other forms of sarcoidosis appears to be minimal.[21] In addition, serum amyloid A (SAA) accumulates in granulomas as a result of the hyperimmune T helper (Th)1 cell response response necessary for killing the infecting agent.[26] This mechanism could be an explanation for the ongoing chronic granulomatous inflammation seen in many patients with sarcoidosis.[27] Furthermore, blood levels of SAA correlate with the disease stage and thus could serve as a biomarker and/or therapeutic target.[28]

## GENETICS

Compelling studies of familial clustering and the incidence of sarcoidosis among different racial groups indicate that sarcoidosis susceptibility is influenced by the interplay of genetic factors. In the ACCESS study, first-degree relatives were reported to have a fivefold increased risk of developing the disease.[17] Associations have been found between risk of sarcoidosis and class I and II human leukocyte antigen (HLA) gene products, which have essential roles in antigen presentation. It is considered likely that a susceptibility locus for sarcoidosis exists within the HLA gene region, as is the case with other autoimmune diseases and cancers such as Hodgkin's lymphoma. An intriguing analysis of data from the ACCESS study identified associations between genetic factors (HLA alleles), environmental factors, and sarcoidosis phenotypes. In considering together several of the factors postulated to cause sarcoidosis, a compelling argument can be made for a genetic factor that predisposes individuals to the disease and a subsequent environmental exposure that triggers the onset of sarcoidosis. Specifically, Rossman and colleagues found that HLA-DRB1*1101 and insecticide exposure at work are significantly associated with cardiac sarcoidosis and hypercalcemia.[29] A similar relationship between HLA-DRB1*1101, mold and musty odors, and pulmonary sarcoidosis was described.[29]

Certain HLA alleles have been found to be protective or associated with increased susceptibility or different disease phenotypes. In Caucasian populations, HLA-DRB1*01 and HLA-DRB1*04 were protective against the disease, whereas HLA-DRB1*03, *11, *12, *14, and *15 were risk factors for sarcoidosis.[30] Interestingly, HLA-DRB1*11 was found to be related to presentation of the mycobacterial antigens ESAT-6 and mKatG (previously described), thus providing a possible mechanistic link between a genetic background and reaction to a pathogen.[31]

Moreover, HLA-DRB1*03 was associated with spontaneous resolution, whereas HLA-DRB1*14 and HLA-DRB1*15 were associated with a chronic course of the disease.[32] In European patients with Löfgren's syndrome, which is an acute form of sarcoidosis characterized by bilateral hilar lymphadenopathy (BHL), fever, and erythema nodosum and/or periarticular ankle inflammation, HLA-DRB1*03 is four times more common than in healthy individuals[33] and has been associated with favorable prognostic factors. In one study, most DRB1*03$^+$ patients experienced resolution of Löfgren's syndrome within 2 years after diagnosis. By contrast, nearly half of patients without this allele had chronic disease.[34] The mechanism underlying this difference in disease course remains unknown. A relationship between HLA-DRB1*03 and interferon (IFN)-γ homozygosity has been suggested in sarcoidosis. Wysoczanska and colleagues reported that when combined, these two genetic factors increase the risk of Löfgren's syndrome, which may be indicative of a complex gene-gene interaction underlying this sarcoidosis phenotype.[35]

Genetic studies of non-HLA genes have been inconclusive.[30] The two candidate genes that have been most consistently associated with sarcoidosis are TNF and the IL-23 receptor (IL-23G) gene. Other possible candidate genes have produced conflicting results. The TNF G-308A polymorphism was found to be predictive of response to treatment with TNF inhibitors,[36] which might help tailor treatment based on pharmacogenetics in the near future.

Analysis of gene-expression profiles to distinguish between tuberculosis and sarcoidosis has revealed similarities

between the two diseases; however, matrix metallopeptidase 14 could be identified as most distinctive marker for sarcoidosis.[46] Ongoing genome-wide association studies are seeking to identify additional genes that may be linked to sarcoidosis onset and susceptibility. To date, these studies have identified several novel gene candidates. In particular, *BTNL2*, *ANXA11*, *RAB23*, *OS9*, *CCDC88B*, *PRDX5*, and *NOTCH4* have been identified as potential loci for sarcoidosis susceptibility.[30] Most of these genes play a role in the immune system, but their functional role has yet to be characterized.

More recently, epigenetic changes (such as DNA methylation) that influence gene expression without changing the primary nucleotide sequence have been identified as an important mechanism in many diseases, such as cancer and kidney disease, but also in autoimmune diseases.[38] In sarcoidosis, methylation changes of telomeres and specific microRNAs (such as miR-144, miR-20a, miR-302c, miR-92b, and miR-206) have been found. Their functional role and the clinical consequences remain to be elucidated.[39]

Taken together, it is likely that sarcoidosis might be further subclassified depending on the genetic backgrounds and their respective immunologic responses,[32] thus enabling us to identify individual therapeutic targets.

## PATHOGENESIS

Granuloma formation is a hallmark of sarcoidosis disease. The underlying immunologic events include (1) exposure to one or more (unknown) antigens, (2) activation of antigen-presenting cells (macrophages and/or dendritic cells), (3) a T cell response in an effort to eliminate the antigen, and (4) granuloma formation.

Physiologically, granulomas act as shields, protecting tissues from pathogens and thereby pre-empting inflammatory reactions. Granulomas consist of a core of mononuclear phagocytes, such as epithelioid cells, multinucleated giant cells, and macrophages, which are encased by lymphocytes, including B cells, $CD4^+$ T cells, and $CD8^+$ T cells.[12] The immunologic reaction is influenced by a genetic background that renders individuals more or less susceptible to the development of a "sarcoidosis phenotype" (described in the subsequent text).

Granuloma formation occurs in different stages, divided into the initiation, accumulation, and effector phases and resolution.[40] After antigen exposure, macrophages detect antigens via pattern recognition receptors (e.g., Toll-like receptors [TLRs]) and then internalize and process these antigens. In sarcoidosis, it is mainly TLR2 and TLR4 that are involved.[41] They then present these antigen(s) to the T cell receptor via HLA molecules. This step requires the presence of co-stimulatory molecules (such as CD80, CD83, and CD86) to determinate the immune response (activation or inhibition). One of these inhibitory signals may be mediated by the *butyrophilin-like 2 (BTNL2)* gene. Locally activated naïve $CD4^+$ Th cells differentiate into activated $CD4^+$ Th1-like cells during the accumulation phase, causing subsequent elevations in Th1-associated inflammatory mediators, such as IL-2, IFN-$\alpha$, IFN-$\gamma$, monocyte chemotactic protein-1, macrophage inflammatory protein-1, and granulocyte-macrophage colony-stimulating factor. $CD4^+$ Th cells also interact with antigen-presenting cells to initiate the development of granulomas and to preserve granulomas.[3] Thus T cells undergo oligoclonal proliferation in areas of immune system activity. At this point in the disease process, lymphocyte levels are typically increased at the sites of inflammation, but peripheral blood lymphopenia may occur as a result of sequestration. The ratio of CD4/CD8 cells becomes elevated in the lungs and other affected organs. During the effector phase, T cells and macrophages release mediators (IL-12, IL-15, IL-18, and TNF), which amplifies the Th1-driven inflammatory response.

Macrophage-derived cytokines contribute to the external signaling milieu that favors the differentiation of $CD4^+$ cells to Th1 cells.[42,43] Ultimately, a feedback loop from a downstream-produced cytokine cascade induces macrophages to differentiate into epithelioid cells, which gain secretory capability, lose phagocytic capacity, and fuse to form multinucleated giant cells.[3] These epithelioid cells form the cellular basis of granulomas. Also contributing to granuloma formation are Th2 cells. These cells synthesize fibronectin and C-C motif ligand 18 (CCL18). During resolution, release of these mediators results in a positive feedback loop of CCL18-activated and macrophage-mediated collagen formation. Although granulomas spontaneously resolve without causing damage in most cases, this cycle leads to fibrosis in up to 25% of patients with sarcoidosis.[3] As patients' fibrosis becomes more extensive, their prognosis worsens. Although the mechanisms responsible for granuloma fibrosis have not been fully characterized, patterns of cytokines change (upregulation of transforming growth factor [TGF]-$\beta$), and Th2 cells may lead to an increased ratio of CD8 to CD4 cells. Fewer than 5% of patients die of sarcoidosis, but fibrosis leading to respiratory failure is a contributing factor in many sarcoidosis-related deaths. In recent years, additional cell populations such as regulatory T cells (Tregs) and Th17 cells that release IL-17A (see Chapter 12) have been found to be upregulated in individuals with sarcoidosis.[44-46] Interestingly, nicotine treatment improves TLR responsiveness and normalizes the numbers of Tregs in individuals with sarcoidosis,[47] supporting the anecdotal observation that the incidence of sarcoidosis is diminished in smokers.[48]

Overall, there appear to be two possible immunologic scenarios in sarcoidosis: an intense immune reaction in patients with active disease, finally resulting in antigen clearance, or chronic disease with less inflammation but the inability to eradicate the inciting agent and subsequent chronic stimulation of the immune response, resulting in organ damage such as lung fibrosis.[41]

## CLINICAL FEATURES

Sarcoidosis can affect every organ system, with the lungs the most commonly affected organ. Joint symptoms are quite common in sarcoidosis, affecting up to 25% of patients.[2] The eyes (uveitis and retinal vasculitis), liver (abnormal liver function tests), lymph nodes (enlargement), and skin (lupus pernio, papules, nodules, plaques, and scar sarcoidosis [tattoos]) are the most frequent extrapulmonary organs affected (see Table 117-1).[32] However, rheumatologists are more likely to be confronted with patients who have systemic, atypical, or primarily musculoskeletal manifestations or disease that is refractory to

**TABLE 117-1** Extrapulmonary Localizations of Sarcoidosis

| | Prevalence | Symptoms | Investigations |
|---|---|---|---|
| Skin | ~15% | Papules, nodules, plaques, scar sarcoidosis (tattoos), lupus pernio, subcutaneous sarcoidosis, erythema nodosum | Biopsy (except for lupus pernio and erythema nodosum*) |
| Lymph nodes | 10%-20% | Mostly cervical or supraclavicular; inguinal, axillary, epitrochlear, submandibular sites also possible; painless and mobile | Biopsy |
| Eye | 10%-30% | Anterior, intermediate or posterior uveitis, retinal vasculitis, conjunctival nodes, lacrimal gland enlargement | Systematic ophthalmology examination, slit-lamp examination, fluorescein angiography |
| Liver | 20%-30% | Often symptom-free, abnormal liver function tests in 20%-30%, hepatomegaly, rarely hepatic insufficiency, chronic intrahepatic cholestasis, portal hypertension | Liver function tests, abdominal ultrasound or CT, rarely biopsy if moderate or severe abnormalities on liver tests or if the diagnosis is uncertain |
| Spleen | ~10% | Splenomegaly, rarely pain or pancytopenia, very rarely splenic rupture | Ultrasound and CT |
| Heart | 2%-5% | Atrioventricular or bundle branch block, ventricular tachycardia or fibrillation, congestive heart failure, pericarditis, impairment of sympathetic nerve activity, sudden cardiac death | Electrocardiography, echocardiography, 24-h Holter monitoring, MRI, perfusion scintigraphy, 18F-FDG-PET; biopsies are rarely performed because of low yield |
| Nervous system | ~5% | Facial nerve palsy, optic neuritis, leptomeningitis, diabetes insipidus, hypopituitarism, seizures, cognitive dysfunction, deficits, hydrocephalus, psychiatric manifestations, spinal cord disease, polyneuropathy, small-fiber neuropathy | CSF investigation, MRI, intraepidermal nerve fiber density, SFN screening list |
| Joints† | 15%-25% | Acute or chronic arthritis, in acute arthritis often bilateral ankle swelling with periarthritis/tenosynovitis, rarely dactylitis; chronic arthritis oligo- or polyarticular, most often associated with systemic disease; Jaccoud deformity, trabecular changes, phalangeal cysts, or granulomatous erosions possible | Plain radiographs, ultrasound, MRI |
| Kidney | 0.5%-2% | Rare symptoms, acute kidney injury, chronic renal insufficiency, hypercalcemia, hypercalciuria, nephrocalcinosis, kidney stones, granulomatous interstitial nephritis | 24-h urinary calcium, creatinine, proteinuria (spot urine), kidney biopsy, ultrasound |
| Parotitis | 4% | Symmetrical parotid swelling, Heerfordt's syndrome when associated with uveitis, fever, and facial nerve palsy | |
| Nose | 0.5%-6% | Nasal stuffiness, nasal bleeding, crusting, anosmia | Sinus CT, rhinoscopy, biopsy |
| Larynx | 0.5%-1% | Hoarseness, breathlessness, stridor, dysphagia | Laryngoscopy, biopsy |
| Skeletal muscles | 1%-2% | Proximal muscle weakness, amyotrophy, myalgia, intramuscular nodes | Creatine phosphokinase test, MRI, 18F-FDG-PET, electromyography, biopsy |
| Genitourinary tract | | All organs can be involved, including uterus, epididymis, and testicle | Biopsy; imaging as appropriate |
| Gastrointestinal tract | 1% | Most often symptom-free; esophagus, stomach, small intestine, and colon can be involved | Endoscopy, biopsy |

*Biopsies of erythema nodosum do not demonstrate granulomas.

†From Sweiss NJ, et al: Rheumatologic manifestations of sarcoidosis. *Semin Respir Crit Care Med* 31:463–473, 2010.

CSF, Cerebrospinal fluid; CT, computed tomography; 18F-FDG-PET, 18F fluorodeoxyglucose-positron emission tomography; MRI, magnetic resonance imaging; SFN, small fiber neuropathy.

Modified from Valeyre D, Prasse A, Nunes H, et al: Sarcoidosis. *Lancet* 383:1155–1167, 2014.

standard treatment. Additional symptoms are sarcoidosis-associated fatigue (present in about 50% to 70% of patients),[49] small-fiber neuropathy (44%), which is difficult to diagnose and treat,[50] and cognitive dysfunction.[51] Musculoskeletal involvement can present as osseous sarcoidosis (axial manifestation or sacroiliitis) in up to 13% of patients[52] or sarcoid myopathy in up to 75%, the latter of which is only rarely symptomatic.[2] Between 15% and 25% of patients with sarcoidosis have arthritis, which is the most frequently observed rheumatologic symptom of sarcoidosis. In patients with sarcoidosis, arthritis can be acute or chronic; the acute form is most common. Chronic arthritis is typically associated with multisystem sarcoidosis.[53]

## Acute Sarcoid Arthritis

Febrile arthropathy is the most frequently observed joint involvement in sarcoidosis. Although any joint may be involved, the condition usually is symmetric, involving ankles, knees, wrists, and elbows, and exists with BHL and erythema nodosum. Of note, ankle swelling is most often due to periarthritis or tenosynovitis rather than true synovitis when assessed by ultrasound or MRI.[53a,53b] Patients with acute sarcoid arthritis experience pain and stiffness, and their joints may be swollen or tender (see supplemental Figure 117-1 on ExpertConsult.com). Acute sarcoid arthritis sometimes resolves within weeks of onset, but occasionally

symptoms last for several months. Once this condition resolves, it usually does not recur.

In a study of patients with acute sarcoid arthritis, Visser and colleagues[53] prospectively evaluated patients and published criteria to assist in diagnosis. Of 579 participants, 55 patients (9%) eventually were diagnosed with sarcoid arthritis. Diagnoses were made after it was established that patients had a combination of arthritis and BHL, as determined by chest radiography. From the findings of their study, investigators established criteria with 93% sensitivity and 99% specificity to guide physicians in differentiating between sarcoid and other causes of arthritis (Table 117-2).

In addition to Visser's criteria, most symptoms of sarcoid arthritis vary among patients. Patients with sarcoid arthritis frequently have elevated erythrocyte sedimentation rates,[53,54] but other symptoms, such as fever, are observed only in up to 66% of patients.[53-56] Of note, in a small number of patients with acute sarcoid arthritis, abnormal alterations may develop in the bones that can be seen on radiographs of the hands or feet. In some patients, sarcoid dactylitis may be the presenting symptom (see Figure 117-1C and E), characterized by swollen soft tissues surrounding affected fingers, erythematous skin, or tenderness. Individuals with dactylitis also may have nail abnormalities, such as dystrophy. Therefore, it is important to rule out other conditions that affect the nails, such as psoriatic arthritis, when making sarcoid arthritis diagnoses.

Other skeletal areas that may be affected by sarcoidosis include nasal bones, pelvic girdle structures, ribs, and the skull. Patients with lupus pernio may be particularly at risk for the development of abnormalities in their nasal bones. Because of similarities between a patient with sarcoidosis who has nasal bone involvement and a patient who has granulomatosis with polyangiitis (GPA), it is important to distinguish between these conditions.[57] Serology may be useful because most patients with GPA will have a positive anti-neutrophil cytoplasm antibody (ANCA), but sarcoidosis does not lead to a positive ANCA.[58] On the other hand, about 60% of patients with sarcoidosis will have an elevated angiotensin-converting enzyme (ACE) level at presentation, which is not seen in the presence of vasculitis.[57] Lesions in the pelvic bones (see Figure 117-1D) or in the spine (see Figure 117-1F) may at first lead one to suspect

**TABLE 117-2** Visser's Criteria for Sarcoidosis in Patients with Arthritis and Bihilar Lymphadenopathy

| Of the following criteria, patients must have three of four characteristics to establish the diagnosis (sensitivity 93%, specificity 99%): |
|---|
| • Age <40 yr |
| • Erythema nodosum |
| • Symmetric ankle arthritis |
| • Symptom duration <2 mo |

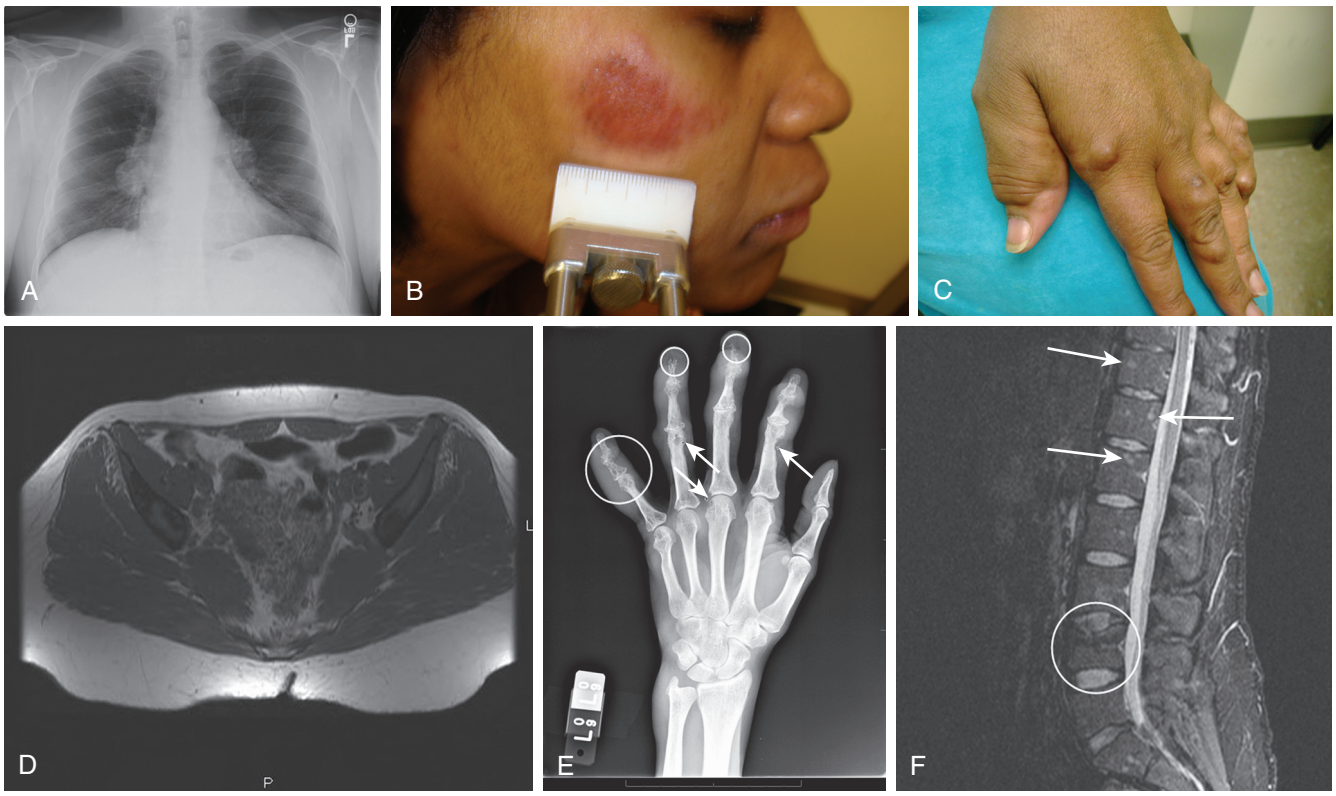



**Figure 117-1** Various manifestations of sarcoidosis. **A,** Bilateral hilar adenopathy and right paratracheal lymph node enlargement demonstrated on a posterior anterior chest roentgenogram, Scadding stage 1.[29] **B,** Facial lesion consistent with lupus pernio.[60] **C,** Hand changes consistent with sarcoidosis in the fingers. **D,** Noncontrast MRI scan of the pelvis demonstrating bone marrow replacement by granulomatous tissue. **E,** Cystic changes *(arrows)* within the bones of the fingers of a patient with sarcoidosis. **F,** Gadolinium enhancement of lesions of the spine *(arrows)* seen on an MRI scan of a patient with sarcoidosis. ***(B,** Reproduced with permission from the patient.)*



**Supplemental Figure 117-1** Cutaneous granuloma of sarcoidosis. *(From Habboushe JP, Newman DH: Images in emergency medicine: woman with painful swelling in fingers. Cutaneous granuloma of sarcoidosis.* Ann Emerg Med *57:434–441, 2011.)*

cancer metastases. Therefore, a comprehensive assessment is warranted in patients who present with changes in their skeletal bones, even if they exhibit other symptoms typical of sarcoidosis.

## Chronic Sarcoid Arthritis

The chronic form of sarcoid arthritis is less common than the acute form, and in the United States it affects more African-American than white people. It most often affects people with systemic sarcoidosis and multiorgan involvement and typically manifests as oligoarthritis or polyarthritis. Sarcoid arthritis can have a varied presentation. Joint destruction (granulomatous erosions) or Jaccoud's deformity (i.e., deformity without obvious destruction) can occur when inflammation persists, and phalangeal cysts or a trabecular pattern can be present on plain radiographs.

In some patients, joint symptoms manifest early in the course of sarcoidosis, whereas other patients experience sarcoid arthritis several years after onset of the disorder. Lupus pernio and chronic uveitis may manifest concomitantly with the chronic form of arthritis.

As is the case with other organ system manifestations, several conditions may mimic sarcoid arthritis and should be excluded. For example, symmetric oligoarthritis or polyarthritis and an elevated rheumatoid factor are common features of sarcoid arthritis, but they may also result from reactive arthritis or rheumatoid arthritis (RA). Furthermore, a subset of patients with RA may experience some lung dysfunction, which can cause confusion in differentiating RA from sarcoidosis. In addition, it has been reported that coincident sarcoidosis eventually develops in some patients with RA, although it is rare for sarcoidosis to coexist with connective disease disorders. Therefore, it is crucial for physicians to distinguish between the two conditions or to determine whether the two diseases are comorbid within the same patient.[59]

In patients with migratory polyarthritis, rheumatic fever may be suspected, particularly when joint symptoms manifest before additional sarcoidosis-related symptoms are noted. A biopsy of the synovium or tendon sheaths may be helpful, with characteristic granulomas appearing upon pathologic examination. Differential diagnoses for patients with monarthritis include gout, septic arthritis, and calcium pyrophosphate dihydrate–positive arthritis (see Tables 117-3, 117-4, and supportive content for detailed consideration of the diagnostic approach).[2]

## DIAGNOSIS AND DIAGNOSTIC TESTS

The diagnosis of musculoskeletal disease related to sarcoidosis must be examined on the basis of two different presentations. The first is a patient with known sarcoidosis who presents with musculoskeletal symptoms. The other is a patient with musculoskeletal disease in whom sarcoidosis is a possibility. Although the evaluations may be similar, a patient with known sarcoidosis elsewhere who has musculoskeletal manifestations consistent with sarcoidosis is usually assumed to have sarcoidosis in that area. However, in a patient with musculoskeletal involvement and no known sarcoidosis elsewhere, a biopsy usually will be required to confirm the diagnosis. The biopsy specimen may be from the lung or skin, but in some cases it will be from muscle.

**TABLE 117-3** Evaluation of a Patient with Sarcoidosis

| |
|---|
| **Initial Evaluation Suggested for All Patients** |
| History, including occupational and environmental exposures |
| Physical examination |
| Posterior-anterior chest roentgenogram |
| Spirometry |
| Complete blood cell count |
| Liver function studies and serum calcium |
| Routine ophthalmic examination |
| **Evaluation Considered in Selected Patients** |
| Computed tomography scan of chest |
| Holter monitor and/or electrocardiogram |
| Urine analysis |
| Diffusion capacity of lung of carbon monoxide |
| Radiograph of involved joints and/or ultrasound/MRI |
| **Follow-up Every 6 to 12 Months** |
| Any abnormality noted on initial evaluation |
| Chest roentgenogram |
| Liver function studies and serum calcium |
| Spirometry |

Modified from American Thoracic Society: Statement on sarcoidosis: joint statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 160:736–755, 1999.

Sarcoidosis is a diagnosis of exclusion. In clinical practice, other conditions often are suspected first when considering only clinical symptoms (e.g., Hodgkin's lymphoma or tuberculosis with pulmonary findings and/or systemic symptoms). The main goal of the workup is therefore to gather enough clinical clues and compatible features to diagnose sarcoidosis with sufficient certainty and to exclude other diagnoses that warrant a totally different treatment approach. Figure 117-1 presents some features consistent with the disease.[60] In many instances, the diagnosis will rely heavily on the finding of granulomas in a tissue biopsy. However, many other conditions can lead to a granulomatous reaction, as summarized in Table 117-5. Although sarcoidosis granulomas tend to be noncaseating and nonnecrotizing, a significant number of patients with sarcoidosis exhibit evidence of some necrosis in part of their granulomatous response. A biopsy is usually not required for some presentations such as Löfgren's syndrome, Heerfordt's syndrome (defined as fever, uveitis, parotitis, and cranial nerve palsy), BHL with uveitis, and asymptomatic BHL.[61]

The heterogeneity of the disease leads to varied clinical presentations, which may result in delayed diagnosis. In one study of 189 patients with sarcoidosis, it was found that only 15.3% of patients received a diagnosis during their first visit to a physician.[60] Moreover, sarcoidosis can affect any organ, which may cause a patient to be referred to a specialist who may not commonly manage sarcoidosis.[60] Patients with pulmonary symptoms are often misdiagnosed as having asthma. For individuals with musculoskeletal manifestations, the presentation of sarcoidosis may be similar to that of other conditions, most notably autoimmune disorders.[62]

For the patient who presents with musculoskeletal disease and possible sarcoidosis, a vigilant approach is mandatory.

**TABLE 117-4** Comparison of the Features of Sarcoidosis and Other Rheumatologic Diseases

| | Sarcoidosis | SLE | RA | PsA | Systemic Vasculitis |
|---|---|---|---|---|---|
| Clinical findings | Lungs, eyes, and skin most common; any other organ (e.g., heart, brain, skin, bone, and liver) also may be affected | Joints, kidneys (nephritis), mucous membranes, circulatory system, nervous system, lymph nodes, spleen | Joints (most common), lungs, blood vessels | Joints (oligoarthritis, polyarthritis), skin (psoriatic lesions), nails (dystrophy, dactylitis) | Blood vessels (wall thickening), skin (purpura, infarct ulcers), nervous system (headache, meningitis, seizures), joints (arthritis), kidneys (hypertension), heart, GI system |
| Radiographic findings | Bilateral and mediastinal hilar adenopathy, reticulonodular opacities | Bilateral, diffuse air space opacity; diaphragm elevation ("shrinking lung syndrome") | Joint space narrowing, joint erosions, inflammation | Joint space narrowing, erosions, ossification near joints | Granulomatous lesions; multiple bilateral nodules; patchy areas of consolidation |
| Pathologic findings | Noncaseating granulomas | Inflammation, blood vessel abnormalities such as vasculitis, immune complex deposits | Swollen synovium; presence of fibroblast-like and macrophage-like synoviocytes, macrophages, T and B cells | Increased synovial vascularity, dilated blood vessels, neutrophil infiltration | ANCA staining pattern on neutrophils and monocytes; patchy infiltrates, vessel wall granulomas, fibrous tissue |
| Laboratory findings | Elevated ACE; hypercalcemia; presence of ANAs, ANCAs, and anti-dsDNA | Anti-dsDNA, anti-Smith, ANAs, RNP, antithrombin, anti-Ro/SS-A, anti-La/SS-B, anti-topoisomerase, aPL | Rheumatoid factor, ACPA, aPL | ANA (common), anti-dsDNA, rheumatoid factor, anti-Ro, anti-RNP (rare), aPL | ANCA, aPL, inflammatory markers (CRP) |

| | MCTD | Scleroderma | Anti-phospholipid syndrome | Sjögren's syndrome | Ankylosing spondylitis | Reactive arthritis |
|---|---|---|---|---|---|---|
| Clinical findings | Raynaud's phenomenon; joints, muscles, lungs, heart, kidneys, and nervous system may be affected | Skin (tightening, thickening, induration), joints, GI system, lung, heart, kidney | Blood vessels (arterial/venous thrombosis, thrombocytopenia, fetal loss) | Eyes, mouth, and mucous membrane dryness; joints (arthritis), skin, kidneys, nervous system, and lymph nodes may be affected | Vertebral arthritis, back pain, stiffness, synovitis, peripheral arthritis, pulmonary symptoms, iritis | Arthritis, conjunctivitis, urethritis; usually following a genitourinary or GI infection |
| Radiographic findings | Diffuse periarticular osteopenia; swelling of soft tissue; joint erosions, joint space narrowing, tuft resorption, and soft tissue atrophy | Pulmonary fibrosis, diffuse reticulonodular pattern | Patchy infiltrates | Mild joint space narrowing | Vertebral inflammation; sacroiliitis; bone erosions, syndesmophytes | Proliferation at tendon insertion; sacroiliitis; syndesmophytes |
| Pathologic findings | Autoantigen modifications, B and T cell activation | Excess matrix deposition, fibrosis; endothelial cell dysfunction and death; destruction of small vessels | Structural glomerular changes | B cell infiltration | Capsular fibrosis; ossification | Bacterial infection *(Shigella, Salmonella, Campylobacter)* |
| Laboratory findings | ANA, RNP, aPL | ANA (anti-topoisomerase), aPL, ACAs | Lupus anticoagulant, aPL, anti–protein C, anti-prothrombin, anti–protein S, anti-annexin | ANA, anti-Ro/SS-A, rheumatoid factor, aPL | Elevated ESR, leukocytosis, HLA-B27 | ANCA, HLA-B27; elevated ESR, CRP |

ACA, Anti-cardiolipin antibody; ACE, angiotensin-converting enzyme; ACPA, anti-citrullinated protein antibody; ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasm antibody; aPL, anti-phospholipid antibody; CRP, C-reactive protein; dsDNA, double-stranded DNA; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; HLA, human leukocyte antigen; MCTD, mixed connective tissue disease; PsA, prostate-specific antigen; RA, rheumatoid arthritis; RNP, ribonucleotide protein; SLE, systemic lupus erythematosus.

**TABLE 117-5** Major Causes of Granulomatous Reaction besides Sarcoidosis

| |
|---|
| **Infection** |
| *Mycobacterium tuberculosis* |
| Fungal |
| Mycoplasma |
| *Pneumocystis jiroveci* |
| Brucellosis |
| Cat-scratch fever |
| Atypical mycobacteria |
| Toxoplasmosis |
| **Environmental** |
| Berylliosis |
| Hard metal |
| Zirconium |
| Tattoo |
| Hypersensitivity pneumonitis |
| Drugs (e.g., methotrexate) |
| **Miscellaneous** |
| ANCA-associated vasculitis |
| Necrotizing sarcoid granuloma |
| Lymphoma |
| Cancer |
| Granulomatous lesions of unknown significance |
| Crohn's disease |
| Lymphocytic interstitial pneumonia |
| Behçet's disease |
| Rheumatoid nodules |

ANCA, Anti-neutrophil cytoplasm antibody.

Modified from American Thoracic Society: Statement on sarcoidosis: joint statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 160:736–755, 1999.

**TABLE 117-6** Characteristic Features of Sarcoidosis

| | More Likely to Be Sarcoidosis | Less Likely to Be Sarcoidosis |
|---|---|---|
| Chest x-ray | Bilateral hilar adenopathy<br>Upper lobe disease | Pleural effusion |
| Computed tomography of chest | Subpleural reticulonodular infiltrates<br>Mediastinal adenopathy<br>Peribronchial thickening<br>Traction bronchiectasis of upper lobe | Subpleural honeycombing |
| Skin lesions | Erythema nodosum<br>Lupus pernio<br>Maculopapular lesions | |
| Ocular disease | Uveitis<br>Optic neuritis | Episcleritis |
| Neurologic disease | Cranial seventh nerve paralysis | |
| Renal disease | Nephrocalcinosis<br>Granulomatous or non-granulomatous interstitial nephritis | Glomerulonephritis |
| Laboratory data | Elevated angiotensin-converting enzyme<br>Elevated serum calcium<br>Elevated alkaline phosphatase<br>Elevated soluble IL-2 receptor | Positive anti-neutrophil cytoplasm antibody |

A comprehensive multisystem evaluation, as is commonly performed in rheumatology practice, should prove informative. For example, hilar adenopathy on a posterior chest roentgenogram is seen in about two-thirds of patients. Scadding proposed a staging system of the chest roentgenogram[63]: stage 1 disease consists of hilar adenopathy alone (see Figure 117-1A); stage 2 disease is hilar adenopathy plus infiltrates; stage 3 is infiltrates alone; and stage 4 is fibrosis. Although this scoring is commonly used, differences may be noted in staging of the roentgenogram, even among sarcoidosis experts.[64] Patients may have evidence of disease on a chest computed tomography (CT) scan. Table 117-6 lists some of the features that, when present, support or refute the diagnosis.[65] These features include erythema nodosum, lupus pernio (see Figure 117-1B), cranial seventh nerve paralysis, and hypercalcemia.

For patients with sarcoidosis, several tests (summarized in Table 117-3) have been proposed to serve as a minimal evaluation.[66] These tests reflect the fact that sarcoidosis is a multiple-organ disease and that one needs to look for evidence of specific organ involvement. Because serum ACE levels may or may not be elevated in patients with sarcoidosis and therefore are not accurate for the diagnosis,[67] other biomarkers for the diagnosis (chitotrisodase[68]) or activity (soluble IL-2 receptor [sIL-2R], neopterin, chitotriosidase, lysozyme, KL-6, and amyloid A[32]) are being evaluated. sIL-2R and neopterin correlate with 18F-fluorodeoxyglucose-positron emission tomography (18F-FDG-PET) uptake,[69] but its use in clinical practice has not been finally determined. Because no biomarkers with sufficient sensitivity or specificity have been identified for sarcoid arthritis, we suggest regular evaluation with routine clinical examination (observing for signs of inflammation, swelling, or radiographic changes) and standard inflammatory markers (erythrocyte sedimentation rate and C-reactive protein) to assess for severity/progression in arthritis.

MRI with administration of gadolinium may identify involvement of sarcoidosis in the brain[70] or the heart.[71] MRI findings also may be useful in identifying bone sarcoidosis or sarcoid arthritis and may identify asymptomatic lesions.[71a]

Several lines of evidence have indicated that 18F-FDG-PET may be useful for diagnosis and assessment in (extra) pulmonary sarcoidosis,[72] but it usually is not recommended as a first diagnostic tool. 18F-FDG-PET can be used to determine which organs are affected, particularly when no evidence of lung involvement is found; this includes cardiac sarcoidosis.[71] Furthermore, 18F-FDG-PET can point investigators to appropriate organs to biopsy when no distinct organ system involvement is obvious.[73] For pulmonary sarcoidosis, endobronchial ultrasound is used to help the bronchoscopist select appropriate biopsy sites for transbronchial needle aspiration.[74]

Criteria for specific organ involvement in patients with known sarcoidosis have been proposed and updated.[75,76] Definitive organ involvement may be obvious or can be established by positive results of a granuloma biopsy from the affected tissue. For example, it is possible that arthritis with no other cause may be attributed to underlying

sarcoidosis. However, patients with phalangeal cystic changes almost definitely can consider their arthritis to be a manifestation of their sarcoidosis[75] (Table 117-4).

Highly probable sarcoid arthritis includes the typical features of the trabecular pattern, osteolysis, cyst formation, and punched-out lesions. Signs of sarcoidosis that is at least probable include dactylitis, nodular tenosynovitis, and positive findings of PET, MRI, or gallium-67 bone imaging. Arthralgias are a sign of possible sarcoid arthritis, and patients who present with nonspecific arthritis are categorized under "no consensus." This classification has limitations and has not been validated in a prospective clinical setting.[76]

# TREATMENT

## General Approach

Prednisone and adrenocorticotropic hormone have both been approved for treatment of pulmonary sarcoidosis, but no treatment for extrapulmonary sarcoidosis, including sarcoid arthritis, has been approved by the U.S. Food and Drug Administration (FDA). With the advent of 18F-FDG-PET scanning and other imaging modalities, it is likely that a larger proportion of patients with asymptomatic but widespread disease will be diagnosed. Decisions to begin immunosuppressive therapy, however, depend on symptoms (e.g., persistent cough or dyspnea) and organ dysfunction (e.g., abnormal pulmonary function tests) and not on the mere number of organs affected (sometimes termed "systemic sarcoidosis"). As a general rule, current treatment options are divided into first-, second-, and third-line therapies.[77] First-line therapy for most manifestations (with the exceptions of sarcoidosis-associated fatigue and small-fiber neuropathy) are corticosteroids. A dose of 20 to 40 mg per day should be maintained for 1 to 3 months and then tapered to a maintenance dose of 5 to 10 mg/day, which, empirically, is usually maintained for 1 year.[78] Second-line therapies include so-called "disease-modifying antisarcoid drugs" (DMASDs), including methotrexate, azathioprine, leflunomide, hydroxychloroquine, chloroquine, mycophenolate, and cyclosporine. For refractory cases, TNF inhibitors can be used as third-line therapy. The two best studied agents of this class of drugs are infliximab and adalimumab. INF has been studied in two placebo-controlled trials and improved FVC and dyspnea, especially in patients with longer disease duration and more dyspnea at baseline.[78a,78b] ADA has shown some effectiveness in pulmonary sarcoidosis.[79] Etanercept has proven to be ineffective for pulmonary and ocular sarcoidosis.[80,81] In a recent trial, the TNF inhibitor golimumab and ustekinumab, an antibody against IL-12/IL-23, were found to be ineffective in the treatment of pulmonary sarcoidosis.[82] B cell inhibition by rituximab had mixed results in individuals with refractory sarcoidosis in a small clinical trial,[83] but it has been effective in singular cases.[84] A definite recommendation cannot be made for using rituximab to treat sarcoidosis.

In addition, it must be remembered that paradoxical reactions to biologic agents can occur, including positivity for anti-nuclear antibody, drug-induced lupus erythematosus, demyelinating disorders, and sarcoid-like granulomatosis.[85,86] A sarcoid-like reaction also has been reported with ustekinumab,[87] suggesting that other mechanisms than upregulation of interferon-α through TNF inhibition[88] might play a role. A genetic predisposition (possibly TNF polymorphisms) is likely, but at present, it is impossible to predict which patients will experience this reaction. Therefore, a high index of suspicion is mandatory when using this type of drug. Tables 117-7 and 117-8 provide an overview of treatment recommendations for the various disease manifestations.

## Management of Sarcoid Arthritis

No randomized trials have been conducted that can guide clinical decision making. To help guide clinicians in management, we have proposed a treatment algorithm, which is shown in Figure 117-2.[2] NSAIDs, methotrexate, and local or low-dose systemic corticosteroids are our preferred first-line therapies. Alternatively, hydroxychloroquine and other DMASDs may be used. Using information obtained from the diagnostic evaluation, the clinician usually makes treatment decisions after carefully considering the disease severity and its probable clinical course, as revealed by its radiographic progression.

The proposed algorithm requires regular patient visits to monitor disease activity and therapeutic efficacy and tolerability. Responders continue to take first-line agents until disease resolution or, alternatively, treatment failure. Before nonresponders are prescribed second-line therapies, higher doses of corticosteroids may be used, depending on tolerability and manifestations of toxicity. For patients who require second-line medications, two options are available: (1) methotrexate may be administered to methotrexate-naïve patients, or (2) nonmethotrexate disease-modifying antisarcoid drugs (e.g., hydroxychloroquine, and azathioprine) as monotherapy or combinations thereof may be prescribed to patients who have an inadequate response to first-line methotrexate.

For patients for whom this treatment approach fails, one can consider an alternative third-line biologic therapy or an aggressive course of systemic corticosteroids with careful monitoring of toxicity. Alternatively, participating in a clinical trial may be appropriate for some patients.

Vitamin D metabolism represents a more complex issue in sarcoidosis than in other rheumatic diseases.[89] Patients with sarcoidosis might be more sensitive to calcium and vitamin D substitution,[90] with the associated risks of hypercalcemia and hypercalciuria, which are present in up to 10% of patients who don't receive vitamin D supplements. The mechanism usually attributed to hypercalcemia has been increased production of 1,25-dihydroxyvitamin D (1,25-$OH_2D$) by epithelioid cells in the granuloma (1α-hydroxylase activity). This risk must be balanced against the adverse effects of potential chronic vitamin D deficiency, and patients often require treatment for osteoporosis because of long-term glucocorticoid use. Bisphosphonates alone may be adequate to treat corticosteroid-induced osteoporosis.[91] Patients who do not receive glucocorticoid therapy do not seem to have a lower bone mineral density per se because of sarcoidosis.[92] Recently, higher levels of 25-hydroxyvitamin D (25-OHD) were associated with a higher frequency of fractures in patients with sarcoidosis.[93] The authors found that levels between 10 and 20 ng/mL were associated with

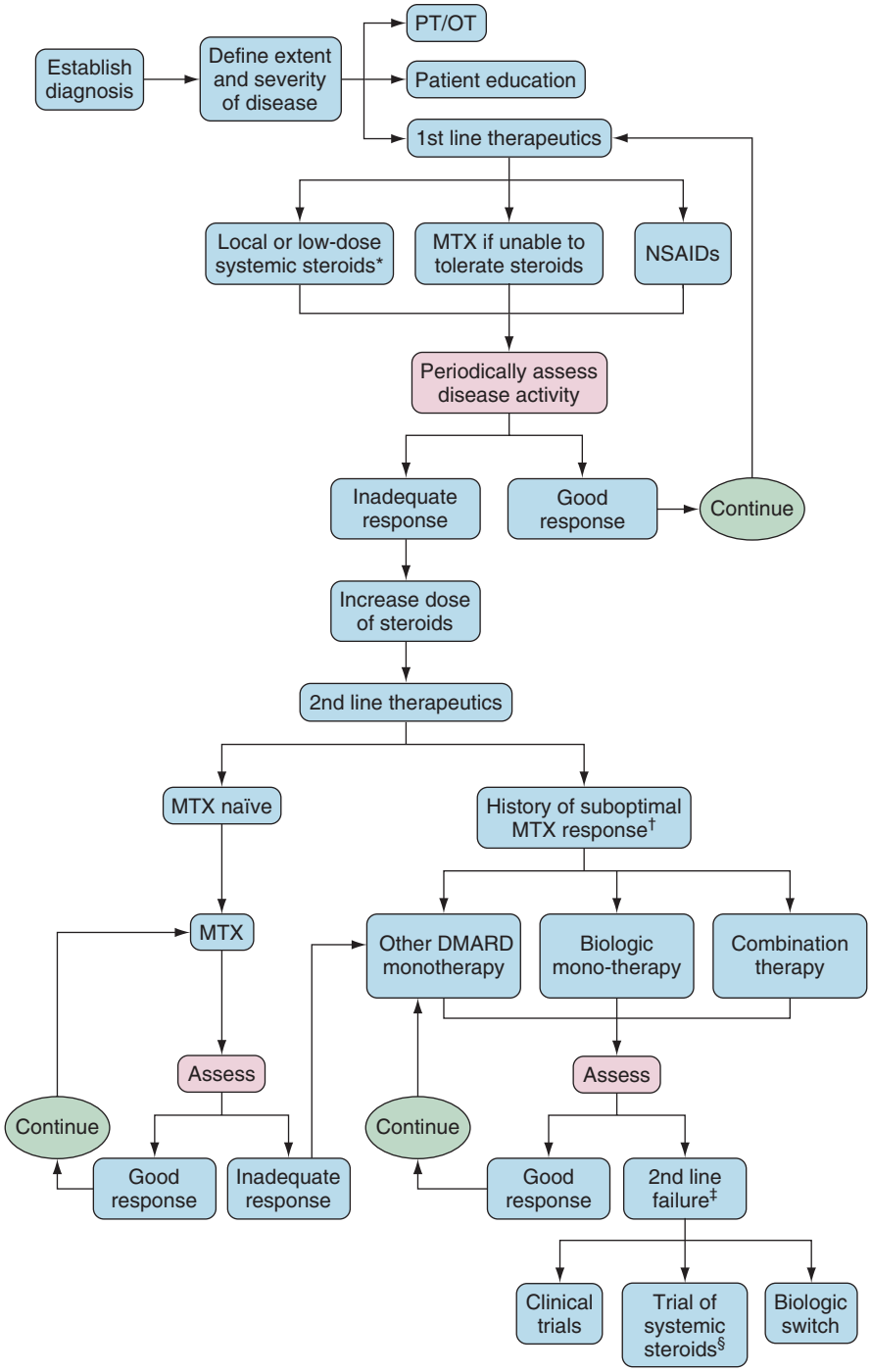



**Figure 117-2** Algorithm describing an approach to treating sarcoidosis arthritis. *Low-dose steroids: <10 mg prednisone daily. †Suboptimal response to methotrexate (MTX): intolerance to drug, lack of satisfactory efficacy with dosage up to 25 mg/wk, or a contraindication to medication use. ‡Disease-modifying anti-rheumatic drug (DMARD) failure: progressive disease or drug intolerance. §Methylprednisone preferred over prednisone if prednisone has been used previously. OT, Occupational therapy; PT, physical therapy. *(From Sweiss NJ, Patterson K, Sawaqed R, et al: Rheumatologic manifestations of sarcoidosis.* Semin Respir Crit Care Med *31:463–473, 2010.)*

**TABLE 117-7** Overview of Treatment Recommendations for the Various Manifestations of Sarcoidosis

| | TREATMENT RECOMMENDATIONS FOR SARCOIDOSIS | | |
|---|---|---|---|
| | First Line | Second Line | Third Line |
| Pulmonary | Corticosteroids<br>ACTH analogue | MTX<br>AZA<br>LEF<br>HCQ | INF<br>ADA |
| Ocular | Corticosteroids | MTX<br>AZA<br>LEF | INF<br>ADA |
| Cutaneous | Corticosteroids<br>NSAID for erythema nodosum associated with Löfgren's syndrome | MTX<br>AZA<br>LEF<br>HCQ<br>MMF | INF<br>ADA<br>Apremilast<br>THA |
| Lymph node | Corticosteroids | MTX<br>AZA<br>LEF | INF<br>ADA |
| Musculoskeletal | Corticosteroids<br>NSAID | MTX<br>HCQ<br>AZA<br>LEF | INF<br>ADA |
| Neurosarcoidosis | Corticosteroids | MTX<br>LEF<br>AZA<br>MMF | ADA<br>INF<br>RTX<br>CYC |
| Cardiac | Corticosteroids | MTX<br>LEF<br>AZA<br>MMF | INF<br>ADA<br>CYC |
| Renal | Corticosteroids | HCQ<br>AZA<br>MMF | INF |
| Liver | Corticosteroids | MTX<br>AZA<br>LEF<br>HCQ<br>MMF | INF<br>ADA |
| Small fiber neuropathy | Consider pregabaline, amitryptiline | INF<br>ADA<br>IVIG | |
| Sarcoidosis-associated fatigue | Methylphenidate<br>Armodafinil<br>CBT<br>Rehabilitation | INF | |

ACTH, Adrenocorticotropic hormone; ADA, adalimumab; AZA, azathioprine; CBT, cognitive behavioral therapy; CYC, cyclophosphamide; HCQ, hydroxychloroquine; INF, infliximab; IVIG, intravenous immunoglobulin; LEF, leflunomide; MMF, mycophenolate mofetil; MTX, methotrexate; RTX, rituximab; THA, thalidomide.

Data from Valeyre D, Prasse A, Nunes H, et al: Sarcoidosis. *Lancet* 383:1155–1167, 2014; Korsten P, Mirsaeidi M, Sweiss NJ: Nonsteroidal therapy of sarcoidosis. Curr Opin Pulm Med 19:516–523, 2013; Beegle SH, Barba K, Gobunsuy R, et al: Current and emerging pharmacological treatments for sarcoidosis: a review. *Drug Des Devel Ther* 7:325–338, 2013; Baughman RP, Nunes H, Sweiss NJ, et al: Established and experimental medical therapy of pulmonary sarcoidosis. *Eur Respir J* 41:1424–1438, 2013.

the lowest risk for fractures. Because of the disassociation between 25-OHD and 1,25-$OH_2$D in sarcoidosis, it seems reasonable to measure both levels to ascertain which patients should receive vitamin D supplements.[89,94,95]

## OUTCOME AND PROGNOSIS

The overall mortality in sarcoidosis is low (about 5%),[96] and many patients with spontaneous regression will never require treatment. In people who need treatment, important differences exist between African-American and white patients. African-American patients with sarcoidosis are known to experience a more severe and systemic disease; mortality is 12 times higher in this population.[97]

## CONCLUSION

Much remains to be learned about sarcoidosis. The causes of this disease remain unclear, and no medications have been approved by the FDA for its treatment with the exception of prednisone and ACTH gel, which was approved in the 1950s on the basis of a few case reports. Clinical trials are currently ongoing to evaluate the safety and efficacy of Acthar gel in various sarcoidosis phenotypes. Although increasing attention has been directed to the underlying mechanisms of granuloma formation and the cause (including an infectious cause), full details of sarcoidosis immunopathogenesis have yet to be determined. Appropriate animal models and candidate genes are needed to help advance our understanding of this disease. Large clinical trials are warranted.

We have presented an algorithm that can be used in treating patients with an established diagnosis of sarcoidosis. However, treatment approaches vary by institution and by individual clinicians as a result of a myriad of conflicting studies that have been published about sarcoidosis management. The diversity of clinical presentations and the lack of large, well-designed, randomized, controlled clinical trials addressing the various clinical phenotypes currently make it impossible to provide more specific treatment recommendations. Furthermore, therapeutic choices will likely differ according to the type and extent of organ system involvement observed in individual patients. It is probable that symptoms such as sarcoidosis-associated fatigue or small-fiber neuropathy are underrecognized because patients must be specifically assessed for their presence. The systemic nature of this disease coupled with the complexity of phenotypic presentation among various ethnic groups and within the same patient makes it difficult to study this complex disease.

For relapsed and refractory disease, steroid-sparing agents including cytotoxic drugs and novel biologic therapies such as TNF inhibitors and B cell depleting therapy increasingly have been used. TNF inhibitors have been investigated in numerous sarcoidosis studies because of the potential role of TNF and other pro-inflammatory factors in sarcoidosis pathogenesis. Limiting their usefulness in this setting are mounting reports of granulomatous reactions to TNF blocking therapies in patients treated with these agents for nonsarcoidosis indications. In other words, TNF inhibitors may help treat *and may cause* sarcoidosis.[98-101] With this point in mind, it is relevant to our discussion

**TABLE 117-8** Overview of Drugs Used to Treat Sarcoidosis

| Drug | Dose Recommendation | Required Monitoring | Comments |
|---|---|---|---|
| Corticosteroids | 20-40 mg/day initial<br>Taper to 5-10 mg/day<br>Lower doses may be considered | Physical examination<br>BP<br>Blood glucose | |
| Methotrexate | 5-25 mg/wk | CBC<br>LFT<br>Kidney function | Lower doses usually sufficient<br>Risk of hypersensitivity pneumonitis 5% in people with RA; in people with sarcoidosis, possibly lower |
| Azathioprine | 1-2 mg/kg/day | CBC<br>LFT | Consider TPMT testing before treatment<br>Concomitant use of allopurinol with a high risk of toxicity |
| Leflunomide | 10-20 mg/day | CBC<br>LFT | Lung toxicity reported<br>Silent liver fibrosis |
| Hydroxychloroquine | 200-400 mg/day (max 5 mg/kg/day) | Ophthalmologic examination once a year (may be extended to every 5 years) | |
| Mycophenolate mofetil | 500-3000 mg/day | CBC<br>LFT | |
| Cyclophosphamide | 500-1000 mg IV or 500 mg/m$^2$ IV every 3-4 wk | CBC, especially after 10-14 days<br>Hematuria<br>Kidney function | Rarely used<br>Typically 6-8 cycles are used |
| Infliximab | 3-5 mg/kg IV at weeks 0, 2, and 6, then every 4-8 wk | CBC<br>Liver and kidney function test<br>Infections | Test for tuberculosis/hepatitis before treatment<br>Heart failure<br>Concomitant use with another drug (MTX) recommended to prevent antibody formation |
| Adalimumab | 40 mg SC every 1-2 wk | Infections<br>CBC<br>Liver and kidney function test | Test for tuberculosis/hepatitis before treatment<br>Possibly higher dose preferred<br>Heart failure |
| Rituximab | 1 g IV on day 0 and 14 | Serum immunoglobulins every 3-6 mo | Progressive multifocal leukoencephalopathy is a rare complication<br>Test for hepatitis before treatment |
| Thalidomide | 50-200 mg/day | CBC | Birth defects<br>Polyneuropathy relatively common |
| ACTH analogue | 40-80 units IM or SC | Blood pressure<br>Electrolytes | Adrenal suppression with rapid withdrawal<br>Cushing's syndrome with prolonged use<br>GI ulcers/bleeding |

ACTH, Adrenocorticotropic hormone; BP, blood pressure; CBC, complete blood cell count; GI, gastrointestinal; IM, intramuscular; IV, intravenous; LFT, liver function test; MTX, methotrexate; RA, rheumatoid arthritis; SC, subcutaneous; TPMT, thiopurine S-methyltransferase.

Data from Valeyre D, Prasse A, Nunes H, et al: Sarcoidosis. *Lancet* 383:1155–1167, 2014; Korsten P, Mirsaeidi M, Sweiss NJ: Nonsteroidal therapy of sarcoidosis. Curr Opin Pulm Med 19:516–523, 2013; Beegle SH, Barba K, Gobunsuy R, et al: Current and emerging pharmacological treatments for sarcoidosis: a review. *Drug Des Devel Ther* 7:325–338, 2013; Baughman RP, Nunes H, Sweiss NJ, et al: Established and experimental medical therapy of pulmonary sarcoidosis. *Eur Respir J* 41:1424–1438, 2013.

that the presentation of TNF inhibitor–induced sarcoidosis, similar to all phenotypes of sarcoidosis, is a unique entity, but it does overlap with that of other autoimmune diseases. Furthermore, TNF inhibitors have been reported to induce autoimmune diseases other than sarcoidosis, including systemic lupus erythematosus, vasculitis, interstitial lung disease, and demyelinating disorders. Diagnosing these conditions in patients with TNF inhibitor–induced sequelae is critical.

Collaborative multicenter studies utilizing a translational personalized approach are required to best serve patients with sarcoidosis, ensure safety, and prevent long-term toxicity while improving quality of life.



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 118

# Hemochromatosis



Gaye Cunnane

**KEY POINTS**

- Elevated ferritin levels (>200 µg/L) and transferrin saturation (>45%) in the absence of other causes are useful screening measures for hereditary hemochromatosis (HHC).
- Genetic testing should be reserved for patients with suggestive biochemical abnormalities, a positive family history of HHC, or both.
- Disease phenotype varies greatly among individuals with similar HHC genotypes including genes encoding *HFE,* hepcidin, hemojuvelin transferrin receptor 2, and ferroportin.
- Diet, alcohol intake, and other risk factors for chronic liver disease all influence the clinical expression of HHC.
- Phlebotomy is an effective treatment for decreasing iron stores.
- Some clinical manifestations of disease improve with treatment (e.g., constitutional symptoms, diabetes mellitus, and liver enzyme abnormalities), whereas others are not affected (e.g., arthritis, hypogonadism, and cirrhosis).
- Atypical osteoarthritis or chondrocalcinosis should trigger a search for an underlying metabolic disorder.
- Early diagnosis and treatment improve outcome.

Hemochromatosis refers to the presence of excess iron in body tissues because of increased iron absorption. Primary or hereditary hemochromatosis (HHC) is an autosomal recessive disease, whereas secondary hemochromatosis refers to iron overload as a result of increased iron availability, ineffective erythropoiesis, or inherited abnormalities of iron metabolism.

Hemochromatosis was first recognized in the 1880s in a series of case reports that described "bronze diabetes" and "pigmented cirrhosis," but von Recklinghausen[1] is credited with the first use of the term in 1889. In 1935, the familial pattern of HHC was described by Sheldon,[2] who suggested that the disease was due to an inborn error of metabolism. In 1955, Finch and Finch[3] showed that HHC was caused by abnormal iron absorption in the presence of a normal diet. At that time, premorbid recognition of the problem was uncommon, and most cases were diagnosed at autopsy. In 1972, serum ferritin became available as a measure of iron stores. Three years later, Simon and colleagues[4] discovered that the HHC gene was present on chromosome 6, close to the human leukocyte antigen (HLA)-A locus. It took 21 more years for the mutated gene, *HFE*, to be described, and in the past decade, it has been recognized that other gene mutations also can cause iron overload.[5] Genetic testing has revolutionized the diagnosis of HHC, although the phenotype of any given mutation may vary greatly.[6,7] Nevertheless, the detection of such genes has greatly enhanced the overall prognosis of this condition by allowing the disease to be diagnosed at a pre-clinical stage in high-risk individuals. This discovery, along with improved knowledge at a molecular level, has further aided our understanding of the pathogenesis and treatment of HHC and helped many patients achieve a normal life expectancy.

## IRON METABOLISM IN HEALTH AND DISEASE

Iron has an essential role in erythropoiesis and in normal cellular function. Along with anemia, chronic iron deficiency results in a variety of other clinical manifestations such as changes to the nails, tongue, esophagus, and muscles.[8] In normal adults, the average total body iron content is 3 to 4 g, mostly contained within hemoglobin, but also present in myoglobin and cytochromes, in addition to the storage proteins, ferritin and hemosiderin (Table 118-1). Of a typical daily Western diet of 10 to 20 mg of iron, 1 to 2 mg is absorbed by duodenal enterocytes each day.[9,10] Heme dietary sources from fish and meat have a higher bioavailability than nonheme sources, such as vegetables. The addition of ascorbic acid to the meal increases absorption of nonheme iron, whereas tannins, bran, and phytates inhibit iron absorption.[11,12]

Iron homeostasis, which is tightly controlled at cellular and molecular levels, is influenced by regulatory feedback between body iron requirements and intestinal iron absorption.[13] Dietary iron enters the body in the duodenum, where, in the ferrous state, it gains access to the enterocyte via divalent metal transporter 1. It is then either stored intracellularly and eventually excreted through the feces or absorbed via ferroportin transfer out of the enterocyte, aided by ferroxidase activity of the ceruloplasmin homologue, hephaestin.[14] Circulating iron is then either used (mainly by erythroid cells) or stored (in the liver or macrophages).

Communication between sites of iron uptake, utilization, and storage is tightly controlled. Ferroportin facilitates release of intra-cellular iron and is a major player in iron homeostasis, modulated by hepcidin, which plays a central role in the process.[13,15] Hepcidin is produced by the liver in response to changes in iron status and negatively regulates iron levels.[16] It acts directly on ferroportin by binding to it and causing it to be internalized by the cell and destroyed.[17] A signaling pathway for bone morphogenetic protein (BMP)-6 regulates hepcidin by binding to a specific receptor

**TABLE 118-1** Mechanisms of Iron Metabolism

| Term | Role in Iron Metabolism |
|---|---|
| Ferritin | Major iron storage protein in iron storage diseases and inflammation<br>Markedly increased in adult-onset Still's disease<br>Plasma levels reflect iron stores (e.g., 1 ng/mL ferritin = 10 mg iron) |
| Transferrin | Transporter protein for iron in plasma<br>Synthesized in liver<br>Increased in iron deficiency states |
| Transferrin saturation | Serum iron (μg/dL) ÷ total iron-binding capacity (μg/dL) × 100 in iron deficiency anemia/chronic disease/ferroportin mutation in hemochromatosis/ineffective erythropoiesis/iron overload states/severe liver failure |
| Iron regulatory proteins | Maintain iron homeostasis by modulating synthesis of transferrin receptors/ferritin/duodenal iron transporter |
| HFE protein | Identified in cells of deep crypts of duodenum and in Kupffer cells<br>Thought to modulate uptake of transferrin-bound iron into duodenal crypt cells<br>May regulate iron absorption via interaction with hepatocytes expressing hepcidin transferrin receptor 2 or JFV genes |
| Iron exporter proteins | Ferroportin/hephaestin/divalent metal transporter 1 |
| Hepcidin | Acute phase reactant produced by liver<br>Intrinsic anti-microbial activity<br>Negative regulator of iron absorption<br>Reduces iron release from macrophages<br>Prevents iron loss by reducing entry of iron into bloodstream via inhibition of ferroportin<br>Mutations found in some families with juvenile hereditary hemochromatosis |
| Hemojuvelin (HJV) | Expressed in liver, heart, and skeletal muscle<br>Membrane-bound HJV is a co-receptor for bone morphogenetic proteins and activates hepcidin<br>Soluble HJV is produced by furin cleavage in hypoxia and iron deficiency and downregulates hepcidin |
| Hemosiderin | Histologic identification of iron stain in tissues |

**TABLE 118-2** Hereditary Hemochromatosis

| Name | Gene | Gene Product | Pattern of Inheritance | Phenotype |
|---|---|---|---|---|
| **HFE-Related HHC** | | | | |
| Type 1 | *HFE,* 6p21.3 | HFE | Autosomal recessive | Variable clinical outcome |
| **Juvenile-Type HHC** | | | | |
| Type 2A | *HJV,* 1q21 | Hemojuvelin | Autosomal recessive | Earlier onset, ↑organ damage |
| Type 2B | *HAMP,* 19q13.1 | Hepcidin | | |
| **TfR2-Related HHC** | | | | |
| Type 3 | *TFR2,* 7q22 | Transferrin receptor 2 | Autosomal recessive | Similar to type 1 |
| **Ferroportin-Related HHC** | | | | |
| Type 4 | *SLC40A1,* 2q32 | Ferroportin | Autosomal dominant | Hepatic type<br>Macrophage type |

HHC, Hereditary hemochromatosis.

on hepatocytes.[18] Hepcidin expression is normally induced by excess iron or inflammation and downregulated by iron deficiency, ineffective erythropoiesis, or hypoxia.[19-21] Persistently high levels of hepcidin are associated with the anemia of chronic inflammation; iron is retained within the enterocytes and macrophages, leading to low transferrin saturation and reduced availability of iron for erythropoiesis. In contrast, inappropriately low hepcidin expression permits increased absorption of iron from the duodenum, high transferrin saturation, and iron overload in sites of tissue storage.

## GENETICS

Four types of HHC have now been described, all of which are linked to gene mutations (Table 118-2). Classic HHC (type 1) is an autosomal recessive disorder caused by mutations of the *HFE* gene located on chromosome 6.[22] The most common mutation is a single amino acid substitution of tyrosine for cysteine at position 282 (C282Y), particularly in people originating from Northern Europe. Homozygosity for this mutation is a risk factor for organ damage as a result of iron overload, although phenotypic expression varies widely. Other mutations of the *HFE* gene include the replacement of histidine with aspartic acid at position 63 (H63D) and the substitution of serine for cysteine at position 65 (S65C). Clinical manifestations of the latter mutations are less significant, although compound heterozygosity may be associated with evidence of iron overload, particularly if comorbid conditions such as excess alcohol intake or steatosis are present.[23,24]

Unlike mutations of the *HFE* gene that may become clinically obvious in middle age, hemojuvelin (HJV-related HHC, type 1A) or hepcidin (HAMP-related HHC, type

2B) mutations result in juvenile HHC (type 2), with evidence of iron overload in the teens or twenties. The affected gene has been mapped to the long arm of chromosome 1.[22] In such cases, the rate of iron accumulation seems to be greater than in adult HHC and is associated with widespread organ involvement and early mortality.[25] In these patients, cardiomyopathy, diabetes mellitus (DM), and hypogonadism are more common than severe liver disease.[22,26] Unlike the association of HHC with people of Northern European ancestry, juvenile HHC has been most frequently reported in Italy.[27] Transferrin receptor (TfR) mutations (TfR2-related HHC, type 3) cause clinical disease similar to classic HFE-related HHC. Such mutations are very rare.[28] Type 4 or ferroportin-related HHC is an autosomal dominant condition that is described in European and Australian families.[29,30] Two types of ferroportin mutations have been reported. In the first type, loss of surface localization of ferroportin results in a decreased ability of cells to export iron, causing iron to build up predominantly in macrophages. In the second type, hepcidin-induced ferroportin dysfunction leads to iron accumulation in parenchymal cells of the liver and other tissues. Phenotypic expression varies, with some patients manifesting the effects of iron overload in a similar manner to classic HHC, and others showing minimal evidence of organ damage.[31]

### Other Inherited Iron-Overload Syndromes

Several other inherited iron-overload syndromes have been described. In people of African descent, a common polymorphism in the ferroportin 1 gene is associated with mild anemia and a tendency to iron loading.[32] Aceruloplasminemia is caused by homozygous mutation of the ceruloplasmin gene and results in anemia, evidence of iron excess, glucose intolerance, and neurologic symptoms.[33] Familial atransferrinemia is an autosomal recessive condition associated with severe anemia in the presence of tissue iron deposition.[34] A number of ferritin mutations have been described that result in hyperferritinemia, some with specific organ damage. Patients with hereditary hyperferritinemia-cataract syndrome have elevated ferritin levels and bilateral congenital cataracts.[35] Neuroferritinopathy is an autosomal dominant movement disorder in which iron preferentially deposits in the brain despite variable serum ferritin levels.[36] In contrast, the entity of benign hyperferritinemia has also been reported, in which, despite a mutation in the coding region of L-ferritin, no clinical manifestations develop.[37]

A congenital form of iron overload is neonatal hemochromatosis in which excess iron deposition results from immune-mediated liver injury and is ameliorated by administration of intravenous Ig during pregnancy.[38]

## EPIDEMIOLOGY

Although HHC was previously thought to be a rare condition, genetic testing has revealed that it is one of the most common heritable disorders. Although 5 out of every 1000 individuals of Northern European origin are homozygous for the *HFE* mutation, phenotypic expression varies, and clinical cases are much fewer in number.[22] In a study of nearly 100,000 individuals from primary care practices in the United States, the prevalence of C282Y homozygosity was as follows: white, 0.44%; Native American, 0.11%; Hispanic, 0.027%; African American, 0.014%; Pacific Islander, 0.012%; and Asian, 0.0004%.[39] Peak age at the time of diagnosis is 40 to 60 years for classic HHC.

## INFLUENCE OF OTHER GENES AND ENVIRONMENT

Clinical manifestations of HHC vary greatly between people with similar mutations, suggesting that other factors influence disease expression. One study demonstrated that among C282Y homozygotes, up to 82% have hyperferritinemia, whereas approximately 28% of males and 1% of females ultimately experience clinical manifestations of HHC—defined as the presence of liver disease, hepatocellular carcinoma, and arthritis of the second and third metacarpophalangeal joints—by age 65 years.[40] In contrast, compound heterozygotes with *C282Y/H63D* mutations exhibit higher serum ferritin and transferrin saturation levels compared with normal control subjects but are at very low risk of clinical HHC.[41]

Other genes may play a role in modifying the phenotypic expression of iron overload. The presence of the gene *CYBRD1*, which encodes duodenal reductase DCYTB, is associated with lower serum ferritin levels in C282Y homozygotes.[42] Furthermore, mutations of other iron-related genes, such as hepcidin, hemojuvelin, haptoglobin, and BMP, may influence disease manifestations.[43-47] In addition, profibrotic genes (e.g., transforming growth factor) may accelerate the onset of cirrhosis in susceptible people.[48]

The reason that HHC disease penetrance is more evident in C282Y homozygote males than females may be explained by recurrent menstrual blood loss and consequent slower accumulation of iron stores in women. However, genetically determined sex differences in ferritin levels may occur because distinct HLA A*03B*07 and A*03B*14 haplotypes have been reported in men and women with clinical evidence of HHC.[49]

Environmental factors, including diet, smoking, alcohol intake, and comorbid diseases, also influence clinical expression of HHC. The metabolic syndrome is associated with insulin resistance–associated iron overload, which, in the presence of HHC, may have a synergistic effect on liver damage.[16] Concurrent liver disease due to hepatitis or steatosis may exacerbate the process of fibrogenesis.[50] In addition to the known toxic effect of alcohol on hepatocytes, it also downregulates hepcidin expression and increases iron absorption, contributing to end-organ damage in patients with innate iron overload.[51,52] Furthermore, meat consumption and high citrus fruit intake also contribute to increased iron loading. However, ingestion of noncitrus fruits may have a protective effect.[53]

## PATHOGENESIS

The clinical expression of HHC may be influenced by several pathophysiologic mechanisms, including increased duodenal absorption of dietary iron; decreased expression of the key regulatory peptide, hepcidin; altered function of the HFE protein; and the direct and indirect toxic effects of iron deposition in target organs.[19] Recent insight has been gained into the function of hepcidin. Human mutations and murine

knockout models of genes for hepcidin, hemojuvelin, TfRs or HFE result in reduced hepcidin levels and increased duodenal iron absorption.[54] The HFE protein also plays a role, and it has been suggested that interactions between HFE and TfR-1 and -2 influences hepcidin expression by acting as a hepatocyte membrane-based iron sensor.[55]

Chronic iron overload is thought to cause tissue damage by several mechanisms, including impairment of mitochondrial function and weakening of lysosomal membranes with consequent discharge of enzymes into the cytoplasm.[56] Increased free radical formation contributes to lipid peroxidation of cell membranes, and profibrogenic cytokines are released.[19] The extent and duration of iron deposition correlate with the development of fibrosis, and it is thought that substantial hepatocyte and Kupffer cell iron accumulation precedes organ damage.[16] In HHC, iron first accumulates in parenchymal cells, with reticuloendothelial (RE) involvement a late feature, in contrast to transfusional iron overload, in which RE cells are primarily targeted. Values of serum ferritin exceeding 1000 μg/L are associated with significantly increased risks of liver fibrosis and cirrhosis.[7,19,57]

## CLINICAL FEATURES

### Extra-Articular Manifestations

HHC presents more commonly in men than in women and typically manifests in middle-aged adults when iron stores gradually accumulate, often reaching 20 to 30 g.[19,40] The classic findings of hepatic abnormalities, skin pigmentation, arthritis, DM, erectile dysfunction, heart enlargement, and/or conduction defects indicate late disease.[58] Organ involvement varies and is unpredictable, although the liver, as the major site of iron storage, is usually affected. Not infrequently, abnormalities of liver enzymes, checked as part of a routine health check, identify the problem.[59] However, with the recent availability of genetic testing, presentation by asymptomatic relatives of probands is now more common.

In the liver, increasing iron deposition results in eventual fibrosis and cirrhosis. It is worth noting that some patients with HHC and iron overload may progress to fibrosis even in the presence of normal levels of serum transaminases.[60] Therapeutic phlebotomy can cause regression of some of these changes, particularly in early disease.[61] Additional causes of significant liver disease are common in patients with HHC and may contribute to hepatocellular damage.[60] In people with established cirrhosis, the risk of malignant hepatoma is 20-fold greater than the general population, with a higher risk in affected men than women.[62,63]

Iron deposition in the heart can result in conduction system abnormalities and heart failure. Several large population studies of HHC and atherosclerosis have failed to find a link. However, elevated ferritin levels, particularly in the setting of nonalcoholic fatty liver disease, may be associated with vascular damage via hepcidin upregulation.[64-66] Glucose intolerance tends to be a late finding in HHC and is due to progressive iron accumulation in pancreatic β cells, causing low C-peptide and insulin levels.[67] α Cell function is usually preserved, and serum glucagon levels are normal or increased. In patients with HHC who present symptomatically, DM has been noted in approximately 50%. However, for patients with HHC who have early insulin resistance and no evidence of cirrhosis, venesection may reverse iron deposition–induced β cell dysfunction.[68,69] The risk of DM is higher in C282Y heterozygotes with no clinical evidence of HHC compared with control subjects.[70,71]

Pituitary involvement in HHC is due to iron deposition causing reduced serum levels of secreted hormones, mainly from the anterior aspect of this gland, resulting in loss of libido and erectile dysfunction.[72,73] Primary hypogonadism from testicular iron overload is much less common.[74] Therapeutic phlebotomy may reduce these symptoms, particularly in younger men.[75] Hypothyroidism in HHC is thought to be due to a direct toxic effect of iron on thyroid cells and is associated with low thyroxine and elevated thyroid-stimulating hormone.[76] Such endocrine abnormalities may contribute to the development of osteoporosis in these individuals.

Skin discoloration occurs as a result of extra melanin and iron in the epidermis. It is a late finding, and the development of "bronze diabetes" represents the end stage of years of iron accumulation in the tissues.

Patients with HHC have increased susceptibility to certain infections. High serum iron concentrations may increase bacterial virulence, and excess iron in macrophages is thought to reduce phagocytosis.[77] Particular caution is advised with uncooked seafood because of the risk of septicemia from *Vibrio vulnificus*. In addition, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Salmonella enteritidis* serotype *typhimurium*, *Klebsiella pneumoniae*, *Escherichia coli*, *Rhizopus arrhizus*, and *Mucor* species all have been reported to cause severe illness in patients with iron overload.[9]

Apart from the increased risk of malignant hepatoma in patients with established cirrhosis, an independent association of nonhepatic cancers, particularly breast and colorectal tumors, in HFE C282Y homozygotes has been reported.[78] H63D homozygosity confers a threefold increased risk of colorectal cancer in carriers of the MMR gene mutation.[79] Iron is potentially carcinogenic via several mechanisms, including its immunosuppressive properties and its role as an essential cofactor for tumor cell growth and in catalyzing the formation of hydroxyl radicals. Furthermore, cancer risk is lower with reduced iron stores.[80]

### Articular Features

Arthralgia/arthritis is a common presentation in HHC, affecting 50% to 80% of patients and significantly interfering with quality of life.[81-87] Although it tends to be a late feature, joint pain nevertheless may be the presenting symptom of HHC, alerting a diligent physician to the presence of an underlying metabolic disorder. Articular involvement may be widespread, but changes to the second and third metacarpophalangeal joints are most characteristic[87] (Figures 118-1A and B). Arthritis may be present in the proximal interphalangeal joints, wrists, shoulders, hips, knees, and ankles.[86] Patients notice pain and stiffness of the involved joints, but clinical evidence of synovitis is usually absent. Hip damage develops in approximately 25% of people with HHC, and after hip arthroplasty, the risk of aseptic loosening of the prosthesis is increased.[88,89] The differential diagnosis of HHC-related arthropathy includes severe osteoarthritis, rheumatoid arthritis, other forms of

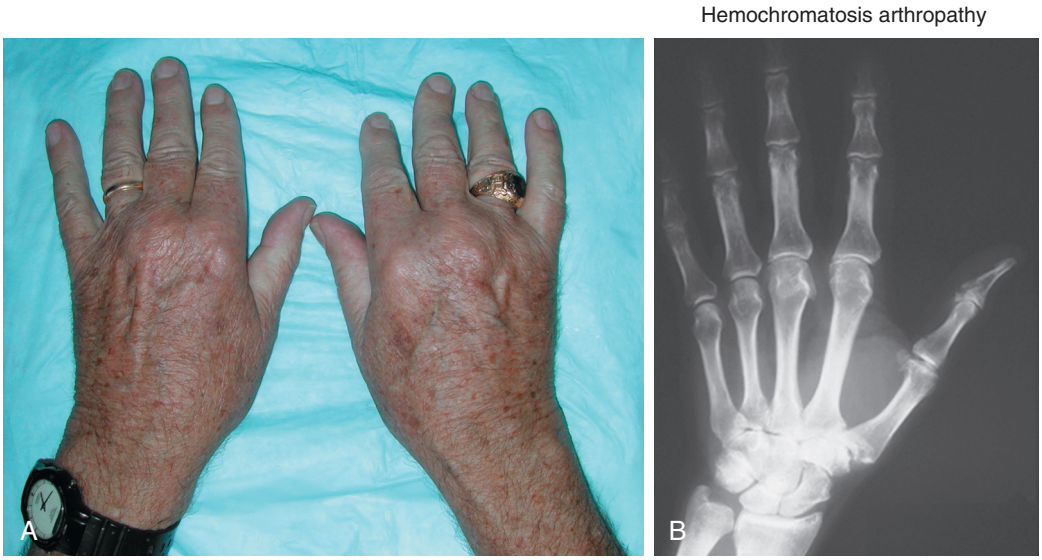



**Figure 118-1** Arthritis of the second and third metacarpophalangeal joints, characteristic of hereditary hemochromatosis.

inflammatory arthritis, and crystal arthritis. Rheumatoid factor is typically negative, however, and in established cases, radiographs show distinctive findings, such as joint space narrowing of the second and third metacarpophalangeal joints, hook-like osteophytes on the radial aspect of the metacarpal heads, and chondrocalcinosis, particularly of the triangular fibrocartilage adjacent to the ulnar styloid. HHC-related arthritis is more common in male C282Y homozygotes than in other genotypes.[7]

The pathogenetic mechanisms underlying HHC-related arthritis are unknown, and the prevalence of joint pain in this condition has not been found to correlate with body iron stores. Toxic effects from local iron deposition, acceleration of cartilage defects, and immunologic mechanisms have been implicated.[83,84,90] Under light microscopy, the involved synovium demonstrates iron deposits, particularly in the lining cells, but inflammatory cell infiltration is not typical.[91-93] Neutrophil infiltration of the synovial sublining layer has been described.[94] Apatite and calcium pyrophosphate dihydrate crystals may be observed, but it is not known why they are preferentially expressed in HHC. In association with the increased incidence of calcium pyrophosphate dihydrate deposition disease in HHC, a putative role for a parathyroid hormone fragment (PTH 44-68) has been suggested.[95] Joint symptoms due to HHC tend not to improve after therapeutic phlebotomy.

**TABLE 118-3** Differential Diagnosis of Iron Overload

| |
|---|
| **Hereditary Hemochromatosis** |
| HFE-associated hemochromatosis |
| C282Y homozygosity or C282Y/H63D heterozygosity (type 1) |
| Non–HFE-associated hemochromatosis |
| Hemojuvelin/hepcidin mutations (type 2) |
| Transferrin receptor 2 mutations (type 3) |
| Ferroportin mutations (type 4) |
| Other |
| African iron overload syndrome |
| Aceruloplasminemia |
| Familial atransferrinemia |
| Hyperferritinemia-cataract syndrome |
| Neuroferritinopathy |
| **Secondary Iron Overload** |
| Iron-loading anemias |
| Ineffective erythropoiesis |
| • Thalassemias/sideroblastic anemia/myelodysplasia |
| Increased erythropoiesis |
| • Chronic hemolytic anemia |
| Parenteral iron overload |
| Multiple blood transfusions |
| Multiple iron injections |
| Chronic liver disease |
| Hepatitis B and C |
| Alcoholic liver disease |
| Steatosis |
| Porphyria cutanea tarda |

## DIFFERENTIAL DIAGNOSIS

Because of the lack of a physiologic mechanism to enhance iron excretion, the inevitable result of increased iron absorption is iron overload, which may be due to inherited defects in iron metabolism, as previously described, or secondary to other causes (Table 118-3). A full history of medication use, including over-the-counter iron tablets, and a review of any blood transfusions should be elicited. Ineffective erythropoiesis in conditions such as the thalassemias or sideroblastic anemia also results in the buildup of iron stores. Porphyria cutanea tarda (PCT) is associated with hyperferritinemia, high transferrin saturation levels, and an increased incidence of *HFE* mutations.[96] However, the clinical findings, particularly dermatologic findings, distinguish this disease from HHC. Elevated ferritin levels may result from severe inflammation. IL-6 is a potent stimulator of hepcidin via the STAT3 pathway, resulting in reduced iron absorption and elevation of tissue and serum ferritin levels, although these changes are accompanied by low transferrin saturation.[97] Chronic liver disease resulting from hepatitis, alcohol

excess, or fatty infiltration is associated with hyperferritinemia and normal or elevated transferrin saturation values.[98] Alcohol, independent of liver damage, induces ferritin synthesis and iron overload in people who consume more than two alcoholic drinks per day.[63,99]

## DIAGNOSTIC TESTS

A high index of suspicion is helpful when a patient presents with joint pain and abnormal liver enzymes. Although the differential diagnosis is wide, the presence of elevated ferritin and transferrin saturation levels (serum iron × 100/total iron-binding capacity) strongly points to the answer. Serum iron should be measured with the patient fasting because concentrations may be increased after a meal. High ferritin levels also may be caused by systemic inflammation or malignancy, but these conditions tend to be associated with reduced transferrin saturation. Other causes of elevated transferrin saturation include high serum iron as a result of hepatic cytolysis or low transferrin levels as a result of liver failure, and these possibilities should be excluded. If ferritin measures greater than 200 μg/L and transferrin saturation is greater than 45%, genetic screening is recommended. The finding of homozygosity for the C282Y mutation or compound heterozygosity for C282Y/H63D confirms the diagnosis. However, if these mutations are not present and evidence of iron overload exists, investigation for more rare genetic defects, such as alterations of the genes that encode hemojuvelin, hepcidin, TfR2, or ferroportin, can be considered in the context of the pertinent clinical features (see Table 118-2).

A liver biopsy may be considered for prognostic purposes in established cases. Patients most likely to show evidence of cirrhosis are those who have the combination of a ferritin level greater than 1000 μg/L, elevated transaminases, and thrombocytopenia.[57,100] In contrast, patients with a serum ferritin level less than 1000 μg/L and no additional risk factors for liver disease are unlikely to have cirrhosis. A liver biopsy is therefore considered for those with advanced disease or if the diagnosis is in question, particularly in people with evidence of iron overload who are HFE negative. HHC can be distinguished histologically from alcoholic cirrhosis by the preferential distribution of iron in the hepatocytes in the former and in the Kupffer cells in the latter.[101] MRI of the abdomen also can be used to determine iron overload in the internal organs. Gradient T2-weighted sequences show decreased signal intensity and correlate highly with liver iron concentrations.[102,103] This imaging method also can identify other locations of iron deposition (e.g., in the spleen, pancreas, lymph nodes, and heart). Because HHC is a systemic condition, other investigations should include a search for diabetes, thyroid disease, hypogonadism, osteoporosis, and cardiomyopathy. Disease mimickers, such as PCT, ineffective erythropoiesis, chronic alcohol excess, and the metabolic syndrome should be excluded.

## SCREENING

Greater disease awareness and the availability of genetic screening have meant that HHC is now likely to be diagnosed before the classic triad of cirrhosis, DM, and skin hyperpigmentation develops. However, late presentation can occur, particularly in people with additional risk factors for iron overload or liver disease. For individuals in whom genetic testing has identified a risk for HHC, but with no clinical evidence of disease, yearly biochemical screening is recommended, measuring serum ferritin, transferrin saturation, and liver enzymes. Such monitoring facilitates early detection of organ compromise and timely initiation of treatment.

### Population Screening

HHC is an attractive target for population screening because of its high prevalence, potential disease severity, availability of cost-effective treatment, and impact of early diagnosis on the morbidity and mortality of affected patients. However, the clinical penetrance of HHC is low and the negative consequences (e.g., insurance related and psychological) of finding an associated gene mutation in an asymptomatic person without any evidence of disease would be considerable. General screening for HHC is therefore not recommended.[19,102,104]

### Screening People with a Positive Family History

Individuals with a first-degree relative who has been diagnosed with HHC should be tested for *HFE* mutations and evidence of iron overload.[19] For children of a patient with HHC, it is recommended that the other parent be screened first, because offspring will be affected only if both parents carry a relevant mutation. In first-degree relatives of C282Y homozygotes, the degree of iron overload is associated with disease severity in the proband.[105] The risk of clinically relevant iron toxicity is extremely low for people who are found to have C282Y or H63D heterozygosity. Infrequently, H63D homozygosity is associated with mild iron excess.[24]

### Screening in Patients with Related Diseases

The European Association for the Study of Liver Diseases has recommended HFE testing in patients with unexplained chronic liver disease and raised transferrin saturation levels (see Figure 118-2). Furthermore, HFE testing should be considered in people with PCT, well-defined chondrocalcinosis, and hepatocellular carcinoma and those with late-onset type 1 DM because C282Y homozygosity has a higher prevalence in these conditions.[102] HHC-related genotypes are a risk factor for the development of liver disease in the presence of significant comorbid illness, particularly chronic infectious hepatitis, PCT, or steatosis.[19,106]

## TREATMENT

Removal of iron from the body can be undertaken by phlebotomy, erythrocytapheresis, or the use of iron chelators. Treatment is divided into the "depletion" stage, which aims to reduce ferritin levels to normal, and the "maintenance" stage, in which the frequency of iron removal is decreased and ferritin levels are monitored. Some, but not all, consequences of iron deposition are reversed by removing iron. Most patients note an improvement in well-being and skin

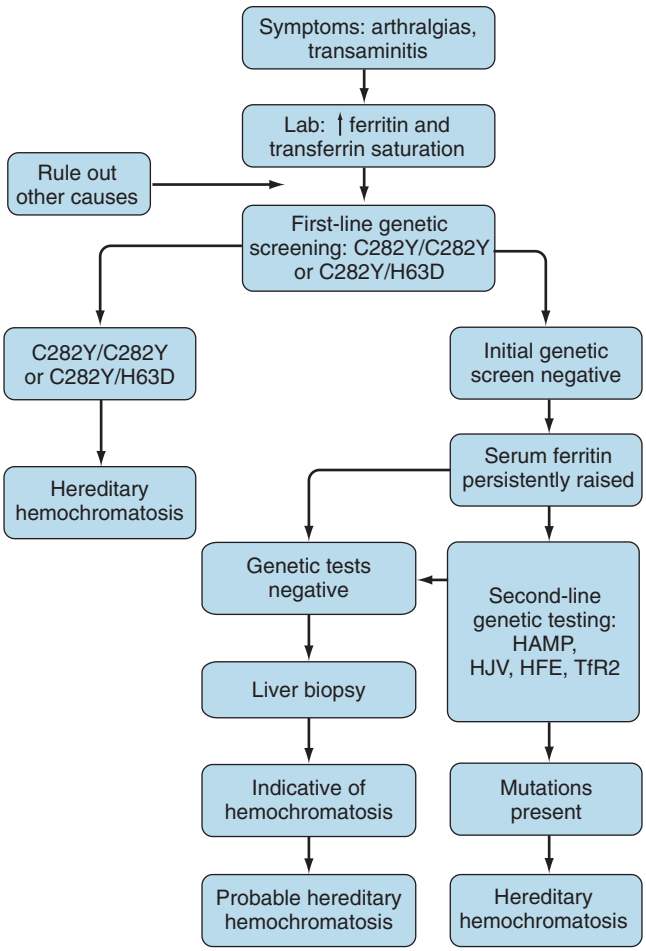



**Figure 118-2** Algorithm for the diagnosis of hereditary hemochromatosis.

pigmentation; furthermore, amelioration of cardiac function and glucose control, in addition to hepatic fibrosis, has been documented.[61,102] However, symptoms of arthritis do not change, and established cirrhosis is irreversible.[19]

Phlebotomy is the mainstay of treatment for iron overload in the setting of HHC. Every 500 mL of whole blood contains up to 250 mg of iron, and thus it can take more than 1 year for iron stores to normalize with weekly venesection. It is recommended that treatment be started when serum ferritin levels are consistently and progressively above normal local reference ranges.[104] Weekly phlebotomy of 500 mL of blood should be guided by ferritin and hemoglobin/hematocrit levels, aiming for serum ferritin of 50 µg/L in the absence of anemia. Transferrin saturation levels are not an accurate measure of therapeutic efficacy because they are relatively resistant to change in iron stores in C282Y homozygotes.[107] In the maintenance phase, the frequency of venesection depends on the response to treatment. Anemia should be avoided because it may trigger further lowering of hepcidin levels and encourage intestinal iron absorption.[104] Although generally safe and well tolerated, adverse effects of blood removal in HHC include cardiac arrhythmias that may develop during rapid mobilization of iron stores.[108] Vitamin C supplementation may exacerbate such problems by facilitating iron release and increasing pro-oxidant and free radical activity.[19,108,109] In many countries, therapeutic phlebotomy from patients with HHC may contribute to the blood donation pool.[102,107]

Erythrocytapheresis removes more red blood cells per session than phlebotomy and preserves platelets, plasma proteins, and coagulation factors.[110] The target of normal iron levels is achieved more rapidly with this technique in comparison with phlebotomy, but the financial costs are greater. The additional technical expertise means that erythrocytapheresis is not commonly used to treat iron overload in individuals with HHC.[107]

Iron chelators may be administered orally (e.g., deferasirox) or parenterally (e.g., deferoxamine). However, these drugs are associated with more adverse effects than phlebotomy and thus are reserved for patients who are intolerant of regular venesection.[107,111] Proton pump inhibitors (PPIs) can potentially contribute to reduced iron absorption because reduced gastric acid secretion lowers the solubility of inorganic iron, thereby decreasing its absorption. Furthermore, they may influence iron uptake into red blood cells, a process that requires proton cotransport.[107] However, currently no evidence shows that chronic use of PPIs significantly affects iron levels in patients with HHC.

High doses of vitamin C facilitate iron release and increase pro-oxidant and free radical activity.[19,108,109] Therefore patients with HHC should not take extra vitamin C but can continue to eat fresh produce containing this substance. Other dietary recommendations include reduction or avoidance of food containing high doses of iron, such as red meat and internal organs. Uncooked shellfish is a particular hazard because of the risk of contamination with *V. vulnificus*.[112] Some alcoholic drinks contain iron, and all are potentially hepatotoxic. Alcohol should be consumed only occasionally because it seems to have a synergistic effect in the presence of iron overload on the development of cirrhosis and hepatocellular carcinoma.[113] Maintenance of normal body weight is important to avoid the hepatic damage associated with steatosis.

Just as the pathogenesis of joint pain in HHC is unclear, treatment of arthritis in this condition is unsatisfactory, and joint symptoms may progress despite effective phlebotomy. NSAIDs, colchicine, and intra-articular corticosteroids may be helpful in some cases. Osteoporosis is a potential disease complication, particularly in the setting of hypogonadism or reduced thyroid function. Hormone replacement, if indicated, should be instituted, although some patients may require additional treatment with calcium and bisphosphonates.

Although animal studies suggest that iron overload contributes to an overall risk of carcinogenesis, little epidemiologic information to date suggests that this phenomenon applies to humans with HHC. Nevertheless, age-appropriate screening for malignancy may be considered in the holistic approach to such patients.[114]

## OUTCOME

The earlier HHC is diagnosed, the better is the prognosis, because morbidity and mortality are directly related to the extent of iron overload and consequent organ compromise. The absolute risk of liver damage is approximately 5% in

homozygous males and 1% in females.[115] However, the development of cirrhosis is a serious indicator of reduced longevity. For patients with HHC-related hepatic failure who undergo liver transplantation, survival rates are lower compared with people who receive liver transplants for other reasons. Post-operative death in these circumstances is typically due to cardiac complications or infection.[116]

In the absence of cirrhosis or diabetes, patients with HHC have a normal life expectancy.[104] Given the importance of timely recognition of this common metabolic problem, a vigilant physician can make an enormous difference in the lives of patients who present with early symptoms of this disease. In this context, the rheumatologist has a particularly relevant role in keeping a high index of suspicion for the diagnosis of HHC in patients with atypical osteoarthritis or chondrocalcinosis.

## CONCLUSION

Cost-effective screening focused on people at greatest risk of clinical disease, in association with a high level of diagnostic suspicion in patients with relevant symptoms and signs, should reduce the number of patients presenting with irreversible organ damage from HHC. In addition, greater understanding of the genetic and environmental modifiers of clinical disease expression may help to delay or mitigate the consequences of iron overload in susceptible individuals. How comorbid disease influences the onset and extent of disease is particularly relevant in the context of the growing numbers of patients with obesity, metabolic syndrome, and hepatic steatosis. Although phlebotomy has been the mainstay of treatment for many years, exact targets for iron stores and ferritin levels warrant further investigation. New chelators are under review, and the development of hepcidin peptides/agonists, which may be able to correct the physiologic deficit linked to increased iron absorption and deposition, hold promise for the future and may contribute to our understanding of clinical penetrance in patients with genetic susceptibility to iron overload.[104] In the meantime, the predictive value of symptom combinations in relevant people may influence screening guidelines and facilitate a cost-effective way of reducing the disease burden in populations at risk of HHC.[13]



The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 119

# Hemophilic Arthropathy



Lize F.D. van Vulpen • Goris Roosendaal • Roger E.G. Schutgens • Floris P.J.G. Lafeber

**KEY POINTS**

- The most common rheumatologic complication in severe hemophilia is hemophilic arthropathy, which mainly affects the elbows, knees, and ankles.
- A combined cascade of degenerative and inflammatory processes initiated by recurrent joint bleeds leads to hemophilic arthropathy.
- Other musculoskeletal complications of hemophilia are muscle and soft tissue hemorrhage, chronic synovitis resulting from the inflammatory processes, pseudotumors, and osteoporosis.
- Treatment aims at prevention of recurrent hemarthrosis through prophylactic factor replacement therapy.
- If conservative measures fail, orthopedic surgery, including total joint replacement, is indicated and can be performed safely with clotting factor replacement therapy.

Spontaneous joint bleeding is a characteristic manifestation of the inherited coagulation disorder hemophilia,[1] but such bleeding also can occur as a consequence of other bleeding disorders such as von Willebrand disease,[2] as a complication of anticoagulant treatment,[3] or upon experiencing trauma[4] or undergoing major joint surgery.[5] Irrespective of the underlying cause, hemarthrosis can lead to significant joint tissue damage and subsequent major morbidity.

Hemophilia is an X-linked recessive coagulation disorder caused by deficiency of coagulation factor VIII (hemophilia A) or factor IX (hemophilia B; Christmas disease). Hemophilia has an estimated frequency of 1 in 5000 to 10,000 male births,[6,7] with approximately 80% to 85% representing hemophilia A. The lack of clotting factor activity leads to inadequate thrombin generation and a tendency for bleeding to occur. The clinical phenotype is strongly correlated with the amount of clotting factor present, expressed as a percentage compared with normal clotting factor activity in blood. Patients with severe hemophilia have less than 1% clotting factor activity, whereas patients with moderate and mild hemophilia have clotting factor activity of 1% to 5% and 5% to 40%, respectively.[8] Spontaneous bleeding mainly affects patients with severe hemophilia; it occurs less frequently in individuals with moderate hemophilia, and patients with mild hemophilia experience bleeding only after sustaining a major trauma or undergoing surgery. The phenotype is also affected by several other mechanisms, including genetic factors, age at the first episode of joint bleeding, and concomitant thrombotic factors.[9,10] In individuals with severe hemophilia, 70% to 80% of all bleeding events occur in the joints, spontaneously as well as in response to stress or trauma.[11,12] The predilection for bleeding in the joint compared with other tissue sites might be caused by the relatively low expression of tissue factor, which is the initiator of the coagulation cascade,[13,14] together with a high level of tissue factor pathway inhibitor in the normal joint[15] and increased local fibrinolysis in the hemophilic joint.[16] On the other hand, mechanical factors damaging the richly vascularized synovial tissue, specifically after being triggered after a first joint bleed, might be causative in this respect.[17]

Musculoskeletal bleeding and treatment-related complications are the most important complications in hemophilia. Development of alloantibodies directed against administered clotting factor (inhibitors) is common, and the transmission of blood-borne virus infections as a result of the use of plasma-derived products in the 1980s caused major morbidity.

## CLINICAL FEATURES

Bleeding into the musculoskeletal system causes a spectrum of clinical features with involvement of joint, muscle, and bone. The distribution of the most affected joints and muscles is shown in Figure 119-1.

### Acute Hemarthrosis

Nearly all patients with severe hemophilia and half of patients with moderate disease activity experience hemarthroses. The most affected joints are the large synovial joints, especially the ankles, knees, and elbows.[12] The hips and shoulders are affected to a lesser extent, and bleeds in smaller joints are rare. In individuals with severe hemophilia the first joint bleed often occurs when they begin walking or running, at a median age of 1.8 years.[10] At this age, early signs of bleeding include irritability and decreased use of the affected limb. Older children and adults frequently describe the onset of a joint bleed as a tingling sensation and tightness within the joint, followed by rapid swelling, loss of range of motion (ROM), pain, and warmth of the skin over the joint.[1] Flexion is the most comfortable position, and disuse (i.e., preventing movement to avoid pain) can cause secondary muscle spasm. Pain rapidly

reduces after clotting factor replacement, with full recovery of joint function within 8 to 24 hours.

Joint bleeds often have a migratory pattern, moving from one joint to the other.[12] A so-called "target joint" develops in about 25% of patients with severe hemophilia—that is, a joint that is more susceptible to subsequent bleeds compared with other joints. It is defined as a joint in which three or more hemarthroses have occurred in the prior 6 months.[17,18] Nowadays, in youngsters with hemophilia, the target joint mostly involves the ankle joint rather than the elbow or the knee, with the latter most commonly affected before the introduction of prophylactic clotting factor replacement therapy.

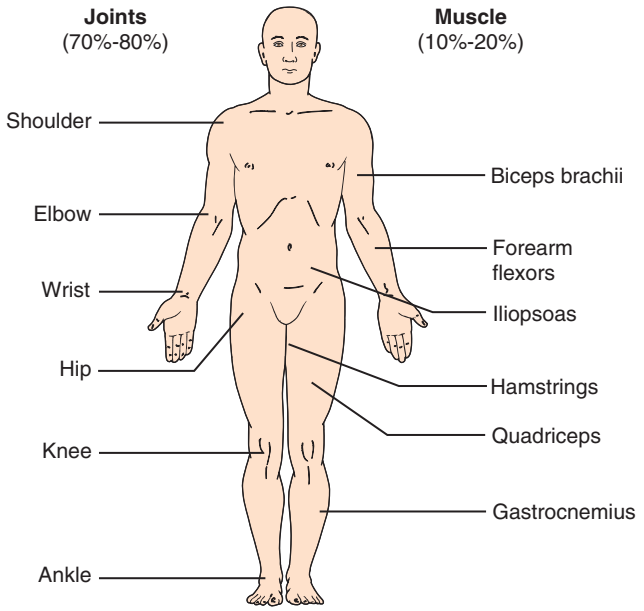



**Figure 119-1** Joints and muscles commonly affected by bleeding in hemophilia. Besides hemarthrosis and muscle bleeding, 5% to 10% of the episodes consist of other major bleeds (e.g., mucous membranes and gastrointestinal), and fewer than 5% of the bleeds occur in the central nervous system.

## Synovitis

Recurrent bleeding into a joint can lead to a vicious circle in which the synovial tissue is unable to remove blood remnants completely, triggering synovial inflammation and proliferation.[19,20] It also induces the development of a rich network of new, fragile blood vessels underneath the synovial tissue, making the joints vulnerable to subsequent (repeated) bleeding.[21] In this condition the joint appears swollen but usually not tense, is often painless, and is only slightly warm. In early stages of synovitis, ROM is preserved, but in chronic stages a mild limitation and flexion deformity can develop. Administration of clotting factor is considered necessary to break the self-perpetuating cycle of hemarthrosis-synovitis-hemarthrosis and to prevent the progressive degeneration of the joint. In contrast to its effect on acute hemarthrosis, clotting factor replacement does not modify the clinical findings immediately, and long-term treatment is indicated.[1]

## Hemophilic Arthropathy

Ultimately, recurrent joint bleeding may lead to hemophilic arthropathy (Figure 119-2). The number of hemarthroses required to cause irreversible damage is unknown and is likely to differ between patients. Progressive degeneration of cartilage, synovial inflammation, and bone changes cause chronic pain, joint stiffness, and a severely limited ROM. Without proper physiotherapy it is often accompanied by muscle weakness and contractures. Joint deformity, subluxation, joint laxity, malalignment, and spontaneous arthrodesis develop in the most severe cases. Physical activity and quality of life are severely affected by hemophilic arthropathy.[22] Progressive synovial fibrosis decreases the frequency of hemarthroses in end-stage arthropathy.

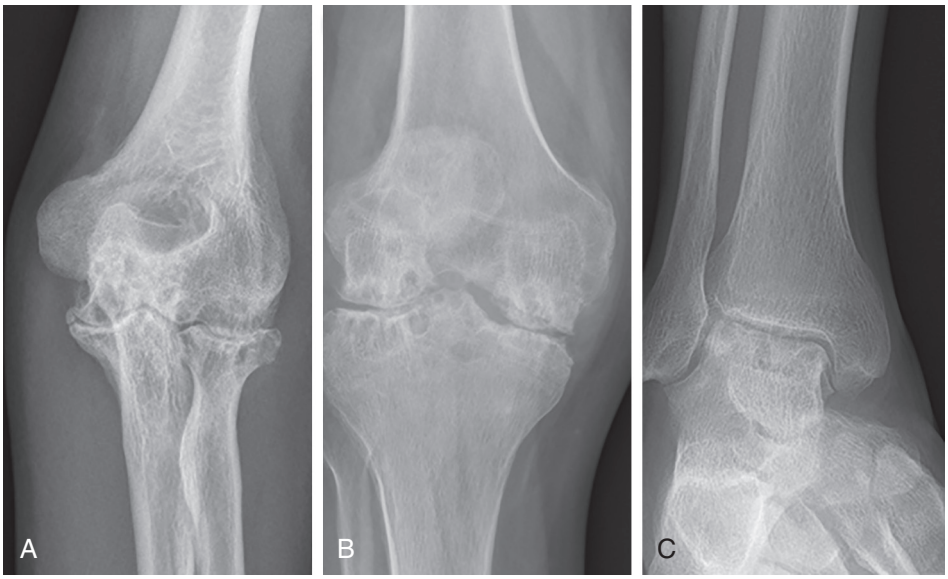



**Figure 119-2** Radiographic changes of hemophilic arthropathy in a 30-year old patient with severe hemophilia. **A,** Left elbow. **B,** Right knee. **C,** Right ankle. All of the radiographs show joint space narrowing and subchondral cysts, which are most pronounced in the elbow and knee.

### Muscle and Soft Tissue Hemorrhage

The muscle is the second most common site of bleeding in individuals with hemophilia, accounting for approximately 10% to 20% of bleeds.[1] The bleeding usually results from a direct blow or sudden stretch and can occur in any muscle of the body. The clinical features depend on the muscle involved, but overall muscle bleeding emerges more insidiously than do hemarthroses, and prodromal symptoms are rare. A tender swelling in the muscle with severe pain upon stretching or active contraction rapidly progresses into protective spasm and a flexed position. Physical examination shows a palpable tender hematoma with swelling, warmth, and bruising. Bleeding into deeper compartments can be difficult to diagnose because visible symptoms are lacking. An iliopsoas hemorrhage usually presents with pain in the lower abdomen, groin, and/or lower back, with pain on extension but not rotation of the hip.[23] A life-threatening complication of ongoing bleeding is compartment syndrome with femoral neuropathy and possible muscle necrosis. Other sites of muscle bleeding associated with neuropathy are the posterior compartments of the lower leg and the flexor group of the forearm. Muscle hemorrhage can result in permanent contracture, rebleeding, and the formation of pseudotumors.[24]

### Pseudotumor

The formation of pseudotumors is a rare but serious complication unique to hemophilia that mainly occurs after inadequate replacement therapy or after development of an inhibitor. Repeated and unresolved muscle bleeding or a progressive subperiosteal hemorrhage causes an expanding encapsulated and calcified hematoma with radiographic features mimicking tumor-like conditions; hence, they are called pseudotumors (Figure 119-3).[1,25] Progressive enlargement of the pseudotumor can lead to erosion of the adjacent bone, compression of neurovascular structures, spontaneous rupture, fistula formation, and pathologic fractures. Pseudotumors have two distinct pathologic forms. In adults, pseudotumors occur proximally, (mainly in the pelvis or femur) expand slowly, and usually require surgical excision. In contrast, pseudotumors affecting young patients occur distal to the elbow or knee, result from direct trauma, develop rapidly, and often are amenable to conservative treatment with immobilization and factor replacement.[26]

### Osteoporosis

Patients with hemophilia have a significantly lower bone mineral density compared with age-matched control subjects.[27] The pathogenesis is multifactorial, with several predisposing factors occurring frequently in the hemophilic population: reduced weight-bearing activity, arthropathy, muscle atrophy, a lower body mass index, presence of an inhibitor, and the influence of blood-borne virus infections and their treatment.[28,29] To maintain bone mass, weight-bearing activities should be promoted and supplementation of calcium and vitamin D in selected cases should be considered.[1]

## DIAGNOSIS

In the majority of patients, the diagnosis "hemophilia" is made in the presence of a positive family history. Up to one-third of the patients have a negative family history, and the disease is the result of a de novo germline mutation, but somatic mosaicism in a (grand)parent also has been described.[30,31] Clinical features raising suspicion of hemophilia are easy bruising in early childhood, spontaneous bleeding (particularly into the joints, muscles, and soft tissues), and excessive bleeding after trauma or surgery. Screening tests in patients with a bleeding diathesis of unknown cause include platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT). Individuals with hemophilia characteristically have a normal platelet count, normal PT, and a prolonged aPTT, although the aPTT might be near normal in individuals with mild hemophilia. Specific clotting factor assays are performed to determine the type and severity of hemophilia and to distinguish it from von Willebrand disease. The diagnosis can be confirmed by DNA-based techniques. Given the rarity and complexity of the disease, a doctor specializing in hemostasis should be consulted.

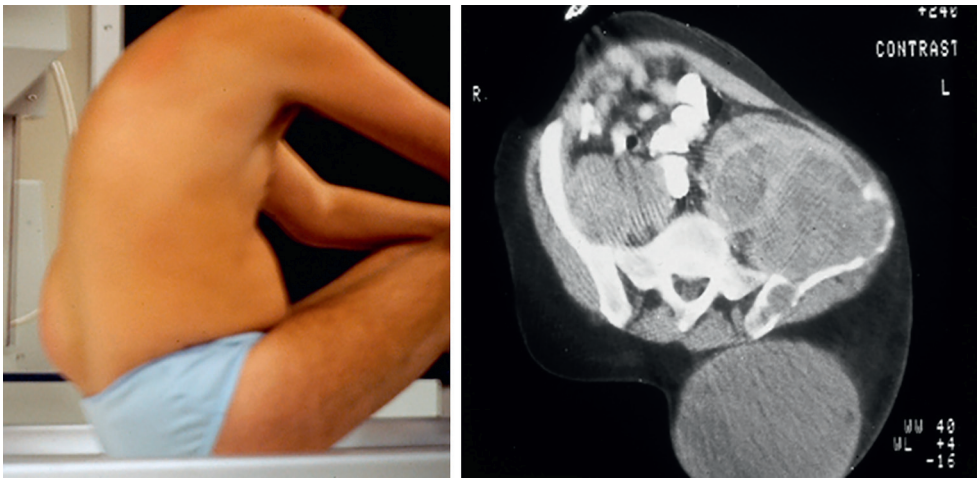



**Figure 119-3** A pseudotumor invading the left wing of the ilium in a patient with severe hemophilia and an inhibitor.

Frequent monitoring of musculoskeletal status is indicated in patients with hemophilia to diagnose complications at an early stage because prevention is the cornerstone of treatment. Use of physical examination assessment tools can help identify subtle early signs of joint damage, monitor joint health over time, and evaluate treatment efficacy. Several hemophilia-specific instruments have been developed and validated.[32-34]

Clinical assessment of the musculoskeletal system at the time of an acute bleed should include evaluation of warmth, bruising, swelling, tenderness, muscle tone, pain, ROM, gait, and function.[1,35]

## Conventional Radiography

Conventional radiology is most frequently used for imaging in hemophilia and is mainly suitable for late osteochondral changes. Radiologic classification of hemophilic arthropathy is usually performed via the Pettersson score (Table 119-1).[36] This additive scoring system is based on assessment of the knees, ankles, and elbows, with eight criteria being scored: osteoporosis, epiphyseal enlargement, irregularity of the subchondral surface, joint space narrowing, subchondral cyst formation, erosion of joint margins, incongruence of the articulating bone ends, and joint deformity. The Pettersson score correlates well to function[37] but is only able to diagnose late arthropathic changes. Typically it is used to evaluate progression of arthropathy and for planning of arthrodesis or joint replacement. It has been discussed whether digital scoring systems are more objective and have a higher interobserver variability than the Pettersson method. Digital analysis of hemophilic arthropathy of the knee is feasible,[38] but adaptation for the specific characteristics of hemophilic arthropathy is necessary.

Radiography has poor sensitivity in demonstrating early soft tissue changes that occur before irreversible cartilage and bone damage. Acute hemarthrosis shows as joint effusion and displacement of fat pads, but it is difficult to distinguish effusion from synovial hyperplasia.

**TABLE 119-1** Pettersson Score[36]

| Joint: Elbow/Knee/Ankle | | Score |
|---|---|---|
| Osteoporosis | Absent<br>Present | 0<br>1 |
| Enlargement of epiphysis | Absent<br>Present | 0<br>1 |
| Irregularity of subchondral surface | Absent<br>Partially involved<br>Totally involved | 0<br>1<br>2 |
| Narrowing of joint space | Absent<br>Joint space >1 mm<br>Joint space <1 mm | 0<br>1<br>2 |
| Subchondral cysts formation | Absent<br>1 cyst<br>>1 cyst | 0<br>1<br>2 |
| Erosion of joint margins | Absent<br>Present | 0<br>1 |
| Gross incongruence of articulating bone ends | Absent<br>Slight<br>Pronounced | 0<br>1<br>2 |
| Joint deformity (angulation and/or displacement) | Absent<br>Slight<br>Pronounced | 0<br>1<br>2 |
| Total (maximum 13 points per joint) | | |

Radiographs of the six index joints (both knees, both ankles, and both elbows) are scored separately. The sum of these scores, with a maximum of 78 per patient, is the Pettersson score.

## Magnetic Resonance Imaging

As prophylactic treatment improves and joint damage diminishes, more sensitive MRI has obvious advantages compared with radiography in evaluating treatment efficacy. MRI enables visualization of small alterations, such as hemosiderin depositions, synovial hypertrophy, and minor cartilage damage without joint space narrowing.[39] The clinical implications of small changes, especially in the absence of reported joint bleeds, remains to be established,[40] but as in osteoarthritis (OA), it may be predictive of subsequent rapid progressive damage. MRI can also provide more detailed information about advanced changes, such as erosions, subchondral cysts, and cartilage destruction. It is valuable in detailed evaluation of a pseudotumor, synovitis, or diagnosis and follow-up of a hemorrhage in deeper compartments (e.g., the abdomen and iliopsoas). Widespread use of MRI is restrained by its costs, availability, and requirement of sedation in young children.

## Ultrasonography

With ultrasonography becoming increasingly standard in rheumatology practices, it is possible to diagnose joint effusion, synovial hypertrophy, abnormalities involving osteochondral surfaces, pseudotumors, and acute hemarthroses.[39,41] Ultrasonography is less costly than MRI, is readily available, does not require sedation in young children, and enables dynamic investigation such as assessment of vascularity of a pseudotumor. Disadvantages are the interobserver variability, complexity of image analyses, difficulty in identifying changes in deeper structures, and a lack of validated scoring systems for hemophilic arthropathy.

## Biochemical Markers

In the field of OA and rheumatoid arthritis (RA), considerable effort is directed toward the identification of biochemical markers in blood and/or urine that can assist in diagnosis, prognosis, and response to treatment.[42] Such markers are as yet sparsely used in clinical trials and still are not used in clinical practice. Also, in the field of hemophilic arthropathy, such markers may become helpful in identifying the tissue-destructive activity of a joint bleed.[43,44]

# PATHOGENESIS

Although it is clear that the presence of blood has devastating effects on the joint, the exact pathogenetic mechanisms of hemophilic arthropathy, and in particular the earliest changes induced by acute hemarthrosis, are not completely understood. Surgical specimens of end-stage arthropathy in patients with hemophilia, together with in vitro experiments and animal models, have indicated that

two major processes are involved: synovial inflammation and cartilage degeneration (Figure 119-4).[20,45] In this respect, hemophilic arthropathy has characteristics of both the RA and the OA joint (Figure 119-5).[46]

## Synovial Inflammation and Proliferation

Synovial tissue is highly vascularized, and in hemophilia even minimal forces can lead to hemarthroses. Filling of the joint space with blood leads to an influx of inflammatory cells into the synovial tissue. Synoviocytes and invading macrophages evacuate blood from the joint cavity completely over a period of 3 to 4 weeks.[17] Four hours after the induction of hemarthrosis, erythrophagocytosis already can be observed.[47] Adaptive changes in the expression of iron regulation proteins in the synovium of patients with hemophilia after repeated joint bleedings are demonstrated.[48] With successive hemarthroses the synovial capacity is overloaded and blood remnants, especially iron, accumulate as hemosiderin depositions in synovial tissue and even cartilage.[19] The presence of iron triggers an inflammatory response and stimulates proliferation of synoviocytes (Figure 119-6). Pro-inflammatory cytokines, including IL-1β, IL-6, and TNF, are produced by the iron-laden synoviocytes.[19,46]

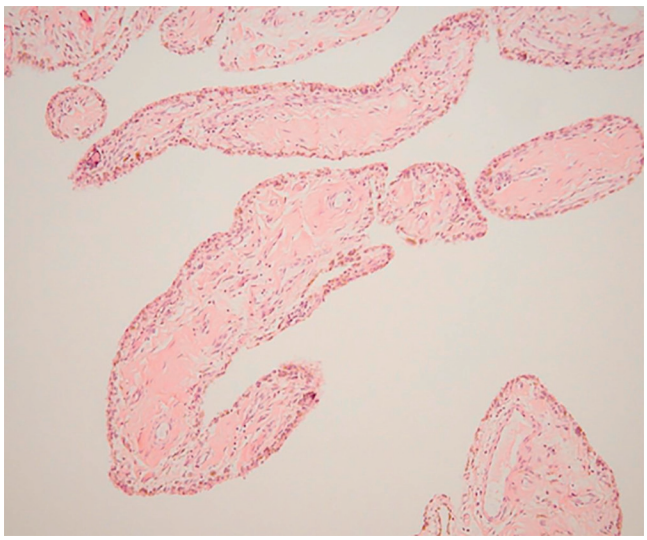


**Figure 119-4** Synovial tissue of hemophilic synovitis of the knee showing fibrous hypertrophy, thickened vascular structures, and iron pigment deposition in superficial synoviocytes and in macrophages in the deeper layers. Hematoxylin–eosin stain, ×1000.

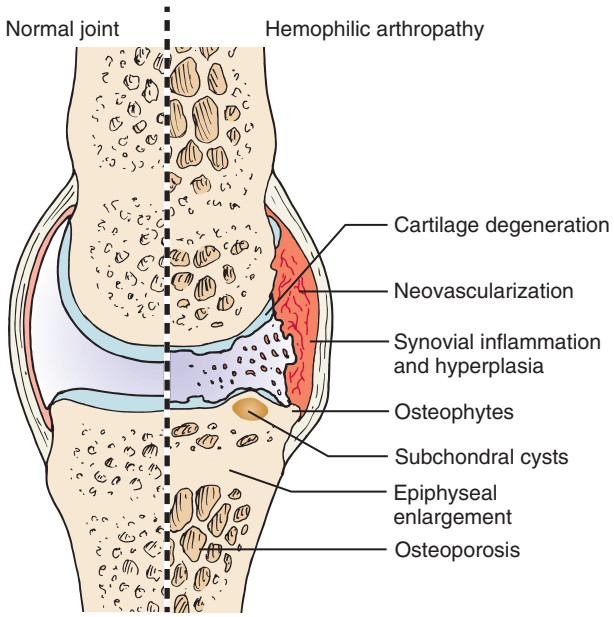



**Figure 119-5** Changes that occur in the synovial joint affected by hemophilic arthropathy.

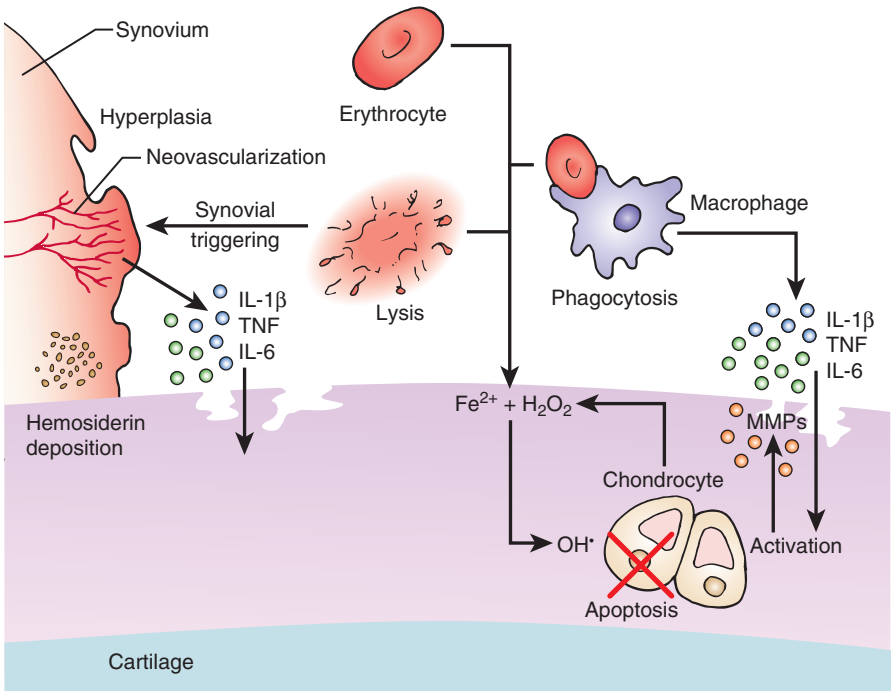



**Figure 119-6** Proposed mechanism of blood-induced joint damage. MMPs, Matrix metalloproteinases.

The normally thin synovial membrane becomes irregular, grossly hypertrophied, villous, friable, and highly vascular.[49,50] Synovial proliferation is postulated to result from an aberrant gene expression induced by the overload of iron.[51] It induces an overexpression of the proto-oncogene *c-Myc*, which is associated with synovial cell proliferation, and of the p53 tumor suppressor binding protein *mdm2*, leading to the abrogation of apoptosis of synovial cells.[52,53] An increased oxygen demand of the hypertrophied synovium causes hypoxia and thus potentially induces the release of growth factors such as vascular-derived endothelial growth factor and the formation of a rich network of brittle capillaries underneath the hypertrophied synovium.[21,54] This process sets up a vicious circle because these fragile vessels are more susceptible to recurrent bleeds. Over time chronic synovitis develops, with pannus invading and eroding marginal cartilage,[55] and ultimately the synovial tissue becomes fibrotic. Clearly there are similarities to the rheumatoid joint.

## Cartilage Degeneration

Cartilage destruction results from both synovial-dependent and synovial-independent processes. Synovial pannus invades cartilage at the periphery of inflamed joints. The inflamed hemosiderotic synovium produces cartilage-destructive pro-inflammatory cytokines and cartilage matrix degrading proteases.[19,50] These destructive properties of synovial tissue on cartilage are already present within 24 to 48 hours after a joint bleed and cause long-lasting damage.[56,57]

Hemarthroses also exert harmful effects directly on the cartilage (see Figure 119-6). Exposure of cartilage explants to whole blood or the combination of erythrocytes and mononuclear cells severely affects cartilage matrix turnover and results in chondrocyte apoptosis.[58,59] The pro-inflammatory mediators IL-1β and TNF produced directly after blood exposure transiently decrease proteoglycan turnover. These cytokines activate chondrocytes to increase $H_2O_2$ production, which in combination with iron derived from erythrocytes, leads to oxidative stress.[60,61] Iron in combination with $H_2O_2$ reacts via the Fenton reaction to form hydroxyl radicals, resulting in chondrocyte apoptosis.[62] Because chondrocytes hardly proliferate and have the responsibility to produce and maintain the extra-cellular matrix, apoptosis of chondrocytes will result in long-lasting impaired matrix turnover. These effects are more pronounced on immature articular cartilage compared with mature cartilage,[63] and impaired cartilage is at least as susceptible to blood-induced joint damage as healthy cartilage.[64] These findings imply that it is important not only to prevent joint bleeds in children but also in patients with already affected joints. These direct effects on cartilage with chondrocyte death represent characteristics of OA.

# TREATMENT OF HEMOPHILIA

Treatment of hemophilia consists of substitution of the deficient clotting factor. This substitution was first performed through whole blood transfusion and the use of fractions from plasma. The introduction of plasma-derived factor concentrates in the 1960s dramatically improved treatment and quality of life in patients with hemophilia. Use of these concentrates enabled prophylactic clotting factor replacement therapy, thereby converting the disease from a severe to a milder form.[65] It reduced the risk of bleeds with the setback of blood-borne infections. The introduction of highly purified, safe concentrates and recombinant products led to a marked reduction in the risk of viral transmissions.

Traditionally, treatment is provided at the time of clinically evident bleeding (known as episodic treatment or on-demand therapy). Nowadays, in developed countries, most patients with severe hemophilia are treated prophylactically, that is, via regular intravenous injection to prevent anticipated bleeding.[1] This treatment is called *primary prophylaxis* because it is initiated before the second clinically evident large joint bleed and before the age of 3 years. With secondary prophylaxis, regular treatment is started after two or more bleeds into large joints, but before the onset of "joint disease" (documented by physical examination and conventional radiography). Prophylactic factor replacement therapy started after the onset of overt "joint disease" is called *tertiary prophylaxis*. The optimum time to initiate prophylaxis, the optimum dosage, the frequency of injection, and whether prophylaxis should be given indefinitely remain unclear.[66-68] Addressing these questions is important because prophylaxis is a highly expensive treatment.

Prophylactic factor VIII replacement typically requires infusions two to three times a week, whereas factor IX can be given less frequently because of a longer half-life. The burden of regular venipunctures in young children is high, and central venous access devices, such as Port-A-Cath and Hickman lines, are often inserted for reliable long-term venous access.[69]

In patients with mild hemophilia A or in hemophilia A carriers, desmopressin, a synthetic analogue of vasopressin, can be administered to release endogenous factor VIII, von Willebrand factor, and tissue plasminogen activator from endothelial storage sites and possibly from platelets.[70] On average a threefold increase in factor VIII levels can be expected, and therefore a residual factor VIII activity of greater than 10% is required. Responsiveness to desmopressin should be tested prior to therapeutic treatment because it varies largely between patients.

Antifibrinolytic therapy is useful to treat or prevent bleeds in areas of increased fibrinolysis, such as skin and mucosal surfaces (epistaxis, menorrhagia, or oral bleeding).[71] It promotes clot stability by inhibiting plasminogen activation in the fibrin clot. Tranexamic acid, the most commonly used antifibrinolytic, acts by reversibly binding to plasminogen and thereby blocking its activation and transformation to plasmin. Epsilon aminocaproic acid is similar to tranexamic acid but is less widely used because it has a shorter half-life, is less potent, and is more toxic.[72]

To improve hemophilia treatment, current research focuses on factor modifications to extend the half-life, along with the development of gene-based therapy.[73,74] Improved bioavailability could be achieved by PEGylation, sialylation, and fusion of the clotting factor to albumin or a fragment of an immunoglobulin. Especially for factor IX, prolongation of the half-life to up to 100 hours could be achieved, allowing substitution intervals of 1 to 2 weeks.

Development of gene therapy is also most promising in hemophilia B because the factor IX gene is considerably smaller than the factor VIII gene, and antifactor VIII immunity is far more frequent. In a trial for hemophilia B, long-term expression of factor IX in levels sufficient to improve the bleeding phenotype has been observed after adeno-associated viral (AAV) vector-mediated gene transfer to the liver.[75] The main concern with this approach is that immune-mediated clearance of AAV-transduces hepatocytes. Moreover, about 40% of men with severe hemophilia B will be excluded from this therapy because of the presence of pre-existing neutralizing antibodies to AAV.[76] Although the initial studies seem promising, a number of significant hurdles have yet to be overcome.

## Complications of Treatment

### Inhibitors

Inhibitors are alloantibodies that neutralize clotting factor activity after infusion of factor VIII or factor IX, these antibodies are primarily of the $IgG_4$ subclass. Risk of inhibitor development is highest in the first period of treatment, especially in the first 50 to 75 exposure days. Patients with severe hemophilia A have a 20% to 30% lifetime risk of inhibitor development, compared with a risk of 5% to 10% in individuals with moderate or mild disease.[77,78] Inhibitors are encountered much less frequently in individuals with hemophilia B, occurring in fewer than 5% of affected individuals.[79] Other risk factors are age and number of exposures to factor, family history of inhibitor development, ethnicity, the causative factor VIII genotype, polymorphisms in the immune system, and intensive factor replacement therapy related to surgery or trauma.[78] Development of factor IX inhibitors is associated with severe allergic or anaphylactic reactions to factor administration; however, these reactions are almost never encountered in patients with hemophilia A.[80] Inhibitor development can be suspected in patients who fail to respond to clotting factor infusion, especially if they were previously responsive. In patients with mild or moderate hemophilia, the presence of an inhibitor can result in conversion of the bleeding phenotype to a more severe form because the inhibitor may neutralize endogenously synthesized clotting factor.

Patients with inhibitors are classified according to the inhibitor titer, expressed in Bethesda units. Patients with titers above five Bethesda units at any time are considered high responders and will show an increase in antibody titer after each exposure to clotting factor. In low responders, antibody titers are below five Bethesda units and do not increase after factor infusion. In these patients, the inhibitor may be transient and replacement therapy may be continued with minimal dose change.

Inhibitor eradication by immune tolerance induction (ITI) through frequent administration of clotting factor is successful in up to 70% of patients with hemophilia A and 30% of patients with hemophilia B.[81,82] ITI in patients with hemophilia B carries the risk of anaphylactic reactions and the development of nephrotic syndrome as a result of complex formation and deposition in the kidney. In patients with hemophilia A who have refractory disease, rituximab may be useful.[83,84] Other immunosuppressive treatments are high-dose prednisolone, cyclophosphamide, or azathioprine.[85]

Control of bleeding in patients with low-titer inhibitors could be achieved through high doses of factor concentrates or in patients with high-titer inhibitors with bypassing agents such as recombinant factor VIIa and (activated) prothrombin complex concentrates.[86,87] These agents act by boosting thrombin generation independently of the presence of factor VIII.

### Viral Infections

The use of non–virus-inactivated plasma, cryoprecipitate, and plasma-derived factor concentrates from multiple human blood donations resulted in high mortality and morbidity as a result of transmission of blood-borne virus infections. More than 90% of patients with hemophilia who were treated with these products before 1985-1987 became infected with hepatitis C.[88-90] A chronic infection developed in the majority of these patients, and in 20% to 30%, it progressed to liver cirrhosis. The cumulative incidence of HIV infection in the United States in the peak years was 78% of the factor VIII recipients and 37% of the factor IX recipients.[91] Other viruses transmitted are hepatitis A, B, or G and cytomegalovirus.

Different measures are taken to ensure safety of plasma-derived factor concentrates, including selection of healthy donors, screening donations for the absence of relevant infectious blood-borne viruses, and screening for viral markers by serologic and nucleic acid testing. Additionally, various viral inactivation procedures are required, such as pasteurization, exposure to solvent-detergent, dry heat, chemical disruption with sodium thiocyanate, or ultrafiltration.[92]

The introduction of recombinant products and the implementation of these measures almost completely eliminated HIV and hepatitis C virus transmission through factor concentrate.[93,94] Hepatitis A and B immunization is still recommended to reduce infection risk, although the risk of infection nowadays is low.[95]

# TREATMENT OF MUSCULOSKELETAL COMPLICATIONS

## Comprehensive Care

Maintaining musculoskeletal health and preventing complications requires encouragement of physical activities to promote physical fitness and normal neuromuscular development. For optimal management of musculoskeletal complications, multidisciplinary care is required. Because prevention is the cornerstone of maintaining musculoskeletal health, the care provided by hemophilia specialists is important to prevent and adequately treat bleeds and provide early recognition of musculoskeletal issues. Physiotherapy is required for recovery after musculoskeletal bleeds and to maintain muscle strength in individuals with established hemophilic arthropathy. A physiatrist can help provide patient education to prevent disabling injuries, care during bleeding episodes, long-term rehabilitation, and orthotics and shoe adaptations.[96] In individuals with end-stage arthropathy, orthopedic surgery is often inevitable.

## Acute Hemarthrosis

The primary aim of therapy for acute hemarthrosis is to stop the bleeding as quickly as possible by administering clotting factor concentrates. The efficacy of joint aspiration along with adequate factor replacement is unclear. Theoretically, shortening the exposure of cartilage to blood might limit blood-induced damage.[97] However, aspiration bears the risk of infection or repeat bleeds, and even when the joint is washed, some blood will always be left in the joint. No data are available on the long-term benefit of aspiration. The most recent in vitro experimental data suggest that limitation of blood exposure to cartilage may prevent tissue damage later.[97] However, these data must be confirmed by clinical studies that will be difficult to perform. Clearly, if the suspicion of infection is high, arthrocentesis is mandatory for diagnosis, but in general it should be avoided. According to the World Federation of Hemophilia guidelines, arthrocentesis may be considered in a bleeding, tense, and painful joint that shows no improvement 24 hours after conservative treatment and after exclusion of inhibitor development.[1] Arthrocentesis should be performed only after appropriate clotting factor replacement and under strictly aseptic conditions. In recurrent or massive bleeds that are unresponsive to appropriate clotting factor replacement, therapeutic embolization could be considered.

Pain relief could be achieved by analgesics, but RICE (rest, ice, compression, elevation) is also commonly recommended to decrease pain, inflammation, and bleeding. Immobilization and avoiding or minimizing weight bearing on the affected joint is important during active bleeding and might be helpful in protecting against cartilage damage.[98] As soon as the swelling and pain subside, active rehabilitation is indicated to minimize muscle atrophy, prevent contractures, and regain functional ability.

## Chronic Synovitis

In chronic synovitis the joint is vulnerable to repeated bleeding, and therefore the goal of treatment is to reduce synovial irritability. This goal might be achieved by (more intensive) prophylactic clotting factor concentrate replacement for 6 to 8 weeks combined with active physiotherapy and cooling. Use of cyclooxygenase (COX)-2 inhibitors may reduce inflammation.[99] Other NSAIDs should be avoided because of the possible impairment of platelet function. If conservative measures fail and chronic synovitis persists with frequent recurrent bleeding, synovectomy might be indicated either surgically, arthroscopically, or through intra-articular injection of radiopharmaceutical agents or chemicals. Radioisotopic synovectomy using a pure beta emitter (yttrium-90 or phosphorus-32) is preferred because it is minimally invasive and requires little factor replacement and less intense rehabilitation afterward. This procedure is very successful in reducing hemarthroses; 60% to 100% are reduced in the majority of patients.[100] If necessary, the procedure can be repeated after 6 months. However, potential direct harmful effects to cartilage in the long term should be taken into consideration.[101] Chemical synovectomy using rifampicin or oxytetracycline chlorhydrate is painful and needs to be repeated weekly until the synovitis is controlled, but it is an appropriate alternative if a radioisotope is not available.[102] Surgical options are preferred in cases in which MRI reveals severe synovial hypertrophy or when bone cysts are present because they carry the risk of dispersing radioactive material outside the intra-articular space. Performing orthopedic surgery in hemophilic patients requires a large supply of clotting factor and intensive rehabilitation.[103]

## Hemophilic Arthropathy

In individuals with established hemophilic arthropathy, the goal of treatment is to improve joint function, relieve pain, and assist with the continuation or resumption of normal activities of daily living. With use of conservative procedures, remarkable benefit can be achieved and surgical interventions can be postponed. For pain relief, adequate analgesic treatment, manual traction of the joint, and in some cases transcutaneous electrical nerve stimulation are indicated.[104] Physiotherapy is crucial for muscle strengthening and stretching, joint stability, and functional training. In patients with involvement of more than one joint, hydrotherapy enables functional training with minimal weight bearing. Orthotics and shoe adaptations provide immobilization, support, stability, compensation for deformities, and reduced weight bearing.[105] In individuals with advanced ankle arthropathy, immobilization with a cast can provide insight about whether arthrodesis would be helpful.

In individuals with end-stage arthropathy, orthopedic surgery is often indicated. Different surgical options could be considered depending on the joint or specific condition requiring correction. Joint replacement therapy of the knee and hip and less commonly of the elbow and shoulder and ankle arthrodesis are the most performed procedures. Joint replacement therapy is very successful in relieving pain and increasing functional activities and participation. Hip replacement usually improves ROM, whereas after knee replacement therapy, a restricted ROM may limit functional recovery.[106] Ankle arthrodesis is very effective in diminishing pain and stopping recurrent joint bleeding. Drawbacks of arthrodesis are the loss of mobility of the joint and the possibility of overloading other joints of the lower limb/foot, necessitating surgical repair of other joints.[107,108] Total ankle replacement has the advantage of preserving motion, but long-term follow-up data are scarce. Higher rates of aseptic loosening and deep infections due to poor bone quality and increased risk of micro-bleeds at the prosthesis-bone interface are concerns.[109,110] In this respect, a new surgical approach for the ankle could be joint distraction. Joint distraction in the treatment of OA has gained increasing attention in recent years.[111] This technique also seems very helpful for treatment of hemophilic ankle arthropathy,[112] although larger and prospective studies are needed.

Other procedures performed in end-stage arthropathy or in case of severe contractures include soft tissue contracture release, arthroscopic débridement for intra-articular adhesions and impingement, tendon reconstruction, osteotomy to correct angular deformity, and radial head excision combined with synovectomy if enlargement and erosion of the radial head cause mechanical blockage of forearm rotation.

Major orthopedic procedures can be performed safely and successfully, even in patients with inhibitors.[113] Surgical interventions should always be performed at or in

consultation with a comprehensive hemophilia treatment center.[114] Adequate quantities of clotting factor concentrates should be available, as well as blood bank support and laboratory facilities for reliable monitoring of clotting factor level and inhibitor testing. In patients who have arthropathy of more than one joint of the lower extremities, multiple procedures could be combined during one in-hospital stay, either in a single session or staged.[115] With this approach, reduced quantities of clotting factor concentrate are required and a faster rehabilitation is expected. Careful assessment by a multidisciplinary team is a prerequisite to ensure that recovery will not be compromised for any of the procedures.

## CONCLUSION

Despite the use of prophylactic infusions of clotting factor starting at an early age, it is not possible to completely prevent hemarthroses and the consequent joint damage. Patients with severe hemophilia who receive intermediate dose prophylaxis still experience 0.8 to 2.7 joint bleeds per year.[116] Patients at risk of the development of joint damage are those who do not have access to clotting factor treatment, experience the development of inhibitors, are not compliant with their prophylactic clotting factor treatment, have unrecognized and thus untreated joint bleeds, have post-traumatic bleeds, and receive on-demand therapy. Diagnostic strategies are needed to detect early changes within a joint and to evaluate joint damage progression regularly; biochemical markers of joint tissue damage are very promising in this respect.

Gene therapy to "cure" hemophilia faces many challenges regarding efficacy and safety. Specific therapies opposing inflammatory cytokines[117,118] or reducing synovial iron deposition[119] to limit synovitis and cartilage damage and procedures aiming at cartilage restoration[112,120] in overt joint damage may become plausible new approaches, although introduction of these therapeutic strategies into clinical practice remains challenging. In the meantime, emphasis should be placed on preventing hemarthrosis by clotting factor replacement, education, adequate pain relief, physical therapy, and maintaining physical activity to reduce development of end-stage arthropathy.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 120



# Rheumatic Manifestations of Hemoglobinopathies

Carlos J. Lozada • Elaine C. Tozman

**KEY POINTS**

- Hemoglobinopathies are inherited diseases caused by mutations in globin genes.
- Thalassemias are inherited defects in globin chain synthesis resulting in hypochromia and microcytosis.
- Skeletal changes in thalassemias can result in cortical thinning, osteoporosis, and vertebral compression fractures.
- Sickle cell disease is a single gene disorder caused by homozygous inheritance for sickle cell hemoglobin.
- Vaso-occlusive crises of bone occur in people with sickle cell disease and result in severe pain at the site of bony infarction, typically of long bones.
- The index of suspicion for septic arthritis should be high in patients with sickle cell disease who present with fever and joint pain.
- Hyperuricemia can be seen in people with sickle cell disease and thalassemias, but gout is uncommon.

Hemoglobin (Hb) has a tetrameric structure consisting of two pairs of α-globin and two non–α-globin polypeptide chains, each associated with a heme group. The interaction of these chains is responsible for the quaternary structure of the Hb molecule and its normal function. The fundamental role of Hb is oxygen transport.

Hemoglobinopathies are inherited diseases caused by mutations in globin genes. Abnormalities in Hb can alter red blood cell shape, deformability, and viscosity. Approximately 1000 mutations have been described[1]; however, most are *not* associated with clinical disease. The gene mutations that result in sickle cell disease and thalassemia syndromes cause diseases that often involve the musculoskeletal system and will be discussed in this chapter.

## THALASSEMIAS

The term *thalassemia* is derived from a Greek term that roughly means "the sea" (Mediterranean) in the blood and was used to describe anemias in people from Italy and Greece. The term is now used to refer to inherited defects in globin chain biosynthesis.[2] Individual syndromes are named according to the globin chain whose synthesis is adversely affected. For example, α-globin chains are absent or reduced in patients with α-thalassemia; β-globin chains are absent or reduced in patients with β-thalassemia; δ- and β-globin chains are absent or reduced in patients with δβ-thalassemia; and so on. Thalassemias are inherited as pathologic alleles of one or more of the globin genes located on chromosomes 11 and 16 and range from total deletion or rearrangement of the loci to point mutations that impair transcription, processing, or translation of globin messenger RNA.

As a consequence of reduced globin chain production, a reduction of functioning Hb tetramers occurs. Hypochromia and microcytosis are found in all patients with thalassemia. In the milder forms of the disease, these changes may be barely detectable. Impaired globin synthesis leads to an imbalance of the individual α- and β-subunits. Free or "unpaired" α-, β-, and γ-globin chains are either highly insoluble or form homotetramers (Hb H and Hb Bart's) that cannot release oxygen normally or are relatively unstable and precipitate as the cell ages. Excess α-globin chains, for example, continue to accumulate and precipitate in individuals with β-thalassemia. Unpaired subunits are the major sources of morbidity and mortality (Tables 120-1 and 120-2).

Thalassemias have been encountered in virtually every ethnic group and geographic location, but they are most common in the Mediterranean basin and tropical or subtropical regions of Asia and Africa. The "thalassemia belt" extends from the Mediterranean across the Arabian Peninsula and through Turkey, Iran, and India to southeastern Asia and southern China. The prevalence of thalassemia in these regions ranges from 2.5% to 15%. As with sickle cell anemia, thalassemia is most common in areas affected by endemic malaria. Malaria infection in thalassemia heterozygotes results in milder disease and confers a selective advantage on reproductive fitness. The gene frequency of thalassemia has become fixed and is high in populations exposed to malaria over many centuries.

## SICKLE CELL ANEMIA AND RELATED DISORDERS

Sickle cell anemia is a single gene disorder caused by homozygous inheritance for sickle Hb (Hb S). Hb S is identical to normal Hb (Hb A) except for the replacement of glutamic acid, the sixth amino acid in β-globin, with valine. Hb S is capable of carrying oxygen, but once the oxygen is released, the Hb joins with other Hb S molecules to form rigid rods that bend the normally flexible, disk-shaped red blood cells into distorted narrow crescents. The sickled cells impede blood flow when they stick to the walls of blood vessels and to other blood cells. About 10% of black Americans are heterozygotes—that is, they inherit a sickle globin

**TABLE 120-1** α-Thalassemias

| No. of Missing α Genes | | Clinical Presentation |
|---|---|---|
| 1 | Silent carrier | No overt manifestations |
| 2 | Thalassemia trait | Hypochromic, microcytic with mild anemia |
| 3 | Hemoglobin H disease | Hypochromic, microcytic with hemolytic anemia, hepatosplenomegaly, and jaundice |
| 4 | Hydrops fetalis syndrome (hemoglobin Bart's) | Severe anemia, ascites, edema, hepatosplenomegaly, cardiovascular and skeletal malformations; death in utero |

**TABLE 120-2** β-Thalassemia

| No. of Affected Genes | | Clinical Presentation |
|---|---|---|
| 1 | β-thalassemia minor | Heterozygous, asymptomatic, silent carrier |
| 2 | β-thalassemia trait | Mild anemia |
| 3 | β-thalassemia intermedia | Mild anemia, typically diagnosed in late childhood |
| 4 | β-thalassemia major | Homozygous, severe anemia, extramedullary hematopoiesis, hepatosplenomegaly, skull changes, iron overload from transfusions |

gene from one parent and a normal gene from the other parent and have sickle cell trait. They have no significant clinical problems unless they are subjected to marked dehydration or hypoxia.[3] Rarely these individuals experience splenic infarction, stroke, or sudden death after strenuous physical activity, particularly at a high altitude. Individuals with sickle cell trait may have impaired ability to form concentrated urine (hyposthenuria), and a few have episodes of painless hematuria as a result of medullary infarction. Other internal organ damage is extremely uncommon, and heterozygotes have a normal life expectancy.

One-fourth of the children of parents with sickle trait will be homozygotes and have red blood cells containing primarily Hb S and no Hb A. These individuals have severe hemolytic anemia and episodes of vaso-occlusion that cause acute bouts of pain and progressive organ damage. A similar clinical phenotype is encountered in individuals who are compound heterozygotes—that is, those who inherit the sickle gene from one parent and, from the other parent, either a β-thalassemia gene or a gene that encodes Hb C, another β-globin structural mutant. Patients with $S/\beta^0$ thalassemia[1] are unable to make any Hb A and are as severely affected as those with Hb SS disease. In contrast, patients with Hb $S/\beta^+$-thalassemia or with Hb SC disease have less morbidity and a longer life span.

The hallmark of sickle cell disease is the vaso-occlusive pain crisis,[4] which is the most common clinical manifestation but can occur with varying frequency in different individuals. It results from the complex interplay between sickled red blood cells, neutrophils, endothelium, and plasma factors. The result is tissue hypoxia, leading to tissue death and associated pain. Episodes may be precipitated by dehydration, infection, and cold weather, although in half of the cases, no precipitating factor is found. Repeated splenic infarctions in childhood result in "autosplenectomy" and loss of splenic function by age 6 to 8 years in approximately one-half of patients.[5]

## DIAGNOSIS OF HEMOGLOBINOPATHIES

One in 12 African-Americans has sickle-cell trait, one in 40 carries Hb C, and one in 40 carries the trait for β-thalassemia.[6] Latin Americans are an underappreciated population affected by sickle cell disease.[7] For example, it is estimated that 30,000 people in Brazil have sickle cell disease. African and African-American patients are at increased risk of carrying Hb S and other hemoglobinopathies and should be offered preconception or prenatal screening. Hb S is also more common among individuals of Mediterranean, Middle Eastern, and Asian Indian descent. The American College of Obstetricians and Gynecologists recommends that patients at risk for having a child with hemoglobinopathy be offered prenatal genetic counseling and a variety of tests to document the Hb abnormality.[8] In the United Kingdom, the Royal College of Obstetricians and Gynaecologists provides clinical guidelines for the management of sickle cell disease in pregnancy and standards of care from both hematology and the sickle cell community.[9] Prenatal diagnosis can be performed with either chorionic villus sampling or amniocentesis.

Because detection of α-thalassemia or α-thalassemia trait is based on molecular genetic testing and is not detectable using Hb electrophoresis, routine carrier screening is not offered. If microcytic anemia is present in the absence of iron deficiency and the Hb electrophoresis is normal, then testing for α-thalassemia should be considered, particularly among individuals of Southeast Asian descent. Sickle cell anemia can be accurately diagnosed with high-performance liquid chromatography and isoelectric focusing. Rapid methods such as solubility testing and sickling of red blood cells using sodium metabisulfite are less reliable tests. These tests are suitable for large field studies because they require only a drop of blood and use stable reagents, but they do not distinguish between homozygotes and heterozygotes. Newborn screening is mandatory in most of the United States and is well established, although not mandatory, for both sickle cell disease and thalassemias in the United Kingdom. Such screening tests vary in other areas of the world and may not be performed.[7] Polymerase chain reaction is the method of choice for prenatal diagnosis.

## TREATMENT

Hydroxycarbamide (hydroxyurea) is the only drug approved by the U.S. Food and Drug Administration to treat sickle cell disease. It can reduce the number of painful episodes and hospitalizations, but only about two-thirds of adult patients have a response to this drug.[10]

Bone-marrow transplants can provide a cure because they replace the faulty hematopoietic stem cells that produce sickle cells with ones that make healthy red blood cells.[11]

However, transplants are expensive, not widely available, and have associated risks such as infections and life-threatening immune-system reactions.

Gene therapy is another potentially successful treatment for sickle cell disease.[12] Conventional gene therapy, also known as gene addition, typically involves inserting new genes. Usually, a harmless virus is modified with the gene to be inserted, and this "viral vector" is mixed with cells from the patient in vitro. The virus searches out the cells and inserts the gene into the cells' DNA, after which the cells are transplanted into the patient. Gene editing is more specific when the faulty DNA sequence is removed and a piece of laboratory-created DNA is inserted. In both approaches, the modified DNA dictates the formation of a normal, working protein. A team led by Alan Flake at the Children's Center for Fetal Research at the Children's Hospital of Philadelphia has successfully treated sickle-cell disease in utero in mice and dogs, and trials in monkeys are under way.[13] Stem cells from the mother are collected and injected into the bloodstream of the fetus. No destruction of the fetal immune system is required, because the fetal and maternal immune systems are naturally tolerant of one another.

## MUSCULOSKELETAL INVOLVEMENT: SICKLE CELL DISEASE

### Vaso-Occlusive Crisis of Bone

Bone involvement in patients with sickle cell disease is the most common clinical manifestation of the disease, both as an acute process and as a chronic symptom[14] (Table 120-3). Painful osteoarticular crisis was the most common reason for emergency care of children with sickle cell disease in an African population (58.6%).[15] All patients experience painful vaso-occlusive crises, which usually begin in late infancy and continue throughout life. Patients typically report pain in the chest, low back, and extremities. Abdominal pain may mimic acute abdomen of other causes. Fever is often present, even in the absence of infection. Although microvascular occlusion can occur in any organ, it is common in tissues with low blood flow, such as the bone marrow, where deoxygenation and polymerization of Hb S leads to bone thrombosis, infarction, and necrosis. Spinal cord infarction can occur.[16] The medullary cavity or the epiphysis of the bone is affected. Patients have severe pain in one or more areas of their bones, with tenderness, erythema, and swelling of the infarcted bone. Generalized symptoms of fever and leukocytosis occur, and most patients recover without sequelae (Figure 120-1).

When infarction involves the vertebral body, collapse and a fish mouth deformity can result.[17] Bone infarctions can occur in any bone, with a predilection for long bones. The most common sites are the tibia/fibula (30%), the femur (25%), and the radius, ulna, and humerus (21%).[18] MRI is sensitive in detecting bone and bone marrow infarction, but this technology is not useful in distinguishing acute infarction from osteomyelitis.[19]

**TABLE 120-3** Bone and Joint Involvement in Sickle Cell Disease

| |
|---|
| Vaso-occlusive crisis of bone |
| Dactylitis |
| Osteomyelitis |
| Acute synovitis |
| Septic arthritis |
| Growth disturbance |
| Osteopenia, osteoporosis |
| Osteonecrosis |
| Stress fracture |
| Orbital compression |
| Dental complications |
| Vertebral collapse |
| Sickle cell arthropathy |
| Diffuse chondrolytic arthritis |
| Gouty arthritis |

### Dactylitis

In young children, in the range of 1 to 2 years of age and younger than 7 years, vaso-occlusion manifests in the bone marrow of the small bones of the hands and feet and dactylitis occurs.[20] Dactylitis is also called hand-foot syndrome. Patients have painful swellings of one or more digits (Figure 120-2) as extensive infarction of the marrow, medullary trabeculae, and inner layer of cortical bone occur.



**Figure 120-1** Bony infarcts in the femur of a patient with sickle cell disease. *(From The Radiology Assistant.)*



**Figure 120-2** Hand of a child with sickle cell disease. *(Courtesy Larry B. Mellick. From https://www.youtube.com/watch?v=AChPlzPEA7Y.)*

Subperiosteal new bone formation follows, and as the episode resolves within 2 weeks, radiographs show a "moth-eaten" appearance of the involved digits (Figure 120-3). Rarely, involvement of the epiphysis causes premature fusion and shortened digits.

## Osteomyelitis

Patients with sickle cell disease have an increased risk of infections, including osteomyelitis,[21] particularly from encapsulated organisms that are presumably secondary to loss of functional splenic tissue. The most common cause of osteomyelitis is *Salmonella*, followed by *Staphylococcus aureus* and Gram-negative enteric bacteria.[22] One proposed explanation for the frequency of bowel organism–derived infections has been intravascular sickling leading to bowel microinfarctions.[23] Diagnosis of osteomyelitis in the context of sickle cell disease is an important management issue because the clinical symptoms of pain, swelling, tenderness, and fever are similar to those of bone vaso-occlusive crisis. Elevated markers of inflammation occur in both conditions. No single laboratory test or imaging modality reliably differentiates osteomyelitis from infarction. Until a definite diagnosis can be made, treatment with anti-microbial therapy should be provided.[24]

## Acute Synovitis and Septic Arthritis

When the epiphysis is involved by infarction of sickle cell crisis, a joint effusion may develop. This presentation is an acute synovitis and is clinically indistinguishable from a septic joint.[25] Synovial fluid should be examined and cultured for accurate diagnosis. In a retrospective review of 2000 consecutive adult patients with sickle cell disease, 3% had septic arthritis.[26] The majority of these patients (56/59) had Hb SS. Thirty-six of the 59 infections involved the hip. Symptoms were pain, swelling, and fever higher than 38.2° C, a peripheral white blood cell count of 15,000/mm$^3$, erythrocyte sedimentation rate greater than 24 mm/hour, and C-reactive protein greater than 20 mg/L. Cultures were positive in 96% of the joint aspirates, with staphylococcus and Gram-negative infections the most common. Prior diagnosis of osteonecrosis, osteomyelitis, and comorbid medical conditions of diabetes mellitus and use of corticosteroids and hydroxyurea were associated with septic arthritis. It is suggested that a clinician should have a high index of suspicion for septic arthritis in patients with sickle cell disease who present with fever and joint pain. Distant osteomyelitis was more often the source of a joint infection than contiguous osteomyelitis. If a joint infection is suspected, the patient should undergo aspiration and culture of the synovial fluid.



**Figure 120-3** Radiograph of the hand of a child with sickle cell disease. *(From Medscape. Available at http://img.medscape.com/pi/emed/ckb/radiology/336139-413542-8885tn.jpg.)*

Another form of arthritis in patients with sickle cell disease is polyarticular (80%) and symmetric (60%) in the majority of patients and generally involves the large joints of the lower extremity.[27] Symptoms last less than 1 week, and radiographic changes are consistent with periarticular osteopenia, erosions of bone, and joint space narrowing.

## Growth Disturbance

A majority of children with SS disease will show decline in growth compared with normal peers.[28] Puberty is delayed on average by 12 to 24 months, as is skeletal age. Factors such as nutritional status and inactivity could be modified and decrease the effect on growth. Delayed skeletal maturation in children also can occur.[29]

## Osteopenia and Osteoporosis

Osteopenia and osteoporosis are prevalent in Hb SS disease, especially given the young age of the patients. The lumbar spine appears to be the site most often affected. Low body

mass index is a consistent correlate of decreased bone density, as are low vitamin D levels.[30]

### Osteonecrosis

Vaso-occlusion resulting in infarction of articular surfaces of long bone occurs most commonly in the femur, followed by the humerus.[31] It was previously thought to occur with increased frequency in Hb SC disease as opposed to Hb SS disease. However, because of the increased longevity of patients with Hb SS disease, its prevalence is greatest in patients with Hb SS disease.[32] By age 33 years, 50% of patients will have avascular necrosis of the femoral head. The presence of concurrent deletional α-thalassemia and a history of frequent vaso-occlusive crises are classic risk factors for femoral avascular necrosis. Patients present with chronic joint pain with progressive decrease in range of motion of affected joints. Multiple joints are commonly involved. The vast majority of untreated patients with avascular necrosis will progress to femoral head collapse within 5 years.

Symptoms always preceded hip collapse. The duration to collapse was shorter with more advanced stages at study onset, and the majority of patients progress to symptomatic avascular necrosis that requires intervention.[33]

Avascular necrosis has been treated with a number of modalities including core decompression, osteotomy, bone grafting, surface arthroplasty, and joint replacement.[34] The only randomized trial in avascular necrosis compared core decompression and physical therapy versus physical therapy alone and did not show a difference in outcome between the two arms; however, follow-up was short, and a significant number of stage III hips were included. Core decompression is a useful option in early stage avascular necrosis. Several studies associate total hip replacement in individuals with sickle cell disease with a higher rate of orthopedic and medical complications.[35,36] However, other studies show a lower rate of orthopedic complications.[37] Structural bone diseases in people with sickle cell disease make joint replacement technically challenging (see Chapter 103).

### Iron Metabolism

Red blood cell transfusions are a mainstay in the treatment of both acute and chronic sickle cell disease complications.[38] Most patients receive at least one transfusion in their lifetime, usually for acute complications. Blood transfusions increase arterial oxygen pressure and Hb oxygen affinity, thereby reducing red blood cell sickling, and they also improve microvascular perfusion. Furthermore, regular blood transfusion regimens suppress endogenous erythropoiesis and therefore the production of red blood cells containing sickle Hb. Iron metabolism and consequently patterns of iron loading differ in sickle cell disease and thalassemia.[39]

Erythropoiesis is variably increased in sickle cell disease, but it is not ineffective. Unlike patients with thalassemia, patients with nontransfused sickle cell disease do not develop systemic iron overload because of increased iron absorption, and they may actually present with iron deficiency, possibly related to intravascular hemolysis and the resulting excessive urinary loss of iron. Inflammation, which is part of the pathophysiology of sickle cell disease, increases synthesis of hepcidin and consequently decreases iron absorption and enhances retention of iron within the reticuloendothelial system. As a result, tissues and organs affected by iron overload are different in sickle cell disease and thalassemia. Iron-induced cardiac and endocrine dysfunction is less common in sickle cell disease. Musculoskeletal manifestations of iron overload include a symmetrical, polyarticular arthropathy with bony enlargement and minimal inflammation that can develop in 25% to 50% of patients. It notably affects the second and third metacarpophalangeal joints but also the proximal interphalangeal joints, wrists, elbows, shoulders, and hips. Osteoporosis can affect up to 25% of patients.

## MUSCULOSKELETAL INVOLVEMENT: THALASSEMIAS

### Decreased Bone Density, Osteoporosis

Skeletal changes in people with untreated thalassemia are related to bone marrow hyperplasia, resorption of cortex, rarefaction of cancellous bone with coarsening of the trabeculae, and a generalized decrease in bone density.[40] In patients with thalassemia major, onset of the radiographic changes can be seen in their first year of life. Involvement of the spine, skull and facial bones, ribs, and metaphyses of long bones are typical (Figure 120-4).

In the spine, osteoporosis and cortical thinning are associated with vertebral compression fractures (Table 120-4). After an initial increase in the height-to-width ratio of the vertebral body, thinning of the subchondral bone plates occurs and the bones become biconcave and wedge shaped as multiple compression fractures occur. Patients can experience scoliosis and kyphosis.[41] Lateral plain radiographs of the spine may show a "bone-within-a-bone" appearance as the end plate depression is evident (Figure 120-5). MRI studies show predominance of red marrow in the vertebrae and early degeneration of the intervertebral disks of the lower thoracic and lumbar spine.[42] Abnormal intravertebral iron deposition also can be seen.

### Extramedullary Hematopoiesis

Extramedullary hematopoiesis is seen as extraosseous extension of red marrow with symmetrical lobulated confluent masses expanding in the paravertebral and presacral spaces. These soft tissue masses have intermediate T1 signal, variable intensity of T2-weighted sequences, and mild gadolinium enhancement. Radionuclide imaging with

**TABLE 120-4** Bone and Joint Involvement in Thalassemias

| |
|---|
| Osteopenia, osteoporosis |
| Bone pain |
| Bone fractures |
| Iron chelator arthropathy |
| Intervertebral disk disease |
| Spinal deformity |




**Figure 120-4** Spinal involvement with vertical trabeculations in a person with thalassemia. *(Case courtesy Dr. Chris O'Donnell, Radiopaedia .org, rID: 16592.)*

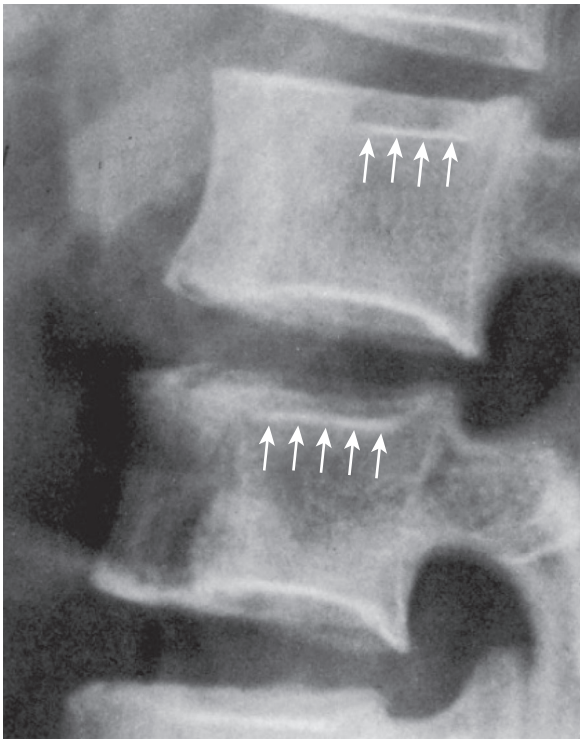


**Figure 120-5** Vertebral end plate changes in thalassemia. Vertebral end plate depression highlighted by *arrows*. *(From Cockshot P, Middlemiss H: Clinical radiology in the tropics, Edinburgh, 1979, Churchill Livingstone.)*

technetium-labeled sulfur colloid may help define the hematopoetic nature of the masses.[43] Spinal cord compression occasionally occurs as a result of epidural marrow extension. In the skull, marrow expansion causes widening with thinning of the outer table and thickening of the inner table, with remodeling of the skull (tower skull; Figure 120-6).

## Iron Metabolism

The anemia of thalassemia major becomes symptomatic between 6 months and 2 years of age, requiring the institution of a regular transfusion program. As a consequence of continuous anemia, erythropoiesis, although inefficient, can be intense; the bone marrow undergoes an enormous expansion with consequent distortion of facial features, and the plasma volume increases. In addition, hepatosplenomegaly develops. A regimen maintaining a minimum Hb concentration of 9.5 to 10.5 g/dL prevents all the aforementioned complications and fosters normal growth at least until puberty. Transfusion therapy results in iron overload in various tissues, and iron chelation therapy prevents accumulation of iron, promotes normal growth, and prevents death.[44] Deferoxamine chelation is effective but challenging to use because of its cost and the inconvenience of prolonged subcutaneous infusions. Deferiprone, an oral iron chelator, has improved compliance and access to iron chelation therapy. However, serious complications can occur when it is used, including agranulocytosis. Mild arthralgias and/or arthritis may occur in up to 38% of patients[45] and usually resolve on their own or with NSAIDs. In some patients, however, severe arthropathy related to deferiprone develops, requiring discontinuation of iron chelation therapy. In addition, erosive epiphyseal, physeal, and

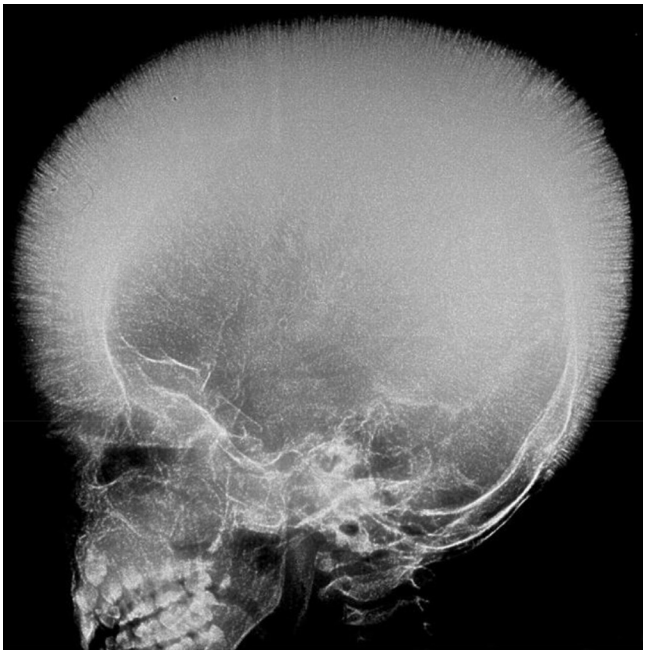


**Figure 120-6** Skull of a child with thalassemia. *(From Azam M, Bhatti N: Hair-on-end appearance. Arch Dis Child 91:735, 2006.)*

metaphyseal changes are seen on radiographs of the long bones of the hands of symptomatic children.[46]

## COMORBID RHEUMATIC DISEASE

### Autoimmune Disorders

The occurrence of systemic autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus, has occasionally been reported in patients with hemoglobinopathies.[47] Some authors have suggested that their association has an increased risk.[48] Several cases of mild recurrent arthritis have been reported in patients with thalassemia. Rheumatoid arthritis has rarely been described in patients with sickle cell disease.[49] The prevalence and clinical features of rheumatoid arthritis in patients with thalassemia is unknown. A review of medical records of patients with either thalassemia or sickle cell disease followed up over a decade in Greece provides insight.[50] Rheumatoid factor was detected in 23% of 90 patients, and anti-citrullinated protein antibodies were detected in only 2%. A low titer of positive anti-nuclear antibody (ANA) was present in nearly half of patients, and double-stranded DNA antibodies were found in one patient. Four percent of the patients with thalassemia major fulfilled American College of Rheumatology criteria for rheumatoid arthritis and received low-dose corticosteroids to control synovitis. One patient underwent methotrexate therapy. Patients with rheumatoid arthritis had no specific features that distinguished them from the other patients with hematologic disease. The nature of the rheumatoid arthritis was mild, and extra-articular features were uncommon.

Abnormalities of the immune system can occur in patients with sickle cell disease. Immune deficiency and the presence of autoantibodies have been described in frequencies higher than that of control groups of Africans and Caucasians, and up to 20% of patients are positive for ANAs.[51] The presence of these autoantibodies could be related to genetic factors and/or environmental influences. Although it has been suggested that a deficiency of the alternative complement pathway in patients with sickle cell disease was a predisposing factor in the development of immune complex diseases, this mechanism has not been confirmed.[52]

Autoimmune disease in adults with sickle cell disease may fail to be diagnosed or diagnosis may be delayed as a result of shared clinical manifestations. Bone and joint, lung, cardiac, renal, and central nervous systems may be involved in either autoimmune disease or sickle cell disease. Recognition of an autoimmune disease in patients with sickle cell disease may require a change in therapy, and the use of immunosuppressive medications may result in exacerbation of sickle cell disease.[53]

### Gout

Hyperuricemia and gout are uncommon in people with primary red blood cell disorders but have been reported in patients with sickle cell disease, Hb SC and CC disease, and thalassemias.[54] Increased red blood cell turnover in sickle cell disease can lead to hyperuricemia, as can renal tubular involvement and a decrease in uric acid excretion. Two patients with tophaceous gout and sickle cell disease have been reported.[55] Appropriate therapy is treatment of the underlying hematologic disease and typical measures for acute gout. In studies from the 1970s, hyperuricemia was reported as a common feature of sickle cell disease, occurring in up to 41% of patients.[56] To assess the recent prevalence of hyperuricemia and gout in adult patients with sickle cell disease, 65 consecutive patients with sickle cell disease in one center in France were studied during a 2-year period.[57] Hyperuricemia was observed in only 9.2% of the patients in the cohort, and none had gout.

## CONCLUSION

Hemoglobinopathies, particularly sickle cell disease and the thalassemias, are often associated with significant and varied musculoskeletal manifestations, including osteonecrosis, arthropathies, and vertebral fractures. Awareness of the clinical presentations associated with these manifestations is essential so they are recognized by the physician and an effective management strategy can be formulated.


The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 121



# $IgG_4$-Related Disease

John H. Stone • Shiv Pillai

**KEY POINTS**

- **Immunoglobulin $G_4$-related disease ($IgG_4$-RD) is an immune-mediated condition capable of affecting essentially any organ. The most commonly affected tissues are the major salivary glands, pancreas, orbits, lymph nodes, and retroperitoneum.**
- **Most patients have elevated serum $IgG_4$ concentrations, often to extremely high levels, but a significant number of patients have normal serum $IgG_4$ levels.**
- **The hallmark pathologic features of $IgG_4$-RD are a lymphoplasmacytic infiltrate with a high percentage of plasma cells staining for $IgG_4$, storiform fibrosis, obliterative phlebitis, and mild to moderate tissue eosinophilia. The foundation of the diagnosis rests upon robust clinicopathologic correlation.**
- **The peripheral blood of patients is characterized by high numbers of plasmablasts, regardless of whether the serum $IgG_4$ concentration is elevated. These cells appear to correlate with disease activity.**
- **Nearly all patients respond well to glucocorticoids, but most patients ultimately require long-term maintenance therapy or intermittent retreatment.**
- **B cell depletion strategies appear to be effective, perhaps in part through their interference with the presentation of antigen by B cells to T cells; the importance of this pathway is becoming more clear in disease pathophysiology.**

$IgG_4$-related disease ($IgG_4$-RD) is a multiorgan immune-mediated condition that mimics many malignant, infectious, and inflammatory disorders.[1-4] The diagnosis links numerous conditions once regarded as isolated, single-organ diseases that existed outside of any known underlying systemic condition (Table 121-1). $IgG_4$-RD has many similarities to sarcoidosis and certain forms of systemic vasculitis. These diseases are all protean conditions in which the histopathologic findings are consistent across a wide range of organ systems.

Awareness of $IgG_4$-RD is essential because it generally responds well to treatment. Moreover, the approaches to the treatment of $IgG_4$-RD differ significantly from those of its mimics. Regardless of the organ affected, $IgG_4$-RD has a tendency to form mass lesions and therefore is often confused with cancer. In addition, autoimmune disorders such as Sjögren's syndrome, granulomatosis with polyangiitis, and idiopathic membranous nephropathy can also be difficult to distinguish from $IgG_4$-RD. Understanding the immune dysregulation associated with $IgG_4$-RD will likely provide important insights into the processes of fibrosis, B and T lymphocyte cross-talk, and processes central to the pathophysiology of other conditions.

## EPIDEMIOLOGY

Mapping the epidemiology of $IgG_4$-RD has been hampered by insufficient awareness of this relatively newly recognized disease. The disease first crept into the medical literature in 2003.[5,6] Definitive diagnosis generally requires a biopsy, insightful interpretation of the pathologic features, and rigorous clinicopathologic correlation. $IgG_4$-RD was recognized first in the pancreas, and although the overall prevalence of type 1 ($IgG_4$-related) autoimmune pancreatitis (AIP) was estimated to be 2.2 cases per 100,000 population in Japan,[7] this figure certainly underestimates the true prevalence of AIP because the study was conducted only shortly after the appearance of the first publications about the disease.

Moreover, the pancreas is only one of more than a dozen organs affected by $IgG_4$-RD. Among the different organs affected, AIP, sialadenitis (particularly of the submandibular gland [Figure 121-1]), a variety of orbital and periorbital lesions (e.g., dacryoadenitis), lymphadenopathy, and $IgG_4$-related retroperitoneal fibrosis (RPF) (Figure 121-2) are among the most common disease features. A variety of lesions in the lung (Figure 121-3), kidney (Figure 121-4), and aorta (Figure 121-5) are also well described.

The typical patient with $IgG_4$-RD is a middle-aged to elderly man.[7,8] In individuals with AIP, the mean age at diagnosis 67 years and the male : female ratio is 3 : 1.[7] When all types of $IgG_4$-RD organ involvement are considered, however, this ratio may be closer to 3 : 2.[9] The male predilection contrasts with the classic autoimmune diseases but does not appear to affect disease severity; when women are afflicted by $IgG_4$-RD, the disease severity is generally no less than the phenotype in men.[9] HLA associations and other potential genetic links of $IgG_4$-RD remain incompletely characterized. Detailed genetic studies have not yet been undertaken in populations of sufficient size, but familial cases appear to be very rare. Features of the epidemiology of $IgG_4$-RD will remain elusive until the disease is recognized more broadly.

## ETIOLOGY AND PATHOGENESIS

Most features of the current understanding of the pathophysiology of $IgG_4$-RD remain highly theoretical, but the field is changing quickly. The theory that $IgG_4$ itself is a

crucial player in cause of disease, which held sway for many years after the first recognition of the elevated serum $IgG_4$ concentrations in "sclerosing pancreatitis," is swiftly falling away. Figure 121-6 outlines current thinking regarding many of the different pathways contributing to the disease.

## The $IgG_4$ Molecule: Central or Not?

Many patients with $IgG_4$-RD have striking and disproportionate elevations of serum $IgG_4$ concentrations. In addition, except for advanced cases characterized primarily by fibrotic lesions, $IgG_4^+$ plasma cells are nearly always present in abundance at sites of disease. Substantial elevations of plasmablasts have also been reported in $IgG_4$-RD, and these cells appear to mirror disease activity.[10,11] Consequently, the $IgG_4$ antibody and humoral immunity have been emphasized most prominently in discussions of pathophysiology thus far.

$IgG_4$ itself is unlikely to drive the pathogenesis of $IgG_4$-RD. The subclasses of IgG differ from each other in important ways, defined by the sequences of their heavy chain constant domains. $IgG_4$, normally the least abundant of all of the subclasses, accounts for approximately 4% of the total immunoglobulins among healthy individuals. The $IgG_4$ molecule has unique chemical properties that render it unlikely to play a central inflammatory role. $IgG_4$ demonstrates weak binding to C1q and to Fc-γ receptors because of a critical few amino acid differences in its CH2 domain.[12,13] As a result, the ability of $IgG_4$ to activate the classic

**TABLE 121-1** Previously Recognized Conditions that Are Part of the $IgG_4$-Related Disease Spectrum

| |
|---|
| Mikulicz's disease (affecting the salivary and lacrimal glands) |
| Küttner's tumor (affecting the submandibular glands) |
| Riedel's thyroiditis |
| Eosinophilic angiocentric fibrosis (affecting the orbits and upper respiratory tract) |
| Lymphomatoid granulomatosis, grade 1 (commonly affecting the lungs) |
| Multifocal fibrosclerosis (commonly affecting the orbits, thyroid gland, retroperitoneum, mediastinum, and other tissues/organs) |
| Lymphoplasmacytic sclerosing pancreatitis/autoimmune pancreatitis |
| Inflammatory pseudotumor (affecting the orbits, lungs, kidneys, and other organs) |
| Mediastinal fibrosis |
| Retroperitoneal fibrosis |
| Sclerosing mesenteritis |
| Periaortitis/periarteritis |
| Inflammatory aortic aneurysm |
| Idiopathic hypocomplementemic tubulointerstitial nephritis with extensive tubulointerstitial deposits |

From Stone JH, Zen Y, Deshpande V: IgG4-related disease. *N Engl J Med* 366:539–551, 2012. Modified with permission from the Massachusetts Medical Society.

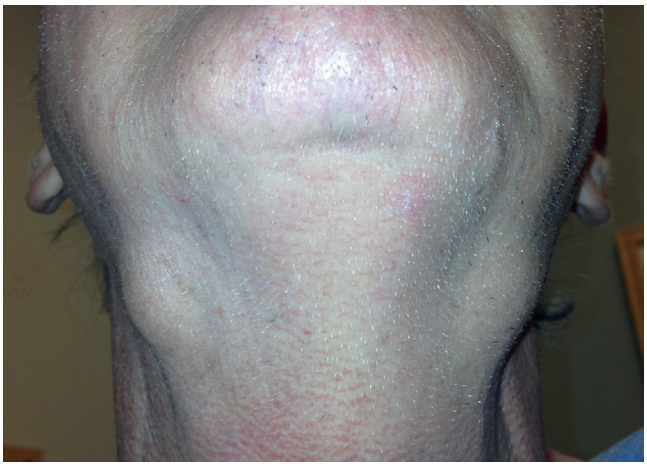


**Figure 121-1** Nontender enlargement of both submandibular glands in a patient with $IgG_4$-related sialadenitis of the submandibular gland. The patient had previously undergone a modified Whipple procedure (partial pancreatectomy) for what turned out to be type 1 ($IgG_4$-related) autoimmune pancreatitis.

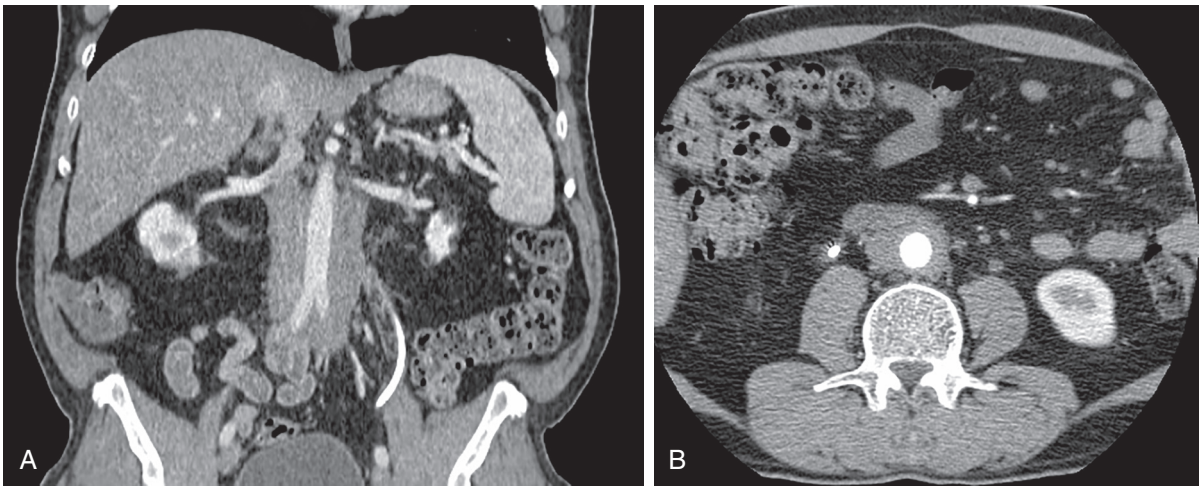



**Figure 121-2** Retroperitoneal fibrosis and periaortitis. **A,** Computed tomographic angiogram showing encasement of the infrarenal abdominal aorta by retroperitoneal fibrosis, extending inferiorly as far as the iliac arteries. **B,** This patient had also experienced right hydronephrosis caused by entrapment of the right ureter by the fibroinflammatory process. His right ureteral stent is visible on the left side of the figure.

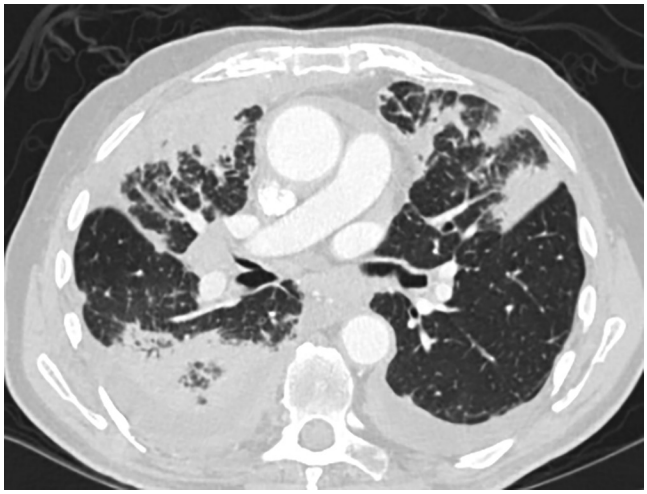


**Figure 121-3** $IgG_4$-related lung disease. Computed tomographic study of the lung showing thickening of the bronchial walls centrally, extensive pleural fibrosis anteriorly and posteriorly, and interstitial lung changes.

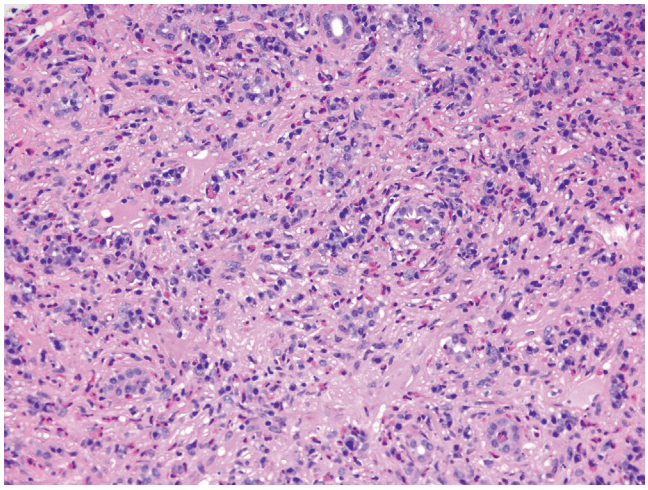


**Figure 121-4** $IgG_4$-related tubulointerstitial nephritis. A renal biopsy specimen demonstrates a decreased number of tubules in the renal interstitium, extensive lymphoplasmacytic infiltrate, numerous eosinophils, and storiform fibrosis throughout the tissue.

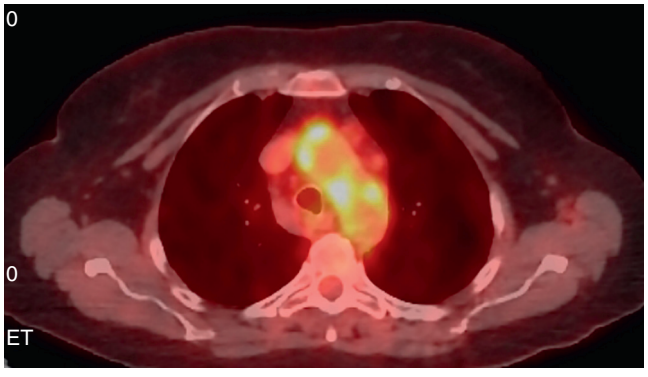



**Figure 121-5** A positron emission tomography study demonstrating increased 18F-fluorodeoxyglucose uptake in the aortic arch. The patient also had a subglottic mass and an inflammatory lesion encircling the left pulmonary artery. Results of the subglottic lesion biopsy are shown in Figure 121-9A and B.

complement pathway and participate in antibody-dependent cell-mediated cytotoxicity is attenuated substantially in comparison, for example, with the capabilities of $IgG_1$.[13]

Another unique feature of $IgG_4$ is its ability to form "half antibodies" through the process of Fab arm exchange. Fab arm exchanges result in the formation of IgG molecules with two different binding specificities.[13] Amino acid variation at the hinge region of $IgG_4$ permits the reduction of the disulfide bonds that join the two halves of an $IgG_4$ molecule. Recombination of the dissociated arms leads to the random formation of "asymmetric antibodies" composed of half-antibody fragments directed against different antigens.[12,13] The consequence is a reduced ability to cross-link antigens and form immune complexes.

In summary, the properties of the $IgG_4$ molecule appear to exert a suppressive effect on inflammation. Indeed, it has been demonstrated that the $IgG_4$ response develops after chronic antigen exposure as a part of a tolerogenic response.[12,13] Therefore, $IgG_4$ may play the role of a noninflammatory "antigen sink," the purpose of which is to "mop up" antigen through its monovalent binding in a process that tends to downregulate inflammation.[1]

## B Cell Lineage

Judging from the response of patients to rituximab, B cells and the cells of their lineage likely play an important role in $IgG_4$-RD.[14,15] The rapidity with which the serum $IgG_4$ concentration declines after B cell depletion suggests that the cells making most of the serum $IgG_4$ are short-lived plasmablasts and plasma cells. Plasmablasts are found in high concentrations in $IgG_4$-RD, regardless of the serum $IgG_4$ concentration.[9-11] Both total plasmablasts and $IgG_4^+$ plasmablasts appear to be good biomarkers for disease activity in $IgG_4$-RD, and their measurement is also useful in diagnosis. Flow cytometry gating for these cells is not widely available at this time, even though transcriptional profiling of whole blood has proved useful in other disorders. B cells and their lineage may play a variety of roles in $IgG_4$-RD, including the production of $IgG_4$ (although the centrality of $IgG_4$ to disease pathophysiology is debated). It is speculated that B cells and plasmablasts play important roles in antigen presentation to T cells.

## T Cells

More recent evidence suggests that T cells play a crucial and directory role in the pathogenesis of $IgG_4$-RD. $CD_4^+$ T cells are typically dispersed throughout localized $IgG_4$-RD lesions and are the most abundant cell found within affected tissues. The circulating plasmablasts in $IgG_4$-RD demonstrate intense somatic hypermutation, a hallmark of interaction with T cells within the germinal centers of lymph nodes.[16] Conflicting reports have implicated T helper (Th)1 cells and Th2 cells in the pathophysiology of $IgG_4$-RD.[17,18] Recent studies suggest that Th2 cells accumulate in the blood only in subjects with concomitant atopy.[19,20] The recent identification of a clonally expanded population of $CD4^+$ cytotoxic T lymphocytes in both the peripheral blood and fibrotic lesions of patients with $IgG_4$-RD suggests that these cells may be central to the disease.[16] These cytotoxic T cells make products such as granzyme B and perforin that

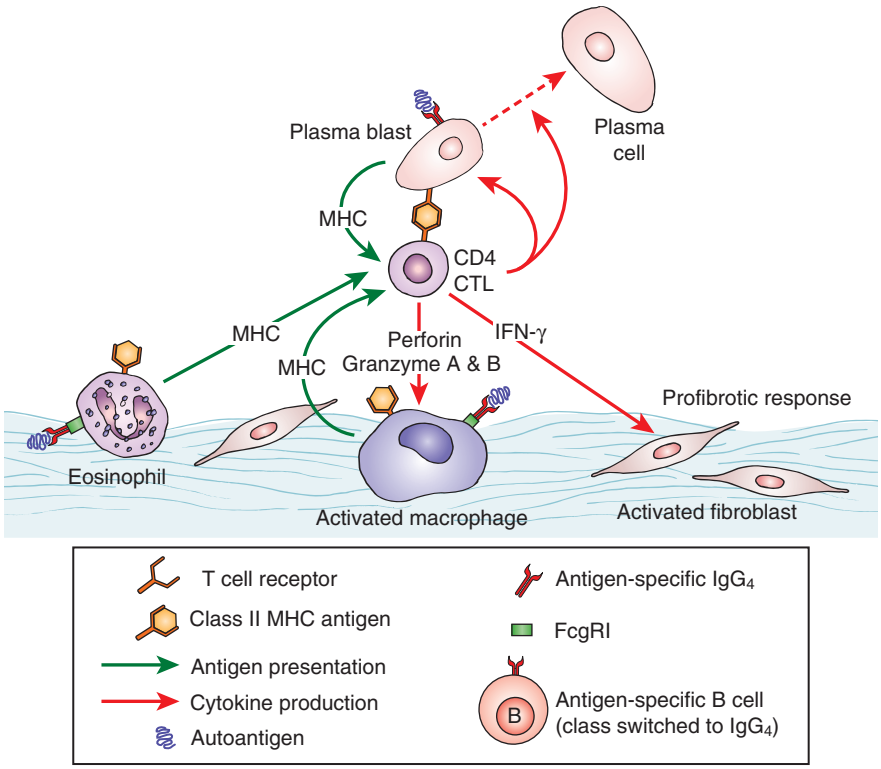



**Figure 121-6** Scheme of pathophysiology. CTL, Cytotoxic T lymphocyte; MHC, major histocompatibility complex.

are associated more commonly with $CD8^+$ T cells. Moreover, the interleukin (IL)-1 elaborated by these $CD4^+$ $SLAMF7^+$ cells may be an important mediator of the fibrosis.

It is attractive to consider that $CD4^+$ T cells orchestrating the disease are sustained by continuous antigen presentation by B cells. A T follicular helper cell response that is separate from the $CD4^+$ cytotoxic T lymphocytes is likely to be responsible for the development of germinal centers within lymph nodes (and involved organs). In theory, such T follicular helper cells could produce the cytokines (e.g., IL-4) that drive the $IgG_4$ class switch, culminating in the creation of $IgG_4$-secreting plasmablasts and long-lived plasma cells. The fact that B cell depletion does not lead to the complete normalization of serum $IgG_4$ concentrations implies the presence of long-lived plasma cells that continue to make this Ig.[14]

## Serum $IgG_4$ Concentrations

Serum $IgG_4$ measurements continue to play an important role in the diagnosis and management of $IgG_4$-RD, but $IgG_4$ levels must be considered from several angles.

### Dramatic $IgG_4$ Elevations in a Minority of Patients

A serum $IgG_4$ level is often the first diagnostic test ordered when $IgG_4$-RD is suspected. Large elevations of serum $IgG_4$ concentrations—for example, on the order of six to eight times the upper limit of normal for a given assay—strongly suggest the diagnosis. Patients with multiorgan disease can have dramatic elevations in serum $IgG_4$ concentrations, occasionally exceeding 4 mg/dL.[21] Patients with $IgG_4$-RD also often have elevations of other IgG subclasses (particularly $IgG_1$, $IgG_2$, and $IgG_3$), but the elevations of $IgG_4$ are usually disproportionate.[9]

### A Diagnostic Paradox

Despite the frequently striking serum $IgG_4$ concentrations observed in patients with this disease, an important paradox exists. Among patients with active disease whose cases are identified on the basis of histopathology rather than by elevations in serum $IgG_4$ concentrations, just over half of the patients have elevated serum $IgG_4$ concentrations before treatment.[9] In contrast, when cases were sought by querying a hospital laboratory database about the number of patients with abnormally high serum $IgG_4$ measurements, the sensitivity of an elevated serum $IgG_4$ concentration was 90%.[22] Two major points should be drawn from these studies. First, a large proportion of patients with biopsy-proven, clinically validated $IgG_4$-RD have normal serum $IgG_4$ concentrations, even before beginning treatment. Reliance upon serum $IgG_4$ concentration elevations for diagnosis, therefore, is certain to lead to a dangerous degree of underdiagnosis. Second, among patients with elevated serum $IgG_4$ levels, the range of values is extremely broad. The factors contributing to such variability in the $IgG_4$ response remain poorly defined.

### $IgG_4$ as a Biomarker of Disease Activity

Similar questions pertain to the ability of elevations in serum $IgG_4$ concentrations to predict disease flares. In a

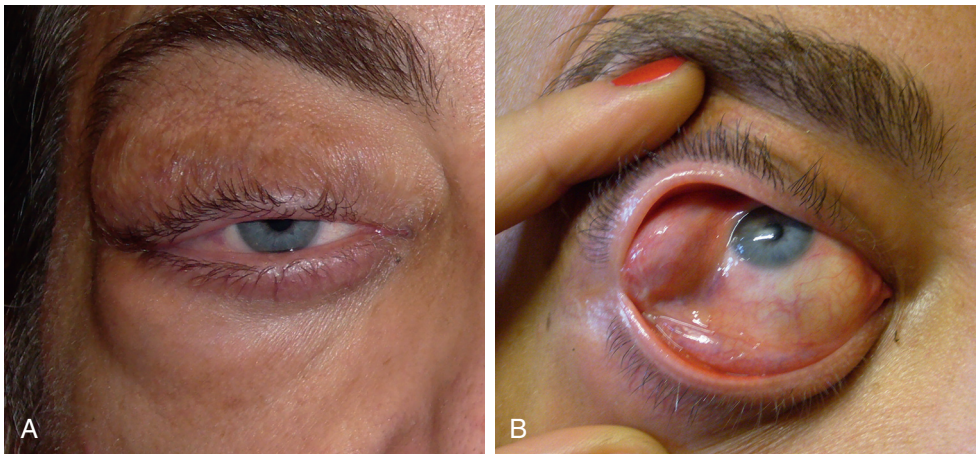



**Figure 121-7** Dacryoadenitis. **A,** Bulge of the superolateral portion of the right eye caused by lacrimal gland enlargement and enlargement of the extraocular muscles. **B,** The upper eyelid is everted, revealing the enlarged lacrimal gland.

large retrospective study of type 1 AIP from 17 centers in Japan,[23] serum $IgG_4$ levels declined in all patients after treatment with glucocorticoids but remained above the upper limit of normal in 63% of the patients despite good clinical responses. Thirty percent of the patients who relapsed had normal serum $IgG_4$ levels at the time of relapse. Thus serum $IgG_4$ concentrations should not be relied upon exclusively as an indicator of disease activity.

### Serum $IgG_4$ in Other Conditions

Mild $IgG_4$ elevations are common in a variety conditions that can mimic $IgG_4$-RD. Vasculitides such as granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome), bronchiectasis, biliary diseases such as primary sclerosing cholangitis, pancreatic cancer, and numerous others can have confounding high serum $IgG_4$ concentrations, although they rarely if ever reach the highest values achieved by some patients with $IgG_4$-RD.[24] This phenomenon imposes additional limitations on the diagnostic interpretation of an elevated serum $IgG_4$ level.

In summary, substantial elevations in serum $IgG_4$ levels are useful in suggesting the diagnosis of $IgG_4$-RD but are not diagnostic in and of themselves. A large minority of patients with histopathologically confirmed disease—on the order of 40%—have normal serum $IgG_4$ concentrations. Finally, $IgG_4$ concentrations correlate only loosely with disease activity and are imperfect predictors of the need for additional treatment.

## CLINICAL MANIFESTATIONS

The presentation of $IgG_4$-RD is typically subacute. Symptoms and signs of organ dysfunction can be evident for months or even years before the diagnosis. Although weight losses of 10 to 30 lb can occur over months, fevers and hectic presentations are unusual. Fatigue commonly accompanies $IgG_4$-RD, particularly when the disease affects multiple organ systems. Many patients have arthralgias and other musculoskeletal symptoms, particularly enthesopathy, but frank arthritis is highly atypical and should signal other diagnoses.

### Orbits

Lacrimal gland enlargement (dacryoadenitis [Figure 121-7]) is the most common feature of ophthalmic disease in $IgG_4$-RD.[25] Proptosis can result from combinations of orbital lesions, such as pseudotumors that do not affect the lacrimal gland and inflammation and thickening of the extraocular muscles. Scleritis and nasolacrimal duct disease (obstruction) also can occur in $IgG_4$-RD, thereby mimicking granulomatosis with polyangiitis.

### Nervous System Disease

The brain parenchyma is seldom if ever involved, but $IgG_4$-RD is among the most common causes of "idiopathic" hypertrophic pachymeningitis. $IgG_4$-RD is also an established cause of hypophysitis.[26] $IgG_4$-related hypophysitis can lead to hormone deficiencies from both the anterior and posterior pituitary. MRI shows sellar enlargement and thickening of the pituitary stalk. MRI studies also often reveal perineural inflammation of peripheral nerves in the area of the orbit, particularly the trigeminal and infraorbital nerves.[27] Peripheral nerve lesions typically consist of perineural masses, often up to 3 cm in diameter. These masses are often observed on MRI in the absence of overt clinical manifestations but can lead to concern about the possibility of malignant growths.

### Salivary Glands

The triad of dacryoadenitis and enlargement of both the parotid and submandibular glands is a classic presentation of $IgG_4$-RD that for more than 100 years was known as Mikulicz's disease.[28-29] Isolated submandibular gland enlargement (see Figure 121-1) is also a common finding in people with $IgG_4$-RD, but parotid and sublingual gland disease can also be prominent. Xerostomia often accompanies $IgG_4$-RD, but this condition improves with immunosuppression (in contrast to Sjögren's syndrome). Biopsies of minor salivary glands can reveal $IgG_4$-RD, although the sensitivity of this procedure is lower than that of a major salivary gland biopsy (e.g., the submandibular gland). Fine-needle aspirates of major salivary glands are useful for excluding malignancy,

but excisional biopsies are usually required for diagnostic confirmation.

## Ear/Nose/Throat

Allergic rhinitis, nasal polyps, chronic sinusitis, nasal obstruction, and rhinorrhea are common in people with IgG4-RD. Mild to moderate peripheral eosinophilia and serum IgE concentration elevations that sometimes exceed ten times the upper limit of normal are common. The prevalence of atopy among patients with IgG4-RD is similar to that of the general population, but a subset of nonatopic patients has peripheral blood eosinophilia and elevated IgE levels,[19] which suggests that processes inherent to IgG4-RD itself rather than atopy per se contribute to the eosinophilia and IgE elevation. Circulating Th2 memory cells are found only in patients with IgG4-RD who have a pre-existing history of atopy.[20]

Mass lesions can occur in the sinuses, and destructive lesions in the middle ear and facial bones have been reported.[30,31] IgG4-RD can also lead to diffuse inflammation in the pharynx, hypopharynx, and Waldeyer's ring, frequently associated with mass lesions.[32] Tracheal inflammation and vocal cord involvement have also been described.

## Thyroid Gland

Riedel's thyroiditis has been linked convincingly to IgG4-RD.[33] Fibrosing Hashimoto's thyroiditis may also fall within the spectrum of IgG4-RD disease, but further study of this entity is required.[34] More controversial is the assertion that a substantial proportion of individuals with Hashimoto's thyroiditis also have an IgG4-related disorder. A form of thyroid disease referred to as "IgG4-related thyroiditis," distinct from Hashimoto's thyroiditis, is purported to exist,[35] but this assertion requires confirmation in other studies.

## Lymphadenopathy

The lymphadenopathy associated with IgG4-RD is typically either a generalized lymphadenopathy or localized disease adjacent to an affected organ.[36] The lymph nodes involved are generally 1 to 3 cm in diameter and nontender. Involvement of the cervical, supraclavicular, submandibular, axillary, hilar, mediastinal, para-aortic, retroperitoneal, and inguinal nodes is described. Establishing the diagnosis of IgG4-RD through lymph node biopsy is often difficult because it is unusual for lymph nodes to undergo the degree of fibrosis observed in other organs. At least five histopathologic variants of IgG4-related lymphadenopathy have been described.[37] One of these variants closely mimics Castleman's disease.

## Thoracic Aorta, Branches of the Aorta, and Coronary Lesions

IgG4-related aortitis, which often is suspected on the basis of an incidental radiologic finding, is also sometimes an unexpected finding at surgery. IgG4-related aortitis can lead to aneurysms or dissections in the thoracic aorta.[38-40] In contrast to giant cell and Takayasu's arteritis, which have a predilection for the primary aortic branches—particularly the subclavian arteries—IgG4-RD tends to spare these vessels, at least in terms of clinical manifestations. Small case series substantiate the concept that medium-sized blood vessels can also be affected by IgG4-RD. The relationship between the obliterative arteritis and obliterative phlebitis observed microscopically (see the Pathologic Features section) and larger vessel disease remains unclear. Definitive histopathologic investigations of primary aortic branch vessels have not been undertaken.[41] Coronary artery lesions in IgG4-RD, sometimes associated with aneurysm formation, are documented.[42]

## Chronic Periaortitis and Retroperitoneal Fibrosis

"Idiopathic" RPF is now considered to belong to a larger disease spectrum known as chronic periaortitis. The three major components of chronic periaortitis are IgG4-related RPF (see Figure 121-2), IgG4-related abdominal aortitis, and IgG4-related perianeurysmal fibrosis.[43] IgG4-RD is responsible for up to two-thirds of cases of "idiopathic" RPF.[43,44] Some cases of idiopathic RPF that do not appear to be part of the IgG4-RD spectrum can have similar presentations and can be distinguished from IgG4-RD through careful histopathologic and immunostaining studies.

Common presentations of IgG4-related RPF and chronic periaortitis are poorly localized pain in the back, flanks, lower abdomen, or thighs; lower extremity edema; and hydronephrosis from ureteral involvement. These presentations are generally subtle at first, subacute in onset, and nonspecific, leading to diagnostic delay. The disease targets three sites: (1) periaortic/arterial regions involving connective tissue around the abdominal aorta or its first branches; (2) periureteral areas, leading to ureteral obstruction and hydronephrosis; and (3) a plaque-like mass that broadly involves the retroperitoneum.

The classic lymphoplasmacytic infiltrate may no longer be evident in cases of longstanding duration, but both storiform fibrosis and obliterative phlebitis can often still be identified. The ratio of $IgG_4^+$ plasma cells to the total number of plasma cells in tissue may be more helpful diagnostically than the overall number of $IgG_4^+$ plasma cells per high-power field (hpf) in patients with advanced fibrotic disease.

## Lung

The greatest diversity of clinical and radiologic presentations occurs in the lung.[45] Thickening of the bronchovascular bundle, best appreciated on CT examination, underscores the tendency of IgG4-RD to track along bronchi and blood vessels, which course together.[46] Other radiologic features of IgG4-RD include pulmonary nodules, ground-glass opacities, pleural thickening, and interstitial lung disease. The interstitial lung disease mimics nonspecific interstitial pneumonitis and other forms of interstitial fibrosis (see Figure 121-3).

## Kidney

Tubulointerstitial nephritis (TIN) is the most characteristic form IgG4-related kidney disease (Figure 121-4). IgG4-

related TIN recapitulates the histopathology found in other organs.[47] $IgG_4$-related TIN is often distinguished from other organ manifestations of $IgG_4$-RD by profound hypocomplementemia. The basis of this hypocomplementemia remains poorly understood, but it appears unlikely to be explained by $IgG_4$ itself, because $IgG_4$ does not bind complement effectively. The hypocomplementemia could result from the formation of immune complexes that contain $IgG_1$ or $IgG_3$, subclasses that bind complement more effectively and are often elevated to a lesser degree.

CT scanning in $IgG_4$-related TIN can reveal substantial renal enlargement and hypodense lesions within the renal parenchyma. Advanced renal dysfunction and even end-stage renal disease can result. Proteinuria can develop, but the levels are usually subnephrotic. Kidneys affected by $IgG_4$-RD can undergo atrophy, even in the setting of good clinical responses to therapy.[48]

Membranous glomerulonephropathy also occurs in $IgG_4$-RD, but the pathophysiology of this entity appears to have a pathophysiology different from that of $IgG_4$-related TIN.[49] The anti-phospholipase A2 ($PLA_2$) receptor antibody now known to cause "idiopathic" membranous glomerulonephropathy is not associated with $IgG_4$-related membranous glomerulonephropathy.[50] It is interesting to note, however, that the anti-$PLA_2$ receptor antibody is primarily of the $IgG_4$ subclass.[51]

## Pancreas

The pancreas was the first organ recognized to have an association with elevated serum $IgG_4$ concentrations.[5,6,52] Two AIP subtypes exist, only one of which (type 1) is associated with $IgG_4$-RD. Type 1 AIP demonstrates the classic histopathologic findings of lymphoplasmacytic sclerosing pancreatitis. The histologic features of type 2 AIP, which is less common than type 1, include neutrophilic infiltration into the pancreatic duct epithelium.[53-55]

The most common clinical presentation of type 1 AIP is obstructive jaundice, induced by concomitant $IgG_4$-related sclerosing cholangitis. Secondary diabetes mellitus occurs in approximately half of all cases. The nearly diagnostic CT features of AIP include diffuse pancreatic enlargement with delayed enhancement and a capsule-like low-density rim (Figure 121-8).[55] Diffuse, irregular narrowing of the main pancreatic duct on endoscopic retrograde and magnetic resonance cholangiopancreatography is also highly specific for AIP. Endoscopic ultrasonography-guided fine-needle aspiration is a useful diagnostic approach to exclude pancreatic cancer and should be attempted before any empiric glucocorticoid trial is undertaken.[56] Pancreatic stones occur with increased frequency among patients with type 1 AIP.

## $IgG_4$-Related Sclerosing Cholangitis and Cholecystitis

$IgG_4$-related sclerosing cholangitis frequently occurs with type 1 AIP.[57] The histology of $IgG_4$-related sclerosing cholangitis includes obliterative phlebitis and transmural fibrosis with dense infiltration of $IgG_4^+$ plasma cells and T cells. $IgG_4$-related sclerosing cholangitis must be differentiated from both primary sclerosing cholangitis and hilar cholangiocarcinoma. Neither serum $IgG_4$ concentrations nor cholangiographic or cholangioscopic findings differentiate these diseases with clarity,[58-61] and thus an endoscopic transpapillary biopsy is usually required. Although cholangiocarcinoma can be excluded by endoscopic biopsy, the superficial nature of endoscopic biopsies limits their utility for diagnosing $IgG_4$-related sclerosing cholangitis.[61] $IgG_4$-related cholecystitis sometimes occurs with $IgG_4$-related sclerosing cholangitis. Thickening of the gallbladder wall is detected on imaging but is usually asymptomatic.

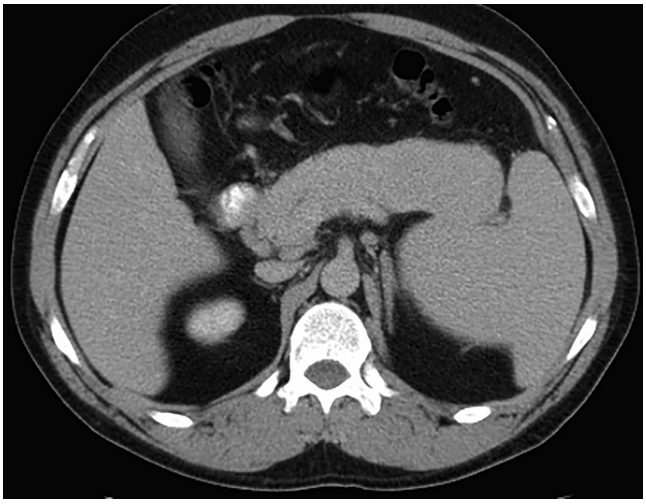


**Figure 121-8** Type 1 ($IgG_4$-related) autoimmune pancreatitis. Computed tomographic study showing an enlarged, sausage-shaped pancreas that has lost its normal markings.

## Other

Sclerosing lesions of both the mesentery and mediastinum are described.[62,63] Sclerosing mesenteritis often appears to originate at the mesenteric root. The ensuing process often merges imperceptibly with RPF and can evolve in a devastating manner, encasing vital organs and obviating any attempt at surgical resection. Fibrosing mediastinitis can lead to the compression of vital mediastinal structures from proliferation of invasive fibrous tissue. A review of 15 patients with fibrosing mediastinitis demonstrated that a substantial proportion of cases fall within the $IgG_4$-RD spectrum.[63] The relationship between these cases and antecedent histoplasmosis infections, if any, remains unclear.

Several clinical presentations of $IgG_4$-related skin disease have been reported. The most common cutaneous manifestation is the presence of erythematous papules. These lesions typically affect the region of the head and neck but have also been described on the trunk and extremities.[64] Among individuals with darkly pigmented skin, hyperpigmented lesions have been observed.

Biopsy-proven $IgG_4$-related prostatic disease leading to prostatic enlargement has been reported.[65] The diagnosis of $IgG_4$-related prostate disease is often made presumptively when the initiation of treatment for $IgG_4$-RD in other organs mediates abrupt symptomatic relief of "benign prostatic hypertrophy."

## PATHOLOGIC FEATURES

### Histologic Features

A defining feature of $IgG_4$-RD is the similarity of the histologic appearance regardless of the organ affected.[4] The hallmark morphologic findings include a dense lymphoplasmacytic infiltrate rich in $IgG_4^+$ plasma cells (Figure 121-9A and B). This lymphoplasmacytic infiltrate is enmeshed by an irregularly whorled fibrotic process that is termed "storiform" fibrosis (Figure 121-9C).[66,67] Obliterative phlebitis, resulting in destruction of the venous lumen, is another characteristic lesion observed in the majority of cases (Figure 121-10).[66] Obliterative arteritis is also observed in some organs, particularly the lung. Eosinophilic infiltration occurs in approximately 50% of cases, regardless of the organ involved.[66]

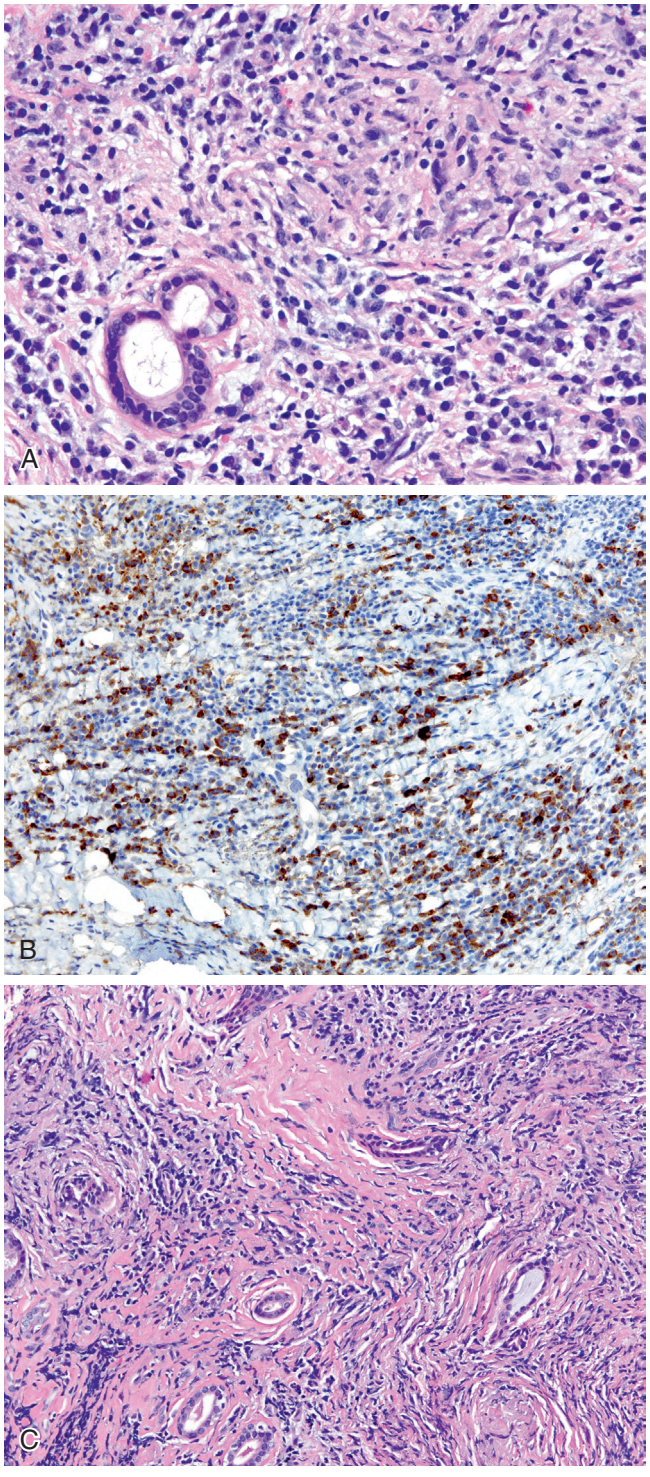



**Figure 121-9** A biopsy specimen of a subglottic lesion from the patient whose aortitis is shown in Figure 121-5 revealed a dense lymphoplasmacytic infiltrate (**A**), extensive infiltration by $IgG_4^+$ plasma cells (**B**), and storiform fibrosis (**C**). The tissue has a swirling pattern, characteristic of the type of fibrosis that is classic for $IgG_4$-RD.

### Immunostaining

The preponderance of plasma cells within tissue lesions stain for $IgG_4$. An increase in the number of $IgG_4^+$ plasma cells per hpf and an elevated $IgG_4^+/IgG^+$ plasma cell ratio can therefore support the diagnosis of $IgG_4$-RD, but careful clinicopathologic correlation and sound clinical judgment must be exercised in rendering the diagnosis of $IgG_4$-RD. An important issue pertains to the total number of $IgG_4^+$ plasma cells needed to regard a biopsy as being "diagnostic" of $IgG_4$-RD. In fact, no number of $IgG_4^+$ plasma cells within a biopsy is sufficient to support the diagnosis of $IgG_4$-RD on its own. Neither the number of $IgG_4^+$ plasma cells per hpf nor the $IgG_4^+/IgG^+$ plasma cell ratio is specific for the diagnosis of $IgG_4$-RD. Rather, the finding of the characteristic histopathology in the right clinical context allows the pathologist and clinician to reach the diagnosis in collaboration. "Positive" $IgG_4$ immunostaining lends additional support to the diagnosis of $IgG_4$-RD but is not diagnostic in and of itself.

One may expect fewer $IgG_4^+$ plasma cells in biopsy specimens from patients with fibrotic lesions such as RPF because of the overwhelmingly fibrotic nature of the disease at the time of biopsy in most patients. In such patients, as few as 10 $IgG_4^+$ plasma cells per hpf may be regarded as supportive of the diagnosis of $IgG_4$-RD, particularly if the remainder of the clinical and pathologic features are consistent with that diagnosis. In contrast, the number of $IgG_4^+$ plasma cells per hpf is generally much higher in biopsy specimens of the major salivary glands, lacrimal glands, pancreas, lungs, kidneys, and many other organs. Therefore, different criteria for appropriate cutoffs have been proposed.[66-68] A finding

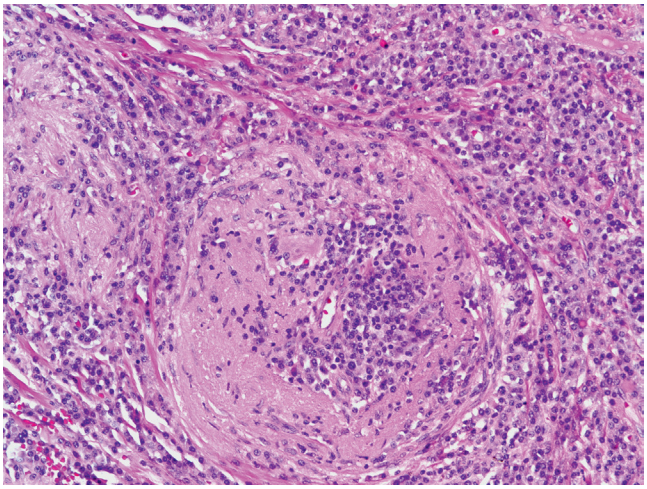


**Figure 121-10** Obliterative phlebitis. A vein observed within a lung biopsy specimen from a patient with $IgG_4$-RD. The lymphoplasmacytic infiltrate has completely obliterated the lumen of the lung.

of more than 30 $IgG_4^+$ plasma cells per hpf has good specificity for type 1 AIP, whereas more than 10 $IgG_4^+$ plasma cells per hpf has reasonable specificity for $IgG_4$-related TIN.[68,69]

The $IgG_4^+/IgG^+$ plasma cell ratio can also be a useful metric to aid in diagnosis. Most cases of documented $IgG_4$-RD have an $IgG_4^+/IgG^+$ plasma cell ratio greater than 40%. This value has been proposed as a criterion for diagnosis across all organs.[68] The $IgG_4^+/IgG^+$ plasma cell ratio can be particularly helpful in the setting of advanced fibrosis (e.g., in RPF), when the paucity of cells makes large concentrations of $IgG_4^+$ plasma cells unlikely.

### Morphologic Change of Affected Organs

Transformations in the gross pathology of affected organs occur. The pancreas and kidneys become diffusely enlarged. In contrast, ductal organs (e.g., the bile duct and bronchus) assume the appearance of a pipe stem, with diffuse wall thickening.[57]

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of $IgG_4$-RD varies according to the sites of organ involvement. Table 121-2 shows the broad differential diagnosis of this condition, by organ.

## TREATMENT

No randomized clinical trials have been performed to evaluate the comparative effectiveness of different treatment regimens, and the optimal treatment of $IgG_4$-RD is unknown. Current approaches to management are based on observational case series. The decision to treat is predicated upon patients' symptoms and the organ systems involved. Glucocorticoids are currently the first-line treatment for $IgG_4$-RD. Most data on the use of glucocorticoids are derived from the treatment of $IgG_4$-related pancreatitis (type 1 AIP) and have been extrapolated to the treatment of extrapancreatic $IgG_4$-RD. Treatment of $IgG_4$-related pancreatitis with glucocorticoids typically results in a symptomatic response within 2 weeks and remission at some point within 2 to 3 months in most cases.[21]

Many patients experience a relapse as glucocorticoids are tapered or discontinued, however, and the optimal approach to maintenance of remission remains unclear.[21] In a large cohort of patients with $IgG_4$-related pancreatitis, 34% of patients experienced a relapse after withdrawal of glucocorticoids. This figure probably underestimates the true number of relapses because patients often have recurrent manifestations of $IgG_4$-RD many years after the initial presentation.[70]

Because $IgG_4$-RD tends to involve a middle-aged to elderly patient population, extended treatment with glucocorticoids is particularly problematic because of heightened concerns for osteoporosis, diabetes, and infections. For this reason, there has been an interest in steroid-sparing agents, particularly in the subset of patients with refractory or recurrent disease. Few data suggest that conventional "steroid-sparing" agents such as azathioprine and mycophenolate mofetil are effective in the absence of concurrent glucocorticoids.[53]

**TABLE 121-2** Differential Diagnosis of $IgG_4$-Related Disease by Organ System

| Organ | Differential Diagnosis |
|---|---|
| Orbits and periorbital tissues | Lymphoma; Graves' disease; granulomatosis with polyangiitis; sarcoidosis |
| Ears, nose, and sinuses | Allergic disease; eosinophilic granulomatosis with polyangiitis (churg-strauss syndrome); granulomatosis with polyangiitis; sarcoma |
| Salivary glands | Lymphoma; Sjögren's syndrome; sarcoidosis; sialodocholithiasis |
| Meninges | Idiopathic hypertrophic pachymeningitis; inflammatory myofibroblastic tumor; granulomatosis with polyangiitis; giant cell arteritis; langerhans cell histiocytosis; sarcoidosis |
| Pituitary | Neoplasms; histiocytosis; hypophysitis: primary and secondary (sarcoidosis, ipilimumab-induced) |
| Lymph nodes | Castleman's disease; lymphoma |
| Thyroid gland | Thyroid lymphoma; differentiated thyroid carcinoma (mainly the papillary variant); other malignancy |
| Lungs | Malignancy (adeno- or bronchioloalveolar carcinoma); inflammatory myofibroblastic tumor; sarcoidosis; Castleman's disease; lymphomatoid granulomatosis; idiopathic interstitial pneumonia; Erdheim-Chester disease; inflammatory myofibroblastic tumor; granulomatosis with polyangiitis |
| Aorta | Primary large-vessel vasculitis (giant cell or Takayasu's arteritis); sarcoidosis; Erdheim-Chester disease; histiocytosis; lymphoma |
| Retroperitoneum | Lymphoma; sarcoma; Erdheim-Chester disease |
| Kidney | Lymphoma; renal cell carcinoma; drug-induced tubulointerstitial nephritis; pauci-immune necrotizing and crescentic glomerulonephritis; sarcoidosis; Sjögren's syndrome; systemic lupus erythematosus; mixed cryoglobulinemia |
| Pancreas | Pancreatic cancer; type 2 autoimmune pancreatitis |
| Biliary tree | Pancreatic cancer; cholangiocarcinoma; primary sclerosing cholangitis |
| Liver | Cholangiocarcinoma; hepatocellular carcinoma; primary sclerosing cholangitis |

From Stone JH, Zen Y, Deshpande V: $IgG_4$-related disease. *N Engl J Med* 366:539–551, 2012. Modified with permission from the Massachusetts Medical Society.

Treatment with rituximab to induce B cell depletion has yielded promising results.[14,71] A recent prospective trial of rituximab appears to validate smaller case series indicating that B cell depletion is an effective approach to the treatment of IgG4-RD.[15] Further investigation is needed to establish the optimal use of rituximab in IgG4-RD and to test methods of monitoring the clinical response.

The references for this chapter can also be found on ExpertConsult.com.

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CHAPTER 122

# Arthritis Accompanying Endocrine and Metabolic Disorders



Soumya D. Chakravarty • Joseph A. Markenson

**KEY POINTS**

- **Endocrine disorders can initially present or manifest with a multitude of musculoskeletal signs and symptoms. Additionally, endocrine manifestations of underlying autoimmune disorders are common and need to be recognized promptly.**
- **Whereas Cushing's disease arises from excessive secretion of adrenocorticotropic hormone as a result of a pituitary adenoma, exogenous Cushing's syndrome can result from chronic corticosteroid therapy and should be vigorously sought and promptly treated.**
- **Many musculoskeletal complications observed in people with diabetes mellitus can be attributed to modifications in connective tissue, leading to fibrosis and tendinopathies. Underlying diabetic neuropathy can result in a debilitating and irreversible osteoarthropathy.**
- **Prompt and thorough evaluation for underlying thyroid disease in patients presenting with musculoskeletal symptoms is critical because in most instances appropriate therapy can alleviate such manifestations.**
- **Primary hyperparathyroidism often causes a crystal-induced arthropathy consistent with calcium pyrophosphate dihydrate deposition.**

Rheumatic manifestations are often the initial presentation of systemic illnesses. Each endocrine disorder has its own set of arthritic symptoms that can mimic or present as definitive rheumatic diatheses, requiring a high degree of vigilance and a thorough assessment of patients presenting with such varied symptoms. Both rheumatologists and general internists should be well versed in identifying the manner in which components of the musculoskeletal system are affected by diseases of the endocrine system. Additionally, recognition that endocrinopathies can arise in the course of treating rheumatic illnesses, such as with the use of corticosteroids, is critical in minimizing or reversing any potential adverse sequelae in these patients. This chapter will describe the common musculoskeletal manifestations of endocrine disease encountered in the clinical setting.

## ADRENAL GLAND DISORDERS

In 1932, Harvey Cushing described several rheumatic conditions, including osteoporosis, avascular necrosis, myopathy, and synovitis, later to be termed collectively as part of Cushing's syndrome. Since then, our understanding of the syndrome has evolved, with identification of two major categories. The first category, termed *primary Cushing's disease*, is caused by a pituitary adenoma, a benign malignancy that results in oversecretion of adrenocorticotropic hormone (ACTH).[1,2] The second and more common manifestation, exogenous Cushing's syndrome (also termed *hypercortisolism* or *hyperadrenalism*), occurs as a result of the use of chronic corticosteroid therapy and can have a variety of adverse effects on the musculoskeletal system.[3] Avascular necrosis generally occurs after prolonged use of high-dose corticosteroids but can be observed months or even years after the discontinuation of steroids.[4,5] Less commonly, it can be associated with short courses of therapy or intermittent high-dose intravenous therapy; consequently, the risk for avascular necrosis should always be considered during one's assessment.[5-7]

Steroid myopathy can occur in patients treated with corticosteroids and presents as extreme muscle weakness and pain that is most often pronounced around the pelvic girdle. This muscle weakness and pain may either occur suddenly or be insidious in onset.[8-10] Usually a muscle biopsy demonstrates type 2 fiber atrophy, whereas electromyography shows a myopathic picture. Muscle enzymes are usually not elevated, and the condition gradually improves as corticosteroids are tapered or discontinued completely.[11] A similar picture has been reported in Cushing's disease.[12] As part of this clinical presentation, osteopenia, as well as progression to osteoporosis, can be related to the corticosteroid dose and duration of disease, more frequently occurring at dosages greater than 7.5 mg/day of prednisone or its equivalent[13-15]; several treatment regimens reported including the use of calcium, vitamin D ($D_3$), bisphosphonates, and teriparatide (recombinant parathyroid hormone [PTH]).[16,17] Interestingly, recent work performed to elucidate whether allelic variants in the vitamin D receptor (VDR), which has been associated with osteopenia, may have an effect on bone mineral density (BMD) in people with Cushing's disease was unable to demonstrate an association between BMD and common VDR polymorphisms.[18] Finally, the myopathy associated with Cushing's disease can mimic other primary musculoskeletal disorders, such as statin-induced myopathy, polymyalgia rheumatica, or polymyositis, and thus should also be included in the differential diagnosis.[19]

Adrenal insufficiency can be categorized into three subgroups depending on the level at which the hypothalamic-pituitary-adrenal (HPA) axis is involved.[19] Primary adrenal insufficiency, or Addison's disease, is caused by damage to

the adrenal gland. The secondary form results from insufficient ACTH production by the anterior pituitary gland, whereas the tertiary form is a consequence of inadequate corticotropin-releasing hormone (CRH) secretion by the hypothalamus. Acute adrenal insufficiency, also known as adrenal crisis, can be a life-threatening condition and is characterized by shock. The cause of primary adrenal insufficiency is believed to be primarily autoimmune in nature and is associated with hypotension, nausea, abdominal pain, weight loss, generalized weakness, and hyperpigmentation.[20] Secondary adrenal insufficiency usually results from destruction of the anterior pituitary gland or ACTH deficiency, with precipitating events such as hemorrhage, thrombosis, or granulomatous diseases like sarcoidosis. The chronic use of corticosteroids and their subsequent cessation can trigger secondary or tertiary adrenal insufficiency.[21] The mechanisms underlying corticosteroid-induced adrenal insufficiency are varied and include decreased production of CRH and adrenal atrophy, particularly after chronic ACTH deficiency. Addisonian crisis, which can be a life-threatening event, has been observed in patients receiving even physiologic doses of corticosteroids. Because the likelihood of HPA axis suppression and the inability to respond to medical and surgical stressors is very high in individuals treated with more than 5 mg/day of prednisone or its equivalent, transiently increasing steroid dosing in the acute setting is advisable.[22-24]

## ACROMEGALY

Acromegaly is caused by hypersecretion of growth hormone (GH) and subsequently of insulin-like growth factor (IGF)-I as a result of a GH-secreting pituitary tumor. Other causes, such as increased GH-releasing hormone (GHRH) production from hypothalamic tumors, ectopic GHRH production, and ectopic GH secretion from nonendocrine tumors, are rare. Acromegaly occurs most commonly in middle-aged men and women. Its prevalence is 4.6 cases per million population, and the incidence is 116.9 new cases per million per year.[25] Hypersecretion of GH affects all organs, causing disturbances in structure, endocrine function, and metabolism. GH released into circulation stimulates production of IGF-I, which is the primary mediator of GH and stimulates growth of soft tissue and fibroblast proliferation, causing an increased thickening of connective tissue. Local manifestations also occur with growth of a pituitary adenoma as it compresses local structures and causes neurologic symptoms.[26,27]

Musculoskeletal manifestations of acromegaly present as arthralgias of the large joints (i.e., knees, hips, and shoulders). Patients may experience proximal muscle weakness, although muscle enzymes are generally normal and biopsy specimens may demonstrate variation in fiber size without inflammation. Fibrous synovial thickening and/or bony overgrowth in the proximal interphalangeal (PIP) and distal interphalangeal joints is reported in 50% of patients.[28] Radiographic changes include an enlarged sella turcica, increased thickness and enlargement of the frontal sinuses, elongation of the mandible, soft tissue thickening, broad metacarpals and phalanges, prominent distal tufts, widened joint spaces (due to cartilage hypertrophy), increased thickness of the heel pad, broadening of the sternal ends of the ribs, kyphosis, and enlargement of the vertebral bodies.

Morning stiffness and joint swelling are rare, and the severity of musculoskeletal complaints seems to correlate with the severity of disease. Carpal tunnel syndrome (CTS) occurs in approximately 60% of patients with acromegaly and is frequently bilateral.[29,30] GH secreted by the anterior pituitary acts on hepatocytes to secrete somatomedins, which have stimulatory effects on bone and cartilage, including chondrocyte replication, synthesis of proteoglycans, collagen, osteoblastic proliferation, and increased bone collagen (resulting in increased bone mass).[31] Somatomedins also have insulin-like activity on muscle and fat. Treatment is aimed at decreasing the size of the adenoma (surgically, using ultrasound or laser), and success may not result in changes in the musculoskeletal findings or symptoms.

Arthralgia, as originally described by Pierre Marie in 1886, is one of the most commonly reported symptoms of people with acromegaly, affecting up to 75% of patients. Involvement of large peripheral joints (i.e., the shoulder, knee, and hip) is experienced by 60% to 70% of patients. Back pain may be a particularly troubling feature of acromegaly. Approximately 50% of patients have axial arthropathy, mainly affecting the lumbar area. Patients report a dull, diffuse pain in the lumbar region.[32] Other reported symptoms relate to limited range of movement, joint instability, and joint deformation. Ossification of the anterior surface of vertebral bodies is common and in more severe cases can bridge the disk space, resembling the diffuse idiopathic skeletal hyperostosis syndrome. Biermasz and colleagues[33] reported that a high prevalence of self-reported joint symptoms persisted despite successful long-term treatment of acromegaly. These joint problems were an important indicator of impaired quality of life.[33]

### Bone Mass and Quality in Acromegaly

Although acromegaly is often included in lists of endocrinopathies associated with osteoporosis because of increased bone turnover, some investigators have reported normal or increased bone mass in individuals with this disorder.[34] During childhood, GH secretion stimulates linear bone growth via its effect on IGF-I. The effects of GH secretion due to acromegaly on skeletal mass and bone metabolism in adults have been somewhat conflicting. GH and IGF-I have a stimulatory effect on osteoblast function, and several studies indicate a potential anabolic effect of GH, at least on the cortical bone. People with acromegaly had significantly higher levels of osteocalcin than did control subjects. Osteoporosis occurs rarely in individuals with acromegaly, and if present, it may be a result of hypogonadism. Acromegaly causes an increase both in bone apposition and resorption, and as a result, data on bone mass are controversial, generally because different skeletal sites are reported, different equipment is used for measurement, and data for groups have been reported without regard to comparison of sex and gonad status. Data on cortical bone generally show a normal or even increased BMD (regardless of the gonadal status), whereas discrepant data are seen with measurement of trabecular bone. However, acromegaly does confer an increased risk of morphometric vertebral fractures, which seems to correlate with acromegaly activity and its duration

but can persist after biochemical control is achieved.[35] Unfortunately, especially in post-menopausal women, these osteoporotic vertebral fractures often can be asymptomatic and thus are underdiagnosed.[35,36]

## HYPOTHALAMIC-GONADAL AXIS

Female-to-male ratios differ among connective tissues diseases. The ratio for rheumatoid arthritis (RA) is 3:1, with the exception of juveniles and older adults. Systemic lupus erythematosus (SLE) has a female-to-male ratio of 9:1, and in RA, the incidence is higher in post-menopausal as opposed to pre-menopausal women.[37] Adult men have a significant delayed onset of disease. Masi and Kaslow[38] and Petri[39] have suggested that these findings may be due to gonadal hormones with protection against RA in younger men (as a result of testosterone) and a predisposition to SLE in women (as a result of estrogens). In a longitudinal study of the levels of total testosterone, sex hormone–binding globulin, and luteinizing hormone (LH) in 41 males with early RA (naïve to disease-modifying anti-rheumatic agents [DMARDs] at study entry) over 2 years,[40] it was only in male patients younger than 50 years that lower baseline levels of testosterone were found compared with control subjects. When treated with DMARDs, the males who responded with decreased disease activity (as measured by Disease Activity Score–28 and the Health Assessment Questionnaire) also demonstrated significantly increased levels of testosterone. Changes in testosterone levels in patients older than 50 years were marginally better after DMARD treatment, and LH levels in both groups were unchanged (although lower in men older than 50 years). The consequence was improvement of the hypothalamic–pituitary–gonadal (HPG) axis at the gonadal level but not at the HPA level. In patients with RA, both the HPG and HPA axes have been reported to respond abnormally to inflammation and stress. The important question of whether inflammation decreased the HPA axis (increasing the risk for RA) and whether treatment reversed this risk was not answered. As pointed out by Masi et al.,[40] addressing this question would have required calculating baseline levels for these hormones prior to the onset of disease.

Adrenal glucocorticoids, secreted in response to ACTH stimulation, are considered to be among the key factors involved in the regulation of immune responses. It follows that dysfunction of the HPA axis caused by its inherited or acquired downregulation could create a predisposing environment for autoimmunity development.[41] Specific gene variants have been associated with several changes in HPA axis reactivity, resulting in suboptimal cortisol levels during challenges.[42] In addition to genetic predisposition, some early life events appear to program HPA function in adulthood. Hence this process could contribute to the development of various diseases, including RA, as seen in animal models of arthritis.[43,44]

### Adrenal Androgens in Rheumatoid Arthritis

Glucocorticoids play a role in regulating the expression of peptide genes in corticotropin-releasing hormone neurons that encode ACTH, thereby functioning as negative feedback to the HPA axis.[45] However, considerations of trophic (cell mass/competence) influences on the amount of hormones may occur at the adrenal gland level.[46] Adrenal glands of patients with pre-menopausal onset RA may have relatively deficient mass capacity of their endocrine function, particularly in the zona reticularis production of adrenal androgens, resulting in dissociation of glucocorticoids and adrenal androgens.[47] This phenomenon could result in functional and anatomic changes in the zona reticularis of the adrenals. Evaluation of androgen concentrations in patients with RA showed decreased dehydroepiandrosterone sulfate (DHEAS) in pre-menopausal women and decreased testosterone levels in men.[48] In a study of 50 human leukocyte antigen RA identical post-menopausal discordant sibling pairs, significantly lower levels of DHEAS correlated with disease severity and duration.[49] A prospective study showed decreased DHEAS levels in 35 women before disease development.[48] Another study reported no significant differences between 116 patients and 329 control subjects.[50] In addition, patients not treated with glucocorticoids have altered steroidogenesis of DHEA and DHEAS. Combined lower DHEAS and lower baseline cortisol levels could indicate adrenocortical hypocompetence. In a previous study, 40% of pre-menopausal women with RA were identified with combined relatively lower basal cortisol and lower DHEAS levels.[51] This finding might suggest decreased basal adrenal function and a consequent impact on immune function.

## DIABETES MELLITUS

Among individuals with diabetes mellitus (DM), an increased frequency in rheumatologic disorders has been observed compared with healthy control subjects.[52,53] In people with long-standing diabetes, connective tissue is subjected to various modifications, ranging from glycosylation of proteins to microvascular damage to blood vessels and nerves and deposition of extra-cellular matrix proteins in skin and periarticular areas. Cumulatively, this modification can result in a wide spectrum of musculoskeletal disorders and complications.[52,53]

### Shoulder

Adhesive capsulitis, or frozen shoulder, represents the most common form of shoulder involvement in diabetes, reportedly occurring in approximately 20% of diabetic patients.[54] A recent longitudinal study demonstrated that the hazard ratio of developing adhesive capsulitis for the diabetes group compared with the nondiabetes group was 1.333 (95% confidence interval [CI], 1.24 to 1.44, $P$ <0.0001).[55] Patients generally report shoulder stiffness and impaired external and internal range of motion. Another manifestation to consider with diabetics includes calcific shoulder tendonitis, which occurs three times more often in these patients compared with individuals without diabetes and involves periarticular calcium hydroxyapatite deposition, predominantly in the area of the rotator cuff tendons.[56]

### Hands

Involvement of the hands in diabetic patients is observed in more than 30% of patients and can present in a myriad

of forms, including trigger finger, flexor tenosynovitis, Dupuytren's contracture, and/or "stiff hand" syndrome.[57-60] Incidentally, the onset of these manifestations often predicts increased renal, ophthalmologic, and other complications of DM. Trigger finger results from the occurrence of stenosing flexor tenosynovitis, which drives proliferation of fibrous tissue in the tendon, particularly when the tendon passes through the fibrous ring or pulley. De Quervain's tenosynovitis entails the same mechanism at the radial styloid with thickening of the extensor pollicis brevis or the adductor pollicis longus tendon and occurs in 17% to 23% of patients with DM.[61] Dupuytren's contracture (palmer thickening of the flexor tendons) initially was reported in 15% to 21% of diabetic patients.[62] Flexion contractures of the fingers can occur, usually involving the fourth digit, but the second through fifth digits also can be involved. Interestingly, a study demonstrated a positive result with injections of collagenase clostridium histolytic for the treatment of Dupuytren's contracture.[63] Finally, diabetic "stiff hand" syndrome (or diabetic cheiroarthropathy)—a fibrosing syndrome often resembling scleroderma with features of thick, tight, and waxy skin—results in contractures at the metacarpophalangeal (MCP) and PIP joints.[57,64] This symptom is classically manifested in the advanced stages of the process by the patient's inability to press his or her palms together completely without a gap remaining between opposed palms and fingers and is known as the "prayer sign."

CTS can be a frequent complication of diabetes, occurring in up to 20% of diabetic patients. The mechanisms underlying median nerve entrapment involve the same aspects of connective tissue modulation in diabetes as previously mentioned.[65] Associated motor weakness also may occur as a consequence of median nerve compression and should be assessed. Testing in the form of an electromyographic (EMG)/nerve conduction velocity study can confirm the diagnosis of CTS.[66] More recently, ultrasonography has also become a valuable tool for confirming the diagnosis of CTS.[67,68] Ultrasonography permits detection of changes in the shape of the median nerve, can assist in ruling out anatomic variants, and can determine whether any space-occupying lesions such as ganglion cysts or tenosynovitis are also present.[67,68]

### Feet

Patients with DM often present with a diabetic neuropathy and its associated arthropathy, known as diabetic osteoarthropathy or Charcot's arthropathy.[69,70] Diabetic osteoarthropathy is rare; it is seen in people with long-standing disease and in both type 1 and type 2 diabetes.[69,70] The pathogenesis is believed to be neurovascular rather than neurotraumatic (resulting from decreased sensitivity of nerve endings) or caused by reduced flow as a result of arterial sclerosis of small vessels.[71] Because of neuropathy involving the sympathetic nervous system, patients with diabetes have increased blood flow to subchondral bone, resulting in increased osteoclastic activity and bone resorption. This phenomenon occurs even in the absence of peripheral vascular disease, resulting in bone fatigue and disorganization. It appears radiographically as progression from localized osteopenia to osteolysis of subchondral bone, fragmentation of bone and cartilage (part of which may be come embedded in synovial tissue), and sclerosis.[72] In addition to plain radiography, MRI and bone scintigraphy can be useful studies in evaluating the extent of disease. Joints involved in order of frequency include the ankle, metatarsophalangeal, and tarsometatarsal. This distribution differentiates diabetic arthropathy from tabes dorsalis in which the knee is more commonly involved. The pathogenic mechanism active in DM that can cause tissue damage is multifactorial. One possibility with regard to chondrocytes includes the role of receptors for advanced glycation end products (which are increased in individuals with diabetes and are mediators of inflammation and increased atherosclerosis), thereby upregulating matrix metalloproteinases that are involved in inflammation.[73-75]

### Muscles

Diabetic muscle infarction, a rare condition marked by spontaneous infarction of muscle tissue with no preceding injury, occurs largely in patients who are considered "brittle diabetics."[76] Characteristically, these patients will have accumulated numerous microvascular sequelae from their uncontrolled disease. Clinically, patients will present with abrupt onset of pain and swelling, usually involving the thigh or calf muscles, and creatine phosphokinase (CPK) levels may be normal or elevated.[76] MRI using T2-weighted imaging may be useful in arriving at the diagnosis, with confirmatory testing involving an incisional muscle biopsy and demonstration of muscle edema and necrosis.

### Diffuse Idiopathic Skeletal Hyperostosis

Diffuse idiopathic skeletal hyperostosis (DISH) is characterized by ossification of the anterior longitudinal ligament of the spine, can involve various other extraspinal ligaments, and is generally accompanied by osteophyte formation (Figure 122-1). However, intervertebral disk spaces and sacroiliac joints are commonly spared. A high incidence of DISH has been demonstrated in a cohort of patients affected by both clinically active and subclinical DM (both type 1 and type 2), as well as in obese patients.[77] A more recent study found that individuals in their fifth decade of life are more likely to be affected by DISH if they were obese, had a first-degree relative with either DM or hypertension, had lumbar or thoracic spinal pain, and were affected by enthesopathies or tendonitis.[78] The likelihood of relatively young patients with three or more of these clinical parameters being affected by DISH was six times higher compared with age- and sex-matched control subjects ($P = 0.004$).[78]

## HYPOTHYROIDISM

The most common cause of primary hypothyroidism in the United States is autoimmune (Hashimoto's thyroiditis [HT]; also known as chronic autoimmune thyroiditis), with an occurrence of 3.6/100 person-years in females and 0.8/1000 person-years in men.[79-81] Other causes include pituitary adenoma or Sheehan's syndrome, surgical resection, iodine deficiency, deficiency in the iodothyronine synthetase pathway, or use of certain drugs (lithium or

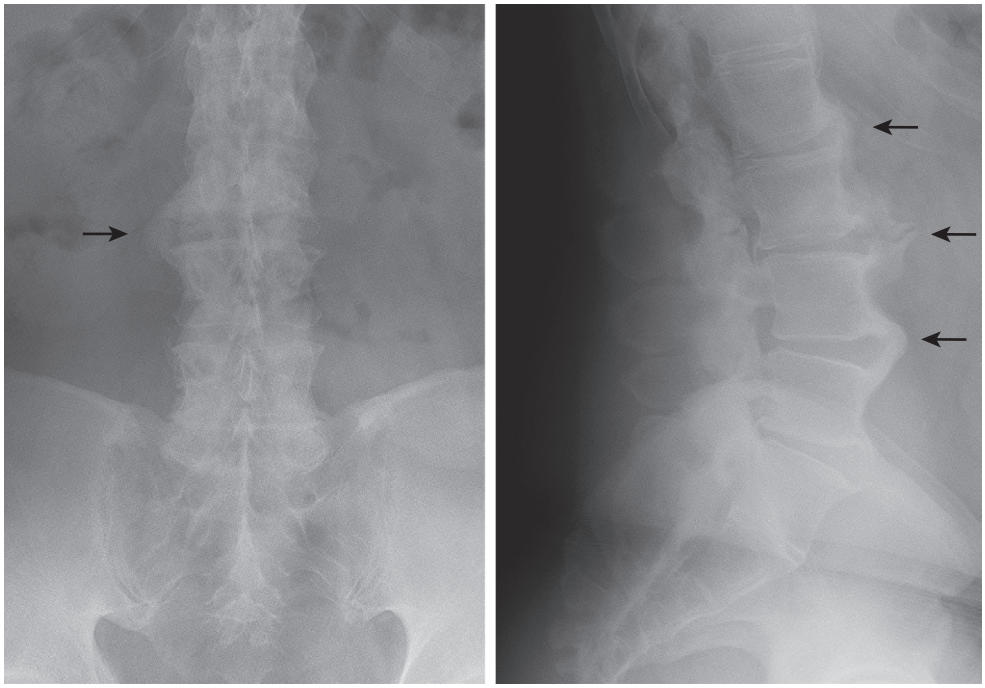


**Figure 122-1** A patient with diffuse idiopathic skeletal hyperostosis, as seen on anterior-posterior *(left)* and lateral *(right)* views. Calcifications of the anterior longitudinal spinal ligament are observed *(arrows)*. With advanced disease, these calcifications will typically progress into osteophytes that can extend across multiple vertebrae.

amiodarone). Hypothyroidism often presents with a characteristic symmetrical arthropathy involving stiffness of the joints of the hands and knees. Upon palpation, the joints feel "gelatinous," and aspiration of fluid is usually non-inflammatory and viscous, with high levels of hyaluronic acid. Calcium pyrophosphate dehydrate (CPPD) crystals are often found, and hypothyroidism should be included in the differential diagnosis of joints that demonstrate CPPD crystals upon aspiration.[82] The conditions associated with CPPD crystal deposition include:

- Hyperparathyroidism
- Familial hypocalciuric hyperkalemia
- Hemochromatosis
- Hemosiderosis
- Hypophosphatasia
- Hypomagnesemia
- Hypothyroidism
- Gout
- Neuropathic joints
- Aging
- Amyloidosis
- Trauma, including surgery

In patients with hypothyroidism, a myopathy has been reported that presents with pain, cramps, stiffness, proximal weakness, fatigue, elevated CPK levels, abnormal muscle disease on biopsy (atrophy of type II fibers and an increase in type I fibers), and hypercholesterolemia.[83,84] Hoffman's syndrome is a rare disorder of hypothyroidism manifesting with increased muscle mass (pseudohypertrophy in addition to the other aforementioned manifestations). Kocher-Debré-Semelaigne syndrome is a disorder of infancy (cretinism) presenting with similar pseudohypertrophy in mothers who have deficient levels of iodine.[85] Most symptoms of myopathy resolve upon treatment with thyroid hormone. Explanations for the effect of thyroid hormone on muscle include delayed contraction and relaxation (related to a change from fast to slow muscle fibers), alteration in myosin heavy chain gene forms, and impaired glycogenosis.[86] A neuropathy such as CTS can also be the initial manifestation of hypothyroidism. The mechanism for compression is thought to be a result of the accumulation of glycosaminoglycans within the surrounding tissues. One systematic review of 4908 patients with CTS found a pooled odds ratio of 1.4 (95% CI, 1.0 to 2.0) for the prevalence of concomitant hypothyroidism compared with healthy control subjects.[87]

## THYROID DISEASE

Patients with hyperthyroidism because of Graves' disease may present with pretibial myxedema and ophthalmopathy. The myxedema appears as nodules (usually 1 cm or larger) over the pretibial surface. They are composed of hyaluronic acid, are pink to purple in color, and are painless.[88,89] Patients with Graves' disease can also have proximal muscle weakness, associated shoulder adhesive capsulitis, loss of muscle mass, and weight loss. Most of these manifestations will correct with treatment.[90] Nail changes, onycholysis, and clubbing (thyroid acropachy) may be associated with periostitis around the metacarpal joints, as well as soft tissue swelling of the digits. Unfortunately, this condition may not return to normal when the patient is treated.[91,92]

A common and serious manifestation of hyperthyroidism is osteopenia and osteoporosis. Treatment with thyroid

hormone to achieve a normal level of thyroid-stimulating hormone (TSH), as well as adjusting the dose of thyroid replacement in hypothyroid patients, is essential. Achieving improvement in BMD (as measured by bone densitometry) in patients with thyroid disease is important.[93,94]

## Thyroid Disorders in Patients with Rheumatic Disease

In a recent prospective trial of 201 patients with rheumatic disease and 122 age matched healthy control subjects, mean TSH values of the patients with rheumatic disease and the control subjects were 3.1 ± 2.7 mIU/L and 1.9 ± 0.8 mIU/L, respectively ($P$ = 0.004).[95] The mean fT4 value of the patients with rheumatic disease was 1.43 ± 0.67 ng/dL, whereas that of the control subjects was 1.58 ± 0.68 ng/dL ($P$ <0.001), and thyroid autoantibodies were significantly higher in patients with rheumatic disease versus control subjects ($P$ <0.001).[95] A cohort of patients with and without RA from the Mayo Clinic was studied. No difference was found in the incidence of hypothyroid disease between patients with RA and non-RA control subjects. However, the presence of hypothyroidism in patients with RA was positively correlated with increased cardiovascular disease in the RA population even after adjustment for other traditional risk factors.[96] In a group of euthyroid patients with HT and no well-defined connective tissue disease, 98% presented with arthralgia and 59% with fibromyalgia, 28% had Raynaud's disease, and 26% had sicca symptoms.[97] Arthritis was radiographically present in 88% of these patients (arthritis of the spine accounted for 45%). TSH levels correlated with anti-thyroid peroxidase (anti-TPO) antibodies, but not erythrocyte sedimentation rate nor anti-thyroglobulin (anti-TG) antibody levels. The authors concluded that rheumatic manifestations frequently occur in patients with CTL in the absence of overt thyroid dysfunction.[97]

The occurrence of chronic HT, as well as hypothyroidism, is common in the general population, making it difficult to ascertain whether the incidence of either is increased in patients with connective tissue disease.[81] Two exceptions may be scleroderma in which fibrosis is the cause of hypothyroidism and congenital heart block in children of hypothyroid anti-Ro/SS-A mothers. Medications used to treat various connective diseases, as well as the disease itself, can affect thyroid function. For example, glucocorticoids inhibit TSH secretion, thereby slightly lowering serum thyroid hormone concentrations. Aspirin and NSAIDs may lower serum thyroid hormone by interfering with the binding to their carrier proteins.

## Systemic Lupus Erythematosus

Anti-thyroid antibodies are present in 15% to 20% of patients with SLE.[98] Concomitant hypothyroidism is estimated at 5.7% (five times greater than expected), as opposed to hyperthyroidism, occurring at 1.7% (similar to the normal population). This association may be genetic, as noted in a study of multiplex SLE families in which 35 of them who also had autoimmune thyroid disease demonstrated a linkage with 5q14.3-15 (the major susceptibility locus for SLE also found in autoimmune thyroid disease). This finding suggests that 5q14.3-15 harbors a susceptibility gene shared by patients with SLE and autoimmune thyroid disease.[99]

A common reason for referral to a rheumatologist is a young female patient with arthralgias from whom the referring physician has obtained a blood sample testing positive for anti-nuclear antibody (ANA). Studies of patients with Graves' disease and chronic autoimmune thyroiditis have demonstrated upward of 26% positivity for ANA and 34% for anti–single-stranded DNA antibodies. No patients in these studies had antibodies to double-stranded DNA or extractable nuclear antigens (anti-Ro/SS-A, anti-La/SS-B, anti-SM, or anti-RNP).[100] With respect to polymyalgia rheumatica and giant cell arthritis, few studies are available, with some demonstrating a relationship[101] but most reporting no association.[102,103] However, the occurrence of polymyalgia rheumatica or giant cell arthritis may be more common in patients with autoimmune thyroid disease.[104] In people with fibromyalgia, an attenuated response of TSH to thyrotropin-releasing hormone, as well as an increased prevalence of anti-thyroid antibodies compared with control subjects, has been reported (the clinical significance of which is unknown).[105,106]

## Rheumatoid Arthritis and Psoriatic Arthritis

In both RA and psoriatic arthritis, an increase in subclinical and clinical disease and an increase in the frequency of anti-thyroid antibodies has been demonstrated compared with control subjects.[107-109] Even though this increase in anti-TPO and anti-TG antibodies and abnormal variation in TSH level was demonstrated, no significant alteration of hormone levels was found, nor was there any association with any particular RA phenotype.[110]

## Sjögren's Syndrome

Prevalence of thyroid disease runs from a low of 10% to a high of 70%; however, this phenomenon may be related to the demographics of Sjögren's syndrome, which usually affects older women. In one study, the occurrence of hypothyroidism was 14%, with the occurrence of thyrotoxicosis being 1.8%.[111] Symptoms of Sjögren's syndrome, such as conjunctivitis sicca and xerostomia, have been reported in up to 32% of patients with autoimmune thyroiditis.[112] In a large study of 479 patients with Sjögren's syndrome, the frequency of HT (6.26%) was found to be higher than in the normal population (1% to 2%); however, this increased frequency was not seen for Graves' disease. It was also observed that the presence of Graves' disease and HT could appear either before or after the occurrence of Sjögren's syndrome, making it difficult to ascertain if primary Sjögren's syndrome was a predisposing factor for the development of autoimmune thyroiditis.[113]

## Scleroderma and Mixed Connective Tissue Disease

As previously discussed, fibrosis of the gland can lead to hypothyroidism, and in this group only 50% of patients had positive tests for anti-thyroid antibodies.[114] Unfortunately, few data are available for mixed connective tissue disease. Anti-thyroid antibodies were found in a quarter of the

patients, and overt hypothyroidism was found in fewer than 20% of patients.[109]

### Juvenile Idiopathic Arthritis

The occurrence of anti-thyroid antibodies (anti-TG and anti-TPO), as well as thyroid function, was assessed in a small group of children with juvenile idiopathic arthritis (JIA) compared with control subjects. The investigators concluded that children with JIA have a higher incidence of anti-thyroid antibodies and subclinical hypothyroidism than do normal children. The children who were positive for antibodies all had oligoarticular JIA.[115]

### Spondyloarthritis

To evaluate the prevalence of HT in a group of patients ($n$ = 357) with spondyloarthritis (SpA) and normal age-matched control subjects ($n$ = 318), serum levels of TSH, free triiodothyronine, and free thyroxine, along with titers of anti-TG and anti-TPO antibodies, were assayed.[116] Indices of thyroid autoimmunity were significantly more frequent in patients with SpA than in control subjects (24.1% vs. 10.7%, $P$ <0.05). In the SpA group, a higher prevalence of HT was found in patients with active disease than in those with low to moderate disease levels. Also in the SpA group, patients with a disease duration greater than 2 years had a higher prevalence of HT and anti-TPO antibody positivity than did patients with a disease duration less than 2 years. Ultrasonography of the thyroid gland was performed in all subjects, and rheumatic activity was evaluated. Ultrasonography detected a significantly higher frequency of thyroid nodules and hypoechoic pattern in patients with SpA than in control subjects. HT and anti-TPO antibodies were significantly more frequent in patients with SpA who had peripheral involvement (68.6%) than in patients with axial involvement (31.4%; $P$ <0.05). This study demonstrated a significantly higher prevalence of thyroid autoimmunity in patients with SpA compared with control subjects. Thyroiditis occurs more frequently in patients with longer disease duration and active rheumatic disease. The authors suggested that thyroid function tests be part of the clinical evaluation in patients with SpA.[116]

### Hashimoto's Thyroiditis

HT frequently overlaps with autoimmune disease. To evaluate for the prevalence of various non–organ-specific autoantibodies in patients with HT, 80 patients with SLE and RA and a control group of 34 healthy subjects were examined for serum FT3 and FT4, TSH, and antibodies against thyroglobulin and thyroperoxidase; in addition, ultrasound of the thyroid gland was performed.[117] Patients with HT had a significantly higher prevalence of ANAs than did control subjects (45% vs. 14.7%, $P$ <0.001). There were no significant differences in the prevalence of other antibodies between the groups. $ANA^+$ patients were younger than $ANA^-$ patients and had significantly higher anti-TG values ($P$ <0.05). The prevalence of HT in people with RA and SLE was significantly higher than in the control subjects (24% vs. 8%, $P$ <0.05). No significant differences in the prevalence of HT were detected between patients with SLE and RA. The investigators concluded that ANA is the most frequent non–organ-specific antibody associated with HT, whereas the other antibodies occur rarely. The prevalence of HT in patients with SLE and RA was 24%. These results indicate that it is clinically important to screen patients with SLE and RA for the coexistence of thyroid autoimmune disease.[117]

## HYPOPARATHYROIDISM

A disorder of PTH is usually caused by either autoimmune destruction of the parathyroid glands (isolated endocrine deficiency syndrome or failure of other endocrine glands) or neck surgery with extensive removal of parathyroid tissue. Two other lesser causes are a congenital disorder with failure of derivatives of the third and fourth pharyngeal pouch (DiGeorge syndrome) and intra-cellular processing defects or mutations of the calcium sensing receptor. Bone structure and bone turnover is different from normal and may result in enthesopathy, as well as paravertebral and or subcutaneous calcifications.[118] The clinical manifestations (circumoral numbness, paresthesias, carpal and pedal muscle spasms, laryngeal spasm, tetany, and/or seizures), which are seen infrequently, are a result of hypocalcemia.

Albright's osteodystrophy or pseudo-hypoparathyroidism (pseudo HoPT) results from end organ resistance (bone and kidney) to PTH and presents with elevated levels of PTH, hypocalcemia, and hyperphosphatemia. Type 1a HoPT (autosomal dominant) is inherited maternally and characterized by calcification of the paraspinal ligaments, short stature, and mental retardation. Patients clinically have shortened metatarsal and metacarpal bones and a defect in the genes encoding the α-subunit of the cell membrane–associated guanine nucleotide stimulating unit of adenyl cyclase.[119] Type 1b HoPT also has resistance to PTH but has a normal phenotype and is inherited paternally.[120,121] Soft tissue calcifications not clinically relevant (in basal ganglia, cataracts, shoulder joints, or subcutaneous tissues of the hands) have been reported in HoPT and infrequently in pseudo HoPT.[122] Surgically induced HoPT may also be accompanied by muscle weakness that is usually related to the degree of hypocalcemia and is responsive to treatment with vitamin D and calcium.

Renal disease can result in crystal deposition disease (sodium urate, calcium pyrophosphate, or basic calcium phosphate hydroxyapatite) as a result of hyperphosphatemia from reduced glomerular filtration and secondary hyperparathyroidism.[123] Sodium urate deposition results in acute gout seen more commonly in people with renal insufficiency. Gout is rare in patients undergoing dialysis but can be seen after renal transplantation with decreased creatinine clearance and use of calcineurin inhibitors, such as cyclosporine. CPPD deposition is seen less often in renal disease than gout or hydroxyapatite and is rare in individuals undergoing dialysis. Hydroxyapatite deposition can cause acute synovitis and periarticular inflammation. Painful subcutaneous nodules or chronic asymptomatic nodules (uremic humoral calcinosis) can occur. Prevention is achieved through phosphate restriction, adequate dialysis, and oral phosphate binding agents.

Renal osteodystrophy is a result of osteomalacia, osteitis fibrosa cystica, osteosclerosis, aluminum toxicity, osteoporosis, and β2 microglobulin amyloid deposition and presents with bone pain, muscle wasting, and myalgias, as well as bone fractures.[124] To define the abnormalities in skeletal microstructure and to establish the potential efficacy of PTH replacement in hypoparathyroidism, standard histomorphometric and micro–computed tomography analyses were performed on iliac crest bone biopsies obtained from patients with hypoparathyroidism. Participants were treated with teriparatide for 2 years and compared with age- and sex-matched control subjects with no abnormalities in parathyroid function. Initially bone density was increased and skeletal features reflected the low turnover state with greater bone volume/trabecular bone volume), trabecular width, and cortical width, as well as suppressed mineralizing surface and bone formation rate compared with control subjects. With teriparatide therapy, bone turnover and BMD increased in the lumbar spine. Requirements for calcium and vitamin D fell, whereas serum and urinary calcium concentrations did not change. The investigators concluded that the abnormal microstructure of the skeleton in hypoparathyroidism reflects the absence of PTH. Replacement therapy with PTH has the potential to correct these abnormalities and to reduce the requirements for calcium and vitamin D.[125]

### Similarities to Other Rheumatic Diseases

The skeletal abnormalities of hypoparathyroidism are caused by calcification, which can simulate ankylosing spondylitis with clinical signs, including morning stiffness, gait, and posture.[126] Sacroiliitis is not expected, although it is the earliest manifestation in most patients with ankylosing spondylitis. The patterns of syndesmophytes in patients with hypoparathyroidism can resemble those of ankylosing spondylitis with origin from the vertebral margin and preserved disk space, but more often there is also involvement of the posterior paraspinal ligament. Spinal changes in hypoparathyroidism have also been described to be similar to those in DISH, which is characterized by ossification of the anterior longitudinal ligament of the spine and of various extraspinal ligaments, but is rarely reported before 50 years of age. The pain is not responsive to immunosuppressive agents and NSAIDs but may resolve completely upon treatment with calcitriol.[127] Serum calcium may need to be included in the diagnostic workup of patients with inflammatory back pain, especially if they present with atypical features as previously described. It is important to differentiate hypoparathyroid-related spondylitis from ankylosing spondylitis because the management for the two disorders is different. In fact, some of the drugs used for ankylosing spondylitis, such as bisphosphonates, may worsen hypocalcemia. The mechanism underlying these skeletal changes in hypoparathyroidism is not well defined. Decreased intestinal calcium absorption caused by a defect in the action of 1,25-dihydroxyvitamin D has been suggested to play a role in a controlled study of paravertebral ligamentous ossification.[128] Okazaki and colleagues[129] suggested that the ossifying diathesis of paravertebral ligaments, which is the origin of DISH, might be initiated or aggravated by hypoparathyroidism.

### Systemic Lupus Erythematosus

SLE is known to potentially affect any organ system, and hypoparathyroidism has been found to occur in 4.0% to 5.7% of patients with SLE compared with 1.0% in the general population.[130] Hypoparathyroidism is underestimated because it is poorly manifested clinically and can be precipitated by certain aggravating factors, such as corticosteroid treatment or vitamin D deficiency. Although acute hypocalcemia typically gives symptoms and signs of musculoskeletal irritability, chronic hypocalcemia usually gives mild symptoms and can even be asymptomatic. Despite the possible lack of outward manifestations, hypocalcemia may be a significant health risk as a result of QT interval prolongation and the consequent risk of sudden death. These cases raise the question of whether periodic calcium and phosphate screening should be routine care for patients with SLE.[131]

## HYPERPARATHYROIDISM

PTH along with vitamin D and calcitonin controls calcium homeostasis. These hormones are responsible for bone metabolism and remodeling in addition to maintaining serum calcium in a very narrow range. In primary hyperparathyroidism (HPT), this balance is altered as a result of changes in the parathyroid gland, whereas changes in other organs of the body that result in calcium loss and vitamin D deficiency with increased secretion of PTH are called secondary HPT.[132]

Approximately 1% to 2% of the population has hypercalcemia, with 10% to 20% of these individuals having HPT.[133] Most patients with HPT have a single adenoma; carcinoma is rare, and in secondary HPT, diffuse hyperplasia related to hypocalcemia or resistance to PTH is seen. Secondary HPT is most commonly the result of osteomalacia, vitamin D deficiency, and renal failure.[123] Skeletal symptoms include arthralgias, as well as radiographic findings such as osteitis fibrosa cystica and subperiosteal resorption along the radial side of the phalanges. In addition, the distal tufts of the phalanges may also reveal subperiosteal resorption.

Arthritis associated with HPT involves the small joints of the hand (possibly becoming erosive) and spares the PIP joints. In this respect, it can mimic RA, but the presence of subperiosteal resorption is very distinctive. In contrast to RA, these patients generally have low erythrocyte sedimentation rates, negative rheumatoid factors and anti-citrullinated protein antibodies, and laboratory values consistent with HPT.[134] HPT more commonly involves the radiocarpal, inferior radioulnar, and MCP joints compared with RA. Lytic lesions resulting from HPT in the skeleton (Brown's tumors) are localized areas of fibrous tissue resulting from increased osteoclastic activity, giant cells, and decomposing blood.[135] CPPD (pseudo gout or chondrocalcinosis) is also associated with HPT.[136] Attacks of gout also can be seen in HPT, and the concomitant occurrence of uric acid and CPPD crystals can be found in synovial fluid aspirated from acutely inflamed joints. In addition to the acute attacks of CPPD, chronic CPPD disease may result in erosive disease.[137] Muscle involvement can occur in HPT, presenting with proximal muscle weakness. Muscle enzymes are not

elevated, and both EMG studies and muscle biopsy results demonstrate a picture of denervation.

Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 123



# Musculoskeletal Syndromes in Malignancy

Samera Vaseer • Eliza F. Chakravarty

**KEY POINTS**

- Musculoskeletal and rheumatic syndromes can be the first presentation of an underlying malignancy. Older age at onset, prominent constitutional symptoms, atypical features of rheumatic disease, and limited response to glucocorticoids or other conventional therapy suggests of a paraneoplastic process.
- Data from numerous cohorts have confirmed a threefold increase in the incidence of malignancy associated with dermatomyositis, including clinically amyopathic dermatomyositis and, to a lesser extent, polymyositis. Risk factors include age greater than 45 years, male gender, rapid onset, rapid weight loss, cutaneous necrosis, high erythrocyte sedimentation rate/C-reactive protein, dysphagia, anti–p155-140 (anti-transcriptional intermediary factor-1γ) antibody, and anti-NXP2 (anti-MJ) antibody.
- Chronic autoimmune conditions including Sjögren's syndrome, rheumatoid arthritis, and systemic lupus erythematosus are associated with an increased risk of lymphoid malignancies compared with the general population. This increased risk is believed to be attributed, at least in part, to chronic inflammation and immune stimulation.
- Patients with systemic sclerosis are at increased risk for developing solid organ tumors that primarily involve tissues affected by the fibrotic process. Positivity for anti-RNA polymerase III confers higher odds for developing cancer within 36 months of systemic sclerosis onset compared with individuals who do not have this antibody.

Musculoskeletal syndromes may be associated with malignancy in a variety of ways, although cause and effect may be difficult to define clearly in many situations. Certain chronic rheumatic diseases have been associated with an increased risk of the subsequent development of malignancy; one example is the development of lymphoma in an individual with primary Sjögren's syndrome. The converse situation also exists, in that certain rheumatic diseases are seen more frequently in the presence of an underlying malignancy, such as dermatomyositis (DM). Little is understood regarding the pathogenesis of connective tissue disease in association with neoplastic disease. Other factors can contribute to the association of musculoskeletal syndromes and malignancy; for example, many of the medications used to treat rheumatic diseases modulate the immune system and may be associated directly or indirectly with an increased risk for the development of malignancy. In unusual circumstances, musculoskeletal involvement occurs as a paraneoplastic process, defined as a hormonal, neurologic, hematologic, or biochemical disturbance that is associated with malignancy but is not directly related to invasion by the neoplasm or its metastases.[1]

## PARANEOPLASTIC SYNDROMES

Musculoskeletal syndromes can develop as a manifestation of a paraneoplastic process and occasionally can be the first presentation of an underlying malignancy. Hematologic malignancies, lymphoproliferative disorders, and solid tumors are associated with a wide variety of paraneoplastic rheumatic syndromes. Older age at onset, atypical features of rheumatic disease, and absence of response to glucocorticoids or other conventional therapy may suggest a paraneoplastic process. Knowledge of the associations with rheumatic syndromes and underlying malignancy is crucial when caring for these patients. Hypertrophic osteoarthropathy, amyloidosis, and secondary gout are reviewed in Chapters 87, 93, and 106. Table 123-1 lists common paraneoplastic associations.

### Carcinomatous Polyarthritis

The term *carcinomatous polyarthritis* is used to describe the development of arthritis in association with malignancy, but it is distinct from arthritis associated with metastasis or direct tumor invasion. Table 123-2 lists common features of carcinomatous polyarthritis. This disease generally occurs in patients who are older than 50 years, has an explosive onset, and often develops in close temporal correlation with the discovery of the malignancy. Although it can have various presentations and may mimic the appearance of rheumatoid arthritis (RA)[2] or asymptomatic migratory polyarthritis,[3] carcinomatous polyarthritis is more often a seronegative asymmetric disease with predominant involvement of the lower extremities and some sparing of the small joints of the hands. There is no evidence of direct tumor extension or metastasis and no specific identifying histologic or radiographic appearance. Carcinomatous polyarthritis can occur in association with many types of malignancy, but it has been reported most frequently in association with breast, colon, lung, and ovarian cancers and with lymphoproliferative disorders.[4] The underlying pathogenesis of this process has not been elucidated; however, the arthritic symptoms may be improved with successful treatment of the malignancy.[5]

**TABLE 123-1** Paraneoplastic Syndromes

| Connective Tissue Disease | Malignancy | Clinical Setting | Clinical Alert |
|---|---|---|---|
| Carcinomatous polyarthritis | Multiple types of solid tumors, including breast; lymphoproliferative disorders | See Table 123-2 | See Table 123-2 |
| Vasculitis | Lymphopoietic and hematopoietic malignancies | Cutaneous vasculitis most common; systemic vasculitis rare | Vasculitis not related to infections, medications, or autoimmune disease |
| Mixed cryoglobulinemia | Non-Hodgkin's lymphoma | Immune complex–mediated disease with cutaneous vasculitis, neuropathy, fatigue, and visceral organ involvement | Usually appears 5-10 yr after diagnosis of cryoglobulinemia |
| Panniculitis | Hematologic malignancies; pancreatic, breast, and prostate cancers | Induration of skin and deeper tissues; eosinophilia often present | Usually refractory to prednisone |
| Fasciitis | Ovarian, breast, gastric, and pancreatic cancers | Palmar fasciitis with inflammatory polyarthritis; similar in presentation to reflex sympathetic dystrophy | Bilateral presentation; severe fibrosis and contractures; aggressive course |
| Reflex sympathetic dystrophy syndrome | Multiple cancer types; Pancoast tumors | Tumors may invade stellate ganglionar brachial plexus on affected side | Absence of typical antecedent factors; failure to respond to conventional therapies |
| Erythromelalgia | Myeloproliferative disorders | Often seen in setting of thrombocytosis | — |
| Atypical polymyalgia rheumatica | Renal, lung, and colon cancer; multiple myeloma | — | Age <50 yr; asymmetric involvement; poor response to prednisone |
| Digital necrosis | Gastrointestinal and pulmonary tumors | Severe Raynaud's phenomenon with onset after age 50 yr | Asymmetric features; digital necrosis |
| Remitting seronegative symmetric synovitis with pitting edema | Several tumor types | Abrupt onset of arthritis and edema surrounding wrists and small joints of hands | Presence of fever, weight loss; poor response to prednisone |
| Multicentric reticulohistiocytosis | Lung, stomach, breast, cervix, colon, and ovarian carcinomas | — | — |
| Lupus-like syndromes | Variety of solid tumors and lymphoproliferative disorders | — | Rare associations with malignancy limited to case reports |
| Anti-phospholipid antibodies | Multiple cancer types | Association between antibodies, cancer, and risk of thrombosis unclear | Higher presence of antibodies found in patients with malignancy |
| Osteogenic osteomalacia | Solid tumors and tumors of mesenchymal origin | Bone pain and muscle weakness | Diligent search is indicated in all patients with late-onset apparent idiopathic osteomalacia |
| Sarcoidosis | Cervical, bladder, gastric, lung, breast, and renal cancers and cutaneous and pulmonary squamous cell carcinomas | Highest incidence of "malignancy" during first 4 yr after detection of granulomas | Malignant tumors can cause sarcoid-like tissue reactions leading to mistaken diagnosis of sarcoidosis before recognition of malignancy |
| Lymphomatoid granulomatosis | Lymphoma | Unusual granulomatous form of vasculitis with angiodestructive infiltration of various tissues | — |

## Vasculitis

Vasculitis in association with malignancy is uncommon and has a reported prevalence of only 8% in patients with a malignancy.[6] The association seems to be significantly higher with lymphoproliferative and myeloproliferative disorders than with solid tumors, and vasculitis commonly predates the identification of malignancy. The vasculitic process usually affects small vessels and is cutaneous and only rarely involves significant organs. Up to 5% of patients with cutaneous vasculitis have an underlying neoplasm.[7,8] Treatment often requires the use of glucocorticoids in addition to therapy directed against the underlying malignancy.

Table 123-1 shows malignancies associated with vasculitis. In the setting of malignancy, it is believed that persistent antigen stimulation from the tumor results in T cell activation or immune complex formation and deposition.

The development of small vessel vasculitis has been reported to ante-date and post-date the development of lymphoproliferative and myeloproliferative diseases. One group of investigators retrospectively examined the cases of 421 adult patients with cutaneous vasculitis and identified 16 in whom an associated malignancy had developed.[8] Of these 16 patients, 9 had hematologic neoplasia and 7 had malignant solid tumors. Similar findings were reported by

**TABLE 123-2** Features of Carcinomatous Polyarthritis

| |
|---|
| Close temporal relationship between onset of arthritis and discovery of malignancy |
| Late age of onset |
| Asymmetric joint involvement |
| Explosive onset |
| Predominant lower extremity involvement with sparing of wrists and small joints of hands |
| Absence of rheumatoid nodules |
| Absence of rheumatoid factor |
| No family history of rheumatoid disease |
| Nonspecific histopathologic appearance of synovial lining |
| No periosteal reaction |

investigators who found an underlying malignancy in 8 of 192 patients with cutaneous vasculitis. Most malignancies (six of eight) were hematologic, and five of eight pre-dated the diagnosis of cancer.[9] In a retrospective analysis of 23 patients with cutaneous vasculitis and hematologic malignancies, the authors were able to attribute the presence of vasculitis to the malignancy itself in 61% of cases.[10]

Systemic vasculitis is much less commonly associated with underlying malignancy. In case reports and small series it has been reported that anti-neutrophil cytoplasmic antibody (ANCA)$^-$ and ANCA$^+$ vasculitis is associated with hematologic malignancies.[11-13] Granulomatosis with polyangiitis likewise has been associated with the development of several types of malignancies, including lymphoproliferative disorders, bladder cancer, and renal cell carcinoma.[14,15] In some cases the malignancy was diagnosed within months of the diagnosis of granulomatosis with polyangiitis,[14] and in other reports, cancer developed many years after diagnosis and treatment of vasculitis,[15] and thus it is unclear whether the malignancies were a result of the vasculitis or possibly of the treatment. A group of investigators from the Cleveland Clinic published a retrospective study of the temporal relationship between vasculitis and cancer.[16] During an 18-year study period, the investigators found only 12 cases of vasculitis and cancer diagnosed within the same 12-month period: Six patients had lymphoproliferative disorders, and six had solid tumors. In most cases, the vasculitis responded partially to immunosuppressive therapy, but the investigators observed a more impressive improvement of vasculitis with definitive treatment for the underlying malignancy. In a more recent study, investigators found 20 cases of malignancy among 200 patients with ANCA$^+$ vasculitis; 6 malignancies were diagnosed concurrently with diagnosis of vasculitis, and 14 malignancies pre-dated vasculitis by a median of 96 months.[17] Only 4 of 20 malignancies in this series were lymphoproliferative; the others were solid organ tumors.

Vasculitis associated with underlying malignancy often responds poorly to conventional therapy directed against the vasculitis. In one series of 13 patients with cutaneous vasculitis and lymphoproliferative or myeloproliferative disorders, symptoms of vasculitis had a poor response to therapy with NSAIDs, glucocorticoids, antihistamines, and antiserotonin agents. Although the investigators reported a lessening of the severity of the vasculitis after chemotherapy was directed against the malignancy, they generally found chemotherapy to be ineffective. Of the 13 patients identified, 10 died as a direct result of the malignancy.[18] Similarly, Hutson and Hoffman[16] found a general concurrence between improvement in vasculitic syndrome and definitive treatment for the associated underlying malignancy. More recently, in a study of cutaneous small-vessel vasculitis associated with solid tumors (most commonly lung, breast, and prostate), investigators found a significant response to immunosuppressive therapy directed against the vasculitis, although concurrent treatments for the underlying malignancy were undertaken.[19]

## Cryoglobulinema

Cryoglobulins are immunoglobulins that precipitate at a reduced temperature. Cryoglobulinemia manifests with symptoms of hyperviscosity or vasculitis. Patients often present with fatigue, arthralgia or arthritis, cutaneous vasculitis or purpura, neuropathy, digital ischemia, and visceral organ involvement (renal or pulmonary).[20] The following three types of cryoglobulins have been identified:

- *Type I:* Monoclonal Ig, either IgG or IgM; this type is associated with lymphoproliferative disorders.
- *Type II:* Monoclonal IgM directed against polyclonal IgG; type II cryoglobulins were initially thought to be idiopathic and were known as mixed essential cryoglobulinemia. With the identification of hepatitis C virus (HCV), it has been discovered that most of these patients have HCV infection that is directly involved in the pathogenesis of the cryoglobulins. Specific epitopes of HCV antigens are recognized by IgG components of immune complexes, and viral particles are found in the cryoprecipate.[21] In one study, investigators found clonal B cell populations in the peripheral blood of 48% of HCV$^+$ patients with type II cryoglobulinemia, many of whom were eventually diagnosed with a B cell malignancy.[22] Overall, it is estimated that non-Hodgkin's lymphoma may develop in approximately 5% to 8% of patients who have mixed cryoglobulinemia, usually after 5 to 10 years of the diagnosis of cryoglobulinemia.[23,24] The risk of developing non-Hodgkin's lymphoma among patients with HCV$^+$ cryoglobulinemia may be 35 times higher than that in the general population.[25] Other data suggest that HCV infection may be associated with other hematologic malignancies.[26,27] At this time, the subset of patients with mixed cryoglobulinemia in whom lymphoma will develop cannot be predicted.
- *Type III:* Mixed polyclonal IgG and IgM; type III cryoglobulins are commonly seen with a variety of illnesses, including connective tissue diseases (systemic lupus erythematosus [SLE] and RA) and infections. In one study of 607 patients diagnosed with mixed cryoglobulinemia, 27 cases of hematologic malignancies were identified. Of these, systemic autoimmune diseases were detected in 56% of the cases of non-Hodgkin's lymphoma.[27]

## Panniculitis

Fasciitis-panniculitis syndrome, which includes eosinophilic fasciitis, is characterized by swelling and induration of the skin that extends into deeper subcutaneous tissues

and is associated with fibrosis and chronic inflammation. Patients may experience arthritis and subcutaneous nodules similar to those seen in people with erythema nodosum. The arthropathy seems to be secondary to periarticular fat necrosis, can be monarticular or polyarticular,[28] and may mimic RA. This syndrome can be idiopathic and have a benign course, or it can be secondary to a variety of infectious, vascular, or traumatic etiologies. Subcutaneous panniculitis-like T cell lymphoma, derived from a/b T cells, is considered a primary cutaneous T cell lymphoma, with biopsy required to distinguish it from autoimmune panniculitis.[29] In a few patients, the fasciitis-panniculitis syndrome is associated with an underlying malignancy. Hematologic malignancies are most often associated with this syndrome and are usually diagnosed concurrently or within the first year.[30,31] Pancreatic cancer and pancreatitis also can be associated with this syndrome.[28] Patients with cancer-associated fasciitis-panniculitis syndrome are predominantly female and generally have a refractory response to prednisone.[30]

## Palmar Fasciitis

Palmar fasciitis and arthritis is a syndrome characterized by progressive bilateral contractures of the digits, fibrosis of palmar fascia, and inflammatory polyarthritis.[32-34] The metacarpophalangeal and proximal interphalangeal joints are most commonly affected; other affected joints include the elbows, wrists, knees, ankles, and feet. Indurated reticulate palmar erythema also can be seen as part of the palmar fasciitis spectrum.[35] Palmar fasciitis is almost uniformly associated with the presence of an underlying malignancy, most often ovarian, breast, gastric, and pancreatic tumors.[33,34,36,37] Although initially thought to be an atypical variant of reflex sympathetic dystrophy, the severity of manifestations, bilateral presentation, and strong association with occult malignancy suggest that, in these cases, palmar fasciitis is a distinct entity that behaves as a paraneoplastic syndrome (Figure 123-1). Neither glucocorticoids nor chemotherapy appear to result in significant improvement, although fasciitis occasionally regresses with treatment of the underlying malignancy.[32]

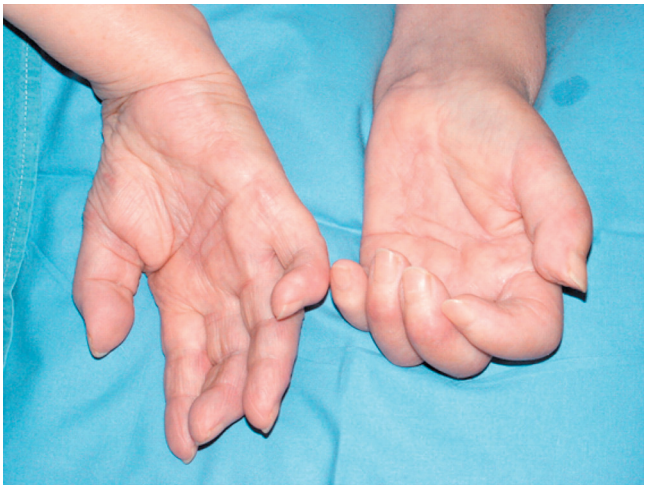


**Figure 123-1** Palmar fasciitis and polyarthritis as a paraneoplastic syndrome associated with tubal carcinoma in a 73-year-old woman. *(From Denschlag D, Riener E, Vaith P, et al: Palmar fasciitis and polyarthritis as a paraneoplastic syndrome associated with tubal carcinoma: a case report.* Ann Rheum Dis *63:1177–1178, 2004.)*

## Reflex Sympathetic Dystrophy

Reflex sympathetic dystrophy (complex regional pain syndrome) and a variant, shoulder-hand syndrome, are characterized by regional pain, swelling, vasomotor instability, and focal osteoporosis in a given limb; this condition is thought to be caused by sympathetic dysfunction. The absence of associated antecedent factors such as stroke, myocardial infarction, or trauma and failure to respond to conventional therapy may warrant a search for an underlying malignancy. A variety of malignancies have been associated with the development of reflex sympathetic dystrophy or its variants.[38,39] Pancoast tumor of the lung apices or other malignancies that infiltrate the stellate ganglion or brachial plexus have been described in patients with reflex sympathetic dystrophy.[40-42] Therapy directed against the underlying malignancy may lead to some amelioration of symptoms.

## Erythromelalgia

Erythromelalgia is an enigmatic condition characterized by attacks of severe burning, erythema, and warmth of the extremities, with symptoms predominantly involving the feet.[43,44] Symptoms are often exacerbated when the extremities are placed in a dependent position, during ambulation, or during exposure to increased temperatures. Partial relief can be obtained through elevation or cooling of the extremity. This disorder can occur idiopathically (60%) or secondary to another disease (40%).[43,45] Myeloproliferative disorders, including polycythemia vera and essential thrombocytosis, are common primary causes and have been found to precede the diagnosis of erythromelalgia by several years.[7,44,46] The underlying pathophysiology of erythromelalgia remains unknown; however, it is often associated with thrombocythemia. In the largest published retrospective cohort, 168 patients at the Mayo Clinic were identified with this diagnosis between 1970 and 1994.[47] Investigators found that after a mean follow-up of 8.7 years, 31.9% of patients reported worsening of disease, 26.6% reported no change, 30.9% reported improvement, and 10.6% reported complete resolution of symptoms. Kaplan-Meier survival curves revealed a significant decrease in survival compared with control subjects. A history of myeloproliferative disease was found in 15 of 168 patients. The exact cause of the symptoms is unclear, but microvascular arteriovenous shunting has been hypothesized.[48] In cases of secondary erythromelalgia, platelet breakdown products and platelet microthrombi may underlie disease pathogenesis.[7] The most effective therapy seems to be the use of daily aspirin, leading to a significant relief of symptoms. A host of other therapies have been tried with varying success.[49] Because of the association with myeloproliferative diseases, routine monitoring with complete blood cell counts is prudent.

## Polymyalgia Rheumatica

Polymyalgia rheumatica, a disorder affecting older adults, manifests with discomfort and stiffness in the shoulder and

hip girdle, fatigue, anemia of chronic disease, and elevated erythrocyte sedimentation rate (ESR). Classically this condition responds to moderate doses of prednisone within 48 hours. A variety of other conditions can have presentations that mimic polymyalgia rheumatica, including other rheumatic disorders, systemic infections, and malignancy.[50] Although the association between polymyalgia rheumatica and malignancy has been controversial, atypical features of polymyalgia rheumatica may suggest the presence of occult malignancy, including age younger than 50 years, limited or asymmetric involvement of typical sites, ESR less than 40 mm/hour or greater than 100 mm/hour, severe anemia, proteinuria, and poor or delayed response to 20 mg of prednisone daily. Kidney, lung, and colon cancer and multiple myeloma are most often found in patients presenting with atypical polymyalgia rheumatica.[51-53] In one study of patients undergoing evaluation for possible polymyalgia rheumatica, it was found that 10% had a diagnosis of malignant neoplasms.[54] In contrast, several prospective studies have shown that patients presenting with classic polymyalgia rheumatica or giant cell (also known as temporal) arteritis do not have an increased risk of the development of malignancy compared with age-matched control subjects.[55-59]

## Raynaud's Phenomenon and Digital Necrosis

The development of digital necrosis or profound Raynaud's phenomenon may suggest the presence of infection, inflammatory disease, or an underlying malignancy. In patients older than 50 years, the development of Raynaud's phenomenon, particularly in an asymmetric fashion or in association with digital necrosis, should raise the possibility of a paraneoplastic process. In one study, underlying malignancy was associated with digital ulcers in 15% of patients presenting with digital ischemia.[60] These features often antedate the diagnosis of the malignancy by an average of 7 to 9 months.[61,62] A variety of solid tumors and lymphoproliferative disorders have been associated with this syndrome.[61-67] Certainly, the presence of digital necrosis in patients with DM is highly suggestive of the presence of an underlying malignancy. Mechanisms proposed include cryoglobulinemia, immune complex–induced vasospasm, hypercoagulability, marantic endocarditis with emboli, and necrotizing vasculitis.[67] Therapy with interferon-α also has been reported in association with the development of Raynaud's phenomenon and digital necrosis.[68-70]

## Remitting Seronegative Symmetric Synovitis with Pitting Edema

Remitting seronegative symmetric synovitis with pitting edema is an uncommon disorder primarily affecting the metacarpophalangeal joints and the wrists. Although the underlying cause and pathogenesis are unclear, lymphoma, myelodysplastic syndrome, and several solid tumors, mostly adenocarcinoma, all have been reported in association with this illness.[71-75] In one retrospective study from a single center in the United States, it was found that cancer developed in 3 of 14 patients who had remitting seronegative symmetric synovitis with pitting edema.[76] Characteristics that suggest possible underlying malignancy include the presence of systemic features, such as fever or weight loss, and a poor response to glucocorticoids.[73,74]

## Multicentric Reticulohistiocytosis

Multicentric reticulohistiocytosis is a rare condition characterized by the presence of cutaneous papules. This condition is often associated with a destructive arthritis of the interphalangeal joints of the hands. The papules are flesh colored to brown-yellow and classically are present in the periungual region and on the dorsal aspect of the hands and face. Arthritis mutilans may develop in 50% of cases. The characteristic histologic appearance of tissue infiltration with histiocytes and multinucleated giant cells can be found in affected skin, joints, and occasionally internal organs.[77] Multicentric reticulohistiocytosis has been reported in association with hyperlipidemia, malignancies, and autoimmune diseases. Malignancy has been associated in 25% to 31% of cases, although most literature consists of individual case reports.[78,79] The most frequently seen malignancies include carcinoma of the lung, stomach, breast, cervix, colon, and ovary.[78]

## Lupus-like Syndromes

Lupus-like syndromes are rarely associated with underlying malignancy. Isolated case reports have described lupus-like syndromes with ovarian carcinoma[80,81] and hairy cell leukemia[82]; subacute cutaneous lupus was reported in a patient with breast carcinoma.[83] Studies of the presence of antinuclear antibodies (ANAs) in patients with cancer have yielded mixed results. In two studies, investigators were unable to find a significantly increased prevalence of ANAs in patients with solid tumors or lymphomas compared with healthy control subjects.[84,85] In contrast, in a smaller study, investigators found an increased prevalence in patients with non-Hodgkin's lymphoma compared with control subjects (21% versus 0),[86] and in another study, investigators found a 27.7% prevalence of ANAs in 274 patients with various malignancies compared with a prevalence of 6.45% in 140 healthy control subjects.[87] There do not seem to be any predictive features that suggest occult malignancy in patients presenting with lupus-like syndromes or positive ANAs.

## Anti-phospholipid Antibody Syndrome

Anti-phospholipid antibodies (aPLs) and their association with thromboses have been described as a primary syndrome and a secondary phenomenon in autoimmune diseases, primarily SLE. More recently, aPLs have been associated with a variety of malignancies. Correlations between aPLs in individuals with cancer and thromboembolic events have been less clear, however.

Several studies have shown the presence of aPLs in patients with solid tumors and lymphoproliferative disorders at a higher frequency than the 1% to 5% seen in the general population.[88,89] In an early study of 216 consecutive patients with cancer, it was found that 22% were positive for anticardiolipin antibodies compared with 3.4% of control subjects. In this study, a twofold increase was found in the development of thromboembolism in patients with positive

antibodies compared with patients who had negative serologies; study findings also indicated that most thromboembolic events occurred in patients with higher antibody titers.[90] Other studies have confirmed the association between malignancy and aPLs (12.5% to 68%) but have been unable to show a correlation with thromboembolic events.[86,88,91-95] A correlation between antibody titer and disease activity has been shown in some studies,[83,85] and decreased survival has been shown in other studies.[95] In a recent survey of 95 patients with cancer who were hospitalized in the intensive care unit, an aPL seropositivity rate of 74% was found; however, autoantibody status was not associated with mortality.[96] Upon a review of the literature, it was concluded that aPLs resolve in one-third of patients with cancer after treatment for the underlying malignancy.[97]

Studies of the prevalence of aPLs in unselected patient populations have shown an association with underlying malignancy. A prospective study in France found that 7% of 1014 consecutive patients admitted to a medical ward had aPLs.[98] In antibody-positive patients, cancer was the most frequently associated disease. In a more recent study in patients presenting with a first ischemic stroke, a significantly higher rate of the development of cancer within 12 months was found in patients who had anti-cardiolipin antibodies compared with patients without these antibodies (19% vs. 5%).[99]

## Osteomalacia

Osteomalacia is the softening of bones that is often associated with the failure of adequate calcification as a result of renal dysfunction or a lack of vitamin D. Osteomalacia has been associated with benign and malignant solid tissue and mesenchymal tumors.[100] Tumors causing oncogenic osteomalacia overproduce fibroblast growth factor 23 (FGF-23), and elevated serum levels of FGF-23 can be detected in patients with this paraneoplastic condition.[101,102] Octreotide scintigraphy may be a useful tool for identifying occult tumors.[103,104] Upon removal of the tumor, there often seems to be resolution of the osteomalacia and normalization of serum FGF-23 levels.[102]

## Sarcoidosis

Noncaseating granulomas can occur in numerous settings and are not pathognomonic for sarcoidosis. Granulomas resembling those of sarcoidosis may be found in lymph nodes that drain sites of malignancy. These tumor-related tissue reactions resulting in granuloma formation have been described with many types of malignant lesions, including solid tumors and lymphomas.[105,106] Because the clinical and radiographic presentation of sarcoidosis can be virtually indistinguishable from cancer, it is important to pursue aggressive evaluation in patients with sarcoidosis.[107,108]

The risk of the development of malignancy in patients with established diagnoses of sarcoidosis is controversial. Some studies have shown an increased risk of the development of lung cancer and lymphoma,[109-111] whereas other studies have shown no increased risk of cancer compared with the general population.[107,112,113]

## Lymphomatoid Granulomatosis

Lymphomatoid granulomatosis is a rare disorder with angiodestructive and lymphoproliferative features involving the lung and, less often, the skin and central nervous system. Although lymphocytic infiltration of vessels is a hallmark of the disease, lymphomatoid granulomatosis now seems to fall within the spectrum of lymphoproliferative disorders.[114] Despite the predominance of T cells within inflammatory infiltrates, studies have suggested that an Epstein-Barr virus (EBV)-associated B cell proliferation may underlie the pathogenesis of the disease.[115,116] Prognosis is generally poor, with a median survival from diagnosis of 14 months,[117] although more recent reports suggest some response to rituximab therapy.[118,119] Frank lymphomas evolve in 25% of cases.[120]

## Inflammatory Myopathies

The inflammatory myopathies in adult populations encompass a group of illnesses characterized by an idiopathic immune-mediated attack on skeletal muscle resulting in muscle weakness. An association between myositis and cancer was first proposed in 1916. The association between inflammatory myopathy and cancer is well described in adults, although the pathophysiologic mechanisms underlying the association remain elusive. Many epidemiologic studies substantiate this association, which appears stronger for DM than for other subtypes. Associations between polymyositis (PM) and inclusion body myositis are becoming increasingly recognized. New data have emerged regarding the pathologic changes observed in muscle tissue and cancer. Zampieri et al.[121] hypothesized that in genetically predisposed patients, cancer is accompanied by subclinical tumor-induced myopathy, which can later evolve to full-blown autoimmune myositis. These investigators compared muscle biopsies of 13 patients with myositis, three of them with cancer-associated myositis, 14 with colorectal cancer unrelated to myositis, and 19 healthy control subjects. They found a higher number of fibers with internally located nuclei, a sign of muscle damage, in patients affected with myositis and in those with colorectal cancer, but not in control subjects.[121] These investigators suggest that myositis-specific antigens are expressed in regenerating muscle fibers and trigger an aberrant autoimmune response that acts as a positive feedback loop. These findings are intriguing and require further investigation.[122]

On average, the prevalence of malignancy in association with the inflammatory myopathies has been approximately 15% to 25%, which appears to be consistent across populations studied. However, the frequency of malignancy has ranged from 6% to 60% in patients with DM and from 0 to 28% in patients with PM.[123,124] Other estimates have placed the incidence of cancer in patients with inflammatory myopathies at five to seven times that of the general population.[123]

One of the first large population-based studies was the National Swedish Registry of 788 PM/DM cases. A moderate but significant increase was found in the incidence of cancer in male and female patients with PM. A clear increase in the number of cancers in patients with DM was found, especially in female patients. The risk of cancer was

high during the first 5 years in patients with DM, which strongly suggests its association with cancer. The risk of cancer of the ovary was increased nearly 17-fold.[125] Hill and colleagues[126] studied a pooled cohort of patients from Sweden, Denmark, and Finland and found 198 cases of cancer in 618 patients with DM. The standardized incidence ratio (SIR) for malignancy with DM was 3. Similar results were found in a Scottish cohort of 286 patients with DM.[127] Of patients, 77 were found to have underlying malignancies, with an SIR of 3.3 to 7.7. In a National Australian Registry of 537 myositis cases, increased rates of cancers were found in many phenotypes. Cases were restricted to biopsy-proven myositis. Many type of cancers were seen, but the most frequent were breast and lung cancer (Figure 123-2A and B).[128]

DM has been associated with a wide range of malignancies; interestingly, solid tumors are most often seen in cancer-associated myositis as opposed to lymphoid malignancies. Most common tumor types are ovarian, lung, and gastric tumors in European populations and nasopharyngeal malignancies in Asian populations. All of these studies have suggested that cancers are most commonly diagnosed within 1 to 2 years of diagnosis of DM. In a nationwide Taiwanese study, 9.4% of 1012 patients with DM were found to have an underlying malignancy, the most common of which was nasopharyngeal cancer, followed by lung cancer.[129,130] In a smaller study, 66.6% of 15 patients with DM in Singapore had malignancies, most of which were nasopharyngeal carcinoma.[131] Eight Caucasian patients found to have nasopharyngeal carcinoma within 1 year of diagnosis of DM were reported in Tunisia.[132] A similar study from Japan found that cancer was diagnosed in 24% of patients with DM; the majority had gastric cancer, which is a common malignancy in the Japanese population.[133]

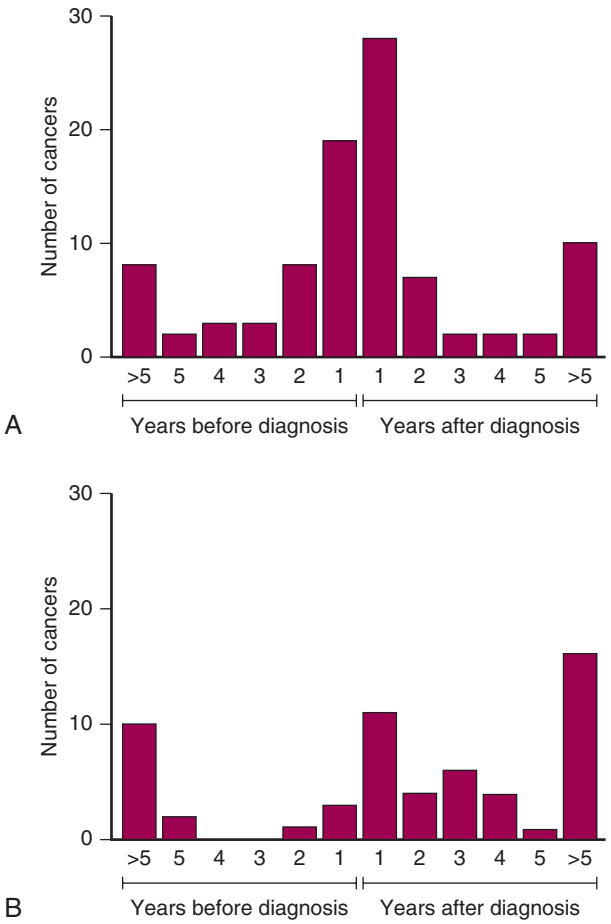



**Figure 123-2** **A,** Temporal relation between the diagnosis of dermatomyositis and the diagnosis of cancer. **B,** Temporal relation between the diagnosis of polymyositis and the diagnosis of cancer. *(From Sigurgeirsson B, Lindelöf B, Edhag O, Allander E: Risk of cancer in patients with dermatomyositis or polymyositis: a population-based study.* N Engl J Med *326:363–367, 1992.)*

In people with PM, the relative risk (RR) of developing internal malignancies seems to be lower than that for people with DM, but it is consistently increased compared with that expected in the general population. Studies have found a 14% to 30% prevalence of cancer among patients with PM, with SIRs increased to 1.2 to 2.1.[126-129] The nationwide Taiwanese study found a 4.4% incidence of cancer among 643 patients with PM, with an SIR of 2.15 compared with the general population.[130] Small numbers of many types of cancers were found in these studies. These studies confirm results found in previous large studies of Swedish and Finnish populations and a 1994 meta-analysis of all published case-control and cohort studies of malignancy and myositis that identified odds ratios for the association of cancer with DM and PM of 4.4 and 2.1, respectively.[134]

In amyopathic DM, a variant of DM in which typical cutaneous manifestations are present with subclinical or no identifiable muscle disease, the association with underlying malignancy is becoming clearer. Whereas earlier reports were limited to small groups of patients,[131,135-137] more recent cohort studies and a systematic review of the amyopathic DM literature have suggested similar associations between amyopathic DM and internal malignancies.[133,138,139]

Far less is known about the association of inclusion body myositis and underlying malignancy. In Buchbinder's study, 52 patients with inclusion body myositis were identified.[128] Of these patients, 12 were found to have internal malignancies, with an SIR of 2.4.

Not all studies concur regarding the association between the inflammatory myopathies and malignancy.[140,141] In a study performed at the Mayo Clinic, patients with myositis did not seem to have a statistically significant risk for the development of malignancy. No clinical differences were seen between patients in whom a malignancy did and did not develop.[141] Despite the negative results of some studies, most work appears to support the notion of an increased risk of malignancy in association with DM and PM.

The types of malignancy associated with the inflammatory myopathies have been varied, including adenocarcinomas of the breast, ovaries, and stomach. Most cases of DM and malignancy seem to occur within 1 year of each other, with myositis diagnosed first in most cases.[123] When a malignancy is identified, removal of the malignancy may result in improvement of the myopathic process, which further supports the paraneoplastic nature of myositis in some cases.[142]

The risk of malignancy may be higher in certain cohorts, including individuals older than 50 years at diagnosis,[143] individuals with evidence of distal extremity weakness,[144] and individuals with prominent pharyngeal and diaphragmatic involvement.[145] Patients who have myositis-associated

**TABLE 123-3**

| Factors That Predict Malignancy in Myositis | Factors Associated with Reduced Risk of Malignancy |
|---|---|
| Age >45 yr | Interstitial lung disease |
| Male gender | Arthritis |
| Dysphagia | Raynaud's phenomenon |
| Cutaneous necrosis | Anti-Jo-1 |
| Cutaneous vasculitis | Anti-ENA |
| Rapid onset of myositis (<4 wk) | Other myositis-specific antibody |
| Shawl sign | |
| Elevated ESR/CRP | |
| Anti–p155-140 (TIF-1γ) and anti-NXP2 (anti-MJ) antibodies | |
| Refractory disease | |

Data from references 147 through 151.

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TIF-1, transcriptional intermediary factor 1.

autoantibodies may be at less risk for the development of malignancy.[122,144,145] More recent work has suggested that the presence of leukocytoclastic vasculitis,[146] cutaneous ulceration,[145] and an absence of pulmonary involvement[143] further increases the possibility that an underlying malignancy is present. Similar findings were reproduced in a meta-analysis by Lu et al.,[147] who looked at factors predicting malignancy for patients with PM/DM and found that age greater than 45 years, male sex, dysphagia, cutaneous necrosis, cutaneous vasculitis, rapid onset of myositis (<4 weeks), an elevated creatine kinase level, a higher ESR (>35), higher C-reactive protein levels (above the upper limit of normal), and anti–P-155 antibody are all associated with an increased risk of malignancy. Several factors were found to be related to reduced risk, including the presence of interstitial lung disease, arthritis/arthralgia, Raynaud's phenomenon, or anti–Jo-1 antibody and the presence of anti-ENA antibodies (Table 123-3).

In 2006, Targoff et al. described a novel autoantibody against a 155 kDa protein in a large series of patients with myositis.[147a] This antibody was then identified as human transcriptional intermediary factor 1-γ (TIF-1γ), a member of the TIF1 gene family. A recent study has proposed that TIF-1γ is involved in the transforming growth factor-β (TGF-β) signaling pathway, which is inactivated in some malignancies. TIF-1γ acts on TGF-β and is essential in chromatin-mediated transcriptional regulation, functioning as a positive (agonist) or negative (antagonist) regulator of this pathway, which seems to be related to carcinogenesis.[148] The association between cancer and anti-p155 has been confirmed by other investigators, reinforcing this link.[149,150] Antibodies to nuclear matrix protein 2 (NXP-2; anti-MJ) have been found to be specifically associated with cancer in males.[151]

For patients in whom an inflammatory myopathy has been diagnosed, a workup for the presence of malignancy should be performed. The extent of this workup has been debated, however, because extensive undirected searches often result in a very low yield. It is probably rare for an undirected workup to yield evidence of malignancy in patients with PM and DM[152]; any workup should be tailored to the individual patient's age, symptoms, and signs. Studies have suggested that imaging of the chest, abdomen, and pelvis may increase the potential for the discovery of an underlying malignancy.[153,154] Other investigators have suggested the use of serum tumor markers (CA125 and CA19-9) to augment detection of patients with DM or PM who are at highest risk for associated malignancy.[155] More recently, a prospective study of whole-body PET/CT was found to be comparable with broad conventional screening (including chest, abdominal, and pelvic CT scans, among other tests).[156] Malignancies associated with inflammatory myopathies have been known to develop many years after the diagnosis of muscle disease, and thus continued vigilance and repeated screening for malignancy are warranted.

Clinical suggestions include performing a careful history, physical examination, general laboratory screening, stool heme occult ×3, prostate examination/prostate-specific antigen test in men, and Papanicolaou (Pap) smear, mammogram, gynecological examination, and possibly transvaginal sonography and the CA-125 test in women.

## RISK OF MALIGNANCY IN SYSTEMIC AUTOIMMUNE DISEASES

### Lymphoproliferative Disorders

Since the 1960s, reports of the association between rheumatic diseases and the development of malignancies, particularly lymphoproliferative disorders, have been increasing. Table 123-4 shows pre-existing connective tissue diseases that have been associated with malignancy. Much of what is known about associations between rheumatic disease and malignancy is drawn from retrospective and prospective cohort studies, registry-linkage studies, small series, and case reports. This association is thought to be mediated, at least in part, by chronic immune stimulation and hyperactivity that may lead to malignant transformation.[157] In addition, certain confounding factors need to be considered when assessing the risk of the development of malignancy, including the potential oncogenic properties of many of the immunosuppressive and cytotoxic medications prescribed to treat autoimmune diseases. Lymphoproliferative disorders have developed in patients with rheumatic diseases and in recipients of solid organ transplantations treated with immunosuppressive agents. EBV has been implicated in the development of lymphoid neoplasia in immunosuppressed patients. In the following sections, several rheumatic diseases and the therapies used to treat them are discussed.

### Sjögren's Syndrome

Sjögren's syndrome, an autoimmune exocrinopathy, is characterized by a benign lymphocytic infiltrate of salivary and lacrimal glands that leads to the development of sicca syndrome (keratoconjunctivitis and xerostomia).[158] The development of lymphoproliferative disorders in the setting of Sjögren's syndrome is perhaps the prototypic example of chronic autoimmune disease and an increased risk of malignancy. In 1964, investigators first reported the development of four cases of lymphoproliferative disorders in a cohort of

**TABLE 123-4** Pre-existing Systemic Autoimmune Diseases Associated with Malignancy

| Systemic Autoimmune Disease | Malignancy | Associated Factors | Clinical Alert |
|---|---|---|---|
| Sjögren's syndrome | Lymphoproliferative disorders | Glandular features—lymphadenopathy, parotid or salivary enlargement<br>Extraglandular features—purpura, vasculitis, splenomegaly, lymphopenia, low C4 cryoglobulins | Clues to progression from pseudolymphoma to lymphoma include worsening of clinical features, disappearance of rheumatoid factor, and decline of IgM |
| Rheumatoid arthritis | Lymphoproliferative disorders | Presence of paraproteinemia, greater disease severity, longer disease duration, immunosuppression, Felty's syndrome | Rapidly progressive, refractory flare in long-standing rheumatoid disease may suggest an underlying malignancy |
| SLE | Lymphoproliferative disorders | — | Non-Hodgkin's lymphoma should be considered in patients with SLE who have adenopathy or masses; lymphoma of the spleen is another cause of splenic enlargement in SLE |
| Discoid lupus erythematosus | Squamous cell epithelioma | Found in oldest plaques, ≥20 yr after onset of discoid lesion, primarily in men 30-60 yr old | Poorly healing skin lesion within discoid plaques should be evaluated |
| Systemic sclerosis (scleroderma) | Alveolar cell carcinoma<br>Nonmelanoma skin cancer<br>Adenocarcinoma of the esophagus | Pulmonary fibrosis, interstitial lung disease<br>Areas of scleroderma and fibrosis in the skin<br>Barrett's metaplasia | Annual chest radiograph after fibrosis is detected<br>Change in skin features or poorly healing lesions should be evaluated<br>Esophagoscopy and biopsy, if indicated, of distal esophageal constricting lesions |
| Paget's disease of bone | Osteogenic sarcoma | Development of severe pain; increasing incidence with age | Swelling and bone destruction in pre-existing Paget's disease may be sarcoma; diagnosis may require biopsy |
| Dermatomyositis | Ovarian, lung, and gastric cancer in Western populations; nasopharyngeal carcinoma in Asian populations | Older in age, presence of cutaneous vasculitis; less likely in setting of myositis-specific antibodies or interstitial lung disease | Malignancy evaluation needs to be tailored to individual patient's age, symptoms, and signs |

SLE, Systemic lupus erythematosus.

58 patients with Sjögren's syndrome.[159] In 1978, 7 of 136 patients with sicca syndrome were identified as having developed non-Hodgkin's lymphoma. Compared with the expected incidence of cancer among women of the same age range, there was a 44-fold increased risk of developing non-Hodgkin's lymphoma.[160] These findings have been reproduced numerous times in other cohorts. Lymphoproliferative disorders are complications in approximately 4% to 10% of cases of primary Sjögren's syndrome.[161-169] The RR for development of lymphoproliferative disorders in patients with primary Sjögren's syndrome ranges from 6 to 44,[160,169-175] and meta-analyses of cohort studies have found pooled SIRs of 13.8[176] to 18.8.[172] Most lymphoproliferative disorders were non-Hodgkin's lymphoma, specifically diffuse large B cell lymphoma, mucosa-associated lymphoid tissue lymphoma, and other marginal-zone lymphomas.[157,166,167] Waldenström's macroglobulinemia, chronic lymphocytic leukemia, and multiple myeloma were more rarely reported.[160,164,165,171]

Generally, the development of lymphoma is a late manifestation of Sjögren's syndrome; it often is seen after the disease has been present for 6.5 years.[162,163,177] Several clinical and laboratory features seem to be associated with or predictive of the development of lymphoproliferative disorders, including palpable purpura,[159,164,165,175] cutaneous ulcerations,[161] cryoglobulinemia,[165,166] low serum complement levels,[164-166,178] monoclonal gammopathies,[179,180] cytopenias,[159,166,175] splenomegaly,[159,166] and adenopathy.[161] Newer studies have suggested that elevated levels of serum Fms-like tyrosine kinase-3 ligand (Flt-3-L)[181] and evidence of germinal center–like formations in minor salivary gland biopsies[182] are strongly associated with later progression to lymphoma. Progression to high-grade lymphoma portends a poor prognosis.[136,138,139,156,163-165,175] In contrast, with the exception of thyroid cancer (SIR, 2.6; 95% confidence interval [CI], 1.1 to 4.0),[176] the incidence of other malignancies or all-cause mortality was not increased in patients with Sjögren's syndrome compared with the general population.[171,175,177,183] It is believed that chronic B cell stimulation may lead to the malignant transformation of clonal lines characteristic of Sjögren's syndrome.[158] The presence of a viral trigger accounting for malignant transformation is one possible theory. EBV, among other viruses, has been implicated, but studies have failed to find EBV or other viral particles in lymphoma specimens associated with Sjögren's syndrome.[184]

It also has been reported that chromosomal translocations are present with increased frequency in patients with Sjögren's syndrome in whom lymphoma has developed. One group of investigators identified the presence of translocations of the proto-oncogene Bcl-2[185] in five of seven patients

who had Sjögren's syndrome and lymphoma through the use of polymerase chain reaction. Such translocations were found in peripheral blood or bone marrow in 5% of unselected patients with Sjögren's syndrome who did not have evidence of lymphoma in another study.[186] Conversely, no evidence of Bcl-2 translocations was present in 50 salivary gland biopsy specimens of patients with Sjögren's syndrome who did not have evidence of lymphoma.[187] Analysis of biopsy specimens taken before the development of lymphoma from the seven patients previously mentioned showed no evidence of Bcl-2 translocation. Translocation seemed to correlate with the development of lymphoma in at least a subset of patients with Sjögren's syndrome, and the use of polymerase chain reaction technology may allow for early detection of malignant transformation.[186-188]

## Rheumatoid Arthritis

Since the 1970s it has been well established that RA is associated with a twofold to threefold increased risk for the development of lymphoproliferative disorders, the magnitude of which has remained constant despite dramatic changes in therapy. Many factors, including chronic inflammation and immune dysregulation, in addition to potential oncogenic properties of immunosuppressive therapies for the treatment of RA, must be considered when evaluating the risk of the development of hematologic malignancies. It is often difficult to separate the effects of medication use from the underlying severity of inflammation that makes medication use necessary or indicated, a concept termed *confounding by indication*.[189]

In 1978, an SIR of 2.7 for lymphoma was reported in a group of 46,101 Finnish patients with RA compared with the general population.[190] A similarly increased risk of 2.4 for lymphoma was seen later in a group of 20,699 Danish patients,[191] and an SIR of 1.9 to 2 was reported in a large cohort of 76,527 Swedish patients.[192,193] In the United States, an increased risk of 1.9 was found in a cohort of 18,527 patients,[194] and an SIR of 2.2 was found in a separate cohort of 8458 patients aged 65 years and older.[195] An SIR of 2 to 2.4 for lymphoma was observed in an inception cohort of 2015 patients with inflammatory arthritis in England compared with the general population,[196] and an SIR of 2.04 to 2.39 was seen for non-Hodgkin's lymphoma in a cohort of 26,623 patients with RA in Scotland.[197] In a meta-analysis of nine cohort studies of patients with RA, a pooled SIR of 3.9 was found for lymphoma using a random effects model.[173] Canadian investigators found an increased risk of leukemia (SIR, 2.47) among patients with RA but were unable to confirm elevated rates of lymphomas compared with the general population.[198] Data from case-control studies of patients with non-Hodgkin's lymphoma have shown similar results: Odds ratios of 1.3 to 1.5 were found for underlying RA.[172,174] These associations have been confirmed in other populations, including patients from Japan,[199] Taiwan,[200] California,[201] and Spain.[202] In a more recent study of lymphoma risk in biologic-naïve patients with RA in the United Kingdom who were followed up from 2002-2009, an SIR of 3.12 was found for non-Hodgkin's lymphoma and an SIR of 12.82 was found for Hodgkin's lymphoma[203]; these rates are not strikingly different from those seen in the 1970s. In general, lymphomas in patients with RA seem to be predominately diffuse large B cell type and favor non–germinal center subtypes.[204]

Most studies have suggested that the risk for the development of lymphoma is related to the degree of inflammation. The Swedish group identified high inflammatory activity (defined by ESR, swollen and tender joint counts, and the physician's global assessment of disease activity) as a significant risk factor, with an odds ratio of 25.8 compared with low disease activity.[205] No association between any specific drug and the development of lymphoma was identified; however, the cohort examined was treated between 1965 and 1983, and few of these patients were treated with immunosuppressive drugs. In a 2006 follow-up case-control study of 378 lymphomas in a Swedish group of patients with RA, a 71-fold increased risk of lymphoma in patients with high cumulative disease activity compared with low disease activity was seen.[203] Immunosuppressive therapy did not seem to modify the risk for lymphoma in this study. Patients did not have increased rates of lymphoma development prior to disease onset.[206] In a Veterans Affairs study of 906 men, patients with Felty's syndrome (a variant of RA associated with neutropenia and splenomegaly) were found to have a twofold increase in total cancer incidence but a 12-fold increase in the risk of non-Hodgkin's lymphoma.[207]

### Disease-Modifying Anti-rheumatic Drug Therapy

Several studies have looked at the contribution of disease-modifying anti-rheumatic drug (DMARD) therapy to the elevated risk of malignancies in patients with RA. A prospective, observational study was performed in a group of Canadian patients with RA who were enrolled in a DMARD registry.[208] Although study investigators found an increased rate of lymphoproliferative disorders in this cohort compared with the general Canadian population (SIR, 8.05), no significant differences in DMARD exposure were found between patients in whom malignancy did and did not develop. A second group of Canadian investigators similarly identified an increased risk for the development of lymphoma and myeloma in patients with RA overall compared with control groups.[209] In this study, the risk of lymphoma and myeloma seemed to be fourfold greater in the RA group when DMARD use was not controlled for and 3.4-fold greater when individual DMARD use was controlled for. Despite the low level of DMARD exposure in this population, no strong effect of DMARD use was seen.

Similar effects of DMARD use were seen in a study of Swedish patients with RA and lymphoma: Treatment with any DMARD (odds ratio, 0.9) or specific use of methotrexate (odds ratio, 0.8) did not seem to be associated with increased risk of lymphoma compared with DMARD-naïve patients who had RA; however, no patients had been treated with TNF inhibition.[192] In contrast, a European cohort of patients with RA who were enrolled in a DMARD registry was evaluated longitudinally for the development of malignancies.[210] These investigators found an increased risk of lymphoproliferative disorders in patients with the highest cumulative exposure to DMARDs compared with patients who had less than 1 year of exposure (SIR, 4.82).

Although inconclusive, data from these studies when taken together suggest a possible increased risk for the

development of lymphoproliferative disorders in patients with RA who are treated with DMARDs. However, more recent studies have suggested that this increased risk may be due to the duration and severity of the underlying disorder rather than to specific medication use. The introduction and widespread use of TNF inhibitors for the treatment of RA brought theoretical concerns about an increased risk of malignancy as a result of reducing innate tumor surveillance. After approximately two decades of use, use of TNF inhibitors as a class has not been shown to increase risk of lymphoma above the rates in the general RA population.[211] Associations of specific immunosuppressive therapy for the treatment of autoimmune diseases and malignancy is discussed further in Chapters 61 and 62.

### Risk of Solid Tumors

Despite persuasive evidence of increased risks of lymphoproliferative disorders associated with underlying RA, rates of overall all-site malignancies do not seem to be higher compared with the general population.[190-192,197,198,212,213] The overall "null" result of all malignancies is due to the combination of an increased risk of lymphoproliferative disorders offset by an apparent decreased risk of colorectal malignancies.[190-192,197,198,214] The decreased risk of colorectal cancer has been attributed to long-term use of NSAIDs among patients with RA. Aside from lymphoproliferative disorders, only a few solid tumors have been associated with RA, including lung cancer and skin cancer.

An increased risk of lung cancer in patients with RA has been seen in multiple studies.[190-192,197-203,212-214] In a study evaluating three separate RA cohorts (an inpatient registry of 53,067 prevalent cases of RA, an inception cohort of 3703 incident RA cases, and a registry of 4160 patients with RA who were treated with TNF inhibitors), a consistently increased risk of lung cancer was found in all cohorts (SIR, 1.48 to 2.4) compared with the general population.[84,214] This association may be related to tobacco use, which seems to be a common risk factor for the development of RA, in addition to its well-known association with lung cancer,[215] although the particular association of lung cancer among patients with RA who smoke is unknown. Similarly, a study of lung cancer in a cohort of 8768 U.S. veterans (92% male) with RA found an increased risk of lung cancer (odds ratio, 1.43; 95% CI, 1.23 to 1.65) after adjustment for covariates including age, gender, and tobacco exposure.[216]

A slightly increased risk for the development of nonmelanoma skin cancer (most commonly basal cell carcinoma and squamous cell carcinoma) has been noted in several studies,[190,191,214,217,218] although the significance of these tumors, which carry a low probability of metastasis, is unclear. Nonmelanoma skin cancers are rarely captured in national cancer registries, and thus the incidence among the general population or subpopulations, including individuals with RA, is difficult to quantify or compare. Furthermore, important risk factors for nonmelanoma skin cancer, including ultraviolet light exposure, are almost impossible to quantitate in observational studies. Of greater concern, however, is newer data suggesting an increased rate of melanoma among patients with RA.[195,200,218-220] A recent meta-analysis has shown that the risks of both melanoma and nonmelanoma skin cancer appear to be further increased by the use of TNF inhibitors.[211] Because of a suggestion of increased risk of skin cancer, whether attributed to underlying disease or immunosuppressive therapies, it is reasonable to suggest periodic skin examinations in patients with RA, particularly those who have other risk factors including smoking, increased ultraviolet light exposure, and use of TNF inhibitors. Certainly, all suspicious lesions should be evaluated by a dermatologist, and a biopsy should be strongly considered.

## Systemic Lupus Erythematosus

The risks of developing malignancy in association with SLE have become more clear in recent years, as large multicenter, multinational SLE cohorts have been increasingly studied for associations with different comorbidities.[221] The SIR of individual studies has ranged from 1.1 to 2.6.[222] A meta-analysis of six of the clinical cohort studies found a slightly increased risk of overall malignancies in cohorts of patients with SLE, with an SIR of 1.58.[223] This analysis showed an increased risk of lymphomas in these cohorts, with an SIR of 3.57 for non-Hodgkin's lymphoma and 2.35 for Hodgkin's disease. Investigators who performed a separate meta-analysis of the incidence of lymphoma in patients with SLE found an SIR of 7.4.[173] Individual hospital discharge database studies have shown a consistently higher risk of non-Hodgkin's lymphoma (SIR, 3.72 to 6.7), but in these studies only hospitalized patients with SLE were examined.[222]

A series of studies analyzing nearly 9500 patients with lupus (approximately 77,000 patient-years of observation) in a multinational cohort study has helped to define better potential associations with malignancy.[224,225] The authors found a slightly increased risk of malignancies overall (SIR, 1.15), with higher risks for the development of hematologic malignancies (SIR, 2.75), particularly non-Hodgkin's lymphoma (SIR, 3.64).[224] Forty-two cases of non-Hodgkin's lymphomas were identified, most of which were of aggressive histologic subtypes.[226] The incidence of lymphoma in this study appeared to be early in the course of SLE, rather than after many years of chronic disease activity or use of multiple immunosuppressive medications.[225,227] The elevated risk of non-Hodgkin's lymphoma seemed to be independent of race or ethnicity in this cohort, although white patients seemed to have higher rates of malignancy in general compared with patients of other ethnicities.[228] Cancer risks in this multinational cohort have recently been updated in 2013,[229] with 644 malignancies occurring in 16,409 patients with SLE. Risk estimates were similar to earlier studies, but estimates were more precise. The SIR for all hematologic malignancies was 3.20, and it was increased to 4.39 for non-Hodgkin's lymphoma. In a case-cohort study within the multisite international SLE cohort, age, disease-related damage, and smoking were found to be associated with an increased risk of malignancy; use of immunosuppressant medications (particularly lagged 5 years) may contribute to an increased risk of hematologic malignancies.[230]

Although the exact cause of the association is unknown, several theories have arisen to explain the possible connection between SLE and malignancy, especially B cell

lymphoma. Some authors have postulated that certain immunologic defects may predispose patients to SLE and B cell lymphoma, including apoptosis dysfunction, chronic antigenic stimulation, and overexpression of the Bcl-2 oncogene.[221,231] Viruses, EBV in particular, also have been postulated to be part of the development of SLE and lymphoma.[221,231] Studies have not conclusively validated any of these theories to date, however. More recently, elevated levels of APRIL (A Proliferating-Inducing Ligand) has been found in sera and tumor specimens in patients with SLE who have non-Hodgkin's lymphoma.[232] This cytokine has long been associated with SLE itself and may suggest a common pathway between SLE and lymphoma.[221]

The relative prevalence of cervical cancer in patients with SLE is difficult to estimate because national cancer registries often do not record malignancies in situ. Cervical cancer remains an important issue for women with SLE, and an increased risk may come about for different reasons, including (1) reduced clearance of human papilloma virus, the causal agent in the majority of cervical cancer cases; (2) increased risks of cervical cancer associated with immunosuppressive medications; and (3) reduced rates of routine Pap smears and other screening procedures in a population of patients with chronic illnesses. In a large multinational study performed more recently, the SIR for invasive cervical cancer was found to be elevated at 1.26, albeit with confidence intervals that cross the null.[224,229] Other studies have confirmed an increased risk of abnormal Pap smears and cervical dysplasia in women with SLE.[233-239] In a recent meta-analysis of published studies, a pooled SIR of high-grade squamous intraepithelial lesions of the cervix was found to be 8.66 compared with control subjects.[237] Different studies have implicated increased prevalence of human papillomavirus infection and other sexually transmitted diseases,[235,238-240] and immunosuppression[234,238,240,241] may partly explain this association. As with mammography, women with SLE seem less likely to undergo routine Pap testing than do women in the general population.[242,243] Interestingly, data have suggested a reduced risk of hormone-sensitive tumors among patients with SLE, including breast, endometrial, and possibly ovarian and prostate tumors.[221,229] Reasons for these associations remain unclear.

In several studies, a link has been identified between SLE and the development of lung cancer.[227] Indeed, the largest multinational cohort of subjects with SLE found an increased incidence of lung cancer compared with the general population (SIR, 1.30; 95% CI, 1.04 to 1.60).[229] Further analyses of the cases of lung cancer in this cohort reveal a variety of tumor types, including adenocarcinoma, bronchoalveolar carcinoma, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and carcinoid tumor.[244] The majority of cases (71%) occurred in smokers, 25% of cases were in men, and few (20%) had previous exposure to immunosuppressive agents.[244]

Overall, the presence of SLE carries a risk for the development of cancer, particularly non-Hodgkin's lymphoma, lung cancer, and cervical cancer. The underlying causes of these associations are unknown, but they do not seem to be exclusively related to the use of immunosuppressive or cytotoxic agents.[221,230,245-248] Data also suggest that patients with lupus may be less likely to receive recommended cancer screening.

## Systemic Sclerosis

Although data are conflicting, most evidence suggests that people with systemic sclerosis (SSc) seem to have an increased risk of developing malignancy.[249,250] Estimates of the prevalence of cancer among patients with scleroderma range from 3.6% to 10.7%.[250,251] However, 13% of deaths among patients with SSc are caused by cancer.[252] The malignancies that have been implicated are often in organs affected by inflammation and fibrosis, including the lung, breast, esophagus, and skin. The SIR of malignancy in the scleroderma population is 1.5 to 5.1 compared with that of the general population.[253-258] There is an apparent increase in the observed number of cases of lung cancers that occur in the setting of pulmonary fibrosis but not in association with tobacco use.[257,259] In a different study evaluating 20 lung cancers among 632 patients with scleroderma in Australia, it was found that cigarette smoking, but not underlying pulmonary fibrosis, was associated with an increased risk of lung cancer.[260] Study findings have been mixed regarding an increased risk or temporal relationship between scleroderma and breast cancer.[250,253,261]

A meta-analysis of more than 7000 patients with scleroderma indicated that compared with the general population, the summary RR to develop all invasive cancers in patients with scleroderma was 1.75 (95% CI, 1.41 to 2.18). A strong association with lung cancer (RR, 4.35; 95% CI, 2.08 to 9.09) and a significant increased risk for hematologic neoplasms (RR, 2.24; 95% CI, 1.53 to 3.29) was seen. The relationship with breast cancer, which was suggested in some previous epidemiologic studies, was not confirmed (RR, 1.05; 95% CI, 0.86 to 1.29). Similar results were reported by Zhang et al.[262] in another meta-analysis of 7183 patients with scleroderma, with an SIR of 3.14.[262]

Data are mixed regarding associations between SSc-specific autoantibodies and the development of cancer. Selected studies support potential associations,[256,263] whereas others do not.[257,264] Shah et al.[265] reported a close temporal relationship with cancer (within 1 to 2 years of scleroderma diagnosis) in patients with scleroderma who had positive antibodies for RNA polymerase I/III. Interestingly, expression of RNA polymerase I and RNA polymerase III were detected in the tumors of affected individuals.[265] Similar findings were reported in a large single-center United Kingdom retrospective cohort study of 2177 patients with SSc[266]; 7.1% had a history of cancer, 26% had anticentromere antibodies (ACAs), 18.2% had anti-Scl-70 antibodies, and 26.6% were positive for anti-RNA polymerase III (anti-RNAP) antibody. The major malignancy cancer subtypes were breast (42.2%), hematologic (12.3%), gastrointestinal (11.0%), and gynecologic (11.0%). The frequency of cancers among patients with anti-RNAP (14.2%) was significantly increased compared with patients with anti-Scl-70 antibodies (6.3%) and ACAs (6.8%; $P < 0.0001$ and $P < 0.001$, respectively). Among the patients who were diagnosed with cancer within 36 months of the clinical onset of SSc, there were more patients with anti-RNAP (55.3%) than with other autoantibody specificities (ACA = 23.5%, $P < 0.008$, and anti-Scl-70 antibodies = 13.6%, $P < 0.002$, respectively). Breast cancers were temporally associated with the onset of SSc among patients with anti-RNAP.

The precise pathogenic mechanisms that cause and maintain an autoimmune response in patients with cancer have not been fully elucidated. Autoimmunity in cancer may occur as a consequence of an abnormal self-antigen expression and/or mutated antigens, as well as of tumor-derived biologic substances produced by cancer cells. In addition, the consequences of chronic inflammation and fibrotic processes may damage tissue, cells, and DNA, leading to altered immune responses and the development of malignant transformation, triggering the development of lung cancer or cutaneous malignancies. Researchers from Johns Hopkins recently reported genetic alterations of the POLR3A locus in six of eight patients with antibodies to RNA polymerase III but not in eight patients with scleroderma who had other autoantibody specificities. Analyses of peripheral blood lymphocytes and serum suggested that POLR3A mutations triggered cellular immunity and cross-reactive humoral immune responses.[267]

Although the SIR for all malignancies is 1.5 to 2.4, the incidence ratio for lung cancer and non-Hodgkin's lymphoma can be as high as 7.8 and 9.6, respectively. Cases of non-Hodgkin's lymphoma seem to be more likely to occur within the first year of the diagnosis of SSc.[255] Elevations in incidence also were found for other specific cancers, including nonmelanoma skin cancers (4.2), primary liver cancers (3.3), and hematopoietic cancers (2.3)—all having a higher incidence than that in the general population. The greatest risk seems to correspond to areas commonly affected by fibrosis, particularly the lung and skin. Esophageal involvement, which is common in limited and diffuse SSc, is the likely cause for an increased incidence of Barrett's esophagus (12.7%)[268] and the development of esophageal cancer (SIR, 9.6).[269] In contrast to the aforementioned data, one study found no increase in overall or specific malignancies in patients with SSc (SIR, 0.91 overall).[264] Localized scleroderma, including morphea or linear scleroderma, does not seem to convey an increased risk of malignancy.[270]

Sclerodermatous skin changes have been reported with breast, ovarian, pulmonary, and gastrointestinal cancer. Several reports have described the development of post-irradiation morphea in patients treated for breast cancer.[271]

## PRIMARY TUMORS AND METASTATIC DISEASE

### Primary Musculoskeletal Tumors

This section does not provide in-depth knowledge of the primary tumors of the musculoskeletal system. Rather, it provides a reference to the most common primary malignant musculoskeletal tumors and symptoms that may arise in association with them. The primary tumors of bone, including benign and malignant tumors, are discussed in more detail in Chapter 124.

A primary malignant bone cancer is any neoplasm that develops from the tissues or cells found within bone and has the ability to metastasize. Neoplasms may develop or arise from any of the types of cells present within the bone—osteoblasts, chondrocytes, adipose and fibrous tissue, vascular cells, hematopoietic cells, and neural tissue.[272] A neoplasm developing from any of these tissues is called a sarcoma, which signifies that it is derived from mesenchymal tissue. The bone sarcomas are named for the predominant differentiated tissue type, such as osteosarcomas, chondrosarcomas, liposarcomas, and angiosarcomas.[272]

The most common manifestation of these tumors is the development of pain in the area of the lesion, which may be accompanied by a sympathetic effusion or stiffness in the surrounding joint. This discomfort does not seem to be activity related and is often worse at night. However, these tumors can manifest as painless masses or as pathologic fractures. Systemic features such as fatigue, malaise, weight loss, fevers, and night sweats are rare with all of these tumors except for Ewing's sarcoma.[272] Primary malignant bone tumors are uncommon, particularly compared with other types of cancer. They have their highest incidence in childhood and adolescence and constitute 3.2% of childhood malignancies that occur before age 15 years. The incidence has been reported in this age group as 3 per 100,000 individuals.[237] These tumors commonly arise out of areas of rapid growth, with the most common site of primary bone sarcomas being the metaphysis near the growth plate.[273]

Table 123-5 lists the most common types of primary malignant bone tumors. Osteosarcoma is the most common of the tumors and generally occurs in individuals in the second decade of life or in elderly individuals.[274] Osteosarcoma also can occur as a result of radiation therapy delivered as treatment for other malignancies. Paget's disease of bone can rarely (in <1% of cases) proceed to malignant transformation.[275] Severe pain in the setting of Paget's disease may signal transformation to osteogenic sarcoma. Tumors most frequently affect the femur, humerus, skull, and pelvis and can result in pathologic fractures. Survival is usually less than 1 year. Differentiation of malignancy from Paget's disease may require a biopsy.[276,277]

Chondrosarcoma has been reported as the second most common of the malignant bone tumors. This tumor may occur as a primary tumor or as a malignant transformation in the setting of benign lesions, such as an enchondroma or osteochondroma.[278] Fibrosarcoma is significantly less common than the previously mentioned tumors and accounts for less than 4% of primary malignant bone tumors.[279]

As a group, round cell tumors include primary lymphomas of bone, Ewing's sarcoma, and metastatic neuroblastoma. Ewing's sarcoma is a common primary bone tumor of childhood. Giant cell tumors as a group account for 4.5% of bone tumors. They usually arise from the metaphysis or epiphysis of long bones, generally around the knee. Most are benign, but a few are malignant lesions, usually arising out of a previously irradiated benign giant cell tumor.[272]

In addition to the primary malignancies of bone, a plethora of malignant tumors can arise from mesenchymal connective tissue; these tumors are also known as sarcomas.[279,280] They can result in joint symptoms but more often

**TABLE 123-5** Primary Bone Tumors

| |
|---|
| **Nonosseous Tumors** |
| Multiple myeloma; round cell tumors |
| **Osseous Tumors** |
| Osteosarcoma; chondrosarcoma; giant cell tumors; fibrosarcoma |

result in soft tissue symptoms. They are very rare. Rhabdomyosarcoma is a malignant tumor arising from muscle tissue. It is the fourth most common solid tumor in children and is responsible for more than half of all soft tissue sarcomas in children. Rhabdomyosarcoma rarely occurs in adults. It can appear at any site, and the symptoms are most often referable to the site involved. Most commonly, rhabdomyosarcoma affects the orbits, genitourinary tract, limbs, head and neck, and parameningeal areas. It commonly metastasizes to the lymph nodes, lungs, and bone.[273,281]

### Metastatic Disease

When bone lesions are identified, primary tumors need to be considered, although most malignant lesions in bone are metastatic. Metastasis rarely affects muscles, joints, or adjacent connective tissue; more commonly, it affects bone. The most common sites of metastasis are the spine and pelvis. It is uncommon to find metastatic lesions distal to the elbow, and, although rare, metastasis to the foot is more common than to the hand.[282] When distal or acral metastasis is identified, it is often associated with lung cancer.[283] Primary tumors generally associated with metastases to bone include tumors in the prostate, thyroid, lung, breast, and kidney.[284] Although most skeletal metastases do not produce pain, one of the most common causes of cancer-related pain is the infiltration of bone. The pain can be intense and stabbing or dull. It is often constant rather than intermittent, is worse at night, and often is worse with weight bearing and movement.[285] Rheumatic or arthritic symptoms often can occur before lesions are easily identified on radiographs. Arthritis associated with metastatic carcinoma is most commonly monarticular and usually affects the knee. Metastases to the hip, ankle, wrist, hand, and foot have been reported but occur less frequently. Breast and lung carcinomas are present in most patients.[286] Metastases to the extremities can simulate gout, osteomyelitis, tenosynovitis, or acro-osteolysis. The development of joint involvement can be related to direct synovial implantation or involvement of the juxta-articular or subchondral bone.[287] Table 123-6 presents the clinical features suggestive of underlying metastases.

Radiographic features of bone tumors can be significant when interpreting the duration of disease and the type of malignancy. Lesions may be lytic or blastic, and patterns of destruction often reflect the aggressiveness of tumors. Well-circumscribed lesions may be more indicative of slower growth, whereas a "moth-eaten" pattern with evidence of cortical destruction typically signifies a more rapid rate of growth. What has been described as a permeative pattern suggests an extremely rapid rate of destruction and is often associated with an extraosseous soft tissue mass.[272] CT, MRI, and radionucleotide imaging also can provide significant information for diagnosis, staging, prognosis, and therapy.

**TABLE 123-6** Frequent Features of Arthritis Resulting from Metastatic Carcinoma

| |
|---|
| Presence of constitutional symptoms |
| Prior history of malignancy |
| Protracted clinical course |
| Negative culture results, negative crystal analysis |
| Medical therapeutic failure |
| Rapid reaccumulation of hemorrhagic non-inflammatory effusion |
| Radiologic evidence of destructive process |

## POST-CHEMOTHERAPY RHEUMATIC DISEASE

Several rheumatic or musculoskeletal manifestations can develop in patients after administration of chemotherapy for the treatment of malignancy. Post-chemotherapy rheumatism has been best described in patients treated for breast cancer, but it also has been described in other malignancies, including ovarian cancer and non-Hodgkin's lymphoma.[288-290] The phenomenon has been described as a non-inflammatory, self-limited, migratory arthropathy. Typically symptoms develop several weeks to several months after the completion of chemotherapy and often include myalgia, stiffness, arthralgia, and arthritis involving the small joints of the hands, ankles, and knees.[289] Post-chemotherapy rheumatism can be mistaken for RA based on its symptoms; however, most patients have little or no evidence of synovial thickening and have no radiographic or serologic evidence to suggest RA. The pathogenesis of this process is unknown; however, it is self-limited, usually lasting less than 1 year, and is best treated in a conservative fashion. Evaluation should be performed to exclude recurrent carcinoma or another inflammatory condition. The medications most frequently implicated in this phenomenon are cyclophosphamide, 5-fluorouracil, methotrexate, and tamoxifen.[290-292]

Other immunomodulatory agents also have been linked to the development of musculoskeletal findings. Tamoxifen use has been associated with the development of an acute inflammatory arthritis similar to RA[292]; however, a randomized controlled trial of tamoxifen or placebo in 7145 women at high risk for breast cancer (but without a diagnosis of cancer) did not find an increased prevalence of arthralgia or arthritis symptoms in the tamoxifen arm.[293] More recently, aromatase inhibitors have been used widely for hormone receptor–positive breast cancer, with increasing reports of associated arthralgia and arthritis. A retrospective, exploratory analysis of the Arimidex Tamoxifen Alone or in Combination (ATAC) trial evaluated the development of joint symptoms in 5433 women with early stage breast cancer who were randomized to receive either anastrozole alone or tamoxifen alone.[294] When analysis was limited to women who entered the study without joint symptoms at randomization, 35.2% experienced joint symptoms while taking anastrozole compared with 30.3% who experienced joint symptoms while taking tamoxifen ($P$ <0.0001). However, the intensity of symptoms was not different between groups. A prospective, nonrandomized study evaluated the prevalence of musculoskeletal symptoms in women who switched from anastrozole to letrozole as a result of articular symptoms.[295] The majority (61%) were able to continue with letrozole, with 28.5% discontinuing the second aromatase inhibitor as a result of severe musculoskeletal symptoms.

Therefore, switching aromatase inhibitors may be a reasonable strategy when an initial agent is intolerable because of arthralgia or arthritis; however, many women continue to be intolerant to more than one agent.

Biologic agents used for the treatment of chronic viruses or a variety of malignancies can similarly lead to an autoimmune phenomenon. Use of IL-2 can result in spondyloarthritis or inflammatory arthritis. Interferon-α administration can result in seropositive nodular RA and myalgia and arthralgia.[296,297] The use of interferon also can result in autoantibody formation and features suggestive of SLE and autoimmune thyroid disease.[297-299]

## Lymphoproliferative and Myeloproliferative Diseases

### Leukemia

Leukemia can result in the development of musculoskeletal symptoms. Bone pain is the most common musculoskeletal manifestation and has been reported to occur in 50% of adults with leukemia.[300] Long bone pain is more common in children, whereas axial pain is more common in adults. Generally, the bone pain is more common in the lower than in the upper extremities.[301] Overt synovitis can develop in association with acute and chronic leukemia and can result in the development of monarticular or polyarticular arthritis.[302] The pathogenesis seems to be leukemic infiltration of the synovium and subperiosteal tissue. Bleeding or hemorrhage in the joint also may be associated with the process. Most cases of arthritis associated with leukemia are seen in children (14% to 50% compared with 4% to 16.5% in adults).[303-305]

In a series of adult patients with acute leukemia who were studied over a 10-year period, 5.8% (8 of 139) of the patients presented with rheumatic manifestations. On average, symptoms of arthritis preceded the diagnosis of leukemia by 3.25 months.[306] The most common patterns of presentation were an asymmetric large joint involvement in association with low back pain, followed by symmetric polyarthritis mimicking early RA. Rheumatic manifestations included morning stiffness, low back pain, nonarticular bone pain, pain out of proportion to objective findings, low-grade fever, and elevation of the ESR. The response to NSAIDs, glucocorticoids, and conventional anti-rheumatic therapy was reportedly poor, but tumor-directed chemotherapy resulted in substantial improvement of the rheumatic manifestations. Patients with these manifestations also were more likely to exhibit early osteopenia or lytic bone lesions. Ultimately, prognosis and mortality rates were no different between patients presenting with or without rheumatic manifestations.[306]

In contrast, in a large retrospective study of children with leukemia, it was found that 21.4% (36 of 168) with acute lymphoblastic leukemia and 10.5% (6 of 57) with acute nonlymphoblastic leukemia experienced symptoms associated with bones and joints. In 13 of these patients with acute lymphoblastic leukemia, radiographs showed evidence of bony lesions.[307]

Many of these children had been incorrectly treated for juvenile RA or osteomyelitis before the diagnosis of leukemia. The group with bone lesions seemed to do very well, and their condition might fall into a subgroup of childhood leukemia that has a better prognosis.[302,307] A more recent study found that the presence of subtle blood cell count changes and nighttime pain may help distinguish leukemia from juvenile RA.[305]

### Multiple Myeloma

Multiple myeloma is a neoplastic proliferation of plasma cells—a nonosseous malignant tumor arising in the marrow. In contrast with the other primary tumors of bone, which have their highest incidence in children and adolescents, myeloma is a tumor of adults, occurring most commonly in the fifth and sixth decades of life. The most common musculoskeletal feature of this disease is the development of bone pain. Other hallmark features are diffuse pain and stiffness. Patients characteristically experience osteopenia, and osteolytic lesions are seen on radiographs. The lytic lesions, which can occur in any area of the skeleton, are produced by focal accumulations of plasma cells. Osteosclerotic lesions also have been reported.[308] True arthritis is rare, but cases of arthritis resulting from articular and periarticular invasion with malignant cells have been reported in individuals with multiple myeloma and Waldenström's macroglobulinemia.[309] A secondary feature of the disease, which can often lead to additional musculoskeletal complaints, is the development of hyperuricemia and secondary gout. Sjögren's syndrome and other autoimmune phenomena also have been described in association with multiple myeloma.[310]

### Lymphoma

Musculoskeletal symptoms have been reported in 25% of cases of non-Hodgkin's lymphoma.[311] The most common musculoskeletal problem associated with lymphoma is the development of bone pain associated with metastases or lymphoma in the bone. By report, more than 50% of patients have evidence of bone lesions at autopsy; however, few patients actually present with arthritis or bone pain.[302,312] Nonetheless, non-Hodgkin's lymphoma has been reported to manifest as a seronegative arthritis with or without other features, such as lymphadenopathy and hepatomegaly, typically seen with this disease. Monarticular and polyarticular involvement can occur. Cases have been reported of polyarthritis simulating RA in the setting of non-Hodgkin's lymphoma.[313] Although it is unusual to see direct involvement of the synovium, this finding also has been reported. There have been cases with radiographic evidence of bone destruction associated with non-Hodgkin's lymphomatous arthropathy.[314] Suspicion of lymphoma should be heightened in patients in whom severe constitutional symptoms seem out of proportion to the degree of arthritis, especially in patients who are negative for rheumatoid factor.

### Angioimmunoblastic Lymphadenopathy

Angioimmunoblastic lymphadenopathy is a rare lymphoproliferative disorder marked by the clinical features of lymphadenopathy, hepatosplenomegaly, rash, and hypergammaglobulinemia. Patients can experience a nonerosive, symmetric, seronegative polyarthritis concurrent with other

**TABLE 123-7** Musculoskeletal Manifestations of Hematologic Malignancy

| Malignancy | Pathogenesis |
|---|---|
| Leukemia | Infiltration of synovium |
| Lymphoma | Metastases or invasion of bone, rarely joint |
| Angioblastic lymphadenopathy | Vasculitis, cryoglobulinemia |
| Multiple myeloma | Metastasis or invasion of bone, hyperuricemia |

features or as an initial symptom of the disease.[315-317] Similar features have been reported with intravascular lymphoma, with a report of a patient presenting with a symmetric polyarthritis accompanied by fever.[318] Table 123-7 lists musculoskeletal symptoms found with hematologic malignancy.

### Graft-Versus-Host Disease

Graft-versus-host disease is a complication of bone marrow transplantation and a major cause of morbidity and mortality in the transplant population. Numerous musculoskeletal symptoms arise in the setting of acute graft-versus-host disease (lasting 0 to 3 months) and in chronic graft-versus-host disease (lasting >3 months after transplantation). The most frequent manifestation is the involvement of the skin, which in many cases can progress to resemble the changes of SSc. Skin changes consistent with eosinophilic fasciitis also have been reported.[301] Graft-versus-host disease can lead to symptoms of keratoconjunctivitis sicca and xerostomia resembling Sjögren's syndrome. Other features including arthralgias, arthritis, myositis, Raynaud's phenomenon, and serositis also have been reported.[301]

## CONCLUSION

A plethora of factors contribute to the development of musculoskeletal syndromes in the setting of autoimmune disease and malignancy. A great many autoimmune disorders exist, and for most of them, the underlying cause and pathogenesis have not been elucidated. Because of this incredible diversity, understanding the relationship between associated symptoms is often difficult. To make any generalizations regarding the association between an autoimmune disorder and the subsequent development of malignancy, large numbers of patients must be studied longitudinally for exceptionally long periods. Other confounders complicate the picture. For example, many of the agents used in the treatment of connective tissue and autoimmune disorders modulate the immune system. These agents may have direct carcinogenic potential, whereas others may affect the immune system in a way that may decrease tumor surveillance and subsequently lead to the development of a neoplasm. Intricately entwined are the unique differences of individual immune systems not only in healthy individuals, but also in individuals whose immune systems are already altered based on an underlying autoimmune disorder. Although uncommon, it is plausible that virtually any of the autoimmune-based diseases and the agents used to treat them might be associated with malignancy in certain circumstances. Most important, when musculoskeletal symptoms arise, malignancy or paraneoplastic syndromes should be considered in the differential diagnosis, especially when patients present with atypical features of autoimmune disease or are refractory to conventional treatment. In addition, the potential for any agent to induce a neoplastic process must be weighed against its proposed benefits before initiating it as therapy.



Full references for this chapter can be found on ExpertConsult.com.

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CHAPTER 124



# Tumors and Tumor-like Lesions of Joints and Related Structures

Darcy A. Kerr • Andrew E. Rosenberg

## KEY POINTS

**Most mass lesions that involve joints and synovial-lined structures are benign. Synovial cysts, which are the most common, are not true cysts because they lack an epithelial lining. Synovial cysts may involve joints (e.g., a Baker's cyst) or tendon sheaths, and when the synovial lining is inconspicuous, they are designated ganglion cysts. Treatment depends on the symptoms; observation is a reasonable course of action in many cases.**

**Synovial chondromatosis is an uncommon benign condition characterized by nodules of hyaline cartilage, often ossified, within the subsynovial connective tissue, most frequently involving the knee. These nodules can be confused with osteochondral loose bodies, and treatment consists of removal of the nodules.**

**Tenosynovial giant cell tumor of joints and tendon sheaths is the most common neoplasm of synovial-lined structures. The tumor is most often monoarticular, with the knee involved in 80% of cases. The neoplastic cells overexpress macrophage colony-stimulating factor as a result of a chromosomal translocation. Although the tumor is benign, it can be locally destructive. Treatment consists of removal of the tumor, and new forms of targeted medical therapy are now available.**

**The most common primary malignant tumor of joints is synovial sarcoma, which usually affects children and young adults. The disease has an aggressive course, with a long-term survival of approximately 50%.**

**Lymphoproliferative diseases, especially acute leukemia, may involve joints. Joint involvement is most common in children, for whom the reported incidence ranges from 12% to 65%. Arthritis caused by leukemic infiltration of the synovium can occur at any time in the course of the disease and can be the presenting symptom.**

Synovial-lined joints and periarticular structures are often affected by mass-forming lesions. Arthrosis, arthritis, trauma, and structural derangements produce the most common lesions, which are non-neoplastic and include ganglions, synovial cysts, and loose bodies. In contrast, it is uncommon for neoplasms, whether benign or malignant, to develop in joints and periarticular structures.

Joint neoplasms can be divided into tumors that are primary or arise de novo within the synovium and tumors that are secondary and access the joint by invading from neighboring bones and soft tissues or by spreading from distant sites via the vascular system. Primary joint neoplasms are more common and tend to recapitulate the phenotype of tissues that normally compose the joint and synovial sheath—synovium, fat, blood vessels, fibrous tissue, and cartilage. Regardless of the histologic type, benign variants greatly outnumber their malignant counterparts, and as a group these tumors develop more frequently in the synovium, in comparison with other periarticular structures.

Tumors and tumor-like lesions of the joints and periarticular structures are biologically and morphologically diverse. They often pose significant challenges in diagnosis and treatment, and their clinicopathologic features are the focus of this chapter.

## NON-NEOPLASTIC LESIONS

### Synovial and Ganglion Cysts

Cysts are defined as closed compartments or sacs that are lined by epithelium and are frequently filled with fluid. Neither the synovial cyst nor the ganglion cyst is considered a true cyst because each lacks an epithelial lining.

Synovial cysts are common and develop from the synovial lining of a joint, tendon, or bursa. They are non-neoplastic lesions caused by herniation of the synovium through the joint capsule or tendon sheath into neighboring tissues or by expansion of a pre-existing bursa. In adults, synovial cysts frequently develop in association with a variety of joint disorders, including trauma, osteoarthrosis, crystal arthropathies, infection, and rheumatoid arthritis or one of its variants. Most synovial cysts have an anatomic attachment to a joint, and most originate in the posterior medial aspect of the knees of adults, where they are known as popliteal or Baker's cysts. Anatomically, this region of the knee is prone to the development of synovial cysts because the joint capsule can be structurally insufficient and because people in this age group often have co-existing joint disease.[1,2] Synovial cysts of the posterior knee joint are purported to affect 2.4% of children, who, in contrast to adults, are usually asymptomatic and have an otherwise normal knee joint.[3] Other joints affected by synovial cysts in decreasing order of frequency are the shoulder, hip, and spine. In the spine they originate from the facet joints and are most commonly located in the lower lumbar region.

Synovial cysts can enlarge over time as they become further distended with increasing amounts of synovial fluid.[1,3-5] Consequently, they may manifest as a periarticular mass, produce progressive joint pain and swelling, limit

joint mobility, and compress adjacent neurovascular structures. An example of compression occurs in the spine, where synovial cysts that arise from facet joints may impinge or extend along spinal nerves, causing radicular pain.[6-8] Other complications of synovial cysts, which sometimes can produce dramatic clinical findings, are acute rupture and secondary infection.

A variety of radiographic techniques have been used to create images of synovial cysts. Modalities that provide the greatest diagnostic information include arthrography, ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI).[1,3-5] All of these techniques reveal synovial cysts to be simple or septated thin-walled structures that are filled with fluid, with a density similar to that of water (Figure 124-1).

Grossly, synovial cysts usually range in size from 1 to 10 cm. Their inner surface is smooth, glistening, and translucent; however, it can be distorted by an attached blood clot, inflammatory debris, or granulation tissue resulting from a prior hemorrhage or infection. The inner surface of the cyst wall is lined by flattened or plump cuboidal synoviocytes arranged in one or several cell layers and surrounded by an outer sheath of fibrous tissue (Figure 124-2). Sometimes the synovial lining cells may be hyperplastic and form papillary fronds of varying size; other findings that may develop are scattered subsynovial collections of hemosiderin-laden macrophages from previous hemorrhage and aggregates of mononuclear cells and reactive fibrosis resulting from chronic irritation. Facet joint cysts often contain abundant amorphous debris surrounded by macrophages and are associated with severe degenerative changes of the ligamentum flavum and its elastic fibers and the annulus fibrosus.

The treatment of synovial cysts varies, depending on their location and associated symptoms. They can be managed successfully with conservative therapy; however, in certain clinical situations, surgical excision is required.[1,3-8]

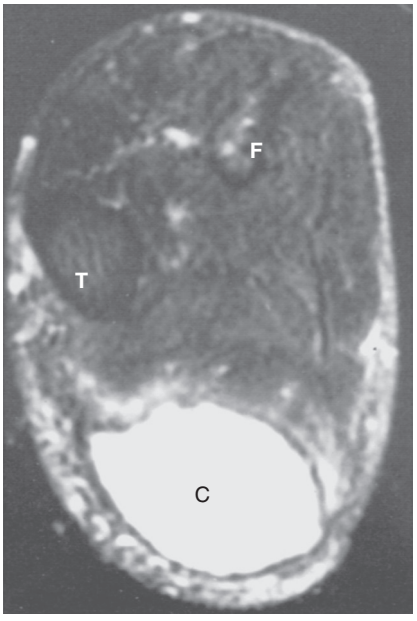



**Figure 124-1** MRI shows a large, oval-shaped synovial cyst with high T2-weighted signal intensity that extends from the knee joint into the posterior calf. C, Synovial cyst; F, fibula; T, tibia.

Ganglion cysts have been recognized for centuries; Hippocrates described them as being composed of "mucoid flesh."[9] They are more common than synovial cysts and arise from tendon sheaths, ligaments, menisci, joint capsules, and bursae.[9] Occasionally they develop de novo in the subchondral areas of bone (intraosseous ganglion), and rarely, they arise within nerve or skeletal muscle and lack communication with a joint. Intraneural ganglia develop from dissection of joint fluid along an articular branch of a nerve.[10] Ganglion cysts are distinguished from synovial cysts by virtue of the fact that they lack a surface lining of synoviocytes.[11] A variety of hypotheses have been proposed to explain their pathogenesis, but none has been proven.[9] The most accepted theory is that ganglia develop from mucoid cystic degeneration of periarticular structures and are lined by fibroblast and myofibroblast-like cells.[11] They are commonly associated with repetitive motion activities, inflammatory arthritides, and trauma.

Most ganglia arise along the dorsal and volar aspects of the wrists and fingers and the dorsum of the feet.[9,12] They are usually asymptomatic and typically manifest as a slowly growing, mobile, firm mass that moves with the structure from which it has arisen (Figure 124-3). Ganglia may be painful if traumatized and can compress adjacent

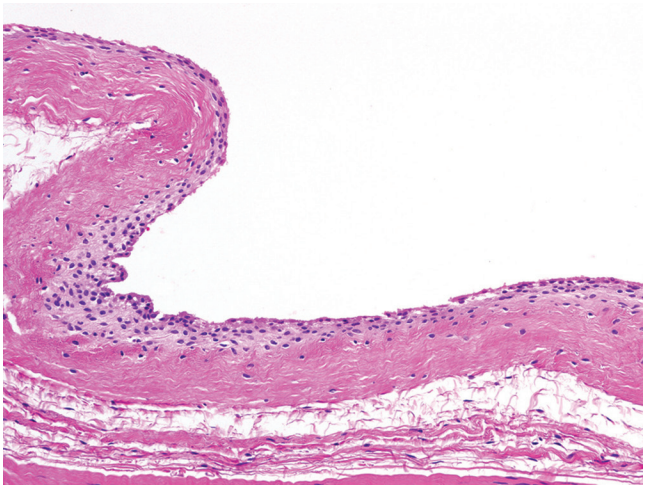


**Figure 124-2** The wall of a synovial cyst that is composed of an inner lining of synoviocytes overlaying a layer of dense, fibrous tissue.



**Figure 124-3** A round, firm ganglion cyst bulging from the dorsal aspect of the hand.

neurovascular structures, producing a variety of symptoms. The radiographic characteristics of ganglia are similar to those of synovial cysts, and they appear on images as small, fluid-filled cystic structures.[1,12] However, with use of ultrasound, ganglion cysts are generally more difficult to compress than synovial cysts because of the thicker, more viscous content and denser capsule of the ganglion cyst; this difference may allow the sonographer to distinguish these two entities through sonocompression characteristics.[2]

Macroscopically, most ganglia are round, but they may form elongate cylindrical structures if they track along a tendon sheath. Ganglia are uniloculated or multiloculated, have thin walls, and are filled with translucent mucoid fluid rich in hyaluronic acid and other mucopolysaccharides (Figure 124-4). The cyst lacks a distinct inner cell lining, and the bulk of the wall consists of dense fibrous tissue, which is usually surrounded by areolar tissue (Figure 124-5).

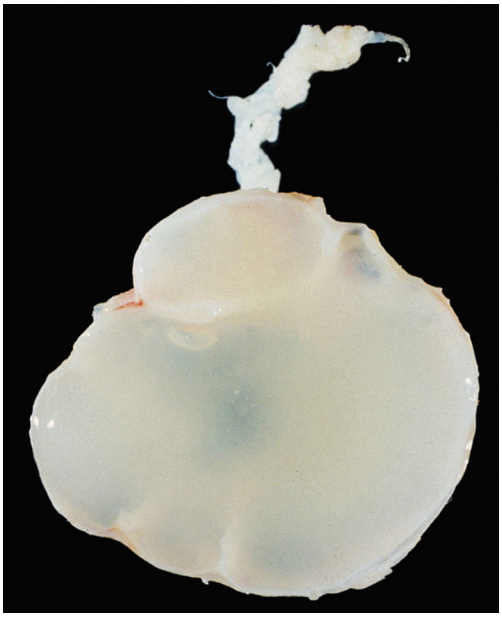


**Figure 124-4** An intact ganglion cyst with a thread-like pedicle that attaches to a periarticular structure.

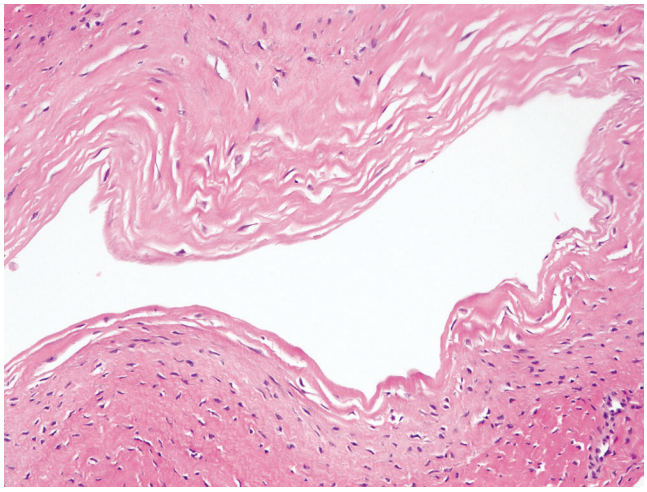


**Figure 124-5** A ganglion cyst wall composed of scattered flattened fibroblasts on the luminal surface and a well-formed layer of fibrous tissue.

In many instances, the cyst wall is distorted by variable quantities of reactive myxoid tissue and muciphages, which result from small ruptures and extravasation of fluid.

When ganglion cysts form, they may remain stable for years or may spontaneously resolve, and ganglia that disappear may subsequently redevelop. Treatment is frequently conservative because of their innocuous nature. Ganglia occasionally require aspiration or surgical excision, however, especially if they are symptomatic.

## Loose Bodies

*Loose bodies* and *joint mice* are generic terms for free-floating structures within a joint cavity. They are the most common tumor-like lesions of joints and may be exogenous, such as fragments of a bullet, or endogenous, such as pieces of articular cartilage, osteophytes, menisci, ligaments, or bone.[13-15] When not otherwise specified, the term *loose bodies* refers to detached pieces of articular cartilage or subchondral bone (osteoarticular loose bodies) or both that lie free within the joint or that have become secondarily embedded in the synovium. Loose bodies can cause pain, crepitance, and locking, and they can limit the range of joint motion.

Osteoarticular loose bodies are a secondary complication of a variety of conditions, including trauma, osteochondritis dissecans, and arthritides of various causes. When dislodged, the sloughed articular cartilage remains viable because it receives its nourishment from the synovial fluid, but the subchondral bone dies because it derives its nutrition solely from blood vessels. Over time, as the loose body tumbles within the joint, its edges become rounded and smooth; however, it eventually becomes embedded within the synovium. Subsequently, the synovium may digest and resorb it, or adjacent subsynovial connective tissue cells may undergo a proliferative and metaplastic response. These cells produce layers of newly formed fibrocartilage and hyaline cartilage, which may undergo endochondral ossification and are deposited on the surface of the loose body (Figure 124-6). These layers of newly formed tissue surround the centrally located dislodged native structure, similar to the cambium layers of a tree, and provide a mechanism for the entire structure to increase gradually in size and become significantly larger than the initial osteochondral defect from which it originated (Figures 124-7 and 124-8). As the




**Figure 124-6** A large, nodular loose body formed from a semilunar-shaped piece of articular cartilage that is surrounded by newly formed cartilage.



**Figure 124-7** A loose body with visible layers of newly formed tissue.

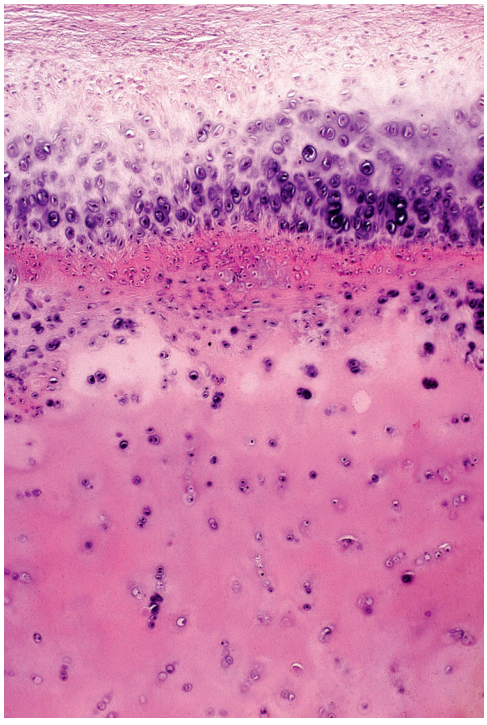


**Figure 124-8** A loose body composed of sloughed articular hyaline cartilage *(bottom)* covered by consecutive layers of newly formed metaplastic hyaline cartilage and bone.

loose body enlarges, the innermost portion of original articular cartilage cannot be supported adequately by diffusion of synovial fluid, and it dies and calcifies. This combination of events causes the loose body to appear on radiographs as dense, speckled, and ring-like calcifications (Figure 124-9). Radiographically and histologically, the differential diagnosis includes synovial chondromatosis. Treatment is simple excision, which can be performed arthroscopically.[14,15]

### Intra-articular Ossicles

Small bony nodules normally occur in the knees of some rodents[16] and other mammals, and they may rarely occur in humans.[17-19] In rodents, small bony nodules are consistently found in the anterior portions of the joint and frequently in the posterior portions as well. In humans, nodules develop within the substance of the meniscus of the knee joint adjacent to its attachments to the tibia.



**Figure 124-9** A knee with an osteoarticular loose body in the suprapatellar region *(arrow)*.

The exact origin of such structures is unknown, although they probably are true sesamoid bones, as seen in rodents. Alternatively, they may represent ossification resulting from local injury. This latter possibility is supported by the fact that previous knee trauma has been noted in numerous reported cases. The main symptom of meniscal ossicles is pain after exertion, such as walking or prolonged standing, with relief when the knee is at rest. Radiographs may reveal an intra-articular calcification that can be confused with a loose body. MRI shows the ossicle to be a corticated marrow-containing structure that has increased signal intensity on T1-weighted images and decreased signal intensity on T2-weighted images.[17,19] The ossicle is located within the lateral or the medial meniscus and appears as a small (~1 cm in diameter), palpable, bony nodule (Figure 124-10).[18] If the ossicle is symptomatic, it should be excised; however, if it is an incidental finding, it can be managed conservatively.[17,19]

## NEOPLASMS

### Fatty Lesions of the Synovium

Although the subsynovial connective tissue of diarthrodial joints is rich in fat, a true lipoma of the synovium is rare. When lipomas develop, they most frequently affect the knee joint and the synovial tendon sheaths of the hands, ankles, and feet, where they are more common in the extensor than in the flexor synovial sheaths.[20,21] Synovial lipomas can be sessile or pedunculated, and when pedunculated, they may produce pain if they twist on their stalks and become secondarily ischemic. Synovial lipoma, similar to

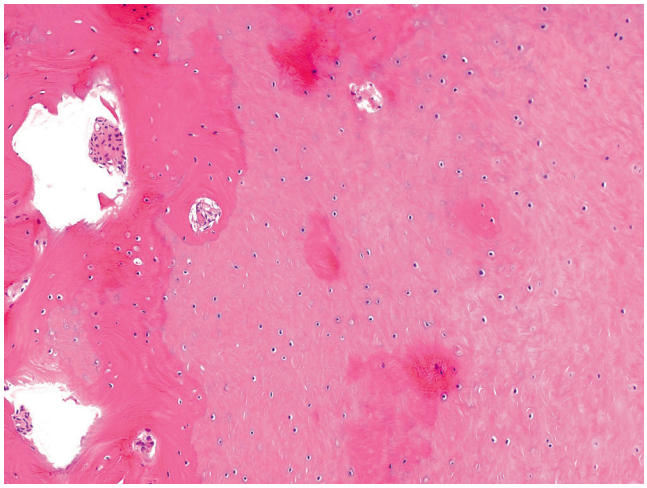


**Figure 124-10** An intra-articular ossicle embedded in the fibrocartilage of the meniscus.



**Figure 124-11** Lipoma arborescens manifesting as a suprapatellar mass.

its subcutaneous counterpart, is composed of lobules of mature white adipocytes that are delineated by a thin fibrous capsule.

A more common but still unusual fatty lesion of the joint is lipoma arborescens, also known as *villous lipomatous proliferation* and *lipomatosis of the synovium*.[22,23] This disorder is characterized by a diffuse increase in the quantity of subsynovial fat, which bulges into the overlying synovial lining, producing a villous architecture. It is uncertain whether the proliferating fat is neoplastic (lipomatosis) or is a manifestation of a hyperplastic or reactive process. Most cases occur in the context of degenerative or inflammatory arthritis, supporting the idea that the fatty proliferation may represent a secondary reactive process in most instances.[24] Affected patients are usually adults, but the lesion sometimes develops in adolescents and rarely in children.[25] In younger patients, lipoma arborescens may occur in the absence of an identifiable etiologic agent and affect joints other than the knee, raising the possibility of a distinct primary form of the disease.[24] Lipoma arborescens causes chronic effusions, pain, and swelling and restricts joint motion.[23] The duration of symptoms is often long, and symptoms have been reported to be present for as long as 30 years; however, acute onset has been documented.

Lipoma arborescens most commonly arises in the knee (Figure 124-11), especially the suprapatellar portion, although it also has been observed in the hip, ankle, and wrist joints. One study found that nearly a third of cases demonstrate an atypical presentation, with multifocality or disease outside of the knee.[24] Lipoma arborescens is typically localized to one joint, but several cases of bilateral knee involvement have been described.[23] Laboratory studies are unremarkable, and the joint fluid is clear and yellow.[23] Plain films show joint fullness, and findings of osteoarthritis are often present. Arthrography reveals multiple lobulated filling defects, which on CT represent a villonodular mass of low signal intensity that on MRI has the density of fat (Figure 124-12).[26] At surgery, the affected synovium is seen to have a prominent villous or villonodular architecture and is tan-yellow (Figure 124-13). Histologically, the lesion is composed of sheets of mature adipocytes admixed with



**Figure 124-12** MRI of lipoma arborescens shows a villonodular mass in the knee joint.

nutrient blood vessels, all of which are partially compartmentalized by fibrous septa, and it is covered on its intra-articular surface by several layers of synovial cells (Figure 124-14). Synovectomy may relieve the symptoms and prevent effusions, but associated osteoarthritis may be progressive.[23]

The clinicoradiologic differential diagnosis includes diffuse tenosynovial giant cell tumor, synovial chondromatosis, and synovial hemangioma. These lesions can be distinguished easily from lipoma arborescens by their distinct histologic features. Another disorder that should be included in the differential diagnosis is Hoffa's disease, which is a



**Figure 124-13** Lipoma arborescens composed of a villonodular mass of fatty tissue covered by glistening synovium.

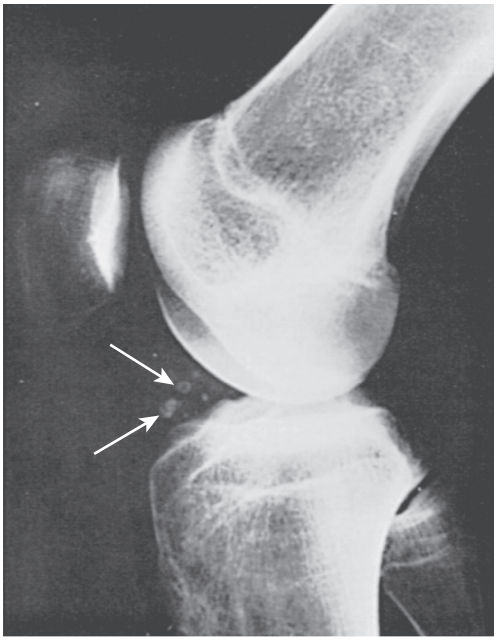


**Figure 124-15** A localized synovial hemangioma with phleboliths prominently seen in the joint *(arrows)*. The patient is 16 years old and has had painful swelling for many years when standing and relief of this pain when the knee is flexed.

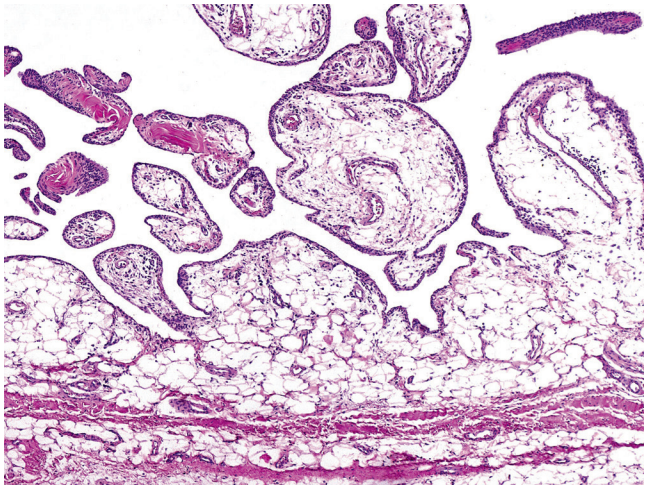


**Figure 124-14** Lipoma arborescens with the subsynovial compartment filled with mature adipocytes and covered by synoviocytes.

condition of irritation, inflammation, and hyperplasia of the synovial lining in regions where fat is normally present, such as adjacent to the patella or patellar ligament.[27]

## Vascular Lesions of the Synovium

Benign vascular tumors of the synovium are rare. Their growth pattern can be localized or diffuse. They tend to predominate in adolescence and young adulthood, but symptoms frequently can be traced back to childhood.[28] The joint most commonly involved is the knee, but hemangiomas also have been described in the elbow, the ankle, the tarsometatarsal and temporomandibular joints, and the tendon sheaths of the wrist and ankle.[29,30] Unusual complications of synovial hemangiomas include a secondary destructive arthritis and Kasabach-Merritt syndrome.

Synovial hemangiomas produce a variety of symptoms, including unilateral, intermittent joint pain and enlargement, which may result in limitation of motion, locking, buckling, and hemarthrosis, especially after minimal trauma.[29] Classically, the affected joint diminishes in size if it is elevated to a sufficient degree to allow the blood to drain out of the lesion. Upon physical examination, the joint is swollen and doughy, and nearby cutaneous hemangiomas may be evident. Joint aspiration frequently yields bloody fluid. Pre-operative diagnosis of localized hemangioma is difficult; the differential diagnosis includes localized tenosynovial giant cell tumor, and in the knee it includes discoid meniscus, meniscal tears, cysts, and ossicles.[23] The diffuse hemangioma is more easily identified, but it can mimic diffuse tenosynovial giant cell tumor and hemophilic arthropathy.

Radiographic evaluation may show nothing more than a vague soft tissue shadow indicative of a swollen synovium and a distended joint capsule or regional osteoporosis in patients who have had long-term symptoms and recurrent hemarthrosis. Rarely, calcified phleboliths are apparent; however, they are more often associated with a soft tissue arteriovenous malformation with secondary joint involvement than with an isolated intra-articular hemangioma (Figure 124-15). Arthrography may show an intra-articular filling defect, and arteriography may be negative in small localized capillary hemangiomas, but contrast material may collect in the more diffuse lesions that contain cavernous or large ectatic vascular spaces (Figure 124-16). CT reveals a lobulated soft tissue mass with mild enhancement after injection of contrast material.[30] MRI may show that the

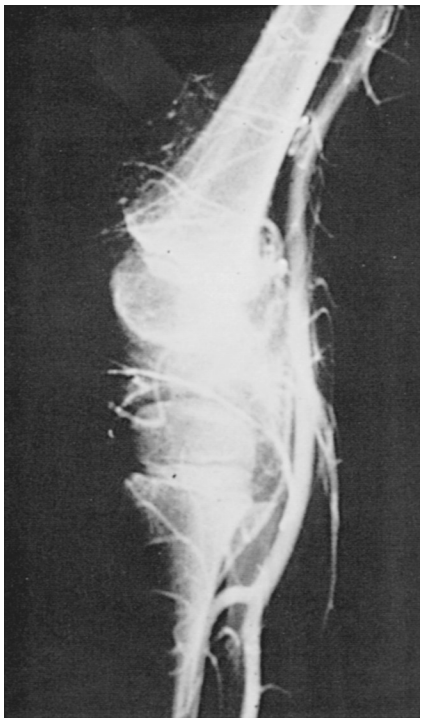


**Figure 124-16** An arteriogram of an arteriovenous malformation in the soft tissues of the thigh and leg with involvement of the knee joint. The extensive vascular blush in the knee indicates aberrant synovial and capsular vasculature.

tumor has a low to isointense signal on T1-weighted images and high signal intensity on T2-weighted images.[31]

Macroscopically, the localized hemangioma tends to be small, but larger lesions (8 cm) have been documented.[31] The hemangioma may be poorly defined or well circumscribed, sessile, or stalked, and it ranges in color from red to dark blue-purple. Microscopically, the hemangioma is usually of the cavernous or venous type, with large dilated blood-filled vessels lined by cytologically benign endothelial cells. In arteriovenous hemangioma, which grows in a diffuse fashion, the entire synovium may be edematous and beefy red or stained brown by hemosiderin, and it consists of prominent tortuous, congested vessels that may penetrate the joint capsule and extend into neighboring soft tissues. Histologically, the vessels recapitulate architecturally abnormal arteries, veins, and capillaries; have abnormal interconnections; and are arranged in a disorganized tangle.

Therapy for a localized hemangioma is marginal surgical excision, which usually is curative. Diffuse lesions frequently are difficult to eradicate because of their extensive nature. Incomplete excision or debulking may be the only surgical option. Radiation therapy is not indicated.

## Fibroma of the Tendon Sheath

Fibromas of the tendon sheath are uncommon benign neoplasms that clinically mimic tenosynovial giant cell tumors but are morphologically distinct. Fibroma of the tendon sheath was first identified as a clinicopathologic entity in 1936, and since that time, more than several hundred cases have been reported.[32,33] A translocation t(2;11) (q31-32;q12) has been identified in this tumor and is likely the driver of the neoplastic process. A translocation t(9;11)(p24;q13-14) also has been documented as an alternative translocation involving 11q in a fibroma of the tendon sheath; therefore, different molecular aberrations are associated with this neoplasm.[34]

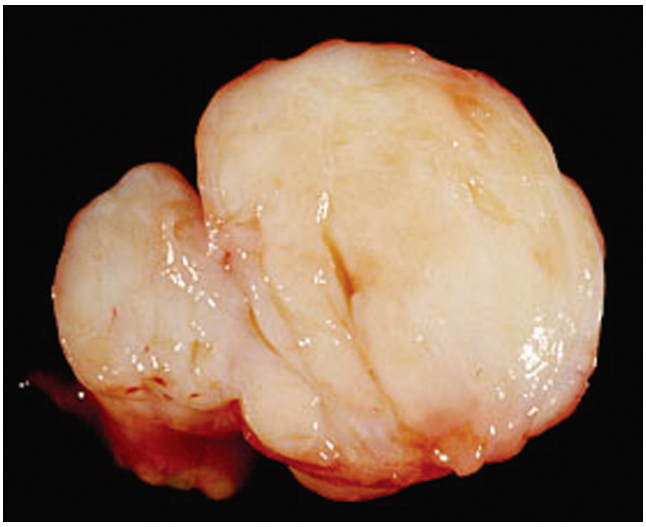


**Figure 124-17** A fibroma of the tendon sheath that formed a well-circumscribed, tan-white mass.

Fibromas of the tendon sheath usually arise from the tendons and sheaths of the flexor surfaces of the distal extremities; approximately 70% of cases involve the fingers or hand. Of the fingers, the thumb is most frequently affected, followed in descending order by the index and middle fingers.[32] Less commonly, large diarthrodial joints such as the knee and rarely the elbow and ankle are sites of origin.[32,33,35] Patients range in age from infants to elderly individuals, but the median age is the early fourth decade of life.[32,33] Most series report a male predominance, with the largest study of 138 cases having a male-to-female ratio of 3:1.[32] Patients present with a slow-growing, painless mass that usually has been noted for several months to a year.[33] In 6% to 10% of cases, a history of antecedent trauma is reported. Tumors developing in large joints can be painful and restrict range of motion, and they may be palpable.[36]

Plain radiographs show soft tissue fullness; rarely is there evidence of bone erosion.[32] CT or MRI shows a solid, well-circumscribed mass of soft tissue density that usually has low signal intensity on T1-weighted images and variable intensity on T2-weighted images. At surgery, the tumors are often attached directly to the tendon or tendon sheath. They are rubbery, oblong, well circumscribed, or encapsulated; average 1.5 to 1.8 cm in their greatest dimension; and have a tan-white cut surface (Figure 124-17).[32,33]

Microscopically, fibromas of the tendon sheath are multilobular, with clefts interposed between adjacent lobules. The lobules are composed of spindle and stellate fibroblasts enmeshed in a collagenous and sometimes myxoid stroma (Figure 124-18). Hypercellular regions reminiscent of nodular fasciitis may be seen. Immunohistochemically, the tumor cells have the staining profile of myofibroblasts, and ultrastructurally, the cells have features of fibroblasts and myofibroblasts.[37]

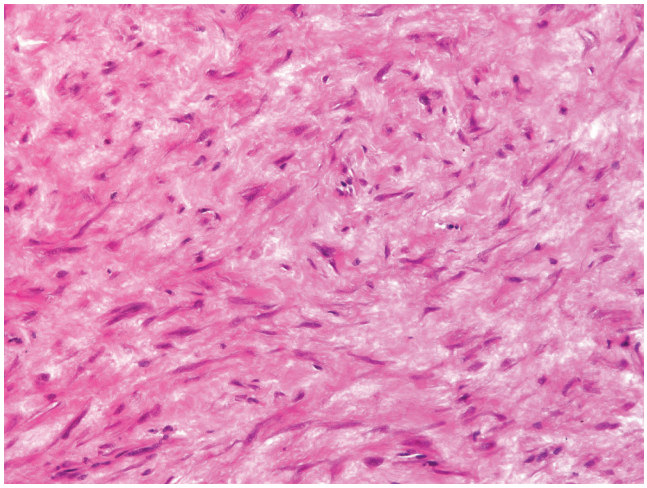


**Figure 124-18** A fibroma of the tendon sheath composed of a hypocellular collagenous mass.

The natural history of a fibroma of the tendon sheath consists of slow growth that eventually ceases. The treatment of choice is surgical excision, but a 24% recurrence rate has been reported.[32]

## Synovial Chondromatosis

Synovial chondromatosis is an uncommon condition characterized by the formation of multiple nodules of hyaline cartilage within the subsynovial connective tissue. If the cartilage nodules undergo endochondral ossification, the term *synovial osteochondromatosis* is appropriate. It is unclear whether the proliferating cartilage is metaplastic or neoplastic; however, more recent cytogenetic abnormalities involving chromosome 6 that are present in the chondrocytes of these lesions support a neoplastic process.[38] Regardless, synovial chondromatosis is benign and does not metastasize.

Synovial chondromatosis most commonly affects middle-aged men, with an average age in the fifth decade of life.[39] In middle-aged women, the disease is more likely to develop in the temporomandibular joint. The genders are equally affected with regard to hand and foot involvement, and patients with hand and foot involvement are usually in their sixth decade.

Patients commonly describe joint pain, swelling, stiffness, crepitance, and limitation of motion with a locking or grating sensation upon movement.[39] Symptoms are usually long-standing, recurrent, and progressive.

Synovial chondromatosis typically arises in large diarthrodial joints. The knee is affected in more than 50% of cases, usually as a monoarticular condition.[39] Other common sites include the hip, elbow, shoulder, and ankle. Infrequently, synovial chondromatosis arises in the small joints of the hands and feet and in the temporomandibular joint.[40] When cartilage nodules develop in the synovial lining of bursae, tendons, and ligaments, this condition is known as *extra-articular synovial chondromatosis*.[36] The extra-articular variant most commonly affects the fingers, followed by the toes, hand, wrist, foot, and ankle, and more than one synovial sheath may be involved.[36]



**Figure 124-19** Synovial chondromatosis of the elbow. Multiple large calcified bodies fill the joint space and are adjacent to bone.

Findings of plain radiographs largely depend on whether the cartilage nodules are calcified or ossified and whether they erode adjacent bony structures. Visible calcifications are absent in 5% to 33% of cases; however, most often, multiple oval intra-articular radiodensities range in size from a few millimeters to several centimeters (Figure 124-19).[41] The pattern of mineralization varies and may appear as irregular flecks that represent calcified cartilage or show a trabecular architecture, which is a manifestation of endochondral ossification. Lesions that are not mineralized can be seen on an arthrogram because they produce multiple filling defects. In approximately 11% of cases, nodules erode the neighboring skeleton, especially along the anterior aspect of the distal femur.

CT may show mass-like nodules in the synovium that have a density similar to skeletal muscle. CT also can detect small calcifications and erosions before they are apparent on plain films. MRI shows that the nodules of cartilage have low signal intensity on T1-weighted sequences and high intensity on T2-weighted sequences, which reflects the high water content of the hyaline cartilage.[41] Areas of calcification or mineralized bone have a low signal intensity on T1-weighted and T2-weighted sequences. CT and MRI scans are helpful in identifying the intra-articular source of the lesion and its anatomic extent. In long-standing disease, the bones adjacent to involved joints may be osteoporotic and may show changes of secondary osteoarthritis.

The cartilage in extra-articular synovial chondromatosis has similar radiographic changes.[41] Nodules of cartilage are frequently mineralized and may appear as a linear arrangement of small calcific densities that are aligned along the sheath and can span many joints (Figure 124-20).

The radiographic differential diagnosis of synovial chondromatosis includes osteochondritis dissecans, osteoarthritis

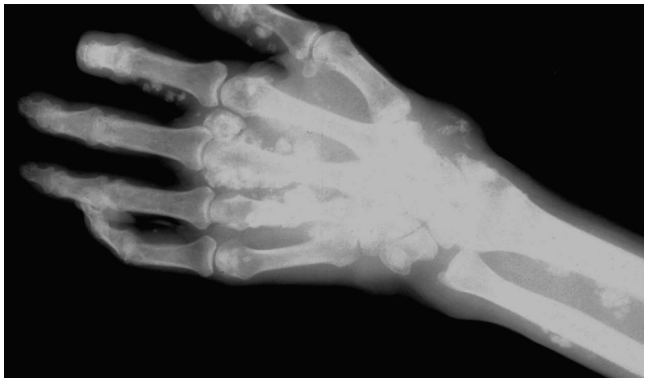


**Figure 124-20** Synovial chondromatosis of the hand and forearm. Multiple calcified nodules of varying size are evident in the soft tissues of the fingers, wrist, and forearm.

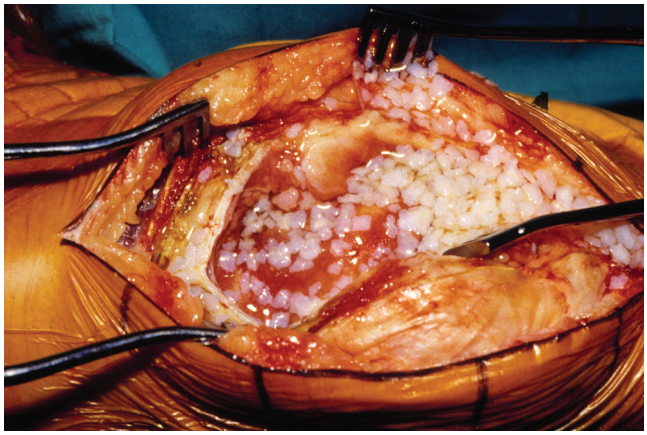


**Figure 124-21** Intraoperative appearance of synovial chondromatosis. Innumerable nodules of cartilage fill the joint.

with loose bodies, tuberculosis, hemopathic arthropathies, pseudogout with extensive synovial calcification, and synovial tumors. In many instances, the clinical presentation and the radiographic picture should lead to the correct diagnosis. However, in a significant proportion of cases, radiographs and the clinical picture are vague, and thus only a biopsy specimen can remove all doubt about the diagnosis.

Characteristic of synovial chondromatosis is a thickened synovium containing numerous opalescent, firm nodules of cartilage that bulge from the surface in a cobblestone pattern (Figure 124-21). These nodules usually measure less than 5 cm and may lose their attachment to the synovium and form loose bodies, sometimes numbering in the hundreds. The calcified cartilage is white, and areas of ossification manifest as gritty tan trabeculae, which may house fatty marrow. The synovium adjacent to the cartilage may show reactive changes, such as edema, hyperemia, hyperplasia, and villous transformation.

The cartilage nodules develop in the connective tissue of the subsynovial compartment (Figure 124-22). Neoplastic chondrocytes produce the hyaline matrix that eventually forms individual nodules, which peripherally abut the surrounding tissues (Figure 124-23). The cartilage varies in its cellularity, and chondrocytes range from small to large; some may be binucleate and hyperchromatic, similar to the

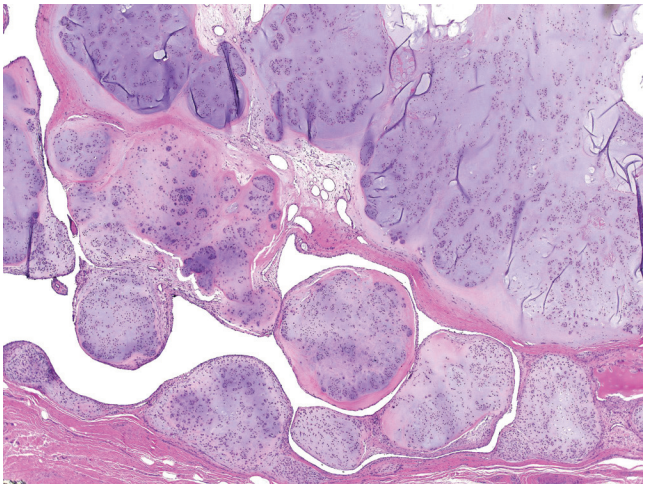


**Figure 124-22** Synovial chondromatosis with nodules of hyaline cartilage in the synovium.

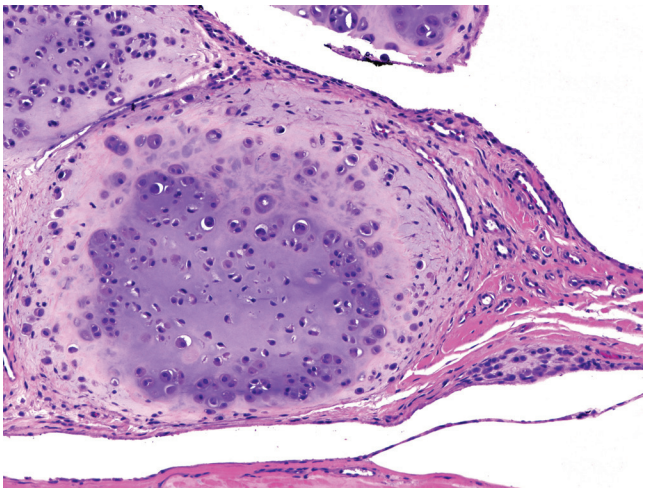


**Figure 124-23** Nodules of hyaline cartilage merging with surrounding connective tissue in synovial chondromatosis.

chondrocytes in intraosseous chondrosarcoma (Figure 124-24). Despite these histologic findings of concern, experience has shown that hypercellular variants with atypical chondrocytes usually behave in a benign fashion. Infrequently, the disease manifests as a single, solitary nodule of cartilage, which may be very large and may undergo partial endochondral ossification (synovial osteochondroma). Intra-articular osteochondroma can severely limit joint motion and may be confused clinically with other types of neoplasms.[42]

Over time, nodules of cartilage attached to the synovium can be invaded by blood vessels, which initiates the process of endochondral ossification, with woven and lamellar bone formation and the development of a medullary cavity with fatty marrow (Figure 124-25). If these nodules lose their synovial attachments and become free-floating, they may continue to increase in size because the cartilage derives its nourishment from synovial fluid, although the osseous portion and the marrow die.

The treatment of choice for synovial chondromatosis consists of excision of the involved synovium and removal of all loose bodies. The prognosis is good, although

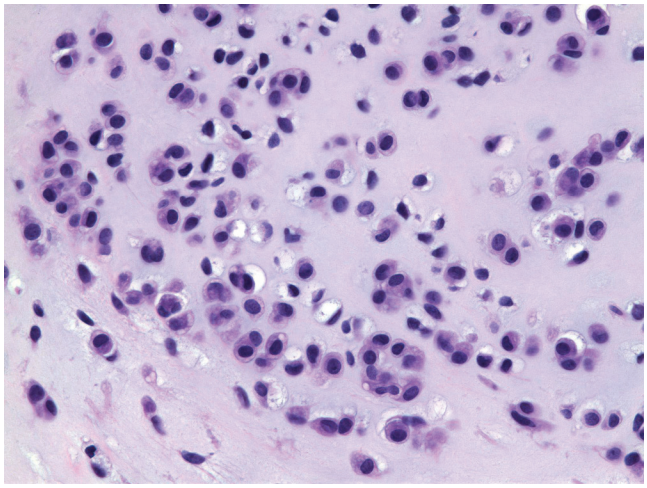


**Figure 124-24** The cartilage in synovial chondromatosis can be cellular, and the chondrocytes may exhibit limited cytologic atypia.

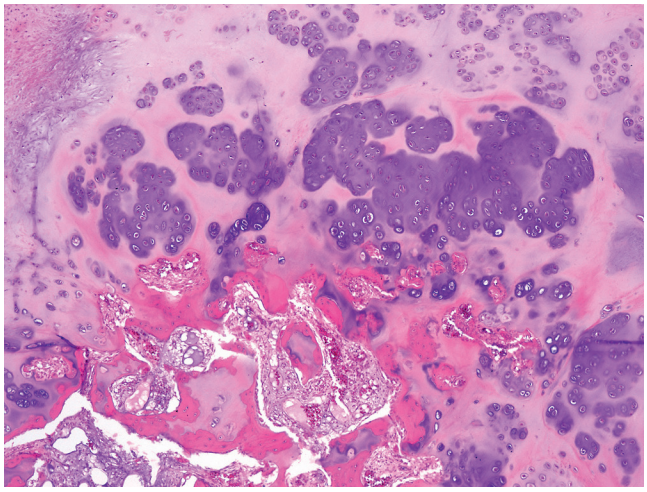


**Figure 124-25** Nodules of cartilage in synovial chondromatosis undergoing endochondral ossification.

recurrences may develop if removal is incomplete. Most recurrences occur in the setting of diffuse involvement of the synovium.

Synovial chondromatosis rarely undergoes malignant transformation into chondrosarcoma, although in one series, this phenomenon occurred in 6.4% of cases.[43] However, a significant percentage of the reported cases of synovial chondrosarcomas have shown evidence of underlying synovial chondromatosis.[44-46] The chondrosarcomatous component exhibits marked hypercellularity, severe cytologic atypia, and prominent mitotic activity; the stroma is often myxoid.

## Chondroma of Tendon Sheath and Periarticular Structures

Solitary soft tissue chondroma is a benign neoplasm that commonly arises in tendon sheaths and infrequently involves joint capsules or other periarticular structures.

Tendon sheath chondromas usually arise in the flexor tendon sheaths of the distal extremities and are about three



**Figure 124-26** An intra-articular solitary chondroma of the knee in the infrapatellar region. The mass is well delineated with amorphous dense calcification, suggesting mineralized cartilage. *(Courtesy Dr. C. Campbell.)*

times more common in the hands than in the feet.[47-49] They affect the sexes equally and are detected in early to mid adulthood, presenting as a painless, slowly growing, firm mass. Radiographically, tendon sheath chondromas appear as an extraosseous, well-delineated soft tissue mass that contains calcifications, which are punctate or ring-like in 33% to 70% of cases.[50,51] Grossly, the tumors are ovoid, firm, blue-white, well-circumscribed masses of hyaline cartilage that are usually 1 to 2 cm in dimension and, in contrast to synovial chondromatosis, are solitary. Histologically, the hyaline cartilage is well formed with occasional small foci of myxoid change. The cartilage can be cellular, and chondrocytes may show limited cytologic atypia, which sometimes causes confusion with chondrosarcoma.[52] The treatment of choice is simple excision, which is infrequently associated with local recurrence.[48,49,52]

The intracapsular and periarticular regions are uncommon sites for soft tissue chondromas. When they occur, they usually originate in the anterior infrapatellar region of the knee (Figure 124-26).[53] In this location, chondromas can achieve a large size (8 cm) and may interfere with knee motion. Their structure and biologic behavior are similar to those of soft tissue chondromas that arise elsewhere. Intracapsular chondromas have been reported in the knees of three members of a family with familial dysplasia epiphysealis hemimelica.[50] Two other cases have been described in which cartilaginous hamartomas of the volar plates of the proximal and distal interphalangeal joints of the hands and feet were associated with peculiar hypertrophic skin lesions of the hand and hemihypertrophy of the limb.

## Tenosynovial Giant Cell Tumor

Tenosynovial giant cell tumors comprise a group of benign tumors that affect the synovial lining of joints, tendon

sheaths, and bursae.[51] These lesions were previously known as *giant cell tumor of the tendon sheath*, *localized nodular synovitis*, and *pigmented villonodular synovitis*. Tenosynovial giant cell tumors may be localized or diffuse and intra-articular or extra-articular. They have the potential to be locally aggressive in that they may invade into bone, grow through joint capsules, extend along tendons, and infiltrate into adjacent soft tissues. Despite their destructive potential, these lesions generally do not have the capacity to metastasize.

The common histologic denominator of these lesions is the neoplastic proliferation of synovial-like cells that may form a localized mass or spread along the synovial surface and invade downward into subsynovial connective tissue. The growing cells expand the subsynovial compartment, producing finger-like extensions, villi, and redundant folds. These projections often fuse into nodules and form convoluted lobulated masses admixed with a tangle of hair-like villi. The process may be a local phenomenon involving only part of the synovial lining, or it may be extensive, with the entire synovial surface affected.

Until recently, the origin of tenosynovial giant cell tumor was unknown. Previously considered a reactive process, possibly in response to repeated hemorrhage, many tenosynovial giant cell tumors result from a translocation between chromosomes 1p13 and 2q35 in which the gene encoding macrophage colony-stimulating factor 1 (M-CSF; also called colony stimulating factor 1 [CSF1]) *(CSF1)* is fused to the collagen VI alpha-3 *(COL6A3)* gene.[51,54,55] Consequently, overexpression of CSF1 occurs in the neoplastic cells, which account for only 2% to 16% of cells in the mass.[54] The remaining cells largely represent nonneoplastic inflammatory cells that are recruited into the tumor because they contain the receptor for MCSF/CSF1 (CSFR1).[54,55] This phenomenon has been termed a *landscape effect* and is also observed in certain types of lymphomas and sarcomas. A subset of tumors demonstrates high M-CSF expression in the absence of the 1p13 translocation, suggesting the presence of alternate mechanisms of M-CSF elevation in a proportion of cases.[56] Indeed, novel M-CSF transcripts and a *CSF1-S100A10* fusion gene resulting from a translocation t(1;1)(q21;p11) have been described, which may help elucidate additional mechanisms of M-CSF overexpression in this disease.[57]

### Tenosynovial Giant Cell Tumor of Joints and Tendon Sheaths: Diffuse Type (Pigmented Villonodular Synovitis)

The diffuse type of tenosynovial giant cell tumor of the joint involves large areas of the synovial lining, although uninvolved areas are invariably present. Its incidence is approximately 1.8 per 1 million. Although it may occur in all age groups, spanning children to elderly people, most of these tumors affect adults who are in the third to fourth decade of life.[58] The sexes tend to be equally affected, although some series have reported a predominance of males or of females.[58-60] Diffuse tenosynovial giant cell tumor of the joint usually manifests as monoarticular arthritis. Bilaterality or involvement of multiple separate sites has been infrequently reported. Some patients with polyarticular disease also have had significant congenital anomalies. Primary complaints include pain and effusions that can be intermittent with repeated bouts of over a long time, ranging from months to years.[58,60] The involved area may be stiff, swollen, and warm, and a palpable mass sometimes can be appreciated. Point tenderness is detected in approximately 50% of patients. Anatomic instability of the involved joint is uncommon.

The knee joint is affected most commonly and is involved in about 80% of cases.[58,60] The next most frequent sites are the hip, ankle, calcaneocuboid joints, elbow, and tendon sheaths of fingers and sometimes toes. Occasionally, the palm, the sole of the foot, and unusual locations such as the temporomandibular joint and posterior elements of the spine are involved. Bursal involvement is rare, but if it happens, it usually occurs in the popliteal and iliopectineal bursae and the bursa anserina. Infrequently, the disease affects large tendon sheaths proximal to the ankle and wrist and produces a periarticular soft tissue mass.[61,62] It is thought that some of these lesions dissect through a joint capsule or a tendon sheath and extend along fascial planes to produce a soft tissue mass.[61]

Invasion of bone on either side of a joint can be seen with intra-articular, bursal, or tendon sheath involvement. This phenomenon most frequently occurs when the tumor involves "tight" joints, such as the hip, elbow, wrist, and feet, or when tendon sheaths are closely apposed to neighboring bones (Figure 124-27).[63,64] Rarely, only one bone may be invaded by an intra-articular lesion, and in this situation, it may be difficult to distinguish this type of lesion from a primary bone tumor (Figure 127-28).[65]

Joint aspiration frequently yields blood-tinged brown fluid that lacks diagnostic abnormalities.[66] Synovial fluid analysis may show a low glucose content, a minimally elevated protein level, and a fair mucin clot. The inflammatory cell count is usually low but may be elevated. Similar findings can be seen in trauma, Charcot's joint, bleeding disorders, sickle cell disease, and Ehlers-Danlos syndrome.



**Figure 124-27** Pigmented villonodular synovitis involving the small joints of the foot with multiple bone erosions. No calcification is present in the lesion.

In at least two-thirds of cases, a soft tissue density due to the tumor or effusion or both can be visualized on a plain film.[67-70] Joint narrowing or calcification is uncommon. Arthrography may show numerous nodular filling defects that extend into an expanded joint space. Arteriograms are unusually striking as a result of the prominent vascularity of the tumor. An inverse correlation tends to exist between the degree of vascularity and the amount of fibrosis or scarring of the lesion.

CT and MRI are useful in delineating the extent of disease and can detect intralesional lipid and hemosiderin deposits, which are important diagnostic features.[67-69] The tumor has low signal intensity (equal to that of skeletal muscle) on T1-weighted images and is heterogeneous on T2-weighted images. Extension into the bone manifests radiographically as multiple, well-marginated, subchondral cyst–like lucencies or juxtacortical oval pressure erosions (see Figures 124-27 and 124-28).[64,65] In the knee, the femoral area adjacent to the intercondylar region is the site most frequently invaded as the tumor grows along the cruciate ligamentous insertions. Periarticular osteopenia, periosteal reactions, and joint destruction are unusual because the joint space is preserved until late in the course of the disease.[68,69] The radiographic differential diagnosis of a given case includes (1) tuberculosis, which generally involves more osteopenia and joint destruction; (2) hemophilia, which is also associated with more extensive joint destruction; (3) synovial chondromatosis, which frequently has calcified radiopaque bodies; and (4) rheumatoid arthritis, which shows more severe osteopenia and joint narrowing.

Grossly, the synovium in diffuse tenosynovial giant cell tumor is red-brown to mottled orange-yellow and looks like a plush Angora rug (Figure 124-29). Matted masses of villous projections and synovial folds are prominent and are admixed with rubbery-to-soft nodules (0.5 to 2 cm in diameter) that are sessile or pedunculated. The synovial membrane is thick and succulent and is often coated with a fibrinous exudate. Red-brown or golden brown tissue may extend deep into subsynovial structures or may invade the joint capsule. If a tendon sheath is involved, a sausage-shaped mass may be evident because the sheath is distended by the proliferating tumor. If the joint capsule is invaded, adjacent soft tissue structures, including nerves and vessels, may be covered by wispy, red-brown tissue. If soft tissue invasion is extensive, the lesion may appear as a soft-to-rubbery, red-brown mass with foci of hemorrhagic cysts. Similar tissue may be present near the chondro-osseous junction or may be wrapped around vascular and ligamentous attachments to bone surfaces, which represent entrance points into the interior of the bone. Although other conditions, such as hemochromatosis and hemosiderosis, also may discolor the synovium brown, the nodular component is usually absent; in addition, microscopic features are definitive for separating these entities (discussed in a subsequent section).

Microscopic examination reveals marked synovial cell hyperplasia with surface proliferation and, more importantly, subsynovial invasion by masses of mitotically active polygonal or round tumor cells with moderate amounts of eosinophilic cytoplasm and round nuclei (Figures 124-30 and 124-31). Included among the invading tumor cells are scattered lymphocytes, multinucleated giant cells (osteoclast, Touton, or foreign body type), hemosiderin-laden macrophages, and fibroblasts. Hemosiderin is usually present and can be located in the stroma or the cytoplasm of synovial lining cells, macrophages, and tumor cells. Foci of hemorrhage are common and are surrounded peripherally by giant cells and macrophages (Figure 124-32). Scattered collections of foamy macrophages (xanthoma cells) filled with



**Figure 124-28** Pigmented villonodular synovitis involving the tibiofibular joint with an adjacent extensive soft tissue mass and eccentric erosion of both bones, simulating a primary bone tumor. The knee joint is normal.

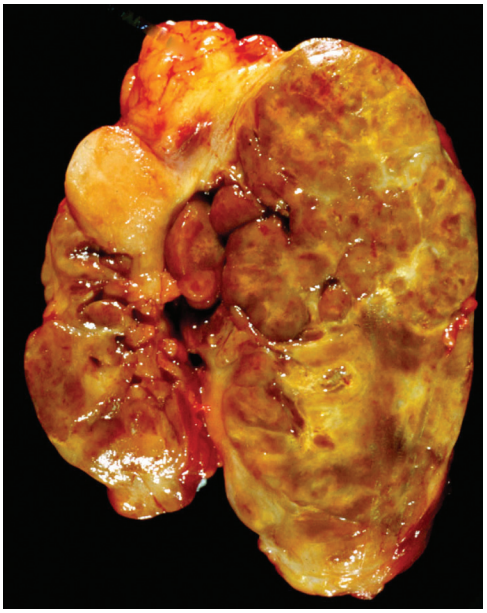


**Figure 124-29** A diffuse type of tenosynovial giant cell tumor consisting of a mottled brown-yellow-red villonodular mass.

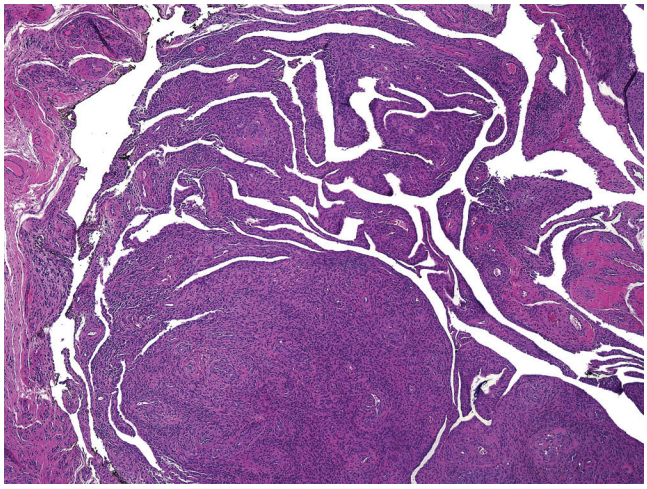


**Figure 124-30** A diffuse type of tenosynovial giant cell tumor growing with a villonodular architecture. Invading cells produce the nodular configuration.

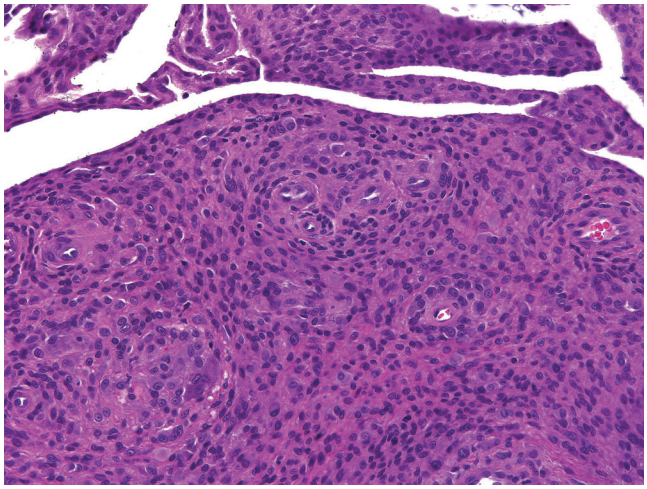


**Figure 124-31** Synovial lining cells covering the mass of proliferating polyhedral cells admixed with multinucleated giant cells.

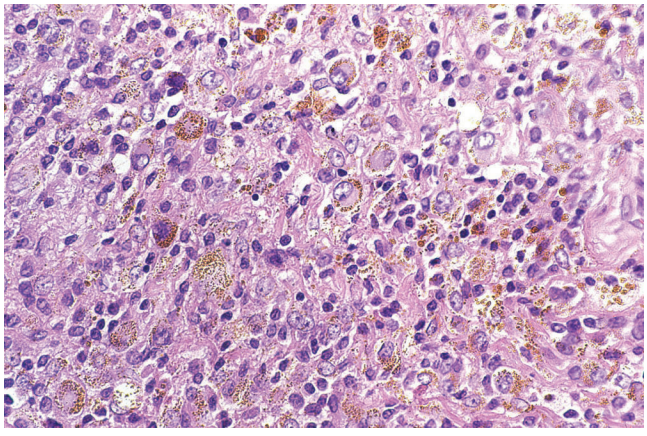


**Figure 124-32** Sheets of macrophages containing abundant hemosiderin.

lipid also are a frequent finding. These different cell populations fill and distend the synovial villi, causing them to fuse with adjacent ones, forming nodules. In some nodules, abundant collagen deposition with hyalinization may cause confusion with neoplastic bone. Rarely, the tumor may contain focally calcified cartilaginous matrix.[71]

Immunohistochemical studies have been interpreted to support a synovial cell or fibrohistiocytic phenotype of the neoplastic cells.[37,70] The tumor cells express clusterin and D2-40, and a minority of large cells stain with antibodies to desmin.[72] It is important to note that the cells that harbor the translocation express CSF1. Flow cytometric analyses have shown that some of these tumors, especially tumors that have large extra-articular soft tissue components, may be aneuploid and have high proliferative indices.[62] Although these flow cytometric findings may help predict which cases would be more locally aggressive, examples of diffuse tenosynovial cell tumors with these attributes have not metastasized.[62]

Treatment of diffuse tenosynovial giant cell tumor of the joint is not standardized and has included radiation therapy, total synovectomy, isotopic synoviorthesis, arthrodesis, bone grafting, and primary arthroplasty.[58,60,73] Although no single therapy has been consistently successful, wide synovectomy is currently the recommended treatment.[58,60,74] It is difficult to perform an actual complete synovectomy, however; residual involved synovium frequently remains, causing a local recurrence rate of 16% to 48%.[75,76] A macroscopically incomplete tumor resection is associated with a more than twofold increased risk for tumor recurrence compared with a macroscopically complete resection.[74] Tumors arising in the knee have a higher rate of recurrence compared with tumors arising in other joints. Rarely, recurrent disease or tumors with large extra-articular components may require more radical surgery, such as ray resection or amputation.[62] Studies have shown that moderate doses of radiation may control disease and even cure patients with such extensive disease, possibly obviating the need for radical surgery or amputation.[77] Finally, drugs targeting the CSF1 pathway are being tested to determine their efficacy in clinically challenging cases.[78] Imatinib mesylate is a tyrosine kinase inhibitor (TKI) with activity against CSFR1, and early studies have shown success in the treatment of progressive or relapsing diffuse tenosynovial giant cell tumor of the joint.[79] Phase II clinical trials with nilotinib, a TKI with similar activity against CSF1R, are currently ongoing.[79] Heterogeneity of tumor responses between different TKI agents has been documented and underscores the need for a better understanding of the associated molecular and genetic mechanisms.[80] Similar targeted approaches are being tested with monoclonal antibodies such as MCS110, a humanized monoclonal antibody directed against CSF1, and RG7115, a monoclonal antibody that inhibits the dimerization of CSFR1; both are currently being investigated in clinical trials.[74]

### Malignant Diffuse Tenosynovial Giant Cell Tumor

Malignant tenosynovial giant cell tumors are rare; only a handful of cases have been reported.[81-83] The knee is the most commonly affected joint, and in many cases, a

co-existing benign-appearing, diffuse tenosynovial giant cell tumor is noted. In the malignant variant, the neoplastic cells may be spindle or polyhedral shaped and cytologically malignant. These tumors have the capacity to behave aggressively; almost 50% of patients die from metastatic disease.[81] Trisomies involving chromosomes 5 and 7 have been reported.[84]

### Localized Tenosynovial Giant Cell Tumor of the Joint (Benign Giant Cell Synovioma, Benign Synovioma, and Localized Nodular Synovitis)

A localized tenosynovial giant cell tumor of the joint manifests as a solitary, well-circumscribed mass. It usually consists of a single sessile or pedunculated, sometimes lobulated, tumor that ranges from 1 to 8 cm in diameter (Figure 124-33). Most commonly, it is unilateral, arises in the knee, and is equally distributed between the sexes.[85]

Symptoms are similar to those of diffuse tenosynovial giant cell tumor, except that in the localized variant, a higher frequency of joint locking is reported because the mass interferes with motion.[85] A few patients may present with acute severe joint pain caused by torsion and infarction of the tumor. Effusions are common, but the synovial fluid tends to be less bloody than in the diffuse variant, and it may be clear.

Imaging studies show a heterogeneous nodular mass that contains lipid and hemosiderin deposits (Figure 124-34). In the knee joint, the tumor frequently arises in the suprapatellar notch, in the femoral notch, and between the meniscus and the joint capsule.[85] Usually, no bone invasion occurs. Marginal excision is generally curative. Small lesions can be extirpated arthroscopically.[85]

Histologically, localized tenosynovial giant cell tumor of the joint is identical to the nodules of the diffuse variant. The main difference is that the prominent synovial villi present in the diffuse variant are absent or sparse.

### Localized Tenosynovial Giant Cell Tumor of the Tendon Sheath (Giant Cell Tumor of the Tendon Sheath and Fibroxanthoma of the Tendon Sheath)

Localized tenosynovial giant cell tumors of the tendon sheath usually involve the hand or wrist and less frequently the foot or ankle.[59] They are the most common soft tissue tumor of the hand and usually arise from the flexor tendon sheaths of the finger; the index finger is affected most frequently, followed in descending order of frequency by the third, fourth, fifth, and first fingers.

Tumors of the digits predominate in females, with a male-to-female ratio of at least 2:1[59,86]; tumors of the toes have an equal sex distribution.[59] On average, patients are in the third to fifth decades of life and present with a painless, palpable, firm, mobile mass.

Clinically, the mass usually is solitary and is located on the flexor surface, but it may bulge into the extensor or lateral aspects of the digits (Figure 124-35). The tumors are slow growing, and the intervals between detection and surgical treatment have ranged from several weeks to longer than a decade, with an average interval of slightly longer than 2 years.[59,86]

Radiographically, the tumors appear as well-circumscribed soft tissue masses, and in about 25% of cases, adjacent extrinsic excavation of the cortical bone has a sclerotic margin (Figure 124-36).[59,86] MRI reveals that the lesions have a hypointense signal on T1-weighted images and either a hypointense or a hyperintense signal on T2-weighted images. These findings are helpful in distinguishing giant cell tumors of the tendon sheath from other soft tissue tumors.[87]

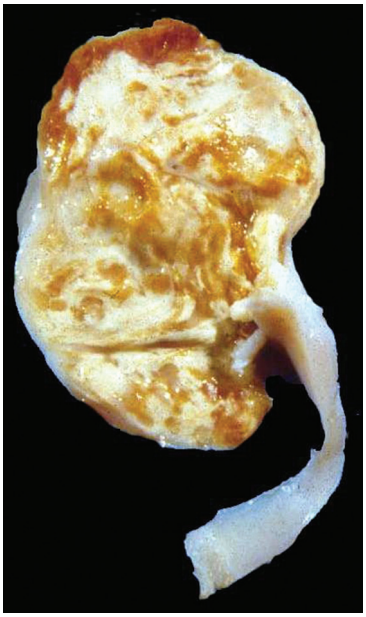


**Figure 124-33** A localized tenosynovial giant cell tumor of the joint with attached pedicle. The mass is well circumscribed and brown-yellow.

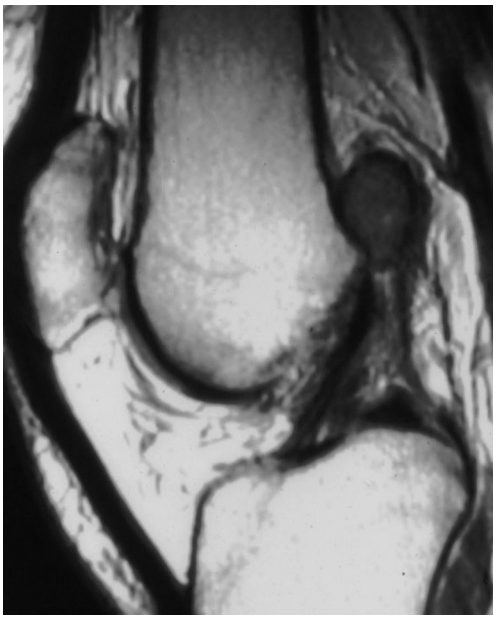


**Figure 124-34** MRI shows a well-delineated dark mass in the posterior knee joint.



**Figure 124-35** A tenosynovial giant cell tumor manifesting as a mobile, solid, firm mass.



**Figure 124-36** The cortex of the phalanx is eroded by the tenosynovial giant cell tumor.

The gross pathologic findings are of a well-circumscribed, multinodular, round, rubbery, variegated red-brown-tan-yellow mass, generally not larger than 5 cm in diameter, which is firmly attached but easily peeled off the involved tendon (Figure 124-37). At surgery, the lesion sometimes may "pop out" of the incision. The cut surface reveals a solid mass that ranges from hues of yellow to orange-red-brown, depending on the quantity of lipid and blood pigments present. Often bands or septa of white fibrous tissue subcompartmentalize the lesion. The structure and immunophenotype of the cell types present are identical to those in the diffuse variant (Figure 124-38).[72,88] Ultrastructurally,

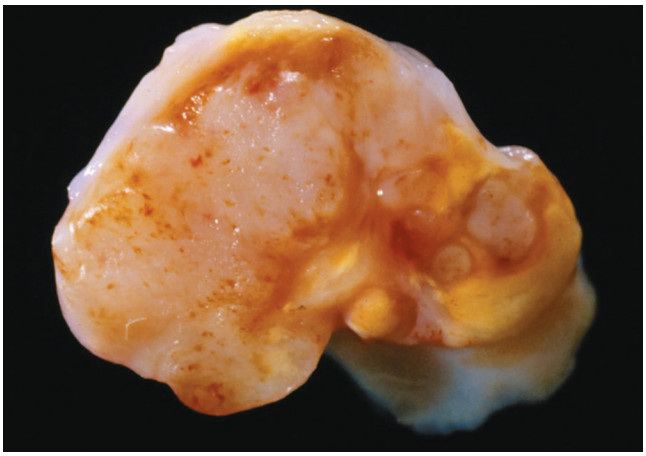


**Figure 124-37** A well-delineated, white-yellow, and focally brown tenosynovial giant cell tumor.

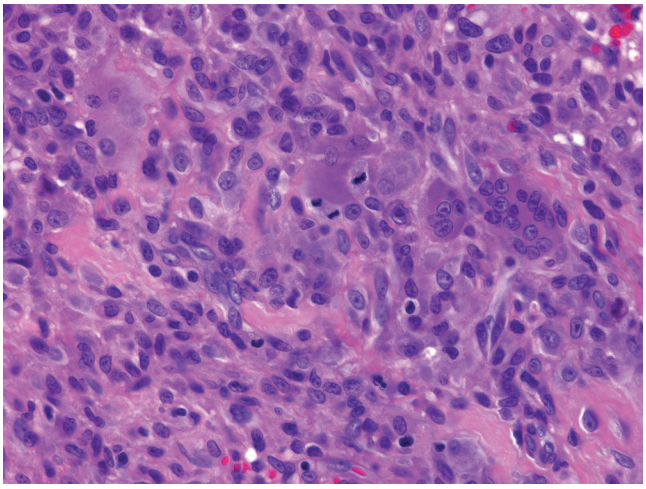


**Figure 124-38** Mitotically active polyhedral cells and scattered osteoclast-type giant cells in a tenosynovial giant cell tumor.

proliferating cells have features similar to type A and type B synovial lining cells.[86-89] Flow cytometry has been performed in a few cases, and all have been diploid.[62] Cytogenetic studies show that many of these tumors have a translocation between 1p13 and 2q35.[54,55,90]

These tumors are benign and do not metastasize. Malignant giant cell tumors of the tendon sheath have rarely been reported.[81,91] The treatment of choice is conservative surgical excision, which is usually curative. Local recurrence may be noted if excision is incomplete.[59,86]

## MALIGNANT TUMORS OF THE JOINT

Malignant tumors of joints are uncommon and are classified into primary and secondary types. Primary malignancies are virtually always sarcomas and usually arise within the synovium of large diarthrodial joints, especially the knee. Patients are adults who present with chronic symptoms of pain, swelling, and effusion, and most tumors are chondrosarcomas or synovial sarcomas. Rarely, other sarcomas originate within a joint; our experience has included cases of intra-articular myxoinflammatory fibroblastic sarcoma,

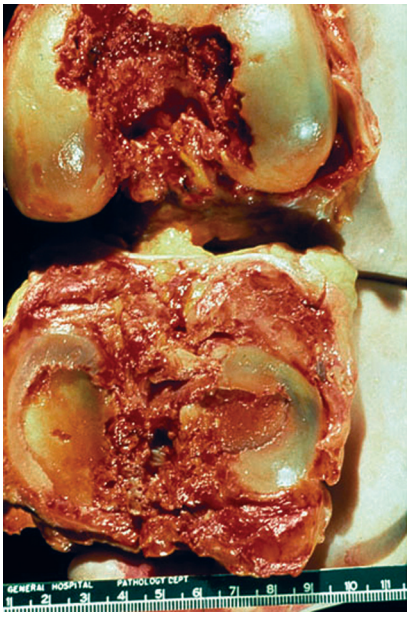



**Figure 124-39** Angiosarcoma of synovium of the knee joint. The hemorrhagic tumor erodes into the distal femur and proximal tibia.

pleomorphic fibrosarcoma, extraskeletal myxoid chondrosarcoma, conventional chondrosarcoma, malignant tenosynovial giant cell tumor, and angiosarcoma (Figure 124-39). Epithelioid sarcoma is another primary sarcoma that has been reported to arise from the joint exceptionally rarely.[92] Secondary malignant tumors of joints, by definition, originate beyond the confines of the joint; most are sarcomas that extend from neighboring bone or surrounding soft tissue. Although synovial tissue is highly vascular, metastases or involvement of the synovium by carcinoma, lymphoma, or leukemia is uncommon.

## Primary Sarcomas of Joints

### Conventional Chondrosarcoma

Conventional chondrosarcoma arising in the synovium is unusual; fewer than 100 cases have been reported in the English language.[43-46,82,93] In approximately 50% of cases, the chondrosarcoma arises in association with pre-existing synovial chondromatosis, and investigators studying a large series of synovial chondromatosis reported malignant transformation into chondrosarcoma in 6.4% of cases.[43,45-47,93,94] Patients are usually in the fifth to seventh decade of life, and distribution between the sexes is equal.[93] Typically, the tumor manifests as a progressively enlarging joint mass that may cause mechanical dysfunction, pain, and stiffness. In patients who have pre-existing synovial chondromatosis, the duration of symptoms is usually long, and in some instances, symptoms may be present for 25 years.[43,93] Most chondrosarcomas arise in the knee joint, followed by the hip and elbow joints.

Radiographic studies usually show a periarticular soft tissue mass that may have dense irregular or ring-like calcifications. Occasionally, invasion into the medullary cavity of adjacent bone is evident. The radiographic differential diagnosis varies according to the presence of calcification and includes synovial chondromatosis, synovial sarcoma, diffuse tenosynovial giant cell tumor, and chronic synovitis.[93]

Grossly, the involved joint is filled with synovium massively thickened by innumerable nodules of opalescent blue-white cartilage. The nodules of cartilage vary in size and may be free-floating in the joint cavity. In several cases, the tumor has extended into adjacent soft tissue and bone.

Microscopically, the tumor is composed of malignant hyaline and myxoid cartilage. Rarely, the matrix is entirely myxoid and has features of extraskeletal myxoid chondrosarcoma.[94] The neoplastic cartilage is cellular and contains cytologically atypical chondrocytes. The periphery of the lobules of cartilage is usually the most cellular, and in this region, some of the tumor cells can be spindled. Other findings include necrosis and permeation of invaded bone.[93] Coexisting synovial chondromatosis can be identified by its well-formed nodules of hyaline cartilage, which are less cellular, containing cytologically banal-appearing chondrocytes and a matrix that is frequently mineralized.

Treatment is usually surgical extirpation with consideration given for chemotherapy in high-grade lesions or lesions that have metastasized. Inadequate surgical removal virtually ensures local recurrence, which may necessitate subsequent radical excision. Metastases have occurred in approximately one-third of reported patients; the lung is the most common site for systemic spread.[43,93]

### Synovial Sarcoma

Synovial sarcoma is a common sarcoma that accounts for approximately 6% to 10% of soft tissue sarcomas. It usually develops in the deep soft tissues and rarely arises in joints (Figure 124-40), but it may secondarily invade articular synovium from neighboring soft tissues. Earlier descriptions of this tumor attest to its wide spectrum of structure because such names as *adenosarcoma* and *synovial fibrosarcoma* were used until the term *synovial sarcoma*, first introduced in 1936, became commonplace. The structure of synovial sarcoma mimics a joint in its early stage of development in that it contains cleft-like spaces and glands delineated by large polygonal (epithelioid) cells surrounded by fascicles of spindle cells. The clefts and glands simulate a microscopic "joint space" that is bounded by synovial lining cells and is supported by subsynovial mesenchymal cells. Because epithelial or spindle cells may predominate, synovial sarcoma has been subtyped into biphasic and monophasic spindle cell and epithelial types, although the last type is exceptionally rare.

Synovial sarcoma commonly affects adolescents and young adults. In a series of 121 cases, the age range was 9 to 74 years, with a median age of 34 years; however, it occurs in children with significant frequency.[95,96]

The term *synovial sarcoma* implies that the tumor originates from the synovium; however, fewer than 10% of cases are intra-articular or show continuity with a synovial lining.[97,98] The cell of origin is thought to be an immature mesenchymal progenitor cell that is thus far incompletely characterized.[99]

Approximately 60% to 70% of synovial sarcomas arise in the extremities, especially the lower limb, in the vicinity of large joints, particularly the popliteal areas of the knee

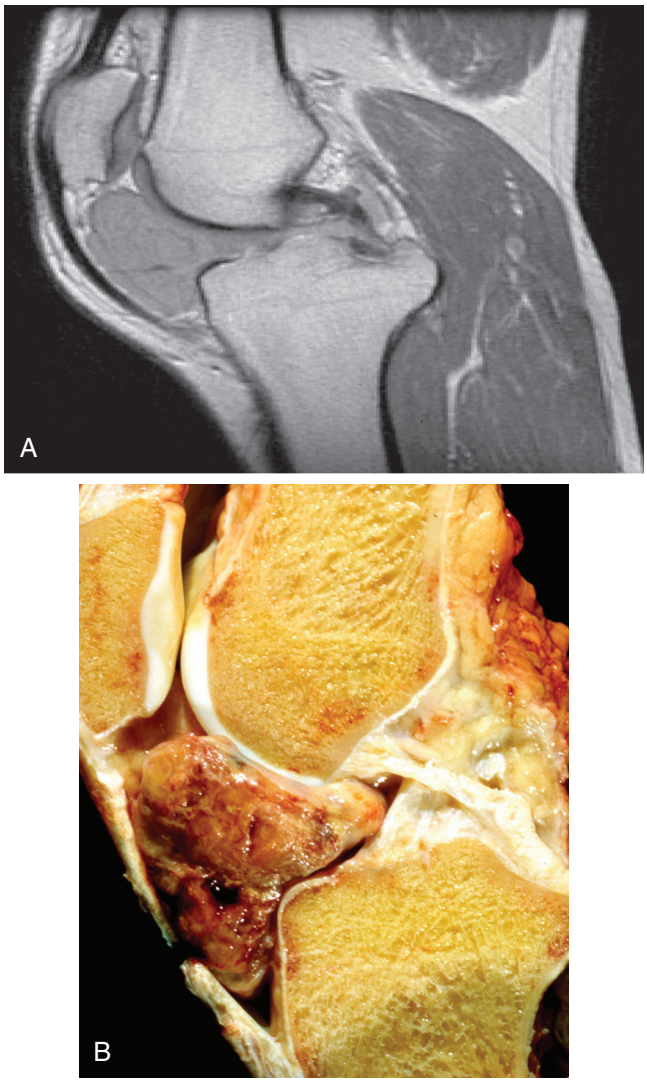


**Figure 124-40** **A,** MRI of a rare example of intra-articular synovial sarcoma. The infrapatellar tumor is well circumscribed and has a focal inhomogeneous appearance. **B,** The gross specimen shows that the tan, hemorrhagic tumor bulges into the joint but is covered by synovium.

and foot.[100,101] Regions of the thigh, hand, leg, and digits may be affected, and in the distal extremities, tumors are often adjacent to joint capsules, tendon sheaths, or both. Tumors also have been reported in the neck, torso, craniofacial region, retropharyngeal space, retroperitoneum, spermatic cord, orbit, tongue, mediastinum, soft palate, heart, liver, kidney, lung, pleura, and prostate.

No clinical features specific to synovial sarcoma distinguish it from other sarcomas. The most common presentation is the development of a slowly enlarging, deep-seated, palpable mass that is painful in about 50% of cases.[96,101] Symptoms may be present for an unusually long time, ranging from months to 25 years, with an average of about 6 months to 2.5 years, before medical evaluation is sought.[96,101] Delay in diagnosis is more frequent with tumors that are located in the deep soft tissues than in tumors that are based in the more superficial and clinically noticeable regions. In some cases involving the knee region, vague mild pain over several months may occur before a mass is appreciated, and if the tumor reaches a large size, limitation of motion finally may be noted. Head and neck lesions produce symptoms related to their specific sites, such as hoarseness and breathing or swallowing difficulties. Rarely, a patient may present with a symptom resulting from pulmonary metastasis, such as hemoptysis.[101]

Classically, plain film findings of synovial sarcoma consist of a well-circumscribed, deep-seated, soft tissue mass. Synovial sarcoma is one of the few primary soft tissue tumors that frequently calcify. Approximately 30% to 50% of cases reveal radiographically detectable calcifications that may have a fine, stippled, or dense appearance (Figure 124-41).[102] The calcification may be focal or may present throughout most of the tumor.[103] Periosteal reaction of adjacent bone is elicited in approximately 20% of cases, but bone is rarely invaded by the tumor.

CT is more sensitive than plain radiography in showing calcification or periosteal reaction. MRI is important in delineating the anatomic extent of the tumor and usually shows a large inhomogeneous mass with areas of hemorrhage. The radiographic differential diagnosis includes

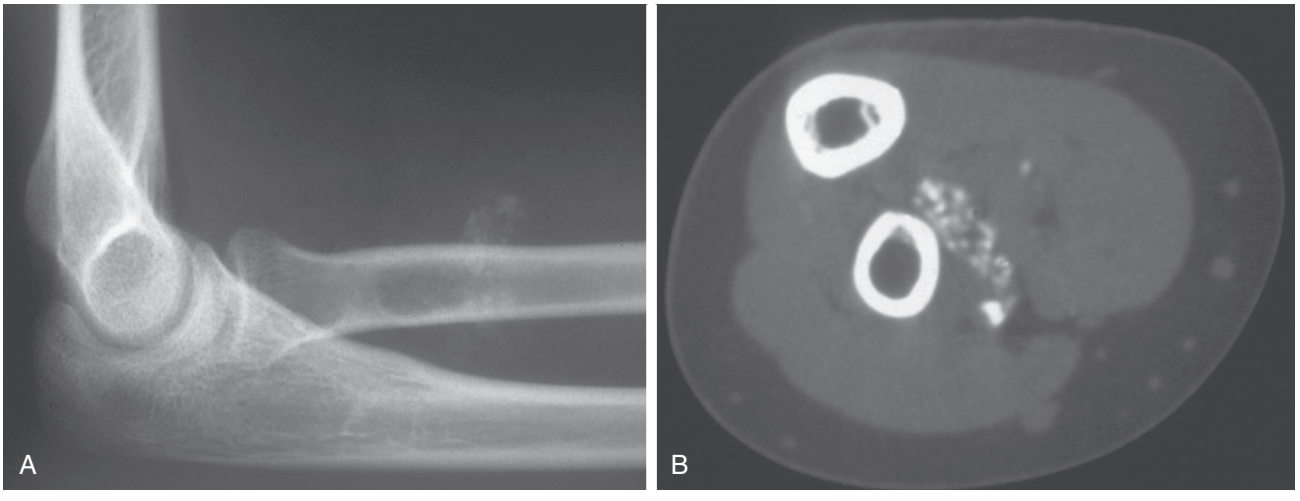


**Figure 124-41** **A,** Focally mineralized synovial sarcoma in the deep soft tissues in the vicinity of the elbow joint. **B,** An axial computed tomography scan shows intratumoral calcification.

hemangioma, lipoma, synovial chondromatosis, soft tissue chondrosarcoma or osteosarcoma, myositis ossificans, aneurysm, and other sarcomas.

The gross pathologic findings of synovial sarcoma reveal a well-demarcated, pink-tan, fleshy mass that easily detaches or "shells out" from its tumor bed (Figure 124-40B). The cut surface is usually uniform, gray-yellow, and rubbery. Calcified areas are gritty and hard. In larger tumors, areas of hemorrhage, necrosis, or both with cystification and gelatinous breakdown of tissue may be seen. A synovial sarcoma sometimes grows between tendons, muscles, and fascial planes or wraps around neurovascular bundles.

Synovial sarcoma is subtyped into three patterns on the basis of predominant microscopic findings: monophasic spindle cell, monophasic epithelial, and biphasic variants. Use of such a classification system requires some subjectivity because many of these tumors have a variable histologic picture. A useful differential observation is that marked cellular pleomorphism and atypia usually are not present in synovial sarcoma and, when observed, tend to point to some other type of neoplasm, such as a pleomorphic fibrosarcoma or an undifferentiated pleomorphic sarcoma.

Microscopically, the hallmark of the more common biphasic synovial sarcoma is the two different populations of neoplastic cells consisting of epithelial and spindle cells (Figure 124-42). Epithelial cells may be cuboidal or columnar and, similar to true epithelium, may have well-defined cytoplasmic borders. These cells may form glandular spaces, line papillae or cleft-like spaces, or grow in cohesive groups (see Figure 124-42). Epithelial cells usually are surrounded by fascicles of uniform, small, and plump spindle cells. Spindle cell fascicles are densely cellular and frequently are arranged in a "herringbone" pattern. In most biphasic synovial sarcomas, the spindle cell component predominates, and calcification of hyalinized stroma most frequently occurs in spindle cell regions. Some tumors may show bone formation, which may be present in spindle or epithelial areas. In the monophasic variants, spindle or epithelial cells may be the only recognizable component (Figure 124-43; also see Figure 124-42).

Immunohistochemistry has shown that epithelial and the spindle cell components frequently stain with antibodies to keratin and epithelial membrane antigen, which usually indicates an association with epithelial neoplasms.[104-106] This pattern of reactivity has helped make it possible to separate synovial sarcomas from morphologically similar tumors, such as fibrosarcomas and malignant peripheral nerve sheath tumors.[106] It has also provided evidence that synovial sarcoma does not arise from or recapitulate the synovium, because normal synovial cells do not stain with these antibodies.[107]

Cytogenetic studies of synovial sarcomas have detected a consistent translocation of t(X;18)(p11.2;q11.2) in almost all cases, regardless of whether the tumor is biphasic or monophasic.[100,108] This finding provides insight into the genesis of synovial sarcoma, which is thought to be related to dysregulation of transcription and can be used as a diagnostic feature. This characteristic translocation leads to the fusion of the SWI/SNF chromatin remodeling complex subunit *SS18* to the C-terminal repression domains of *SSX1* or *SSX2*, disrupting epigenetic control and acting as the central genetic "driver" in this tumor.[99] No relationship between the location of the translocation breakpoint and the prognosis has been discerned.

The prognosis of synovial sarcoma is poor. In one study of 150 patients with nonmetastatic disease, 5-year, 10-year, and 15-year disease-free survival rates were 59%, 52%, and 52%, respectively.[103] Many factors influence prognosis; tumors that are small (<5 cm), arise in patients younger than 25 years, and lack poorly differentiated areas have a high rate of cure.[96] In contrast, large tumors (≥5 cm) that arise in patients 25 years or older and that contain poorly differentiated areas have a dismal outcome.[96] The impact of histologic subtype has been controversial.

The natural history of synovial sarcoma consists of local recurrence, which may be repetitive. Most recurrences manifest within 2 years after initial treatment, but intervals longer than 10 years are not exceptional. Ultimately, metastases occur in many patients, with the lungs the most common site; regional lymph node involvement has been

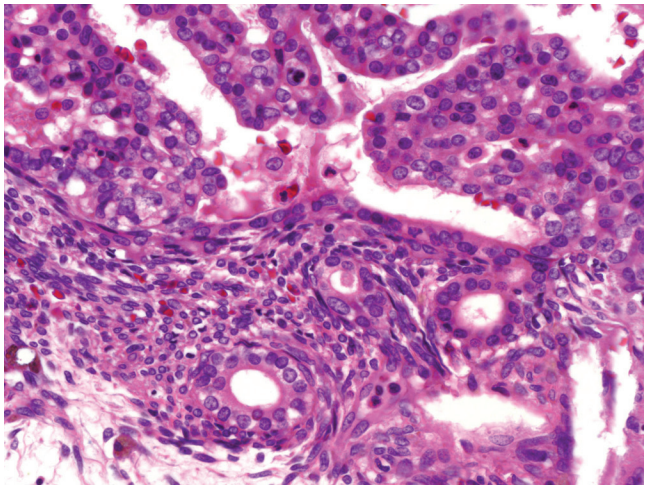


**Figure 124-42** A biphasic synovial sarcoma with epithelial cells forming glands and papillary structures. The spindle cell component surrounds the glands.

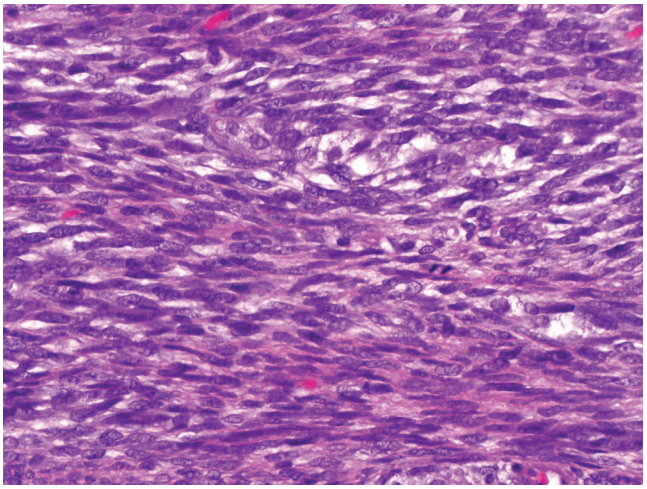


**Figure 124-43** A monophasic spindle cell variant of synovial sarcoma with the fascicles of tumor cells forming a herringbone pattern.

reported in a few patients.[109] About 10% of patients die within 1 year after diagnosis with metastatic disease, and 90% of these patients have massive pulmonary metastases.

Treatment must contend with issues involved with local and systemic therapy. Successful local control usually can be achieved through limb salvage surgery combined with radiation.[109,110] Because regional lymph nodes may be involved, their status should be evaluated carefully, and if enlarged, they should be treated. Systemic treatment consists of various chemotherapy regimens that have provided questionable benefit, although adjuvant chemotherapy usually is recommended for patients who are at high risk—that is, patients with tumors larger than 5 cm.[111]

## Secondary Malignant Tumors of the Joint

### Sarcomas

Primary sarcomas of bone, such as osteosarcoma and chondrosarcoma, infrequently involve a joint because intact articular cartilage usually acts as a barrier to direct tumor extension. However, when it is observed, joint invasion usually occurs via a pathway created by a transarticular fracture, via growth along tendoligamentous structures, or through capsule insertion sites. Because of this circumstance, on histologic grounds alone it may be difficult to distinguish some forms of synovial chondromatosis from a low-grade intraosseous chondrosarcoma that has secondarily spread into the joint.

Similarly, primary soft tissue sarcomas gain access into the interior of a joint by growing through the joint capsule in conduits occupied by pre-existing vascular structures or along tendons and ligaments. This complication can make providing adequate therapy challenging because treatment may require en bloc resection of the joint.

### Metastatic Carcinoma

The synovium, in contrast to other richly vascular tissues, is rarely the site of metastatic carcinoma; this may reflect the fact that only clinical cases in which joint symptoms prevail are reported, because at autopsy, joints are not routinely examined. Most carcinomas that metastasize to the synovium originate in the lung, followed by the gastrointestinal tract and breast.[112-114] Affected patients are usually elderly, and the knee is the most frequently involved joint. In many reported cases, the underlying bone also contains metastatic deposits.

## Malignant Lymphoproliferative Disease

The various types of malignant lymphoproliferative diseases, including leukemia, lymphoma, and myeloma, can involve the synovium and may produce osteoarticular symptoms.[115-117] This complication occurs most frequently in individuals with leukemia and is seen in both acute and chronic forms.[117] Joint symptoms have been observed in 12% to 65% of children and in 4% to 13% of adults with leukemia.[117] Arthritis can develop at any time during the disease course and can be the presenting symptom. Large joints are affected more commonly than are small joints, and the arthritis is often pauciarticular, asymmetric, migratory, and severe. Symptoms may result from leukemic infiltration of the synovium or irritation of the neighboring periosteum. When arthritis is the major presenting symptom, it may cause confusion with septic arthritis, rheumatic fever, subacute bacterial endocarditis, or rheumatoid arthritis.



The references for this chapter can also be found on ExpertConsult.com.

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